

K-122797

Ethicon Endo-Surgery, LLC. 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

510(k) Summary

JAN 25 2013

Company

Ethicon Endo-Surgery, LLC
475 Calle C
Guaynabo, PR 00969

Contact

Emily Kruetzkamp, Regulatory Affairs Associate
Ethicon Endo-Surgery, Inc
Telephone: (513) 337-1546
Fax: (513) 337-2802
Email: ekruetzk@its.jnj.com

Date Prepared: September 10, 2012

Device Name

Trade Name: ENSEAL® G2 Articulating Tissue Sealers
Common Name: Electrosurgical Cutting and Coagulation Instruments

Classification Names

- Electrosurgical, Cutting & Coagulation & Accessories
(21 CFR 878.4400, Product code GEI)
- Electrocautery, Gynecologic and Accessories
(21 CFR 884.4120, Product code HGI)

Predicate Device

ENSEAL® G2 Tissue Sealers, K112033

Device Description:

The Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers are sterile, single-use surgical instruments designed to seal and cut vessels, and to cut, grasp and dissect soft tissue during open and laparoscopic surgery.

The energy activation and knife lock are activated by a single button. The instrument shaft can be rotated using the rotation knob to facilitate visualization and enable easy access to targeted tissue. The power cord is permanently attached to the device and connects the instrument to the generator. The ENSEAL G2 Articulating Tissue Sealers include an articulation capability that allows the user to articulate the end-effector during surgical procedures.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Indication for Use:

The ENSEAL® G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with the Generator GEN11 (GEN11). They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7mm and tissue and/or vascular bundles as large as will fit in the jaws of the instruments.

The ENSEAL® G2 Articulating Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures.

Technological Characteristics:

The Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers components are similar to the Predicate. The ENSEAL G2 Articulating Tissue Sealers have a 5 mm diameter shaft and are available in two shaft lengths: 35 and 45cm; and two jaw types: 3mm curved jaw and 5mm straight jaw. The jaws are normally in the opened position and can be partially or fully closed by squeezing the closing handle. The jaws have atraumatic teeth for grasping and holding targeted tissue when clamped. The handles of the Subject ENSEAL G2 Articulating Tissue Sealers have an ergonomic interface for the user. Like the Predicate, the Subject devices include an integrated energy activation button. The devices use a combination of the application of bipolar electrosurgical energy in conjunction with the I-BLADE knife, to compress, coagulate, and transect tissue.

In addition to the similar technological characteristics as the Predicate, the ENSEAL G2 Articulating Tissue Sealers instrument shaft device articulates approximately 60° to the left and the right of center. The instrument shaft can be articulated using the articulation wheel to gain additional access to tissue and facilitate the user's ability to target tissue and vascular bundles up to 60°. The top of the articulation wheel has a white arrow to indicate when the device shaft is straight and not articulated. When the articulation wheel is rotated clockwise, the shaft articulates to the right; when the articulation wheel is rotated counterclockwise, the shaft articulates to the left. While articulated, the device jaws seal and cut vessels, and cut, grasp and dissect soft tissue as intended. The Subject ENSEAL G2 Articulating Tissue Sealers performance is equivalent to the Predicate ENSEAL G2 Tissue Sealers.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Performance Data:

Ex-vivo and in-vivo tests were performed to verify that the performance of the ENSEAL G2 Articulating Tissue Sealers meets the definition of substantial equivalence to the Predicate device, ENSEAL G2 Tissue Sealers. Device performance was assessed against design requirements, including a 30-day chronic survival study, to demonstrate that the Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers perform as intended.



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center - WO66-G609
Silver Spring, MD 20993-0002

Ethicon Endo-Surgery, LLC
% Ethicon Endo-Surgery, Incorporated
Mrs. Emily Kruezkamp
Regulatory Affairs Associate
4545 Creek Road
Cincinnati, Ohio 45242

January 25, 2013

Re: K122797

Trade/Device Name: ENSEAL® G2 Articulating Tissue Sealers
Regulation Number: 21 CFR 878.4400
Regulation Name: Electrosurgical cutting and coagulation device and accessories
Regulatory Class: Class II
Product Code: GEI, HGI
Dated: January 16, 2013
Received: January 22, 2013

Dear Mrs. Kruezkamp:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you; however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical

Page 2 – Mrs. Emily Kruezkamp

device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please go to <http://www.fda.gov/AboutFDA/CentersOffices/CDRH/CDRHOffices/ucml15809.htm> for the Center for Devices and Radiological Health's (CDRH's) Office of Compliance. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to <http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm> for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address <http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm>.

Sincerely yours,

Peter D. Rumm-S

Mark N. Melkerson
Acting Director
Division of Surgical Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Indications for Use Form

Indications for Use

510(k) Number (if known): _____

Device Name: ENSEAL® G2 Articulating Tissue Sealers

Indications for Use:

The ENSEAL® G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with the Generator GEN11 (GEN11). They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7mm and tissue and/or vascular bundles as large as will fit in the jaws of the instruments.

The ENSEAL® G2 Articulating Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures.

Prescription Use X AND/OR Over-The-Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE
OF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Page 1 of 1

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(Division Sign-Off)

Division of Surgical Devices

510(k) Number K122797



U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Control Center WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

November 02, 2012

ETHICON ENDO-SURGERY, LLC
C/O ETHICON ENDO-SURGERY, INC.
4545 CREEK ROAD
CINCINNATI, OHIO 45242
ATTN: EMILY KRUEZKAMP

510k Number: K122797

Product: ENSEAL G2 ARTICULATING TISSUE

On Hold As of 10/31/2012

We are holding your above-referenced Premarket Notification (510(k)) for 30 days pending receipt of the additional information that was requested by the Office of Device Evaluation. Please remember that all correspondence concerning your submission MUST cite your 510(k) number and be sent in duplicate to the Document Mail Center at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review. Please refer to this guidance for information on current fax and e-mail practices at

<http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089402.htm>.

The deficiencies identified represent the issues that we believe need to be resolved before our review of your 510(k) submission can be successfully completed. In developing the deficiencies, we carefully considered the statutory criteria as defined in Section 513(i) of the Federal Food, Drug, and Cosmetic Act for determining substantial equivalence of your device. We also considered the burden that may be incurred in your attempt to respond to the deficiencies. We believe that we have considered the least burdensome approach to resolving these issues. If, however, you believe that information is being requested that is not relevant to the regulatory decision or that there is a less burdensome way to resolve the issues, you should follow the procedures outlined in the "A Suggested Approach to Resolving Least Burdensome Issues" document. It is available on our Center web page at:

<http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Overview/MedicalDeviceProvisionsofFDAModer nizationAct/ucm136685.htm>.

In accordance with 21 CFR 807.87(l), FDA may consider a 510(k) to be withdrawn if the submitter fails to provide additional information within 30 days of an Additional Information (AI) request. FDA generally permits submitters additional time to respond to such requests. FDA intends to automatically grant a maximum of 180 calendar days from the date of the AI request, even if the submitter has not requested an extension. Therefore, submitters are no longer required to submit written requests for extension. However, submitters should be aware that FDA intends to issue a notice of withdrawal under 21 CFR 807.87(l) if FDA does not receive, in a submission to the appropriate Document Control Center, a complete response to all of the deficiencies in the AI request within 180 calendar days of the date that FDA issued that AI request. In this instance, pursuant to 21 CFR 20.29, a copy of your 510(k) submission will remain in the Office of Device Evaluation. If you then wish to resubmit this 510(k) notification, a new number will be assigned and your submission will be considered a new premarket notification submission.

Records Processed under FOIA Request 2015-6323. Released by CDRH on 1-30-2017
For further information regarding how various FDA and industry actions that may be taken on 510(k)s should affect the review clock for purposes of meeting the Medical Device User Fee Amendments of 2012 (MDUFA III), to the Federal Food, Drug, and Cosmetic Act, you may refer to our guidance document entitled "Guidance for Industry and Food and Drug Administration Staff - FDA and Industry Actions on Premarket Notification (510(k)) Submissions: Effect on FDA Review Clock and Goals". You may review this document at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089735.htm>.

Please remember that the Safe Medical Devices Act of 1990 states that you may not place this device into commercial distribution until you receive a decision letter from FDA allowing you to do so.

If you have procedural questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301)796-7100 or at their toll-free number (800)638-2041, or contact the 510k staff at (301)796-5640.

Sincerely yours,

Marjorie Shulman
Director, 510(k) Program
Premarket Notification Section
Office of Device Evaluation
Center for Devices and Radiological Health



U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Control Center WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

September 13, 2012

ETHICON ENDO-SURGERY, LLC
C/O ETHICON ENDO-SURGERY, INC.
4545 CREEK ROAD
CINCINNATI, OHIO 45242
ATTN: EMILY KRUETZKAMP

510k Number: K122797

Received: 9/12/2012

Product: ENSEAL G2 ARTICULATING TISSUE

The Food and Drug Administration (FDA), Center for Devices and Radiological Health (CDRH), has received the Premarket Notification, (510(k)), you submitted in accordance with Section 510(k) of the Federal Food, Drug, and Cosmetic Act (Act) for the above referenced product and for the above referenced 510(k) submitter. Please note, if the 510(k) submitter is incorrect, please notify the 510(k) Staff immediately. We have assigned your submission a unique 510(k) number that is cited above. Please refer prominently to this 510(k) number in all future correspondence that relates to this submission. We will notify you when the processing of your 510(k) has been completed or if any additional information is required. **YOU MAY NOT PLACE THIS DEVICE INTO COMMERCIAL DISTRIBUTION UNTIL YOU RECEIVE A LETTER FROM FDA ALLOWING YOU TO DO SO.**

Please remember that all correspondence concerning your submission **MUST** be sent to the Document Mail Center (DMC) at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official 510(k) submission.

On September 27, 2007, the President signed an act reauthorizing medical device user fees for fiscal years 2008 - 2012. The legislation - the Medical Device User Fee Amendments of 2007 is part of a larger bill, the Food and Drug Amendments Act of 2007. Please visit our website at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Overview/MedicalDeviceUserFeeandModernizationActMDUFMA/default.htm>

for more information regarding fees and FDA review goals. In addition, effective January 2, 2008, any firm that chooses to use a standard in the review of ANY new 510(k) needs to fill out the new standards form (Form 3654) and submit it with their 510(k). The form may be found at <http://www.fda.gov/AboutFDA/ReportsManualsForms/Forms/default.htm>.

We remind you that Title VIII of the Food and Drug Administration Amendments Act of 2007 (FDAAA) amended the PHS Act by adding new section 402(j) (42 U.S.C. § 282(j)), which expanded the current database known as ClinicalTrials.gov to include mandatory registration and reporting of results for applicable clinical trials of human drugs (including biological products) and devices. Section 402(j) requires that a certification form <http://www.fda.gov/AboutFDA/ReportsManualsForms/Forms/default.htm> accompany 510(k)/HDE/PMA submissions. The agency has issued a draft guidance titled: "Certifications To Accompany Drug, Biological

Product, and Device Applications/Submissions: Compliance with Section 402(j) of The Public Health Service Act, Added By Title VIII of The Food and Drug Administration Amendments Act of 2007"

<http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/PremarketNotification510k/ucm134034.htm>. According to the draft guidance, 510(k) submissions that do not contain clinical data do not need the certification form.

Please note the following documents as they relate to 510(k) review: 1) Guidance for Industry and FDA Staff entitled, "Interactive Review for Medical Device Submissions: 510(k)s, Original PMAs, PMA Supplements, Original BLAs and BLA Supplements". This guidance can be found at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089402.htm>. Please refer to this guidance for information on a formalized interactive review process. 2) Guidance for Industry and FDA Staff entitled, "Format for Traditional and Abbreviated 510(k)s": This guidance can be found at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm084365.htm>. Please refer to this guidance for assistance on how to format an original submission for a Traditional or Abbreviated 510(k).

In all future premarket submissions, we encourage you to provide an electronic copy of your submission. By doing so, you will save FDA resources and may help reviewers navigate through longer documents more easily. Under CDRH's e-Copy Program, you may replace one paper copy of any premarket submission (e.g., 510(k), IDE, PMA, HDE) with an electronic copy. For more information about the program, including the formatting requirements, please visit our web site at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/ucm134508.html>. In addition, the 510(k) Program Video is now available for viewing on line at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/PremarketNotification510k/ucm070201.htm>.

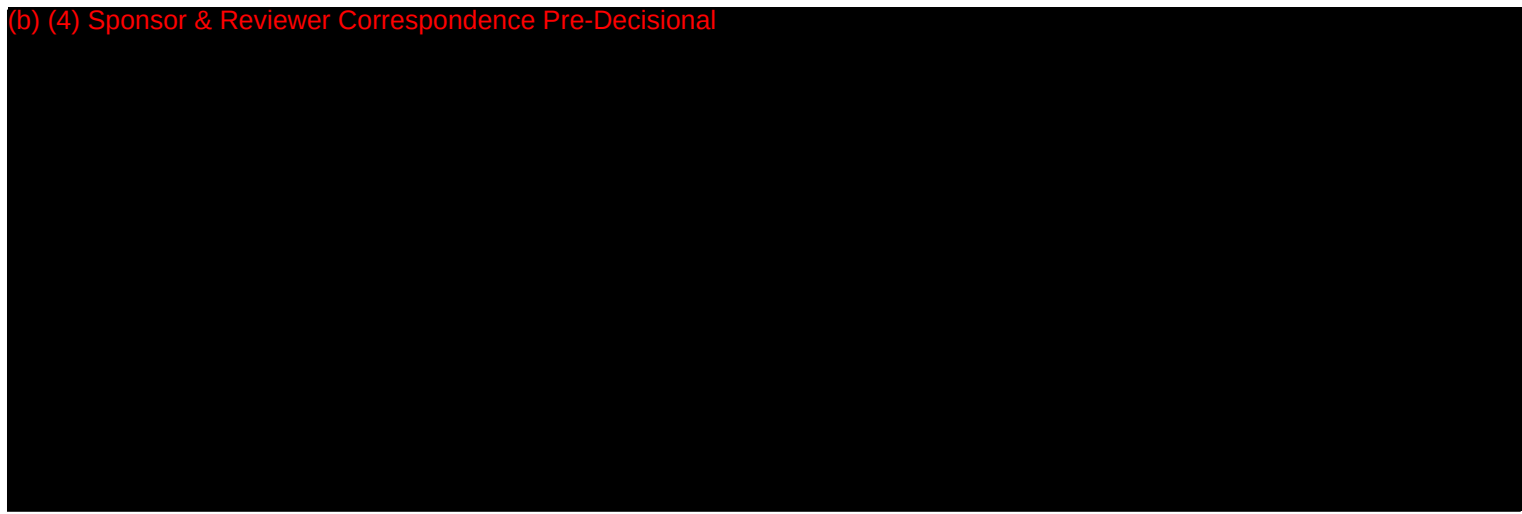
Please ensure that whether you submit a 510(k) Summary as per 21 CFR 807.92, or a 510(k) Statement as per 21 CFR 807.93, it meets the content and format regulatory requirements.

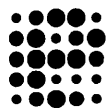
Lastly, you should be familiar with the regulatory requirements for medical devices available at Device Advice <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm>. If you have questions on the status of your submission, please contact DSMICA at (301)796-7100 or the toll-free number (800)638-2041, or at their internet address <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm>. If you have procedural questions, please contact the 510(k) Staff at (301)796-5640.

Sincerely,

510(k) Staff

(b) (4) Sponsor & Reviewer Correspondence Pre-Decisional





**Ethicon
Endo-Surgery**

PART OF THE JOHNSON & JOHNSON FAMILY OF COMPANIES

SEP 12 2012

Received 6260

K122797

Ethicon Endo-Surgery, Inc.
4545 Creek Road
Cincinnati, OH 45242

September 10, 2012

Food and Drug Administration
Center for Devices and Radiological Health
Office of Device Evaluation
Division of General and Plastic Surgery Devices
Document Mail Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

Re: Traditional 510(k) Premarket Notification, ENSEAL® G2 Articulating Tissue Sealers

Dear Sir or Madam:

Pursuant to 21 CFR 807.90, Ethicon Endo-Surgery is submitting two copies of this 510(k) notification for the Enseal. The design of the device is based upon the Predicate device ENSEAL® G2 Tissue Sealers, cleared under K112033. The basis of this submission is that the ENSEAL® G2 Articulating Tissue Sealers are new devices substantially equivalent to a legally marketed device.

The following information is provided in this cover letter per the guidance document *Format for Traditional and Abbreviated 510(k)s*:

Submission Type:	Traditional 510(k)
Submission Date:	September 10, 2012
510(k) Submitter:	Ethicon Endo-Surgery, LLC
Contact Information:	Emily Kruetzkamp, Regulatory Affairs Associate Phone: 513.337.1546 Fax: 513.337.2802 Email: ekruetzk@its.jnj.com
Common Name:	Electrosurgical Cutting and Coagulation Instruments
Trade Name:	ENSEAL® G2 Articulating Tissue Sealers
Classification Names:	Electrosurgical, Cutting & Coagulation & Accessories Electrocautery, Gynecologic and Accessories
Device Class:	Class II
Panel:	General & Plastic Surgery
Classification Code:	GEI, HGI
Classification Regulation:	21 CFR 878.4400, 21 CFR 884.4120
Predicate:	ENSEAL® G2 Tissue Sealers, K112033
Establishment Registration:	3005075853

Confidentiality Preference: Please keep this submission confidential per 21 CFR 807.9
following table contains answers to general questions regarding this submission.

Questions	Yes	No
Is the device intended for prescription use (21 CFR 801 Subpart D)?	X	
Is the device intended for over-the-counter use (21 CFR 807 Subpart C)?		X
Does the device contain components derived from tissue or other biologic source?		X
Is the device provided sterile?	X	
Is the device intended for single use?	X	
Is the device a reprocessed single use device?		X
If yes, does the device type require reprocessed validation data?		
Does the device contain a drug?		X
Does the device contain biologic?		X
Does the device use software?		X
Does the submission include clinical information?		X
Is the device implanted?		X

The unique payment identification number (PIN) assigned to this submission is MD6063192-956733. A copy of the Medical Device User Fee Cover Sheet is included in Section 1.

Per the instructions accessed at 'Electronic Copies for Pre-Market Submissions', an electronic copy is provided with this submission and it is an exact duplicate of the original paper submission. The electronic copy is accompanied by extra copies of the 510(k) cover letter and the 510(k) Truthful and Accuracy Statement with original signatures.

(b) (4), (b) (6)

Sincerely,

(b) (4), (b) (6)

Emily Krutzkamp
Regulatory Affairs Associate

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

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Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers**Screening Checklist for Traditional 510(k)**

Title	Present	Inadequate	N/A
MDUFMA Cover Sheet	Pg 3		
CDRH Premarket Review Submission Cover Sheet	Pg 6		
510(k) Cover Letter	Pg 13		
Indications for Use Statement	Pg 16		
510(k) Summary or 510(k) Statement	Pg 18		
Truthful and Accuracy Statement	Pg 22		
Class III Summary and Certification	N/A	N/A	N/A
Financial Certification or Disclosure Statement	N/A	N/A	N/A
Declarations of Conformity and Summary Reports (Abbreviated 510(k)s)	N/A	N/A	N/A
Executive Summary	Pg 27		
Device Description	Pg 31		
Substantial Equivalence Discussion	Pg 43		
Proposed Labeling	Pg 52		
Sterilization/Shelf Life	Pg 96		
Biocompatibility	Pg 100		
Software	N/A	N/A	N/A
Electromagnetic Compatibility/Electrical Safety	Pg 113		
Performance Testing – Bench	Pg 117		
Performance Testing – Animal	Pg 128		
Performance Testing – Clinical	N/A	N/A	N/A
FORM FDA 3654, Standards Data Report for 510(k)s ²⁵	Pg 160		
Kit Certification	N/A	N/A	N/A

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Section 1: Medical Device User Fee Cover Sheet (Form FDA 3601)

The Medical Device Use Fee Cover Sheet for this device is provided on the following page.

Form Approved: OMB N (b) (4)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION
MEDICAL DEVICE USER FEE COVER SHEETPAYMENT IDENTIFICATION NUMBER
Write the Payment Identification number

A completed cover sheet must accompany each original application or supplement subject to fees. If payment is sent by U.S. mail or courier, please include a copy of this completed form with payment. Payment and mailing instructions can be found at:
<http://www.fda.gov/oc/mdufma/coversheet.html>

(b) (4)

3. TYPE OF PREMARKET APPLICATION (Select one of the following in each column; if you are unsure, please refer to the application descriptions at the following web site: <http://www.fda.gov/oc/mdufma>)

Select an application type:

- ☒ Premarket notification(510(k)); except for third party
☐ 513(g) Request for Information
☐ Biologics License Application (BLA)
☐ Premarket Approval Application (PMA)
☐ Modular PMA
☐ Product Development Protocol (PDP)
☐ Premarket Report (PMR)
☐ Annual Fee for Periodic Reporting (APR)
☐ 30-Day Notice

3.1 Select a center

- ☒ CDRH
☐ CBER

3.2 Select one of the types below

☒ Original ApplicationSupplement Types:

- ☐ Efficacy (BLA)
☐ Panel Track (PMA, PMR, PDP)
☐ Real-Time (PMA, PMR, PDP)
☐ 180-day (PMA, PMR, PDP)

4. ARE YOU A SMALL BUSINESS? (See the instructions for more information on determining this status)

- ☐ YES, I meet the small business criteria and have submitted the required qualifying documents to FDA
☒ NO, I am not a small business

4.1 If Yes, please enter your Small Business Decision Number:

5. FDA WILL NOT ACCEPT YOUR SUBMISSION IF YOUR COMPANY HAS NOT PAID AN ESTABLISHMENT REGISTRATION FEE THAT IS DUE TO FDA. HAS YOUR COMPANY PAID ALL ESTABLISHMENT REGISTRATION FEES THAT ARE DUE TO FDA?

☒ YES (All of our establishments have registered and paid the fee, or this is our first device, and we will register and pay the fee within 30 days of FDA's approval/clearance of this device.)

☐ NO (If "NO," FDA will not accept your submission until you have paid all fees due to FDA. This submission will not be processed; see <http://www.fda.gov/cdrh/mdufma> for additional information)

6. IS THIS PREMARKET APPLICATION COVERED BY ANY OF THE FOLLOWING USER FEE EXCEPTIONS? IF SO, CHECK THE APPLICABLE EXCEPTION.

- ☐ This application is the first PMA submitted by a qualified small business, including any affiliates
☐ The sole purpose of the application is to support conditions of use for a pediatric population
☐ This biologics application is submitted under section 351 of the Public Health Service Act for a product licensed for further manufacturing use only
☐ The application is submitted by a state or federal government entity for a device that is not to be distributed commercially

7. IS THIS A SUPPLEMENT TO A PREMARKET APPLICATION FOR WHICH FEES WERE WAIVED DUE TO SOLE USE IN A PEDIATRIC POPULATION THAT NOW PROPOSES CONDITION OF USE FOR ANY ADULT POPULATION? (If so, the application is subject to the fee that applies for an original premarket approval application (PMA).)

- ☐ YES
☒ NO

PAPERWORK REDUCTION ACT STATEMENT

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Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, 1350 Piccard Drive, 4th Floor Rockville, MD 20850

[Please do NOT return this form to the above address, except as it pertains to comments on the burden estimate.]

(b) (4)

AMOUNT SUBMITTED FOR THIS PREMARKET APPLICATION

02-Aug-2012

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Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

Section 2: CDRH Premarket Review Submission Cover Sheet

The CDRH Premarket Review Submission Cover Sheet for this device is provided on the following page.

DEPARTMENT OF HEALTH AND HUMAN SERVICES FOOD AND DRUG ADMINISTRATION CDRH PREMARKET REVIEW SUBMISSION COVER SHEET		Form Approval OMB No. 0910-0120 Expiration Date: December 31, 2013 See OMB Statement on page 5.
Date of Submission September 10, 2012	User Fee Payment ID Number (b)(4)	FDA Submission Document Number (if known)
SECTION A TYPE OF SUBMISSION		
PMA ☐ Original Submission ☐ Premarket Report ☐ Modular Submission ☐ Amendment ☐ Report ☐ Report Amendment ☐ Licensing Agreement	PMA & HDE Supplement ☐ Regular (180 day) ☐ Special ☐ Panel Track (PMA Only) ☐ 30-day Supplement ☐ 30-day Notice ☐ 135-day Supplement ☐ Real-time Review ☐ Amendment to PMA & HDE Supplement ☐ Other	PDP ☐ Original PDP ☐ Notice of Completion ☐ Amendment to PDP
IDE ☐ Original Submission ☐ Amendment ☐ Supplement	Humanitarian Device Exemption (HDE) ☐ Original Submission ☐ Amendment ☐ Supplement ☐ Report ☐ Report Amendment	Class II Exemption Petition ☐ Original Submission ☐ Additional Information
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> 510(k) ☒ Original Submission: ☒ Traditional ☐ Special ☐ Abbreviated (Complete section I, Page 5) ☐ Additional Information ☐ Third Party </div> <div style="width: 45%;"> Meeting ☐ Pre-510(K) Meeting ☐ Pre-IDE Meeting ☐ Pre-PMA Meeting ☐ Pre-PDP Meeting ☐ Day 100 Meeting ☐ Agreement Meeting ☐ Determination Meeting ☐ Other (specify): </div> </div>		
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> Evaluation of Automatic Class III Designation (De Novo) ☐ Original Submission ☐ Additional Information </div> <div style="width: 45%;"> Other Submission ☐ 513(g) ☐ Other (describe submission): </div> </div>		
Have you used or cited Standards in your submission? ☒ Yes ☐ No (If Yes, please complete Section I, Page 5)		
SECTION B SUBMITTER, APPLICANT OR SPONSOR		

(b) (4), (b) (6)

SECTION D1			REASON FOR APPLICATION - PMA, PDP, OR HDE		
☐ New Device ☐ Withdrawal ☐ Additional or Expanded Indications ☐ Request for Extension ☐ Post-approval Study Protocol ☐ Request for Applicant Hold ☐ Request for Removal of Applicant Hold ☐ Request to Remove or Add Manufacturing Site	☐ Change in design, component, or specification: ☐ Software / Hardware ☐ Color Additive ☐ Material ☐ Specifications ☐ Other (<i>specify below</i>)	☐ Location change: ☐ Manufacturer ☐ Sterilizer ☐ Packager			
☐ Process change: ☐ Manufacturing ☐ Packaging ☐ Sterilization ☐ Other (<i>specify below</i>)	☐ Labeling change: ☐ Indications ☐ Instructions ☐ Performance Characteristics ☐ Shelf Life ☐ Trade Name ☐ Other (<i>specify below</i>)	☐ Report Submission: ☐ Annual or Periodic ☐ Post-approval Study ☐ Adverse Reaction ☐ Device Defect ☐ Amendment			
☐ Response to FDA correspondence:		☐ Change in Ownership ☐ Change in Correspondent ☐ Change of Applicant Address			
☐ Other Reason (<i>specify</i>):					
SECTION D2			REASON FOR APPLICATION - IDE		
☐ New Device ☐ New Indication ☐ Addition of Institution ☐ Expansion / Extension of Study ☐ IRB Certification ☐ Termination of Study ☐ Withdrawal of Application ☐ Unanticipated Adverse Effect ☐ Notification of Emergency Use ☐ Compassionate Use Request ☐ Treatment IDE ☐ Continued Access	☐ Change in: ☐ Correspondent / Applicant ☐ Design / Device ☐ Informed Consent ☐ Manufacturer ☐ Manufacturing Process ☐ Protocol - Feasibility ☐ Protocol - Other ☐ Sponsor	☐ Response to FDA Letter Concerning: ☐ Conditional Approval ☐ Deemed Approved ☐ Deficient Final Report ☐ Deficient Progress Report ☐ Deficient Investigator Report ☐ Disapproval ☐ Request Extension of Time to Respond to FDA ☐ Request Meeting ☐ Request Hearing			
☐ Report submission: ☐ Current Investigator ☐ Annual Progress Report ☐ Site Waiver Report ☐ Final					
☐ Other Reason (<i>specify</i>):					
SECTION D3			REASON FOR SUBMISSION - 510(k)		
☒ New Device	☐ Additional or Expanded Indications	☐ Change in Technology			
☐ Other Reason (<i>specify</i>):					

SECTION E ADDITIONAL INFORMATION ON 510(K) SUBMISSIONS

Product codes of devices to which substantial equivalence is claimed

1	GEI	2	HGI	3		4	
5		6		7		8	

Summary of, or statement concerning, safety and effectiveness information

- ☒ 510 (k) summary attached
☐ 510 (k) statement

Information on devices to which substantial equivalence is claimed (if known)

	510(k) Number		Trade or Proprietary or Model Name		Manufacturer
1	K112033	1	ENSEAL G2 Tissue Sealers	1	Ethicon Endo-Surgery, LLC
2		2		2	
3		3		3	
4		4		4	
5		5		5	
6		6		6	

SECTION F PRODUCT INFORMATION - APPLICATION TO ALL APPLICATIONS

Common or usual name or classification name

	Trade or Proprietary or Model Name for This Device		Model Number
1	ENSEAL G2 Articulating Tissue Sealers, straight jaw	1	NSLG2S35A, NSLG2S45A,
2	ENSEAL G2 Articulating Tissue Sealers, curved jaw	2	NSLG2C35A, NSLG2C45A
3		3	
4		4	
5		5	

FDA document numbers of all prior related submissions (regardless of outcome)

1	K112033	2		3		4		5		6	
7		8		9		10		11		12	

Data Included in Submission

☒ Laboratory Testing☒ Animal Trials☐ Human Trials**SECTION G PRODUCT CLASSIFICATION - APPLICATION TO ALL APPLICATIONS**

Product Code

GEI, HGI

C.F.R. Section (if applicable)

21 C.F.R. 878.4400, 21CFR 884.4120

Device Class

☐ Class I☒ Class II☐ Class III☐ Unclassified

Classification Panel

General & Plastic Surgery

Indications (from labeling)

The ENSEAL® G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures. The devices are bipolar electrosurgical instruments for use with the Generator G11 (GEN11). They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery. Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7mm and tissue and/or vascular bundles as large as will fit in the jaws of the instruments.

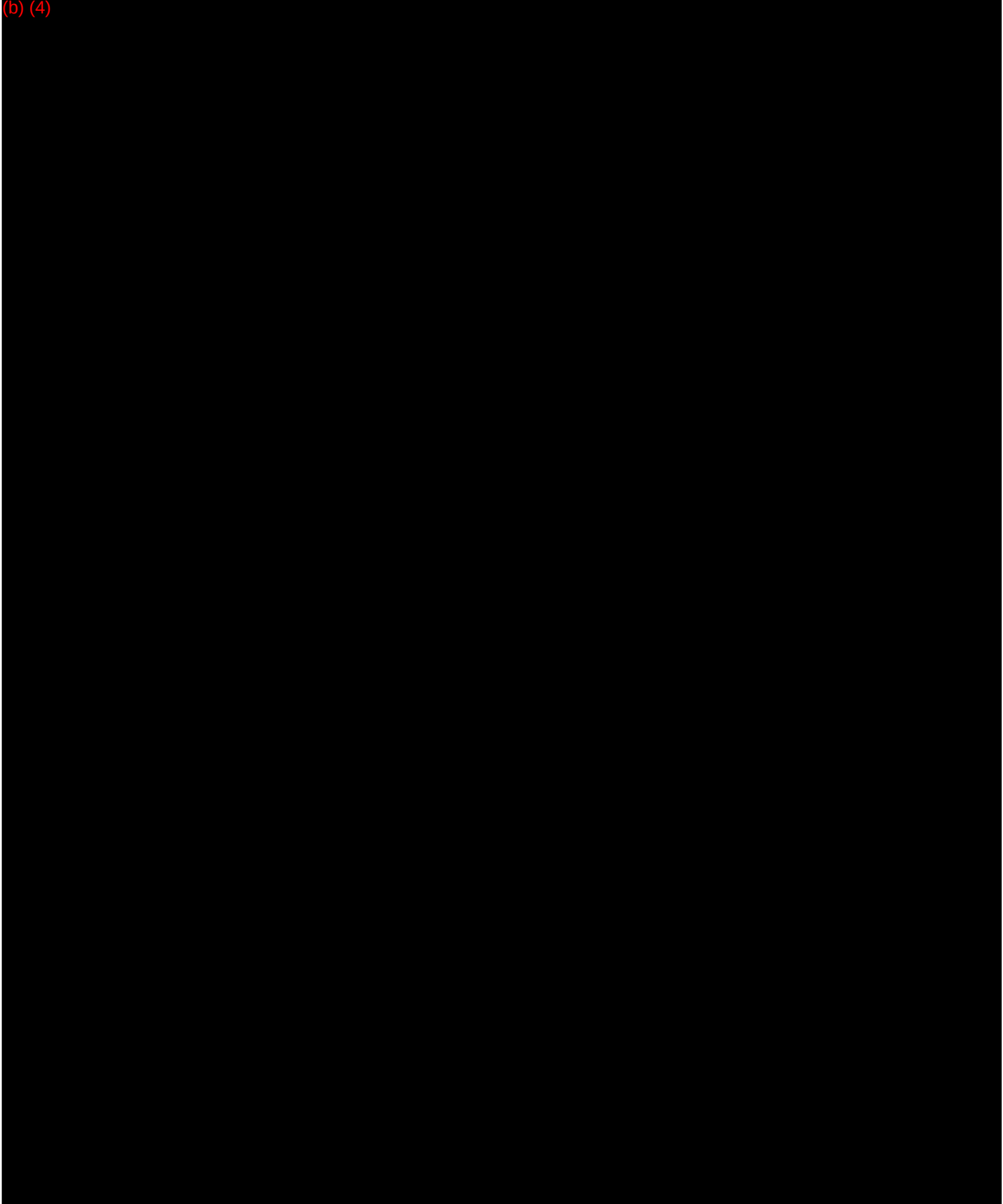
Note: Submission of the information entered in Section H does not affect the need to submit device establishment registration.

FDA Document Number *(if known)*

SECTION H

MANUFACTURING / PACKAGING / STERILIZATION SITES RELATING TO A SUBMISSION

(b) (4)



Note: Submission of this information does not affect the need to submit a 2891 or 2891a Device Establishment Registration form.

FDA Document Number (if known)

SECTION II (Continued)

(b) (4)

☐ Original ☐ Add ☐ Delete		Facility Establishment Identifier (FEI) Number		☐ Manufacturer ☐ Contract Sterilizer ☐ Contract Manufacturer ☐ Repackager / Relabeler	
Company / Institution Name			Establishment Registration Number		
Division Name (if applicable)			Phone Number (including area code)		
Street Address			FAX Number (including area code)		
City			State / Province	ZIP Code	Country
Contact Name		Contact Title		Contact E-mail Address	

☐ Original ☐ Add ☐ Delete		Facility Establishment Identifier (FEI) Number		☐ Manufacturer ☐ Contract Sterilizer ☐ Contract Manufacturer ☐ Repackager / Relabeler	
Company / Institution Name			Establishment Registration Number		
Division Name (if applicable)			Phone Number (including area code)		
Street Address			FAX Number (including area code)		
City			State / Province	ZIP Code	Country
Contact Name		Contact Title		Contact E-mail Address	

SECTION I**UTILIZATION OF STANDARDS**

Note: Complete this section if your application or submission cites standards or includes a "Declaration of Conformity to a Recognized Standard" statement.

1	Standards No. AAMI/ANS/ISO 10993-1	Standards Organization AAMI/ANSI/ISO	Standards Title Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process	Version 2009	Date 10/15/2009
2	Standards No. AAMI/ANS/ISO 11137-1	Standards Organization AAMI/ANSI/ISO	Standards Title AAMI/ANS/ISO 11137-1:2006 Sterilization of health Care Products - Radiation - Part 1: Requirements for Development, Validation and Routine Control of A Sterilization Process for Medical Devices.	Version 2006	Date 04/15/2006
3	Standards No. AAMI/ANS/ISO 11137-2	Standards Organization AAMI/ANSI/ISO	Standards Title AAMI/ANS/ISO 11137-2:2006 Sterilization of Health Care Products - Radiation - Part 2: Establishing the Sterilization Dose.	Version 2006	Date
4	Standards No. AAMI/ANS/ISO 11137-3	Standards Organization AAMI/ANSI/ISO	Standards Title AAMI/ANS/ISO 11137-3:2006 Sterilization of Health Care Products - Radiation - Part 3: Guidance on Dosimetric Aspects	Version 2006	Date
5	Standards No. 60601-1	Standards Organization IEC	Standards Title Medical Electrical Equipment - Part 1: General requirements for basic safety and essential performance.	Version	Date
6	Standards No. 60601-1-2	Standards Organization IEC	Standards Title Medical Electrical Equipment - Part 1-2: General requirements for basic safety and essential performance - Collateral standard: Electromagnetic compatibility - Requirements and tests	Version	Date
7	Standards No. 60601-2-2	Standards Organization IEC	Standards Title Medical Electrical Equipment - Part 2-2: Particular requirements for basic safety and essential performance of high frequency surgical equipment	Version	Date

Please include any additional standards to be cited on a separate page.

Public reporting burden for this collection of information is estimated to average 0.5 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
1350 Piccard Drive, Room 400
Rockville, MD 20850

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

Section 3: 510(k) Cover Letter

The signed cover letter for this submission is provided on the following pages.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

September 10, 2012

Food and Drug Administration
Center for Devices and Radiological Health
Office of Device Evaluation
Division of General and Plastic Surgery Devices
Document Mail Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

Re: Traditional 510(k) Premarket Notification, ENSEAL® G2 Articulating Tissue Sealers

Dear Sir or Madam:

Pursuant to 21 CFR 807.90, Ethicon Endo-Surgery is submitting two copies of this 510(k) notification for the Enseal. The design of the device is based upon the Predicate device ENSEAL® G2 Tissue Sealers, cleared under K112033. The basis of this submission is that the ENSEAL® G2 Articulating Tissue Sealers are new devices substantially equivalent to a legally marketed device.

The following information is provided in this cover letter per the guidance document *Format for Traditional and Abbreviated 510(k)s*:

Submission Type:	Traditional 510(k)
Submission Date:	September 10, 2012
510(k) Submitter:	Ethicon Endo-Surgery, LLC
Contact Information:	Emily Kruetzkamp, Regulatory Affairs Associate Phone: 513.337.1546 Fax: 513.337.2802 Email: ekruetzk@its.jnj.com
Common Name:	Electrosurgical Cutting and Coagulation Instruments
Trade Name:	ENSEAL® G2 Articulating Tissue Sealers
Classification Names:	Electrosurgical, Cutting & Coagulation & Accessories Electrocautery, Gynecologic and Accessories
Device Class:	Class II
Panel:	General & Plastic Surgery
Classification Code:	GEI, HGI
Classification Regulation:	21 CFR 878.4400, 21 CFR 884.4120
Predicate:	ENSEAL® G2 Tissue Sealers, K112033
Establishment Registration:	3005075853

Confidentiality Preference: Please keep this submission confidential per 21 CFR 807.95.
The following table contains answers to general questions regarding this submission.

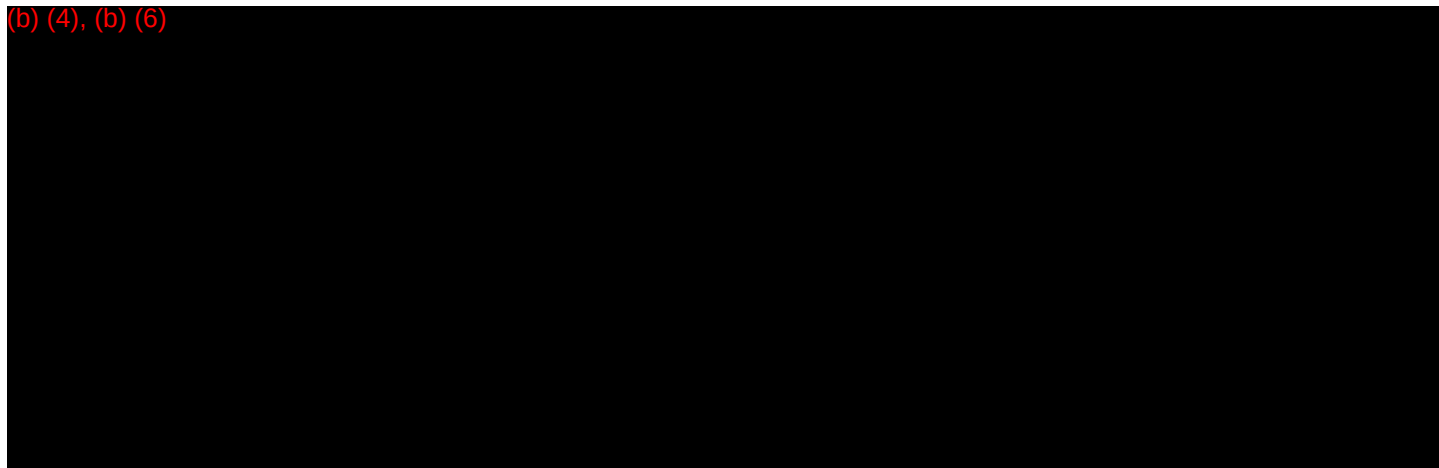
Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Questions	Yes	No
Is the device intended for prescription use (21 CFR 801 Subpart D)?	X	
Is the device intended for over-the-counter use (21 CFR 807 Subpart C)?		X
Does the device contain components derived from tissue or other biologic source?		X
Is the device provided sterile?	X	
Is the device intended for single use?	X	
Is the device a reprocessed single use device?		X
If yes, does the device type require reprocessed validation data?		
Does the device contain a drug?		X
Does the device contain biologic?		X
Does the device use software?		X
Does the submission include clinical information?		X
Is the device implanted?		X

The unique payment identification number (PIN) assigned to this submission is MD6063192-956733. A copy of the Medical Device User Fee Cover Sheet is included in Section 1.

Per the instructions accessed at 'Electronic Copies for Pre-Market Submissions', an electronic copy is provided with this submission and it is an exact duplicate of the original paper submission. The electronic copy is accompanied by extra copies of the 510(k) cover letter and the 510(k) Truthful and Accuracy Statement with original signatures.

(b) (4), (b) (6)



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Section 4: Indications for Use Statement

The Indication for Use statements for the proposed devices are provided on the following page.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Indications for Use Form

Indications for Use

510(k) Number (if known): _____

Device Name: ENSEAL® G2 Articulating Tissue Sealers

Indications for Use:

The ENSEAL® G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with the Generator GEN11 (GEN11). They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7mm and tissue and/or vascular bundles as large as will fit in the jaws of the instruments.

The ENSEAL® G2 Articulating Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures.

Prescription Use X
(Part 21 CFR 801 Subpart D)

AND/OR

Over-The-Counter Use _____
(21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE
OF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Page 1 of 1

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

Section 5: 510(k) Summary

The 510(k) Summary for the Subject devices is on the following pages.

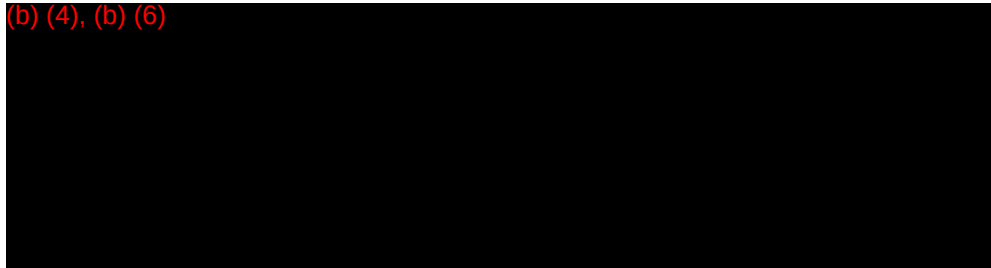
Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

510(k) Summary

Company

Ethicon Endo-Surgery, LLC
475 Calle C
Guaynabo, PR 00969

(b) (4), (b) (6)



Device Name

Trade Name: ENSEAL® G2 Articulating Tissue Sealers

Common Name: Electrosurgical Cutting and Coagulation Instruments

Classification Names

- Electrosurgical, Cutting & Coagulation & Accessories
(21 CFR 878.4400, Product code GEI)
- Electrocautery, Gynecologic and Accessories
(21 CFR 884.4120, Product code HGI)

Predicate Device

ENSEAL® G2 Tissue Sealers, K112033

Device Description:

The Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers are sterile, single-use surgical instruments designed to seal and cut vessels, and to cut, grasp and dissect soft tissue during open and laparoscopic surgery.

The energy activation and knife lock are activated by a single button. The instrument shaft can be rotated using the rotation knob to facilitate visualization and enable easy access to targeted tissue. The power cord is permanently attached to the device and connects the instrument to the generator. The ENSEAL G2 Articulating Tissue Sealers include an articulation capability that allows the user to articulate the end-effector during surgical procedures.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Indication for Use:

The ENSEAL® G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with the Generator GEN11 (GEN11). They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7mm and tissue and/or vascular bundles as large as will fit in the jaws of the instruments.

The ENSEAL® G2 Articulating Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures.

Technological Characteristics:

The Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers components are similar to the Predicate. The ENSEAL G2 Articulating Tissue Sealers have a 5 mm diameter shaft and are available in two shaft lengths: 35 and 45cm; and two jaw types: 3mm curved jaw and 5mm straight jaw. The jaws are normally in the opened position and can be partially or fully closed by squeezing the closing handle. The jaws have atraumatic teeth for grasping and holding targeted tissue when clamped. The handles of the Subject ENSEAL G2 Articulating Tissue Sealers have an ergonomic interface for the user. Like the Predicate, the Subject devices include an integrated energy activation button. The devices use a combination of the application of bipolar electrosurgical energy in conjunction with the I-BLADE knife, to compress, coagulate, and transect tissue.

In addition to the similar technological characteristics as the Predicate, the ENSEAL G2 Articulating Tissue Sealers instrument shaft device articulates approximately 60° to the left and the right of center. The instrument shaft can be articulated using the articulation wheel to gain additional access to tissue and facilitate the user's ability to target tissue and vascular bundles up to 60°. The top of the articulation wheel has a white arrow to indicate when the device shaft is straight and not articulated. When the articulation wheel is rotated clockwise, the shaft articulates to the right; when the articulation wheel is rotated counterclockwise, the shaft articulates to the left. While articulated, the device jaws seal and cut vessels, and cut, grasp and dissect soft tissue as intended. The Subject ENSEAL G2 Articulating Tissue Sealers performance is equivalent to the Predicate ENSEAL G2 Tissue Sealers.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Performance Data:

Ex-vivo and in-vivo tests were performed to verify that the performance of the ENSEAL G2 Articulating Tissue Sealers meets the definition of substantial equivalence to the Predicate device, ENSEAL G2 Tissue Sealers. Device performance was assessed against design requirements, including a 30-day chronic survival study, to demonstrate that the Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers perform as intended.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

Section 6: Truthful and Accuracy Statement

The Truthful and Accuracy Statement for the device is provided on the following page.

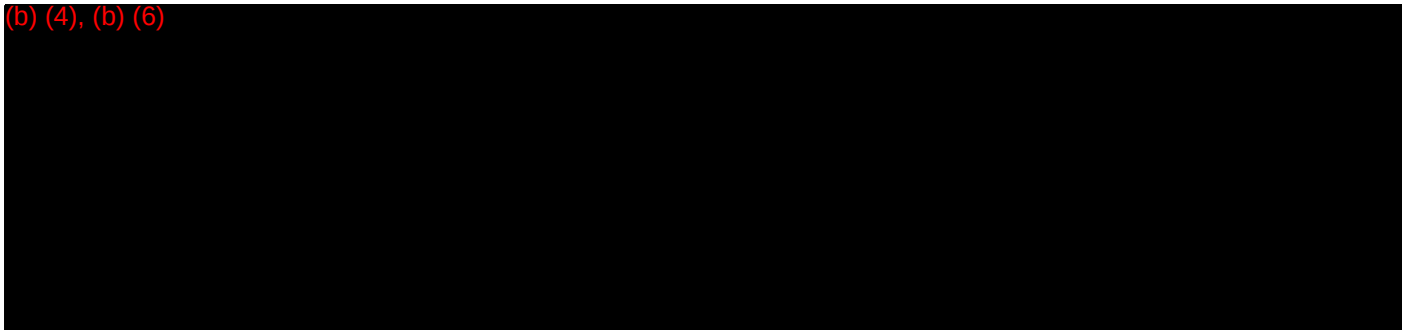
Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Truthful and Accuracy Statement

The Truthful and Accuracy Statement, as required by 21 CFR 807.87(k) is provided below.

I certify that in my capacity as Regulatory Affairs Associate for Ethicon Endo-Surgery Inc., I believe to the best of my knowledge, that all data and information submitted in this premarket notification are truthful and accurate and that no material fact related to a substantial equivalence decision has been omitted.

(b) (4), (b) (6)



Section 7: Class III Summary and Certification

This section does not apply; the ENSEAL[®] G2 Articulating Tissue Sealers are Class II devices.

Section 8: Financial Certification or Disclosure Statement

This section does not apply; no clinical study was performed to support this submission.

Section 9: Declarations of Conformity and Summary Reports

This section does not apply; this submission is a Traditional 510(k).

Section 10: Executive Summary

The purpose of this 510(k) submission is to provide FDA information to review the design of the ENSEAL[®] G2 Articulating Tissue Sealers.

Device Description

The ENSEAL G2 Articulating Tissue Sealers indication is identical to the Predicate device:

The ENSEAL[®] G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with the Ethicon Endo-Surgery Generator G11 (GEN11) previously cleared under K101990. They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7mm and tissue and/or vascular bundles as large as will fit in the jaws of the instruments.

The ENSEAL G2 Articulating Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures.

The Subject device contraindication is identical to the Predicate device statement.

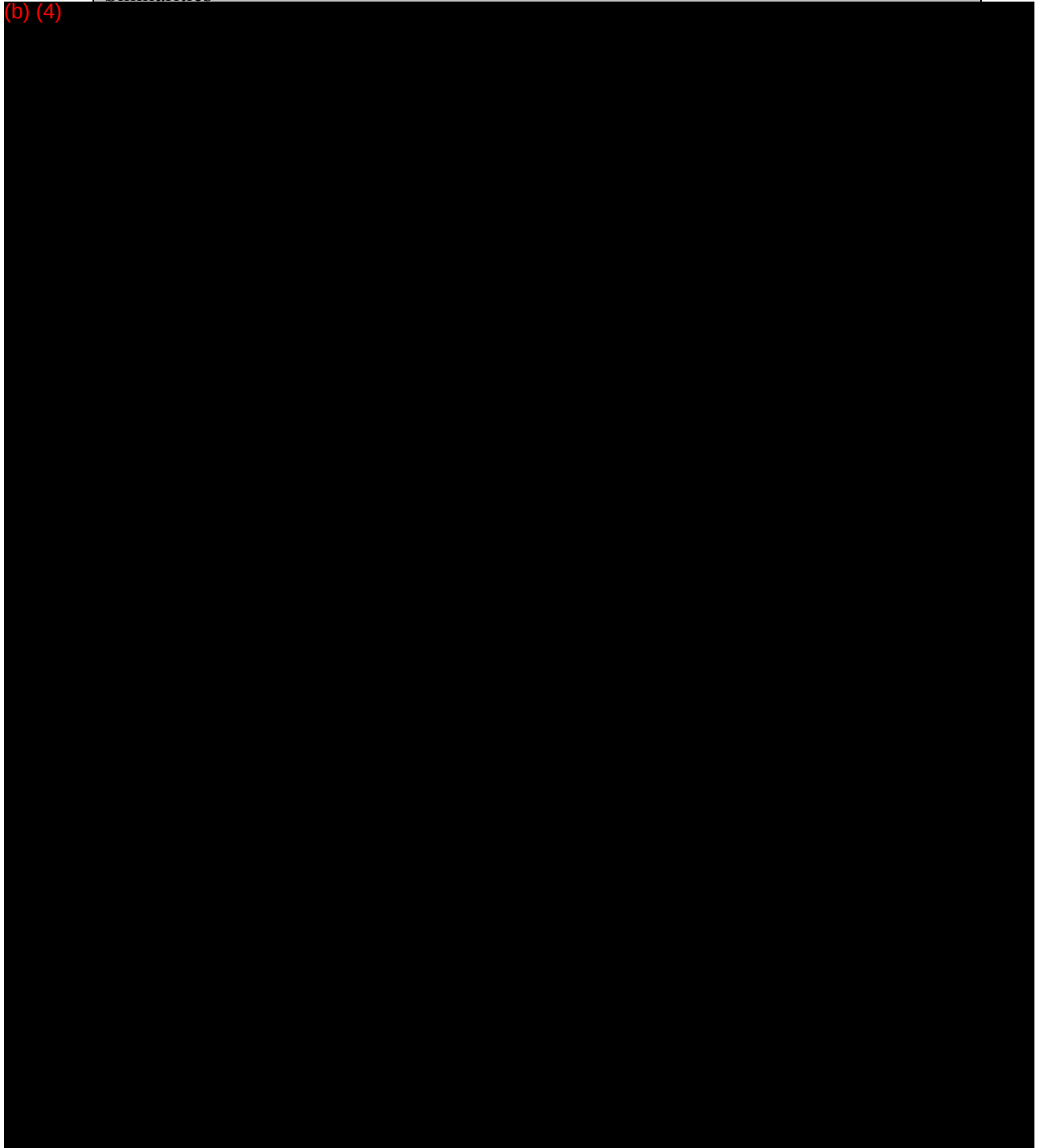
The efficacy of the ENSEAL[®] G2 Articulating Tissue Sealer for the indication of contraceptive tubal coagulation (permanent female sterilization) has not been evaluated and is unknown. The design of the ENSEAL[®] G2 Articulating Tissue Sealer is significantly different from bipolar designs that are marketed for the indication of contraceptive tubal coagulation. The design differences may affect the efficacy of the procedure and failure rates may not be comparable.

The following Table 10.1 provides a detailed side-by-side comparison of the Subject devices and the Predicate devices with respect to technology and device performance.

Table 10.1 Device Comparison Table: Technology and Performance

Characteristic	Subject Device ENSEAL G2 Articulating Tissue Sealers Description	Predicate Device ENSEAL G2 Tissue Sealers Description
Similarities		

(b) (4)



Characteristic	Subject Device ENSEAL G2 Articulating Tissue Sealers Description	Predicate Device ENSEAL G2 Tissue Sealers Description
Packaging	Rigid clear PETG blister package with a heat-sealed Tyvek lid	Identical
Vessel Sealing Performance	Mean Burst Pressure is >240mmHg	Identical
Maximum Indicated Vessel Size	7mm	Identical
Hemostasis, Acute	100% vessels hemostatic through completion of surgery	Identical
Long-Term Seal Quality, Hemostasis,	100% vessels hemostatic 30-days post surgery	Identical
Differences		
Articulation Feature	The device articulates approximately 60° to the left and the right of center.	Not present
Labeling, Package Insert	Additional Warnings and Precautions, Steps to grasp, dissect and cut tissue for the articulation feature	Not present
Shelf Life	1 yr	2 yr
Device Materials	New patient-contacting materials in conjunction with articulation feature	Not present

Summary of Performance Testing

Ex-vivo and in-vivo tests were performed to verify that the performance of the ENSEAL G2 Articulating Tissue Sealers is substantially equivalent to the Predicate device to the Predicate device, ENSEAL G2 Tissue Sealers (K112033). Details concerning the test methods, acceptance criteria, results and discussion are contained in Section 18 Performance Testing – Bench and Section 19 Performance Testing - Animal.

Device performance was assessed against the design requirement for each of the following test requirements:

Ex-vivo Testing, Section 18 Performance Testing – Bench:

Sealed Vessel Burst Test

Energy Activation/Knife Lock Button Functionality

Tissue Sticking

In-vivo Testing, Section 19 Performance Testing – Animal

Hemostasis

Charring

Injury to Adjacent Structures

Thermal Spread

Section 11: Device Description

Device Design and Components

The Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers are sterile, single-use surgical instruments designed to seal and cut vessels, and to cut, grasp and dissect soft tissue during open and laparoscopic surgery. The devices will be available in straight 5mm jaws and curved 3mm jaws, as shown in Figure 11.1.

Figure 11.1 Straight 5mm Jaws and Curved 3mm Jaws



As in the Predicate device, the energy activation and knife lock release are enabled by a single button. The instrument shaft can be rotated using the rotation knob to facilitate visualization and enable easy access to targeted tissue.

The instrument shaft can be articulated using the articulation wheel to gain additional access to tissue and facilitate the user's ability to target tissue and vascular bundles at angles up to 60°. The device articulates at approximately 60° to the left and the right of center. The top of the articulation wheel has a white arrow to indicate when the device shaft is straight and not articulated. When the articulation wheel is rotated clockwise, the shaft articulates to the right; when the articulation wheel is rotated counterclockwise, the shaft articulates to the left. See Figures 11.2 and 11.3.

Whether straight or articulated, the device jaws seal and cut vessels, and cut, grasp and dissect soft tissue as intended. The Subject ENSEAL G2 Articulating Tissue Sealers performance is equivalent to the Predicate ENSEAL G2 Tissue Sealers. Due to the new articulation feature, there are new patient-contacting materials on the Subject device compared to the Predicate. These new materials have been evaluated for Biocompatibility, refer to Section 15 Biocompatibility.

The power cord is permanently attached to the device and connects the instrument to the generator. The ENSEAL G2 Articulating Tissue Sealers are designed to be used exclusively with the Generator GEN11 (K101990), which is packaged separately.

Figure 11.2 Subject Device, Articulated

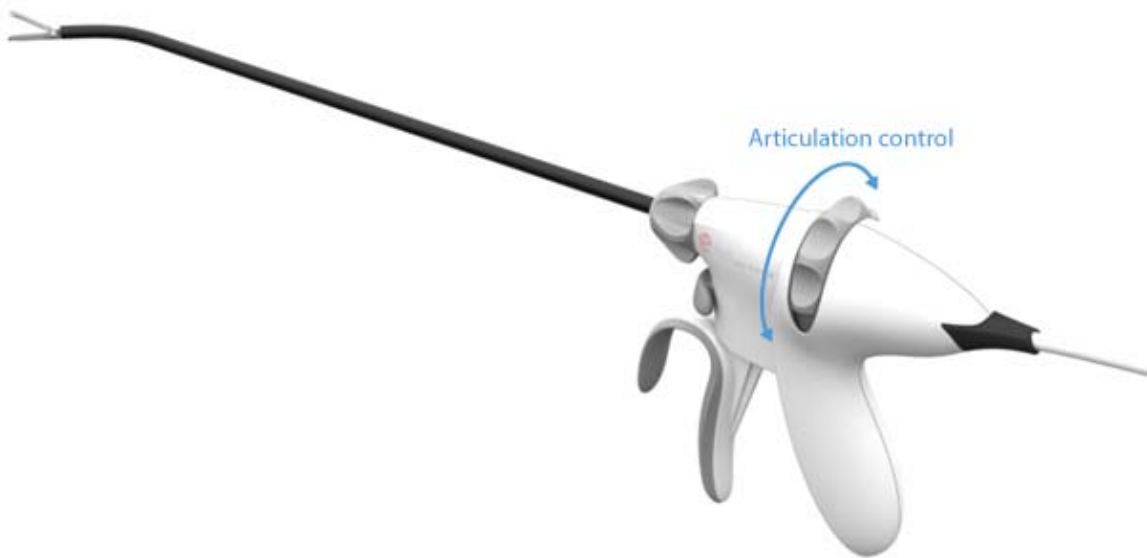
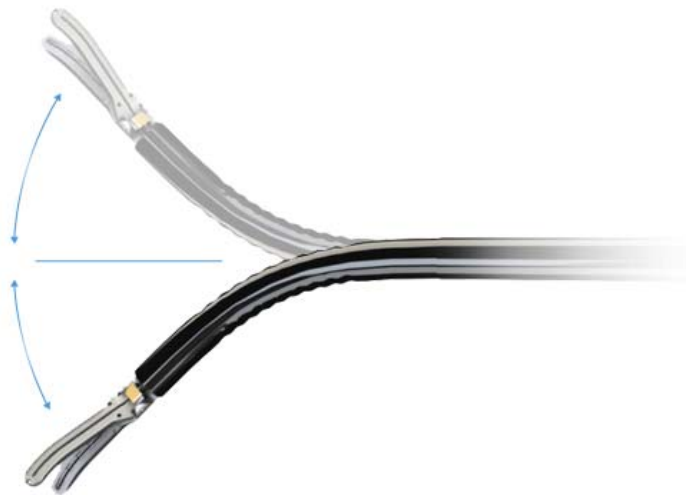


Figure 11.3 Subject Device, Articulated End Effector



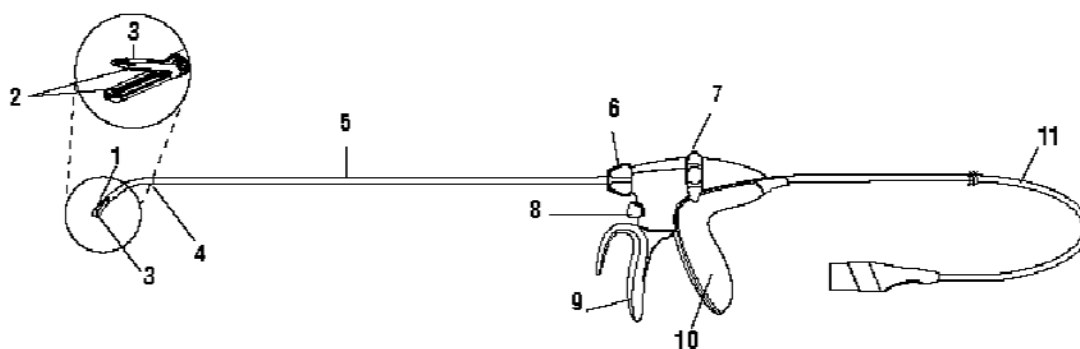
The ENSEAL G2 Articulating Tissue Sealers have a 5 mm diameter shaft and are available in two shaft lengths: 35 and 45cm; and two jaw types: 3mm curved jaw and 5mm straight jaw. See Table 11.1 for a list of all product codes.

Table 11.1 Product Codes and Names

Subject Device Product Code	Subject Device Name	Predicate Device Product Code	Predicate Device Name
NSLG2S35A	ENSEAL G2 Articulating Tissue Sealer, 35 cm Length Straight Jaw	NSLG2S35	ENSEAL G2 Tissue Sealer, 35 cm Length Straight Jaw
NSLG2C35A	ENSEAL G2 Articulating Tissue Sealer, 35 cm Length Curved Jaw	NSLG2C35	ENSEAL G2 Tissue Sealer, 35 cm Length Curved Jaw
NSLG2S45A	ENSEAL G2 Articulating Tissue Sealer, 45 cm Length Straight Jaw	NSLG2S45	ENSEAL G2 Tissue Sealer, 45 cm Length Straight Jaw
NSLG2C45A	ENSEAL G2 Articulating Tissue Sealer, 45 cm Length Curved Jaw	NSLG2C45	ENSEAL G2 Tissue Sealer, 45 cm Length Curved Jaw

The Subject device parts are similar to the Predicate. Figure 11.4 shows the device parts for the Subject device.

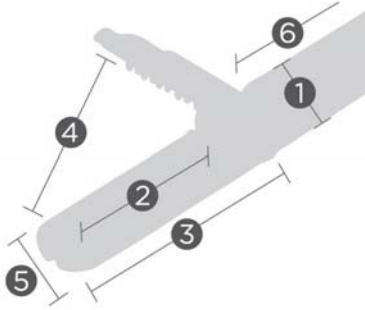
Figure 11.4 Subject Device Parts



- | | |
|--------------------------------|-----------------------------|
| 1. Jaws (curved or straight) | 7. Articulation wheel |
| 2. Atraumatic teeth | 8. Energy activation button |
| 3. I-BLADE knife (not visible) | 9. Closing handle |
| 4. Articulation joint | 10. Grip housing |
| 5. Shaft | 11. Power cord |
| 6. Rotation knob | |

The ENSEAL G2 Articulating Tissue Sealer Subject device dimensions are similar to the Predicate. Table 11.2 lists the device dimensions.

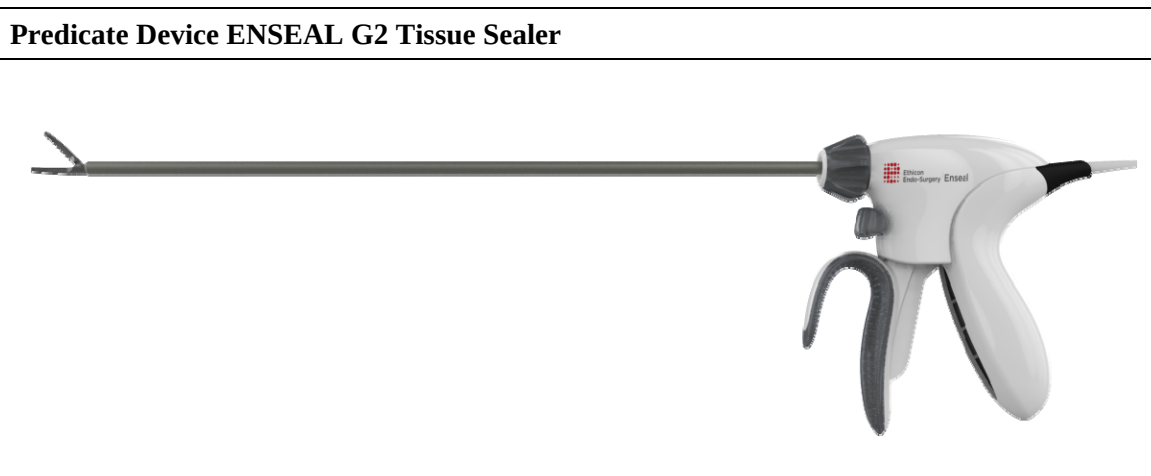
Table 11.2 Device Dimensions

	Subject Device ENSEAL G2 Articulating Tissue Sealers	Subject Device ENSEAL G2 Articulating Tissue Sealers	Predicate Device ENSEAL G2 Tissue Sealers	Predicate Device ENSEAL G2 Tissue Sealers
	Curved	Straight	Curved	Straight
				

(b) (4)

The ENSEAL G2 Articulating Tissue Sealer Subject ergonomic design is similar to the Predicate. The Figure 11.5 shows the Subject and Predicate devices.

Figure 11.5 Subject and Predicate Devices



Principles of Operation

The jaws are provided in the opened position and can be partially or fully closed by squeezing the closing handle. The jaws have atraumatic teeth for grasping and holding targeted tissue when clamped. The handles of the Subject ENSEAL G2 Articulating Tissue Sealers have an ergonomic interface for the user.

The device uses a combination of the application of bipolar electrosurgical energy in conjunction with the I-BLADE knife and compression, to coagulate and transect tissue.

Bipolar energy is delivered to the clamped tissue when the energy activation button is pressed. Coagulation is achieved with bipolar electrosurgical elements and the top jaw that supplies compression to targeted tissue. The jaws of the ENSEAL G2 Articulating Tissue Sealers contain a Positive Temperature Coefficient (PTC) polymeric compound that consists of a thermo-sensitive synthetic material containing embedded conductive particles. The conductive particles within the PTC are arranged in chainlike formations at temperatures below 100°C. As the temperature rises above 100°C, the polymer expands and the conductive chains begin to break apart, thereby reducing the electrical conductivity and preventing the flow of electrical current. As the temperature falls back below 100°C, the conductive chains reform, restoring the flow of electricity. The ability to stop the flow of electrical current at temperatures above 100°C allows the ENSEAL instruments to control heat.

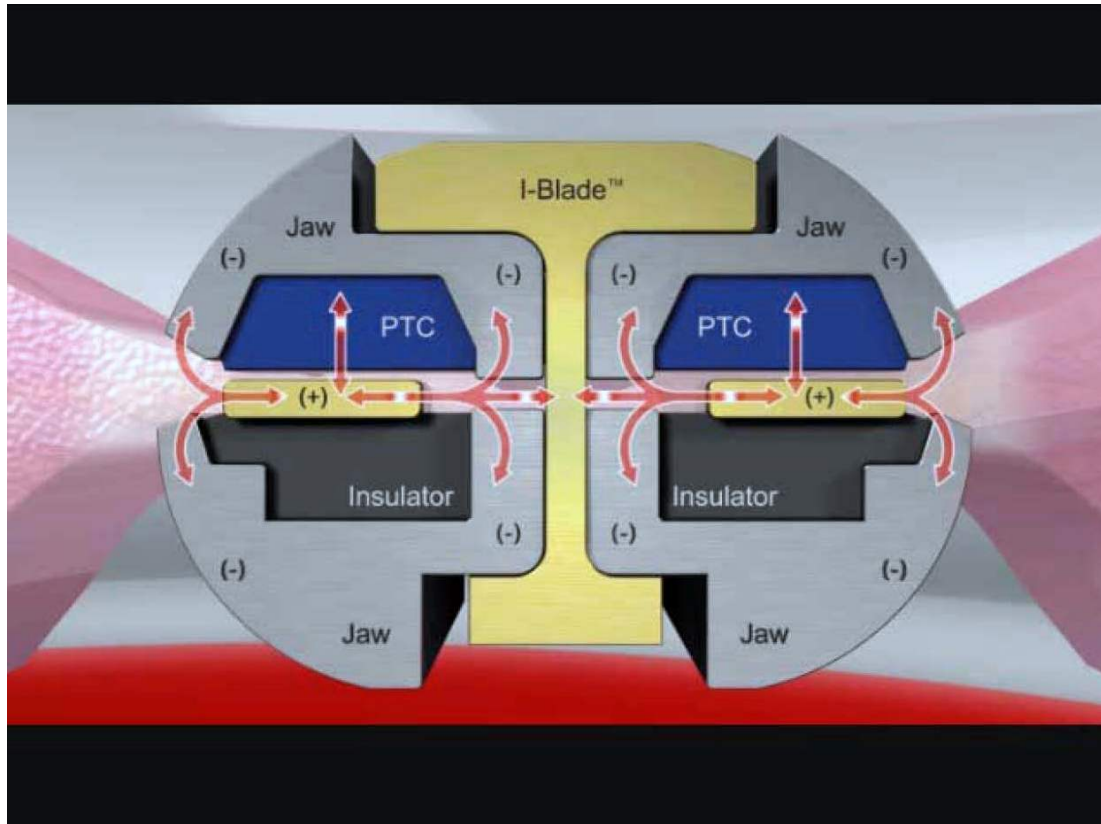
The integrated energy activation and knife lock release functions are integrated into a single button. The energy activation/knife lock release button must be fully depressed before the I-BLADE can advance to transect tissue. The knife cannot be inadvertently advanced prior to energy being delivered to the tissue, as demonstrated in verification testing, refer to Section 18: Performance Testing - Bench.

When the user fully squeezes the closing handle, the I-BLADE advances the length of the jaws. The I-BLADE knife rides within channels located on the top and bottom jaw so that when it advances the jaws are pressed together in order to achieve uniform compression of the targeted tissue. See Figures 11.6 and 11.7.

Figure 11.6 Device Image of I-BLADE Channels, View of Jaw Tip



Figure 11.7 Device Image of I-BLADE, View of Jaw Tip



Summary of Changes

The primary modification between the Subject device and the Predicate device is the addition of the shaft articulation feature. To facilitate the articulation, new patient-contacting materials have been incorporated into the device shaft. Other changes include text changes to the Package Insert Steps for Use and Warnings and Precautions to add articulation steps and enhance clarity to insure more effective use. Additionally, there are component dimension changes to the energy activation button to enhance the sturdiness of the button attachment to the handle. This modification enhances the strength of the device and contributes to a robust manufacturing process.

Assembly Drawing

The following pages include Assembly Drawings that illustrate the device modifications discussed in this submission for the ENSEAL G2 Articulating Tissue Sealers.

Section 12: Substantial Equivalence Discussion

The “510(k) Substantial Equivalence Decision Making Process (Detailed) Decision Tree” (Blue Book Memorandum K86-3, 1986) was used in determining substantial equivalence of the Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers.

New device is compared to predicate devices

Predicate Device: ENSEAL G2 Tissue Sealers, K112033

Subject Device: ENSEAL G2 Articulating Tissue Sealers

Does new device have same indications statement?

Yes, the intended use is the same.

Does the device have same technological characteristics, (e.g., design, materials, etc.)?

No, the Subject devices have articulation capability. There are differences in the dimensions, patient-contacting materials, and steps for use. The devices are both electrosurgical devices so the fundamental technology is the same.

Biocompatibility was evaluated based on the ISO 10993-1 Standard and FDA Guidelines. Refer to Section 15 Biocompatibility.

Could the new characteristics affect safety or effectiveness?

Yes.

Do the new characteristics raise new types of safety or effectiveness questions?

No. The safety or effectiveness questions raised by the design changes are well characterized elements of electrosurgical and electrocautery device performance.

Do accepted scientific methods exist for assessing effects of new characteristics?

Yes. Refer to Section 18 Performance Testing - Bench and Section 19 Performance Testing – Animal which include the testing objectives, test articles, and methods for Subject device testing.

Are performance data available to assess effects of new characteristics?

Yes. Refer to Section 18 Performance Testing – Bench and Section 19 Performance Testing – Animal, which includes the results and discussion for Subject device testing.

Do performance data demonstrate equivalence?

Yes. Refer to Section 18 Performance Testing – Bench and Section 19 Performance Testing – Animal, which includes the conclusion for Subject device verification testing.

Substantially Equivalent Determination

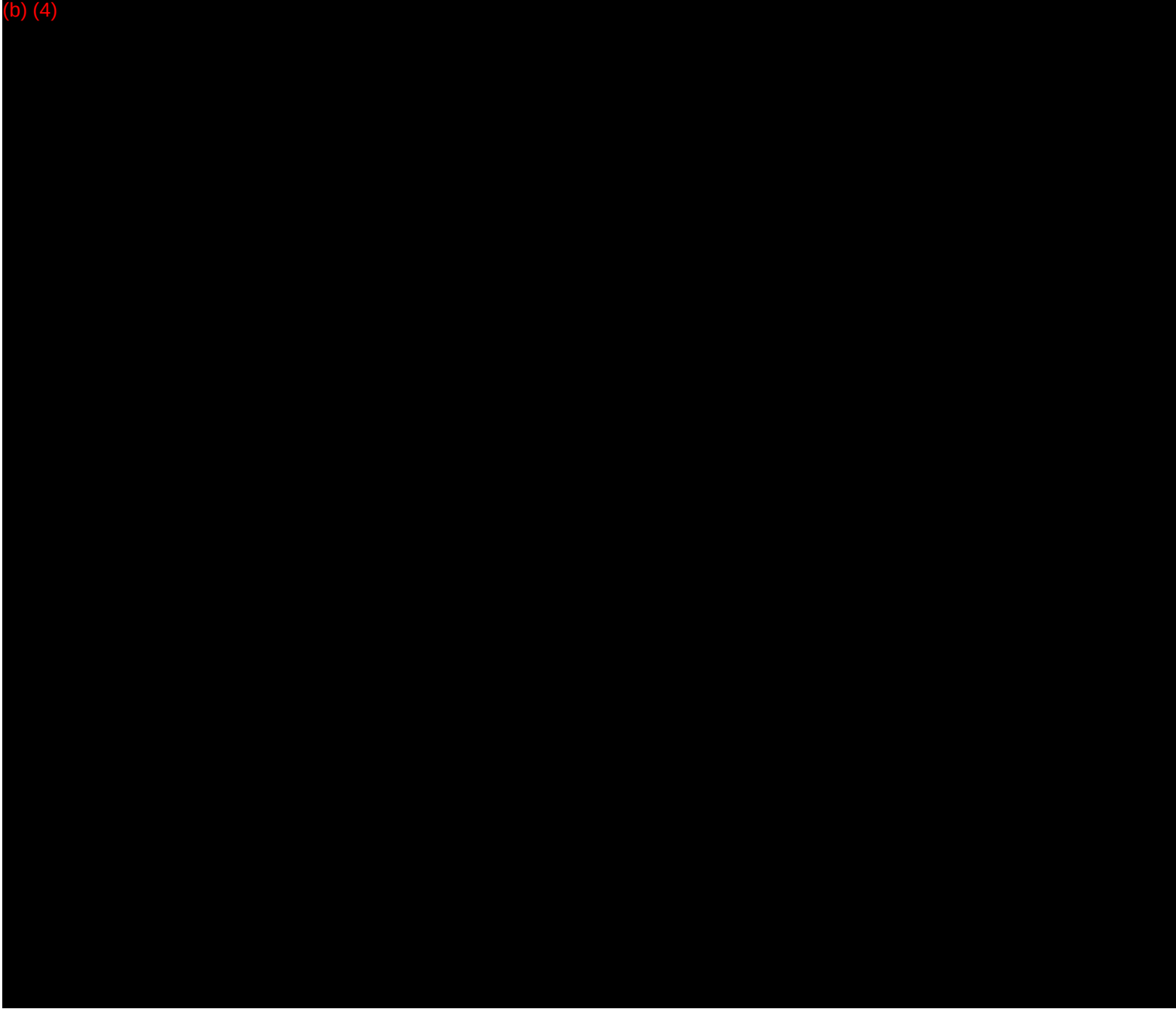
In summary, ENSEAL G2 Articulating Tissue Sealers met the definition of substantial equivalence to the Predicate devices.

In accordance with the Safe Medical Devices Act of 1990 (SMDA), a summary upon which this substantial equivalence determination is based is enclosed in Section 5 510(k) Summary. This summary may be released to the public.

The Substantial Equivalence Decision Making Process Decision Tree, with the path applicable to this submission highlighted, is provided on the following page in Figure 12.1.

**Figure 12.1. 510(k) Substantial Equivalence Decision Making Process (Detailed)
Decision Tree (CDRH 510(k) Manual 92-4158)**

(b) (4)



* 510(k) submissions compare new devices to marketed devices. FDA requests additional information if the relationship between marketed and “Predicate” devices is unclear.

** This decision is normally based on descriptive information alone, but limited testing information is sometimes required.

*** Data may be in the 510(k), other 510(k)'s, the Center’s classification files, or the literature.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL G2 Articulating Tissue Sealers

The ENSEAL G2 Articulating Tissue Sealers indication and contraindication are identical to the marketed Predicate device, refer to Table 12.1.

Table 12.1 Indications

Subject Device ENSEAL G2 Articulating Tissue Sealers	Predicate Device ENSEAL G2 Tissue Sealers
<p>The ENSEAL[®] G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures. The devices are bipolar electrosurgical instruments for use with the Generator G11 (GEN11). They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery. Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7mm and tissue and/or vascular bundles as large as will fit in the jaws of the instruments. The ENSEAL[®] G2 Articulating Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures.</p>	<p>Identical</p>

Table 12.2 Contraindications

Subject Device ENSEAL G2 Articulating Tissue Sealers	Predicate Device ENSEAL G2 Tissue Sealers
<p>The efficacy of the ENSEAL[®] G2 Articulating Tissue Sealer for the indication of contraceptive tubal coagulation (permanent female sterilization) has not been evaluated and is unknown. The design of the ENSEAL[®] G2 Articulating Tissue Sealer is significantly different from bipolar designs that are marketed for the indication of contraceptive tubal coagulation. The design differences may affect the efficacy of the procedure and failure rates may not be comparable.</p>	<p>Identical</p>

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL G2 Articulating Tissue Sealers**Table 12.3 Changes to the Package Insert Text**

IFU Text	Subject Device ENSEAL G2 Articulating Tissue Sealers	Predicate Device ENSEAL G2 Tissue Sealers
Warnings and Precautions	To ensure effective sealing and transection of large vessels, always completely advance the I-BLADE® and wait for Tone 3. Do not stop squeezing the closing handle with the I-BLADE® only partially advanced. •.	To ensure coagulation and transection of tissue, always completely advance the I-BLADE®. Do not stop squeezing the Closing Handle with the I-BLADE® only partially advanced.
Warnings and Precautions	Transection without power application can lead to bleeding in vascular tissue	Transection without power application can lead to bleeding in tissue
Warnings and Precautions	Do not activate the instrument while cleaning to avoid inadvertent user injury.	Do not activate the instrument while cleaning it
Warnings and Precautions	Do not use the top jaw alone to perform tissue dissection.	Not present

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL G2 Articulating Tissue Sealers

IFU Text	Subject Device ENSEAL G2 Articulating Tissue Sealers	Predicate Device ENSEAL G2 Tissue Sealers
Warnings and Precautions	<ul style="list-style-type: none"> • Prior to inserting or removing the device from the trocar, ensure that the device is in the straight, non-articulated position by confirming that the indicator arrow on the articulation wheel is aligned with the top of the instrument. • Removing the device from a trocar while the device is articulated could result in unintentionally removing the trocar from the incision site, a loss of insufflation, or damage to the black shaft covering. • Prior to articulating the shaft, ensure that the jaw and the articulation joint are fully inserted through the trocar. • The ENSEAL® G2 Articulating Tissue Sealer is not a passive articulation device. Do not force the articulation angle by pressing the shaft against a solid body. Use the articulation wheel to achieve the desired articulation angle. • If the rotation force of the articulation wheel seems excessive, discontinue rotation of the wheel. Using excessive force could damage the device. 	Not present

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL G2 Articulating Tissue Sealers

IFU Text	Subject Device ENSEAL G2 Articulating Tissue Sealers	Predicate Device ENSEAL G2 Tissue Sealers
Warnings and Precautions	This device is packaged and sterilized for single use only. Multiple patient use may compromise the device integrity or create a risk of contamination that, in turn, may result in patient injury or illness.	This instrument is packaged and sterilized for single use only. Do not reuse, reprocess or resterilize. Reuse, reprocessing, or resterilization may compromise the structural integrity of the instrument and/or lead to instrument failure that in turn may result in patient injury, illness or death. Also, reprocessing or resterilization of single use instruments may create a risk of contamination and/or cause patient infection or cross-infection, including, but not limited to, the transmission of infectious disease(s) from one patient to another. Contamination of the instrument may lead to injury, illness, or death of the patient.
Steps For Use, Step 3	Before inserting the instrument through the trocar, make certain that the instrument is in the straight (non-articulated) position and the jaws are closed. Insert the instrument into the trocar before articulating the device. To rotate and articulate the device, turn the rotation knob and the articulation wheel to move the shaft to the desired position. When the rotation knob indicator arrow is aligned with the top of the instrument, turn the articulation wheel (clockwise to articulate to the right; counterclockwise to articulate to the left).	Step 3. Turn the rotation knob to move the shaft to the desired position. Position targeted tissue within open jaws.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL G2 Articulating Tissue Sealers

IFU Text	Subject Device ENSEAL G2 Articulating Tissue Sealers	Predicate Device ENSEAL G2 Tissue Sealers
Steps For Use, Steps 4 and 5	Position targeted tissue within open jaws. Squeeze the closing handle to grasp targeted tissue between the jaws.	Step 4. Squeeze the closing handle to grasp targeted tissue between the jaws.
Steps For Use, Step 6	Press the energy activation button to energize the jaws and begin coagulation of the targeted tissue. The generator gives audible feedback (Tone 1) as energy is delivered to the grasped tissue. Affirm tissue effects prior to advancing the I-BLADE through tissue.	Step 5. Press the energy activation button to energize the jaws and begin coagulation of the targeted tissue. The generator gives audible feedback (Tone 1) as energy is delivered to the grasped tissue.
Steps For Use, Step 7	7b. While pressing the energy activation button, continue squeezing the closing handle to advance the I-BLADE and increase tissue compression. Note: For larger vascular bundles it is recommended to advance the I-BLADE(R) slowly (gradually). 7c The generator tone changes pitch (Tone 2) when the tissue impedance threshold is reached, and the I-BLADE is ready to be fully advanced.	Step 6a. Fully depressing the Energy Activation Button unlocks the I-BLADE knife as energy continues to be delivered to the tissue. Step 6b. Continue to hold down the Energy Activation Button. Continue squeezing the Closing Handle to increase compression on the grasped tissue and advance the I-BLADE knife. Step 6c. The tissue is transected as it collapses during coagulation. Audible feedback from the generator changes pitch (Tone 2) when the tissue impedance threshold is reached and the I-BLADE knife is ready to be fully advanced.

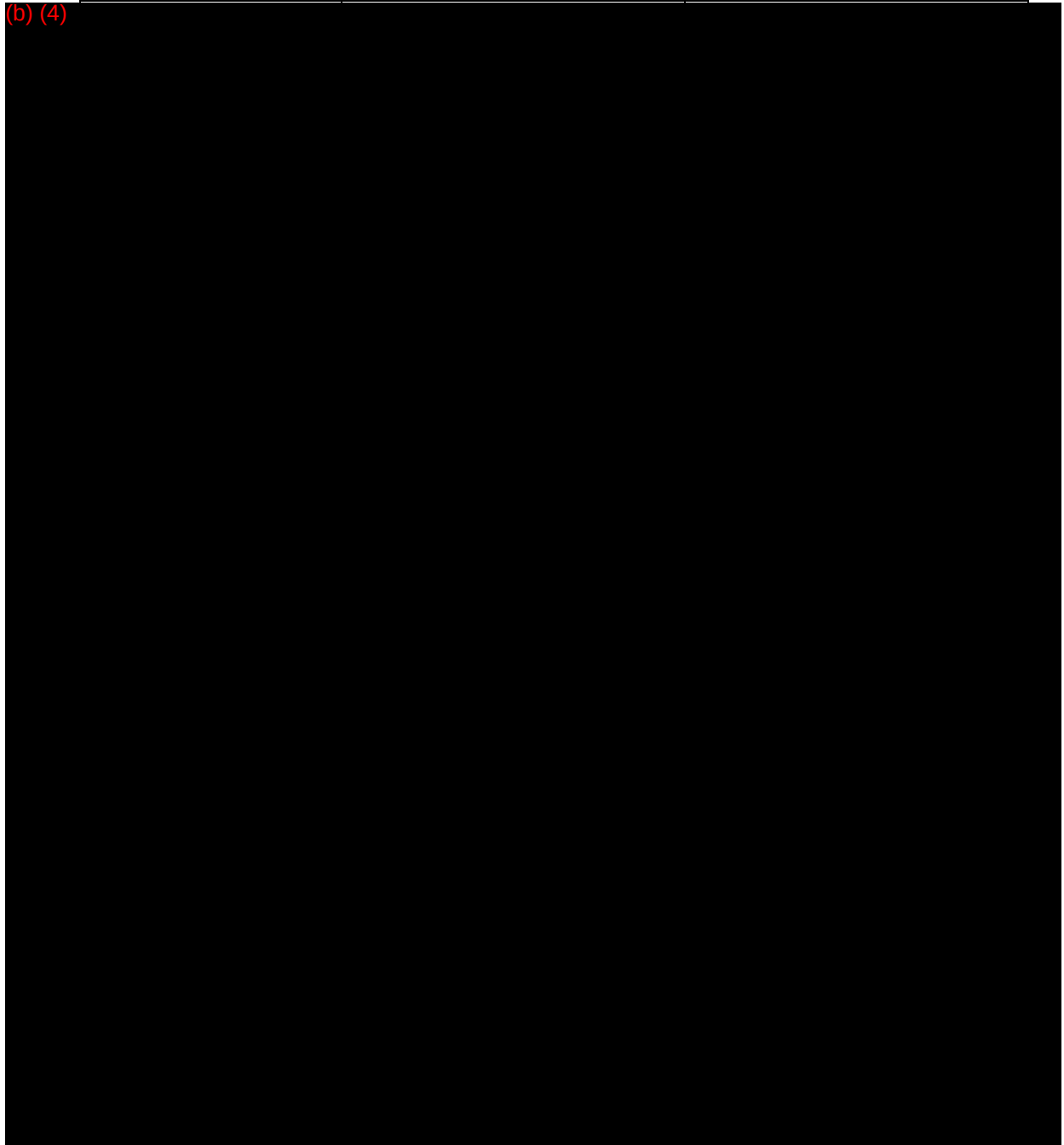
Please note that minor verbiage, grammar and punctuation were adjusted to enhance readability.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

The table below provides a detailed side-by-side comparison of the proposed and Predicate devices with respect to technology and device performance.

Table 12.4 Device Comparison Table: Technology and Performance

Characteristic	Subject Device ENSEAL G2 Articulating Tissue Sealers Description	Predicate Device ENSEAL G2 Tissue Sealers Description
Similarities		



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Characteristic	Subject Device ENSEAL G2 Articulating Tissue Sealers Description	Predicate Device ENSEAL G2 Tissue Sealers Description
Sterility Assurance Level (SAL)	10 ⁻⁶	Identical
Packaging	Rigid clear PETG blister package with a heat-sealed Tyvek lid	Identical
Vessel Sealing Performance	Mean Burst Pressure is >240mmHg	Identical
Maximum Indicated Vessel Size	7mm	Identical
Hemostasis, Acute	100% vessels hemostatic through completion of surgery	Identical
Long-Term Seal Quality, Hemostasis,	100% vessels hemostatic 30-days post surgery	Identical
Differences		
Articulation Feature	The device articulates at approximately 60° to the left and the right.	Not present
Package Insert	Warnings and Precautions, Steps to grasp, dissect and cut tissue for the articulation feature	Not present
Shelf Life	1 yr	2 yr
Device Materials	New materials in conjunction with articulation feature	Not present

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Section 13: Proposed Labeling

This section contains representative labeling for the Subject Device ENSEAL G2 Articulating Tissue Sealers and the Package Insert for the marketed Predicate Device ENSEAL G2 Tissue Sealer.

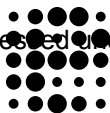
The draft labeling includes:

- Package Inserts
- Primary Labels (also known as film labels)
- Carton Labels

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

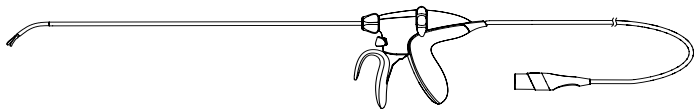
Subject Device ENSEAL G2 Articulating Tissue Sealers Package Insert

The Package Inserts will be supplied in multiple languages. This section includes only the English portions of the Package Insert.



Ethicon
Endo-Surgery

ENSEAL® G2 Articulating Tissue Sealer



Please read all information carefully.

Failure to properly follow the instructions may lead to serious surgical consequences.

Important: This package insert is designed to provide instructions for use of the ENSEAL® G2 Articulating Tissue Sealer. It is not a reference to surgical techniques.

ENSEAL and I-BLADE are trademarks of Ethicon Endo-Surgery.

Instructions

Indications

The ENSEAL® G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with the Generator G11 (GEN11). They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7mm and tissue and/or vascular bundles as large as will fit in the jaws of the instruments.

The ENSEAL G2 Articulating Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures.

Contraindications

The efficacy of the ENSEAL G2 Articulating Tissue Sealer for the indication of contraceptive tubal coagulation (permanent female sterilization) has not been evaluated and is unknown. The design of the ENSEAL G2 Articulating Tissue Sealer is significantly different from bipolar designs that are marketed for the indication of contraceptive tubal coagulation. The design differences may affect the efficacy of the procedure and failure rates may not be comparable.

Device Description

The ENSEAL G2 Articulating Tissue Sealer is a sterile, single patient use surgical instrument used to coagulate and transect vessels up to and including 7mm in diameter and tissue and/or vascular bundles. This device is for soft tissue only. The instrument consists of a grip housing assembly, a rotating and articulating shaft either 35cm or 45cm in length, a moveable jaw and an I-BLADE® in the jaws. The instrument shaft can be rotated to facilitate visualization and enable easy access to targeted tissue. The instrument shaft, normally in the straight position, can be articulated using the articulation wheel to gain additional access to tissue and facilitate additional angles across tissue and/or vascular bundles.

The jaws are in a normally opened position and can be partially or fully closed by squeezing the closing handle. The jaws have atraumatic teeth for grasping and holding targeted tissue when clamped. Bipolar energy is delivered to the clamped tissue when the energy activation button is pressed. Fully squeezing the closing handle advances the I-BLADE the length of the jaws, and the targeted tissue is compressed, coagulated, and transected. The I-BLADE cannot be advanced to transect tissue without first fully depressing the energy activation button. The power cord is permanently attached to the device and connects the instrument to the generator. The ENSEAL G2 Articulating Tissue Sealer is designed for use exclusively with the Generator G11 (GEN11), packaged separately. Dimensions: Jaw Length, Straight: 20mm, Jaw Length, Curved: 19mm, Shaft Length: 35cm from Jaw Tip to Shaft Rotation Knob or 45cm from Jaw Tip to Shaft Rotation Knob, Shaft Diameter: 5mm.

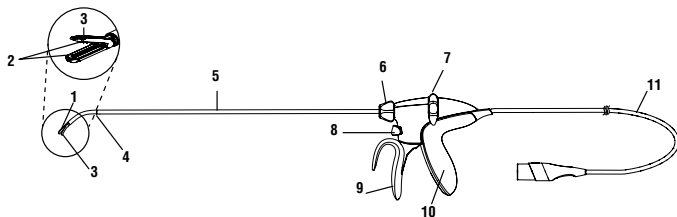
Generator Audible Indicators

Refer to the following table for generator audible feedback tones.

Tone	Description	Action
Tone 1	Continuous steady beeping	Generator On: Device is active.
Tone 2	Continuous steady beeping at increased pitch	Intermediate: Tissue impedance threshold is reached.
Tone 3	One short beep	End of cycle: I-BLADE fully advanced. Energy is stopped.

Illustration and Nomenclature (Illustration 1)

1. Jaws (curved for strapping)
2. Atraumatic teeth
3. I-BLADE[®] knife (not visible)
4. Articulation joint
5. Shaft
6. Activation knob
7. Articulation wheel
8. Energy activation button
9. Closing handle
10. Grip housing
11. Power cord



Transport and Storage Conditions

Temperature: -20° C to +54° C

Relative Humidity: 10% - 80%

How Supplied

The ENSEAL G2 Articulating Tissue Sealer with integrated power cord is supplied sterile for single patient use. Discard after use.

Warnings and Precautions

- Caution: Federal (USA) law restricts this device to sale by or on the order of a physician.
- Examine the shipping carton and instrument for signs of shipping damage. Note any shortages, breakage, or apparent damage, retain the evidence, and notify Ethicon Endo-Surgery Customer Service immediately and replace with a new instrument. Do not use a damaged product.
- A thorough understanding of the principles and techniques involved in laser, electrosurgical, and ultrasonic procedures is essential to avoid shock and burn hazards to both patient and medical personnel and damage to the device or other medical instruments. Ensure that electrical insulation or grounding is not compromised.
- Laparoscopic surgical procedures with the ENSEAL G2 Articulating Tissue Sealer should be performed only by persons with adequate training and preparation. Consult the medical literature regarding techniques, complications, and hazards prior to any surgical procedure.
- Do not immerse the ENSEAL G2 Articulating Tissue Sealer in liquid.
- Pools of conductive fluids (e.g. blood, saline) in direct contact with the instrument jaws may affect device performance. Remove pooled fluid from around the instrument jaws before activating the instrument.
- Verify compatibility with generators. Use device only with the Generator G11 (GEN11).
- The device is at high voltage whenever the generator is active. Activate this device only when the jaws are under direct visual observation to avoid unintended tissue coagulation or necrosis.
- Check if patient has a pacemaker or implanted cardioverter/defibrillator. Consult pacemaker manufacturer for information about the effects of RF energy on these devices.
- This instrument contains a surgically sharp edge. THIS DEVICE CUTS!

- The ENSEAL G2 Articulating Tissue Sealer has not been shown to be effective for sealing large vessels without fully advancing the I-BLADE. Do not attempt to seal large vessels by only clamping the tissue and applying energy.
- Do not use this device on vessels in excess of 7mm in diameter.
- Do not use excessive force on the closing handle to close the jaws; grasp only as much tissue as will fit between the jaws where the current will pass. Greater amounts of tissue require more closing handle force. Excessive force could damage the device.
- Avoid contact with metal and plastic when instrument is active.
- Use surgical gloves specifically designed by their manufacturer for electrosurgical procedures. The use of other gloves can result in burns or shocks.
- In case of system failure, ensure the availability of the appropriate back-up equipment relevant to the specific procedure.
- During and following activation in tissue, the jaws and shaft may be hot. Avoid unintended contact with tissue, drapes, surgical gowns, or other unintended sites at all times.
- After using the instrument to seal and/or transect, always examine the tissue for hemostasis. If hemostasis is not present, appropriate techniques should be used to achieve hemostasis.
- Due to the difficulty of visualizing obscured structures, proceed slowly and do not attempt to transect large masses of tissue in one activation. Avoid the division of large tissue and/or vascular bundles when using the instrument under these conditions.
- Products manufactured or distributed by companies not authorized by Ethicon Endo-Surgery may not be compatible with the ENSEAL G2 Articulating Tissue Sealer instrument. Use of such products may lead to unanticipated results and possible injury to the user or patient.
- Discontinue use and replace device if excessive smoke or sparking is observed.
- Dispose of this device in a "Sharps" container. Dispose of all opened instruments whether used or unused.
- As with all energy sources (Electrosurgery, Laser, or Ultrasound), there are concerns about the carcinogenic and infectious potential of the by-products, such as tissue smoke plume and aerosols. Appropriate measures such as protective eyewear, filtration masks, and effective smoke evacuation equipment should be used in laparoscopic or open procedures.
- Instruments or devices which come into contact with bodily fluids may require special disposal handling to prevent biological contamination.
- To ensure effective sealing and transection of large vessels, always completely advance the I-BLADE and wait for Tone 3.
- Do not stop squeezing the closing handle with the I-BLADE only partially advanced.
- Transection without power application can lead to bleeding in vascular tissue.
- Take care to avoid injury: the I-BLADE is sharp.
- Do NOT immerse distal tip in liquid, including saline solution, to clean.
- Do not clean the instrument with abrasives.
- To avoid inadvertent user injury, do not activate the instrument while cleaning.
-  Prior to inserting or removing the device from the trocar, ensure that the device is in the straight, non-articulated position by confirming that the indicator arrow on the articulation wheel is aligned with the top of the instrument.
- Removing the device from a trocar while the device is articulated could result in unintentionally removing the trocar from the incision site, a loss of insufflation, or damage to the black shaft covering.
- Prior to articulating the shaft, ensure that the jaw and the articulation joint are fully inserted through the trocar.
- The ENSEAL G2 Articulating Tissue Sealer is not a passive articulation device. Do not force the articulation angle by pressing the shaft against a solid body. Use the articulation wheel to achieve the desired articulation angle.
- If the rotation force of the articulation wheel seems excessive, discontinue rotation of the wheel. Using excessive force could damage the device.
- Do not use the top jaw alone to perform tissue dissection.
- This device is packaged and sterilized for single use only. Multiple patient use may compromise the device integrity or create a risk of contamination that, in turn, may result in patient injury or illness.

Instructions for Use

- 1 Using sterile technique, remove the instrument from the package. To avoid damage, do not flip the instrument into the sterile field. Do not use a damaged product.
- 2 Connect the instrument power cord to the generator.
- 3 Before inserting the instrument through the trocar, make certain that the instrument is in the straight (non-articulated) position and the jaws are closed. (**Illustration 2**) Insert the instrument into the trocar before articulating the device.

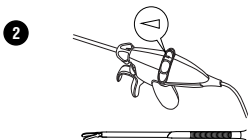
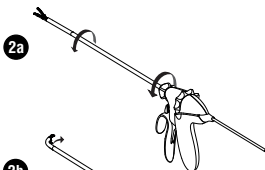
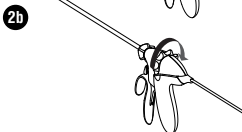
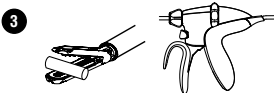
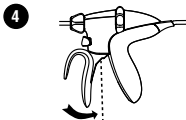
To rotate and articulate the device, turn the rotation knob and the articulation wheel to move the shaft to the desired position. (**Illustration 2a**)

When the rotation knob indicator arrow is aligned with the top of the instrument, turn the articulation wheel (clockwise to articulate to the right; counterclockwise to articulate to the left). (**Illustration 2b**)

- 4 Position targeted tissue within open jaws. (**Illustration 3**).
- 5 Squeeze the closing handle to grasp targeted tissue between the jaws (**Illustration 4**).
- 6 Press the energy activation button to energize the jaws and begin coagulation of the targeted tissue. The generator gives audible feedback (Tone 1) as energy is delivered to the grasped tissue. Affirm tissue effects prior to advancing the I-BLADE through tissue.

Illustrations & Notes

- NOTE: The instrument is straight when the indicator arrow on the articulation wheel is aligned with the top of the instrument.



- NOTE: Tissue between the jaws may be further compressed by squeezing the closing handle to the first stop. Applying additional pressure at the first stop does not increase tissue compression.

Instructions for Use

- 7** Complete the instrument firing cycle (**Illustration 5**).

7a. Fully press the energy activation button to unlock the I-BLADE.

7b. While pressing the energy activation button, continue squeezing the closing handle to advance the I-BLADE and increase tissue compression.
NOTE: For larger vascular bundles, it is recommended to advance the I-BLADE slowly (gradually).

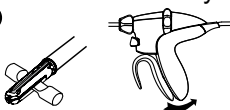
7c. The generator tone changes pitch (Tone 2) when the tissue impedance threshold is reached, and the I-BLADE is ready to be fully advanced. It is recommended to maintain a consistent speed throughout I-BLADE advancement.

7d. Squeeze the closing handle until it stops against the grip housing. This fully advances the I-BLADE (**Illustration 5**).

The generator provides one short beep (Tone 3) to indicate the cycle is complete after the I-BLADE is fully advanced and the tissue impedance threshold has been reached. Energy delivery automatically stops after Tone 3.
- 8** After the cycle is complete, release the closing handle and the energy activation button. (**Illustration 7**)
If the desired cut is longer than the stroke of the blade, transection can be accomplished by repeating Steps 4 - 7.
- 9** Straighten the shaft and close the jaws before removing the device from the trocar. The instrument is straight when the indicator arrow on the articulation wheel is aligned with the top of the instrument.
- 10** Disconnect the instrument from the generator.
- 11** Dispose of the instrument in an appropriate Sharps container. Do not reuse instrument.

Illustrations & Notes

5

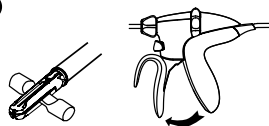


- NOTE: The instrument may be operated with either footswitch or handswitch control.
- NOTE: The I-BLADE cannot be advanced to transect tissue without first fully pressing the energy activation button and then releasing the I-BLADE.
- NOTE: The instrument can be wiped with a sterile moist gauze sponge to remove tissue, if necessary. If tissue is still visible in the jaws, use forceps to remove residue. (**Illustration 6**)

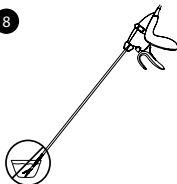
6



7



8



Caution: Do NOT immerse distal tip in liquid, including saline solution, to clean. (**Illustration 8**)

	<p>See Instructions For Use (refer to blue symbol on outer packaging)</p>
<p>STERILE R</p>	<p>Sterilized by Irradiation. Sterility Guaranteed Unless Package Opened or Damaged. Do Not Resterilize.</p>
	<p>Relative Humidity</p>
	<p>Single Patient Use</p>
<p>LOT</p>	<p>Lot</p>
<p>R_x Only</p>	<p>Caution: Federal (USA) law restricts this device to sale by or on the order of a physician.</p>
	<p>Temperature</p>

	
	<p>Manufacturer</p>
<div data-bbox="137 444 241 485" style="border: 1px solid black; padding: 2px; display: inline-block;"> <div style="border-right: 1px solid black; padding: 0 5px;">EC</div> <div style="padding: 0 5px;">REP</div> </div>	<p>Authorized Representative in the European Community</p>
<div data-bbox="137 601 241 642" style="border: 1px solid black; padding: 2px; display: inline-block;"> <div style="border-right: 1px solid black; padding: 0 5px;">USA</div> <div style="padding: 0 5px;">REP</div> </div>	<p>Authorized Representative in the USA</p>

REF
NSLG2S35A, NSLG2C35A
NSLG2S45A, NSLG2C45A



Ethicon Endo-Surgery (Europe) GmbH
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22851 Norderstedt
GERMANY



Ethicon Endo-Surgery, Inc.
Cincinnati, OH 45242-2839 USA
1-800-USE-ENDO



Ethicon
Endo-Surgery



REVISIONS			
LTR	CAF NO.	CHNG	DATE
A			
PRODUCT CODE	ARTWORK NUMBER	PACKAGE COMPONENT NUMBER	
NSLG2S14, NSLG2S25, NSLG2S35, NSLG2S45	A88128P00	P40712P01	



ETHICON ENDO-SURGERY, INC.

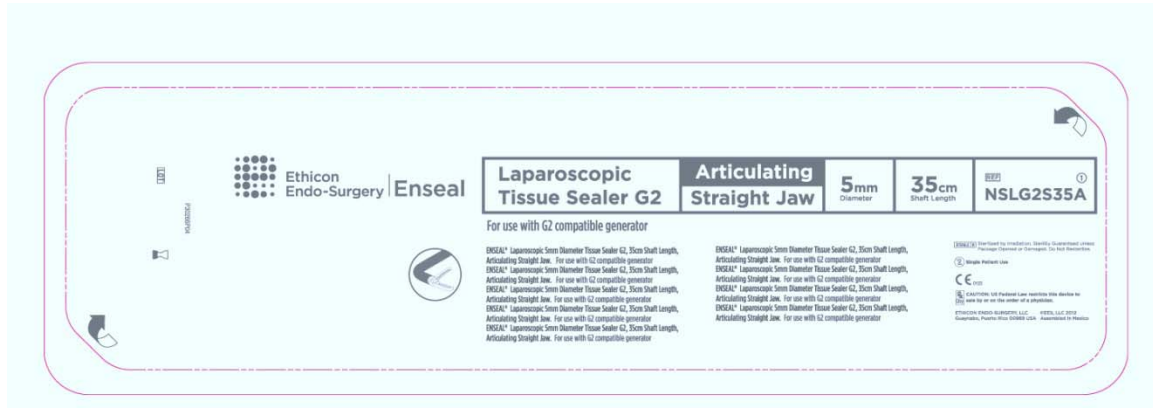
CONFIDENTIAL: NOT TO BE REPRODUCED OR USED IN ANY WAY
WITHOUT WRITTEN ETHICON
ENDO-SURGERY, INC. APPROVAL CINCINNATI, OH

BLACK

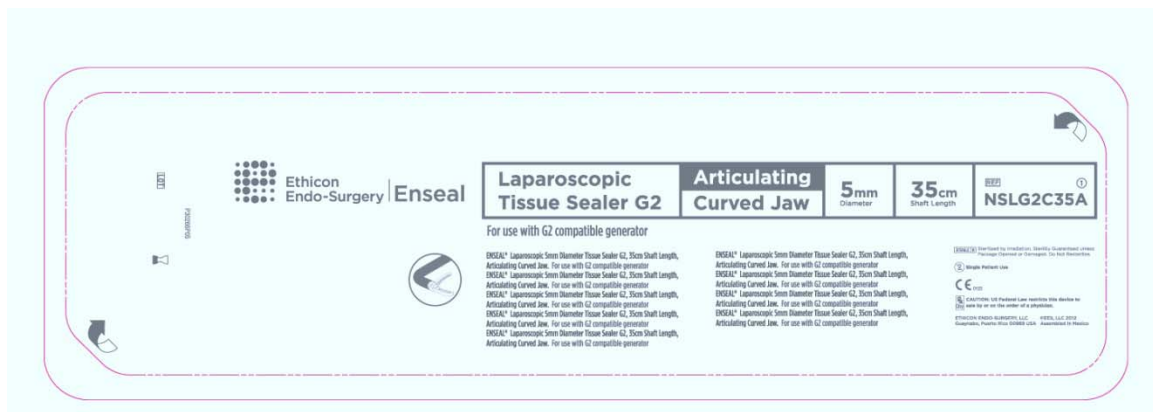
Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Subject Device Primary Package Labels

ENSEAL G2 Articulating Tissue Sealers, Straight Jaw, Code NSLG2S35A



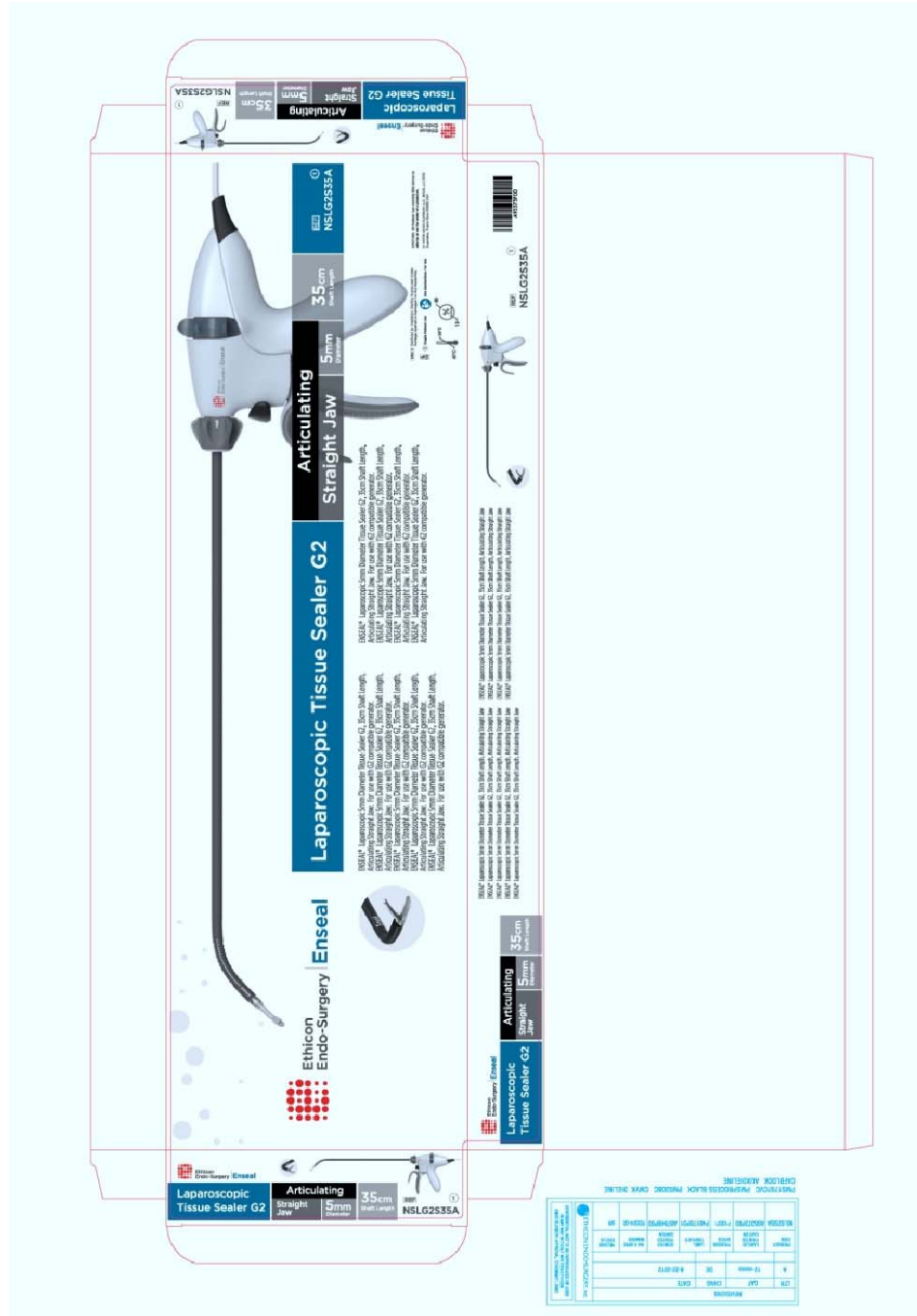
ENSEAL G2 Articulating Tissue Sealers, Curved Jaw, Code NSLG2C35A



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Subject Device Carton Package Labels

ENSEAL G2 Articulating Tissue Sealers, Straight Jaw, Code NSLG2S35A



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

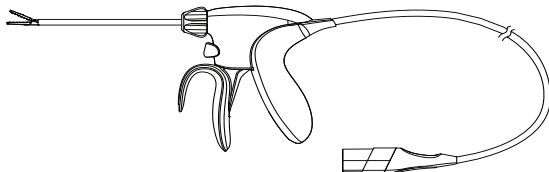
Predicate Device ENSEAL G2 Tissue Sealers Package Inserts

The Package Inserts are supplied in multiple languages. This section includes only the English portions of the Package Inserts, for reference.

- ENSEAL G2 Tissue Sealers, Straight Jaw
- ENSEAL G2 Tissue Sealers, Curved Jaw



ENSEAL® G2 Tissue Sealer



Dispositif de coagulation tissulaire ENSEAL® G2

ENSEAL® Versiegelungsinstrument G2

Dispositivo di chiusura tissutale ENSEAL® G2

Isolador de tecidos ENSEAL® G2

Sellador de tejido ENSEAL® G2

ENSEAL® G2-weefselafdichter

ENSEAL® G2 vævsforsegler

ENSEAL® G2 Tissue Sealer

Εργαλείο στεγανοποίησης ιστού G2 ENSEAL®

ENSEAL® G2 förslutningsinstrument

Zgrzewarka do tkanek ENSEAL® G2

ENSEAL® G2 szövetlezáró eszköz

Nástroj pro uzavěr tkání ENSEAL® Tissue Sealer G2

Nástroj na uzatváranie tkanív ENSEAL® G2 Tissue Sealer

ENSEAL® G2 组织缝合器

Please read all information carefully.

Failure to properly follow the instructions may lead to serious surgical consequences.

Important: This package insert is designed to provide instructions for use of the ENSEAL® G2 Tissue Sealer. It is not a reference to surgical techniques.

ENSEAL is a trademark of Ethicon Endo-Surgery.

Instructions, Instructions, Gebrauchsanweisung, Istruzioni, Instruções, Instrucciones,
Gebruiksaanwijzing, Brugsvejledning, Ohje, Οδηγίες, Bruksanvisning, Instrukcja.

Указания, Návod k použití, Návod, 使用説明

Indications

The ENSEAL® G2 Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with an electrosurgical generator. They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7 mm and bundles as large as will fit in the jaws of the instruments.

The ENSEAL® G2 Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures

Contraindications

The efficacy of the ENSEAL® G2 Tissue Sealer for the indication of contraceptive tubal coagulation (permanent female sterilization) has not been evaluated and is unknown. The design of the ENSEAL® G2 Tissue Sealer is significantly different from bipolar designs that are marketed for the indication of contraceptive tubal coagulation. The design differences may affect the efficacy of the procedure and failure rates may not be comparable.

Device Description

The ENSEAL® G2 Tissue Sealer is a sterile, single patient use surgical instrument used to coagulate and transect vessels up to and including 7mm in diameter and tissue and/or vascular bundles. This device is for soft tissue only. The instrument consists of a grip housing assembly, a rotating shaft, a moveable jaw and an I-BLADE® knife in the jaws. The instrument shaft can be rotated to facilitate visualization and enable easy access to targeted tissue. The jaws are in a normally opened position and can be partially or fully closed by squeezing the closing handle. The jaws have atraumatic teeth for grasping and holding targeted tissue when clamped. Bipolar energy is delivered to the clamped tissue when the energy activation button is pressed. Fully squeezing the closing handle advances the I-BLADE® knife the length of the jaws, and the targeted tissue is compressed, coagulated, and transected. The I-BLADE® knife cannot be advanced to transect tissue without fully pressing the energy activation button and then releasing the I-BLADE® knife. The power cord is permanently attached to the device and connects the instrument to the generator. The ENSEAL® G2 Tissue Sealer is designed for use exclusively with the Generator G11 (GEN11), packaged separately. Dimensions: Jaw Length: 20mm, Shaft Length: 14, 25, 35 or 45 cm from Jaw Tip to Rotation Knob (varies by product code), Shaft Diameter: 5 mm.

Generator Audible Indicators

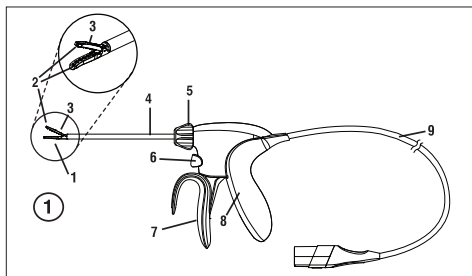
Refer to the following table for generator audible feedback tones.

Tone	Description	Action
Tone 1	Continuous steady beeping	Generator On: Device is active.
Tone 2	Continuous steady beeping at increased pitch	Intermediate: Tissue impedance threshold is reached.
Tone 3	One short beep	End of cycle: I-BLADE® knife fully advanced. Energy is stopped.

Illustration and Nomenclature (Illustration 1)

Processed under FOIA Request 2015-6323. Released by CDRH on 11/19/2015

- | | |
|---------------------------------|-----------------------------|
| 1. Jaws | 6. Energy Activation Button |
| 2. Atraumatic teeth | 7. Closing Handle |
| 3. I-BLADE® knife (not visible) | 8. Grip Housing |
| 4. Shaft | 9. Power Cord |
| 5. Rotation Knob | |



Transport and Storage Conditions

Temperature: -20° C to 60° C

Relative Humidity: 0-80%

How Supplied

The ENSEAL® G2 Tissue Sealer with integrated power cord is supplied sterile for single patient use. Discard after use.

Warnings and Precautions

- Caution: Federal (USA) law restricts this device to sale by or on the order of a physician.
 - Examine the shipping carton and instrument for signs of shipping damage. Note any shortages, breakage, or apparent damage, retain the evidence, and notify Ethicon Endo-Surgery Customer Service immediately and replace with a new instrument. Do not use a damaged product.
 - A thorough understanding of the principles and techniques involved in laser, electrosurgical, and ultrasonic procedures is essential to avoid shock and burn hazards to both patient and medical personnel and damage to the device or other medical instruments. Ensure that electrical insulation or grounding is not compromised.
 - Laparoscopic surgical procedures with the ENSEAL® G2 Tissue Sealer should be performed only by persons with adequate training and preparation. Consult the medical literature regarding techniques, complications, and hazards prior to any endoscopic procedure.
 - Do not immerse the ENSEAL® G2 Tissue Sealer in liquid.
 - Pools of conductive fluids (e.g. blood, saline) in direct contact with the instrument jaws may affect device performance. Remove pooled fluid from around the instrument jaws before activating the instrument.
 - Verify compatibility with generators. Use device only with the Generator G11 (GEN11).
 - The device is at high voltage whenever the generator is active. Activate this device only when the jaws are under direct visual observation to avoid unintended tissue coagulation or necrosis.
 - Check if patient has a pacemaker or implanted cardioverter/defibrillator. Consult pacemaker manufacturer for information about the effects of RF energy on these devices.
- This instrument contains a surgically sharp edge. THIS DEVICE CUTS!

act FDA/CDRH/OCE/DID at CDRH-FOI STATUS.GOV@fda.hhs.gov

- The ENSEAL® G2 Tissue Sealer has not been shown to be effective for sealing vessels without fully advancing the I-BLADE® knife. Do not attempt to seal vessels by only clamping the tissue and applying energy.
- Do not use this device on vessels in excess of 7mm in diameter.
- Do not use excessive force on the Closing Handle to close the jaws; grasp only as much tissue as will fit between the jaws where the current will pass. Greater amounts of tissue require more Closing Handle force. Excessive force could damage the device.
- Avoid contact with metal and plastic when instrument is active.
- Use surgical gloves specifically designed by their manufacturer for electrosurgical procedures. The use of other gloves can result in burns or shocks.
- In case of system failure, ensure the availability of the appropriate back-up equipment relevant to the specific procedure.
- During and following activation in tissue, the jaws and shaft may be hot. Avoid unintended contact with tissue, drapes, surgical gowns, or other unintended sites at all times.
- After utilizing the instrument to seal and transect, always examine the tissue for hemostasis. If hemostasis is not present, appropriate techniques should be used to achieve hemostasis.
- Due to the difficulty of visualizing obscured structures, proceed slowly and do not attempt to transect large masses of tissue in one activation. Avoid the division of large tissue and/or vascular bundles when using the instrument under these conditions.
- Products manufactured or distributed by companies not authorized by Ethicon Endo-Surgery may not be compatible with the ENSEAL® G2 Tissue Sealer instrument. Use of such products may lead to unanticipated results and possible injury to the user or patient.
- Discontinue use and replace device if excessive smoke or sparking is observed.
- Dispose of this device in a "Sharps" container. Dispose of all opened instruments whether used or unused.
- As with all energy sources (Electrosurgery, Laser, or Ultrasound), there are concerns about the carcinogenic and infectious potential of the by-products, such as tissue smoke plume and aerosols. Appropriate measures such as protective eyewear, filtration masks, and effective smoke evacuation equipment should be used in laparoscopic or open procedures.
- Instruments or devices which come into contact with bodily fluids may require special disposal handling to prevent biological contamination.
- This instrument is packaged and sterilized for single use only. Do not reuse, reprocess or resterilize. Reuse, reprocessing, or resterilization may compromise the structural integrity of the instrument and/or lead to instrument failure that in turn may result in patient injury, illness or death. Also, reprocessing or resterilization of single use instruments may create a risk of contamination and/or cause patient infection or cross-infection, including, but not limited to, the transmission of infectious disease(s) from one patient to another. Contamination of the instrument may lead to injury, illness, or death of the patient.
- To ensure coagulation and transection of tissue, always completely advance the I-BLADE®. Do not stop squeezing the Closing Handle with the I-BLADE® only partially advanced.
- Transection without power application can lead to bleeding.
- Take care to avoid injury for the I-BLADE® knife is sharp.
- Do NOT immerse distal tip in liquid, including saline solution, to clean. **(Illustration 7)**
- Do not clean the instrument with abrasives.
- Do not activate the instrument while cleaning to avoid inadvertent user injury.

Instructions for Use

1 Using sterile technique, remove the instrument from the package. To avoid damage, do not flip the instrument into the sterile field. Do not use a damaged product.

2 Connect the instrument power cord to the generator.

3 Turn the rotation knob to move the shaft to the desired position. Position targeted tissue within open jaws. (**Illustration 2**)

4 Squeeze Closing Handle to grasp targeted tissue between the jaws (**illustration 3**).

5 Press the Energy Activation Button to energize the jaws and begin coagulation of the targeted tissue. The generator gives audible feedback (Tone 1) as energy is delivered to the grasped tissue.

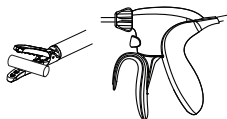
6 Complete the instrument firing cycle. (**Illustration 4**)

6a. Fully depressing the Energy Activation Button unlocks the I-BLADE® knife as energy continues to be delivered to the tissue.

6b. Continue to hold down the Energy Activation Button. Continue squeezing the Closing Handle to increase compression on the grasped tissue and advance the I-BLADE® knife.

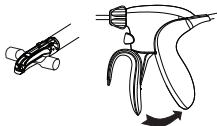
Illustrations & Notes

2

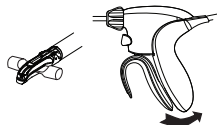


- NOTE: Tissue between the jaws may be further compressed by squeezing the Closing Handle to the first stop.

3



4



- NOTE: The ENSEAL® G2 Tissue Sealer may be operated with either footswitch or handswitch control.
- NOTE: The I-BLADE® knife cannot be advanced to transect tissue without first fully depressing the Energy Activation Button and then releasing the I-BLADE® knife.

Instructions for Use

6c. The tissue is transected as it collapses during coagulation. Audible feedback from the generator changes pitch (Tone 2) when the tissue impedance threshold is reached, and the I-BLADE® knife is ready to be fully advanced.

6d. Once the Closing Handle is fully closed, the I-BLADE® knife is fully advanced, and the tissue impedance threshold has been reached, the generator provides audible feedback (Tone 3) that the cycle is complete and automatically stops delivery of energy.

- 7** After the coagulation and transection cycle is complete, release the Closing Handle and the Energy Activation Button. (**Illustration 5**) If the desired cut is longer than the stroke of the blade, transection can be accomplished by repeating Steps 3 - 6.

- 8** Disconnect the instrument from the generator.

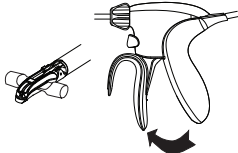
- 9** Dispose of the instrument in an appropriate Sharps container. Do not reuse instrument.

Illustrations & Notes

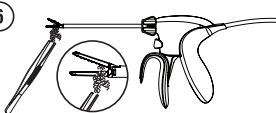
- NOTE: Ensure that the Closing Handle is squeezed to the fully closed position until it stops against the grip housing.

- NOTE: The instrument can be wiped with a sterile moist gauze sponge to remove tissue, if necessary. If tissue is still visible in the jaws, use forceps to remove residue (**Illustration 6**)

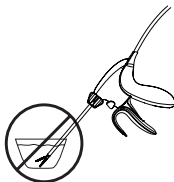
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6



7



STERILE R

Sterilized by Irradiation.
Sterilisé par irradiation.
Do Not Resterilize.
Stérilisé par irradiation.
Stérilité garantie si l'emballage n'a pas été ouvert ou endommagé.
Ne pas restériliser.
Strahlensterilisiert.
Nicht verwenden, wenn die Sterilverpackung geöffnet oder beschädigt ist.
Nicht reesterilisieren.
Sterilizzato con radiazioni.
Sterilità garantita, a meno che la confezione non venga aperta o danneggiata.
Non risterilizzare.
Esterilizado por irradiación.
Esterilização garantida excepto se a embalagem estiver aberta ou danificada.
Não reesterilize.
Esterilizado por radiación.
Esterilización garantizada mientras el envase esté íntegro.
No reesterilizar.
Gesteriliseerd middels straling.
Steriliteit gegarandeerd tenzij de verpakking is geopend of beschadigd.
Niet opnieuw steriliseren.
Strålesteriliseret.
Garanteret steril, med mindre pakken er åbnet eller beskadiget.
Må ikke reesteriliseres.
Steriloitu säteilyttämällä.
Tuote on steriili, kun pakkaus on avaamaton ja ehjä.
Ei saa steriloida uudelleen.
Αποστειρωμένο με ακτινοβολία.
Η στεριότητα είναι εγγυημένη εφόσον δεν ανοιχθεί η συσκευασία ή δεν προκληθεί ζημία σε αυτήν.
Μην επαναποστειρώνετε.
Strålsteriliserad
Steriliteten garanteras under förutsättning att förpackningen inte är öppnad eller skadad.
Får ej omsteriliseras.
Sterylizowane napromienianiem.
Jalowość gwarantowana pod warunkiem, że opakowanie nie zostało otwarte lub uszkodzone.
Nie sterylizować ponownie.
Besugárzással sterilizálva.
A sterilitása addig garantálható, amíg ki nem nyitják, illetve meg nem sérül a csomagolás.
Tilos újra sterilizálni.
Sterilizované ožiarením.
Sterilita je zaručena, pokud nedojde k otevření nebo poškození balení.
Nesterilizujte.
Sterilizované ožiarením.
Sterilita je zaručená, kým balenie nie je otvorené ani poškodené.
Nesterilizujte opakovane.
辐射灭菌。
无菌保证，除非包装打开或损坏。
勿再次灭菌。



Relative Humidity	Suhteellinen kosteus
Humidité relative	Σχετική υγρασία
Relative Feuchte	Relativ fuktighet
Umidità relativa	Wilgotność względna
Humidade relativa	Relativ páratartalom
Humedad relativa	Relativní vlhkost
Relatíve vochtigheid	Relativna vlhkost
Relativ fuktighed	相對濕度

	<p>See instructions for use of the blue symbol on the packaging. Voir notice d'utilisation (se reporter au symbole bleu sur l'emballage extérieur). Bitte Gebrauchsanweisung beachten (siehe blaues Symbol an der äußeren Verpackung). Vedere le Istruzioni per l'uso (vedere il simbolo blu sulla confezione esterna). Consulte as Instruções de utilização (consulte o símbolo azul na embalagem exterior). Ver instrucciones de uso (refiérase al símbolo azul en el envase exterior). Zie de gebruiksaanwijzing (zie het blauwe symbool op de buitenste verpakking). Se betjeningsvejledningen (der henvises til det blå symbol på yderemballagen). Katso käyttöohjeita (katso sinistä symbolia ulkopakkauksessa). Δείτε τις Οδηγίες Χρήσης (ανατρέξτε στο μπλε σύμβολο, στο εξωτερικό της συσκευασίας). Se bruksanvisningen (se den blå symbolen på yttre förpackningen). Należy zapoznać się z instrukcją użytkowania (patrz niebieski symbol na zewnętrznym opakowaniu). Lásd a használati útmutatót (lásd a külső csomagoláson található kék szimbólumot). Viz návod k použití (informace jsou uvedeny u modrého symbolu na vnějším balení). Prečítajte si návod na použitie (vzťahuje sa na modrý symbol na vonkajšom obale). 参见使用说明 (请参阅外包装上的蓝色符号)。</p>	
	<p>Single Patient Use Usage unique Einweg-Instrument, nur für den Einsatz bei einem Patienten Monouso Uso único Uso en un solo paciente Voor gebruik bij één patiënt Kun til engangsbrug</p>	<p>Potilaskohtainen Χρήση σε έναν μόνον ασθενή För enpatientsbruk Do użytku u jednego pacjenta Egyetlen betegnél használható fel Určeno k použití pouze u jednoho pacienta Určené iba pre jedného pacienta 单个患者使用</p>
<p>LOT</p>	<p>Lot Lot Ch.-B. Lotto N°- do Lote N° de lote Partij Parti</p>	<p>Erän koodi Παρτίδα Batchnummer Numer partii produkcyjnej Tétel Šarže Šarža 批号</p>

	<p>Isi: Expire Date À utiliser avant Verw. bis Utilizzare entro Validade Utilizar antes de Gebruik vóór Må ikke anvendes efter udløbsdatoen er overskredet.</p> <p>Κατασκευαστική Används före Koniec okresu przydatności do użytku A feltüntetett dátumig használható fel Použit do data Použitelné do 有效期 :</p>
	<p>Manufacturer Fabricant Hersteller Fabbicante Fabricante Fabricante Fabrikant Producent</p> <p>Valmistaja Κατασκευαστής Tillverkare Producent Gyártó Výrobce Výrobca 制造商</p>
	<p>Authorized Representative in the European Community Représentant agréé dans la Communauté européenne Bevollmächtigter in der Europäischen Gemeinschaft Rappresentante autorizzato nella Comunità Europea Representante autorizado na Comunidade Europeia Representante autorizado en la Comunidad Europea Gemachtigde vertegenwoordiger in de Europese Unie Repræsentant i Det Europæiske Fællesskab Valtuutettu edustaja Euroopan yhteisön alueella Εξουσιοδοτημένος αντιπρόσωπος στην Ευρωπαϊκή Ένωση Auktoriserad återförsäljare inom EU Autoryzowany przedstawiciel we Wspólnocie Europejskiej Meghatalmazott képviselő az Európai Közösségben Autorizovaný zástupce v Evropském společenství Autorizovaný zástupca pre Európske spoločenstvo 欧共体内授权代理</p>



Caution: Federal (USA) law restricts this device to sale by or on the order of a physician.

Attention : La Loi Fédérale (États-Unis d'Amérique) n'autorise la vente de ce dispositif que par un médecin ou sur sa prescription.

Achtung: Laut Gesetz darf dieses Instrument in den USA nur an einen Mediziner oder eine in seinem Auftrag handelnde Person verkauft werden.

Attenzione: la legge federale americana consente la vendita di questo dispositivo solo a medici o dietro prescrizione medica.

Atenção: A lei federal (dos Estados Unidos da América) só permite a venda deste dispositivo a médicos ou por receita destes.

Atención: la ley federal de EE.UU. impone que este producto sólo puede ser vendido por un médico o bajo prescripción médica.

Let op: de federale wetgeving in de VS eist dat dit apparaat uitsluitend wordt verkocht door of in opdracht van een arts.

Forsigtig: I henhold til gældende amerikansk lovgivning må denne anordning kun sælges af eller på ordination af en læge.

Varoitus: Yhdysvaltain liittovaltion lain mukaan tämän tuotteen saa myydä ainoastaan lääkäri tai lääkärin määräyksestä.

Προσοχή: Η ομοσπονδιακή νομοθεσία (των Η.Π.Α.) επιτρέπει την πώληση της συσκευής αυτής μόνον από ιατρό ή κατόπιν εντολής ιατρού.

Viktigt! Enligt amerikansk lag får detta instrument endast säljas till eller på ordination av en läkare.

Przestroga: Prawo federalne (USA) zezwala na sprzedaż tego urządzenia wyłącznie lekarzowi lub na jego zamówienie.

Figyelmeztetés: Az Amerikai Egyesült Államok szövetségi törvényei értelmében ez az eszköz kizárólag orvos által vagy orvos rendelésére értékesíthető.

Upozornění: Federální zákony USA omezují prodej tohoto zařízení pouze na lékaře nebo na jeho předpis.

Upozornenie: Podľa federálnych zákonov (USA) je toto zariadenie možné kúpiť iba od lekára alebo na lekársky predpis.

小心: (美国) 联邦法律规定该器械只能由医师销售或遵医嘱销售。

USA REP

Authorized Representative in the USA

Représentant agréé aux États-Unis d'Amérique

Bevollmächtigter in den USA

Rappresentante autorizzato negli Stati Uniti

Representante autorizado nos EUA

Representante autorizado en los EE.UU.

Gemachtigde vertegenwoordiger in de VS

Autoriseret repræsentant i USA

Valtuutettu edustaja Yhdysvalloissa

Εξουσιοδοτημένος αντιπρόσωπος στις Η.Π.Α.

Auktoriserad återförsäljare i USA

Autoryzowany przedstawiciel w USA

Meghatalmazott képviselő az Amerikai Egyesült Államokban

Autorizovaný zástupce v USA

Autorizovaný zástupca v USA

美国国内授权代理

	<div> <div>Temperature</div> <div>Temperatüre</div> <div>Temperatur</div> <div>Temperatura</div> <div>Temperatura</div> <div>Temperatura</div> <div>Temperatuur</div> <div>Temperatur</div> </div> <div> <div>Lämpötila</div> <div>Θερμότητα</div> <div>Temperatur</div> <div>Temperatura</div> <div>Hőmérséklet</div> <div>Teplota</div> <div>Teplota</div> <div>温度</div> </div>
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P40672P03

REF
NSLG2C14, NSLG2C25, NSLG2C35, NSLG2C45



Ethicon Endo-Surgery (Europe) GmbH
Hummelsbuetteler Steindamm 71
22851 Norderstedt
GERMANY



Ethicon Endo-Surgery, Inc.
Cincinnati, OH 45242-2839 USA
1-800-USE-ENDO



Ethicon
Endo-Surgery



A line drawing of a medical device, likely a catheter or probe. It features a long, thin tube extending from the left. The tube is connected to a complex, multi-lobed head assembly. The head assembly includes a central, elongated, and slightly curved component, possibly a catheter tip, and several smaller, rounded, and lobed structures. A small, rectangular, textured component is attached to the side of the main tube. The overall design suggests a device used for medical procedures, such as catheterization or minimally invasive surgery.

ENSEAL® G2 组织缝合器

ENSEAL is a trademark of Ethicon Endo-Surgery.

Instructions, Instructions, Gebrauchsanweisung, Istruzioni, Instruções, Instrucciones,
Gebruiksaanwijzing, Brugsvejledning, Ohje, Οδηγίες, Bruksanvisning, Instrukcja.
Utis/Διεύθυνση, Návod k použití, Návod, 使用説明 - FOIS ATUS GOV@

Indications

Processed under FOIA Request 2015-6323. Released by CDRH on 1

The ENSEAL® G2 Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with an electrosurgical generator. They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7 mm and bundles as large as will fit in the jaws of the instruments.

The ENSEAL® G2 Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures

Contraindications

The efficacy of the ENSEAL® G2 Tissue Sealer for the indication of contraceptive tubal coagulation (permanent female sterilization) has not been evaluated and is unknown. The design of the ENSEAL® G2 Tissue Sealer is significantly different from bipolar designs that are marketed for the indication of contraceptive tubal coagulation. The design differences may affect the efficacy of the procedure and failure rates may not be comparable.

Device Description

The ENSEAL® G2 Tissue Sealer is a sterile, single patient use surgical instrument used to coagulate and transect vessels up to and including 7mm in diameter and tissue and/or vascular bundles. This device is for soft tissue only. The instrument consists of a grip housing assembly, a rotating shaft, a moveable jaw and an I-BLADE® knife in the jaws. The instrument shaft can be rotated to facilitate visualization and enable easy access to targeted tissue. The jaws are in a normally opened position and can be partially or fully closed by squeezing the closing handle. The jaws have atraumatic teeth for grasping and holding targeted tissue when clamped. Bipolar energy is delivered to the clamped tissue when the energy activation button is pressed. Fully squeezing the closing handle advances the I-BLADE® knife the length of the jaws, and the targeted tissue is compressed, coagulated, and transected. The I-BLADE® knife cannot be advanced to transect tissue without fully pressing the energy activation button and then releasing the I-BLADE® knife. The power cord is permanently attached to the device and connects the instrument to the generator. The ENSEAL® G2 Tissue Sealer is designed for use exclusively with the Generator G11 (GEN11), packaged separately. Dimensions: Jaw Length: 20mm, Shaft Length: 14, 25, 35 or 45 cm from Jaw Tip to Rotation Knob (varies by product code), Shaft Diameter: 5 mm.

Generator Audible Indicators

Refer to the following table for generator audible feedback tones.

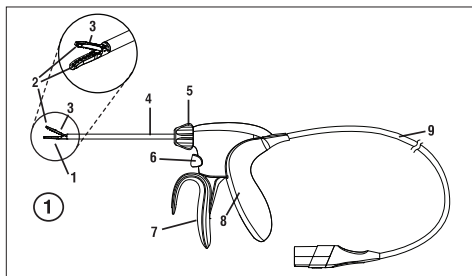
Tone	Description	Action
Tone 1	Continuous steady beeping	Generator On: Device is active.
Tone 2	Continuous steady beeping at increased pitch	Intermediate: Tissue impedance threshold is reached.
Tone 3	One short beep	End of cycle: I-BLADE® knife fully advanced. Energy is stopped.

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Illustration and Nomenclature (Illustration 1)

Processed under FOIA Request 2015-6323. Released by CDRH on 11/19/2015

- | | |
|---------------------------------|-----------------------------|
| 1. Jaws | 6. Energy Activation Button |
| 2. Atraumatic teeth | 7. Closing Handle |
| 3. I-BLADE® knife (not visible) | 8. Grip Housing |
| 4. Shaft | 9. Power Cord |
| 5. Rotation Knob | |



Transport and Storage Conditions

Temperature: -20° C to 60° C

Relative Humidity: 0-80%

How Supplied

The ENSEAL® G2 Tissue Sealer with integrated power cord is supplied sterile for single patient use. Discard after use.

Warnings and Precautions

- Caution: Federal (USA) law restricts this device to sale by or on the order of a physician.
 - Examine the shipping carton and instrument for signs of shipping damage. Note any shortages, breakage, or apparent damage, retain the evidence, and notify Ethicon Endo-Surgery Customer Service immediately and replace with a new instrument. Do not use a damaged product.
 - A thorough understanding of the principles and techniques involved in laser, electrosurgical, and ultrasonic procedures is essential to avoid shock and burn hazards to both patient and medical personnel and damage to the device or other medical instruments. Ensure that electrical insulation or grounding is not compromised.
 - Laparoscopic surgical procedures with the ENSEAL® G2 Tissue Sealer should be performed only by persons with adequate training and preparation. Consult the medical literature regarding techniques, complications, and hazards prior to any endoscopic procedure.
 - Do not immerse the ENSEAL® G2 Tissue Sealer in liquid.
 - Pools of conductive fluids (e.g. blood, saline) in direct contact with the instrument jaws may affect device performance. Remove pooled fluid from around the instrument jaws before activating the instrument.
 - Verify compatibility with generators. Use device only with the Generator G11 (GEN11).
 - The device is at high voltage whenever the generator is active. Activate this device only when the jaws are under direct visual observation to avoid unintended tissue coagulation or necrosis.
 - Check if patient has a pacemaker or implanted cardioverter/defibrillator. Consult pacemaker manufacturer for information about the effects of RF energy on these devices.
- This instrument contains a surgically sharp edge. THIS DEVICE CUTS!

act FDA/CDRH/OCE/DID at CDRH-FOI STATUS.GOV@fda.hhs.gov

- The ENSEAL® G2 Tissue Sealer has not been shown to be effective for sealing vessels without fully advancing the I-BLADE® knife. Do not attempt to seal vessels by only clamping the tissue and applying energy.
- Do not use this device on vessels in excess of 7mm in diameter.
- Do not use excessive force on the Closing Handle to close the jaws; grasp only as much tissue as will fit between the jaws where the current will pass. Greater amounts of tissue require more Closing Handle force. Excessive force could damage the device.
- Avoid contact with metal and plastic when instrument is active.
- Use surgical gloves specifically designed by their manufacturer for electrosurgical procedures. The use of other gloves can result in burns or shocks.
- In case of system failure, ensure the availability of the appropriate back-up equipment relevant to the specific procedure.
- During and following activation in tissue, the jaws and shaft may be hot. Avoid unintended contact with tissue, drapes, surgical gowns, or other unintended sites at all times.
- After utilizing the instrument to seal and transect, always examine the tissue for hemostasis. If hemostasis is not present, appropriate techniques should be used to achieve hemostasis.
- Due to the difficulty of visualizing obscured structures, proceed slowly and do not attempt to transect large masses of tissue in one activation. Avoid the division of large tissue and/or vascular bundles when using the instrument under these conditions.
- Products manufactured or distributed by companies not authorized by Ethicon Endo-Surgery may not be compatible with the ENSEAL® G2 Tissue Sealer instrument. Use of such products may lead to unanticipated results and possible injury to the user or patient.
- Discontinue use and replace device if excessive smoke or sparking is observed.
- Dispose of this device in a "Sharps" container. Dispose of all opened instruments whether used or unused.
- As with all energy sources (Electrosurgery, Laser, or Ultrasound), there are concerns about the carcinogenic and infectious potential of the by-products, such as tissue smoke plume and aerosols. Appropriate measures such as protective eyewear, filtration masks, and effective smoke evacuation equipment should be used in laparoscopic or open procedures.
- Instruments or devices which come into contact with bodily fluids may require special disposal handling to prevent biological contamination.
- This instrument is packaged and sterilized for single use only. Do not reuse, reprocess or resterilize. Reuse, reprocessing, or resterilization may compromise the structural integrity of the instrument and/or lead to instrument failure that in turn may result in patient injury, illness or death. Also, reprocessing or resterilization of single use instruments may create a risk of contamination and/or cause patient infection or cross-infection, including, but not limited to, the transmission of infectious disease(s) from one patient to another. Contamination of the instrument may lead to injury, illness, or death of the patient.
- To ensure coagulation and transection of tissue, always completely advance the I-BLADE®. Do not stop squeezing the Closing Handle with the I-BLADE® only partially advanced.
- Transection without power application can lead to bleeding.
- Take care to avoid injury for the I-BLADE® knife is sharp.
- Do NOT immerse distal tip in liquid, including saline solution, to clean. **(Illustration 7)**
- Do not clean the instrument with abrasives.
- Do not activate the instrument while cleaning to avoid inadvertent user injury.

Instructions for Use

1 Using sterile technique, remove the instrument from the package. To avoid damage, do not flip the instrument into the sterile field. Do not use a damaged product.

2 Connect the instrument power cord to the generator.

3 Turn the rotation knob to move the shaft to the desired position. Position targeted tissue within open jaws. (**Illustration 2**)

4 Squeeze Closing Handle to grasp targeted tissue between the jaws (**illustration 3**).

5 Press the Energy Activation Button to energize the jaws and begin coagulation of the targeted tissue. The generator gives audible feedback (Tone 1) as energy is delivered to the grasped tissue.

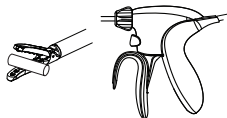
6 Complete the instrument firing cycle. (**Illustration 4**)

6a. Fully depressing the Energy Activation Button unlocks the I-BLADE® knife as energy continues to be delivered to the tissue.

6b. Continue to hold down the Energy Activation Button. Continue squeezing the Closing Handle to increase compression on the grasped tissue and advance the I-BLADE® knife.

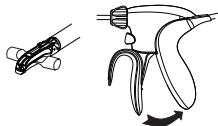
Illustrations & Notes

2

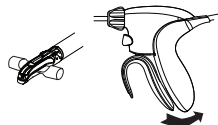


- NOTE: Tissue between the jaws may be further compressed by squeezing the Closing Handle to the first stop.

3



4



- NOTE: The ENSEAL® G2 Tissue Sealer may be operated with either footswitch or handswitch control.
- NOTE: The I-BLADE® knife cannot be advanced to transect tissue without first fully depressing the Energy Activation Button and then releasing the I-BLADE® knife.

Instructions for Use

6c. The tissue is transected as it collapses during coagulation. Audible feedback from the generator changes pitch (Tone 2) when the tissue impedance threshold is reached, and the I-BLADE® knife is ready to be fully advanced.

6d. Once the Closing Handle is fully closed, the I-BLADE® knife is fully advanced, and the tissue impedance threshold has been reached, the generator provides audible feedback (Tone 3) that the cycle is complete and automatically stops delivery of energy.

- 7** After the coagulation and transection cycle is complete, release the Closing Handle and the Energy Activation Button. (**Illustration 5**) If the desired cut is longer than the stroke of the blade, transection can be accomplished by repeating Steps 3 - 6.

- 8** Disconnect the instrument from the generator.

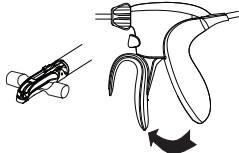
- 9** Dispose of the instrument in an appropriate Sharps container. Do not reuse instrument.

Illustrations & Notes

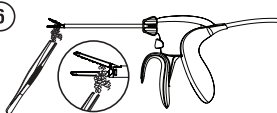
- NOTE: Ensure that the Closing Handle is squeezed to the fully closed position until it stops against the grip housing.

- NOTE: The instrument can be wiped with a sterile moist gauze sponge to remove tissue, if necessary. If tissue is still visible in the jaws, use forceps to remove residue (**Illustration 6**)

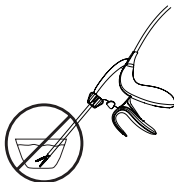
5



6



7



STERILE R

Sterilized by Irradiation.
Sterilisé par irradiation.
Do Not Resterilize.
Stérilisé par irradiation.
Stérilité garantie si l'emballage n'a pas été ouvert ou endommagé.
Ne pas restériliser.
Strahlensterilisiert.
Nicht verwenden, wenn die Sterilverpackung geöffnet oder beschädigt ist.
Nicht reesterilisieren.
Sterilizzato con radiazioni.
Sterilità garantita, a meno che la confezione non venga aperta o danneggiata.
Non risterilizzare.
Esterilizado por irradiación.
Esterilização garantida excepto se a embalagem estiver aberta ou danificada.
Não reesterilize.
Esterilizado por radiación.
Esterilización garantizada mientras el envase esté íntegro.
No reesterilizar.
Gesteriliseerd middels straling.
Steriliteit gegarandeerd tenzij de verpakking is geopend of beschadigd.
Niet opnieuw steriliseren.
Strålesteriliseret.
Garanteret steril, med mindre pakken er åbnet eller beskadiget.
Må ikke reesteriliseres.
Steriloitu säteilyttämällä.
Tuote on steriili, kun pakkaus on avaamaton ja ehjä.
Ei saa steriloida uudelleen.
Αποστειρωμένο με ακτινοβολία.
Η στεριότητα είναι εγγυημένη εφόσον δεν ανοιχθεί η συσκευασία ή δεν προκληθεί ζημία σε αυτήν.
Μην επαναποστειρώνετε.
Strålsteriliserad
Steriliteten garanteras under förutsättning att förpackningen inte är öppnad eller skadad.
Får ej omsteriliseras.
Sterylizowane napromienianiem.
Jalowość gwarantowana pod warunkiem, że opakowanie nie zostało otwarte lub uszkodzone.
Nie sterylizować ponownie.
Besugárzással sterilizálva.
A sterilitása addig garantálható, amíg ki nem nyitják, illetve meg nem sérül a csomagolás.
Tilos újra sterilizálni.
Sterilizované ožiarením.
Sterilita je zaručena, pokud nedojde k otevření nebo poškození balení.
Nesterilizujte.
Sterilizované ožiarením.
Sterilita je zaručená, kým balenie nie je otvorené ani poškodené.
Nesterilizujte opakovane.
辐射灭菌。
无菌保证，除非包装打开或损坏。
勿再次灭菌。



Relative Humidity	Suhteellinen kosteus
Humidité relative	Σχετική υγρασία
Relative Feuchte	Relativ fuktighet
Umidità relativa	Wilgotność względna
Humidade relativa	Relativ páratartalom
Humedad relativa	Relativní vlhkost
Relative vochtigheid	Relativna vlhkost
Relativ fuktighed	相對濕度

	<p>See instructions for use of the blue symbol on the packaging. Voir notice d'utilisation (se reporter au symbole bleu sur l'emballage extérieur). Bitte Gebrauchsanweisung beachten (siehe blaues Symbol an der äußeren Verpackung). Vedere le Istruzioni per l'uso (vedere il simbolo blu sulla confezione esterna). Consulte as Instruções de utilização (consulte o símbolo azul na embalagem exterior). Ver instrucciones de uso (refiérase al símbolo azul en el envase exterior). Zie de gebruiksaanwijzing (zie het blauwe symbool op de buitenste verpakking). Se betjeningsvejledning (der henvises til det blå symbol på yderemballagen). Katso käyttöohjeita (katso sinistä symbolia ulkopakkauksessa). Δείτε τις Οδηγίες Χρήσης (ανατρέξτε στο μπλε σύμβολο, στο εξωτερικό της συσκευασίας). Se bruksanvisningen (se den blå symbolen på yttre förpackningen). Należy zapoznać się z instrukcją użytkowania (patrz niebieski symbol na zewnętrznym opakowaniu). Lásd a használati útmutatót (lásd a külső csomagoláson található kék szimbólumot). Viz návod k použití (informace jsou uvedeny u modrého symbolu na vnějším balení). Prečítajte si návod na použitie (vzťahuje sa na modrý symbol na vonkajšom obale). 参见使用说明 (请参阅外包装上的蓝色符号)。</p>	
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	<p>Isi: Expire Date À utiliser avant Verw. bis Utilizzare entro Validade Utilizar antes de Gebruik vóór Må ikke anvendes efter udløbsdatoen er overskredet.</p> <p>Κατασκευαστική Används före Koniec okresu przydatności do użytku A feltüntetett dátumig használható fel Použit do data Použitelné do 有效期 :</p>
	<p>Manufacturer Fabricant Hersteller Fabbrikante Fabricante Fabricante Fabrikant Producent</p> <p>Valmistaja Κατασκευαστής Tillverkare Producent Gyártó Výrobce Výrobca 制造商</p>
	<p>Authorized Representative in the European Community Représentant agréé dans la Communauté européenne Bevollmächtigter in der Europäischen Gemeinschaft Rappresentante autorizzato nella Comunità Europea Representante autorizado na Comunidade Europeia Representante autorizado en la Comunidad Europea Gemachtigde vertegenwoordiger in de Europese Unie Repræsentant i Det Europæiske Fællesskab Valtuutettu edustaja Euroopan yhteisön alueella Εξουσιοδοτημένος αντιπρόσωπος στην Ευρωπαϊκή Ένωση Auktoriserad återförsäljare inom EU Autoryzowany przedstawiciel we Wspólnocie Europejskiej Meghatalmazott képviselő az Európai Közösségben Autorizovaný zástupce v Evropském společenství Autorizovaný zástupca pre Európske spoločenstvo 欧共体内授权代理</p>



Caution: Federal (USA) law restricts this device to sale by or on the order of a physician.

Attention : La Loi Fédérale (États-Unis d'Amérique) n'autorise la vente de ce dispositif que par un médecin ou sur sa prescription.

Achtung: Laut Gesetz darf dieses Instrument in den USA nur an einen Mediziner oder eine in seinem Auftrag handelnde Person verkauft werden.

Attenzione: la legge federale americana consente la vendita di questo dispositivo solo a medici o dietro prescrizione medica.

Atenção: A lei federal (dos Estados Unidos da América) só permite a venda deste dispositivo a médicos ou por receita destes.

Atención: la ley federal de EE.UU. impone que este producto sólo puede ser vendido por un médico o bajo prescripción médica.

Let op: de federale wetgeving in de VS eist dat dit apparaat uitsluitend wordt verkocht door of in opdracht van een arts.

Forsigtig: I henhold til gældende amerikansk lovgivning må denne anordning kun sælges af eller på ordination af en læge.

Varoitus: Yhdysvaltain liittovaltion lain mukaan tämän tuotteen saa myydä ainoastaan lääkäri tai lääkärin määräyksestä.

Προσοχή: Η ομοσπονδιακή νομοθεσία (των Η.Π.Α.) επιτρέπει την πώληση της συσκευής αυτής μόνον από ιατρό ή κατόπιν εντολής ιατρού.

Viktigt! Enligt amerikansk lag får detta instrument endast säljas till eller på ordination av en läkare.

Przestroga: Prawo federalne (USA) zezwala na sprzedaż tego urządzenia wyłącznie lekarzowi lub na jego zamówienie.

Figyelmeztetés: Az Amerikai Egyesült Államok szövetségi törvényei értelmében ez az eszköz kizárólag orvos által vagy orvos rendelésére értékesíthető.

Upozornění: Federální zákony USA omezují prodej tohoto zařízení pouze na lékaře nebo na jeho předpis.

Upozornenie: Podľa federálnych zákonov (USA) je toto zariadenie možné kúpiť iba od lekára alebo na lekársky predpis.

小心: (美国) 联邦法律规定该器械只能由医师销售或遵医嘱销售。

USA REP

Authorized Representative in the USA

Représentant agréé aux États-Unis d'Amérique

Bevollmächtigter in den USA

Rappresentante autorizzato negli Stati Uniti

Representante autorizado nos EUA

Representante autorizado en los EE.UU.

Gemachtigde vertegenwoordiger in de VS

Autoriseret repræsentant i USA

Valtuutettu edustaja Yhdysvalloissa

Εξουσιοδοτημένος αντιπρόσωπος στις Η.Π.Α.

Auktoriserad återförsäljare i USA

Autoryzowany przedstawiciel w USA

Meghatalmazott képviselő az Amerikai Egyesült Államokban

Autorizovaný zástupce v USA

Autorizovaný zástupca v USA

美国国内授权代理

	<div> <div>Temperature</div> <div>Temperatüre</div> <div>Temperatur</div> <div>Temperatura</div> <div>Temperatura</div> <div>Temperatura</div> <div>Temperatuur</div> <div>Temperatur</div> </div> <div> <div>Lämpötila</div> <div>Θερμότητα</div> <div>Temperatur</div> <div>Temperatura</div> <div>Hőmérséklet</div> <div>Teplota</div> <div>Teplota</div> <div>温度</div> </div>
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P40672P03

REF
NSLG2C14, NSLG2C25, NSLG2C35, NSLG2C45



Ethicon Endo-Surgery (Europe) GmbH
Hummelsbuetteler Steindamm 71
22851 Norderstedt
GERMANY



Ethicon Endo-Surgery, Inc.
Cincinnati, OH 45242-2839 USA
1-800-USE-ENDO

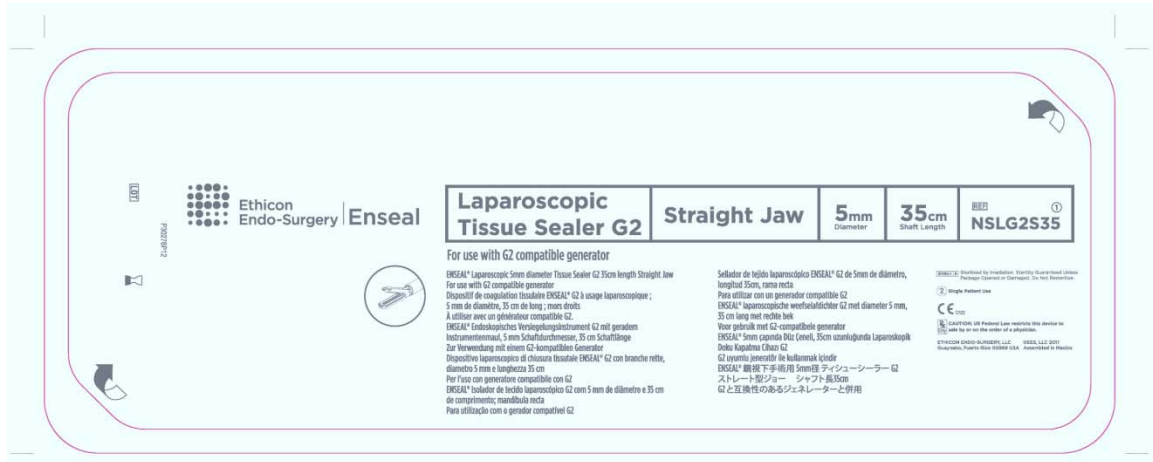


Ethicon
Endo-Surgery

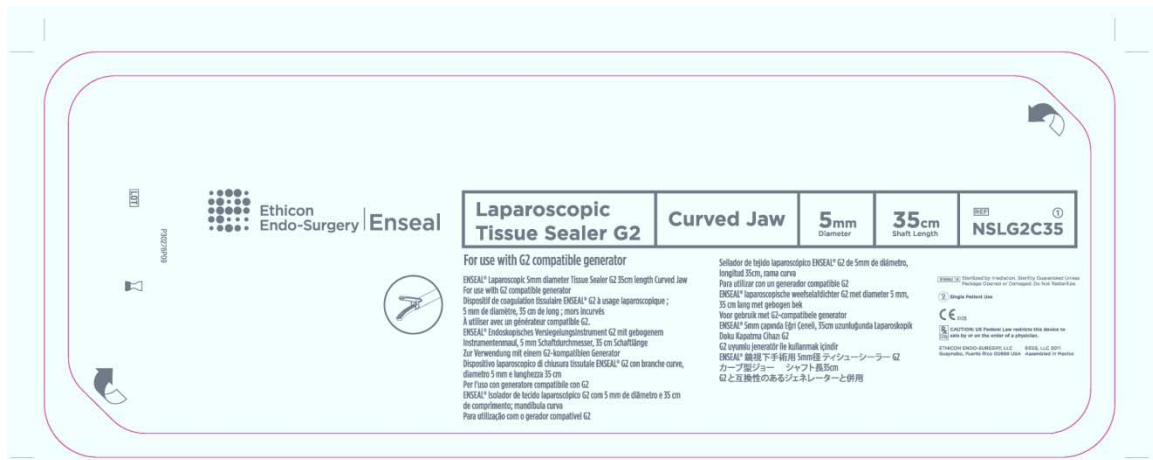
Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Predicate Device Primary Package Labels

ENSEAL G2 Tissue Sealers, Straight Jaw, Code NSLG2S35



ENSEAL G2 Tissue Sealers, Curved Jaw, Code NSLG2C35

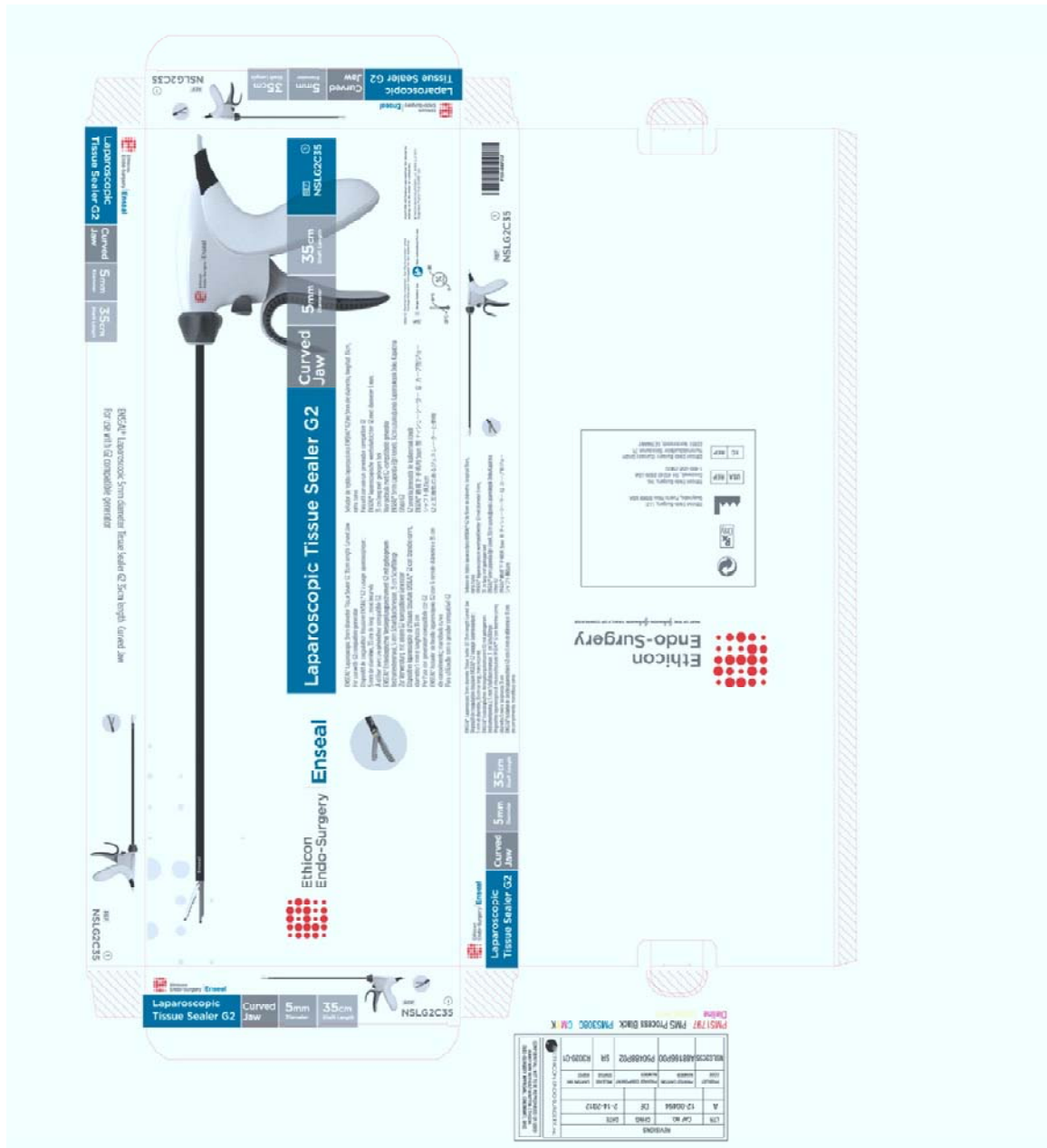


ENSEAL G2 Tissue Sealers, Straight Jaw, Code NSLG2S35



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

ENSEAL G2 Tissue Sealers, Curved Jaw, Code NSLG2C35



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Section 14: Sterilization and Shelf Life

Sterilization

The ENSEAL G2 Articulating Tissue Sealers are sterilized by Cobalt 60 Irradiation. The devices will be validated to 25-45 kGy to achieve a 10^{-6} Sterility Assurance Level (SAL). The dose setting will be performed per Method VD_{max} – Substantiation of 25 kGy as the sterilizing dose.

The sterilization process is validated and the sterilization dose established per the requirements of the following FDA recognized standards:

- ISO 11137-1:2006 – Sterilization of health care products – Radiation – Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices.
- ISO 11137-2:2006 – Sterilization of health care products – Radiation – Part 2: Establishing the sterilization dose.
- ISO 11137-3:2006 – Sterilization of health care products – Radiation – Part 3: Guidance on dosimetric aspects.

Please see Section 21 Other for Forms 3654.

Packaging

The Subject ENSEAL G2 Articulating Tissue Sealers are packaged in a rigid clear PETG blister package with a heat-sealed Tyvek lid. One sealed PETG blister is placed in an SBS sales unit carton, 6 SBS sales unit cartons are placed in a corrugated shipper. The packaging materials and manufacturing methods are the same as the Predicate.

The recommended transportation and storage conditions are:

- Temperature: -20° C to 54° C
- Relative Humidity: 10-80%

Shelf Life

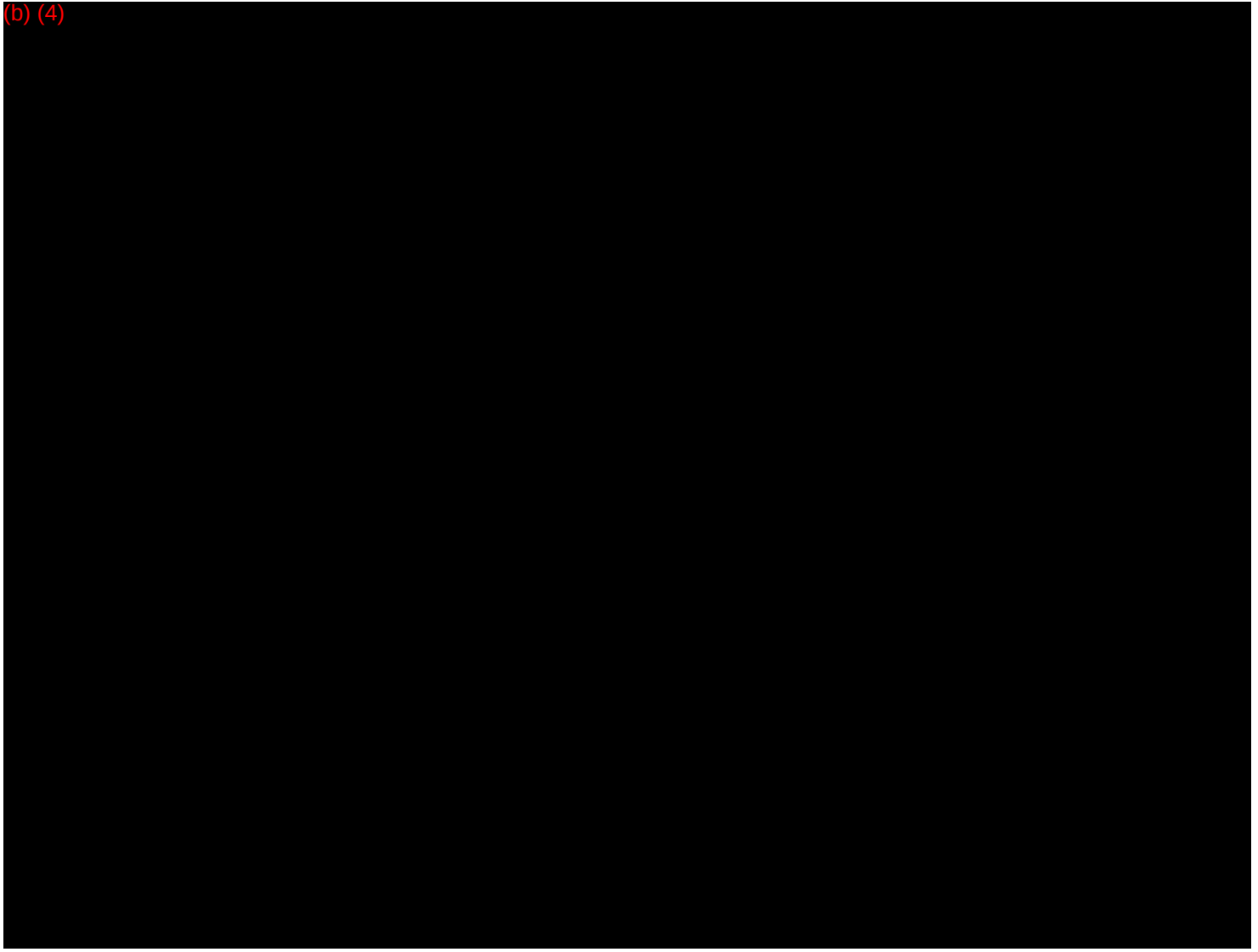
ENSEAL G2 Articulating Tissue Sealers are intended for single patient use and are not intended to be reused or resterilized. At launch, the devices will be labeled with a one-year shelf life. Ethicon Endo-Surgery will have data on file to support the stability and sterility of the device for one (1) year. As additional data becomes available to support a shelf life up to 2 years the expiration date will be adjusted.

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Shelf life will be established by testing of devices that have passed an Accelerated Aging evaluation. The ENSEAL G2 Articulating Tissue Sealers, Curved Jaw 35cm Length (product code NSLG2C35A) will be used for this testing as this configuration is considered representative of all the product codes in this submission. The straight jaw geometry has minimal differences and identical materials as the curved jaw.

Protocol

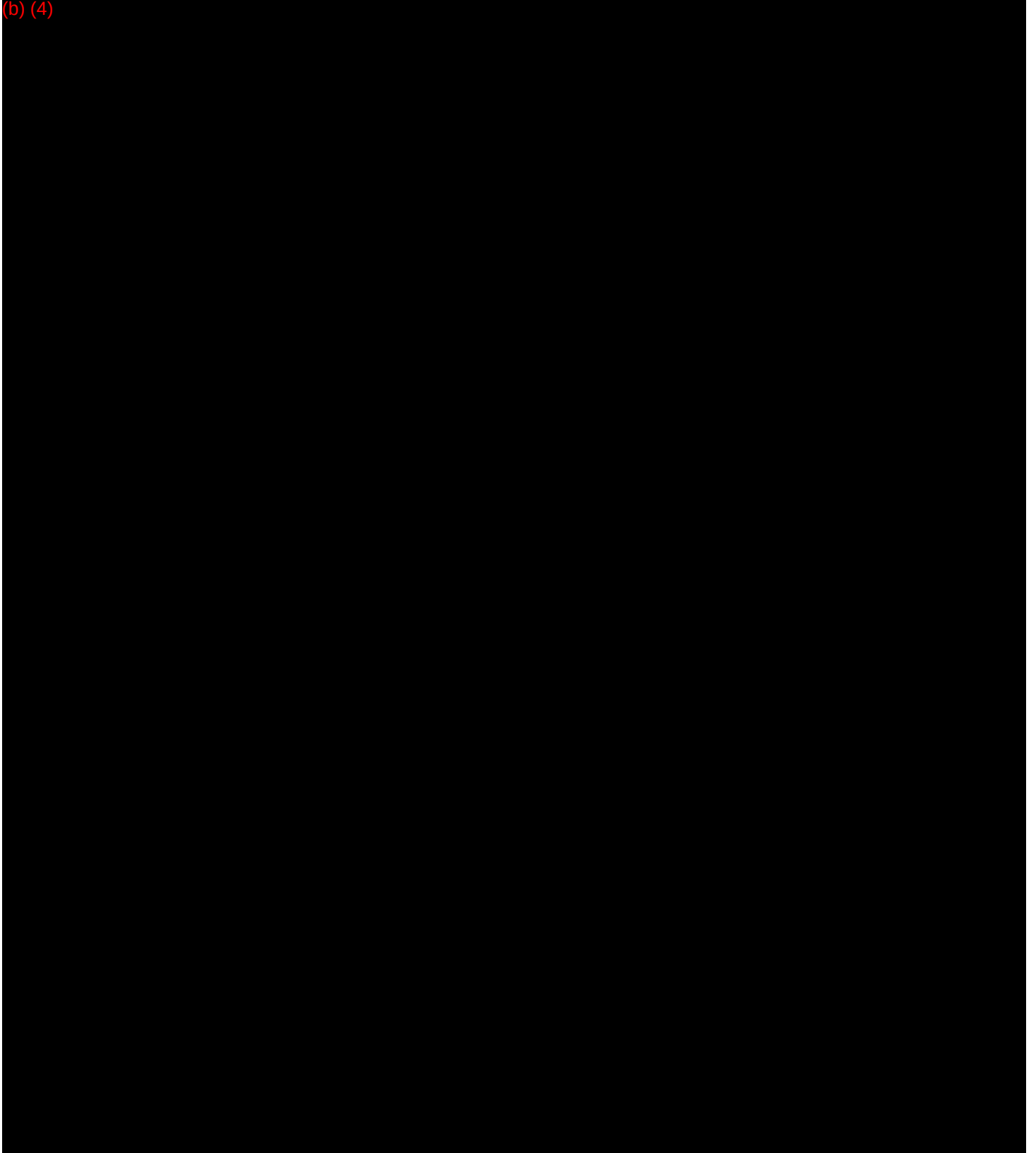
(b) (4)



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

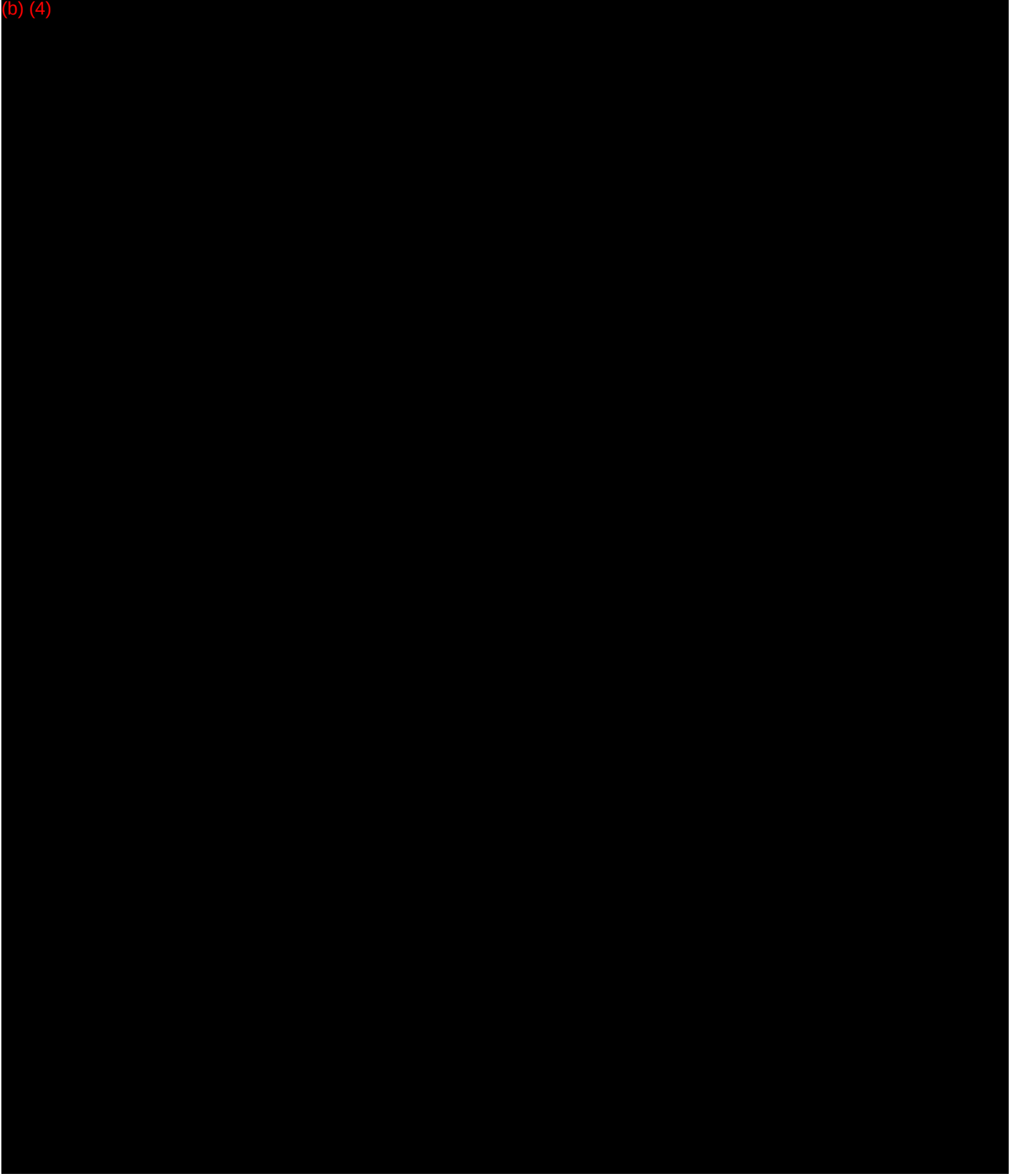
Resistance Test Method:

(b) (4)



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

(b) (4)



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Section 15: Biocompatibility

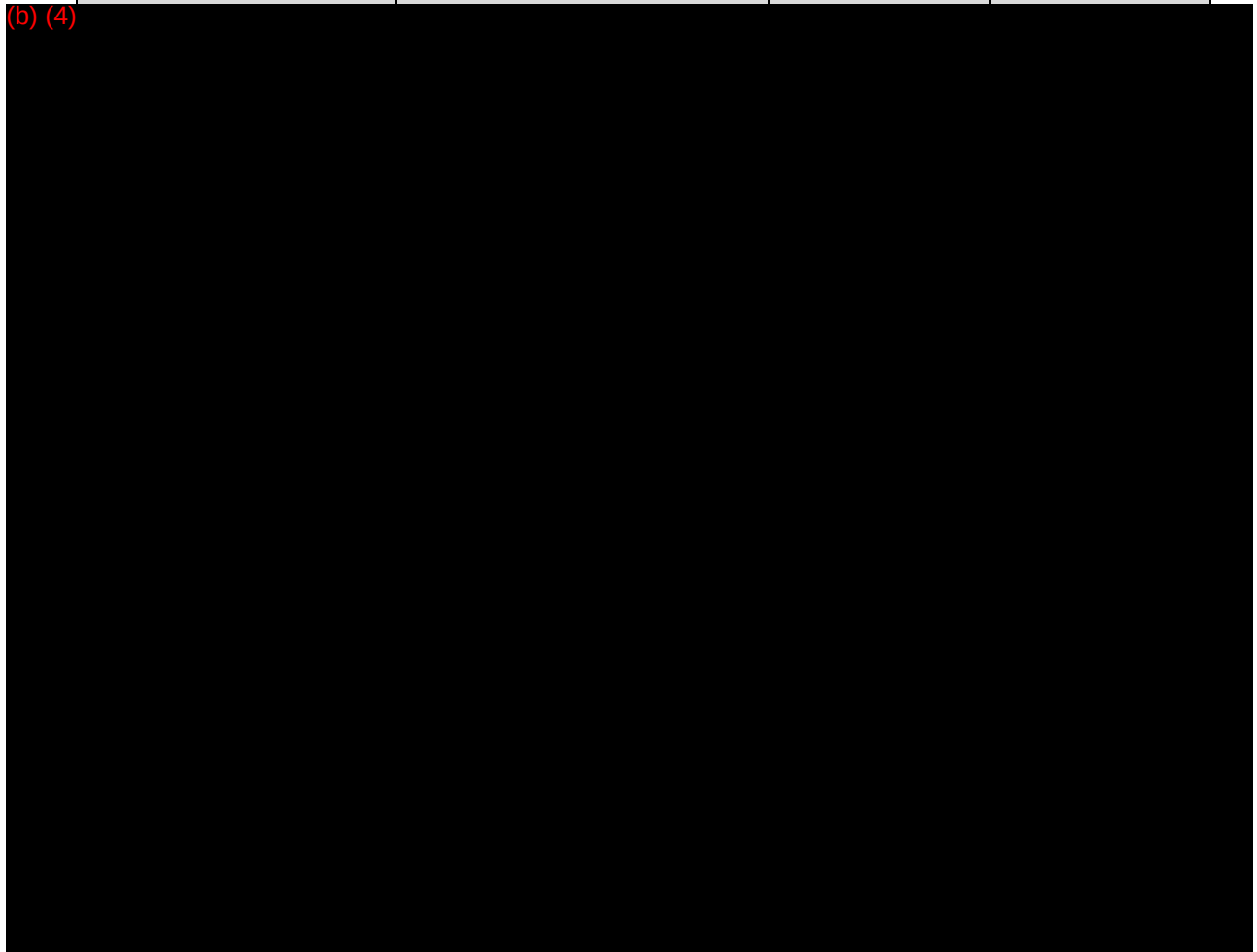
The biocompatibility of materials used in ENSEAL G2 Articulating Tissue Sealers were evaluated based on AAMI/ANSI/ISO 10993-1:2009 “Biological Evaluation of Medical Devices - Part 1: Evaluation and Testing within a Risk Management Process” and on FDA General Program Memorandum #G95-1: Use of International Standard ISO 10993, “Biological Evaluation of Medical Devices - Part 1: Evaluation and Testing.” All tests were performed in accordance with FDA’s Good Laboratory Practice Standard.

The materials in the devices are classified as Surface Devices Breached or Compromised Surfaces and External Communicating Devices Tissue/Bone/Dentin Limited (less than 24 hours) as defined by ISO 10993-1. These materials are listed in Table 15.1. The new materials, associated with the new articulation feature, have been evaluated for biocompatibility.

Table 15.1 Patient-Contacting Materials of the Subject Device Material

Part Name	Subject Device ENSEAL G2 Articulating Tissue Sealers Material	Predicate Device ENSEAL G2 Tissue Sealers	Material used in Previously Cleared Devices (510k)
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(b) (4)



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Part Name	Subject Device ENSEAL G2 Articulating Tissue Sealers Material	Predicate Device ENSEAL G2 Tissue Sealers Material	Material used in Previously Cleared Devices (510k)
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(b) (4)



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Biocompatibility Evaluation of New Material

Biocompatibility Evaluation of Sodium Stearate, Vegetable

Sodium Stearate, Vegetable is used as a lubricant on the flex joint. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. Biocompatibility of this material was evaluated in accordance with ISO 10993-1 as well as by that suggested by the FDA (Acute Systemic Toxicity) for this patient contact category. The test methods and results are summarized in Table 15.2. Although not relevant for this device, additional evaluations were conducted to meet requirements when the material is used in situations of higher patient contact. Biocompatibility of this material was evaluated to show that the material meets or exceeds ISO 10993-1 requirements.

Table 15.2 Biocompatibility Evaluation of Sodium Stearate, Vegetable

Test Type	Test Name	Lab Name	Result
Cytotoxicity	Cytotoxicity (MEM-Elution)	Northview Pacific	Pass
Analytical Test	Analytical Tests (IR)	Evans Analytical	Pass
Pyrogenicity	Pyrogenicity (LAL)	WuXi AppTec Laboratory Services	Pass
Pyrogenicity	Pyrogenicity (Pyrogenicity)	WuXi AppTec Laboratory Services	Pass

The Sodium Stearate, Vegetable material passed all biocompatibility tests recommended under ISO 10993-1 for Externally Communicating Devices in contact with Tissue, Bone, and Dentin for less than 24 hours (“Limited”) as shown in Table 15.2; as well as the test suggested by the FDA (Acute Systemic Toxicity) for this patient contact category, and those considered supplementary tests. Sodium Stearate, Vegetable is considered biologically equivalent to Calcium Stearate. Biocompatibility of Calcium Stearate was evaluated in accordance with ISO 10993-1. These data are consistent with the biocompatibility of Calcium Stearate that has been previously approved for use in Externally Communicating devices in contact with tissue, bone and dentin for less than 24 hours (ISO 10993-1). The test methods and passing results are summarized in Table 15.3. The stearate moiety is expected to be readily metabolized from either the calcium or sodium molecule. In addition, Sodium Stearate is used in the process to manufacture Calcium Stearate. Therefore, Sodium Stearate, Vegetable is considered biologically equivalent to Calcium Stearate.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers**Table 15.3 Biocompatibility Evaluation of Calcium Stearate, Vegetable**

Test Type	Test Name	Lab Name	Result
Implantation	Implantation (119 day with Histopathology)	NAMSA	Pass
Implantation	Implantation (60 day with Histopathology)	NAMSA	Pass
Implantation	Implantation (30 day-muscle)	NAMSA	Pass
Implantation	Implantation (7 day muscle)	NAMSA	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	Northview Laboratories Inc.	Pass
Hemocompatibility	Hemocompatibility (Hemolysis)	Northview Laboratories Inc.	Pass
Cytotoxicity	Cytotoxicity (Agarose overlay)	Northview Laboratories Inc.	Pass
Pyrogenicity	Systemic Toxicity (Material-Mediated Pyrogenicity - rabbit)	Northview Pacific	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute Systemic Toxicity)	Northview Pacific	Pass
Genotoxicity	Genotoxicity (Ames Mutagenicity)	Northview Pacific	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	Northview Pacific	Pass
Irritation/ Intracutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/Irritation)	Northview Pacific	Pass

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers**Biocompatibility Evaluation of Polycarbonate, Calibre 2081-15**

The polycarbonate is used for the flex joint. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. Biocompatibility of this material was evaluated in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.4

Table 15.4 Biocompatibility Evaluation of Polycarbonate, Calibre 2081-15

Test Type	Test Name	Lab Name	Result
Cytotoxicity	Cytotoxicity (MEM-Elution)	Northview Laboratories Inc.	Pass
Cytotoxicity	Cytotoxicity (Agarose overlay)	Northview Laboratories Inc.	Pass
Hemocompatibility	Hemocompatibility (Hemolysis)	Northview Laboratories Inc.	Pass
Physico-Chemical	Physico-Chemical (USP Phys Chem)	Northview Laboratories Inc.	Pass
Irritation/ Intracutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/Irritation)	Northview Pacific	Pass
Implantation	Implantation (Implantation (with Histo.))	Northview Pacific	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute Systemic Toxicity)	Northview Pacific	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	Northview Pacific	Pass
Pyrogenicity	Systemic Toxicity (Material-Mediated Pyrogenicity - rabbit)	Northview Pacific	Pass
Genotoxicity	Genotoxicity (Ames Mutagenicity)	Northview Pacific	Pass
Analytical Test	Analytical Tests (IR)	Northview Pacific	Pass

The polycarbonate, Calibre 2081-15 material passed all biocompatibility tests required under ISO 10993-1 for Externally Communicating Devices in contact with Tissue, Bone or Dentin for less than 24 hours (“Limited”); as well as for the test suggested by the FDA (Acute Systemic Toxicity) for this patient contact category, and those considered supplementary tests.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Biocompatibility Evaluation of PolyEtherEtherKetone (PEEK), Victrex 381G / PEEK

The PolyEtherEtherKetone (PEEK), Victrex 381G / PEEK on the RF Sleeve Insulator. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. Biocompatibility of this material was evaluated in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.5.

Table 15.5 Biocompatibility Evaluation of PolyEtherEtherKetone (PEEK), Victrex 381G / PEEK

Test Type	Test Name	Lab Name	Result
Physico-Chemical	Physico-Chemical (USP Phys Chem)	In House Study	Pass
Hemocompatibility	Hemocompatibility (Prothrombin Time)	Toxikon	Pass
Hemocompatibility	Hemocompatibility (Thrombogenicity)	Toxikon	Pass
Hemocompatibility	Hemocompatibility (<i>In vitro</i> Hemocompatibility)	Toxikon	Pass
Implantation	Implantation (7 day muscle)	N/A	Pass
Hemocompatibility	Hemocompatibility (Hemolysis (Direct))	ViroMED	Pass
Genotoxicity	Genotoxicity (Ames Mutagenicity)	ViroMED	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	ViroMED	Pass
Pyrogenicity	Pyrogenicity (Pyrogenicity)	ViroMED	Pass
Irritation/ Intracutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/Irritation)	ViroMED	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute)	ViroMED	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	ViroMED	Pass

The PolyEtherEtherKetone (PEEK), Victrex 381G / PEEK material passed all biocompatibility tests recommended under ISO 10993-1 for Externally Communicating Devices in contact with Tissue, Bone and Dentin for less than 24 hours (“Limited”); as well as for the test suggested by the FDA (Acute Systemic Toxicity) for this patient contact category, and those considered supplementary tests.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers**Biocompatibility Evaluation of Titanium Nitride**

Titanium Nitride is a well-characterized material used on the I-Blade. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. Biocompatibility of this material was evaluated in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.6. Please note that the PTC Clip Plating, located on the PTC Clip, is not a new material to the Predicate or Subject device, but the material is listed to align with global regulatory standards.

Table 15.6 Biocompatibility Evaluation of Titanium Nitride (I-Blade Plating)

Test Type	Test Name	Lab Name	Result
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	NAMSA	Pass
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	NAMSA	Pass
Analytical Test	Analytical Tests (Gas Chromatography Mass Spectrometry(GCMS))	NAMSA	Pass
Analytical Test	Analytical Tests (Gas Chromatography Mass Spectrometry(GCMS))	NAMSA	Pass
Cytotoxicity	Cytotoxicity (Extract Colony Assay (JMHLW))	WuXi AppTec Laboratory Services	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	WuXi AppTec Laboratory Services	Pass
Hemocompatibility	Hemocompatibility (Hemolysis (Direct))	WuXi AppTec Laboratory Services	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute)	WuXi AppTec Laboratory Services	Pass
Irritation/ Intracutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/Irritation)	WuXi AppTec Laboratory Services	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	WuXi AppTec Laboratory Services	Pass

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Titanium Nitride passed all biocompatibility tests required under ISO 10993-1 for Externally Communicating Devices in contact with Tissue, Bone, and Dentin for less than 24 hours ("Limited"); as well as the test suggested by the FDA (Acute Systemic Toxicity) for this patient contact category. In addition titanium nitride was considered non-hemolytic in a supplementary Hemocompatibility test.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers**Biocompatibility Evaluation of Nickel-Tin Plating**

The Nickel-Tin Plating is used on the on the PTC Clip. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. This material was tested for biocompatibility as a component in a predicate device assembly. Biocompatibility of this material was evaluated in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.7.

Table 15.7 Biocompatibility Evaluation of Nickel-Tin Plating

Test Type	Test Name	Lab Name	Result
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	NAMSA	Pass
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	NAMSA	Pass
Analytical Test	Analytical Tests (ICP-Nickel Analysis)	NAMSA	Pass
Analytical Test	Analytical Tests (Gas Chromatography Mass Spectrometry(GCMS))	NAMSA	Pass
Analytical Test	Analytical Tests (Gas Chromatography Mass Spectrometry(GCMS))	NAMSA	Pass
Analytical Test	Analytical Tests (IR)	NAMSA	Pass
Cytotoxicity	Cytotoxicity (Extract Colony Assay (JMHLW))	WuXi AppTec Laboratory Services	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	WuXi AppTec Laboratory Services	Pass
Analytical Test	Analytical Tests (IR)	WuXi AppTec Laboratory Services	Pass
Hemocompatibility	Hemocompatibility (Hemolysis (Direct))	WuXi AppTec Laboratory Services	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute)	WuXi AppTec Laboratory Services	Pass
Irritation/ Intracutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/Irritation)	WuXi AppTec Laboratory Services	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	WuXi AppTec Laboratory Services	Pass

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

The supplier of the Nickel-Tin Plating changed since the biocompatibility evaluation of the predicate device. Limited testing was performed on samples from each supplier to ensure biological equivalency. Biocompatibility of this material was evaluated in accordance with ISO 10993-1 studies. The test methods and results are summarized in Table 15.8

Table 15.8 Biocompatibility Evaluation of Nickel-Tin Plating

Test Type	Test Name	Lab Name	Result
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	NAMSA	Pass
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	NAMSA	Pass
Analytical Test	Analytical Tests (IR)	NAMSA	Pass
Cytotoxicity	Cytotoxicity (Extract Colony Assay (JMHLW))	WuXi AppTec Laboratory Services	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	WuXi AppTec Laboratory Services	Pass
Analytical Test	Analytical Tests (IR)	WuXi AppTec Laboratory Services	Pass

The Nickel-Tin Plating passed all biocompatibility tests recommended under ISO 10993-1 for Externally Communicating Devices in contact with Tissue, Bone or Dentin for less than 24 hours (“limited”); as well as the test suggested by the FDA (Acute Systemic Toxicity) for this patient contact category, and those considered supplementary tests. The nickel-tin plating of the new supplier is biological equivalent to the plating in the predicate device.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers**Biocompatibility Evaluation of White Acrylic Ink Type PG/NT**

The white acrylic ink is used for the pad printed symbols on the shaft of the device. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. Biocompatibility of this material was evaluated in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.9.

Table 15.9 Biocompatibility Evaluation of White Acrylic Ink Type PG/NT

Test Type	Test Name	Lab Name	Result
Physico-Chemical	Physico-Chemical (USP Phys Chem)	Northview Laboratories Inc.	Pass
Hemocompatibility	Hemocompatibility (Hemolysis)	Northview Laboratories Inc.	Pass
Cytotoxicity	Cytotoxicity (Agarose overlay)	Northview Laboratories Inc.	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	Northview Laboratories Inc.	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute Systemic Toxicity)	Northview Laboratories Inc.	Pass
Implantation	Implantation (Implantation (with Histopathology.))	Northview Pacific	Pass
Pyrogenicity	Systemic Toxicity (Material-Mediated Pyrogenicity - rabbit)	Northview Pacific	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	Northview Pacific	Pass
Genotoxicity	Genotoxicity (Ames Mutagenicity)	Northview Pacific	Pass
Irritation/ Intracutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/Irritation)	Northview Pacific	Pass
Analytical Tests	Analytical Tests (IR)	Northview Pacific	Pass

The White Acrylic Ink Type PG/NT material passed all biocompatibility tests recommended under ISO 10993-1 for Externally Communicating Devices in contact with Tissue, Bone and Dentin for less than 24 hours (“Limited”); as well as the test suggested by the FDA (Acute Systemic Toxicity) for this patient contact category, and those considered supplementary tests.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

**Biocompatibility Testing of Patient –Contacting Components of the Device:
Evaluation for Potential Material Interactions**

The distal end of the Subject device was evaluated to account for potential interaction of patient-contacting materials (ISO 10993-1). The following Cytotoxicity test was completed.

ISO Cytotoxicity

The negative and cell cultures exhibited healthy growth throughout the experiment. The monolayers exposed to CdCl₂ exhibited a toxic response while the black rubber intermediate control induced a mild level of toxicity. Therefore, the test system was responding normally and met the criteria for a valid assay.

When scoring lysis, each sample was rated relative to the amount of lysis displayed by the negative control. The negative and cell control were considered '0', the cells displayed at least 70% viability. The intermediate control displayed a mild cytotoxic reaction resulting in a score of '2'. The positive control displayed a strong cytotoxic reaction, resulting in a score of '4'. Therefore, the test system was responding normally and met the criteria for a valid assay.

In this assay, the test article did not induce cytotoxicity. No abnormal events such as pH change or debris were noted. At the neat concentration and all dilutions, the test article scored '0' at 24, 48, and at 72 ± 4 hours and is considered non-cytotoxic under the conditions of this test.

The test method and result are summarized in Table 15.10.

Table 15.10 Biocompatibility Testing of Patient-Contacting Components of the Device: Evaluation for Potential Material Interactions

Test Type	Test Name	Lab Name	Result
Cytotoxicity	Cytotoxicity (MEM-Endpoint Dilution)	WuXi AppTec Laboratory Services	Pass

Based on the absence of cytotoxicity, no biologically relevant interactions of patient-contacting materials were observed for the device.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

Section 16: Software

This section does not apply; this device does not include software.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Section 17: Electromagnetic Compatibility and Electrical Safety

Electromagnetic Compatibility

Testing to the following standard for Electromagnetic Compatibility was performed and subsequently certified for all applicable sections by the nationally recognized testing facility listed below. An Extent of Standard Conformance Summary Report Table with respect to this standard is included in Section 22: FDA Form 3654 of this submission.

IEC 60601-1-2:2007, Medical Electrical Equipment - Part 1-2: General Requirements for Safety - Collateral Standard: Electromagnetic Compatibility - Requirements and Tests (FDA Recognition Number 5-54)

Electrical Safety

Testing to the following standards for Electrical Safety was performed and subsequently certified for all applicable sections by the nationally recognized testing facility listed below. An Extent of Standard Conformance Summary Report Table with respect to each of these standards is included in Section 22: FDA Form 3654 of this submission.

IEC 60601-1:2005, Medical Electrical Equipment - Part 1: General Requirements for Safety, including Amendment 1, 1991 and Amendment 2, 1995 (FDA Recognition Number 5-4)

IEC 60601-2-2:2009, Medical Electrical Equipment - Part 2-2: Particular requirements for the safety of high frequency surgical equipment (FDA Recognition Number 6-229)

Certification Facility:

TUV Rheinland of North America, Inc.
2100 Golf Rd., Suite 300
Rolling Meadows, IL 60008-4277
Phone: 847.640.5700
www.tuv.com

Excerpts of the test data package, test sample identification, and certification are provided. All IEC testing of the ENSEAL G2 Articulating Tissue Sealers was completed with the Generator G11 (GEN11) which was cleared under K101990.

UL 60601-1, CAN/CSA C22.2 No. 601.

File Number		Manufacturer	Ethicon Endo-Surgery
Engineer		Product Type	Electrosurgical Handpiece
Page	1 of 18	Model	ENSEAL G2 (NSLG2C35A)

TEST DATA PACKAGE

Applicant Information	Name / Address: Ethicon Endo-Surgery, Inc. 4545 Creek Road, Cincinnati, OH 45242
-----------------------	--

Product Information	<p>IEC 60601-1:1988 + A1:1991 + A2:1995; IEC 60601-1: 2005 (Medical Electrical Equipment, Part 1: General Requirements for Safety) UL 60601-1, 1st Edition, 2006-04-26; AAMI ES 60601-1: 2005 (USA) Standard: CAN/CSA-C22.2 No. 601.1-M90, 2005; CSA C22.2 No. 60601-1: 2008 (CANADA) EN 60601-1:1990 + A1:1993 + A2:1995 + A11:1993 + A12:1993 + A13: 1996; EN 60601-1: 2007 (EU) IEC 60601-2-2, Ed.4, Ed. 5</p> <hr/> <p>Product: Electrosurgical Handpiece (Articulating Tissue Sealer)</p> <hr/> <p>Models: ENSEAL G2</p> <hr/>
---------------------	---

	Test Location Name/Address: Medical Equipment Compliance Associates, LLC, 5060 W Ashland Way, Franklin, WI 53132, USA						
Authorized Signatory or TCP Reviewer:	<table><tr><td>Sign</td><td></td></tr><tr><td>Print</td><td>Jeremi Peck</td></tr><tr><td>Date</td><td>2012-07-27</td></tr></table>	Sign		Print	Jeremi Peck	Date	2012-07-27
Sign							
Print	Jeremi Peck						
Date	2012-07-27						

UL 60601-1, CAN/CSA C22.2 No. 601.

File Number		Manufacturer	Ethicon Endo-Surgery
Engineer		Product Type	Electrosurgical Handpiece
Page	3 of 18	Model	ENSEAL G2 (NSLG2C35A)

TEST SAMPLE IDENTIFICATION

The table below is to provide correlation of sample numbers to specific product related information. Refer to this table when a test identifies a test sample by "Sample No." only.

Sample Number	Sample Card Number	Date Received	Manufacturer, Product Identification and Ratings
1	2011M035-1	2011-04-29	Ethicon Endo-surgery, model Gen11, S/N GN1111104286
2	2011M035-2	2011-04-29	Ethicon Endo-surgery handpiece, model NSLG2S35A (straight jaw) 2
3	2011M035-3	2011-04-29	Ethicon Endo-surgery handpiece, model NSLG2S35A (straight jaw) 3
4	2011M035-4	2011-04-29	Ethicon Endo-surgery handpiece, model NSLG2C35A (curved jaw) 4
5	2011M035-5	2011-04-29	Ethicon Endo-surgery handpiece, model NSLG2C35A (curved jaw) 5
6	2011M035-6	2011-04-29	Ethicon Endo-surgery handpiece, model NSLG2C35A (curved jaw) 6
Sampling Procedure (if used) :		Samples represented the full series, specified in the following list NSLG2C35A – ENSEAL G2 Articulating Tissue Sealer, 5mm Curved Jaw 35cm length NSLG2S35A – ENSEAL G2 Articulating Tissue Sealer, 5mm Straight Jaw 35cm length NSLG2C45A – ENSEAL G2 Articulating Tissue Sealer, 5mm Curved Jaw 45cm length NSLG2S45A – ENSEAL G2 Articulating Tissue Sealer, 5mm Straight Jaw 45cm length	

TEST INSTRUMENTS REFERENCE LIST

Code	Instrument_ID	Instrument_Type	Equipment_Make	Equipment_Model	Range_Or_Setting_Use	Last_Calib	Calibration_Due
4	MECA0004	Digital Multimeter	Fluke	111	V, A, W, uF, Hz	10/25/2011	10/31/2012
15	MECA0015	Temp. Meter	Fluke	52	°C	10/25/2011	10/31/2012
20	MECA0020	DWT (Hi-pot)	QuadTech	Sentry 35	kV (ac, dc), mA, Sec.	10/26/2011	10/31/2012
40	MECA0040	Temp. Meter (Oven)	Alnor	Digitherm 6629	°C	10/25/2011	10/31/2012
49	MECA0049	Ball Pressure Tester	MECA	Ball Pressure Tester	20 N, 5 mm dia.	6/14/2012	6/30/2014
54	MECA0054	Force Gauge	Chatillon	DP1P-30	Lbs. Force	11/1/2011	11/30/2012
55	MECA0055	Defib Simulator Volt Divider	MECA0055	Defib Simulator Impulse Generator	Network	4/24/2012	4/30/2013
56	MECA0056	Defib Sim Measure Network	MECA0056	Defib Simulator Measurement Network	Network	4/24/2012	4/30/2013
63	MECA0063	IEC 0.5 j Impact Tool	MECA	20mm dia. ball, tube	0.5 j Impact	6/6/2012	6/30/2014
80	MECA0080	AC Power Source	Elgar	EW 3001	V, A, W, Hz	5/22/2012	5/31/2013
81	MECA0081	Stopwatch	Extech	365510	sec, min, hrs	5/22/2012	5/31/2013
82	MECA0082	Timer Clock	Big Digit	Dual Timer 70-468H	Sec, min, hrs, days	5/22/2012	5/31/2013
85	MECA0085	10x Comparator Loupe	SPI	1983 10x Comparator, #7 Reticle	mm	6/6/2012	6/30/2014
94	MECA0094	Tape Measure	Stanley	30-824	mm, cm, in. (to 3 m)	6/2/2011	6/30/2013
95	MECA0095	Tem. Chart Recorder (Oven)	Dickson	KT603	°C, min, hrs	5/21/2012	5/31/2013
99	MECA0099	Rec./Controller (Hum. Chmbr.)	Comet	H3531	%RH, °C	10/28/2011	10/28/2012
110	MECA0110	Scale	Scalesco Measurement Technologies	SMT-218-006	0-3 kg	5/22/2012	5/31/2013
129	MECA0129	Oscilloscope	Tektronix	TDS 2022	V, sec., 2 CH, 200 MHz	10/25/2011	10/31/2012
A12	MECA0012	Oven (Air Circulating)	Blue M	0V-510A-2	-	3/25/2011	3/31/2012

EnSeal G2
EMC Test Report
ICL Report #1717



Electromagnetic Compatibility Test Report

**Test Specifications: IEC 60601-1-2: 2007, 3rd Edition
(ESD Only)
IEC 60601-2-2: 2009, Edition 5.0
(ESD Only)**

Manufacturer: Ethicon Endo Surgery

Model Name: ENSEAL® G2 Articulating Tissue Sealer

Serial Number: DVI-113

Test Dates: 7/26/2012

Report Issue Date: 7/27/2012

Test Results: MEETs Requirements

Signatures:

Prepared By:

A handwritten signature in black ink, appearing to read 'Daniel M. Mittag', is written over a light blue horizontal line.

**Daniel M. Mittag
ICL EMC Technician**

Reviewed By:

A handwritten signature in black ink, appearing to read 'Ronald W. Zimmerman', is written over a light blue horizontal line.

**Ronald W. Zimmerman, NCE
ICL President**

This report may not be reproduced, except in full, without the written approval of International Compliance Laboratories, LLC. Results related in this document only pertain to those items tested.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS.GOV@fda.hhs.gov or 301-796-8188

Latest Released: YES

State: Released

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

Section 18: Performance Testing – Bench

The information presented in this section represents the bench testing used to evaluate and verify that the ENSEAL G2 Articulating Tissue Sealers meet performance requirements as compared to the Predicate devices. Bench verification testing is described and the results are provided to support a finding of substantial equivalence to the referenced Predicate devices. Ethicon Endo-Surgery Generator GEN11, cleared under K101990, was used to power the devices. Test results show that the Subject device meet the defined design requirements, which supports substantial equivalence. Table 18.1 provides an overview of the performance testing. A summary of bench verification testing is provided on the following pages.

Table 18.1 Summary of Performance Bench Testing

(b) (4)



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

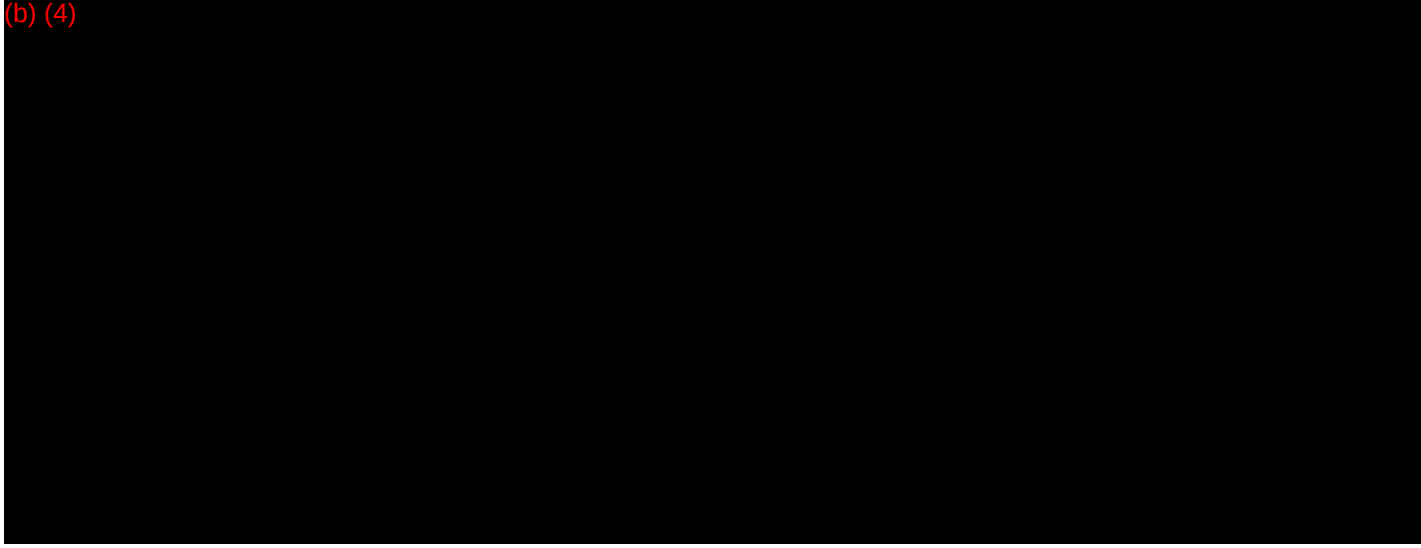
Sealed Vessel Burst Test

Objective:

Confirm burst pressure of vessels sealed with the Enseal G2 Articulating instruments articulated left, straight, and articulated right, powered by the Generator GEN11, is not different than burst pressure values of the Predicate devices.

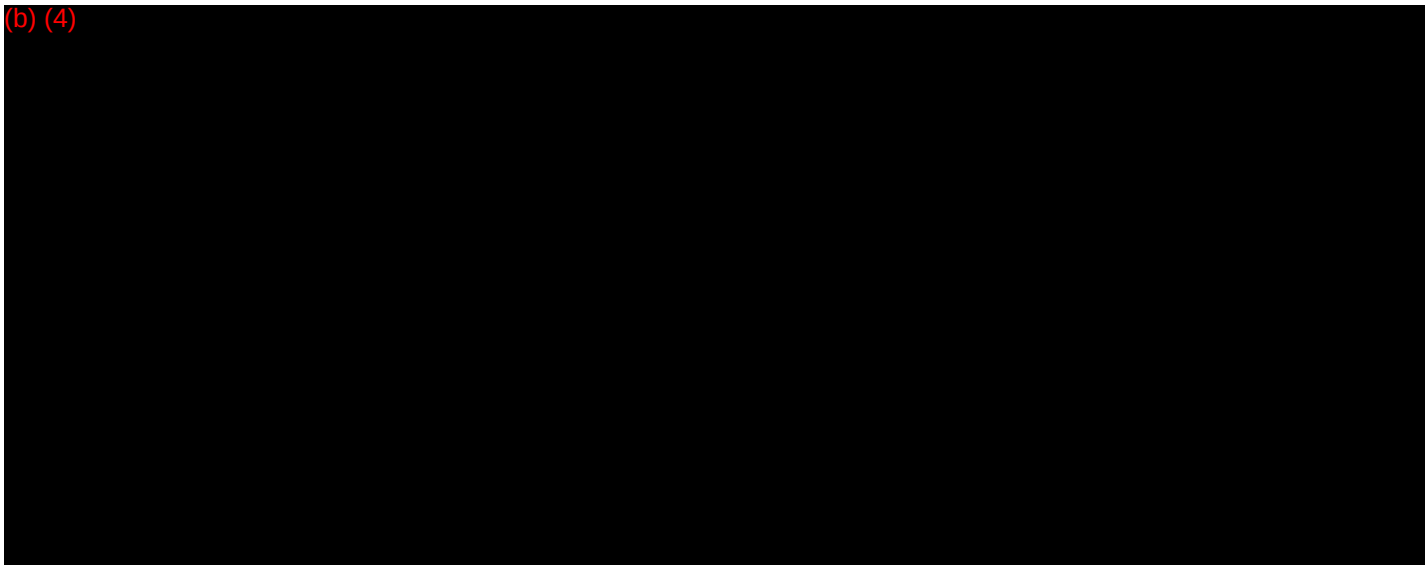
Acceptance Criteria:

(b) (4)



Test Articles:

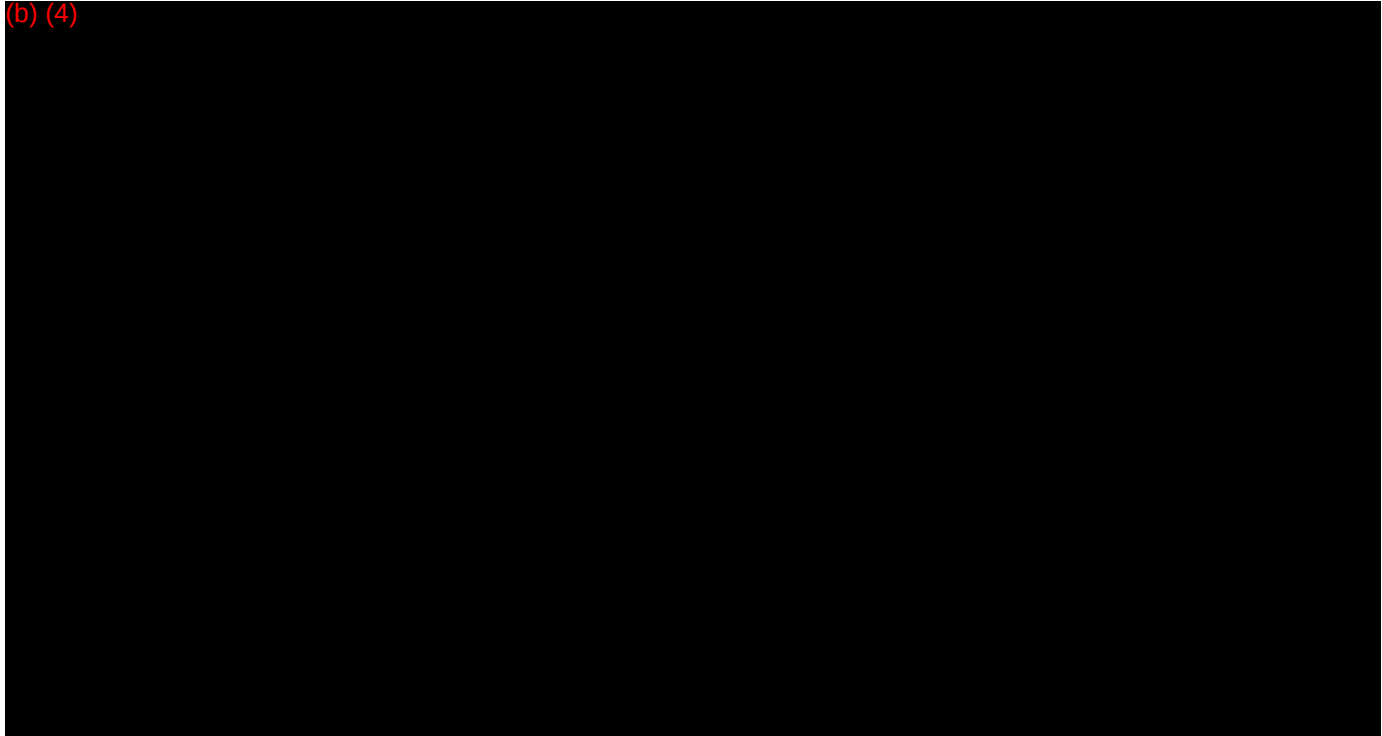
(b) (4)



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

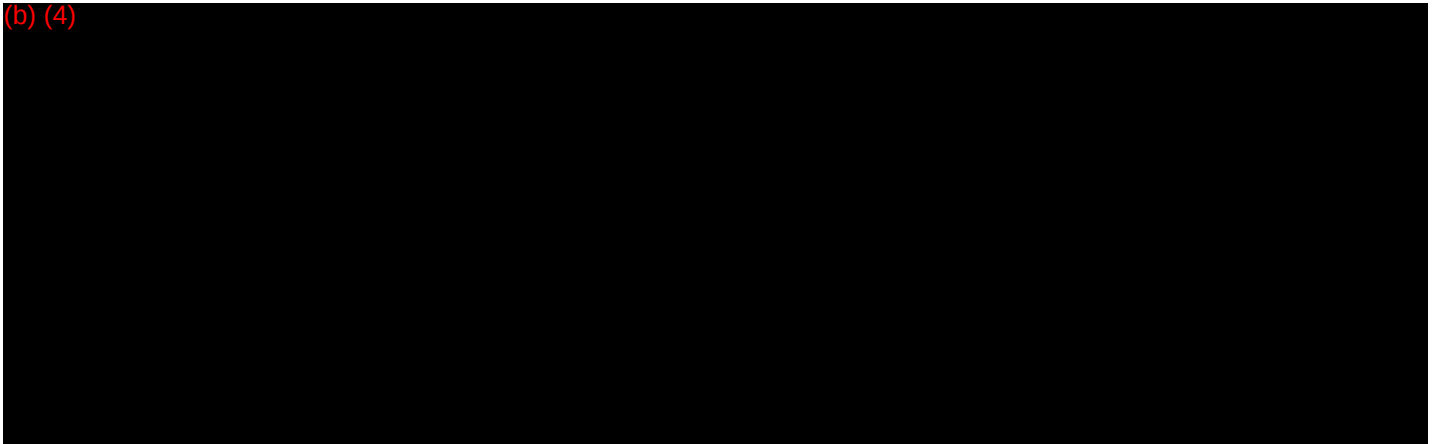
Test Method:

(b) (4)



Results:

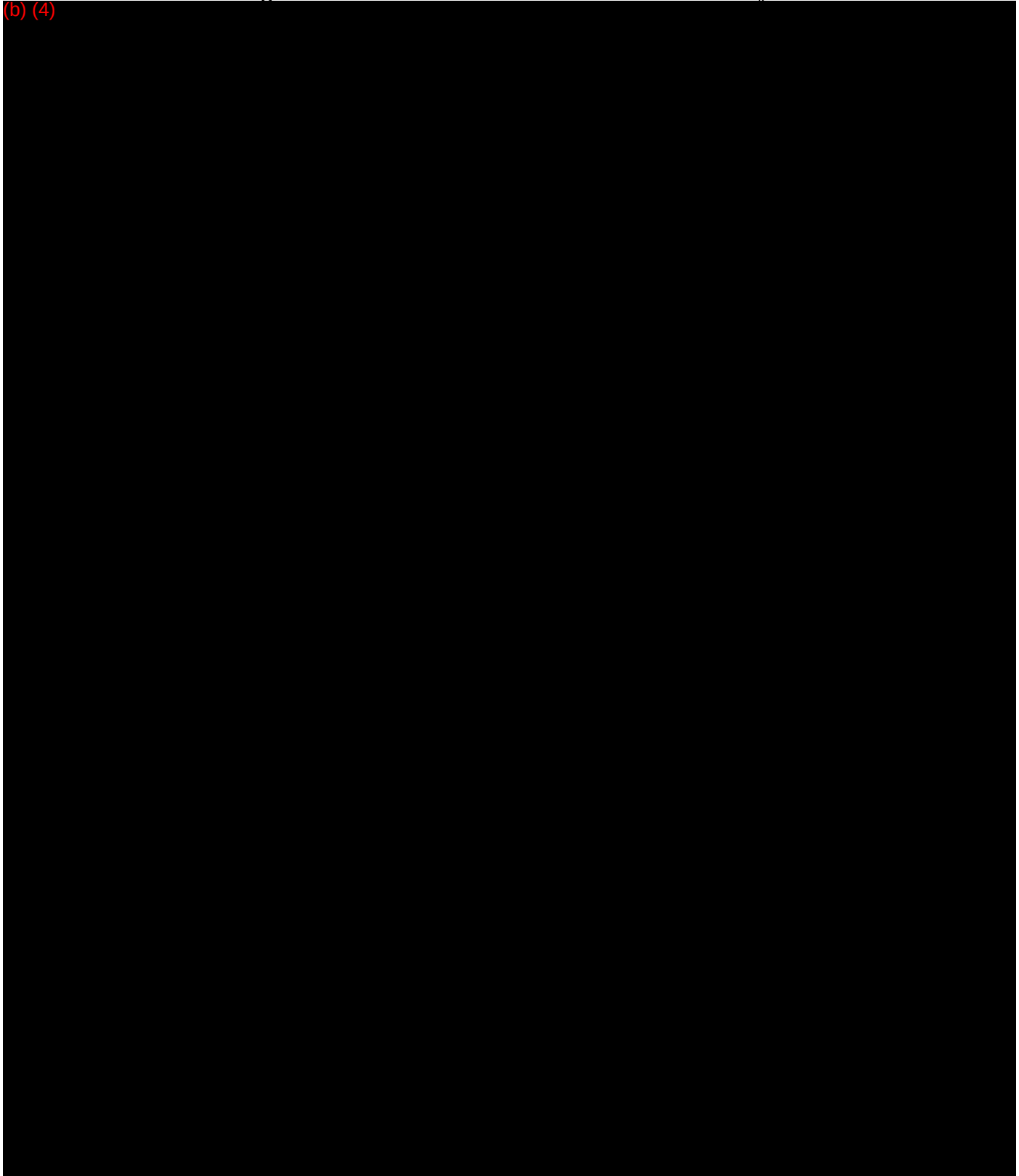
(b) (4)



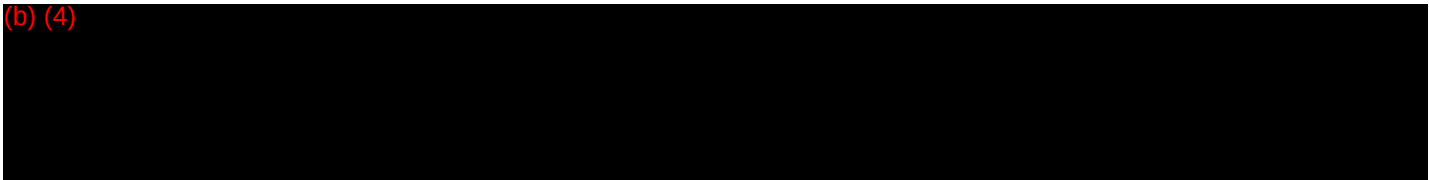
Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

Table 18.2 Straight Jaw Device: Sealed Vessel Burst Test Summary Data

(b) (4)

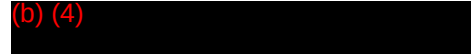


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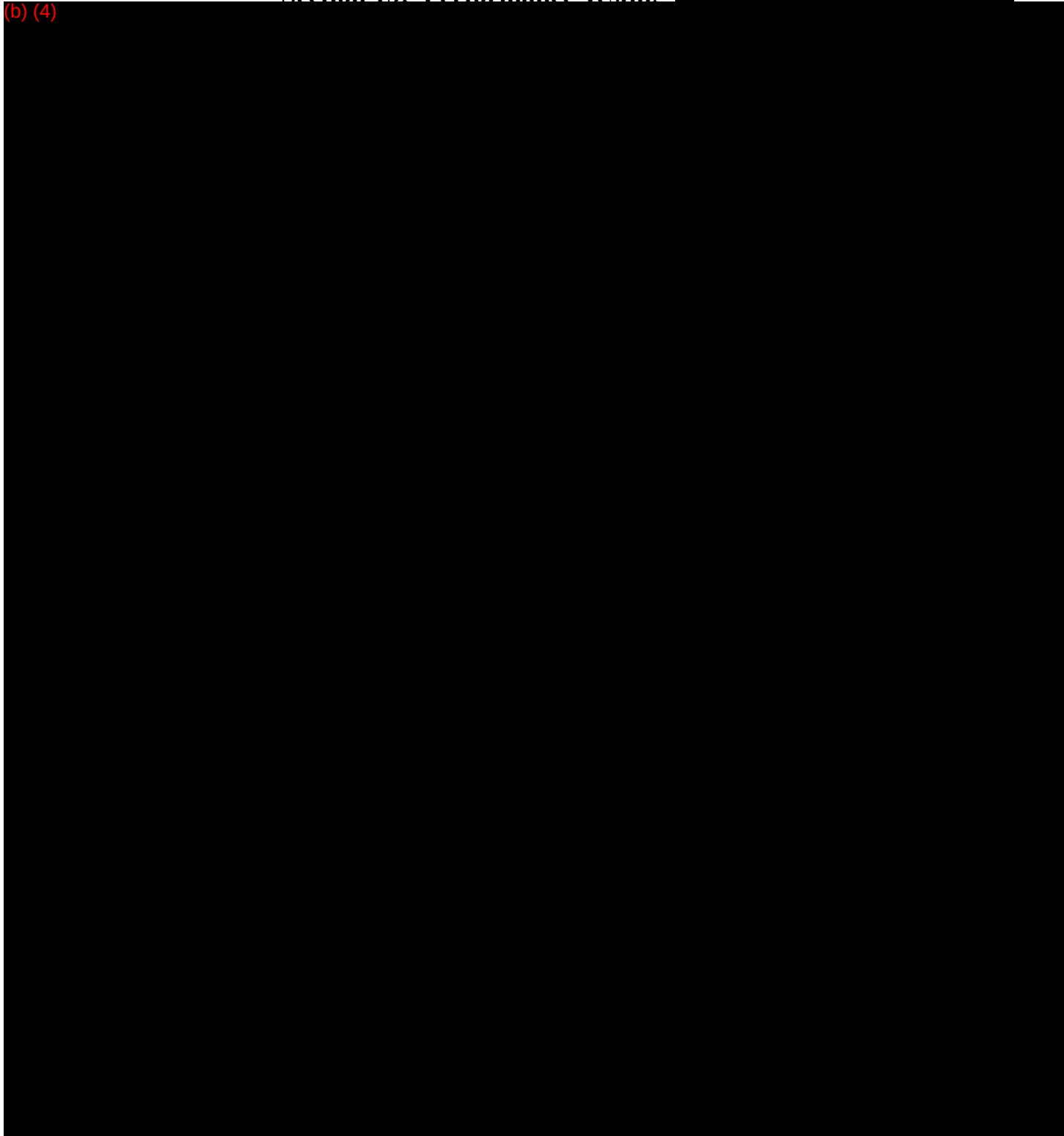
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Section 19: Performance Testing

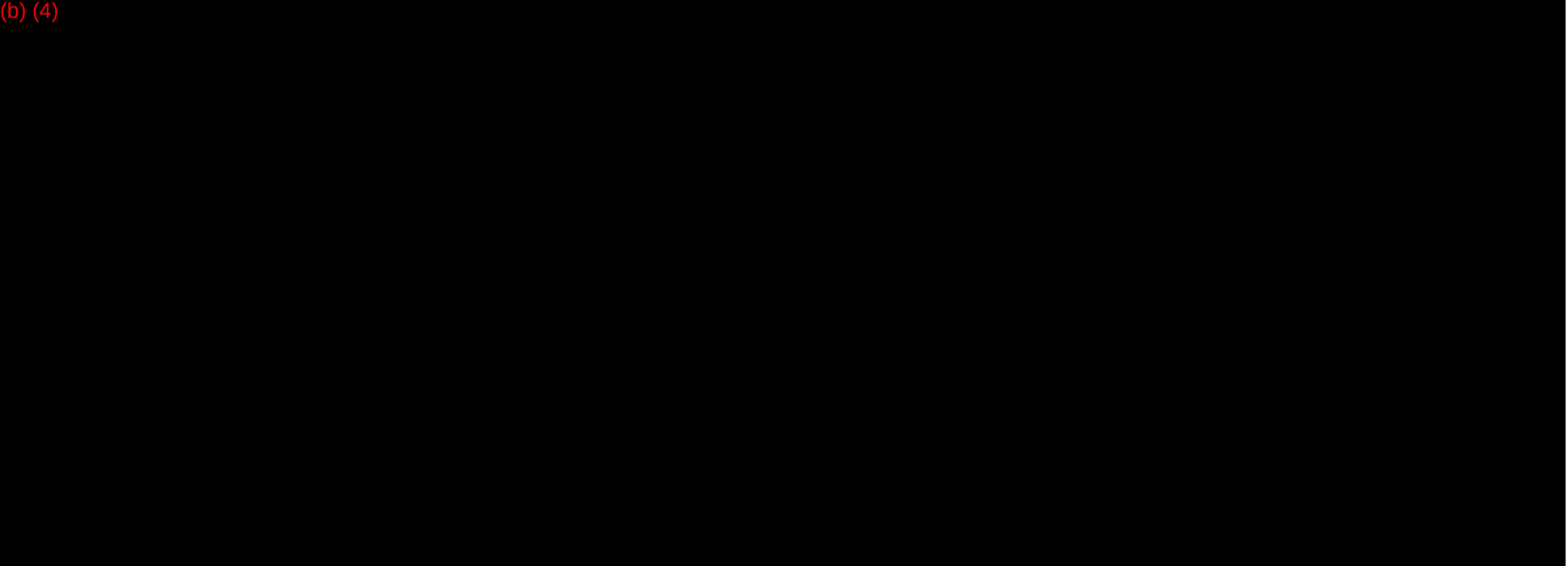
(b) (4)

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(b) (4)

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(b) (4)

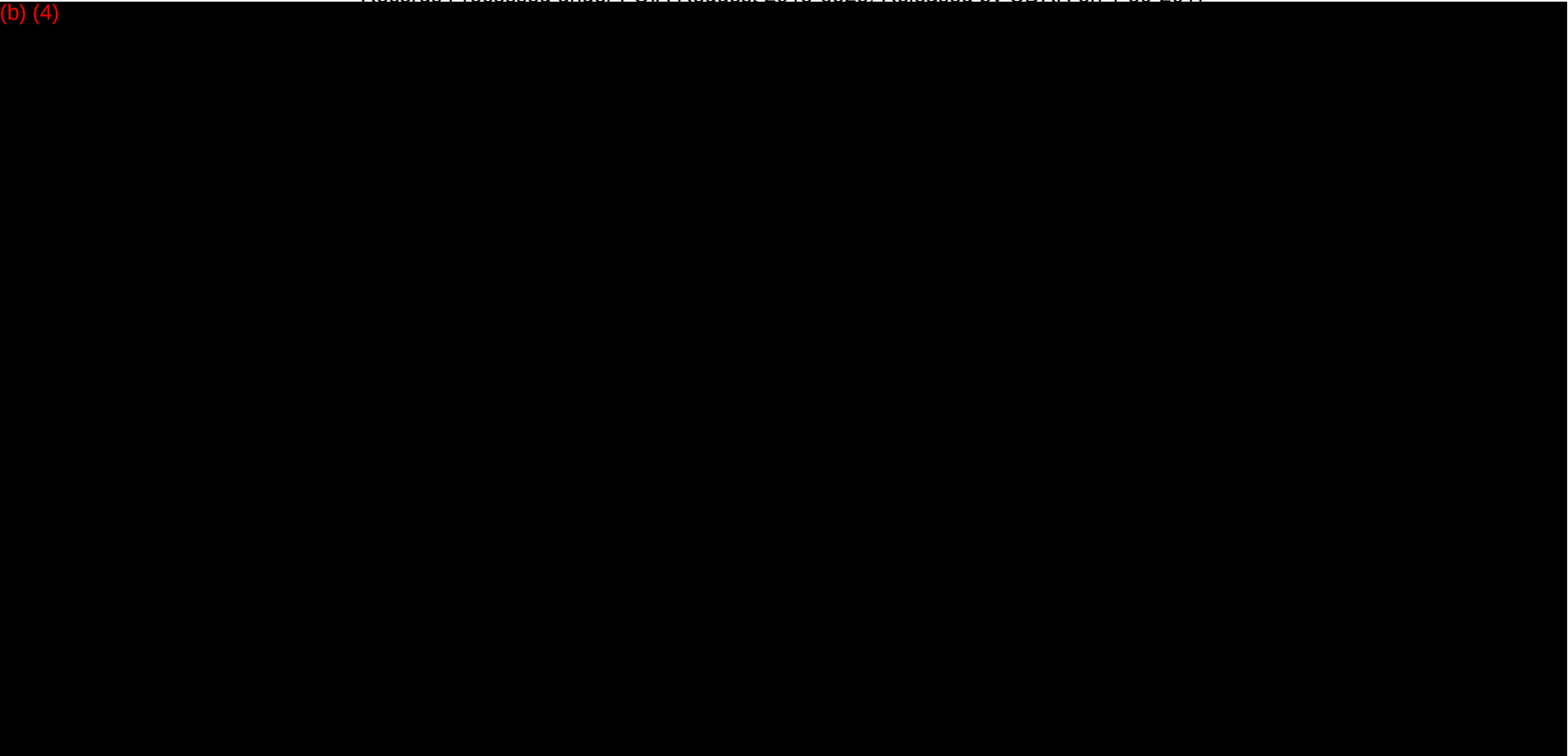


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Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS.GOV@fda.hhs.gov or 301-796-8188

pg. 149

(b) (4)

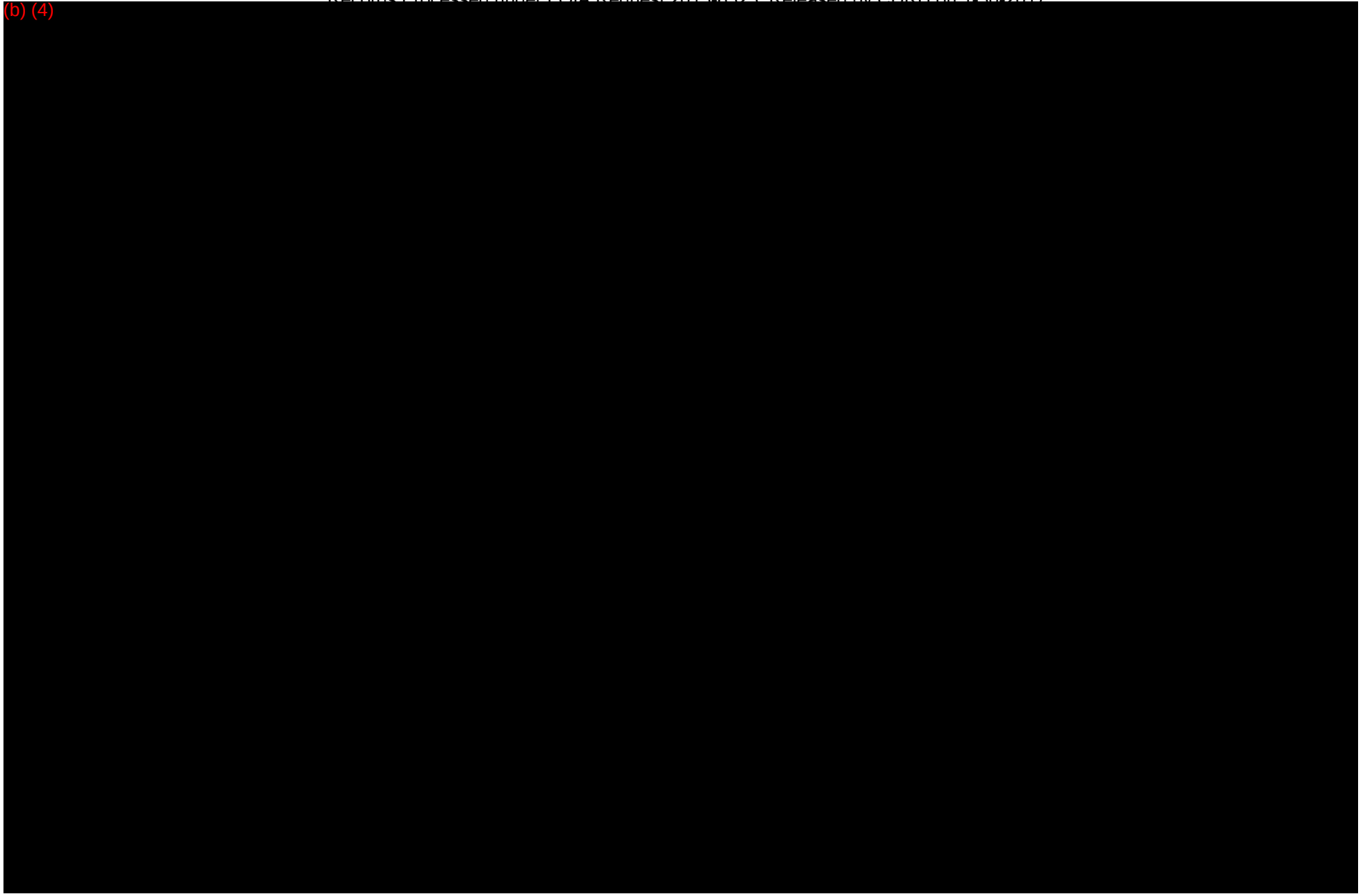


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pg. 150

(b) (4)

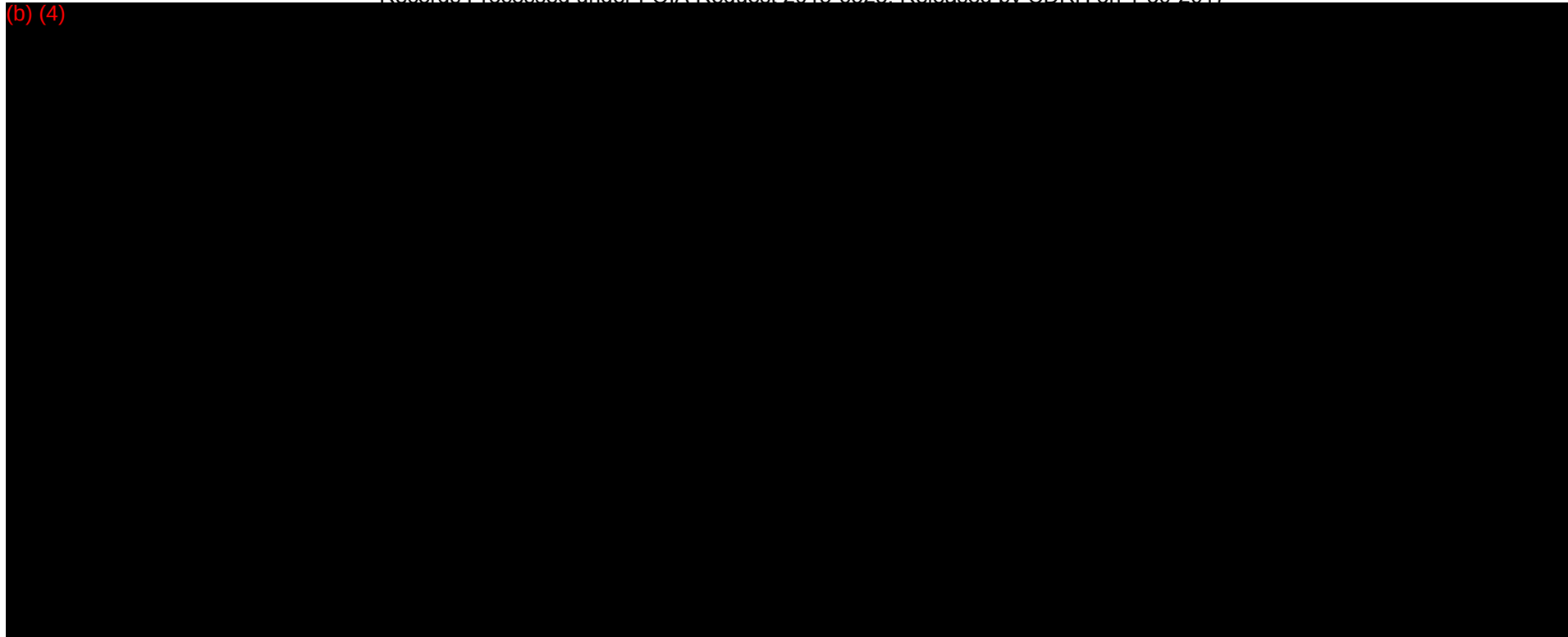


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(b) (4)

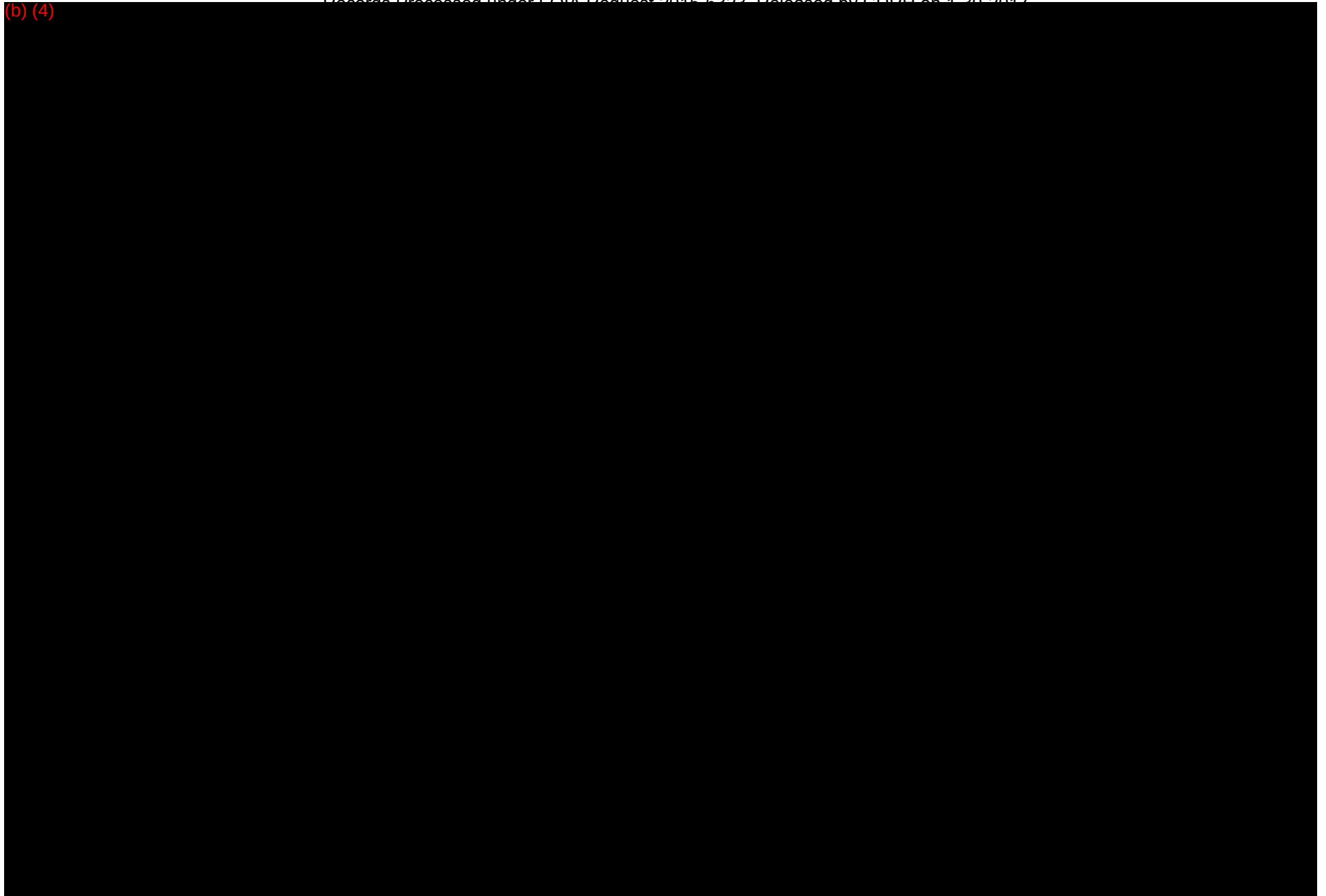


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pg. 152

(b) (4)

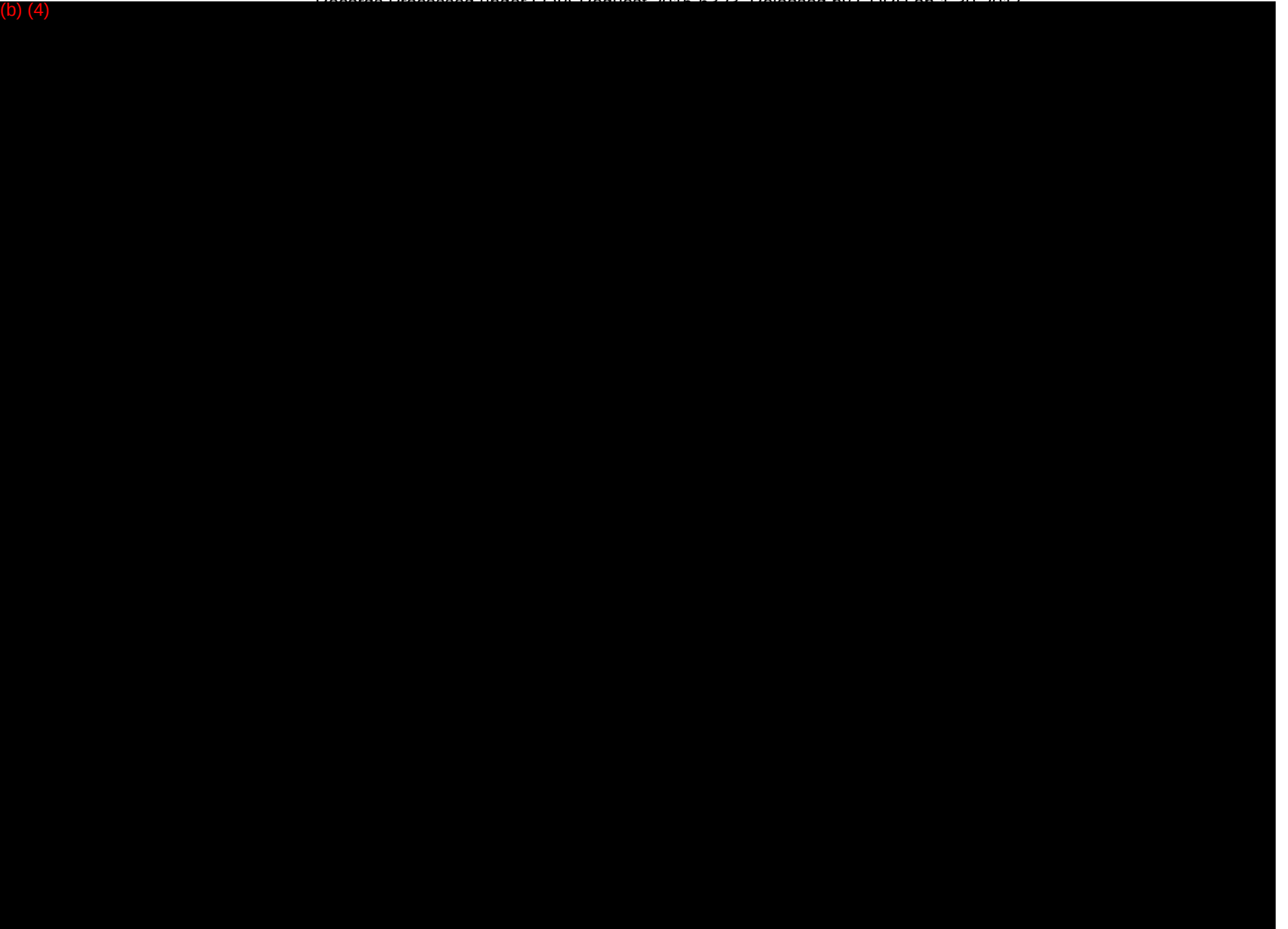


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Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS.GOV@fda.hhs.gov or 301-796-8188

pg. 153

(b) (4)

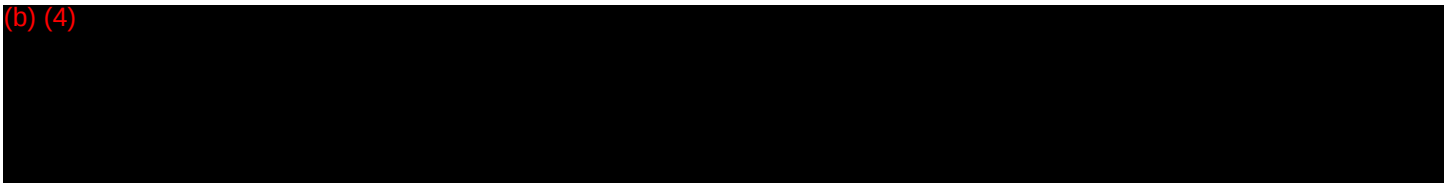


CONFIDENTIAL

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS.GOV@fda.hhs.gov or 301-796-8188

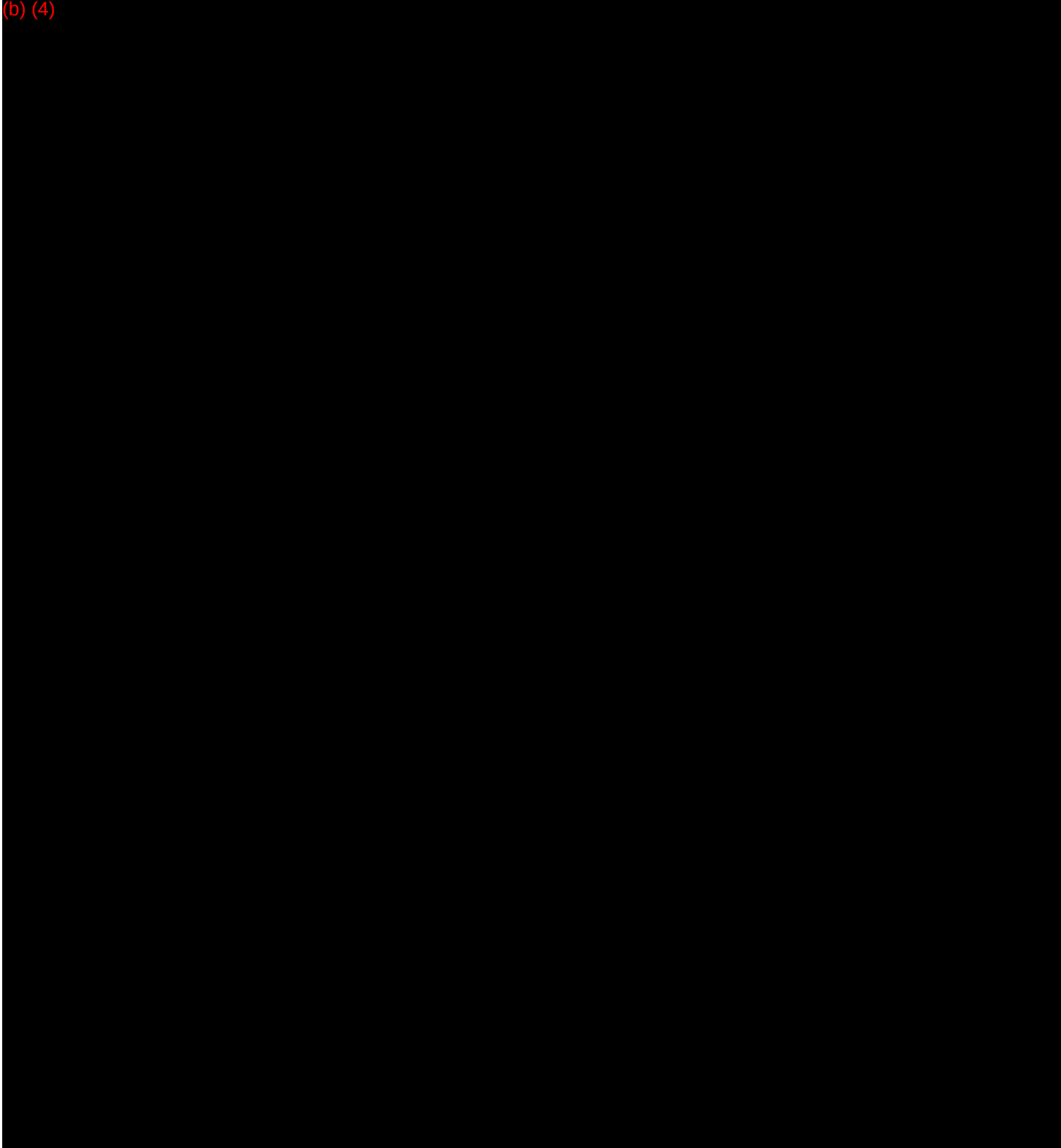
pg. 154

(b) (4)

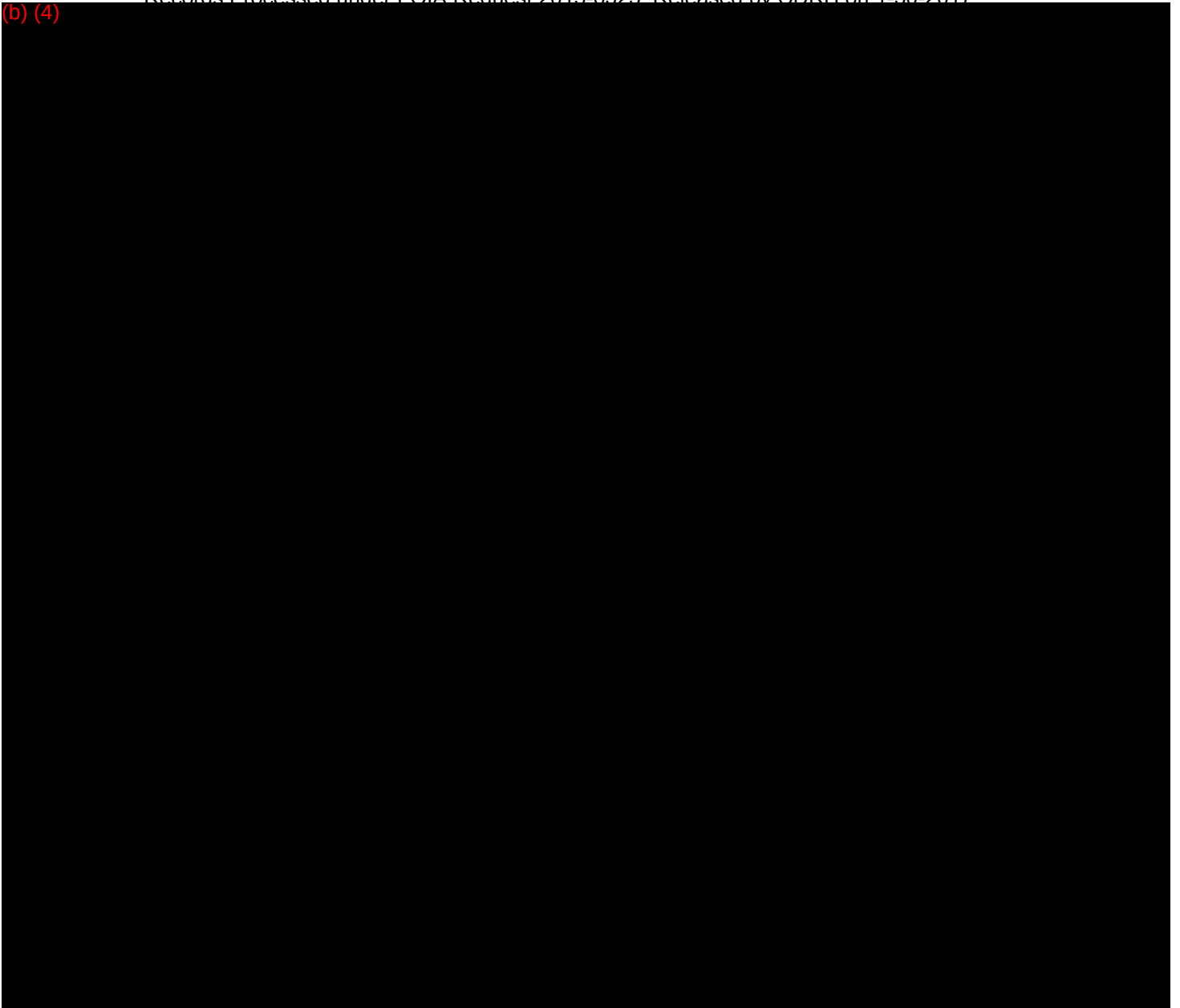
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III. Post-operative Period Results

(b) (4)

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(b) (4)



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Section 20: Performance Testing – Clinical

This section is not applicable. Clinical data is not necessary to evaluate safety and effectiveness for purposes of determining substantial equivalence.

FDA Form 3674

This form does not apply; this 510(k) submission does not reference any clinical trial. Per the FDA guidance document “Guidance for Sponsors, Industry, Researchers, Investigators, and Food and Drug Administration Staff - Certifications To Accompany Drug, Biological Product, and Device Applications/Submissions: Compliance with Section 402(j) of The Public Health Service Act, Added By Title VIII of The Food and Drug Administration Amendments Act of 2007”, FDA Form 3674 is not required for 510(k)s that do not refer to, relate to, or include information on or from a clinical trial.



DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

**Certification of Compliance, under 42 U.S.C. § 282(j)(5)(B), with
Requirements of ClinicalTrials.gov Data Bank (42 U.S.C. § 282(j))**

(For submission with an application/submission, including amendments, supplements, and resubmissions, under §§ 505, 515, 520(m), or 510(k) of the Federal Food, Drug, and Cosmetic Act or § 351 of the Public Health Service Act.)

SPONSOR / APPLICANT / SUBMITTER INFORMATION

1. NAME OF SPONSOR/APPLICANT/SUBMITTER Ethicon Endo-Surgery Inc. c/o Emily Kruetzkamp	2. DATE OF THE APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES September 4, 2012
3. ADDRESS (Number, Street, State, and ZIP Code) 4545 Creek Road Cincinnati, OH, 45242	4. TELEPHONE AND FAX NUMBERS (Include Area Code) (Tel.) (513) 337-1546 (Fax) (513) 337-2802

PRODUCT INFORMATION

5. **FOR DRUGS/BIOLOGICS:** Include Any/All Available Established, Proprietary and/or Chemical/Biochemical/Blood/Cellular/Gene Therapy Product Name(s)
FOR DEVICES: Include Any/All Common or Usual Name(s), Classification, Trade or Proprietary or Model Name(s) and/or Model Number(s)
(Attach extra pages as necessary)

ENSEAL G2 Articulating Tissue Sealers, Straight Jaw, NSLG2S35A,
NSLG2S45A, Class II,

NSEAL G2 Articulating Tissue Sealers, Curved Jaw, NSLG2C35A,
NSLG2C45A, Class II

APPLICATION / SUBMISSION INFORMATION

6. TYPE OF APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES

☐ IND ☐ NDA ☐ ANDA ☐ BLA ☐ PMA ☐ HDE ☒ 510(k) ☐ PDP ☐ Other

7. INCLUDE IND/NDA/ANDA/BLA/PMA/HDE/510(k)/PDP/OTHER NUMBER (If number previously assigned)

8. SERIAL NUMBER ASSIGNED TO APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES

CERTIFICATION STATEMENT / INFORMATION

9. CHECK ONLY ONE OF THE FOLLOWING BOXES (See instructions for additional information and explanation)

☒ A. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, do not apply because the application/submission which this certification accompanies does not reference any clinical trial.

☐ B. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, do not apply to any clinical trial referenced in the application/submission which this certification accompanies.

☐ C. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, apply to one or more of the clinical trials referenced in the application/submission which this certification accompanies and that those requirements have been met.

10. IF YOU CHECKED BOX C, IN NUMBER 9, PROVIDE THE NATIONAL CLINICAL TRIAL (NCT) NUMBER(S) FOR ANY "APPLICABLE CLINICAL TRIAL(S)," UNDER 42 U.S.C. § 282(j)(1)(A)(i), SECTION 402(j)(1)(A)(i) OF THE PUBLIC HEALTH SERVICE ACT, REFERENCED IN THE APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES (Attach extra pages as necessary)

NCT Number(s): _____

The undersigned declares, to the best of her/his knowledge, that this is an accurate, true, and complete submission of information. I understand that the failure to submit the certification required by 42 U.S.C. § 282(j)(5)(B), section 402(j)(5)(B) of the Public Health Service Act, and the knowing submission of a false certification under such section are prohibited acts under 21 U.S.C. § 331, section 301 of the Federal Food, Drug, and Cosmetic Act.

Warning: A willfully and knowingly false statement is a criminal offense, U.S. Code, title 18, section 1001.

(b) (4), (b) (6)

Instructions for Completion of Form FDA 3674

Certification of Compliance, under 42 U.S.C. § 282(j)(5)(B), with Requirements of ClinicalTrials.gov Data Bank (42 U.S.C. § 282(j))

Form 3674 must accompany an application/submission, including amendments, supplements, and resubmissions, submitted under §§ 505, 515, 520(m), or 510(k) of the Federal Food, Drug, and Cosmetic Act or § 351 of the Public Health Service Act.

- 1. Name of Sponsor/Applicant/Submitter** - This is the name of the sponsor/applicant/submitter of the drug/biologic/device application/submission which the certification accompanies. The name must be identical to that listed on the application/submission.
- 2. Date** - This is the date of the application/submission which the certification accompanies.
- 3. & 4.** - Provide complete address, telephone number and fax number of the sponsor/applicant/submitter.
- 5. Product Information - For Drugs/Biologics:** Provide the established, proprietary name, and/or chemical/biochemical/blood product/cellular/gene therapy name(s) for the product covered by the application/submission. Include all available names by which the product is known. **For Devices:** Provide the common or usual name, classification, trade or proprietary or model name(s), and/or model number(s). Include all available names/model numbers by which the product is known.
- 6. Type of Application/Submission** - Identify the type of application/submission which the certification accompanies by checking the appropriate box. If the name of the type of application/submission is not identified, check the box labeled "Other."
- 7. IND/NDA/ANDA/BLA/PMA/HDE/510(k)/PDP/Other Number** - If FDA has previously assigned a number associated with the application/submission which this certification accompanies, list that number in this field. For example, if the application/submission accompanied by this certification is an IND protocol amendment and the IND number has already been issued by FDA, that number should be provided in this field.
- 8. Serial Number** - In some instances a sequential serial number is assigned to the application. If there is such a serial number, provide it in this field. If there is no such number, leave this field blank.
- 9. Certification** - This section contains three different check-off boxes.

Box A should be checked if the sponsor/applicant/submitter has concluded that the requirements of 42 U.S.C. § 282(j), section 402(j) of the Public Health Service Act, do not apply because no clinical trials are included, relied upon, or otherwise referred to, in the application/submission which the certification accompanies.

Box B should be checked if the sponsor/applicant/submitter has concluded that the requirements of 42 U.S.C. § 282(j), section 402(j) of the Public Health Service Act, do not apply at the time of submission of the certification to any clinical trials that are included, relied upon, or otherwise referred to, in the application/submission which the certification accompanies. This means that, even though some or all of the clinical trials included, relied upon, or otherwise referred to in the application/submission may be "applicable clinical trials" under 42 U.S.C. § 282(j)(1)(A)(i), section 402(j)(1)(A)(i) of the Public Health Service Act, on the date the certification is signed, 42 U.S.C. § 282(j), section 402(j) of the Public Health Service Act, does not require that any information be submitted to the ClinicalTrials.gov Data Bank with respect to those clinical trials.

Box C should be checked if the sponsor/applicant/submitter has concluded that the requirements of 42 U.S.C. § 282(j), section 402(j) of the Public Health Service Act, do apply, on the date the certification is signed, to some or all of the clinical trials that are included, relied upon, or otherwise referred to, in the application/submission which the certification accompanies. This means that, as of the date the certification is signed, the requirements of 42 U.S.C. § 282(j), section 402(j) of the Public Health Service Act, apply to one or more of the clinical trials included, relied upon, or otherwise referred to, in the application/submission which this certification accompanies.
- 10. National Clinical Trial (NCT) Numbers** - If you have checked Box C in number 9 (Certification), provide the NCT Number obtained from www.ClinicalTrials.gov for each clinical trial that is an "applicable clinical trial" under 42 U.S.C. § 282(j)(1)(A)(i), section 402(j)(1)(A)(i) of the Public Health Service Act, and that is included, relied upon, or otherwise referred to, in the application/submission which the certification accompanies. Type only the number, as the term "NCT" will be added automatically before number. Include any and all NCT numbers that, as of the date the certification is signed, have been assigned to the clinical trials included, relied upon, or otherwise referred to, in the application/submission which this certification accompanies. Multiple NCT numbers may be required for a particular certification, depending on the number of "applicable clinical trials" included, relied upon, or otherwise referred to, in the application/submission which the certification accompanies. Leave this field blank if you have checked Box 9.C but, at the time the certification is completed, you have not yet received any NCT numbers for the "applicable clinical trial(s)" included, relied upon, or otherwise referred to in the application/submission.
- 11. Signature of Sponsor/Applicant/Submitter or an Authorized Representative** - The person signing the certification must sign in this field.
- 12. Name and Title of Person Who Signed in number 11** - Include the name and title of the person who is signing the certification. If the person signing the certification is not the sponsor/applicant/submitter of the application/submission, he or she must be an authorized representative of the sponsor/applicant/submitter.
- 13. & 14.** - Provide the full address, telephone and fax numbers of the person who is identified in number 11 and signs the certification in number 11.
- 15.** Provide the date the certification is signed. This date may be different from the date provided in number 2.

Paperwork Reduction Act Statement

Public reporting burden for this collection of information is estimated to average 15 minutes and 45 minutes (depending on the type of application/submission) per response, including time for reviewing instructions. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to the address below.

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
1350 Piccard Drive, Room 400
Rockville, MD 20850

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information, unless it displays a currently valid OMB control number.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Section 21: Other

This section contains completed copies of FDA Form 3654 for the standards referenced in this submission. The referenced standards include the following:

AAMI / ANSI / ISO 10993-1:2009, Biological evaluation of medical devices --
Part 1: Evaluation and testing within a risk management process.

AAMI/ANSI/ISO 11137-1: 2006 Sterilization of Health Care Products -
Radiation - Part 1: Requirements for development, validation and routine control
of a sterilization process for medical devices

AAMI/ANSI/ISO 11137-2: 2006 Sterilization of Health Care Products -
Radiation - Part 2: Establishing the sterilization dose

AAMI/ANS/ISO 11137-3:2006 Sterilization of Health Care Products - Radiation
- Part 3: Guidance on Dosimetric Aspects.

Appendix 1 lists the raw data from the Section 18 Performance Testing – Bench, Sealed Vessel Burst Test

Department of Health and Human Services
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STANDARDS DATA REPORT FOR 510(k)s
(To be filled in by applicant)

This report and the Summary Report Table are to be completed by the applicant when submitting a 510(k) that references a national or international standard. A separate report is required for each standard referenced in the 510(k).

TYPE OF 510(K) SUBMISSION

☒ Traditional☐ Special☐ AbbreviatedSTANDARD TITLE ¹

AAMI/ANSI/ISO 10993-1 :2009, Biological evaluation of medical devices - Part I: Eval and testing within a risk mngmt process

Please answer the following questions

Yes No

Is this standard recognized by FDA ²? ☒ ☐FDA Recognition number³ #2-156Was a third party laboratory responsible for testing conformity of the device to this standard identified in the 510(k)? ☒ ☐Is a summary report ⁴ describing the extent of conformance of the standard used included in the 510(k)? ☐ ☒
If no, complete a summary report table.Does the test data for this device demonstrate conformity to the requirements of this standard as it pertains to this device? ☒ ☐Does this standard include acceptance criteria? ☐ ☒
If no, include the results of testing in the 510(k).Does this standard include more than one option or selection of tests? ☐ ☒
If yes, report options selected in the summary report table.Were there any deviations or adaptations made in the use of the standard? ☐ ☒
If yes, were deviations in accordance with the FDA supplemental information sheet (SIS) ⁵? ☐ ☐Were deviations or adaptations made beyond what is specified in the FDA SIS? ☐ ☒
If yes, report these deviations or adaptations in the summary report table.Were there any exclusions from the standard? ☐ ☒
If yes, report these exclusions in the summary report table.Is there an FDA guidance ⁶ that is associated with this standard? ☒ ☐
If yes, was the guidance document followed in preparation of this 510k? ☒ ☐

Title of guidance: FDA Bluebook Memorandum G95-1, Use of Intl Standard ISO-10993, 'Biological Evaluation of Med Dev

¹ The formatting convention for the title is: [SDO] [numeric identifier] [title of standard] [date of publication]

² Authority [21 U.S.C. 360d], <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Standards/default.htm>

³ <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm>

⁴ The summary report should include: any adaptations used to adapt to the device under review (for example, alternative test methods); choices made when options or a selection of methods are described; deviations from the standard; requirements not applicable to the device; and the name and

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⁵ The supplemental information sheet (SIS) is additional information which is necessary before FDA recognizes the standard. Found at <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm>

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE		
STANDARD TITLE AAMI/ANSI/ISO 10993-1 :2009, Biological evaluation of medical devices - Part I: Eval and testing within a risk mngmt process		
CONFORMANCE WITH STANDARD SECTIONS*		
SECTION NUMBER 1	SECTION TITLE Scope	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 2	SECTION TITLE Normative references	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 3	SECTION TITLE Terms and definitions	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
<p>* For completeness list all sections of the standard and indicate whether conformance is met. If a section is not applicable (N/A) an explanation is needed under "justification." Some standards include options, so similar to deviations, the option chosen needs to be described and adequately justified as appropriate for the subject device. Explanation of all deviations or description of options selected when following a standard is required under "type of deviation or option selected," "description" and "justification" on the report. More than one page may be necessary.</p> <p>* Types of deviations can include an exclusion of a section in the standard, a deviation brought out by the FDA supplemental information sheet (SIS), a deviation to adapt the standard to the device, or any adaptation of a section.</p>		
Paperwork Reduction Act Statement		
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**EXTENT OF STANDARD CONFORMANCE
SUMMARY REPORT TABLE**

STANDARD TITLE

AAMI/ANSI/ISO 10993-1 :2009, Biological evaluation of medical devices - Part I: Eval and testing within a risk mngmt process

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER 4	SECTION TITLE General principles applying to biological evaluation of medical devices	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
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TYPE OF DEVIATION OR OPTION SELECTED *

See attached page titled "Attachment to FDA Form 3654, Options of selected ISO 10993-1"

DESCRIPTION

JUSTIFICATION

SECTION NUMBER 5	SECTION TITLE Categorization of medical devices	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
---------------------	--	--

TYPE OF DEVIATION OR OPTION SELECTED *

See attached page titled "Attachment to FDA Form 3654, Options of selected ISO 10993-1"

DESCRIPTION

JUSTIFICATION

SECTION NUMBER 6	SECTION TITLE Biological evaluation process	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
---------------------	--	--

TYPE OF DEVIATION OR OPTION SELECTED *

See attached page titled "Attachment to FDA Form 3654, Options of selected ISO 10993-1"

DESCRIPTION

JUSTIFICATION

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE		
STANDARD TITLE AAMI/ANSI/ISO 10993-1 :2009, Biological evaluation of medical devices - Part I: Eval and testing within a risk mngmt process		
CONFORMANCE WITH STANDARD SECTIONS*		
SECTION NUMBER 7	SECTION TITLE Interpretation of biological eval data and overall biological safety assessment	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER	SECTION TITLE	CONFORMANCE? ☐ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED * See attached page titled "Attachment to FDA Form 3654, Options of selected ISO 10993-1"		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER	SECTION TITLE	CONFORMANCE? ☐ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED * See attached page titled "Attachment to FDA Form 3654, Options of selected ISO 10993-1"		
DESCRIPTION		
JUSTIFICATION		
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Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Attachment to FDA Form 3654

Options Selected for ISO 10993-1

Options Selected from Clause 4 of ISO 10993-1 for Form 3654, Section 4

For Surface Contacting Device the following options were selected: 4.2.2, Surface Contacting Devices (a) Skin Limited Contact (<24 hours); 4.2.3 External Communicating Devices (b) Tissue/bone/dentin Limited Contact (<24 hours); 4.2.4 Implant Devices (a) Tissue Bone Permanent Contact (>30 days).

Options Selected from Clause 5 of ISO 10993-1 for Form 3654, Section 5

For Surface contacting Device – Skin (Limited <24 hours) the following options were selected 5.2.2, Cytotoxicity utilized ISO 10993-5; 5.2.3. Sensitization utilized ISO 10993-10; 5.2.5 Intracutaneous Reactivity utilized ISO 10993-10.

For external communicating devices – Tissue/Bone/Dentin (Limited <24 hours)

The following options were selected: 5.2.2, Cytotoxicity utilized ISO 10993-5; 5.2.3. Sensitization utilized ISO 10993-10; 5.2.5 Intracutaneous Reactivity utilized ISO 10993-10.

Options Selected from Clause 6 of ISO 10993-1

All the tests in Table 1 that were recommended for this device have been conducted as discussed in Section 5 of FDA Form 3654. The following tests from Table 2 were conducted for implant devices tissue/bone, permanent contact >30 days: Chronic Toxicity (per ISO 10993-11) and Carcinogenicity (per ISO 10993-3).

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TYPE OF 510(K) SUBMISSION

☒ Traditional☐ Special☐ AbbreviatedSTANDARD TITLE ¹

AAMI/ANSI/ISO 11137-1:2006, Sterilization of health care products - Radiation Part 1

Please answer the following questions

Yes No

Is this standard recognized by FDA ²? ☒ ☐FDA Recognition number ³ #14-297Was a third party laboratory responsible for testing conformity of the device to this standard identified in the 510(k)? ☒ ☐Is a summary report ⁴ describing the extent of conformance of the standard used included in the 510(k)? ☐ ☒
If no, complete a summary report table.Does the test data for this device demonstrate conformity to the requirements of this standard as it pertains to this device? ☒ ☐Does this standard include acceptance criteria? ☒ ☐
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If yes, was the guidance document followed in preparation of this 510k? ☐ ☐

Title of guidance:

¹ The formatting convention for the title is: [SDO] [numeric identifier] [title of standard] [date of publication]² Authority [21 U.S.C. 360d], <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Standards/default.htm>³ <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm>⁴ The summary report should include: any adaptations used to adapt to the device under review (for example, alternative test methods); choices made when options or a selection of methods are described; deviations from the standard; requirements not applicable to the device; and the name and

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE		
STANDARD TITLE AAMI/ANSI/ISO 11137-1:2006, Sterilization of health care products - Radiation Part 1		
CONFORMANCE WITH STANDARD SECTIONS*		
SECTION NUMBER 1	SECTION TITLE Scope	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 2	SECTION TITLE Normative References	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 3	SECTION TITLE Terms and Definitions	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
<p>* For completeness list all sections of the standard and indicate whether conformance is met. If a section is not applicable (N/A) an explanation is needed under "justification." Some standards include options, so similar to deviations, the option chosen needs to be described and adequately justified as appropriate for the subject device. Explanation of all deviations or description of options selected when following a standard is required under "type of deviation or option selected," "description" and "justification" on the report. More than one page may be necessary.</p> <p>♦ Types of deviations can include an exclusion of a section in the standard, a deviation brought out by the FDA supplemental information sheet (SIS), a deviation to adapt the standard to the device, or any adaptation of a section.</p>		
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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE

AAMI/ANSI/ISO 11137-1:2006, Sterilization of health care products - Radiation Part 1

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER

4

SECTION TITLE

Quality management system elements

CONFORMANCE?

☒ Yes☐ No☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER

5

SECTION TITLE

Sterilizing agent characterization

CONFORMANCE?

☒ Yes☐ No☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER

6

SECTION TITLE

Process and equipment characterization

CONFORMANCE?

☒ Yes☐ No☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE		
STANDARD TITLE AAMI/ANSI/ISO 11137-1:2006, Sterilization of health care products - Radiation Part 1		
CONFORMANCE WITH STANDARD SECTIONS*		
SECTION NUMBER 7	SECTION TITLE Product definition	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED * Option - Validation of a product family		
DESCRIPTION The sterilization was established for the product family		
JUSTIFICATION		
SECTION NUMBER 8	SECTION TITLE Process Definition	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED * Option - Substantiation of a 25kyGy as a sterilization dose		
DESCRIPTION The sterilization dose of 25 kyGy was selected and substantiated		
JUSTIFICATION		
SECTION NUMBER 9	SECTION TITLE Validation	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
<p>* For completeness list all sections of the standard and indicate whether conformance is met. If a section is not applicable (N/A) an explanation is needed under "justification." Some standards include options, so similar to deviations, the option chosen needs to be described and adequately justified as appropriate for the subject device. Explanation of all deviations or description of options selected when following a standard is required under "type of deviation or option selected," "description" and "justification" on the report. More than one page may be necessary.</p> <p>♦ Types of deviations can include an exclusion of a section in the standard, a deviation brought out by the FDA supplemental information sheet (SIS), a deviation to adapt the standard to the device, or any adaptation of a section.</p>		
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**EXTENT OF STANDARD CONFORMANCE
SUMMARY REPORT TABLE**

STANDARD TITLE

AAMI/ANSI/ISO 11137-1:2006, Sterilization of health care products - Radiation Part 1

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER

10

SECTION TITLE

Routine monitoring and control

CONFORMANCE?

☒ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

Option - Gamma irradiator controls

DESCRIPTION

Requirements implemented for gamma irradiator controls

JUSTIFICATION

SECTION NUMBER

11

SECTION TITLE

Product release from sterilization

CONFORMANCE?

☒ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER

12

SECTION TITLE

Maintaining process effectiveness

CONFORMANCE?

☒ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

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TYPE OF 510(K) SUBMISSION

☒ Traditional☐ Special☐ AbbreviatedSTANDARD TITLE ¹

AMI / ANSI / ISO 11137-2:2006, Sterilization of health care products - Radiation - Part 2

Please answer the following questions

Yes No

Is this standard recognized by FDA ²? ☒ ☐FDA Recognition number ³ #14-225Was a third party laboratory responsible for testing conformity of the device to this standard identified in the 510(k)? ☒ ☐Is a summary report ⁴ describing the extent of conformance of the standard used included in the 510(k)? ☐ ☒
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Title of guidance:

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE

AMI / ANSI / ISO 11137-2:2006, Sterilization of health care products - Radiation - Part 2

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER

1

SECTION TITLE

Scope

CONFORMANCE?

☒ Yes☐ No☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER

2

SECTION TITLE

Normative References

CONFORMANCE?

☒ Yes☐ No☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER

3

SECTION TITLE

Abbreviations, terms and definitions

CONFORMANCE?

☒ Yes☐ No☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

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STANDARD TITLE AMI / ANSI / ISO 11137-2:2006, Sterilization of health care products - Radiation - Part 2		
CONFORMANCE WITH STANDARD SECTIONS*		
SECTION NUMBER 4	SECTION TITLE Definition and maintenance of product families	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 5	SECTION TITLE Selection and testing of product for establishing and verifying the ster, dose	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 6	SECTION TITLE Methods of dose establishment	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
<p>* For completeness list all sections of the standard and indicate whether conformance is met. If a section is not applicable (N/A) an explanation is needed under "justification." Some standards include options, so similar to deviations, the option chosen needs to be described and adequately justified as appropriate for the subject device. Explanation of all deviations or description of options selected when following a standard is required under "type of deviation or option selected," "description" and "justification" on the report. More than one page may be necessary.</p> <p>♦ Types of deviations can include an exclusion of a section in the standard, a deviation brought out by the FDA supplemental information sheet (SIS), a deviation to adapt the standard to the device, or any adaptation of a section.</p>		
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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE		
STANDARD TITLE AMI / ANSI / ISO 11137-2:2006, Sterilization of health care products - Radiation - Part 2		
CONFORMANCE WITH STANDARD SECTIONS*		
SECTION NUMBER 7	SECTION TITLE Method 1: dose setting using bioburden information	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 8	SECTION TITLE Method 2: Dose setting using fraction positive information	CONFORMANCE? ☐ Yes ☐ No ☒ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 9	SECTION TITLE Method VDmax: Substantiation of 25 kGy or 15 kGy as the sterilization dose	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED * Option selected was VDmax 25		
DESCRIPTION Substantiation of VDmax 25		
JUSTIFICATION		
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STANDARD TITLE AMI / ANSI / ISO 11137-2:2006, Sterilization of health care products - Radiation - Part 2		
CONFORMANCE WITH STANDARD SECTIONS*		
SECTION NUMBER 10	SECTION TITLE Auditing sterilization dose	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 11	SECTION TITLE Worked examples	CONFORMANCE? ☐ Yes ☐ No ☒ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER	SECTION TITLE	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
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(To be filled in by applicant)

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TYPE OF 510(K) SUBMISSION

☒ Traditional☐ Special☐ AbbreviatedSTANDARD TITLE ¹

AAMI / ANSI / ISO 11137-3:2006, Sterilization of health care products - Radiation - Part 3

Please answer the following questions

Yes No

Is this standard recognized by FDA ²? ☒ ☐FDA Recognition number ³ #14-298Was a third party laboratory responsible for testing conformity of the device to this standard identified in the 510(k)? ☐ ☒Is a summary report ⁴ describing the extent of conformance of the standard used included in the 510(k)? ☐ ☒
If no, complete a summary report table.Does the test data for this device demonstrate conformity to the requirements of this standard as it pertains to this device? ☒ ☐Does this standard include acceptance criteria? ☒ ☐
If no, include the results of testing in the 510(k).Does this standard include more than one option or selection of tests? ☒ ☐
If yes, report options selected in the summary report table.Were there any deviations or adaptations made in the use of the standard?..... ☐ ☒
If yes, were deviations in accordance with the FDA supplemental information sheet (SIS) ⁵? ☐ ☐Were deviations or adaptations made beyond what is specified in the FDA SIS?..... ☐ ☒
If yes, report these deviations or adaptations in the summary report table.Were there any exclusions from the standard? ☐ ☒
If yes, report these exclusions in the summary report table.Is there an FDA guidance ⁶ that is associated with this standard?..... ☐ ☒
If yes, was the guidance document followed in preparation of this 510k? ☐ ☐

Title of guidance:

¹ The formatting convention for the title is: [SDO] [numeric identifier] [title of standard] [date of publication]² Authority [21 U.S.C. 360d], <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Standards/default.htm>³ <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm>⁴ The summary report should include: any adaptations used to adapt to the device under review (for example, alternative test methods); choices made when options or a selection of methods are described; deviations from the standard; requirements not applicable to the device; and the name and

address of the test laboratory or certification body involved in conformance assessment to this standard. The summary report includes information on all standards utilized during the development of the device.

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE		
STANDARD TITLE AAMI / ANSI / ISO 11137-3:2006, Sterilization of health care products - Radiation - Part 3		
CONFORMANCE WITH STANDARD SECTIONS*		
SECTION NUMBER 1	SECTION TITLE Scope	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 2	SECTION TITLE Normative references	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 3	SECTION TITLE Terms and definitions	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE		
STANDARD TITLE AAMI / ANSI / ISO 11137-3:2006, Sterilization of health care products - Radiation - Part 3		
CONFORMANCE WITH STANDARD SECTIONS*		
SECTION NUMBER 4	SECTION TITLE Measurement of dose	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 5	SECTION TITLE Selection and calibration of dosimetry systems	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 6	SECTION TITLE Establishing the maximum acceptable dose	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
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STANDARD TITLE AAMI / ANSI / ISO 11137-3:2006, Sterilization of health care products - Radiation - Part 3		
CONFORMANCE WITH STANDARD SECTIONS*		
SECTION NUMBER 7	SECTION TITLE Establishing the sterilization dose	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 8	SECTION TITLE Installation qualification	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 9	SECTION TITLE Operational qualification	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
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STANDARD TITLE AAMI / ANSI / ISO 11137-3:2006, Sterilization of health care products - Radiation - Part 3		
CONFORMANCE WITH STANDARD SECTIONS*		
SECTION NUMBER 10	SECTION TITLE Performance qualification	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 11	SECTION TITLE Routine monitoring and control	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER	SECTION TITLE	CONFORMANCE? ☐ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
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TYPE OF 510(K) SUBMISSION

☒ Traditional☐ Special☐ AbbreviatedSTANDARD TITLE ¹

IEC 60601-1(1988) Medical Electrical Equipment - Part 1: General Requirements for Basic Safety and Essential Performance

Please answer the following questions

Yes No

Is this standard recognized by FDA ²? ☒ ☐FDA Recognition number ³ #5-4Was a third party laboratory responsible for testing conformity of the device to this standard identified in the 510(k)? ☒ ☐Is a summary report ⁴ describing the extent of conformance of the standard used included in the 510(k)? ☐ ☒
If no, complete a summary report table.Does the test data for this device demonstrate conformity to the requirements of this standard as it pertains to this device? ☒ ☐Does this standard include acceptance criteria? ☒ ☐
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Title of guidance:

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE		
STANDARD TITLE IEC 60601-1(1988) Medical Electrical Equipment - Part 1: General Requirements for Basic Safety and Essential Performance		
CONFORMANCE WITH STANDARD SECTIONS*		
SECTION NUMBER 1	SECTION TITLE General	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 2	SECTION TITLE Environmental Conditions	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 3	SECTION TITLE Protection against electrical shock hazards	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE

IEC 60601-1(1988) Medical Electrical Equipment - Part 1: General Requirements for Basic Safety and Essential Performance

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER 4	SECTION TITLE Protection against Mechanical Hazards	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 5	SECTION TITLE Protection against hazards from unwanted or excessive radiation	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 6	SECTION TITLE Protection against hazards of ignition of flammable anaesthetic mixtures	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		

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**EXTENT OF STANDARD CONFORMANCE
SUMMARY REPORT TABLE**

STANDARD TITLE
IEC 60601-1(1988) Medical Electrical Equipment - Part 1: General Requirements for Basic Safety and Essential Performance

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER 7	SECTION TITLE Protection against excessive temperatures and other safety hazards	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
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TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER 8	SECTION TITLE Accuracy of operating data and protection against hazardous output	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
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TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER 9	SECTION TITLE Abnormal operation and fault conditions; environmental tests	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
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TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE
IEC 60601-1(1988) Medical Electrical Equipment - Part 1: General Requirements for Basic Safety and Essential Performance

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER 10	SECTION TITLE Constructional requirements	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
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TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER	SECTION TITLE	CONFORMANCE? ☐ Yes ☐ No ☐ N/A
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TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER	SECTION TITLE	CONFORMANCE? ☐ Yes ☐ No ☐ N/A
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TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

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Department of Health and Human Services
Food and Drug Administration
STANDARDS DATA REPORT FOR 510(k)s
(To be filled in by applicant)

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TYPE OF 510(K) SUBMISSION

☒ Traditional☐ Special☐ AbbreviatedSTANDARD TITLE ¹

IEC 60601-1-2:2007, Medical Electrical Equipment-Part 1-2:General Requirements for Safety and Performance-Collateral Standard

Please answer the following questions

Yes No

Is this standard recognized by FDA ²? ☒ ☐FDA Recognition number ³ #5-54Was a third party laboratory responsible for testing conformity of the device to this standard identified in the 510(k)? ☒ ☐Is a summary report ⁴ describing the extent of conformance of the standard used included in the 510(k)? ☐ ☒
If no, complete a summary report table.Does the test data for this device demonstrate conformity to the requirements of this standard as it pertains to this device? ☒ ☐Does this standard include acceptance criteria? ☒ ☐
If no, include the results of testing in the 510(k).Does this standard include more than one option or selection of tests? ☐ ☒
If yes, report options selected in the summary report table.Were there any deviations or adaptations made in the use of the standard? ☐ ☒
If yes, were deviations in accordance with the FDA supplemental information sheet (SIS) ⁵? ☐ ☐Were deviations or adaptations made beyond what is specified in the FDA SIS? ☐ ☒
If yes, report these deviations or adaptations in the summary report table.Were there any exclusions from the standard? ☐ ☒
If yes, report these exclusions in the summary report table.Is there an FDA guidance ⁶ that is associated with this standard? ☐ ☒
If yes, was the guidance document followed in preparation of this 510k? ☐ ☐

Title of guidance:

¹ The formatting convention for the title is: [SDO] [numeric identifier] [title of standard] [date of publication]² Authority [21 U.S.C. 360d], <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Standards/default.htm>³ <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm>⁴ The summary report should include: any adaptations used to adapt to the device under review (for example, alternative test methods); choices made when options or a selection of methods are described; deviations from the standard; requirements not applicable to the device; and the name and

address of the test laboratory or certification body involved in conformance assessment to this standard. The summary report includes information on all standards utilized during the development of the device.

⁵ The supplemental information sheet (SIS) is additional information which is necessary before FDA recognizes the standard. Found at <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm>⁶ The online search for CDRH Guidance Documents can be found at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/default.htm>

EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE

IEC 60601-1-2:2001, Medical Electrical Equipment-Part 1-2:General Requirements for Safety and Performance-Collateral Standard

CONFORMANCE WITH STANDARD SECTIONS***SECTION NUMBER**

1

SECTION TITLE

Scope, object, and related standards

CONFORMANCE?☒ Yes☐ No☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER

2

SECTION TITLE

Normative references

CONFORMANCE?☒ Yes☐ No☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER

3

SECTION TITLE

Terms and definitions

CONFORMANCE?☒ Yes☐ No☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE

IEC 60601-1-2:2001, Medical Electrical Equipment-Part 1-2:General Requirements for Safety and Performance-Collateral Standard

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER 4	SECTION TITLE General requirements	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 5	SECTION TITLE Identification, marking, and documents	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 6	SECTION TITLE Electromagnetic compatibility	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		

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TYPE OF 510(K) SUBMISSION

☒ Traditional☐ Special☐ AbbreviatedSTANDARD TITLE ¹

IEC60601-2-2:2009, Medical Electrical Equipment Part 2-2: Particular Requirements for Safety of Hi Frequency Surgical Equipment

Please answer the following questions

Yes No

Is this standard recognized by FDA ²? ☒ ☐FDA Recognition number ³ #6-229Was a third party laboratory responsible for testing conformity of the device to this standard identified in the 510(k)? ☒ ☐Is a summary report ⁴ describing the extent of conformance of the standard used included in the 510(k)? ☐ ☒
If no, complete a summary report table.Does the test data for this device demonstrate conformity to the requirements of this standard as it pertains to this device? ☒ ☐Does this standard include acceptance criteria? ☒ ☐
If no, include the results of testing in the 510(k).Does this standard include more than one option or selection of tests? ☐ ☒
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**EXTENT OF STANDARD CONFORMANCE
SUMMARY REPORT TABLE**

STANDARD TITLE

IEC60601-2-2:2009, Medical Electrical Equipment Part 2-2: Particular Requirements for Safety of Hi Frequency Surgical Equipment

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER 201.1	SECTION TITLE Scope, object, and related standards	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 201.2	SECTION TITLE Normative references	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 201.3	SECTION TITLE Terms and definitions	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE

IEC60601-2-2:2009, Medical Electrical Equipment Part 2-2: Particular Requirements for Safety of Hi Frequency Surgical Equipment

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER 201.4	SECTION TITLE General requirements	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 201.5	SECTION TITLE General requirements for testing of ME Equipment	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		
SECTION NUMBER 201.6	SECTION TITLE Classification of ME equipment and ME systems	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
TYPE OF DEVIATION OR OPTION SELECTED *		
DESCRIPTION		
JUSTIFICATION		

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STANDARD TITLE

IEC60601-2-2:2009, Medical Electrical Equipment Part 2-2: Particular Requirements for Safety of Hi Frequency Surgical Equipment

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER

201.7

SECTION TITLE

ME equipment identification, marking and documents

CONFORMANCE?

☒ Yes☐ No☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER

201.8

SECTION TITLE

Protection against electrical hazards from ME equipment

CONFORMANCE?

☒ Yes☐ No☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER

201.9

SECTION TITLE

Protection against mechanical hazards of me equipment and ME systems

CONFORMANCE?

☒ Yes☐ No☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

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STANDARD TITLE

IEC60601-2-2:2009, Medical Electrical Equipment Part 2-2: Particular Requirements for Safety of Hi Frequency Surgical Equipment

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER

201.10

SECTION TITLE

Protection against unwanted and excessive radiation hazards

CONFORMANCE?

☒ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER

201.11

SECTION TITLE

Protection against excessive temperatures and other hazards

CONFORMANCE?

☒ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER

201.12

SECTION TITLE

Accuracy of controls and instillments and protection against hazardous output

CONFORMANCE?

☒ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE

IEC60601-2-2:2009, Medical Electrical Equipment Part 2-2: Particular Requirements for Safety of Hi Frequency Surgical Equipment

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER 201.13	SECTION TITLE Hazardous situations and fault conditions	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
--------------------------	--	--

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER 201.14	SECTION TITLE Programmable Electrical Medical Systems (PEMS)	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
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TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER 201.15	SECTION TITLE Construction of ME equipment	CONFORMANCE? ☒ Yes ☐ No ☐ N/A
--------------------------	---	--

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

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STANDARD TITLE

IEC60601-2-2:2009, Medical Electrical Equipment Part 2-2: Particular Requirements for Safety of Hi Frequency Surgical Equipment

CONFORMANCE WITH STANDARD SECTIONS***SECTION NUMBER**

201.16

SECTION TITLE

ME Systems

CONFORMANCE?☐ Yes ☐ No ☒ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION**JUSTIFICATION**

Not applicable, this section of the standard refers to separate ME equipments joined as one system

SECTION NUMBER

201.17

SECTION TITLE

Electromagnetic compatibility of ME equipment and ME systems

CONFORMANCE?☒ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION**JUSTIFICATION****SECTION NUMBER**

202

SECTION TITLE

Electromagnetic compatibility - Requirements and tests

CONFORMANCE?☒ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION**JUSTIFICATION**

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Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

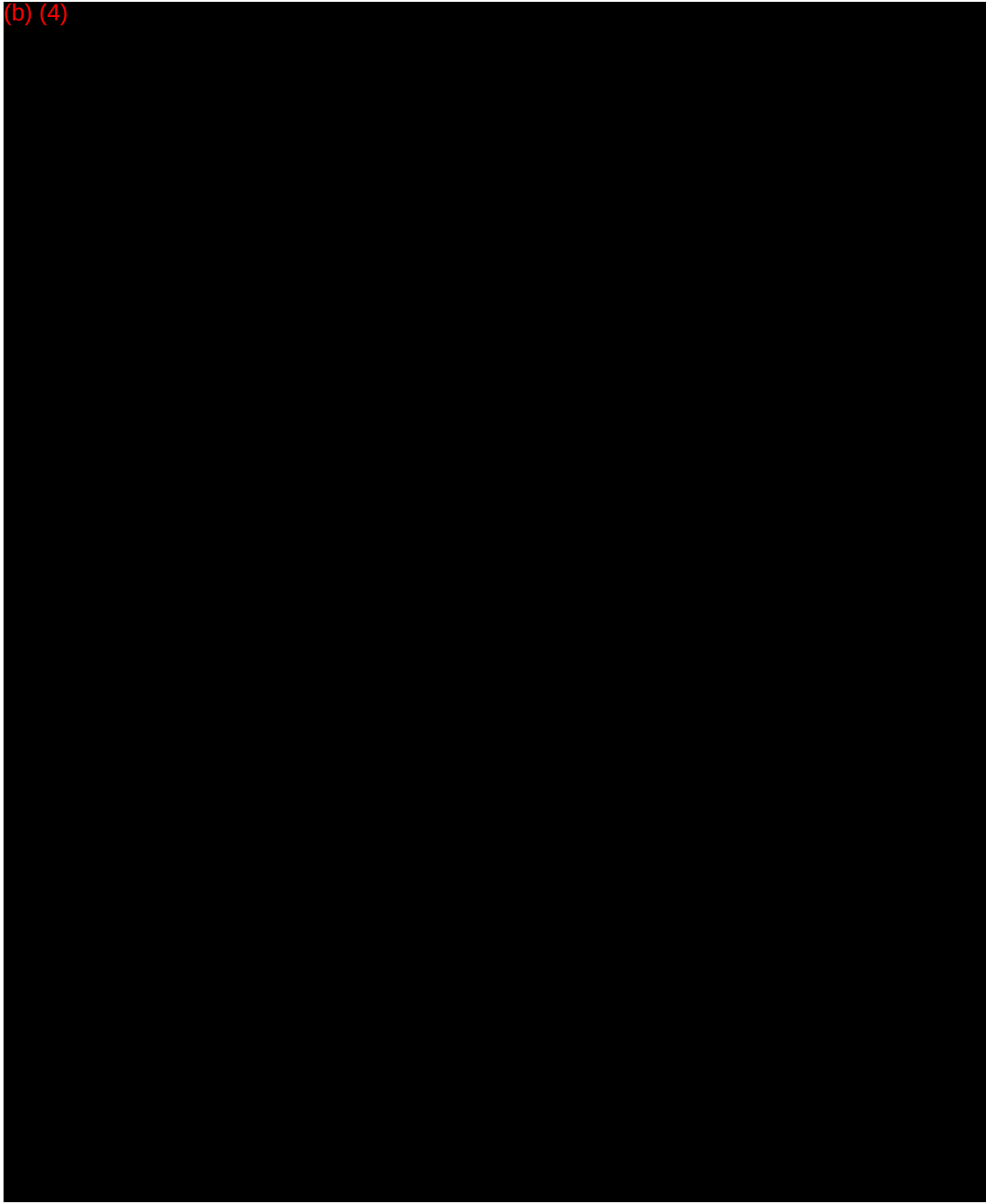
Section 21: Other, Appendix 1:

Raw Data Listing from Section 18 Performance Testing – Bench, Sealed Vessel Burst Test

Abbreviations: C= Carotid artery, T=Thyrocervical vein

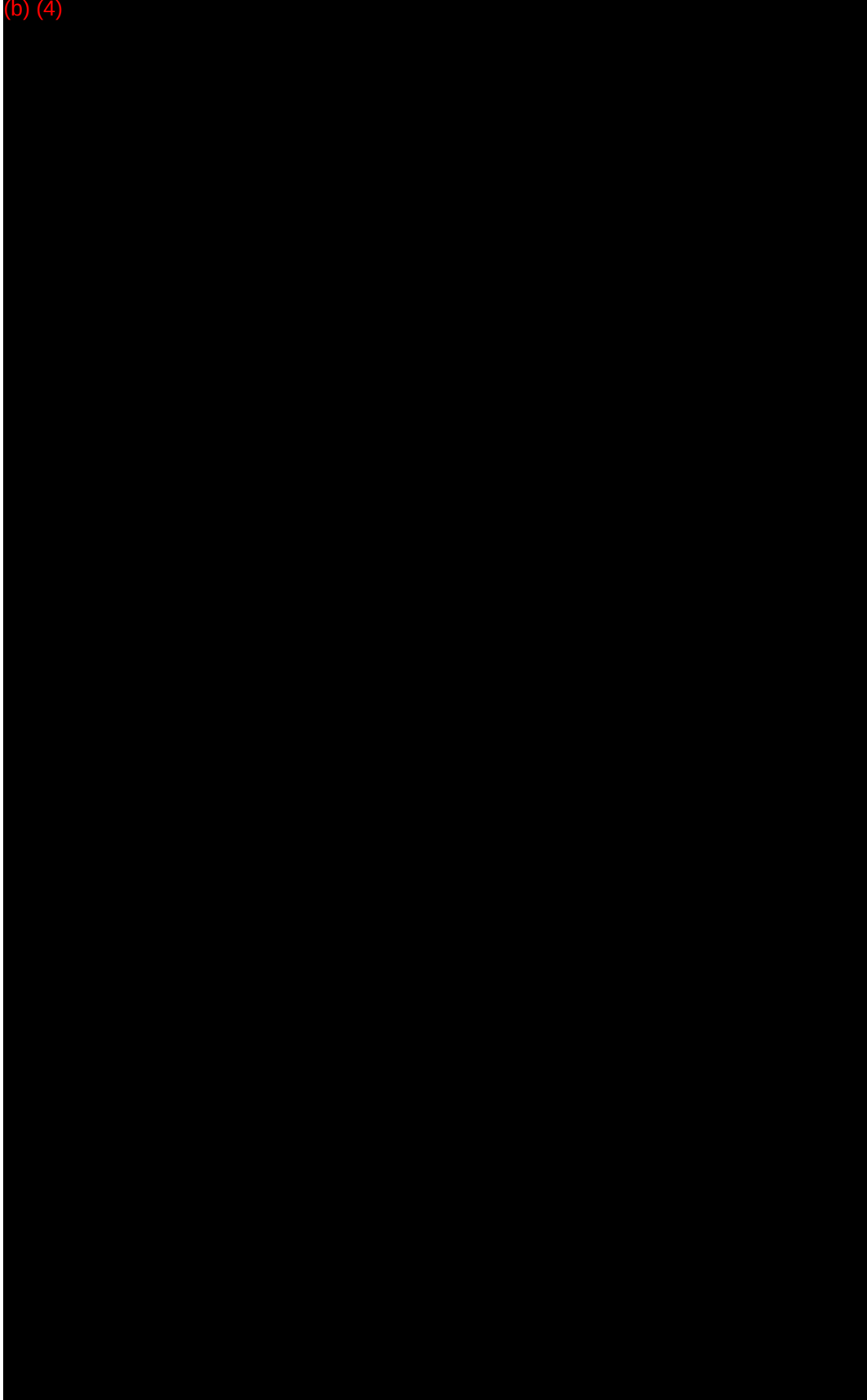
Appendix Table A1.1: Subject Device, Straight Jaw, Bench Data

(b) (4)



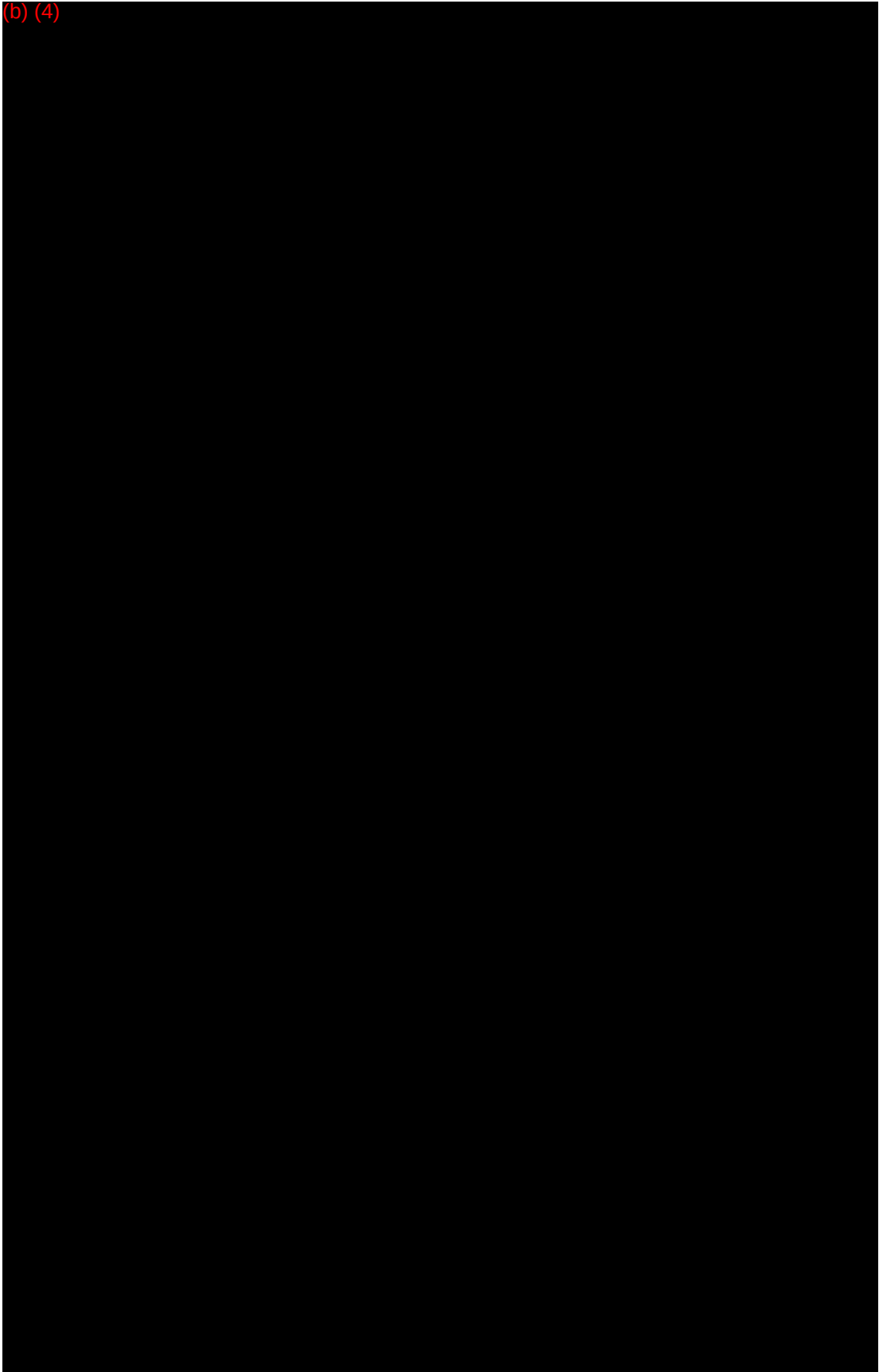
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(b) (4)



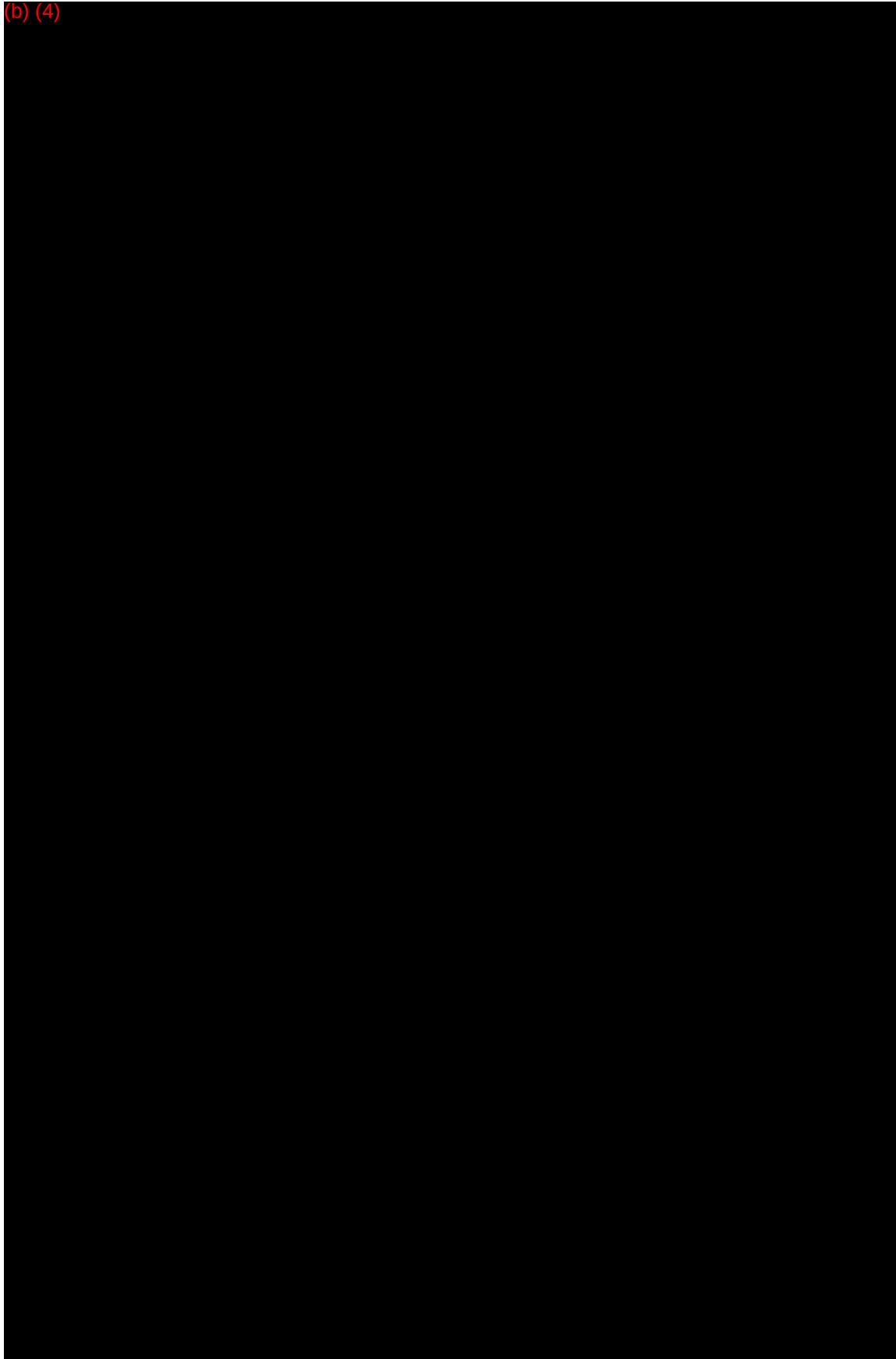
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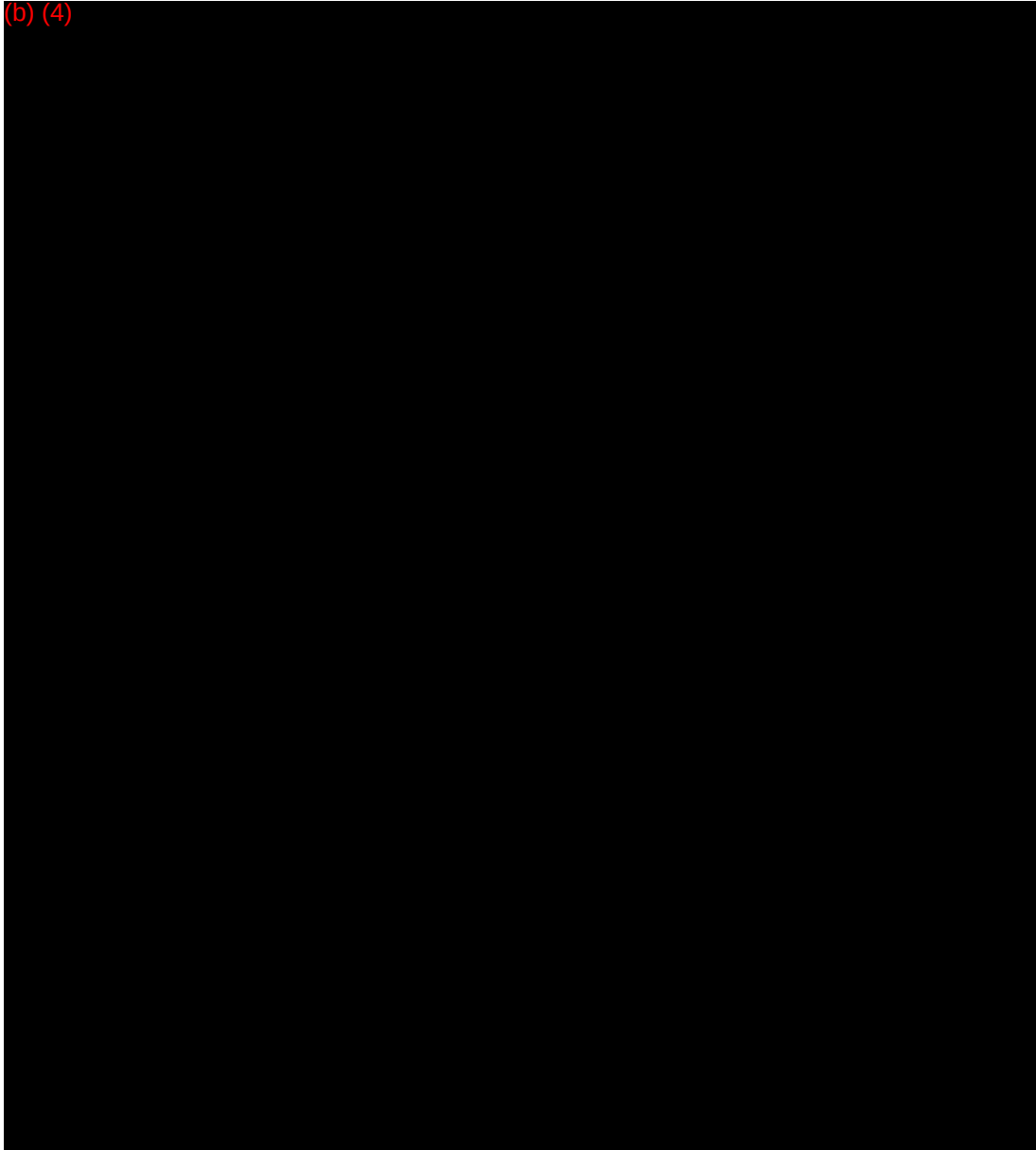
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(b) (4)



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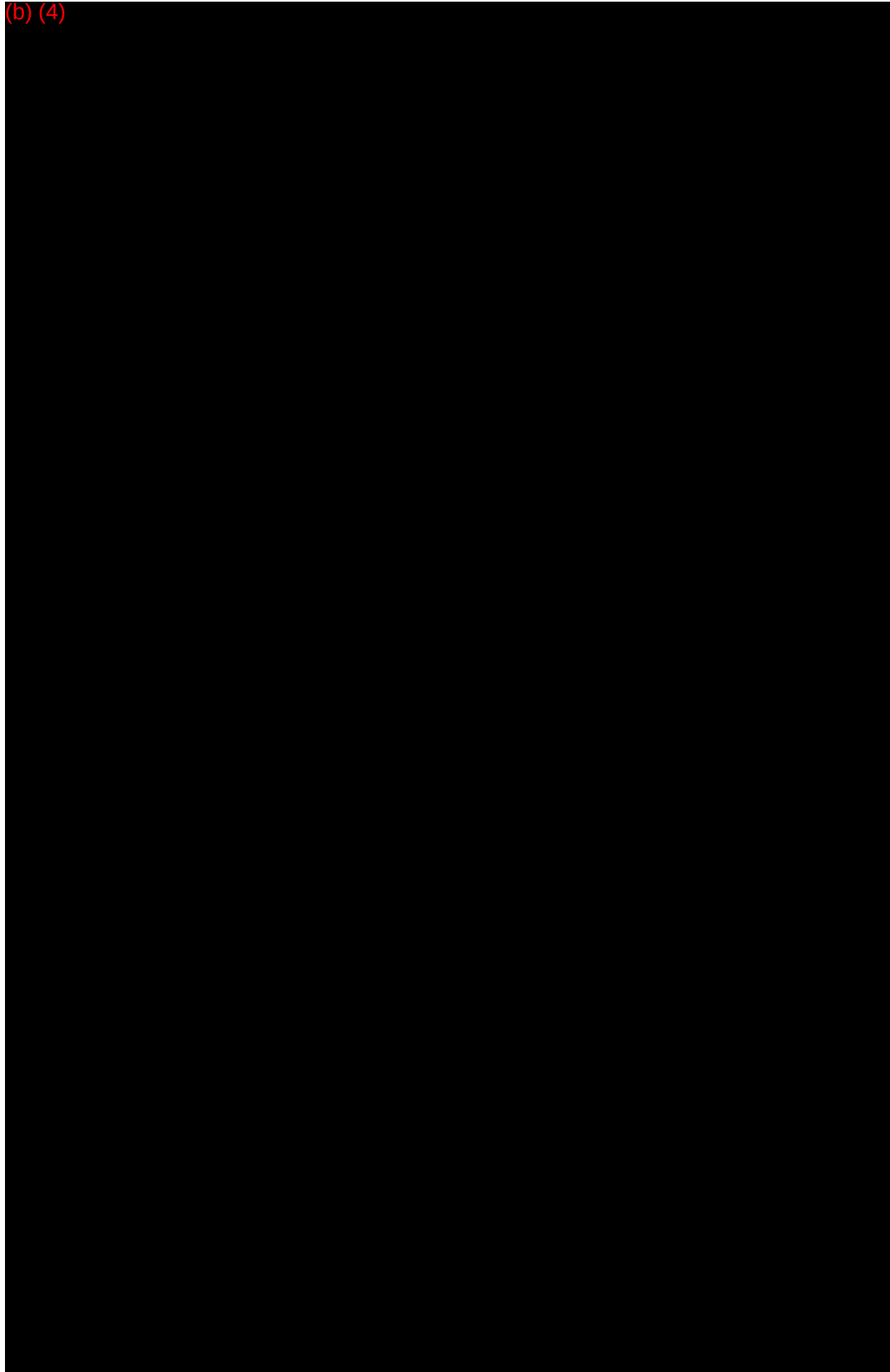
(b) (4)



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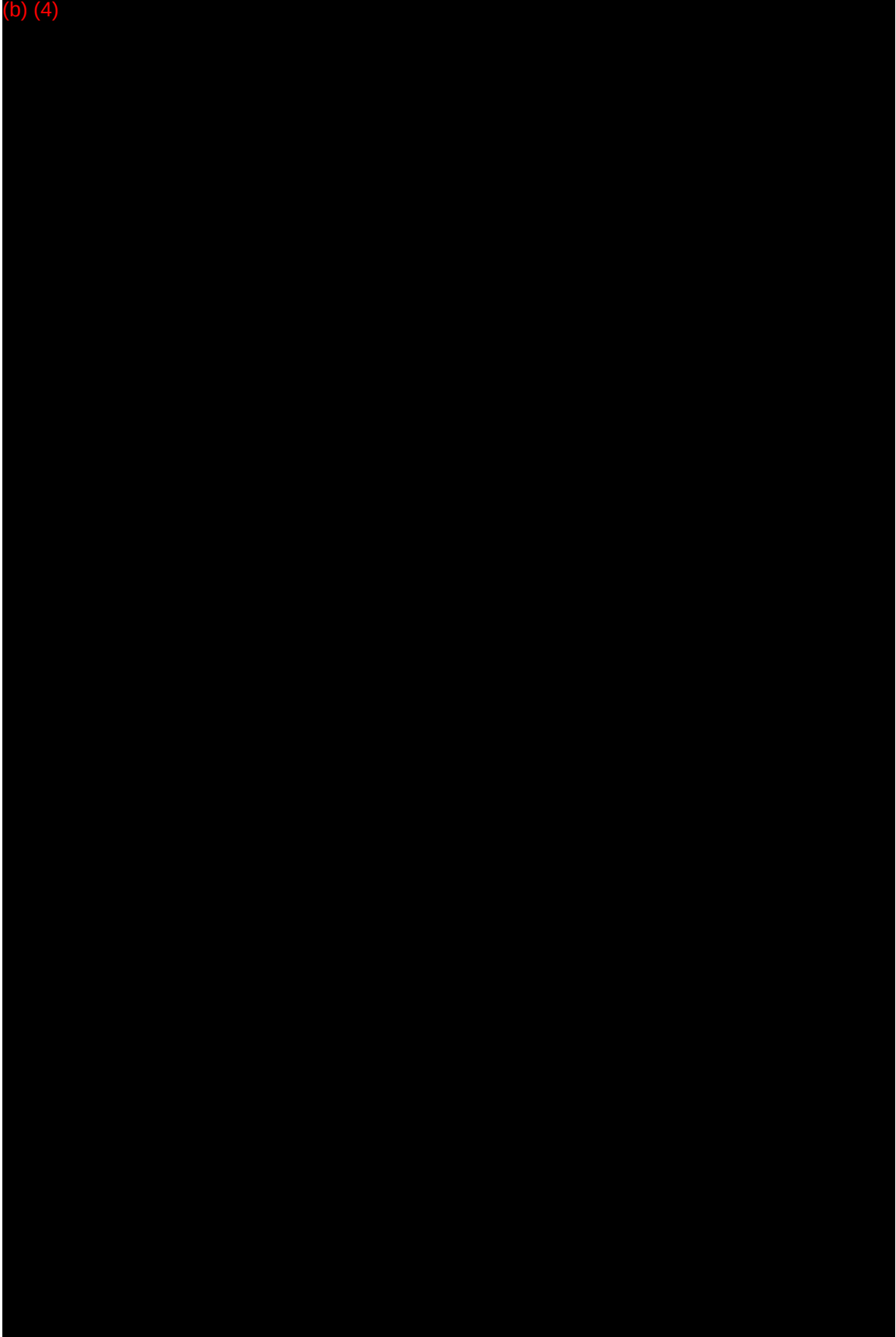
Appendix Table A1.2: Predicate Device, Straight Jaw, Bench Data

(b) (4)



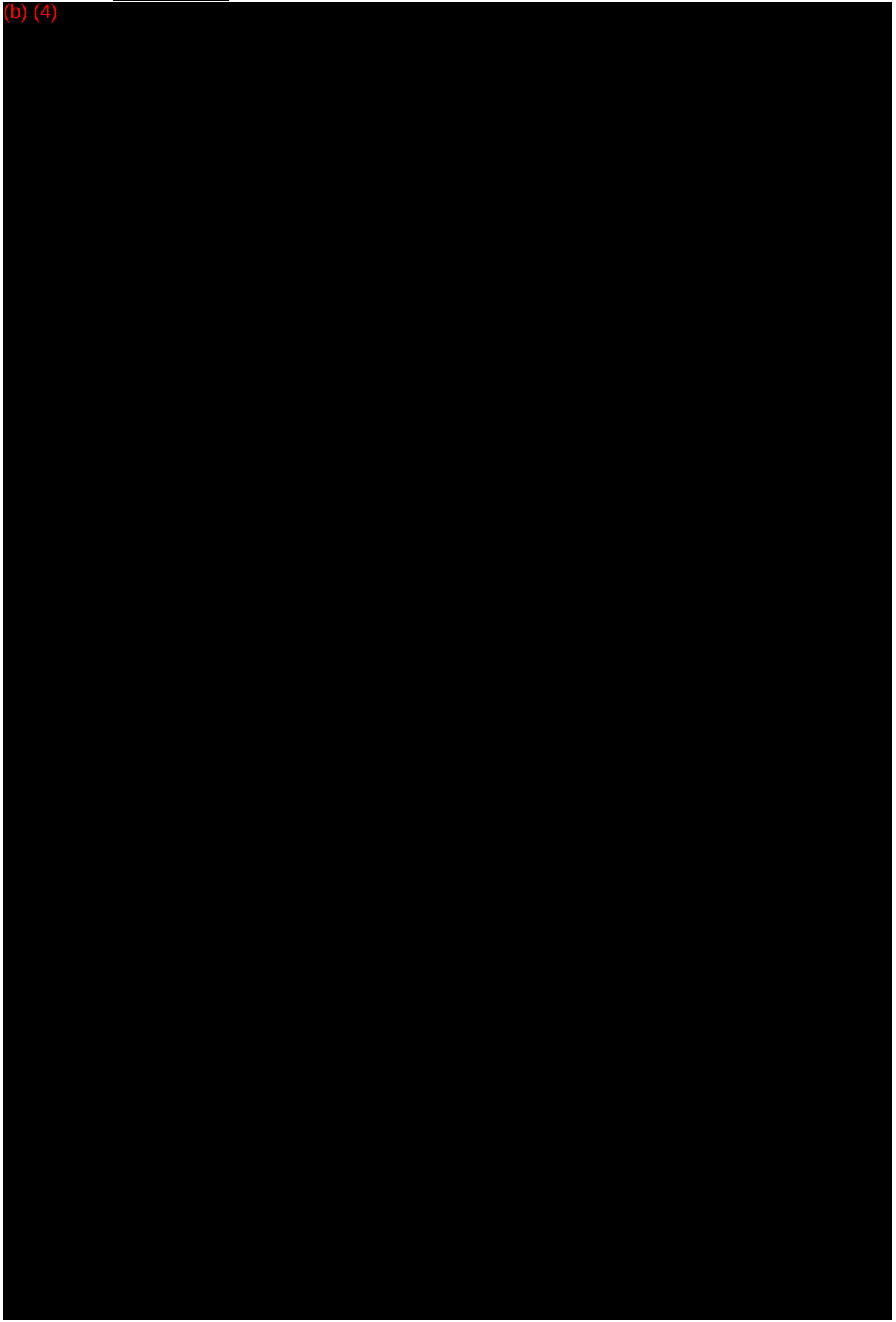
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(b) (4)



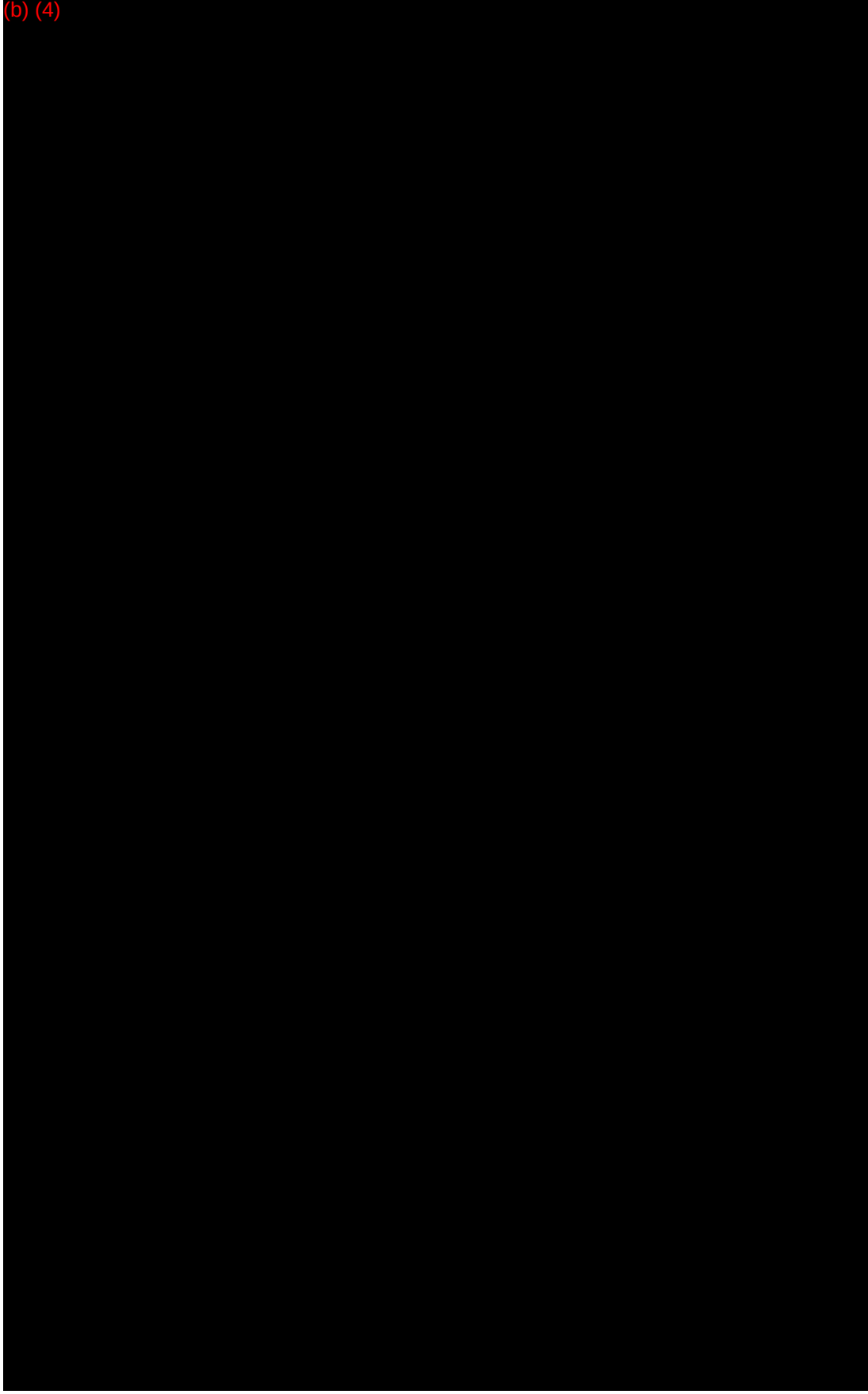
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(b) (4)



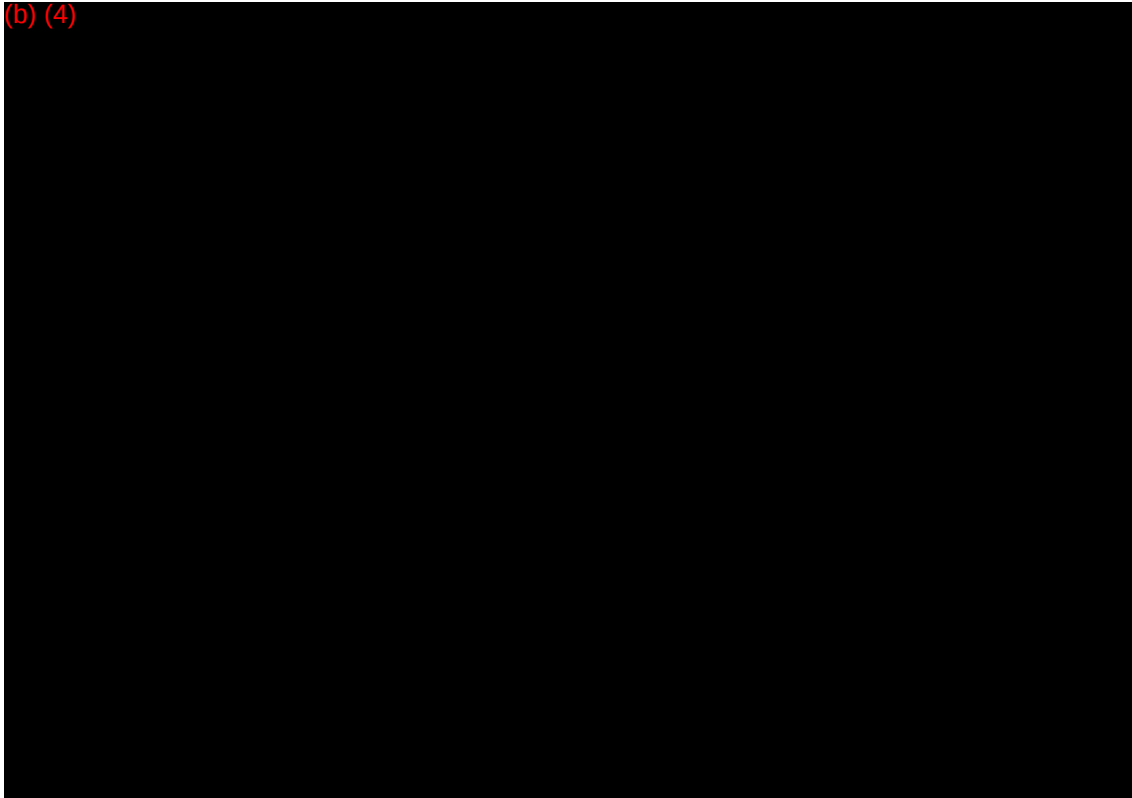
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(b) (4)



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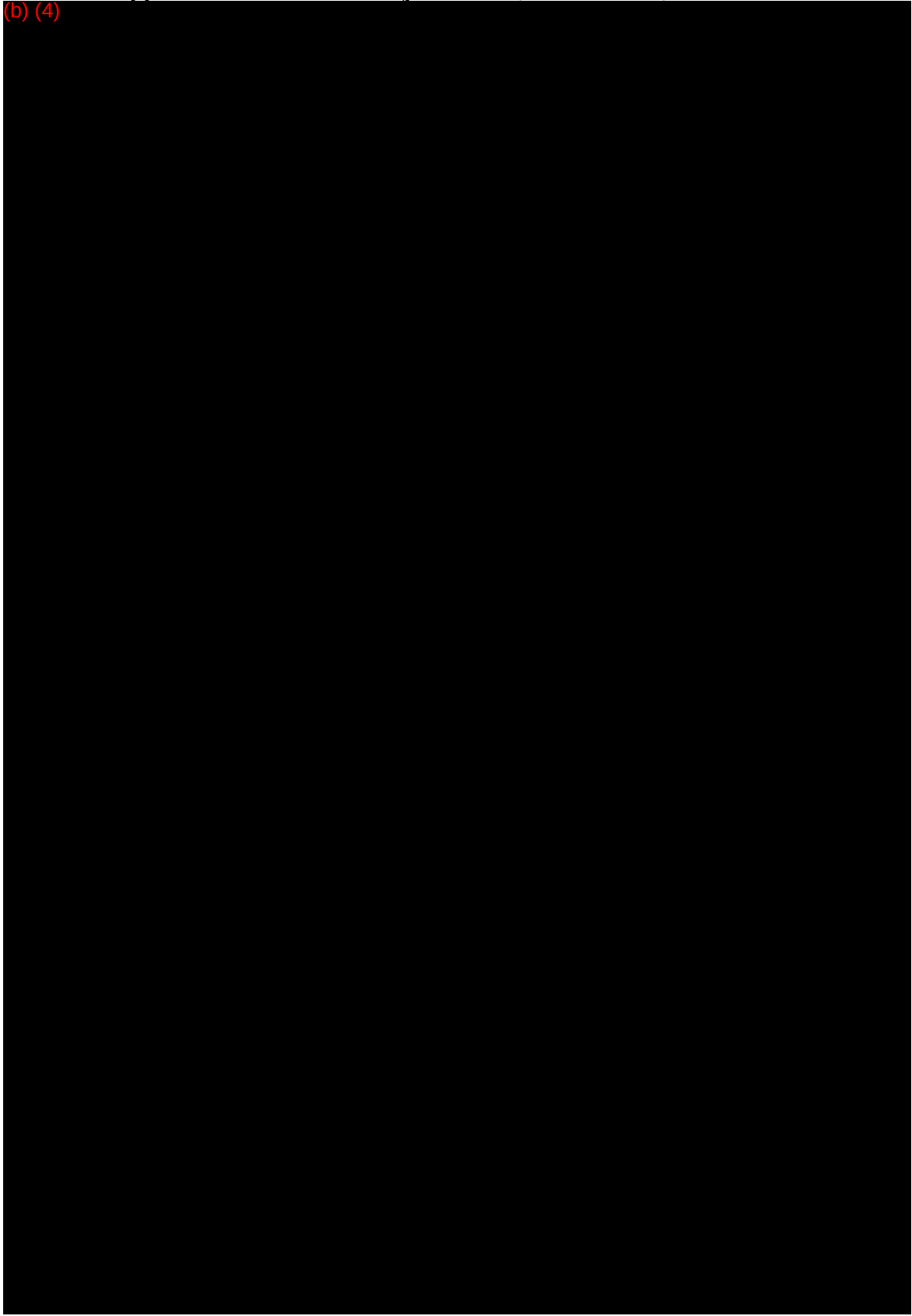
(b) (4)



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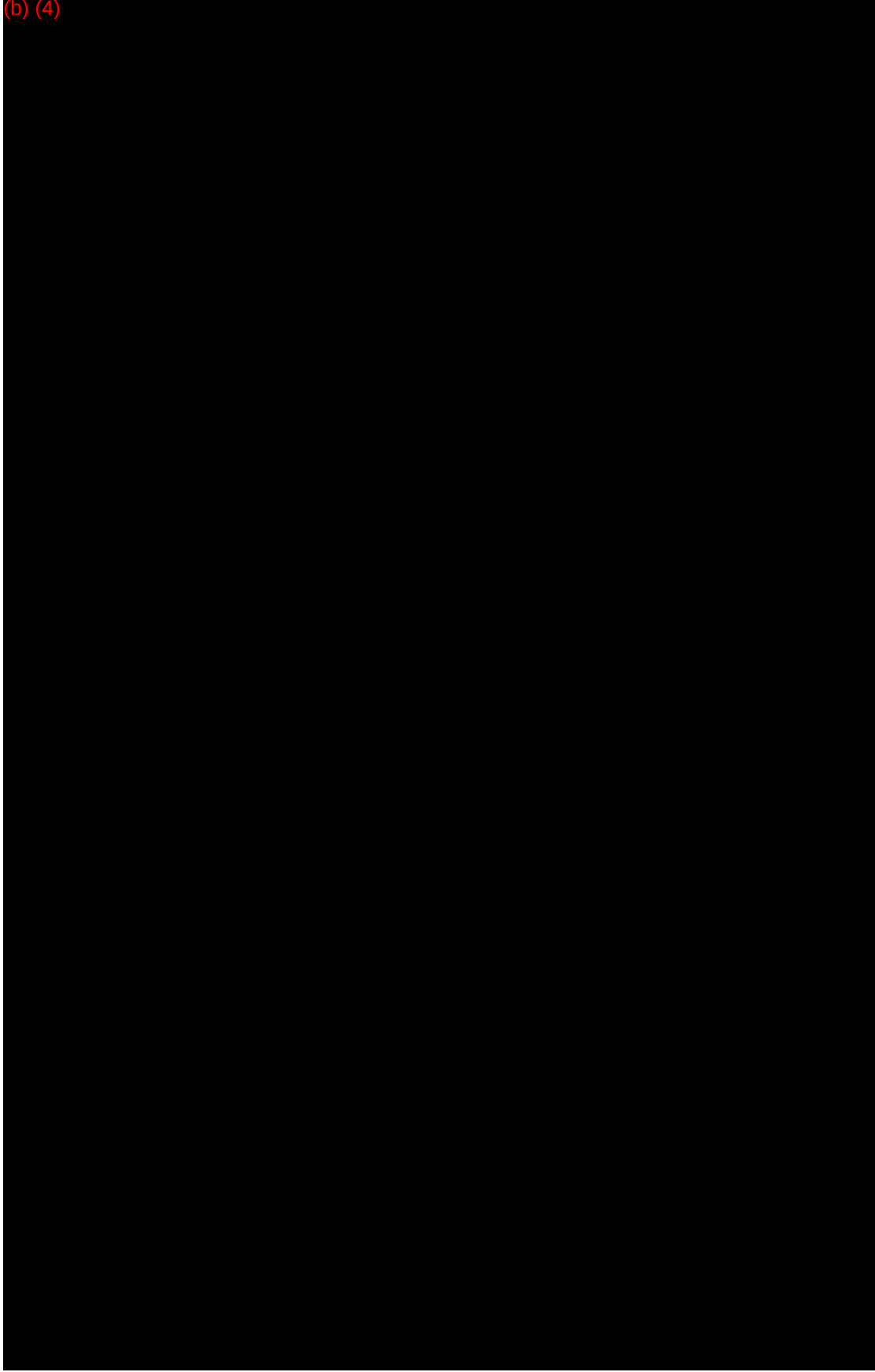
Appendix Table A1.3: Subject Device, Curved Jaw, Bench Data

(b) (4)



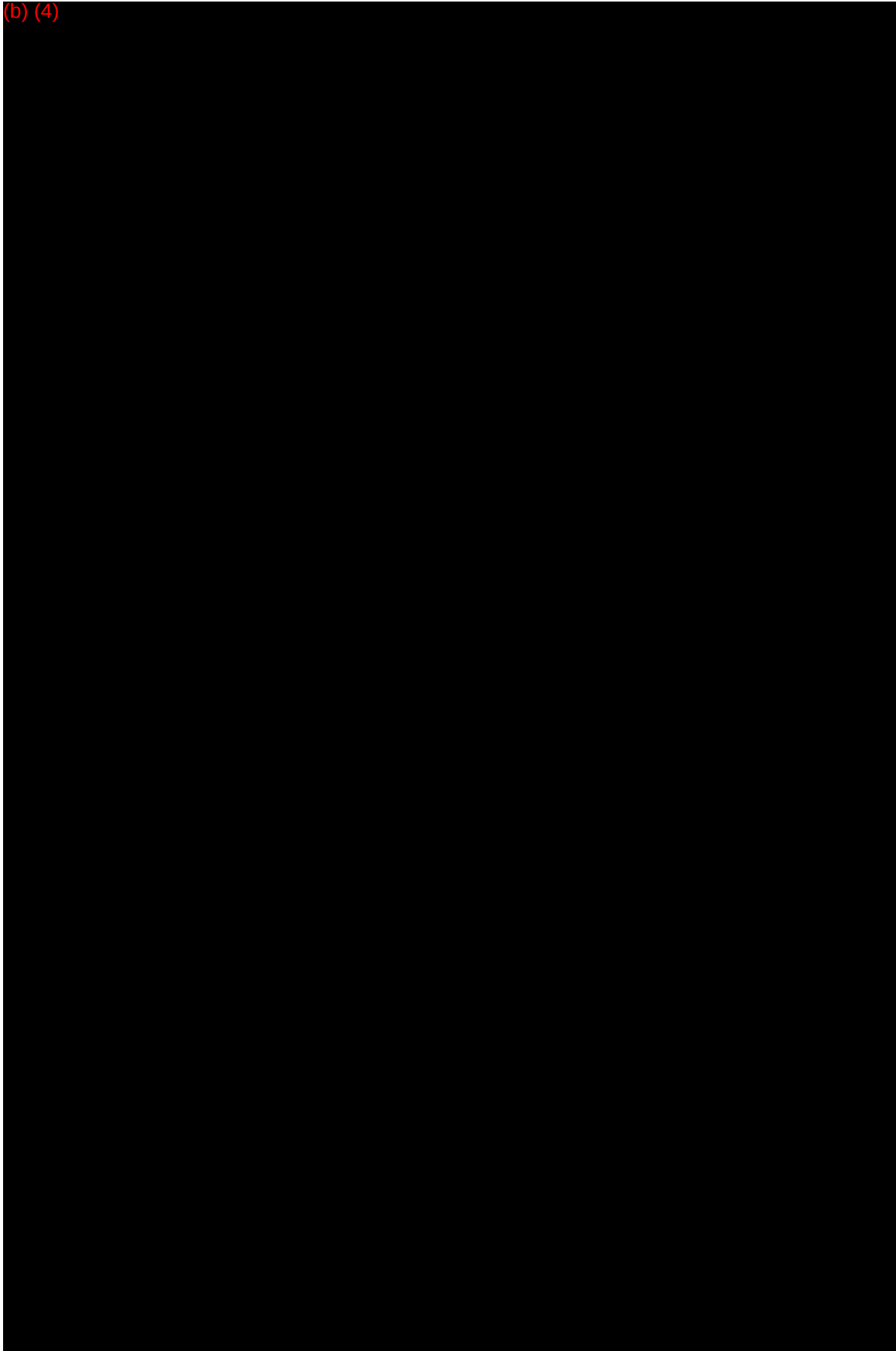
Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

(b) (4)



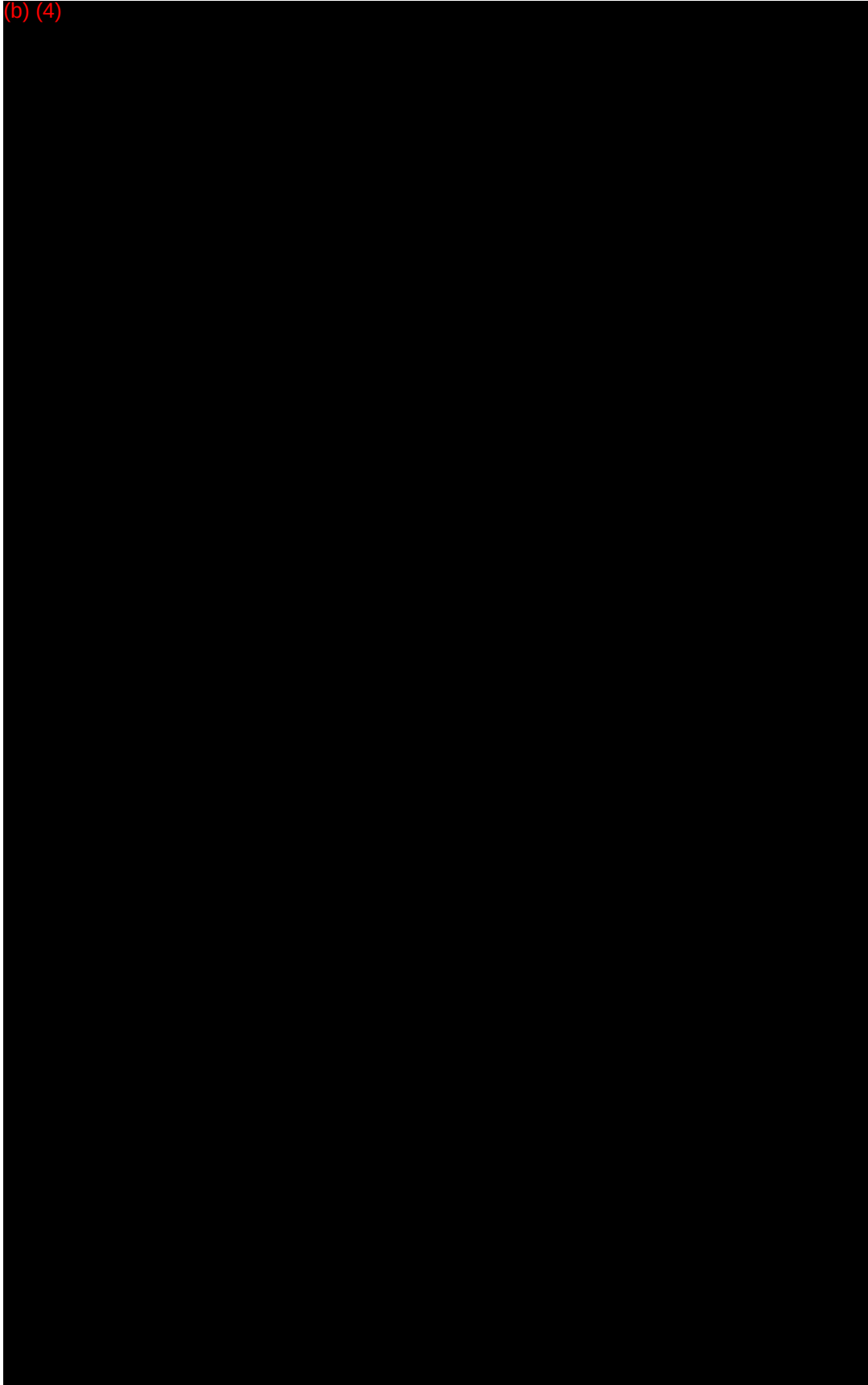
Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

(b) (4)



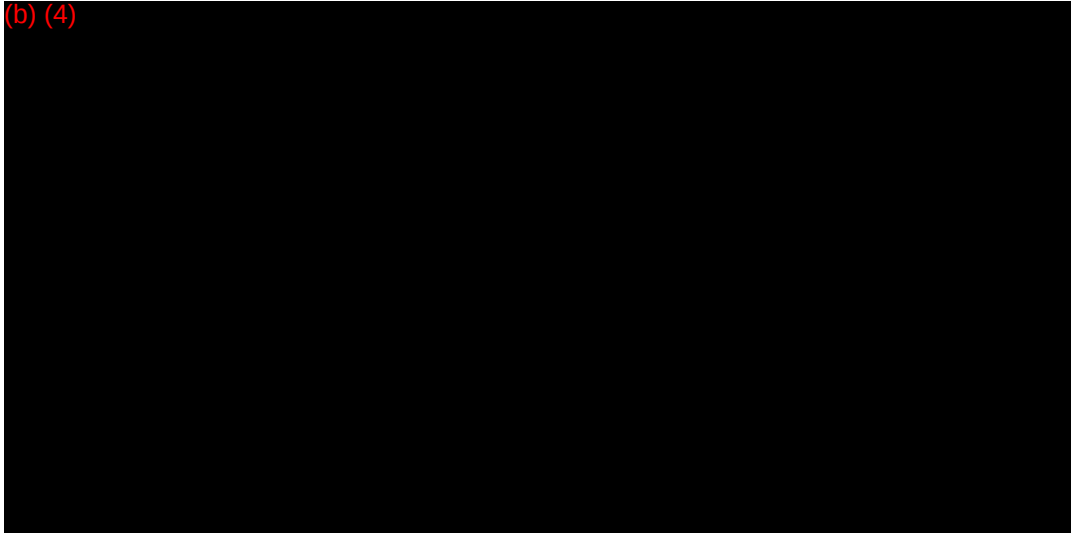
Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

(b) (4)



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

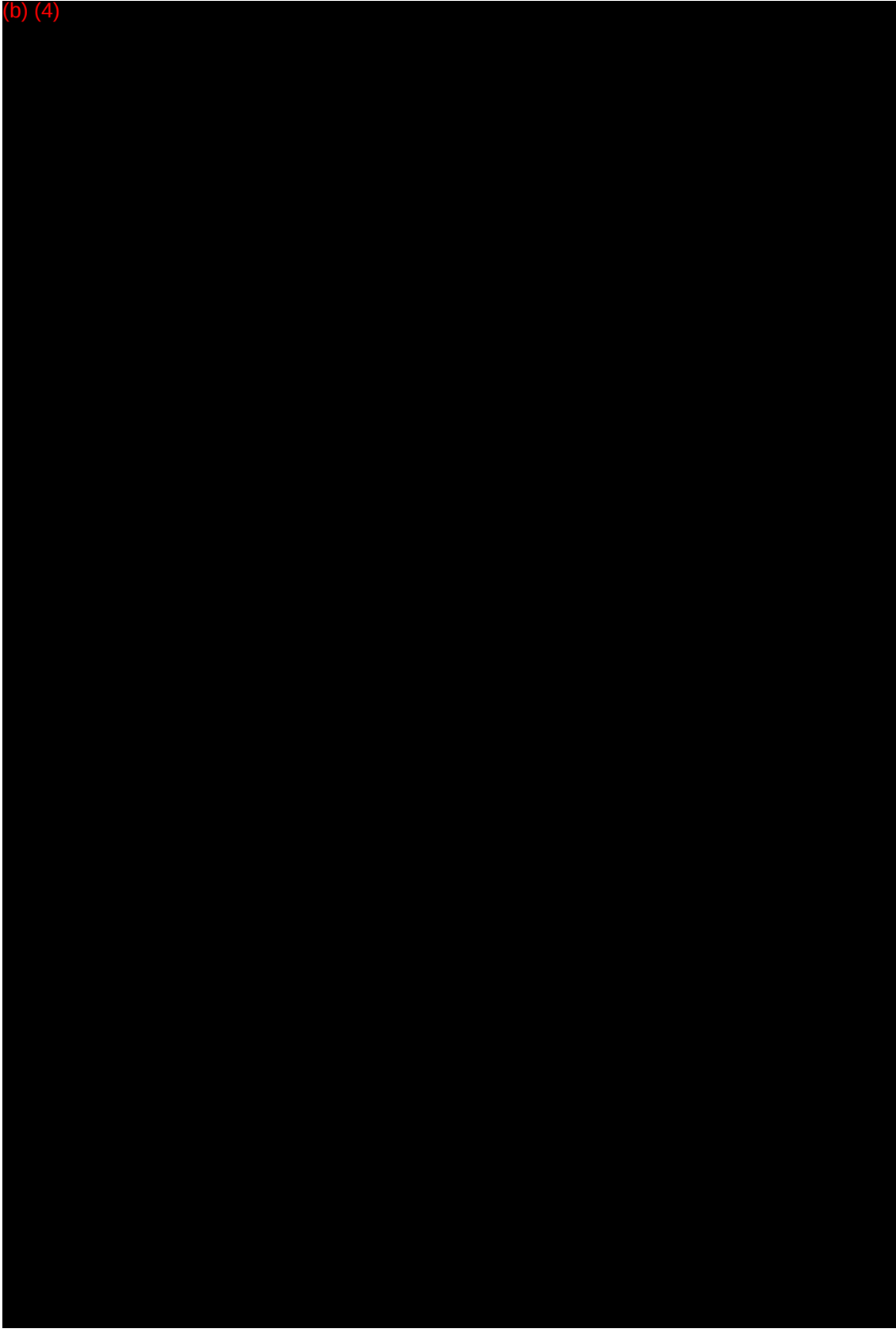
(b) (4)



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

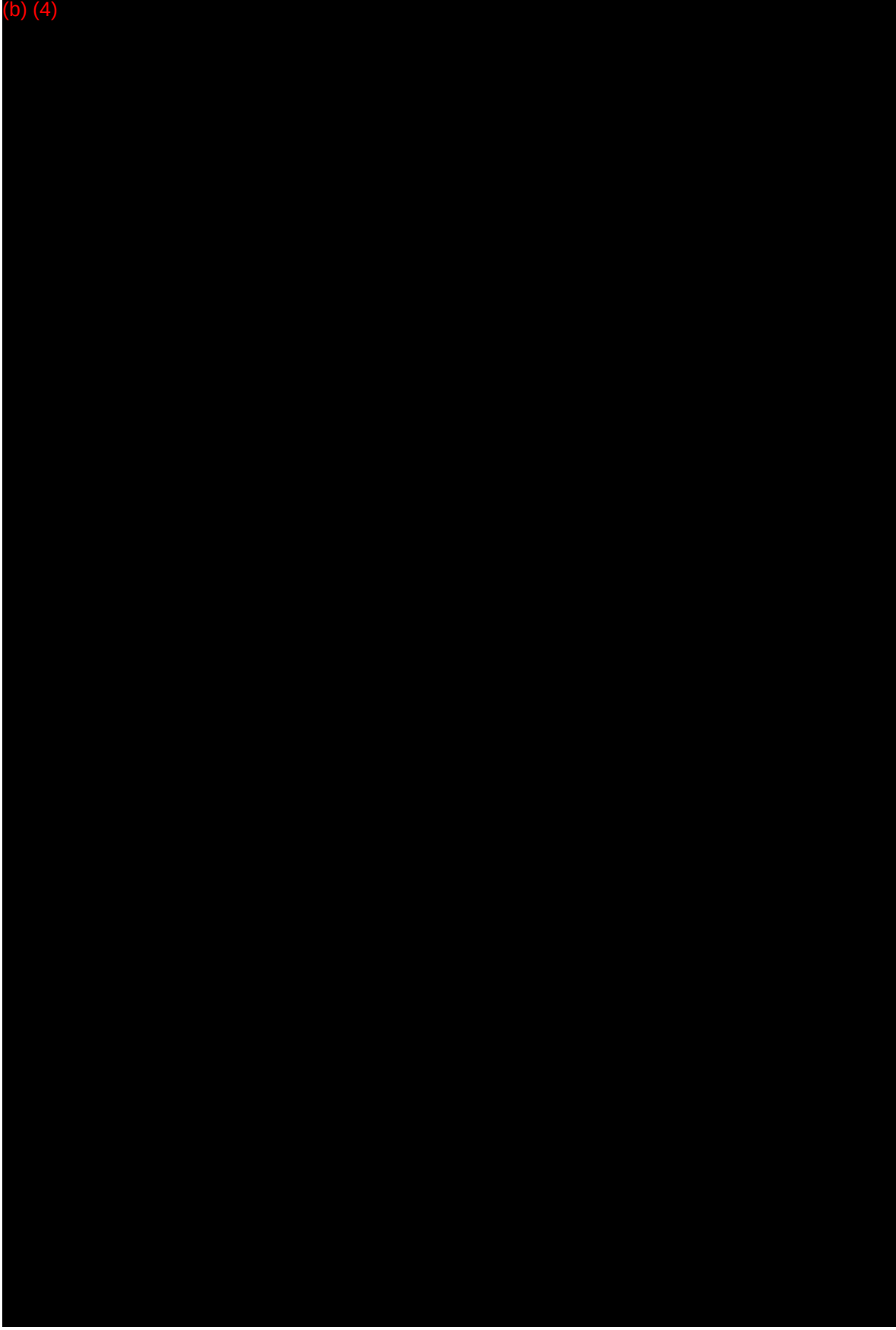
Appendix Table A1.4: Predicate Device, Curved Jaw, Bench Data

(b) (4)



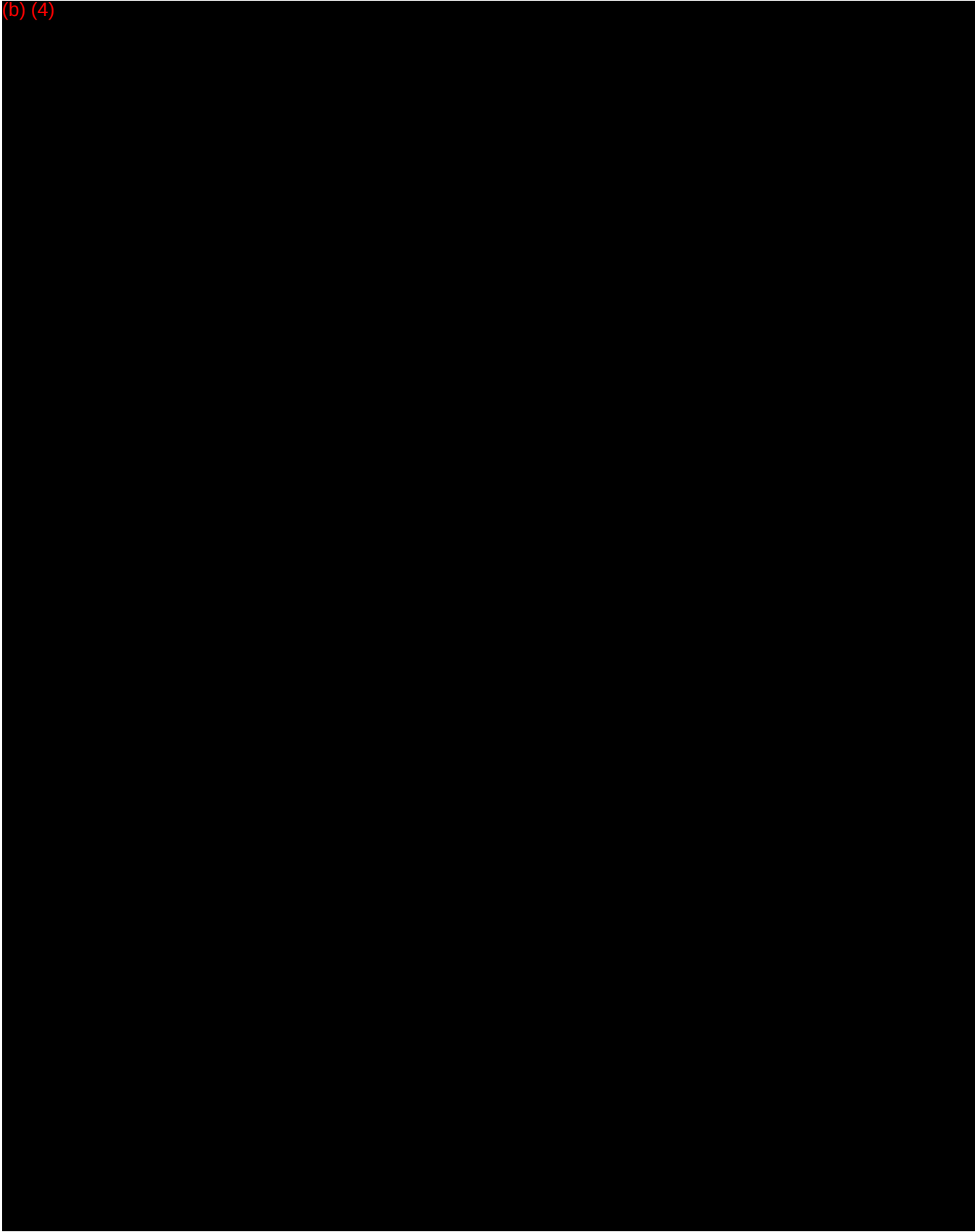
Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

(b) (4)



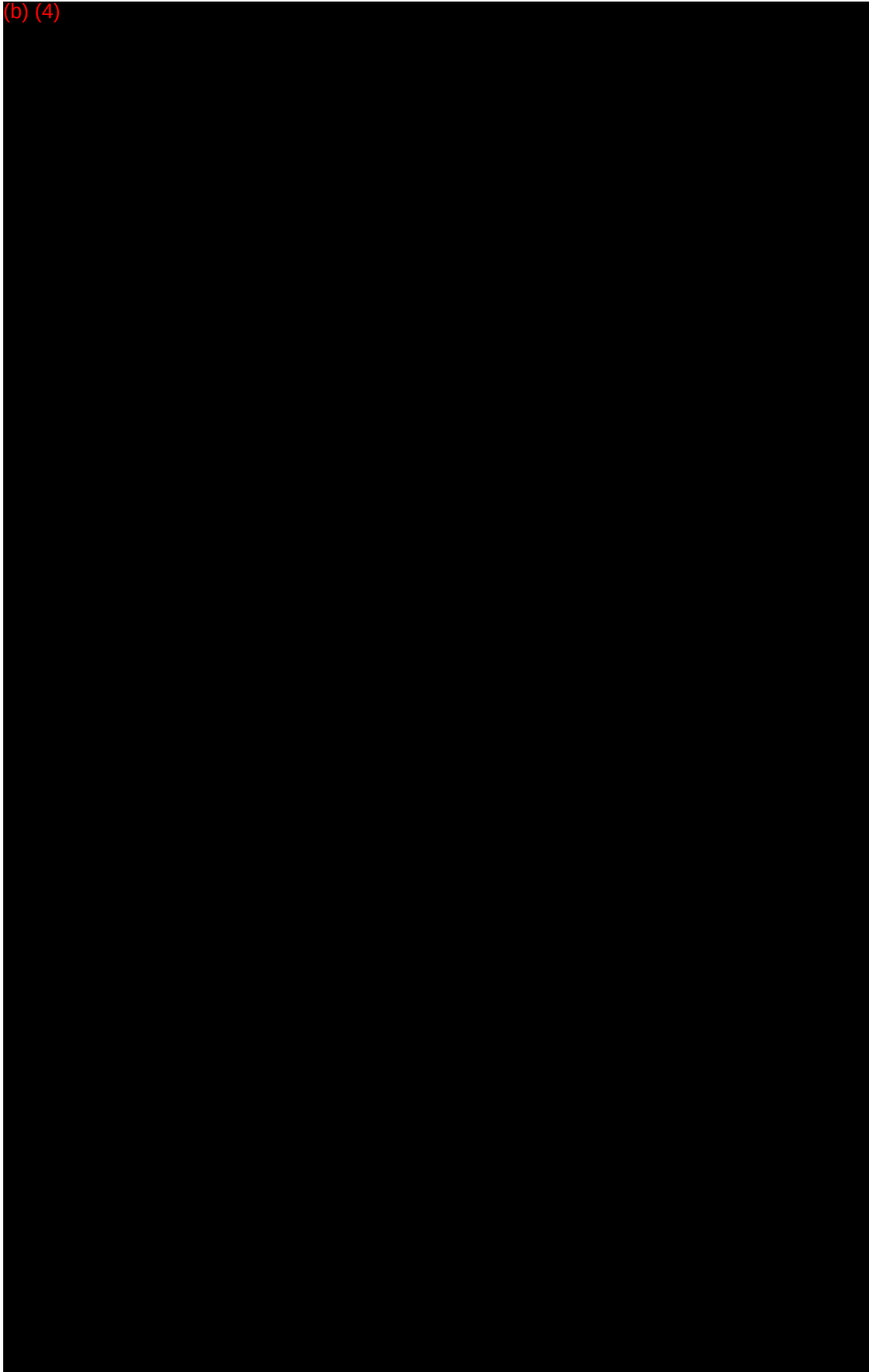
Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

(b) (4)



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

(b) (4)



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL[®] G2 Articulating Tissue Sealers

(b) (4)





COVER SHEET MEMORANDUM

From: Reviewer Name

Subject: 510(k) Number

To: The Record

Nosovskyy, Mykhaylo
K102797

Please list CTS decision code AI

- ☐ Refused to accept (Note: this is considered the first review cycle. See Screening Checklist
http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPreMarketNotification510kProgram/0_5631/Screening%20Checklist%207%202%2007.doc)
- ☒ Hold (Additional Information or Telephone Hold).
- ☐ Final Decision (SE, SE with Limitations, NSE (select code below), Withdrawn, etc.).

Not Substantially Equivalent (NSE) Codes

- | | |
|-----------------------------|---|
| ☐ NO | NSE for lack of predicate |
| ☐ NI | NSE for new intended use |
| ☐ NQ | NSE for new technology that raises new questions of safety and effectiveness |
| ☐ NU | NSE for new intended use AND new technology raising new questions of safety and effectiveness |
| ☐ NP | NSE for lack of performance data |
| ☐ NS | NSE no response |
| ☐ NL | NSE for lack of performance data AND no response |
| ☐ NM | NSE pre-amendment device call for PMAs (515i) |
| ☐ NC | NSE post-amendment device requires PMAs |
| ☐ NH | NSE for new molecular entity requires PMA |
| ☐ TR | NSE for transitional device |

Please complete the following for a final clearance decision (i.e., SE, SE with Limitations, etc.):		YES	NO
Indications for Use Page	Attach IFU		
510(k) Summary /510(k) Statement	Attach Summary		
Truthful and Accurate Statement.	Must be present for a Final Decision		
Is the device Class III?			
If yes, does firm include Class III Summary?	Must be present for a Final Decision		
Does firm reference standards? (If yes, please attach form from http://www.fda.gov/opacom/morechoices/fdaforms/FDA-3654.pdf)			
Is this a combination product? (Please specify category _____, see http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPreMarketNotification510kProgram/0_413b/COMBINATION%20PRODUCT%20ALGORITHM%20(REVISED%203-12-03).DOC)			
Is this a reprocessed single use device? (Guidance for Industry and FDA Staff – MDUFMA - Validation Data in 510(k)s for Reprocessed Single-Use Medical Devices, http://www.fda.gov/cdrh/ode/guidance/1216.html)			
Is this device intended for pediatric use only?			
Is this a prescription device? (If both prescription & OTC, check both boxes.)			
Did the application include a completed FORM FDA 3674, Certification with Requirements of ClinicalTrials.gov Data Bank?			
Is clinical data necessary to support the review of this 510(k)?			
For United States-based clinical studies only: Did the application include a completed FORM FDA 3674, Certification with Requirements of ClinicalTrials.gov Data Bank? (If study was			

conducted in the United States, and FORM FDA 3674 was not included or incomplete, then applicant must be contacted to obtain completed form.)

Does this device include an Animal Tissue Source?

All Pediatric Patients age ≤ 21

Neonate/Newborn (Birth to 28 days)

Infant (29 days - < 2 years old)

Child (2 years - < 12 years old)

Adolescent (12 years - < 18 years old)

Transitional Adolescent A (18 - < 21 years old) Special considerations are being given to this group, different from adults age ≥ 21 (different device design or testing, different protocol procedures, etc.)

Transitional Adolescent B (18 - ≤ 21 ; No special considerations compared to adults $\Rightarrow$ 21 years old)

Nanotechnology

Is this device subject to the Tracking Regulation? (Medical Device Tracking Guidance, <http://www.fda.gov/cdrh/comp/guidance/169.html>)

Contact OC.

Regulation Number

Class*

Product Code

21 CFR 878.4400

II

GEI

(*If unclassified, see 510(k) Staff)

Additional Product Codes:

HGI

Review:

[Signature]
(Branch Chief)

for NRO

GSD8

(Branch Code)

10/31/12

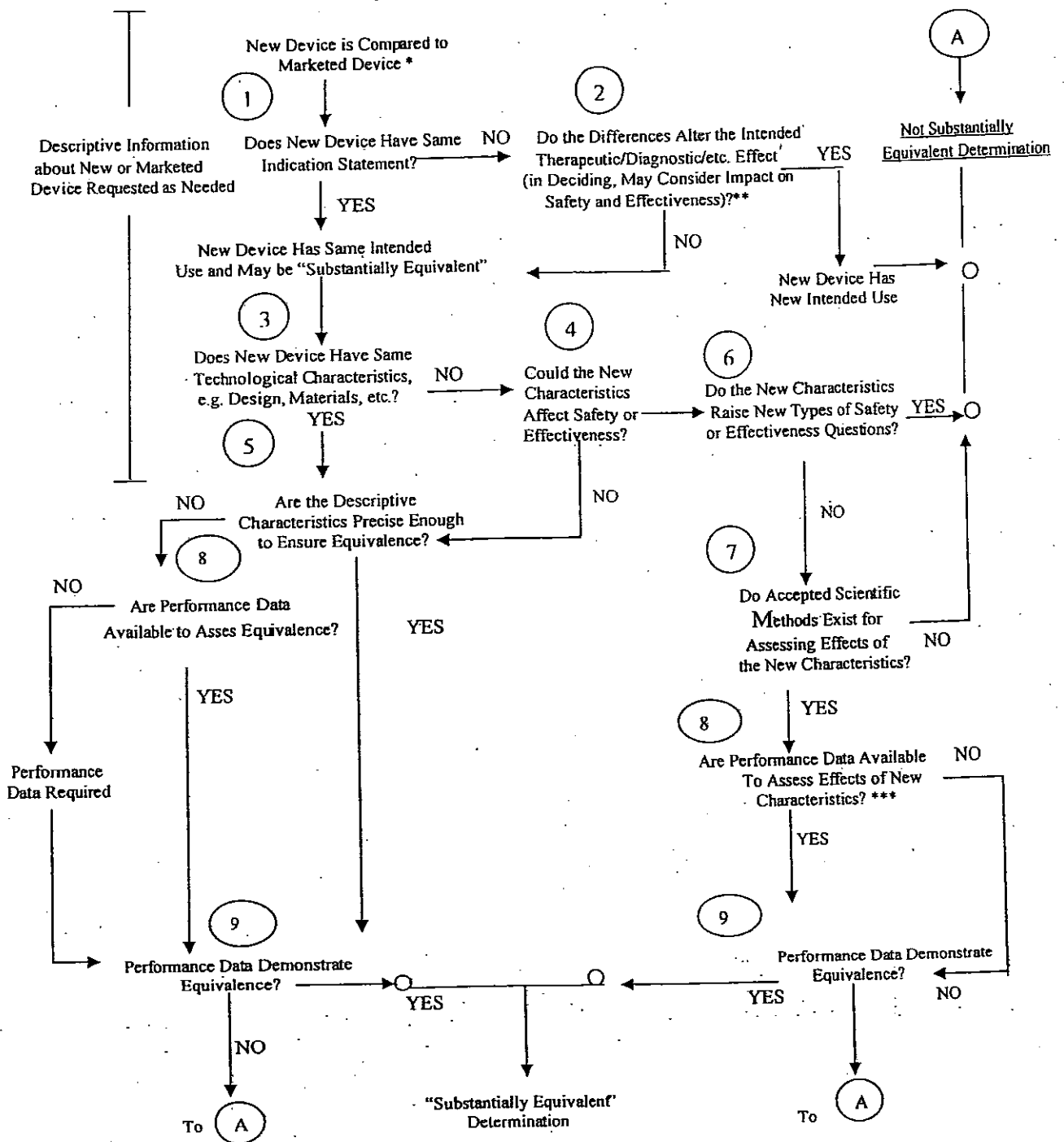
(Date)

Final Review:

(Division Director)

(Date)

510(k) "SUBSTANTIAL EQUIVALENCE" DECISION-MAKING PROCESS



* 510(k) Submissions compare new devices to marketed devices. FDA requests additional information if the relationship between marketed and "predicate" (pre-Amendments or reclassified post-Amendments) devices is unclear.

** This decision is normally based on descriptive information alone, but limited testing information is sometimes required.

*** Data maybe in the 510(k), other 510(k)s, the Center's classification files, or the literature.



DEPARTMENT OF HEALTH AND HUMAN SERVICES

MEMORANDUM

Food and Drug Administration
Office of Device Evaluation
10903 New Hampshire Ave.
Silver Spring, Maryland 20993

**Premarket Notification, [510(k)] Review
Traditional/Abbreviated
K122797**

Date: October 31, 2012
To: The Record
From: Mykhaylo B. Nosovsky
Reviewer (Mechanical Engineer)

Office: ODE
Division: DSORD

510(k) Holder: Ethicon Endo-Surgery, LLC
475 Calle C
Guaynabo, PR 00969
(513) 337-1546
(513) 337-2802

Device Name: ENSEAL® G2 Articulating Tissue Sealers
Contact: Emily Kruetzkamp
4545 Creek Road
Cincinnati, OH 45242
Phone: (513) 337-1546
Fax: (513) 337-2802
E-mail: ekruetzk@its.jnj.com

RECOMMENDATION: AI

I. Purpose and Submission Summary

The 510(k) holder would like to introduce ENSEAL® G2 Articulating Tissue Sealers into interstate commerce. 510(k) submission for ENSEAL® G2 Articulating Tissue Sealers (K122797) includes description of 4 models: straight jaw (NSLG2S35A, NSLG2S45A) and curved jaw (NSLG2C35A, NSLG2C45A). The firm is requesting same indications for use as were granted for Predicate Device (K112033). The firm wants clearance for Prescription Use.

There are a number of deficiencies in the original submission. Please refer to the section XV ('Deficiency') of this memo for more detail. Therefore, the sponsor is being requested additional information and this submission is **placed telephone hold for the 1st time** until the requested information is provided.

II. Administrative Requirements

	Yes	No	N/A
Indications for Use page (Indicate if: Prescription or OTC)	X		
Truthful and Accuracy Statement	X		
510(k) Summary or 510(k) Statement			
Standards Form	X		

510(k) SUMMARY REQUIREMENTS CHECKLIST

All 510(k) summaries shall contain the following information:		Y	N	N/A
1	The submitter's name, address, telephone number, a contact person, and the date the summary was prepared	X		
2	The name of the device, including the trade or proprietary name if applicable, the common or usual name, and the classification name	X		
3	An identification of the legally marketed device(s) to which the submitter claims equivalence.	X		
4	A description of the device that is the subject of the 510(k), including an explanation of how the device functions, the scientific concepts that form the basis for the device, and the significant physical and performance characteristics of the device (e.g., device design, material used, and physical properties)	X		
5	A statement of the indications for use of the device that is the subject of the 510(k), including a general description of the diseases or conditions that the device will diagnose, treat, prevent, cure, or mitigate, including a description, where appropriate, of the patient population for which the device is indicated. Or, if the indication statements are different from those of the legally marketed device(s) identified in paragraph (3) of this section, an explanation as to why the differences are not critical to the intended therapeutic, diagnostic, prosthetic, surgical or other use of the device, and why the differences do not affect the safety and effectiveness of the device when used as indicated.	X		
6	If the device has the same technological characteristics (i.e., design, material, chemical composition, energy source, etc.) as the predicate device(s) identified in paragraph(3) of this section, a summary of the technological characteristics of the new device in comparison to those of the predicate device(s). Or, if the device has different technological characteristics from the predicate device(s), a summary of how the technological characteristics of the device compare to a legally marketed device(s) identified in paragraph (3) of this section.	X		
510(k) summaries for those 510(k)s in which a determination of substantial equivalence is also based on an assessment of performance data shall contain the following information				
7	A brief discussion of the nonclinical tests submitted, referenced, or relied on in the 510(k) for a determination of substantial equivalence		X	
8	A summary discussion of the clinical tests submitted, referenced, or relied on in the 510(k) for a determination of substantial equivalence. This discussion shall include, where applicable, a description of the subjects upon whom the device was tested, a discussion of the safety or effectiveness data obtained from the testing, with specific reference to adverse effects and complications, and any other information from the clinical testing relevant to a determination of substantial equivalence. (There can not be any patient identifier information in the summary.)			X
9	The conclusions drawn from the nonclinical and clinical tests that demonstrate that the device is as safe, as effective, and performs at least as safely and effectively as the legally marketed device identified in paragraph(3) of this section.	X		

In section 21 (pages 160-195) sponsor provided Standards Data Report (Form FDA 3654) for:

- AAMI/ANSI/ISO 10993-1:2009, Biological evaluation of medical devices -- Part 1: Evaluation and testing within a risk management process.
- AAMI/ANSI/ISO 11137-1: 2006 Sterilization of Health Care Products - Radiation – Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices
- AAMI/ANSI/ISO 11137-2: 2006 Sterilization of Health Care Products - Radiation – Part 2: Establishing the sterilization dose
- AAMI/ANS/ISO 11137-3:2006 Sterilization of Health Care Products – Radiation – Part 3: Guidance on Dosimetric Aspects
- IEC 60601-1, Medical Electrical Equipment - Part 1: General Requirements for Safety, 1988; Amendment 1, 1991-11, Amendment 2, 1995.
- AAMI / ANSI / IEC 60601-1-2:2007, Medical electrical equipment - Part 1-2: General requirements for basic safety and essential performance - Collateral standard: Electromagnetic compatibility - Requirements and tests (Edition 3).
- AAMI / ANSI / IEC 60601-2-2:2009, Medical electrical equipment - Part 2-2: Particular requirements for the basic safety and essential performance of high frequency surgery equipment and high frequency surgical accessories.

There is the following deficiency.

You provided 510(k) Summary with the conclusions drawn from the nonclinical and clinical tests that demonstrate that the device is as safe, as effective, and performs at least as safely and effectively as the legally marketed (predicate) device. However, a brief discussion of the nonclinical tests submitted, referenced, or relied on in the 510(k) for a determination of substantial equivalence is missing. Please revise 510(k) Summary to include a brief discussion of the nonclinical tests submitted, referenced, or relied on in the 510(k) for a determination of substantial equivalence.

III. Device Description

	Yes	No	N/A
Is the device life-supporting or life sustaining?		X	
Is the device an implant (implanted longer than 30 days)?		X	
Does the device design use software?	X		
Is the device sterile?	X		
Is the device reusable (not reprocessed single use)?		X	
Are "cleaning" instructions included for the end user?			X

Subject devices are sterile, single use surgical instruments designed to seal and cut vessels up to and including 7mm in diameter, and to cut, grasp and dissect soft tissue during open and laparoscopic surgery. Coagulation is achieved with bipolar electrosurgical elements and an articulated top jaw that supplies compression to targeted tissue. Uniform compression of the targeted tissue is achieved by the I-Blade® knife that forces the jaws together when it is

advanced to transect the tissue in conjunction with the application of bipolar electrosurgical energy.

As in the Predicate device, the energy activation and knife lock release are enabled by a single button. The instrument shaft can be rotated using the rotation knob to facilitate visualization and enable easy access to targeted tissue.

The instrument shaft can be articulated using the articulation wheel at angles up to 60°. The device articulates at approximately 60° to the left and the right of center. The top of the articulation wheel has a white arrow to indicate when the device shaft is straight and not articulated. When the articulation wheel is rotated clockwise, the shaft articulates to the right; when the articulation wheel is rotated counterclockwise, the shaft articulates to the left. See Figures 1 and 2.

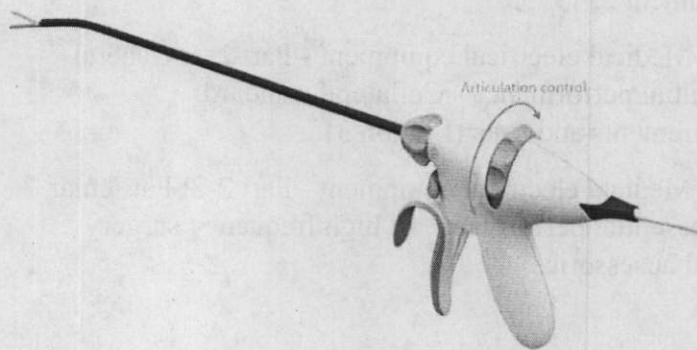


Figure 1.

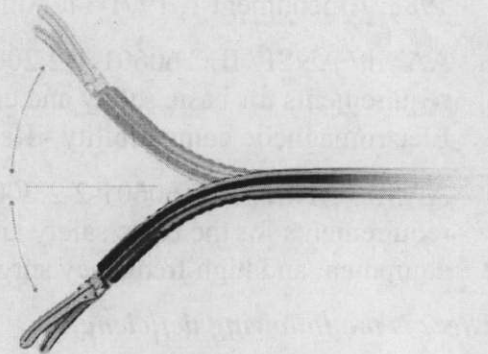


Figure 2.

Whether straight or articulated, the device jaws seal and cut vessels, and cut, grasp and dissect soft tissue as intended. The sponsor states that the Subject ENSEAL G2 Articulating Tissue Sealers performance is equivalent to the Predicate ENSEAL G2 Tissue Sealers. Due to the new articulation feature, there are new patient-contacting materials on the Subject device compared to the Predicate.

The power cord is permanently attached to the device and connects the instrument to the generator. The ENSEAL G2 Articulating Tissue Sealers are designed to be used exclusively with the Generator GEN11 (K101990), which is supplied separately.

The sponsor provided the list of Product Codes in table 11.1 (page 33) which is replicated below.

Subject Device Product Code	Subject Device Name	Predicate Device Product Code	Predicate Device Name
NSLG2S35A	ENSEAL G2 Articulating Tissue Sealer, 35 cm Length Straight Jaw	NSLG2S35	ENSEAL G2 Tissue Sealer, 35 cm Length Straight Jaw
NSLG2C35A	ENSEAL G2 Articulating Tissue Sealer, 35 cm Length Curved Jaw	NSLG2C35	ENSEAL G2 Tissue Sealer, 35 cm Length Curved Jaw
NSLG2S45A	ENSEAL G2 Articulating Tissue Sealer, 45 cm Length Straight Jaw	NSLG2S45	ENSEAL G2 Tissue Sealer, 45 cm Length Straight Jaw
NSLG2C45A	ENSEAL G2 Articulating Tissue Sealer, 45 cm Length Curved Jaw	NSLG2C45	ENSEAL G2 Tissue Sealer, 45 cm Length Curved Jaw

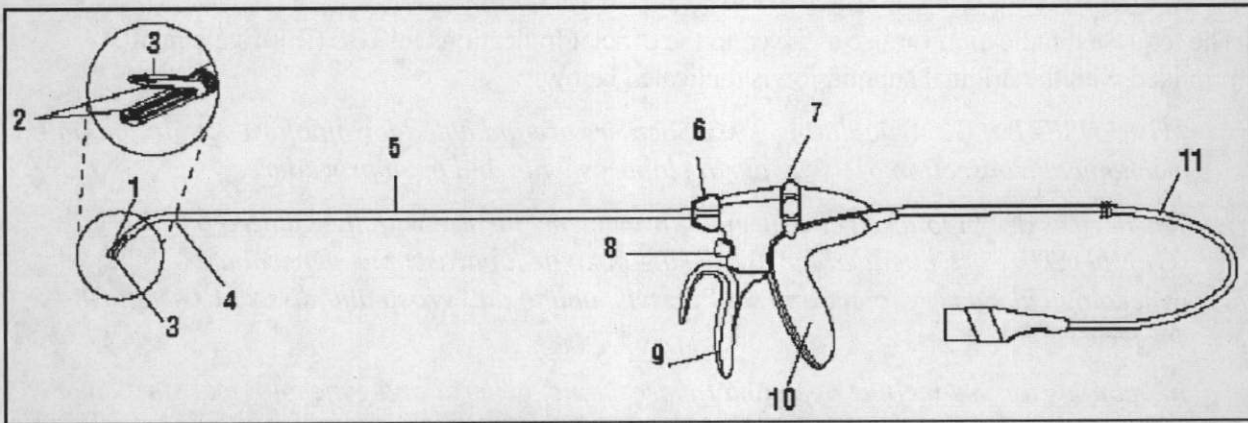


Figure 3 Subject Device Parts

- | | |
|--------------------------------|-----------------------------|
| 1. Jaws (curved or straight) | 7. Articulation wheel |
| 2. Atraumatic teeth | 8. Energy activation button |
| 3. I-BLADE knife (not visible) | 9. Closing handle |
| 4. Articulation joint | 10. Grip housing |
| 5. Shaft | 11. Power cord |
| 6. Rotation knob | |

The sponsor provided device dimensions in table 11.2 (page 34) which is replicated below.

	Subject Device ENSEAL G2 Articulating Tissue Sealers	Subject Device ENSEAL G2 Articulating Tissue Sealers	Predicate Device ENSEAL G2 Tissue Sealers	Predicate Device ENSEAL G2 Tissue Sealers
	Curved	Straight	Curved	Straight

(b) (4)

The sponsor included Assembly Drawings that illustrate the device modifications for the ENSEAL G2 Articulating Tissue Sealers (on pages 39-42).

This was found adequate.

IV. Indications for Use

The requested indication for use as given in the official Indications for Use (IFU) statement provided with the original submission is replicated below:

"The ENSEAL® G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with the Generator GEN11 (GEN11). They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7mm and tissue and/or vascular bundles as large as will fit in the jaws of the instruments.

The ENSEAL® G2 Articulating Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures."

On the bottom of the Indications for Use statement the firm has selected it for **Prescription Use**.

The requested IFU is nearly identical to the one replicated below, cleared for predicate device (K112033):

"The ENSEAL® G2 Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with an electrosurgical generator. They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7 mm and bundles as large as will fit in the jaws of the instruments.

The ENSEAL® G2 Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures."

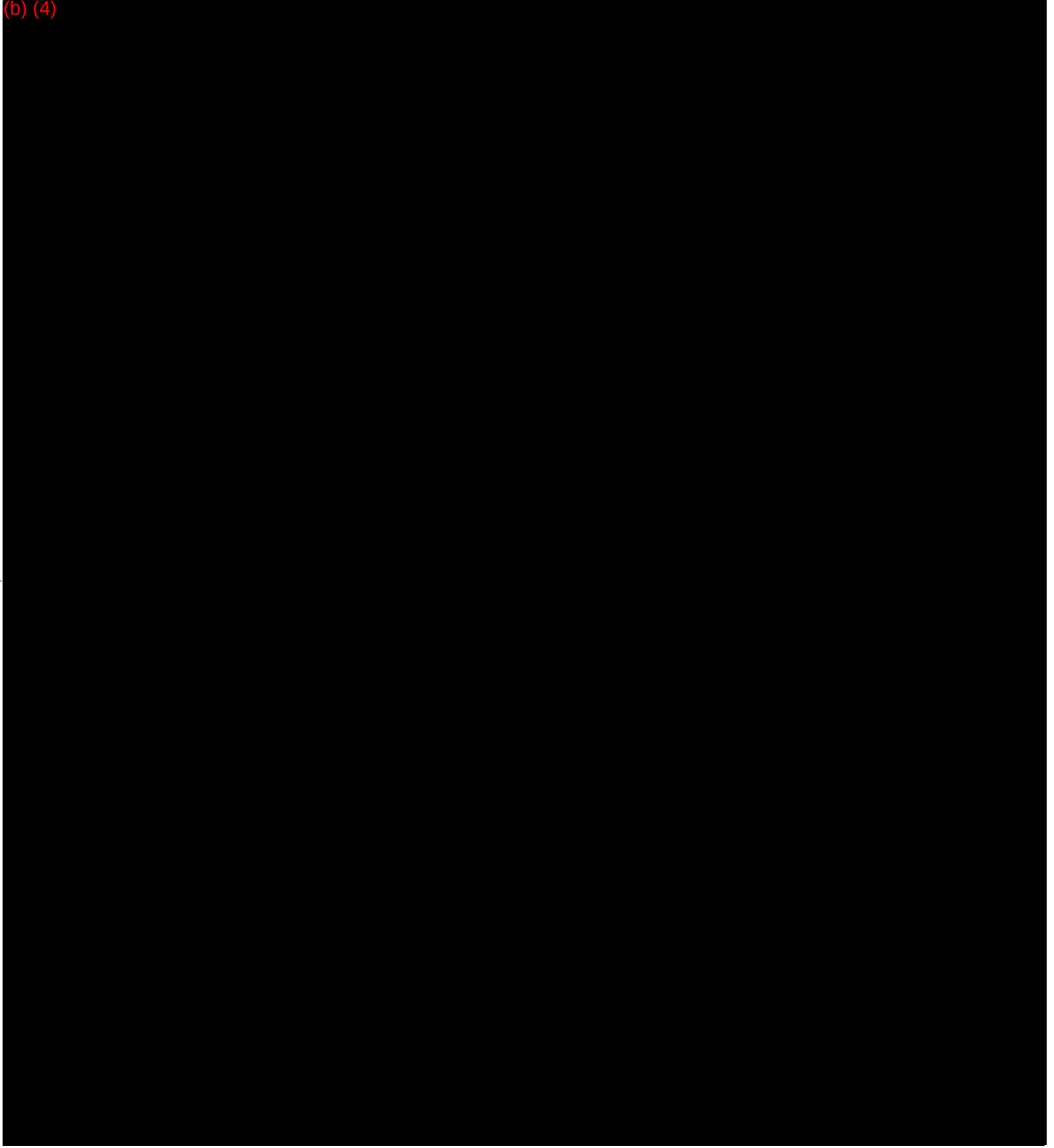
The proposed IFU is nearly identical to that of the K112033 predicate IFU. The addition of "Generator GEN11 (GEN11)" and "tissue and/or vascular bundles" does not raise any new safety and effectiveness questions. The sponsor explains that those statements were added for clarity. This was found adequate.

V. Predicate Device Comparison

The sponsor identified ENSEAL® G2 Tissue Sealers (cleared in K112033) as predicate.

The sponsor provided a comparison table (Table 12.4, pages 50-51) which is replicated below.

(b) (4)



Shelf Life	1 yr	2 yr
Device Materials	New materials in conjunction with articulation feature	Not present

This was found adequate.

VI. Labeling

In Section 13, the sponsor provided following draft labeling materials:

- Package Insert (includes Instructions for Use)
- Primary Labels (for both the curved and straight models)
- Carton Labels (for both the curved and straight models)

The changes to the Package Insert text to compare to the Predicate Device are listed in Table 12.3 (pages 46-49).

(b) (4)

VII. Sterilization/Shelf Life/Reuse

1. Sterilant:	YES	NO
a. Sterilization method description (e.g., Steam (moist heat), EO, Radiation):	Cobalt 60 Irradiation	
b. Dose, for radiation (e.g., 25 – 50 kGy):		
c. Sterilant residuals remaining on the device: For EO, the maximum levels of residuals of EO and ethylene chlorhydrin that remain on the device (note: not to include ethylene glycol residual level because the standards that have been, or currently are recognized, "ANSI/AAMI/ISO 10993-7:1995 and 2008 <i>Biological Evaluation of Medical Devices – Part 7: Ethylene Oxide sterilization residuals</i> ," do not include measurement of ethylene glycol residuals);	N/A	
2. A description of the Validation Method for the sterilization cycle (not data):	ISO 11137-1:2006 – Sterilization of health care products – Radiation – Part 1:	

(Full citation of an FDA recognized standard is recommended including date (e.g., ANSI/AAMI/ISO 11135:2007)),	Requirements for development, validation and routine control of a sterilization process for medical devices. ISO 11137-2:2006 – Sterilization of health care products – Radiation – Part 2: Establishing the sterilization dose. ISO 11137-3:2006 – Sterilization of health care products – Radiation – Part 3: Guidance on dosimetric aspects.”	
3. Sterility assurance level (SAL): (e.g., 10^{-6} for all devices (except 10^{-3} for devices that contact intact skin))	10^{-6}	
4. Is it labeled “Pyrogen Free”? If so, a description of the method: (e.g., LAL (<i>Limulus</i> Amebocyte Lysate test))	N/A	X
5. A description of the packaging (not including package integrity test data):	Rigid clear PETG blister package with a heat-sealed Tyvek lid. One sealed PETG blister is placed in an SBS sales unit carton	

In the ‘Section 14: Sterilization and Shelf Life’ the sponsor stated that:

“The ENSEAL G2 Articulating Tissue Sealers are sterilized by Cobalt 60 Irradiation. The devices will be validated to 25-45 kGy to achieve a 10^{-6} Sterility Assurance Level (SAL). The dose setting will be performed per Method VDmax – Substantiation of 25 kGy as the sterilizing dose.

...
The Subject ENSEAL G2 Articulating Tissue Sealers are packaged in a rigid clear PETG blister package with a heat-sealed Tyvek lid. One sealed PETG blister is placed in an SBS sales unit carton, 6 SBS sales unit cartons are placed in a corrugated shipper. The packaging materials and manufacturing methods are the same as the Predicate.

...
ENSEAL G2 Articulating Tissue Sealers are intended for single patient use and are not intended to be reused or resterilized. At launch, the devices will be labeled with a one year shelf life. Ethicon Endo-Surgery will have data on file to support the stability and sterility of the device for one (1) year. As additional data becomes available to support a shelf life up to 2 years the expiration date will be adjusted.”

There is the following deficiency.

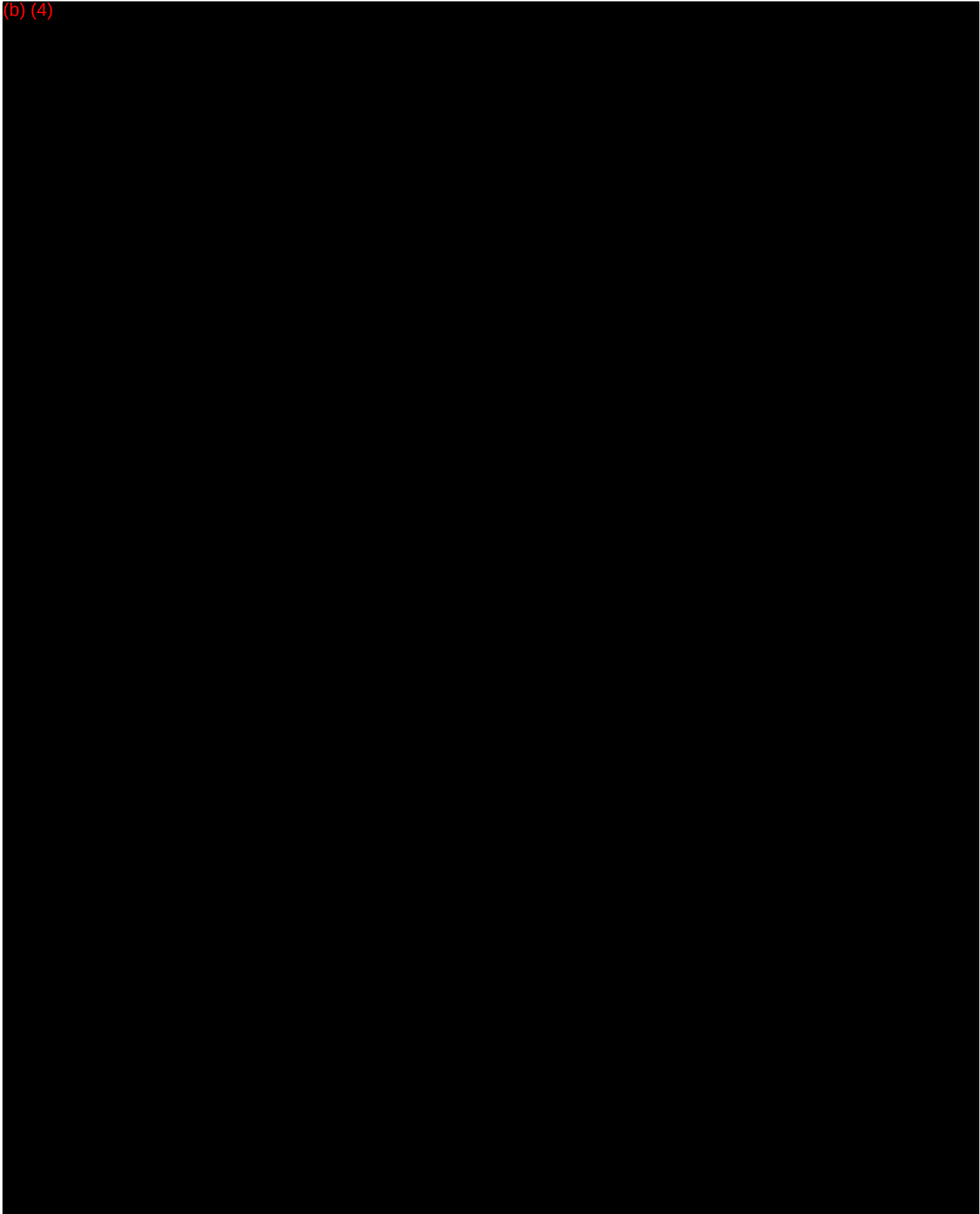
On page 96 of the submission you provided information regarding Sterilization of the subject device. However, from the text of the document it is not clear the dose setting “*will be validated*” or “*is validated*”, also, no particular dose value provided. Please revise the Sterilization section of your submission to clarify the status of the radiation dose verification. If radiation dose verification is not complete, please revise the Sterilization section to include the statement that validation will be completed before subject device will be released to the market.

VIII. Biocompatibility

The materials in the devices are classified as Surface Devices Breached or Compromised Surfaces and External Communicating Devices Tissue/Bone/Dentin Limited (less than 24 hours) as defined by ISO 10993-1.

The sponsor provided a list of patient contacting materials (Table 15.1, pages 100-101) which is

(b) (4)



	Trans Tech America		information is provided herein
--	--------------------	--	--------------------------------

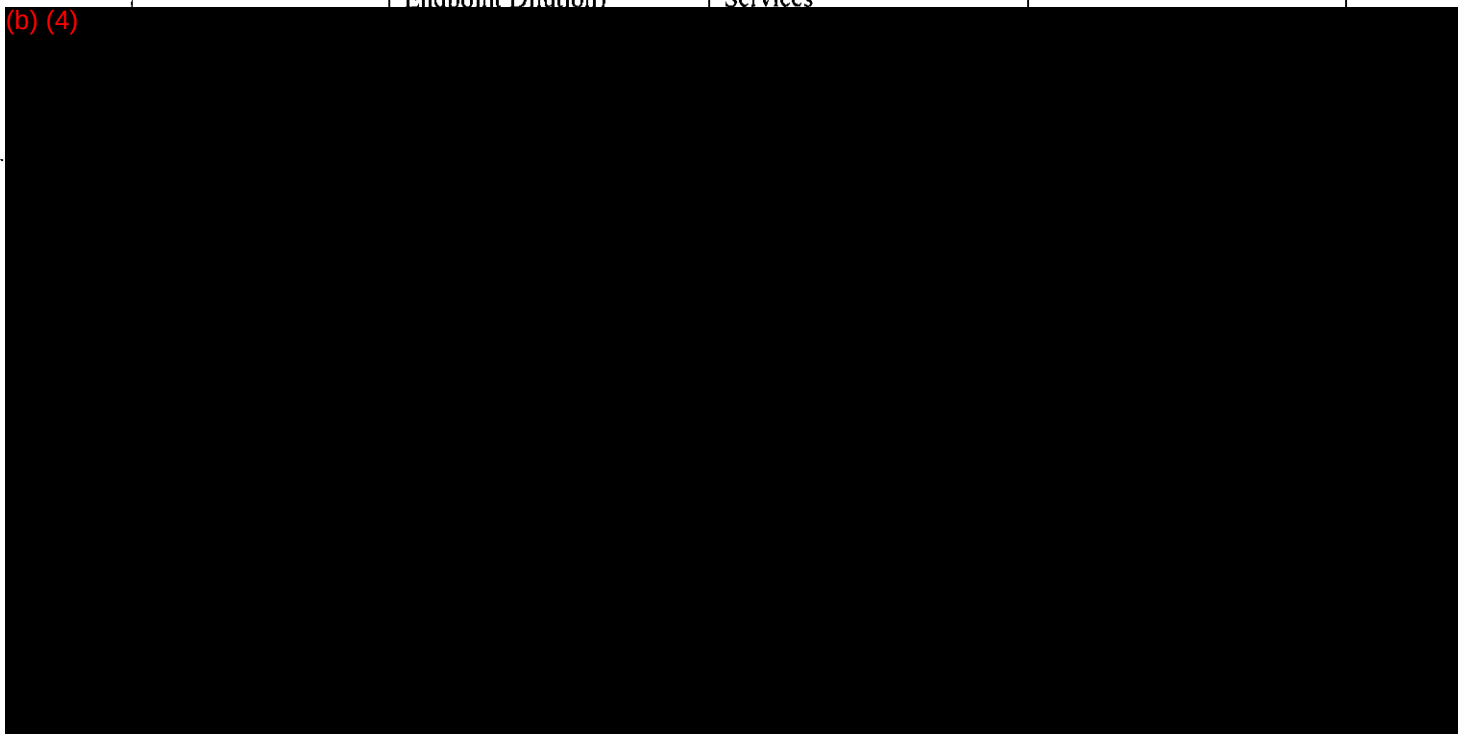
To evaluate biocompatibility of the new materials, associated with the new articulation feature, the sponsor has performed following required testing:

- Cytotoxicity
- Sensitization
- Irritation / Intracutaneous Reactivity
- Acute Systemic Toxicity
- Hemocompatibility

The distal end of the Subject device was evaluated to account for potential interaction of patient-contacting materials (ISO 10993-1). The following test was completed:

Test Type	Test Name	Lab Name	Result
Cytotoxicity	Cytotoxicity (MEM-Endpoint Dilution)	WuXi AppTec Laboratory Services	Pass

(b) (4)



IX. Software

The sponsor stated that this device does not utilize software.

This was found adequate.

X. Electromagnetic Compatibility and Electrical, Mechanical and Thermal Safety

The sponsor provided Standard Data Report Forms for the following:

- **IEC 60601-1: Medical Electrical Equipment - Part 1: General Requirements for Safety: Safety Requirements for Medical Electrical Systems (1998)2005.**
- **IEC 60601-1-2 Medical Electrical Equipment Part 1-2: General Requirements for Safety: Electromagnetic Compatibility (2007)**

- **IEC 60601-2-2** Medical electrical equipment, Part 2-2: Particular requirements for the safety of high frequency surgical equipment **(2009)**

The certification facility used: **TUV Rheinland** of North America, Inc. (www.tuv.com)

The sponsor provided excerpts of the test data package, test sample identification, certification and stated that:

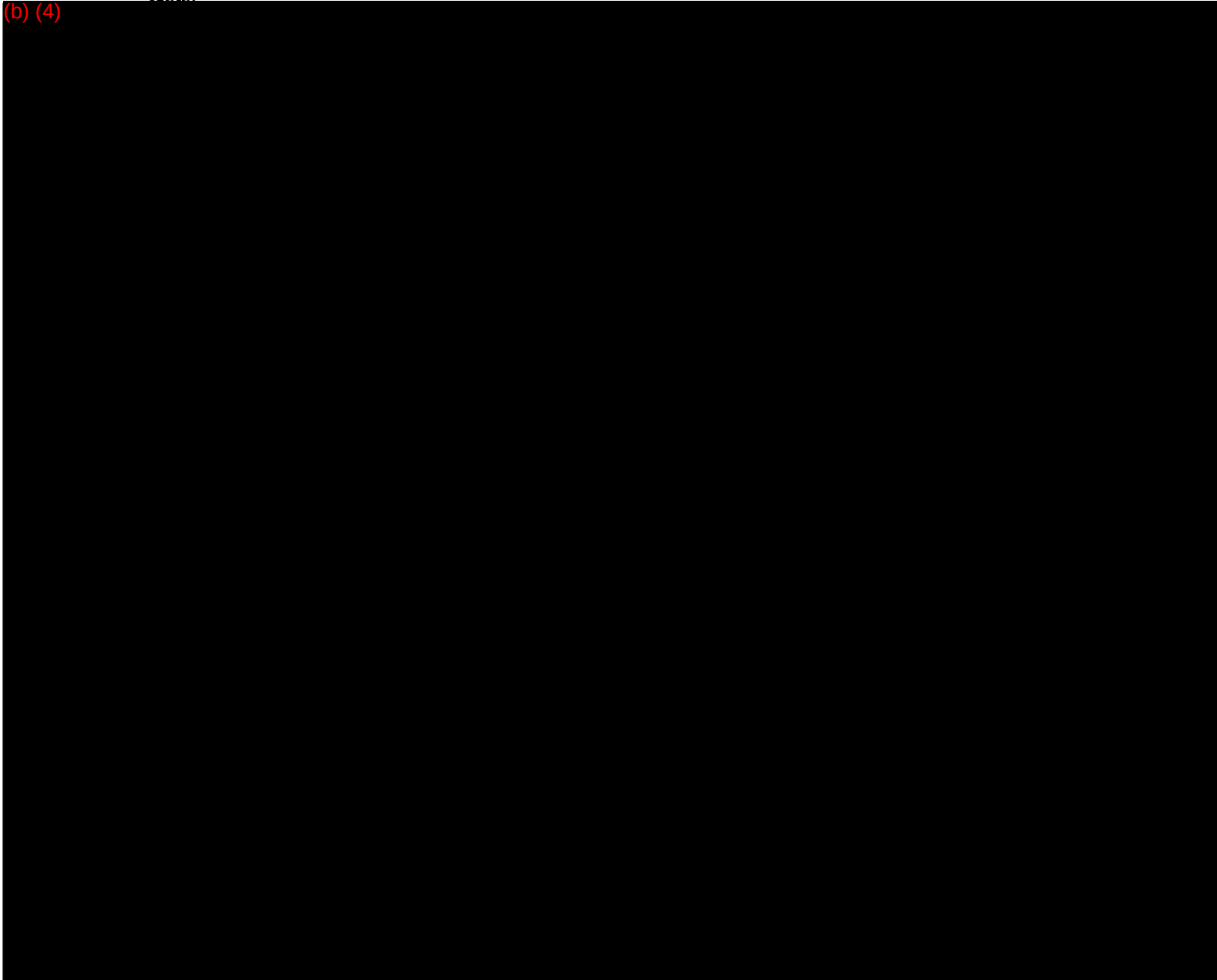
"All IEC testing of the ENSEAL G2 Articulating Tissue Sealers was completed with the Generator G11 (GEN11) which was cleared under K101990."

This was found adequate.

XI. Performance Testing – Bench

In Section 18: Performance Testing – Bench, the sponsor provided information for the following tests:

(b) (4)





K122797/51
V.1
U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Control Center WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

December 20, 2012

ETHICON ENDO-SURGERY, LLC
C/O ETHICON ENDO-SURGERY, INC.
4545 CREEK ROAD
CINCINNATI, OHIO 45242
ATTN: EMILY KRUEZKAMP

510k Number: K122797

Product: ENSEAL G2 ARTICULATING TISSUE

On Hold As of 12/19/2012

We are holding your above-referenced Premarket Notification (510(k)) for 30 days pending receipt of the additional information that was requested by the Office of Device Evaluation. Please remember that all correspondence concerning your submission MUST cite your 510(k) number and be sent in duplicate to the Document Mail Center at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review. Please refer to this guidance for information on current fax and e-mail practices at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089402.htm>.

The deficiencies identified represent the issues that we believe need to be resolved before our review of your 510(k) submission can be successfully completed. In developing the deficiencies, we carefully considered the statutory criteria as defined in Section 513(i) of the Federal Food, Drug, and Cosmetic Act for determining substantial equivalence of your device. We also considered the burden that may be incurred in your attempt to respond to the deficiencies. We believe that we have considered the least burdensome approach to resolving these issues. If, however, you believe that information is being requested that is not relevant to the regulatory decision or that there is a less burdensome way to resolve the issues, you should follow the procedures outlined in the "A Suggested Approach to Resolving Least Burdensome Issues" document. It is available on our Center web page at: <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Overview/MedicalDeviceProvisionsoffDAModer nizationAct/ucm136685.htm>.

In accordance with 21 CFR 807.87(l), FDA may consider a 510(k) to be withdrawn if the submitter fails to provide additional information within 30 days of an Additional Information (AI) request. FDA generally permits submitters additional time to respond to such requests. FDA intends to automatically grant a maximum of 180 calendar days from the date of the AI request, even if the submitter has not requested an extension. Therefore, submitters are no longer required to submit written requests for extension. However, submitters should be aware that FDA intends to issue a notice of withdrawal under 21 CFR 807.87(l) if FDA does not receive, in a submission to the appropriate Document Control Center, a complete response to all of the deficiencies in the AI request within 180 calendar days of the date that FDA issued that AI request. In this instance, pursuant to 21 CFR 20.29, a copy of your 510(k) submission will remain in the Office of Device Evaluation. If you then wish to resubmit this 510(k) notification, a new number will be assigned and your submission will be considered a new premarket notification submission.

Records Processed under FOIA Request 2015-6323. Released by GDRH on 1-30-2017

For further information regarding how various FDA and industry actions that may be taken on 510(k)s should affect the review clock for purposes of meeting the Medical Device User Fee Amendments of 2012 (MDUFA III), to the Federal Food, Drug, and Cosmetic Act, you may refer to our guidance document entitled "Guidance for Industry and Food and Drug Administration Staff - FDA and Industry Actions on Premarket Notification (510(k)) Submissions: Effect on FDA Review Clock and Goals". You may review this document at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089735.htm>.

Please remember that the Safe Medical Devices Act of 1990 states that you may not place this device into commercial distribution until you receive a decision letter from FDA allowing you to do so.

If you have procedural questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301)796-7100 or at their toll-free number (800)638-2041, or contact the 510k staff at (301)796-5640.

Sincerely yours,

Marjorie Shulman
Director, 510(k) Program
Premarket Notification Section
Office of Device Evaluation
Center for Devices and Radiological Health



From: NapperIi, Jesse *
Sent: Thursday, December 20, 2012 8:43 AM
o: Nichols, Karl *
Subject: FW: k122797 HOLD LETTER
Attachments: K1.22797 HOLD.rtf

From: NapperIi, Jesse *
Sent: Thursday, December 20, 2012 8:41 AM
To: 'EKRUETZK@ITS.JNJ.COM'
Subject: k122797 HOLD LETTER

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Control Center WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
December 20, 2012

ETHICON ENDO-SURGERY, LLC
C/O ETHICON ENDO-SURGERY, INC.
4545 CREEK ROAD
CINCINNATI, OHIO 45242
ATTN: EMILY KRUEZKAMP
J10k Number: K122797
Product: ENSEAL G2 ARTICULATING TISSUE
On Hold As of 12/19/2012

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<http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089735.htm>.

**COVER SHEET MEMORANDUM**

From: Reviewer Name Mykhaylo Nosovskyy
Subject: 510(k) Number K122797
To: The Record

Please list CTS decision code AI

- ☐ Refused to accept (Note: this is considered the first review cycle, See Screening Checklist
http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPremarketNotification510kProgram/0_5631/Screening%20Checklist%207%202%2007.doc)
- ☒ Hold (Additional Information or Telephone Hold).
- ☐ Final Decision (SE, SE with Limitations, NSE (select code below), Withdrawn, etc.).

Not Substantially Equivalent (NSE) Codes

- | | |
|-----------------------------|---|
| ☐ NO | NSE for lack of predicate |
| ☐ NI | NSE for new intended use |
| ☐ NQ | NSE for new technology that raises new questions of safety and effectiveness |
| ☐ NU | NSE for new intended use AND new technology raising new questions of safety and effectiveness |
| ☐ NP | NSE for lack of performance data |
| ☐ NS | NSE no response |
| ☐ NL | NSE for lack of performance data AND no response |
| ☐ NM | NSE pre-amendment device call for PMAs (515i) |
| ☐ NC | NSE post-amendment device requires PMAs |
| ☐ NH | NSE for new molecular entity requires PMA |
| ☐ TR | NSE for transitional device |

Please complete the following for a final clearance decision (i.e., SE, SE with Limitations, etc.):		YES	NO
Indications for Use Page	<i>Attach IFU</i>		
510(k) Summary /510(k) Statement	<i>Attach Summary</i>		
Truthful and Accurate Statement.	<i>Must be present for a Final Decision</i>		
Is the device Class III?			
If yes, does firm include Class III Summary?	<i>Must be present for a Final Decision</i>		
Does firm reference standards? (If yes, please attach form from http://www.fda.gov/opacom/morechoices/fdaforms/FDA-3654.pdf)			
Is this a combination product? (Please specify category _____, see http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPremarketNotification510kProgram/0_413b/COMBINATION%20PRODUCT%20ALGORITHM%20(REVISED%203-12-03).DOC)			
Is this a reprocessed single use device? (Guidance for Industry and FDA Staff – MDUFMA - Validation Data in 510(k)s for Reprocessed Single-Use Medical Devices, http://www.fda.gov/cdrh/ode/guidance/1216.html)			
Is this device intended for pediatric use only?			
Is this a prescription device? (If both prescription & OTC, check both boxes.)			
Did the application include a completed FORM FDA 3674, Certification with Requirements of ClinicalTrials.gov Data Bank?			
Is clinical data necessary to support the review of this 510(k)?			

For United States-based clinical studies **only**: Did the application include a completed FORM FDA 3674, *Certification with Requirements of ClinicalTrials.gov Data Bank*? (If study was conducted in the United States, and FORM FDA 3674 was not included or incomplete, then applicant must be contacted to obtain completed form.)

Does this device include an Animal Tissue Source?

All Pediatric Patients age ≤ 21

Neonate/Newborn (Birth to 28 days)

Infant (29 days - < 2 years old)

Child (2 years - < 12 years old)

Adolescent (12 years - < 18 years old)

Transitional Adolescent A (18 - < 21 years old) Special considerations are being given to this group, different from adults age ≥ 21 (different device design or testing, different protocol procedures, etc.)

Transitional Adolescent B (18 - ≤ 21; No special considerations compared to adults ≥ 21 years old)

Nanotechnology

Is this device subject to the Tracking Regulation? (Medical Device Tracking Guidance, <http://www.fda.gov/cdrh/comp/guidance/169.html>) Contact OC.

Regulation Number

21 CFR 878.4400

Class*

II

Product Code

GEI, HGI

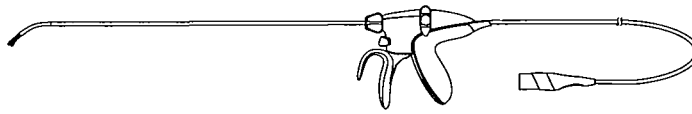
(*If unclassified, see 510(k) Staff)

Additional Product Codes:

Digital Signature Concurrence Table	
Reviewer Sign-Off	Mykhaylo B. Nosovskyy 2012.12.19 12:04:05 -05'00'
Branch Chief Sign-Off	Dwight Yen 2012.12.19 13:49:22 -05'00'
Division Sign-Off	



ENSEAL® G2 Articulating Tissue Sealer



Please read all information carefully.

Failure to properly follow the instructions may lead to serious surgical consequences.

Important: This package insert is designed to provide instructions for use of the ENSEAL® G2 Articulating Tissue Sealer. It is not a reference to surgical techniques.

ENSEAL and I-BLADE are trademarks of Ethicon Endo-Surgery.

Instructions

Indications

The ENSEAL® G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with the Generator G11 (GEN11). They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7mm and tissue and/or vascular bundles as large as will fit in the jaws of the instruments.

The ENSEAL G2 Articulating Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures.

Contraindications

The efficacy of the ENSEAL G2 Articulating Tissue Sealer for the indication of contraceptive tubal coagulation (permanent female sterilization) has not been evaluated and is unknown. The design of the ENSEAL G2 Articulating Tissue Sealer is significantly different from bipolar designs that are marketed for the indication of contraceptive tubal coagulation. The design differences may affect the efficacy of the procedure and failure rates may not be comparable.

Device Description

The ENSEAL G2 Articulating Tissue Sealer is a sterile, single patient use surgical instrument used to coagulate and transect vessels up to and including 7mm in diameter and tissue and/or vascular bundles. This device is for soft tissue only. The instrument consists of a grip housing assembly, a rotating and articulating shaft either 35cm or 45cm in length, a moveable jaw and an I-BLADE® in the jaws. The instrument shaft can be rotated to facilitate visualization and enable easy access to targeted tissue. The instrument shaft, normally in the straight position, can be articulated using the articulation wheel to gain additional access to tissue and facilitate additional angles across tissue and/or vascular bundles.

The jaws are in a normally opened position and can be partially or fully closed by squeezing the closing handle. The jaws have atraumatic teeth for grasping and holding targeted tissue when clamped. Bipolar energy is delivered to the clamped tissue when the energy activation button is pressed. Fully squeezing the closing handle advances the I-BLADE the length of the jaws, and the targeted tissue is compressed, coagulated, and transected. The I-BLADE cannot be advanced to transect tissue without first fully depressing the energy activation button. The power cord is permanently attached to the device and connects the instrument to the generator. The ENSEAL G2 Articulating Tissue Sealer is designed for use exclusively with the Generator G11 (GEN11), packaged separately. Dimensions: Jaw Length, Straight: 20mm, Jaw Length, Curved: 19mm, Shaft Length: 35cm from Jaw Tip to Shaft Rotation Knob or 45cm from Jaw Tip to Shaft Rotation Knob, Shaft Diameter: 5mm.

Generator Audible Indicators

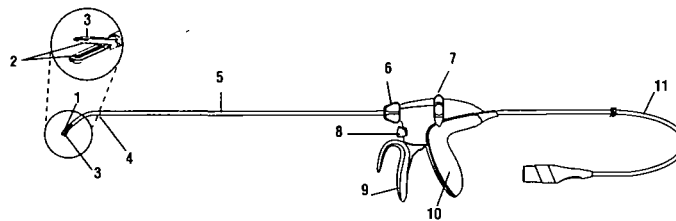
Refer to the following table for generator audible feedback tones.

Tone	Description	Action
Tone 1	Continuous steady beeping	Generator On: Device is active.
Tone 2	Continuous steady beeping at increased pitch	Intermediate: Tissue impedance threshold is reached.
Tone 3	One short beep	End of cycle: I-BLADE fully advanced. Energy is stopped.

Illustration and Nomenclature (Illustration 1)

- | | |
|---------------------------------|-----------------------------|
| 1. Jaws (curved or straight) | 6. Rotation knob |
| 2. Atraumatic teeth | 7. Articulation wheel |
| 3. I-BLADE® knife (not visible) | 8. Energy activation button |
| 4. Articulation joint | 9. Closing handle |
| 5. Shaft | 10. Grip housing |
| | 11. Power cord |

English



Transport and Storage Conditions

Temperature: -20° C to +54° C

Relative Humidity: 10% - 80%

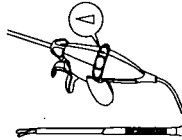
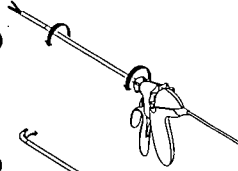
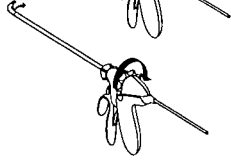
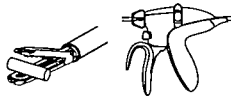
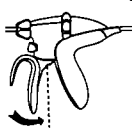
How Supplied

The ENSEAL G2 Articulating Tissue Sealer with integrated power cord is supplied sterile for single patient use. Discard after use.

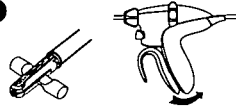
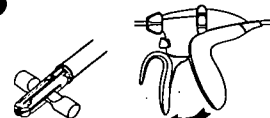
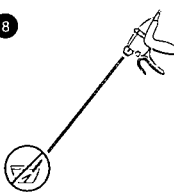
Warnings and Precautions

- Caution: Federal (USA) law restricts this device to sale by or on the order of a physician.
- Examine the shipping carton and instrument for signs of shipping damage. Note any shortages, breakage, or apparent damage, retain the evidence, and notify Ethicon Endo-Surgery Customer Service immediately and replace with a new instrument. Do not use a damaged product.
- A thorough understanding of the principles and techniques involved in laser, electrosurgical, and ultrasonic procedures is essential to avoid shock and burn hazards to both patient and medical personnel and damage to the device or other medical instruments. Ensure that electrical insulation or grounding is not compromised.
- Laparoscopic surgical procedures with the ENSEAL G2 Articulating Tissue Sealer should be performed only by persons with adequate training and preparation. Consult the medical literature regarding techniques, complications, and hazards prior to any surgical procedure.
- Do not immerse the ENSEAL G2 Articulating Tissue Sealer in liquid.
- Pools of conductive fluids (e.g. blood, saline) in direct contact with the instrument jaws may affect device performance. Remove pooled fluid from around the instrument jaws before activating the instrument.
- Verify compatibility with generators. Use device only with Ethicon Endo-Surgery Generator G11 (GEN11) software version X or later. Refer to the Generator G11 (GEN11) Operator's Manual to confirm software versions.
- The device is at high voltage whenever the generator is active. Activate this device only when the jaws are under direct visual observation to avoid unintended tissue coagulation or necrosis.
- Check if patient has a pacemaker or implanted cardioverter/defibrillator. Consult pacemaker manufacturer for information about the effects of RF energy on these devices.
- This instrument contains a surgically sharp edge. THIS DEVICE CUTS!

- The ENSEAL G2 Articulating Tissue Sealer has not been shown to be effective for sealing large vessels without fully advancing the I-BLADE. Do not attempt to seal large vessels by only clamping the tissue and applying energy.
- Do not use this device on vessels in excess of 7mm in diameter.
- Do not use excessive force on the closing handle to close the jaws; grasp only as much tissue as will fit between the jaws where the current will pass. Greater amounts of tissue require more closing handle force. Excessive force could damage the device.
- Avoid contact with metal and plastic when instrument is active.
- Use surgical gloves specifically designed by their manufacturer for electrosurgical procedures. The use of other gloves can result in burns or shocks.
- In case of system failure, ensure the availability of the appropriate back-up eq To articulate the device, turn the articulation wheel. upment relevant to the specific procedure.
- During and following activation in tissue, the jaws and shaft may be hot. Avoid unintended contact with tissue, drapes, surgical gowns, or other unintended sites at all times.
- After using the instrument to seal and/or transect, always examine the tissue for hemostasis. If hemostasis is not present, appropriate techniques should be used to achieve hemostasis.
- Due to the difficulty of visualizing obscured structures, proceed slowly and do not attempt to transect large masses of tissue in one activation. Avoid the division of large tissue and/or vascular bundles when using the instrument under these conditions.
- Products manufactured or distributed by companies not authorized by Ethicon Endo-Surgery may not be compatible with the ENSEAL G2 Articulating Tissue Sealer instrument. Use of such products may lead to unanticipated results and possible injury to the user or patient.
- Discontinue use and replace device if excessive smoke or sparking is observed.
- Dispose of this device in a "Sharps" container. Dispose of all opened instruments whether used or unused.
- As with all energy sources (Electrosurgery, Laser, or Ultrasound), there are concerns about the carcinogenic and infectious potential of the by-products, such as tissue smoke plume and aerosols. Appropriate measures such as protective eyewear, filtration masks, and effective smoke evacuation equipment should be used in laparoscopic or open procedures.
- Instruments or devices which come into contact with bodily fluids may require special disposal handling to prevent biological contamination.
- To ensure effective sealing and transection of large vessels, always completely advance the I-BLADE and wait for Tone 3.
- Do not stop squeezing the closing handle with the I-BLADE only partially advanced.
- Transection without power application can lead to bleeding in vascular tissue.
- Take care to avoid injury: the I-BLADE is sharp.
- Do NOT immerse distal tip in liquid, including saline solution, to clean.
- Do not clean the instrument with abrasives.
- To avoid inadvertent user injury, do not activate the instrument while cleaning.
- Prior to inserting or removing the device from the trocar, ensure that the device is in the straight, non-articulated position by confirming that the indicator arrow on the articulation wheel is aligned with the top of the instrument.
- Removing the device from a trocar while the device is articulated could result in unintentionally removing the trocar from the incision site, a loss of insufflation, or damage to the black shaft covering.
- Prior to articulating the shaft, ensure that the jaw and the articulation joint are fully inserted through the trocar.
- The ENSEAL G2 Articulating Tissue Sealer is not a passive articulation device. Do not force the articulation angle by pressing the shaft against a solid body. Use the articulation wheel to achieve the desired articulation angle.
- If the rotation force of the articulation wheel seems excessive, discontinue rotation of the wheel. Using excessive force could damage the device.
- Do not use the top jaw alone to perform tissue dissection.
- This device is packaged and sterilized for single use only. Multiple patient use may compromise the device integrity or create a risk of contamination that, in turn, may result in patient injury or illness.

Instructions for Use	Illustrations & Notes
<p>1 Using sterile technique, remove the instrument from the package. To avoid damage, do not flip the instrument into the sterile field. Do not use a damaged product.</p> <p>2 Connect the instrument power cord to the generator.</p> <p>3 Before inserting the instrument through the trocar, make certain that the instrument is in the straight (non-articulated) position and the jaws are closed. (Illustration 2) Insert the instrument into the trocar before articulating the device.</p> <p>To rotate the device, turn the rotation knob in the desired direction. (Illustration 2a)</p> <p>To articulate the device, turn the articulation wheel. (Illustration 2b) The illustration provided shows the direction the shaft articulates when the rotation knob arrow is aligned on top.</p> <p>The articulation wheel may be turned even if the rotation knob indicator arrow is NOT aligned on top. The articulation wheel and rotation knob may be used independently.</p> <p>4 Position targeted tissue within open jaws. (Illustration 3).</p> <p>5 Squeeze the closing handle to grasp targeted tissue between the jaws (Illustration 4).</p> <p>6 Press the energy activation button to energize the jaws and begin coagulation of the targeted tissue. The generator gives audible feedback (Tone 1) as energy is delivered to the grasped tissue. Affirm tissue effects prior to advancing the I-BLADE through tissue.</p>	<p>NOTE: The instrument is straight when the indicator arrow on the articulation wheel is aligned with the top of the instrument.</p> <p>2 </p> <p>2a </p> <p>2b </p> <p>3 </p> <p>4 </p> <p>NOTE: Tissue between the jaws may be further compressed by squeezing the closing handle to the first stop. Applying additional pressure at the first stop does not increase tissue compression.</p>

English

Instructions for Use	Illustrations & Notes
<p>7 Complete the instrument firing cycle (Illustration 5).</p> <p>7a. Fully press the energy activation button to unlock the I-BLADE.</p> <p>7b. While pressing the energy activation button, continue squeezing the closing handle to advance the I-BLADE and increase tissue compression. NOTE: For larger vascular bundles, it is recommended to advance the I-BLADE slowly (gradually).</p> <p>7c. The generator tone changes pitch (Tone 2) when the tissue impedance threshold is reached, and the I-BLADE is ready to be fully advanced. It is recommended to maintain a consistent speed throughout I-BLADE advancement.</p> <p>7d. Squeeze the closing handle until it stops against the grip housing. This fully advances the I-BLADE (Illustration 5)</p> <p>The generator provides one short beep (Tone 3) to indicate the cycle is complete after the I-BLADE is fully advanced and the tissue impedance threshold has been reached. Energy delivery automatically stops after Tone 3.</p> <p>8 After the cycle is complete, release the closing handle and the energy activation button. (Illustration 7) If the desired cut is longer than the stroke of the blade, transection can be accomplished by repeating Steps 4 - 7.</p> <p>9 Straighten the shaft and close the jaws before removing the device from the trocar. The instrument is straight when the indicator arrow on the articulation wheel is aligned with the top of the instrument.</p> <p>10 Disconnect the instrument from the generator.</p> <p>11 Dispose of the instrument in an appropriate Sharps container. Do not reuse instrument.</p>	<p>5</p>  <ul style="list-style-type: none"> NOTE: The instrument may be operated with either footswitch or handswitch control. NOTE: The I-BLADE cannot be advanced to transect tissue without first fully pressing the energy activation button and then releasing the I-BLADE. NOTE: The instrument can be wiped with a sterile moist gauze sponge to remove tissue, if necessary. If tissue is still visible in the jaws, use forceps to remove residue. (Illustration 6) <p>6</p>  <p>7</p>  <p>8</p>  <p>Caution: Do NOT immerse distal tip in liquid, including saline solution, to clean. (Illustration 8)</p>

	See Instructions For Use (refer to blue symbol on outer packaging).
STERILE R	Sterilized by Irradiation. Sterility Guaranteed Unless Package Opened or Damaged. Do Not Resterilize.
	Relative Humidity
	Single Patient Use
LOT	Lot
Rx Only	Caution: Federal (USA) law restricts this device to sale by or on the order of a physician.
	Temperature

	Use Until Date
	Manufacturer
	Authorized Representative in the European Community
	Authorized Representative in the USA

REF
NSLG2S35A, NSLG2C35A
NSLG2S45A, NSLG2C45A



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22851 Norderstedt
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4545 Creek Road
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**Ethicon
Endo-Surgery**



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475 Calle C Guaynabo, PR 00969 USA
© Ethicon Endo-Surgery 2012

Rev. 2012-09

CE 0123
P40712P01

REVISIONS			
LTR	CAF NO.	CHNG	DATE
A			
PRODUCT CODE	ARTWORK NUMBER	PACKAGE COMPONENT NUMBER	
NSLG2S35A, NSLG2C35A, NSLG2S45A, NSLG2C45A	A88128P00	P40712P01	



ETHICON ENDO-SURGERY, INC.

 CONFIDENTIAL: NOT TO BE REPRODUCED OR USED IN ANY WAY
 WITHOUT WRITTEN ETHICON
 ENDO-SURGERY, INC. APPROVAL CINCINNATI, OH

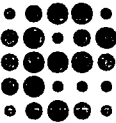
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Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Attachment 2: Draft Primary Labels and Carton Labels

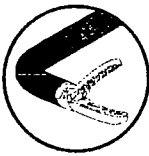
LOT

P30266P05



Ethicon
Endo-Surgery

Enseal



Laparoscopic
Tissue Sealer G2

Articulating
Curved Jaw

5mm
Diameter

35cm
Shaft Length

REF ①
NSLG2C35A

For use with G2 compatible generator

ENSEAL® Laparoscopic 5mm Diameter Tissue Sealer G2, 35cm Shaft Length, Articulating Curved Jaw. For use with G2 compatible generator

ENSEAL® Laparoscopic 5mm Diameter Tissue Sealer G2, 35cm Shaft Length, Articulating Curved Jaw. For use with G2 compatible generator

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ENSEAL® Laparoscopic 5mm Diameter Tissue Sealer G2, 35cm Shaft Length, Articulating Curved Jaw. For use with G2 compatible generator

STERILE

Sterilized by Irradiation. Sterility Guaranteed Unless Package Opened or Damaged. Do Not Resterilize.

②

Single Patient Use

CE

0123

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REVISIONS			
LTR	CAF NO.	CHNG	DATE
A	12-xxxxxx	DE	8-22-2012
PRODUCT CODE	ARTWORK NUMBER	PACKAGE COMPONENT NUMBER	
NSLG2C35A	A88014P00	P30266P05	

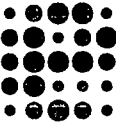
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PMS 424 (Gray)

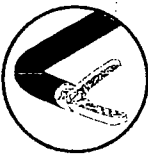
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LOT

P30266P06

Ethicon
Endo-Surgery

Enseal



Laparoscopic
Tissue Sealer G2

Articulating
Straight Jaw

5mm
Diameter

45cm
Shaft Length

REF ①
NSLG2S45A

For use with G2 compatible generator

ENSEAL® Laparoscopic 5mm Diameter Tissue Sealer G2, 45cm Shaft Length, Articulating Straight Jaw. For use with G2 compatible generator

ENSEAL® Laparoscopic 5mm Diameter Tissue Sealer G2, 45cm Shaft Length, Articulating Straight Jaw. For use with G2 compatible generator

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ENSEAL® Laparoscopic 5mm Diameter Tissue Sealer G2, 45cm Shaft Length, Articulating Straight Jaw. For use with G2 compatible generator

STERILE

② Single Patient Use

CE 0123

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LTR	CAF NO.	CHNG	DATE
A	12-xxxxx	DE	9-11-2012
PRODUCT CODE	ARTWORK NUMBER	PACKAGE COMPONENT NUMBER	
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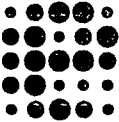
ETHICON ENDO-SURGERY, INC.
STERILIZED BY GAMMA RADIATION
IN A FACILITY THAT IS ISO 9001:2008 CERTIFIED
END-USER: ETHICON ENDO-SURGERY, INC.

PMS 424 (Gray)

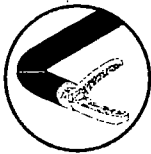
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LOT

P30266P07

Ethicon
Endo-Surgery

Enseal



Laparoscopic
Tissue Sealer G2

Articulating
Curved Jaw

5mm
Diameter

45cm
Shaft Length

REF
NSLG2C45A^①

For use with G2 compatible generator

ENSEAL® Laparoscopic 5mm Diameter Tissue Sealer G2, 45cm Shaft Length, Articulating Curved Jaw. For use with G2 compatible generator

ENSEAL® Laparoscopic 5mm Diameter Tissue Sealer G2, 45cm Shaft Length, Articulating Curved Jaw. For use with G2 compatible generator

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STERILE

Sterilized by Irradiation. Sterility Guaranteed Unless Package Opened or Damaged. Do Not Resterilize.

②

Single Patient Use

CE

0123

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A	12-XXXXX	DE	9-11-2012
PRODUCT CODE		ARTWORK NUMBER	PACKAGE COMPONENT NUMBER
NSLG2C45A		A88016P00	P30266P07

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PMS1797CVC PMSPROCESS BLACK PMS308C CMYK DIELINE
CAFBLOCK AUXDIELINE

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LTR	CAF	CHNG	DATE	
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				RD24-02
				SR

PMS1797CVC PMSPROCESS BLACK PMS308C CMYK DIELINE
CAPBLOCK AUXDIELINE

Laparoscopic Tissue Sealer G2

Articulating Curved Jaw

5mm Diameter

35cm Shaft Length

REF 1

NSLG2C35A

Ethicon Endo-Surgery | Enseal

Laparoscopic Tissue Sealer G2

Articulating Curved Jaw

5mm Diameter

35cm Shaft Length

REF 1

NSLG2C35A

ENSEAL® Laparoscopic 5mm Diameter Tissue Sealer G2, 35cm Shaft Length, Articulating Curved Jaw. For use with G2 compatible generator.

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ENSEAL® Laparoscopic 5mm Diameter Tissue Sealer G2, 35cm Shaft Length, Articulating Curved Jaw. For use with G2 compatible generator.

STERILIZED

Single Patient Use

See Instructions For Use

CAUTION: Use Performed Low retraction this device is not for use on the surface of a pleurothorax.

ETHICON ENDO-SURGERY, LLC, ENSEAL, LLC 2013

Guaymas, Puerto Rico 0989 USA

Laparoscopic Tissue Sealer G2

Articulating Curved Jaw

5mm Diameter

35cm Shaft Length

REF 1

NSLG2C35A

Ethicon Endo-Surgery | Enseal

Laparoscopic Tissue Sealer G2

Articulating Curved Jaw

5mm Diameter

35cm Shaft Length

REF 1

NSLG2C35A

ENSEAL® Laparoscopic 5mm Diameter Tissue Sealer G2, 35cm Shaft Length, Articulating Curved Jaw. For use with G2 compatible generator.

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STERILIZED

Single Patient Use

See Instructions For Use

CAUTION: Use Performed Low retraction this device is not for use on the surface of a pleurothorax.

ETHICON ENDO-SURGERY, LLC, ENSEAL, LLC 2013

Guaymas, Puerto Rico 0989 USA

PMS1797CVC PMSPROCESS BLACK PMS308C CMYK DIELINE
CABLOCK AUXDIELINE

PMS1797CVC PMSPROCESS BLACK PMS308C CMYK DIELINE
CABLOCK AUXDIELINE

PMS1797CVC PMSPROCESS BLACK PMS308C CMYK DIELINE
CABLOCK AUXDIELINE

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Attachment 3: Section 14: Sterilization and Shelf Life

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Section 14: Sterilization and Shelf Life

Sterilization

The ENSEAL G2 Articulating Tissue Sealers are sterilized by Cobalt 60 Irradiation. The devices will be validated to 25-45 kGy to achieve a 10^{-6} Sterility Assurance Level (SAL). The dose setting will be performed per Method VD_{max} – Substantiation of 25 kGy as the sterilizing dose. The dose value will be 25 kGy.

The sterilization process will be validated and the sterilization dose established per the requirements of the following FDA recognized standards:

- ISO 11137-1:2006 – Sterilization of health care products – Radiation – Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices.
- ISO 11137-2:2006 – Sterilization of health care products – Radiation – Part 2: Establishing the sterilization dose.
- ISO 11137-3:2006 – Sterilization of health care products – Radiation – Part 3: Guidance on dosimetric aspects.

The validation will be complete before the Subject device will be released to the market. Please see Section 21 Other for Forms 3654.

Packaging

The Subject ENSEAL G2 Articulating Tissue Sealers is packaged in a rigid clear PETG blister package with a heat-sealed Tyvek lid. One sealed PETG blister is placed in an SBS sales unit carton, 6 SBS sales unit cartons are placed in a corrugated shipper. The packaging materials and manufacturing methods are the same as the Predicate.

The recommended transportation and storage conditions are:

- Temperature: -20° C to 54° C
- Relative Humidity: 10-80%

Shelf Life

ENSEAL G2 Articulating Tissue Sealers are intended for single patient use and are not intended to be reused or resterilized. At launch, the devices will be labeled with a one-year shelf life. Ethicon Endo-Surgery will have data on file to support the stability and sterility of the device for one (1) year. As additional data becomes available to support a shelf life up to 2 years the expiration date will be adjusted.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

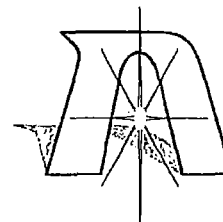
Attachment 4: Biocompatibility Test Reports

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Part Name	Subject Device Material
Articulation Joint Lubricant	Sodium Stearate, Vegetable

Northview Pacific Laboratories, Inc.

551 Linus Pauling Drive ♦ Hercules, CA 94547 ♦ 510/964-9000 ♦ Fax 510/964-0551



SPONSOR 2188-0

Jennifer Reid
Ethicon Endo-Surgery
Mail Location 37
4545 Creek Road
Cincinnati OH 45242

NV REPORT NUMBER

Y2E019G

REPORT DATE

May 30, 2002

TEST ARTICLE

Sodium Stearate NF
Code Identification : RDM-132035500096
Lot Number: 0203200197

TEST PERFORMED

Cytotoxicity - ISO Elution Test (MEM Extract)

STUDY DIRECTOR

Cathy Nguyen, *In Vitro* Analyst
In Vitro Services

PERFORMING LABORATORY

Northview Pacific Laboratories, Inc.
551 Linus Pauling Drive
Hercules, CA 94547

A Northview Biosciences Company



Atlantic, Midwest, and Pacific

F.D.A. REGISTRATION No 29-14117

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STUDY DATES

Study Authorization: Signed Protocol
Date Sample Received: May 13, 2002
Study Initiation Date: May 21, 2002
Date On Test: May 21 2002
Date Off Test: May 24, 2002
Report Date: May 30, 2002

PURCHASE ORDER NUMBER

561389803

TEST ARTICLE IDENTIFICATION

Test Article Identification (Tradename): Sodium Stearate NF
Generic Name: Sodium Stearate
Material Category: Lubricant
Physical Description: Coupons
Total Quantity Received for Testing: 1cm x 1cm metal coupons (1 bag with 30 pcs.)
Total Quantity Used for This Study: 36.0 cm² (18 metal coupons-intact)
Code Identification : RDM-132035500096
Lot Number: 0203200197
Stability Data: Team Responsible for Ensuring Stability
Sterilization (Mrad, Passes, Cycles): Gamma (8-10 Mrad) ETO (2 pass)
Sterilization at NVP: Steam (121°C for 20 minutes)
Storage Condition: Room Temperature

PROTOCOL

This test was conducted according to Annual Protocol File Name: Ethicon Endo-Surgery, which incorporates by reference Northview Standard Operating Procedure 15B-01 and is on file at Northview Pacific Laboratories, Inc. There were no amendments to the protocol.

DEVIATIONS FROM PROTOCOL

There were no deviations.

DATA DISPOSITION

Raw data and the final report from this study are archived at Northview Pacific Laboratories, Inc., 551 Linus Pauling Drive, Hercules, CA 94547, under Northview Report Number Y2E019G. The test article(s) will be disposed of by Northview Pacific Laboratories, Inc., unless the client requests that the test article(s) be returned. It is the responsibility of the client to maintain test articles in accordance with GLP regulations.

SUMMARY OF RESULTS

Under the conditions of this study, the test article, negative control and reagent control were considered non-toxic with a grade of 0 (no reactivity). The positive control was considered moderately toxic with a grade of 3 (moderate reactivity) at 24 hours and severely toxic with a grade of 4 (severe reactivity) at 48 hours. The test article met the requirements of the USP and ISO.



CYTOTOXICITY -ISO ELUTION TEST (MEM EXTRACT)

INTRODUCTION

This test was designed to evaluate the cytotoxic potential of extracts of polymeric materials or any other materials intended to be implanted in the human body or that may come into contact with body fluids or injectable solutions. The test article is subjected to an in vitro cytotoxicity study for biocompatibility based on the USP and ISO guidelines.

MATERIALS AND METHODS

Table 1: Controls

Control	Lot Number	Manufacturer	Expiration Date
Negative Control:			
Whatman® Filter Paper	67384	Whatman®	8/13/02
Positive Control:			
Tygon® F-4040-A	4495	Norton Performance Plastics Corp.	N/A

Table 2: Media and Vehicles

Media/Vehicle	Lot Number	Manufacturer	Expiration Date
Single Strength Minimum Essential Medium (1 x MEM)	1130157	Invitrogen Corporation	3/03
Serum Supreme	1S014F	Bio Whittaker	3/19/05
Fungizone	3060525	Life Technologies	1/03
Penicillin/Streptomycin	1113744	Invitrogen Corporation	9/02



Preparation of Cell Cultures

Multiple cultures of L-929 (ATCC cell line CCL 1, NCTC clone 929) mammalian fibroblast cells were prepared in accordance with NV SOP 15A-02, *Preparation and Maintenance of Cell Cultures for In Vitro Biological Reactivity Tests*, until a uniform near-confluent monolayer was obtained. The cell cultures were plated 24 – 48 hours prior to applying the extract medium in order to allow for a cell monolayer with greater than 80% confluence.

Preparation of Test Article and Controls

Test Article Sterilization – The test article was steam sterilized at 121°C for 20 minutes at Northview Pacific Laboratories, Inc.

Extraction – Single extractions of the test article, positive control, negative control, and reagent control were prepared (see Table 3 for details).

Table 3: Extraction of Test Article and Controls

Article	Extraction Ratio	Amount Extracted	Extracting Medium (Type/Amount)	Extraction Conditions
Test Article	60 cm ² :20 mL	*36.0 cm ²	MEM/12.0 mL	37°C/24 hrs
Positive Control	60 cm ² :20 mL	20.6 cm ²	MEM/6.9 mL	37°C/24 hrs
Negative Control	120 cm ² :20 mL	60.0 cm ²	MEM/10.0 mL	37°C/24 hrs
Reagent Control	N/A	N/A	MEM/20 mL	37°C/24 hrs

*18 metal coupons-intact

Test Procedure

Plating of Test and Control Extracts – After the 24 hour extraction period, the growth medium from triplicate 10 cm² wells each containing the monolayer of L-929 Mouse Fibroblast Cells (ATCC Cell Line CCL1, NCTC Clone 929), were decanted and replaced with 2 mL of test article extract. The control extracts were tested in the same manner as the extracting medium containing the test article. All cell culture wells were incubated for 48 ± 2 hours in a humidified incubator with 4 – 6% CO₂.

Examination – The cells in all of the culture wells were examined under an inverted light microscope with 100x magnification at 24 ± 1 hour and 48 ± 2 hours.

Scoring – The results at the 24 and 48 hour examination points were scored according to the criteria in Table 4. The average score for the three test wells at the 48 hour point was used to determine the cytotoxic response. The average score for the controls at the 48 hour point was used to compare results.



Interpretation and Analysis

The test system is considered valid if the observed effect on the cells exposed to the extracts prepared from the positive and negative controls corresponds to the labeled biological reactivity grade of the relevant Reference Standard.

If the reagent controls do not have the expected response in the test system, the test is repeated.

The test article meets the requirements of the test if the response to the test article preparation is not greater than grade 2 (Mild Reactivity).

If the cultures treated with the test article show a significantly greater reaction than the cultures treated with the negative control, quantitative dilutions may be requested by the client.

RESULTS

Test results for the test article, positive, negative and reagent controls are presented in Table 5.

CONCLUSION

Under the conditions of this study, the test article, negative control and reagent control were considered non-toxic with a grade of 0 (no reactivity). The positive control was considered moderately toxic with a grade of 3 (moderate reactivity) at 24 hours and severely toxic with a grade of 4 (severe reactivity) at 48 hours. The test article met the requirements of the USP and ISO.

Table 4: Reactivity Grades for Elution Test

Grade	Reactivity	Condition of Cell Cultures
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis or empty areas between cells
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed
4	Severe	Nearly complete destruction of the cell layers



Table 5: Test Results

	Reactivity	Grade	Reactivity	Grade
	24 hrs	24 hrs	48 hrs	48 hrs
Test Article #1	None	0	None	0
Test Article #2	None	0	None	0
Test Article #3	None	0	None	0
Controls				
Positive #1	Moderate	3	Severe	4
Positive #2	Moderate	3	Severe	4
Positive #3	Moderate	3	Severe	4
Negative #1	None	0	None	0
Negative #2	None	0	None	0
Negative #3	None	0	None	0
Reagent #1	None	0	None	0
Reagent #2	None	0	None	0
Reagent #3	None	0	None	0

REFERENCES

USP, current revision.

ISO 10993-5; 1993.

NV SOP 15B-01, *Cytotoxicity – Elution Test*.

QUALITY ASSURANCE UNIT GLP INSPECTION AND AUDIT SUMMARY

This study, Y2E019G, was inspected by Quality Assurance at intervals adequate to assure the integrity of the study. The SOP/protocol that the study followed, the phase(s) of the study inspected, and the date(s) of the inspection are provided below.

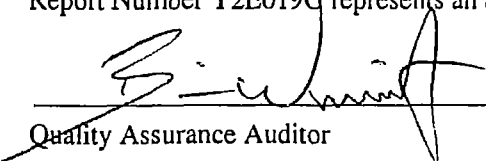
<u>SOP/Protocol</u>	<u>Phase of Study</u>	<u>Date</u>
15B-01	Interpretation of Test Results	5/24/02

QAU inspection findings are routinely reviewed by the management of Northview Pacific Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

QAU inspection findings for this study were reported to the Study Director and Management on the following date(s): May 24, 2002

FINAL REPORT AUDIT

Quality Assurance has conducted a thorough audit of the test data generated during this study. Northview Report Number Y2E019G represents an accurate description of the conduct and final results of the study.



Quality Assurance Auditor

5/31/02

Date



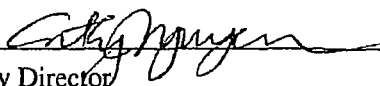
NORTHVIEW PACIFIC LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

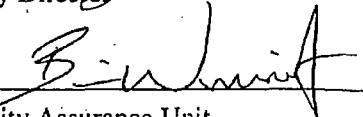
1. Christina Pham, Manager, *In Vitro* Services
2. Cathy Nguyen, *In Vitro* Analyst I

STATEMENT OF COMPLIANCE

This study, Northview Report Number Y2E019G, has been conducted in accordance with applicable Good Laboratory Practice Regulations.


Study Director

5/30/02
Date


Quality Assurance Unit

5/31/02
Date


Management

5/31/02
Date

:cw, 5/30/02 11:29 AM



ORGANIC LABORATORY ANALYSIS REPORT

Requester:	Debbie Brumley
Job Number:	F3730
Analysis Date:	5/16/02

Purpose:

The purpose of this analysis is to identify the lubricant coating, Sodium Stearate NF, on a metal coupon (RDM # 132035500096).

Summary:

The lubricant coating, Sodium Stearate NF, is Sodium Stearate with no evidence of other ingredients or contaminants.

Experimental:

The surface of a metal coupon was gently scraped to remove coating material but not metal. The scrapings were transferred to an infrared transmitting substance for Fourier Transform Infrared (FTIR) Spectroscopic analysis using the FTIR microscope.

Results and Discussion:

The lubricant on the metal coupon labeled Sodium Stearate NF (Spectrum 1) is Sodium Stearate (reference Spectrum 2. See overlay (Spectrum 3) for peak match. There is no evidence of other ingredients.

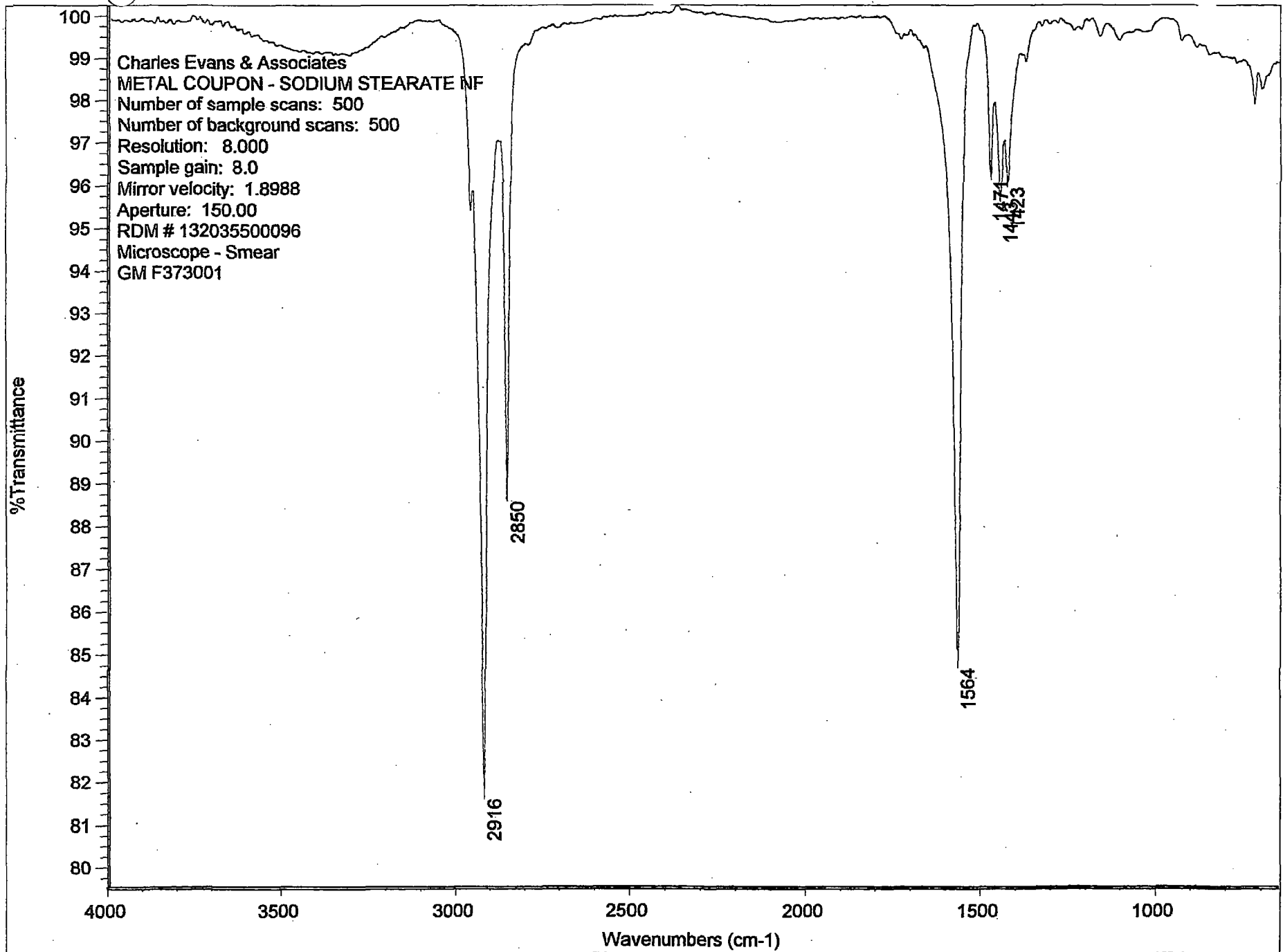
If questions arise as you review the results of this report, principal analyst Gordon or any other member of our technical staff will be available for consultation.

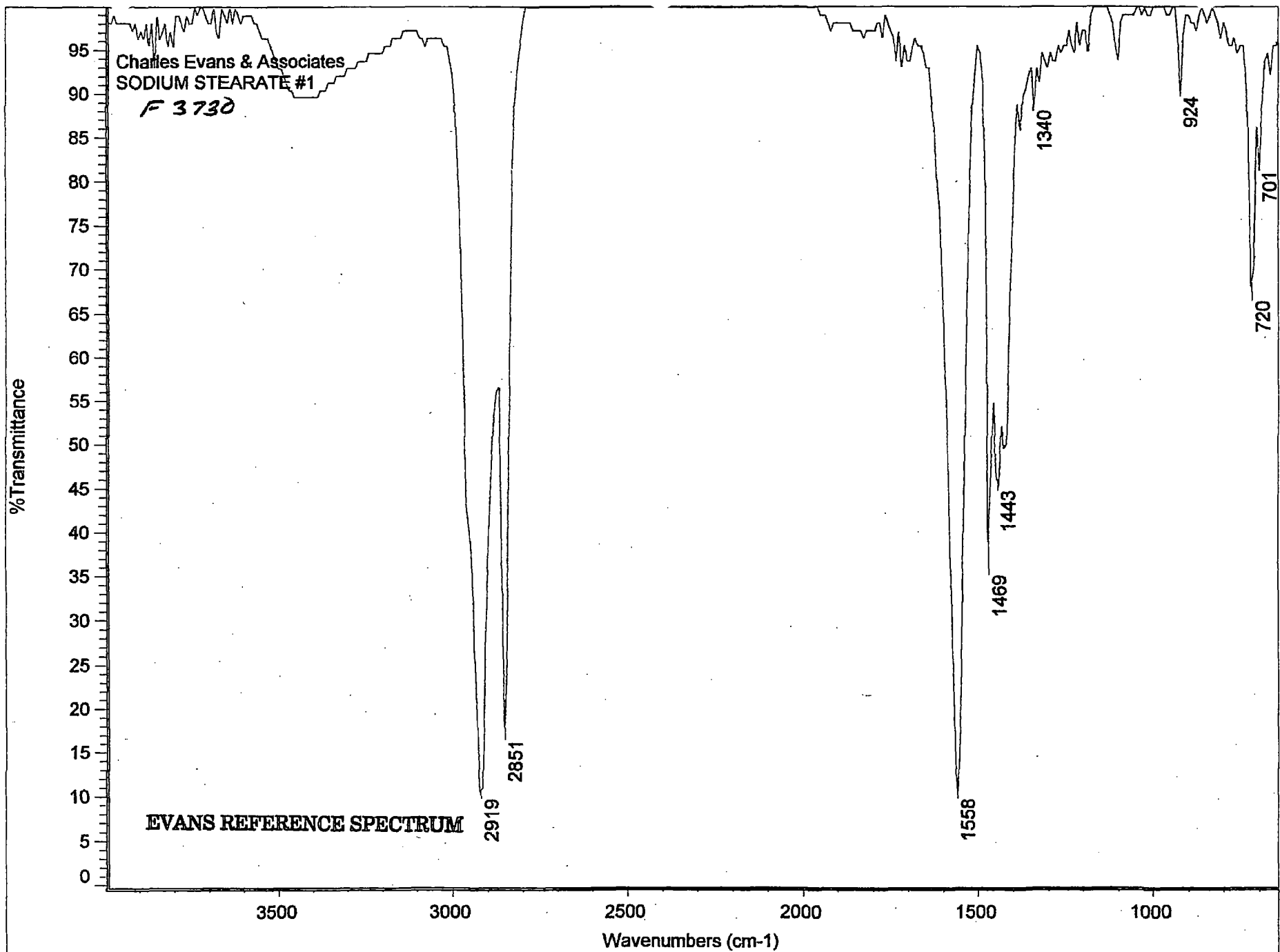
Job number: F3730
Date: May 16, 2002

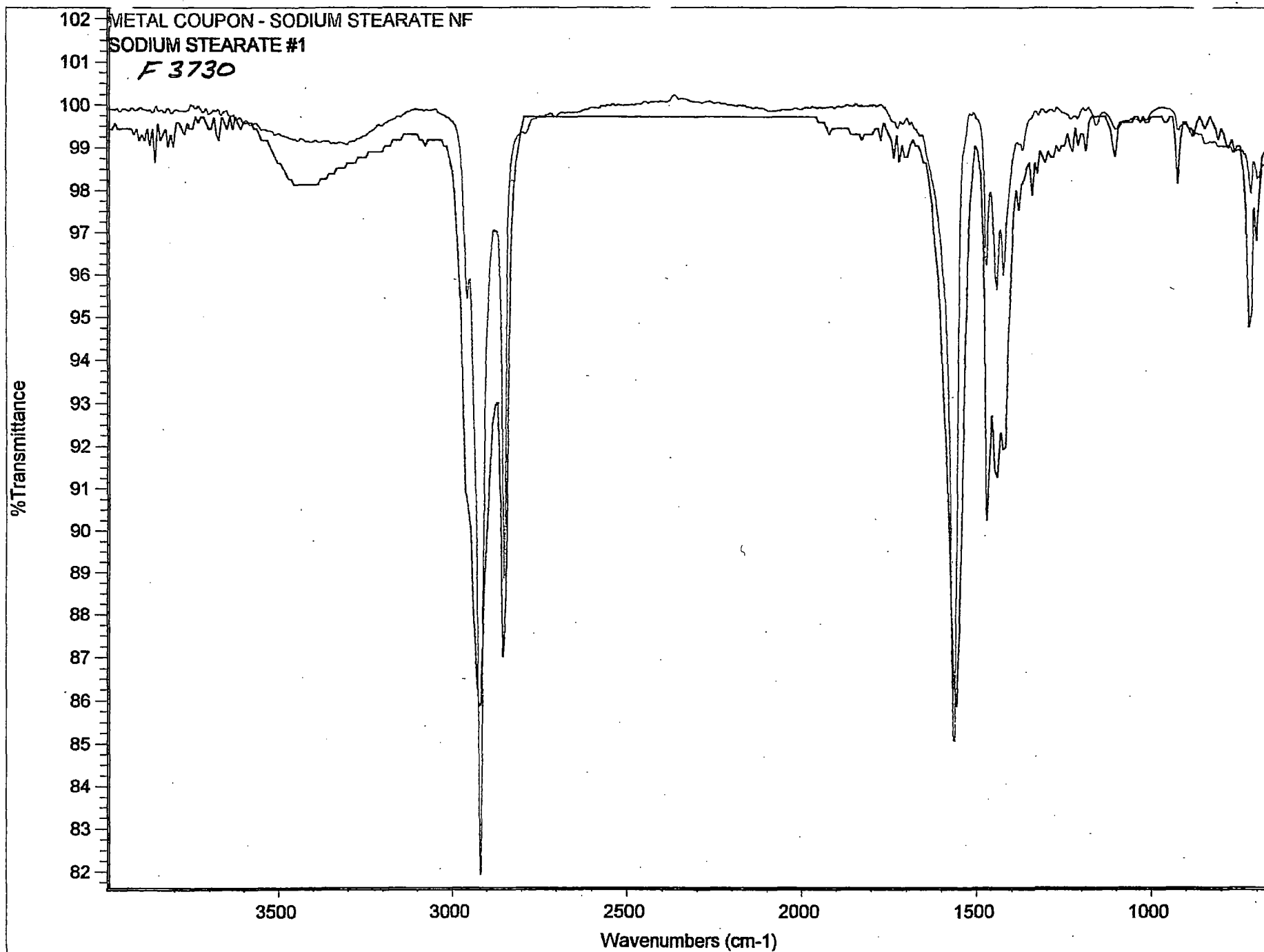
Page: 2

Appendix 1

Infrared Spectroscopy (IR) is the study of molecular vibrations. It is an extremely useful analytical technique, providing specific information about chemical bonding and molecular structure, most suitably for organic materials. The technique is based on the fact that bonds and groups of bonds vibrate at characteristic frequencies. When exposed to infrared radiation, a molecule selectively absorbs infrared frequencies that match those of its allowed vibrational modes. Therefore, the infrared absorption spectrum of a material reveals which vibrations, and thus functional groups, are present in its structure. It should be noted that vibrations that do not involve a change in dipole moment, as in O₂ and N₂, do not absorb infrared radiation. Thus, IR spectra can be collected in air.







SPI TRAM 3



FINAL STUDY REPORT

STUDY TITLE: Bacterial Endotoxin Detection Using the Kinetic Chromogenic LAL Method Inhibition/Enhancement (I/E) Assay

TEST PROTOCOL NUMBER: 130501 Rev. 1

TEST ARTICLE IDENTIFICATION: Ref # EE 2197; Trade name: Ti-3A-2.5V with Vegetable Sodium Stearate; RSPEC: Ti R2017-04, Stearate R1037-30
Generic Name: Titanium alloy staples with lubricant;
Sterilization: 25-45 kGy Gamma;
Sterilized on April 13, 2007; Device Lot # D4GV83

SPONSOR: CANTOX Health Sciences International
2233 Argentia Road
Suite 308
Mississauga, Ontario L5N 2X7
Canada

PERFORMING LABORATORY: AppTec Laboratory Services
1265 Kennestone Circle
Marietta, GA 30066

FINAL REPORT NUMBER: CTX011145.F

STUDY NUMBER: 011145

RESULT SUMMARY: Endotoxin Concentration
of the Test Article = <0.005 EU/mL



QUALITY ASSURANCE UNIT SUMMARY

STUDY: Bacterial Endotoxin Detection Using the Kinetic Chromogenic LAL Method
Inhibition/Enhancement (I/E) Assay

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR part 58) and in accordance to the standard operating procedures and a standard protocol. The Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Each inspection was performed to assure the quality and integrity of the study.

Phase Inspected
Testing Process

Date
April 30, 2007

Study Director
April 30, 2007

Management
May 1, 2007

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance

Date

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58. Test article characterization is the responsibility of the sponsor.

Study Director

Date

Professional personnel involved in study:

Brian Tinnel, BS
Jeretina Mangwana, BS

Study Director
Bacterial Endotoxin Laboratory Section Leader



1.0 PURPOSE

To determine, quantitatively, the amount of bacterial endotoxin present in a test article.

2.0 SPONSOR:

CANTOX Health Sciences International
2233 Argentia Road
Suite 308
Mississauga, Ontario L5N 2X7 Canada

3.0 TEST FACILITY:

AppTec Laboratory Services
1265 Kennestone Circle
Marietta, GA 30066

4.0 TEST ARTICLE IDENTIFICATION:

Ref # EE 2197; Trade name: Ti-3A-2.5V with Vegetable
Sodium Stearate; RSPEC: Ti R2017-04,
Stearate R1037-30
Generic Name: Titanium alloy staples with lubricant;
Sterilization: 25-45 kGy Gamma;
Sterilized on April 13, 2007; Device Lot # D4GV83

5.0 TEST ARTICLE CHARACTERIZATION

The identity, strength, stability, purity, and chemical composition of the test article are the responsibility of the Sponsor.

6.0 SCHEDULING

DATE SAMPLES RECEIVED: April 26, 2007
STUDY INITIATION DATE: April 27, 2007
STUDY COMPLETION DATE: May 3, 2007

7.0 EXPERIMENTAL DESIGN

- 7.1 Ten staples were received for testing. The staples were transferred to a pyrogen-free extraction container and covered with 40 mL of Pyrogen-free water (Lot # J4K230) per staple for a total volume of 400 mL. The test articles were then extracted for at least 15 minutes at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$.
- 7.2 The test article extract was tested undiluted.
- 7.3 The standard curve was prepared by serially diluting 50 EU/mL Control Standard Endotoxin (CSE) to 4 concentrations: 5.0, 0.5, 0.05, and 0.005 EU/mL.
- 7.4 An appropriate automatic pipette was used to fill wells in duplicate with 100 μL for the standard curve, negative controls, test article, and test article Positive Product Control (PPC).
- 7.5 The PPC was prepared by spiking 100 μL of the test article PPC with 10 μL of the 5.0 EU/mL standard, resulting in an approximate 0.5 EU/mL concentration.

7.7 After the assay was completed the pH of the test article extract combined with an equal amount of lysate was measured.

The assay is valid if the linearity of the standard curve is ≥ 0.980 , and the negative control reacts slower than the lowest standard.

The results are valid if the coefficient of variation (CV%) is < 20%, the PPC recovered is between 50% and 200%.

REAGENTS

Control Standard Endotoxin (CSE):	Lot EX74082
LAL Reagent Water (LRW):	Lot 99732055
Endochrome K (Lysate):	Lot W3392E

Linearity of the standard curve: 0.9988

Spike Recovery: 139%

Negative Control (CTRL1): Pass (see table 1)

Standard Curve and Negative Controls are displayed in Table 1.

The endotoxin concentration for the test article and PPC are displayed in Table 2.

TABLE 1: Standard Curve

STANDARD	WELL #	REACTION TIME	MEAN REACTION TIME	CV %
5.0 EU/mL	A1	572.1	580.3	1.42
	A2	588.6		
0.5 EU/mL	B1	908.2	919.8	1.26
	B2	931.4		
0.05 EU/mL	C1	1566.9	1588.5	1.36
	C2	1610.0		
0.005 EU/mL	D1	2795.0	2817.5	0.80
	D2	2840.0		
CTRL1	E1	>2850.0	>2850.0	0.00
	E2	>2850.0		

**TABLE 2: Test Results**

SAMPLE	DILUTION	WELL #	REACTION TIME	MEAN REACTION TIME	CV %	MEAN ENDOTOXIN VALUE (EU/mL)
Ref # EE 2197; Trade name: Ti-3A-2.5V with Vegetable Sodium Stearate; RSPEC: Ti R2017-04, Stearate R1037-30 Generic Name: Titanium alloy staples with lubricant; Sterilization: 25-45 kGy Gamma; Sterilized on April 13, 2007; Device Lot # D4GV83	N/A	G1 G2	>2850.0 >2850.0	>2850.0	0.00	<0.005
Positive Product Control (0.5 EU/mL)	N/A	H1 H2	872.4 894.3	883.3	1.24	0.701

11.0 ANALYSIS / CONCLUSION

The assay and results are valid. The linearity of the standard curve is ≥ 0.980 , the CV % is $< 20\%$, and the PPC is between 50% and 200%.

The test article contained <0.005 EU/mL.

12.0 DATA ANALYSIS / STATISTICAL METHODS

None Used.

13.0 PROTOCOL DEVIATIONS / AMENDMENTS

No deviation or amendments from the protocol were encountered during this study.

14.0 RECORD RETENTION

The testing facility will retain all records for a minimum of seven (7) years involving the study. Including, but not limited to: the signed testing protocol with all amendments, and any written communication concerning the conduct of the study, test article accountability record, raw data worksheets, and an official copy of the final study report and amendments.

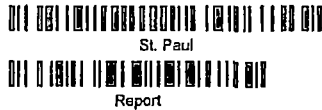
15.0 TEST ARTICLE DISPOSITION

Following completion of testing, any unused portion of the test article was discarded.



16.0 REFERENCES

- 16.1 United States Pharmacopeia, current version, Chapter <85> "Bacterial Endotoxin Testing".
- 16.2 FDA Guideline, "Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices", U.S. Dept. of Health and Human Services, 1987.
- 16.3 AppTec, BE215SOP "LAL – Kinetic Chromogenic Method", current revision.
- 16.4 AppTec, BE216SOP "Inhibition/Enhancement - Kinetic", current revision.



FINAL STUDY REPORT

STUDY TITLE

MATERIALS MEDIATED RABBIT PYROGEN TEST

TEST ARTICLE IDENTIFICATION

Ref.# EE 2197; Trade Name: Ti-3Al-2.5V with Vegetable
Sodium Stearate; RSPEC: Ti R2017-04, Stearate R1037-30
Generic Name: titanium alloy staples with lubricant
Supplier: Ethicon Endosurgery; Sterilization: 25-45 kGy Gamma
Lot # Device lot D4GV83

STUDY COMPLETION DATE

May 30, 2007

PERFORMING LABORATORY

AppTec Laboratory Services
2540 Executive Drive
St. Paul, MN 55120

SPONSOR

CANTOX Health Sciences International
2233 Argentia Road; Suite 308
Mississauga, ONT L5N 2X7 CANADA

PROTOCOL

900770N

PROJECT NUMBER

57611



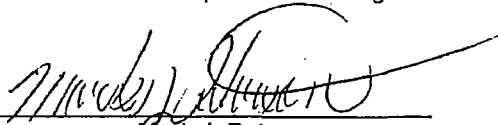
**QUALITY ASSURANCE UNIT SUMMARY****STUDY: Materials Mediated Rabbit Pyrogen Test.**

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the quality and integrity of the study.

<u>Critical Phase</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Test Article Preparation	04/26/07	04/30/07	05/30/07
Dosing	05/23/07	05/23/07	05/30/07
Final Report	05/30/07	05/30/07	05/30/07

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor:


Mark J. Peterson

Date:

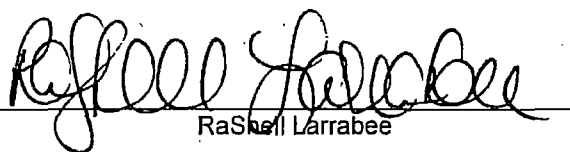
5/30/07

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of AppTec Laboratory Services, are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director:


RaShell Larrabee

Date:

5/30/07

Professional Personnel Involved:

Bonita L. Baskin, Ph.D.

Lisa Olson, BS

Don Palme, Ph.D.

Roxanne Miller, AA, CVT

RaShell Larrabee, BS, CVT

Lynn Wallum, CVT

Jean Mesarich, AA

Chief Executive Officer

Vice President St. Paul Operations

Vice President In-Life Services

Manager, In-Life Technical Services

Study Director

Animal Care Manager, In-Life Facility

Client Relations Manager



PROJECT NUMBER: 57611

SPONSOR: CANTOX Health Sciences International
2233 Argentia Road; Suite 308
Mississauga, ONT L5N 2X7 CANADA

RECORD RETENTION: An exact copy of the original final report and all raw data pertinent to this study will be stored at AppTec Laboratory Services, 2540 Executive Drive, St. Paul, MN 55120. It was the responsibility of the Sponsor to retain a sample of the test article.

CHARACTERIZATION: The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

PURPOSE: The purpose of this study is to determine if a saline extract of the test article causes a febrile response in rabbits.

TEST FACILITY: AppTec Laboratory Services
2540 Executive Drive
St. Paul, MN 55120

DATE SAMPLE RECEIVED: 04/25/07
STUDY INITIATION DATE: 04/25/07
REPEAT TEST START DATE: 05/23/07
STUDY COMPLETION DATE: 05/30/07
IACUC APPROVAL NUMBER: 50503

METHOD: This study was conducted in accordance with the USP Rabbit Pyrogen Test Procedure, United States Pharmacopeia 29 (2006), Pages 2546-2547, with a modification to the sample preparation and extraction procedure to establish a test for materials mediated pyrogens.

EXPERIMENTAL SUMMARY: The Sponsor supplied test article was extracted in 0.9% normal saline and injected into the marginal ear vein of each of three rabbits. Rectal temperatures were recorded for each animal prior to injection and between 1 and 3 hours post injection. The test article is considered non-pyrogenic if none of the animals has an individual temperature difference ≥ 0.5 °C when the baseline temperature is subtracted from the maximum temperature recorded between 1 and 3 hours post injection. One rabbit was observed with an individual temperature rise ≥ 0.5 °C, and therefore a retest was conducted using five other rabbits. If not more than three of the eight rabbits (from the initial test and retest) have individual rises in temperature of 0.5 °C or more and if the sum of the eight individual maximum temperature rises does not exceed 3.3 °C, the test article under examination meets the requirements for the absence of pyrogens. Only one of the eight rabbits had temperatures ≥ 0.5 °C and the sum of all the temperature differences did not exceed 3.3 °C; therefore, based on the requirements of this test, the test article, CANTOX Health Sciences International, Ref.# EE 2197; Trade Name: Ti-3Al-2.5V with Vegetable Sodium Stearate; RSPEC: Ti R2017-04, Stearate R1037-30; Generic Name: titanium alloy staples with lubricant; Supplier: Ethicon Endosurgery; Sterilization: 25-45 kGy Gamma, Lot # Device lot D4GV83 was determined to be non-pyrogenic.

DEVIATIONS/AMENDMENTS: None.

**TEST MATERIAL AND PREPARATION****Test Article Identification :**

Test Article Name: Ref.# EE 2197; Trade Name: Ti-3Al-2.5V with Vegetable Sodium Stearate; RSPEC: Ti R2017-04, Stearate R1037-30; Generic Name: titanium alloy staples with lubricant; Supplier: Ethicon Endosurgery; Sterilization: 25-45 kGy Gamma

Lot/Batch #: Device lot D4GV83

Physical State: Insoluble Material

Stability (Expiration): Not Applicable

Storage Conditions: Room Temperature

Safety Precautions: MSDS Followed

Sterilization: The Sponsor indicated that the test article was sterilized by 25-45 kGy Gamma prior to submitting the sample for testing.

Test Sample Preparation: The test article was extracted intact, placed into an Erlenmeyer Flask and prepared at a ratio of 60 cm² to 20 mL of extraction vehicle. The test article consisted of staples. Only the staples were included for testing, not the cartridge.

EXTRACT VEHICLE	VEHICLE AMOUNT	TEST ARTICLE SURFACE AREA
0.9% Normal Saline (NS)	151.3 mL	454.0 cm ²
0.9% Normal Saline (NS) Retest	198.9 mL	596.6 cm ²

Vehicle Identification:	Lot #	Supplied By:
0.9% Normal Saline (NS)	46-074-JT	Hospira
0.9% Normal Saline (NS) Retest	49-018-JT	Hospira

Extraction: The sample preparation was extracted for 72 ± 2 hours at 50 ± 2 °C.

The extract was cooled, shaken well and decanted into a sterile, dry, glass vessel. The extract was noted to be cloudy and contained particulates; therefore it was filtered with a 0.22 µm filter prior to dosing. The filtered extract was used within 24 hours of preparation.

TEST SYSTEM

Species/Strain: Albino Rabbits, New Zealand White Strain

Source: Charles River Canada, St. Constant, Quebec, Canada

Eligibility: The animals used on this study were used in previous saline extract pyrogen studies.

Age: Unspecified

Animal Identification: Individually numbered ear tags.

Body Weights: The animal body weights are listed in Tables 1 and 2. All rabbits had body weights greater than the protocol specified minimum of 1.5 kg.



HUSBANDRY

Receipt: Animals were received on 01/17/07, 02/28/07 (Initial test and Repeat test) . Each animal was examined for signs of disease and injury prior to entry into the research area.

Housing: One rabbit per suspended stainless steel cage. Housing dimensions complied with AAALAC International and NIH guidelines for this species.

Location: All housing and testing was conducted by AppTec Laboratory Services, St. Paul facility, 2540 Executive Drive, St. Paul, Minnesota 55120.

Environment: Animal rooms were maintained according to AAALAC International recommendations and the "Guide for Care and Use of Laboratory Animals". The laboratory and animal rooms were maintained as limited - access facilities.

Diet: Animals were supplied with certified commercial rabbit chow. No known contaminants present in the feed were expected to interfere with the test results.

Water: Animals were allowed free access (except during testing) to water obtained from the St. Paul municipal water supply. No known contaminants present in the water were expected to interfere with the results.

Compliance: The care, housing and handling of the animals were in compliance with AAALAC International and NIH guidelines as reported in the "Guide for the Care and Use of Laboratory Animals", National Research Council - ILAR, Revised 1996; (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals", Health Research Extension Act of 1985 (Public Law 99-158), Revised 1986, and USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1, 2 and 3, Animal Welfare, Final Rule 1989.

SELECTION OF ANIMALS: Animals were randomly placed in cages upon receipt and were placed on study as available. Animals considered unsuitable due to poor health or outlying body weight were excluded from the study. Only animals with baseline temperatures within a 1°C range from each other were used for the test. No animal with a baseline temperature exceeding 39.8°C was used for this test.

TEMPERATURE RECORDER: Thermistor probes and a Validator automated temperature recorder connected to an IBM compatible computer was used in this study to measure the rectal temperatures from the test rabbits.

PRETEST (SHAM TEST): Prior to using the rabbits for the first time in the pyrogen test, they were conditioned not more than 7 days before use in a Sham Test. This included all the steps and documentation as directed in the pyrogen test, except injection.

TEST ARTICLE ADMINISTRATION: The test article extract was warmed in an incubator set at $37 \pm 1^\circ\text{C}$ prior to dosing the rabbits at 10 mL/kg of body weight. The animals were injected within 30 minutes of recording the baseline temperature. The test sample was injected slowly and steadily into the marginal ear vein of each animal using a sterile pyrogen-free syringe of appropriate size with a sterile pyrogen-free 22 gauge IV catheter needle. Each injection was completed within 10 minutes of initiation.

OBSERVATIONS: The baseline temperature and the temperatures between 1 and 3 hours post injection were recorded at 30 minute intervals. Any restlessness or struggling of the test rabbits was also recorded.

EVALUATION CRITERIA: Temperature decreases were considered as a zero temperature rise. The individual rabbit baseline temperatures were subtracted from the maximum temperature recorded between 1 and 3 hours post injection. If none of the rabbits have a temperature difference $\geq 0.5^{\circ}\text{C}$, then the test article meets the requirements for absence of pyrogens. If any rabbit has an individual temperature rise $\geq 0.5^{\circ}\text{C}$, the Sponsor and Study Director may decide to conduct a retest using five other rabbits. After completion of the repeat test, if not more than three of the eight rabbits (from the initial and retest) have individual rises in temperature $\geq 0.5^{\circ}\text{C}$ and if the sum of the eight individual maximum temperature rises does not exceed 3.3°C , the material under examination meets the requirements for the absence of pyrogens.

REUSING ANIMALS: No rabbit used for this pyrogen test had been used for pyrogen testing within the last 48 hours, nor within 2 weeks following a maximum rise of its temperature of 0.5°C or more while being subjected to the pyrogen test, or following its having been given a test article that was judged pyrogenic.

RESULTS: The animal body weights, dose volumes, baseline temperatures and test temperature results for the initial test and retest are reported in Tables 1 and 2, respectively. During the 3 hour observation period of the initial test, rabbit # 3313 administered the test article extract had a temperature rise $\geq 0.5^{\circ}\text{C}$. Therefore, a retest was conducted using five (5) additional rabbits. During the 3 hour observation period of the retest, none of the rabbits administered the test article extract had a temperature rise $\geq 0.5^{\circ}\text{C}$. The sum of the temperature differences for all eight (8) rabbits did not exceed 3.3°C . See Table 3 for results summary.

ANALYSIS AND CONCLUSION: The baseline temperatures for all the rabbits in this study were within 1°C at the initiation of the test and no animals had a temperature above 39.8°C . During the dosing, rabbit # 3323 broke loose of the restrainer and dosing was interrupted in order to have the rabbit properly restrained. During the course of the study, none of the other animals were observed with undue activity such as struggling. These conditions indicate a valid test.

During the initial test, rabbit # 3313 had an individual temperature rise of 0.7°C from the baseline temperature. This indicated the need for a retest. During the retest, the maximum temperature rise for rabbits administered the test article extract was 0.2°C and the sum of the temperature differences of all eight (8) rabbits was 1.2°C therefore, the response was less than the USP 29 limit. These results indicate that the CANTOX Health Sciences International, Ref.# EE 2197; Trade Name: Ti-3Al-2.5V with Vegetable Sodium Stearate; RSPEC: Ti R2017-04, Stearate R1037-30; Generic Name: titanium alloy staples with lubricant; Supplier: Ethicon Endosurgery; Sterilization: 25-45 kGy Gamma, Lot # Device lot D4GV83 was non-pyrogenic.

STATISTICAL METHODS: None.

TECHNICAL REFERENCES:

ANSI/ AAMI 10993-11: 1993 American National Standard adopted from ISO Biological Evaluation of Medical Devices Part 11: Tests for Systemic Toxicity, pp. 267 – 273.

ISO 10993-12: 2002 Standard, Biological Evaluation of Medical Devices, Part 12-Sample Preparation and Reference Materials.

United States Pharmacopeia, USP 29: 2006, Section 151 Pyrogen Test, p. 2546-2547.



TABLE 1: PYROGEN TEST DATA (INITIAL TEST)

ANIMAL #	3313	3314	2987
Weight (kg)	3.2	3.1	3.8
Dose (mL)	32	31	38
BaselineTemp (°C)	39.1	39.8	39.7
1.0 hr Temp (°C)	39.4	39.8	39.8
1.5 hr Temp (°C)	39.5	39.8	39.8
2.0 hr Temp (°C)	39.5	40.0	39.8
2.5 hr Temp (°C)	39.6	40.0	39.6
3.0 hr Temp (°C)	39.8	40.0	39.8
Max Temp Rise (°C)	0.7 ^a	0.2	0.1

a -- Due to the 0.7 °C temperature rise, the test was repeated.

TABLE 2: PYROGEN TEST DATA (RETEST)

ANIMAL #	3314	3320	3323	2991	2994
Weight (kg)	3.3	3.3	3.9	4.0	4.0
Dose (mL)	33	33	39	40	40
BaselineTemp (°C)	39.8	39.7	39.8	39.7	39.8
1.0 hr Temp (°C)	39.7	39.5	39.5	39.7	38.9
1.5 hr Temp (°C)	39.8	39.4	39.1	39.8	34.0
2.0 hr Temp (°C)	39.8	39.4	38.7	39.8	39.4 ^c
2.5 hr Temp (°C)	39.7	39.5	39.4 ^c	39.9	39.0 ^c
3.0 hr Temp (°C)	39.7	39.3	39.4	39.8	38.9
Max Temp Rise (°C)	0.0	0.0 ^b	0.0 ^b	0.2	0.0 ^b

b -- Negative value reported as zero (0).

c- Animal was re-probed prior to temperature recording.

TABLE 3: PYROGEN TEST RESULTS SUMMARY

ANIMAL #	DATE TESTED	BASILINE TEMP (°C)	HIGHEST TEMP INCREASE (°C)	TEMP DIFFERENCE (°C)	PASS/ FAIL
3313	05/04/07	39.1	39.8	0.7	Fail
3314	05/04/07	39.8	40.0	0.2	Pass
2987	05/04/07	39.7	39.8	0.1	Pass
3314	05/23/07	39.8	39.8	0.0	Pass
3320	05/23/07	39.7	39.5	0.0 ^d	Pass
3323	05/23/07	39.8	39.5	0.0 ^d	Pass
2991	05/23/07	39.7	39.9	0.2	Pass
2994	05/23/07	39.8	39.4	0.0 ^d	Pass
SUM OF TEMPERATURE DIFFERENCES AND INTERPRETATION				1.2	PASS

d -- Negative value reported as zero (0).

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Lab No. 94T 18398 00

TU014-120, TH035-800
TU014-001, TH035-001

P.O. No. A40005
GLP

STUDY TITLE:

USP MUSCLE IMPLANTATION STUDY

(WITH HISTOPATHOLOGY)

IN THE RABBIT

(120 DAYS)

TEST ARTICLE:

CALCIUM STEARATE ON TI6AL-4V-WIRE-TEST

IDENTIFICATION NO.:

CODE RDM# 13-2-0355-00074-TEST

SPONSOR:

MARY K REINHARD D.V.M.
ETHICON ENDO-SURGERY
4545 CREEK ROAD
CINCINNATI, OH 45242



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CERTIFICATE OF QUALITY ASSURANCE INSPECTIONS

Ethicon Endo-Surgery

Representing Lab No(s). 94T-18398-00 and 94T-18399-00

Study: USP Muscle Implantation Test - 120 Day

Study Director: A. Eureste

Phase Inspected	Date	By	Reports to Management	Date
Sample Preparation and Implant	12-15-94	MLW		
Explant and Fixation	4-14-95	DWB		
Histology Preparation and Scoring	4-17-95	MLW	Periodic Status Report for 1-12-95. Periodic Status Report for 2-15-95. Periodic Status Report for 3-1-95. Periodic Status Report for 4-18-95. Periodic Status Report for 5-11-95.	
Final Report Review	5-15-95	DWB		

This study has been conducted in accordance with the provisions of the Good Laboratory Practice Regulations (21 CFR, Part 58).

David W. Briden
Quality Assurance Unit

Date of Release May 15, 1995

TU014-120, TH035-800
TU014-001, TH035-001

-i-

Lab No. 94T 18398 00

SUMMARY

The test article, Calcium Stearate on Ti6AL-4V-Wire-Test, Code RDM# 13-2-0355-00074-Test, was implanted in living muscle tissue of the rabbit. The muscle tissue was evaluated for evidence of irritation or toxicity in accordance with the guidelines of the current USP.

Sterile test and sponsor provided control implant samples were aseptically prepared. USP reference control strips were sterilized by steam in needles. Rabbits were implanted and were then euthanatized 120 days later. Muscle tissues were excised and the implant sites were examined macroscopically. A microscopic evaluation of representative implant sites from each rabbit was conducted to further define any tissue response.

Under the conditions of this study, the macroscopic reaction was not significant as compared to the negative and sponsor control implant material. The implanted test article met the USP requirements. Microscopically, the test article was classified as a nonirritant as compared to the USP reference and the sponsor control materials.

Study and Supervisory
Personnel:

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Don R. Pohl, BS
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David W. Briden, BS, MS
Technologist, Quality Assurance

Study Director:

Anita Eureste
Anita Eureste, BS, ALAT
Study Director, Toxicology

5-15-95
Date Completed

/lms
MMQ

NAMS

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-1-

Lab No. 94T 18398 00

INTRODUCTION

The test article identified below was evaluated for biocompatibility in accordance with the guidelines of the current USP. The purpose of the study was to evaluate the potential for a local irritant or toxic response to material implanted in direct contact with living muscle tissue. The animals were implanted on December 15, 1994, and muscles were explanted on April 14, 1995.

The study, initiated by protocol signature on December 6, 1994, was conducted in accordance with the provisions of the Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Certificate of Quality Assurance Inspections was issued in conjunction with this report.

MATERIALS

The test and control samples provided by the sponsor were identified and handled as follows:

Test Article:	Calcium Stearate on Ti6AL-4V-Wire-Test
Identification No:	Code RDM# 13-2-0355-00074-Test
Stability Testing:	In progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Sponsor Control Article:	Ti6AL4V-Wire-Pristine
Identification No:	Code RDM# 13-2-0355-00074-Control
Stability Testing:	In progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Storage Conditions:	Room temperature
Negative Control Article:	USP plastic 1 mm diameter negative control reference strips purchased by NAMSA from the offices of the US Pharmacopeial Convention and cut into 10 mm sections.
Preparation:	The test and sponsor control articles were received from the sponsor pre-cut and sterile in approximate 10 mm x 1 mm lengths. A minimum of six 16 ga needles (4 test, 2 sponsor control) were steam sterilized for each rabbit. The test and sponsor control article were aseptically loaded at implantation. In addition, a minimum of two USP strips of similar size were loaded into the same size needles as used for the test article for each rabbit. USP control strips were sterilized by steam.
Sample Disposition:	A representative portion of the sample was retained in NAMSA archives and any other sample was returned to the sponsor.



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Lab No. 94T 18398 00

METHODS

Animal Management:

Four healthy rabbits (2 male, 2 female) of the New Zealand White variety were obtained from an approved supplier traceable in NAMSA records. The animals were acclimated to the laboratory as specified in NAMSA SOP. Animals weighed 2.8 to 3.0 kg at the time of implantation; no particular rabbit gender or age was prescribed for this test in the USP. Animals were observed regularly for general health status.

Rabbits, identified by ear tag or tattoo, were individually housed in suspended cages and received a commercially pelleted rabbit feed on a daily basis; tap water was freely available. No diet or water analysis was performed since there were no contaminants suspected that could interfere with this study. Animal husbandry and environmental conditions conformed to current NAMSA SOP's which are based on the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23.

In order to reduce the number of animals used for testing, and to comply with the directives of the NAMSA Institutional Animal Care and Use Committee, rabbits on this study may have been previously used in an unrelated test model. Previously evaluated articles did not cause a response in the animals. Complete history of animal usage is traceable in laboratory records. Animal selection and reuse criteria are detailed in NAMSA Standard Operating procedures. Previously used animals are identified later in the report.

Experimental Procedure:

The fur was clipped from the area over the paravertebral muscles and the rabbits were sedated with acepromazine maleate (0.2 ml/kg dose). After the muscle area was wiped clean with 70% alcohol, each paravertebral muscle was infiltrated with 1% lidocaine to achieve local anesthesia on both sides of the vertebral column.

The test articles were dropped into needles pre-placed into the muscle at a 45° angle. A stylet was placed in the needle and the needle was withdrawn, leaving the sample in the paravertebral muscle. A minimum of four test article sections were implanted in each rabbit.

A minimum of two of the sponsor control articles were implanted in the opposite muscle in the same manner as the test article. A minimum of two needles containing the USP control strips were inserted into this same muscle at a 45° angle. A stylet was placed in the needle and the needle was withdrawn, leaving the sample in the muscle. Rabbits were returned to their respective cages following the procedure.

At 120 days, the rabbits were euthanatized with a sodium pentobarbital based drug. The paravertebral muscles were dissected free and fixed in 10% neutral buffered formalin (NBF) to facilitate cutting. After fixation, the muscles were methodically cut to locate the implanted test articles and the implanted controls in each rabbit. Capsule formation or other signs of irritation were scored and recorded as follows:

- 0 No capsule, no adverse reaction (other than minimal hemorrhage)
- 1 Up to 0.5 mm capsule or reaction area
- 2 0.6 to 1.0 mm capsule or reaction area
- 3 1.1 to 2.0 mm capsule or reaction area
- 4 >2.0 mm capsule or reaction area



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Lab No. 94T 18398 00

Representative tissue implant sites (test and controls) from each rabbit were excised, allowing a sufficient area around the site for proper histological preparation. These sections were routinely embedded, cut, and stained in hematoxylin and eosin (H&E) for evaluation by a board certified pathologist.

Mean macroscopic scores for test implants were compared to mean scores of the control sites. Calculations were rounded off to the nearest 0.1. A Reaction Index difference of 0.0 to 0.5 in scores (test vs. each control) was regarded as "not significant," 0.6 to 1.0 as a "trace," 1.1 to 2.0 as "slight," 2.1 to 3.0 as "moderate" and ≥ 3.1 as "marked." The requirements of the USP test were met if the difference between test and negative control score mean (macroscopic) was not greater than 1.0. The requirements of the test were not met if the difference between the test and negative control score for two (or more) implant sites exceeded "1" for any animal implanted.

A microscopic evaluation of representative implant sites from each rabbit was conducted to further define any tissue response. The histopathology evaluation was conducted by a board certified pathologist. The microscopic irritant response was graded as nonirritant, slight, moderate, or severe.

RESULTS

Macroscopic:

The findings for the macroscopic evaluation are shown below:

Rabbit/ Weight	Sample	Test	USP Control	Sponsor Control
83413♀ 4.1 kg	1	0	0	0
	2	0	0	0
	3	0		
	4	0		
83456♀ 4.4 kg	1	0	0	0
	2	0	0	0
	3	0		
	4	0		
82821♂* 4.4 kg	1	0	0	0
	2	0	0	0
	3	0		
	4	0		
82301♂* 4.0 kg	1	0	0	0
	2	0	0	0
	3	0		
	4	0		

Mean: 0.0 0.0 0.0

♂ = Male

♀ = Female

*Previous use history traceable in laboratory records.

Macroscopically, there was no difference between the reactions of the test and either of the control materials. This resulted in a macroscopic reaction classification of "not significant" tissue contact irritation.



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Lab No. 94T 18398 00

Microscopic:

The microscopic reaction was a nonirritant as compared to the USP negative control implant material and a nonirritant as compared to the sponsor provided control implant material (see report attached).

CONCLUSION

Under the conditions of this study, the macroscopic reaction was not significant as compared to the negative and sponsor control implant material. The implanted test article met the USP requirements. Microscopically, the test article was classified as a nonirritant as compared to the USP reference and the sponsor control materials.

RECORD STORAGE

All raw data, paraffin blocks, and tissue slides pertaining to this study and a copy of the final report are to be stored in the designated archive files at North American Science Associates, Inc. (NAMSA®), 2261 Tracy Road, Northwood, Ohio 43619-1397.



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LAB NO. 94T 18398 00
P.O. NO. A40085
GLP
ID NO. Code RDM#
13-2-0355-00074
-Test

Page 1 of 2

MUSCLE IMPLANTATION TEST IN RABBITS MICROSCOPIC EXAMINATION OF IMPLANT SITES

Test Article: Calcium Stearate on Ti6AL-4V-Wire-Test

Days Implanted: 120

	TEST				USP NEGATIVE CONTROL PLASTIC			
Rabbit Number:	82821	82301	83413	83456	82821	82301	83413	83456
Inflammation								
Polymorphonuclear	0	0	0	0	0	0	0	0
Lymphocytes	1	0	1	0	0	1	0	1
Plasma Cells	0	0	0	0	0	0	0	0
Macrophages	0	0	0	0	0	1	0	0
Giant Cells	1	0	0	0	0	0	0	1
Necrosis	0	0	0	0	0	0	0	0
SUB TOTAL (X2)	4	0	2	0	0	4	0	4
Fibroplasia	0	0	0	0	0	0	0	0
Fibrosis	1	2	1	1	1	1	2	1
Fatty Infiltrate	1	1	2	2	2	2	2	2
SUB TOTAL	2	3	3	3	3	3	4	3
TOTAL	17				21			
AVERAGE* TEST 4.3 (-) CONTROL 5.3 = 0.0								
*Used to determine Irritant Ranking Score shown below as the Conclusion. A negative difference was recorded as zero.								
Traumatic Necrosis	0	0	0	0	0	0	0	0
Foreign Debris	0	0	0	0	0	0	0	0
No. Sites Examined	4	4	4	4	2	2	2	2

Conclusion: Under the conditions of this study, the test article was considered a
X Nonirritant (0.0-2.9), Slight Irritant (3.0-8.9), Moderate Irritant (9.0-15.0),
 Severe Irritant (≥ 15.1) to the tissue as compared to the USP Negative Control Plastic.

Comments: Animal numbers 82821 and 82301 are males; 83413 and 83456 are females.

Daniel J. Hanson, M.D.
Diplomate
American Board of Pathology

Im Histologist

Date

Pathologist

Date



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P.O. NO. A40065
GLP
ID NO. Code RDM#
13-2-0355-00074
-Test

Page 2 of 2

MUSCLE IMPLANTATION TEST IN RABBITS MICROSCOPIC EXAMINATION OF IMPLANT SITES

Test Article: Calcium Stearate on Ti6AL-4V-Wire-Test

Days Implanted: 120

	TEST				REFERENCE CONTROL			
Rabbit Number:	82821	82301	83413	83456	82821	82301	83413	83456
Inflammation Polymorphonuclear	0	0	0	0	0	0	0	0
Lymphocytes	1	0	1	0	1	0	0	0
Plasma Cells	0	0	0	0	0	0	0	0
Macrophages	0	0	0	0	1	1	1	0
Giant Cells	1	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0
SUB TOTAL (X2)	4	0	2	0	4	2	2	0
Fibroplasia	0	0	0	0	0	0	0	0
Fibrosis	1	2	1	1	2	1	2	1
Fatty Infiltrate	1	1	2	2	2	2	2	2
SUB TOTAL	2	3	3	3	4	3	4	3
TOTAL	17				22			
AVERAGE* TEST 4.3 (-) CONTROL 5.5 = 0.0								
*Used to determine Irritant Ranking Score shown below as the Conclusion. A negative difference was recorded as zero.								
Traumatic Necrosis	0	0	0	0	0	0	0	0
Foreign Debris	0	0	0	0	0	0	0	0
No. Sites Examined	4	4	4	4	2	2	2	2

Conclusion: Under the conditions of this study, the test article was considered a
X Nonirritant (0.0-2.9), Slight Irritant (3.0-8.9), Moderate Irritant (9.0-15.0),
 Severe Irritant (≥ 15.1) to the tissue as compared to the Reference Control.

Comments: Animal numbers 82821 and 82301 are males; 83413 and 83456 are females.

Daniel J. Hanson, M.D.
Diplomate
American Board of Pathology

Ime Histologist (See page 1) Date --- Pathologist (See page 1) Date ---

MMG

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS.GOV@fda.hhs.gov or 301-796-8188

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Lab No. 94T 18398 00

TU014-860, TH035-800
TU014-001, TH035-001

P.O. No. A40065
GLP

STUDY TITLE:

USP MUSCLE IMPLANTATION STUDY

(WITH HISTOPATHOLOGY)

IN THE RABBIT

(60 DAYS)

TEST ARTICLE:

Calcium Stearate on Ti6AL-4V-Wire-Test

IDENTIFICATION NO.:

Code RDM# 13-2-0355-00074-Test

SPONSOR:

MARY K REINHARD D.V.M.
ETHICON ENDO-SURGERY
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CERTIFICATE OF QUALITY ASSURANCE INSPECTIONS

Ethicon Endo-Surgery

Representing Lab No(s). 94T-18398-00 and 94T-18399-00

Study: USP Muscle Implantation Test - 60 Day

Study Director: A. Eureste

Phase Inspected	Date	By	Reports to Management	Date
Sample Preparation & Implantation	12-15-94	MLW		
Explant & Fixation	2-13-95	MLW		
Score & Histology Preparation	2-14-95	DWB		
			Periodic Status Report 1-12-95	
			Periodic Status Report 2-15-95	
			Periodic Status Report 3-1-95	
Final Report Review	3-30-95	RTW		

This study has been conducted in accordance with the provisions of the Good Laboratory Practice Regulations (21 CFR, Part 58).

R. Z. Add
Quality Assurance Unit

Date of Release March 30, 1995

All reports are submitted as confidential communications. Authorization for duplication in whole or part is reserved pending our written approval, as a mutual protection.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS.GOV@fda.hhs.gov or 301-796-8188

TU014-860, TH035-800
TU014-001, TH035-001

-i-

Lab No. 94T 18398 00

SUMMARY

The test article, Calcium Stearate on Ti6AL-4V-Wire-Test, Code RDM# 13-2-0355-00074-Test, was implanted in living muscle tissue of the rabbit. The muscle tissue was evaluated for evidence of irritation or toxicity in accordance with the guidelines of the current USP.

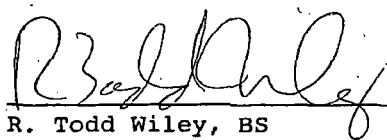
Sterile test and sponsor provided control implant samples were aseptically prepared. USP reference control strips were sterilized by steam in needles. Rabbits were implanted and were then euthanatized 60 days later. Muscle tissues were excised and the implant sites were examined macroscopically. A microscopic evaluation of representative implant sites from each rabbit was conducted to further define any tissue response.

Under the conditions of this study, the macroscopic reaction was not significant as compared to the negative and sponsor control implant material. The implanted test article met the USP requirements. Microscopically, the test article was classified as a nonirritant as compared to the USP reference and the sponsor control materials.

Study and Supervisory
Personnel:

Christina M. Tolles
Don R. Pohl, BS
Paul A. Bell, HT(ASCP)
Randy E. Russell, ALAT
Gregory L. Cox
Jeffrey J. Etue
Maria Ratliff
Kim Waters, BS
Timothy Jansen, BLS

Inspected by QA:


R. Todd Wiley, BS
Supervisor, GLP Compliance

Study Director:


Anita Eureste, BS, ALAT
Study Director, Toxicology

3-30-95
Date Completed

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TU014-860, TH035-800
TU014-001, TH035-001

-1-

Lab No. 94T 18398 00

INTRODUCTION

The test article identified below was evaluated for biocompatibility in accordance with the guidelines of the current USP. The purpose of the study was to evaluate the potential for a local irritant or toxic response to material implanted in direct contact with living muscle tissue. The animals were implanted on December 15, 1994, and muscles were explanted on February 13, 1995.

The study, initiated by protocol signature on December 6, 1994, was conducted in accordance with the provisions of the Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Certificate of Quality Assurance Inspections was issued in conjunction with this report.

MATERIALS

The test and control samples provided by the sponsor were identified and handled as follows:

Test Article:	Calcium Stearate on Ti6AL-4V-Wire-Test
Identification No:	Code RDM# 13-2-0355-00074-Test
Stability Testing:	In progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Sponsor Control Article:	Ti6AL4V-Wire-Pristine
Identification No:	Code RDM# 13-2-0355-00074-Control
Stability Testing:	In progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Storage Conditions:	Controlled room temperature
Negative Control Article:	USP plastic 1 mm diameter negative control reference strips purchased by NAMSA from the offices of the US Pharmacopeial Convention and cut into 10 mm sections.
Preparation:	The test and sponsor control articles were received from the sponsor pre-cut and sterile in approximate 10 mm x 1 mm lengths. A minimum of six 16 ga needles (4 test, 2 sponsor control) were steam sterilized for each rabbit. The test and sponsor control article were aseptically loaded at implantation. In addition, a minimum of two USP strips of similar size were loaded into the same size needles as used for the test article for each rabbit. USP control strips were sterilized by steam.



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-2-

Lab No. 94T 18398 00

METHODS

Animal Management:

Four healthy rabbits (2 male, 2 female) of the New Zealand White variety were obtained from an approved supplier traceable in NAMSA records. The animals were acclimated to the laboratory as specified in NAMSA SOP. Animals weighed 2.5 to 3.3 kg at the time of implantation; no particular rabbit gender or age was prescribed for this test in the USP. Animals were observed regularly for general health status.

Rabbits, identified by ear tag or tattoo, were individually housed in suspended cages and received a commercially pelleted rabbit feed on a daily basis; tap water was freely available. No diet or water analysis was performed since there were no contaminants suspected that could interfere with this study. Animal husbandry and environmental conditions conformed to current NAMSA SOP's which are based on the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23.

In order to reduce the number of animals used for testing, and to comply with the directives of the NAMSA Institutional Animal Care and Use Committee, rabbits on this study may have been previously used in an unrelated test model. Previously evaluated articles did not cause a response in the animals. Complete history of animal usage is traceable in laboratory records. Animal selection and reuse criteria are detailed in NAMSA Standard Operating procedures. Previously used animals are identified later in the report.

Experimental Procedure:

The fur was clipped from the area over the paravertebral muscles and the rabbits were sedated with acepromazine maleate (0.2 ml/kg dose). After the muscle area was wiped clean with 70% alcohol, each paravertebral muscle was infiltrated with 1% lidocaine to achieve local anesthesia on both sides of the vertebral column.

The test articles were dropped into needles pre-placed into the muscle at a 45° angle. A stylet was placed in the needle and the needle was withdrawn, leaving the sample in the paravertebral muscle. A minimum of four test article sections were implanted in each rabbit.

A minimum of two of the sponsor control articles were implanted in the opposite muscle in the same manner as the test article. A minimum of two needles containing the USP control strips were inserted into this same muscle at a 45° angle. A stylet was placed in the needle and the needle was withdrawn, leaving the sample in the muscle. Rabbits were returned to their respective cages following the procedure.

At 60 days, the rabbits were euthanatized with a sodium pentobarbital based drug. The paravertebral muscles were dissected free and fixed in 10% neutral buffered formalin (NBF) to facilitate cutting. After fixation, the muscles were methodically cut to locate the implanted test articles and the implanted controls in each rabbit. Capsule formation or other signs of irritation were scored and recorded as follows:

- 0 No capsule, no adverse reaction (other than minimal hemorrhage)
- 1 Up to 0.5 mm capsule or reaction area
- 2 0.6 to 1.0 mm capsule or reaction area
- 3 1.1 to 2.0 mm capsule or reaction area
- 4 >2.0 mm capsule or reaction area



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-3-

Lab No. 94T 18398 00

Representative tissue implant sites (test and controls) from each rabbit were excised, allowing a sufficient area around the site for proper histological preparation. These sections were routinely embedded, cut, and stained in hematoxylin and eosin (H&E) for evaluation by a board certified pathologist.

Mean macroscopic scores for test implants were compared to mean scores of the control sites. Calculations were rounded off to the nearest 0.1. A Reaction Index difference of 0.0 to 0.5 in scores (test vs. each control) was regarded as "not significant," 0.6 to 1.0 as a "trace," 1.1 to 2.0 as "slight," 2.1 to 3.0 as "moderate" and ≥ 3.1 as "marked." The requirements of the USP test were met if the difference between test and negative control score mean (macroscopic) was not greater than 1.0. The requirements of the test were not met if the difference between the test and negative control score for two (or more) implant sites exceeded "1" for any animal implanted.

A microscopic evaluation of representative implant sites from each rabbit was conducted to further define any tissue response. The histopathology evaluation was conducted by a board certified pathologist. The microscopic irritant response was graded as nonirritant, slight, moderate, or severe.

RESULTS

Clinical Observations: All four animals appeared clinically normal throughout the study.

Macroscopic:

The findings for the macroscopic evaluation are shown below:

Rabbit/ Weight	Sample	Test	USP Control	Sponsor Control
83409♀ 3.8 kg	1	0	0	0
	2	0	0	0
	3	0		
	4	0		
83411♀ 4.5 kg	1	0	0	0
	2	0	0	0
	3	0		
	4	0		
83088♂* 3.5 kg	1	0	0	0
	2	0	0	0
	3	0		
	4	0		
83094♂* 3.7 kg	1	0	0	0
	2	0	0	0
	3	0		
	4	0		

Mean: 0.0 0.0 0.0

♂ = Male

♀ = Female

*Previous use history traceable in laboratory records.



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TU014-860, TH035-800
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Lab No. 94T 18398 00

Macroscopically, there was no difference between the reactions of the test and either of the control materials. This resulted in a macroscopic reaction classification of "not significant" tissue contact irritation.

Microscopic:

The microscopic reaction was a nonirritant as compared to the USP negative control implant material and a nonirritant as compared to the sponsor provided control implant material (see report attached).

CONCLUSION

Under the conditions of this study, the macroscopic reaction was not significant as compared to the negative and sponsor control implant material. The implanted test article met the USP requirements. Microscopically, the test article was classified as a nonirritant as compared to the USP reference and the sponsor control materials.

RECORD STORAGE

All raw data, paraffin blocks, and tissue slides pertaining to this study and a copy of the final report are to be stored in the designated archive files at North American Science Associates, Inc. (NAMSA®), 2261 Tracy Road, Northwood, Ohio 43619-1397.



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LAB NO. 94T 18398 00
P.O. NO. A40065
GLP
ID NO. Code RDM#
13-2-0355-00074
-Test

Page 1 of 2

MUSCLE IMPLANTATION TEST IN RABBITS MICROSCOPIC EXAMINATION OF IMPLANT SITES

Test Article: Calcium Stearate on Ti6AL-4V-Wire-Test

Days Implanted: 60

Rabbit Number:	TEST				USP NEGATIVE CONTROL PLASTIC			
	83094	83088	83409	83411	83094	83088	83409	83411
Inflammation								
Polymorphonuclear	0	0	0	0	0	0	0	0
Lymphocytes	1	0	1	0	0	0	0	0
Plasma Cells	0	0	0	0	0	0	0	0
Macrophages	0	0	0	0	1	0	0	0
Giant Cells	0	0	0	0	1	0	1	1
Necrosis	0	0	0	0	0	0	0	0
SUB TOTAL (X2)	2	0	2	0	4	0	2	2
Fibroplasia	0	0	0	0	0	0	0	0
Fibrosis	1	1	1	2	2	1	1	2
Fatty Infiltrate	1	1	2	2	1	1	2	2
SUB TOTAL	2	2	3	4	3	2	3	4
TOTAL		15				20		
AVERAGE*	TEST	3.8	(-)	CONTROL	5	=	0.0	

*Used to determine Irritant Ranking Score shown below as the Conclusion. A negative difference was recorded as zero.

Traumatic Necrosis	0	0	0	0	0	0	0	0
Foreign Debris	0	0	0	0	0	0	0	0
No. Sites Examined	4	4	4	4	2	2	2	2

Comments: Animal numbers 83094 and 83088 are males; 83409 and 83411 are females.

Conclusion: Under the conditions of this study, the test article was considered a
X Nonirritant (0.0-2.9), Slight Irritant (3.0-8.9), Moderate Irritant (9.0-15.0),
 Severe Irritant (≥ 15.1) to the tissue as compared to the USP Negative Control Plastic.

Daniel J. Hanson, M.D.
Diplomate
American Board of Pathology

Ima Histologist

Date 3/21/95

Pathologist

Date 3/20/96



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ID NO. Code RDM#
13-2-0355-00074
-Test

Page 2 of 2

MUSCLE IMPLANTATION TEST IN RABBITS MICROSCOPIC EXAMINATION OF IMPLANT SITES

Test Article: Calcium Stearate on Ti6AL-4V-Wire-Test

Days Implanted: 60

Rabbit Number:	TEST				REFERENCE CONTROL			
	83094	83088	83409	83411	83094	83088	83409	83411
Inflammation								
Polymorphonuclear	0	0	0	0	1	0	0	0
Lymphocytes	1	0	1	0	1	0	2	1
Plasma Cells	0	0	0	0	0	0	0	0
Macrophages	0	0	0	0	1	1	1	0
Giant Cells	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0
SUB TOTAL (X2)	2	0	2	0	6	2	6	2
Fibroplasia	0	0	0	0	0	0	0	0
Fibrosis	1	1	1	2	2	2	1	2
Fatty Infiltrate	1	1	2	2	1	1	1	1
SUB TOTAL	2	2	3	4	3	3	2	3
TOTAL		15				27		
AVERAGE*	TEST	3.8	(-)	CONTROL	6.8	=	0.0	

*Used to determine Irritant Ranking Score shown below as the Conclusion. A negative difference was recorded as zero.

Traumatic Necrosis	0	0	0	0	0	0	0	0
Foreign Debris	0	0	0	0	0	0	0	0
No. Sites Examined	4	4	4	4	2	2	2	2

Comments: Animal numbers 83094 and 83088 are males; 83409 and 83411 are females.

Conclusion: Under the conditions of this study, the test article was considered a
X Nonirritant (0.0-2.9), Slight Irritant (3.0-8.9), Moderate Irritant (9.0-15.0),
 Severe Irritant (≥ 15.1) to the tissue as compared to the Reference Control.

Daniel J. Hanson, M.D.
Diplomate
American Board of Pathology

Imms Histologist

Date 3/28/95 Pathologist

Date 3/20/95

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TU014-830, TH035-800
TU014-001, TH035-001

Lab No. 94T 18398 00

P.O. No. A40005
GLP

STUDY TITLE:

USP MUSCLE IMPLANTATION STUDY

(WITH HISTOPATHOLOGY)

IN THE RABBIT

(30 DAYS)

TEST ARTICLE:

Calcium Stearate on Ti6AL-4V-Wire-Test

IDENTIFICATION NO.:

Code RDM# 13-2-0355-00074-Test

SPONSOR:

MARY K REINHARD D.V.M.
ETHICON ENDO-SURGERY
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CERTIFICATE OF QUALITY ASSURANCE INSPECTIONS

Ethicon Endo-Surgery

Representing Lab No(s). 94T-18398-00

Study: USP Muscle Implantation Test - 30 Day

Study Director: E. Eureste

Phase Inspected	Date	By	Reports to Management	Date
Sample Preparation	12-13-94	DWB		
Implantation	12-13-94	DWB		
Explant & Fixation	1-12-95	MLW		
Histology Preparation & Scoring	1-13-95	MLW		
			Periodic Status Report 1-12-95	
			Periodic Status Report 2-15-95	
			Periodic Status Report 3-1-95	
Final Report Review	3-7-95	DWB		

This study has been conducted in accordance with the provisions of the Good Laboratory Practice Regulations (21 CFR, Part 58).

David W. Biden
Quality Assurance Unit

Date of Release March 7, 1995

All reports are submitted as confidential communications. Authorization for duplication in whole or part is reserved pending our written approval, as a mutual protection.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS.GOV@fda.hhs.gov or 301-796-8188

TU014-830, TH035-800
TU014-001, TH035-001

-i-

Lab No. 94T 18398 00

SUMMARY

The test article, Calcium Stearate on Ti6AL-4V-Wire-Test, Code RDM# 13-2-0355-00074-Test, was implanted in living muscle tissue of the rabbit. The muscle tissue was evaluated for evidence of irritation or toxicity in accordance with the guidelines of the current USP.

Sterile test and sponsor provided control implant samples were aseptically prepared. USP reference control strips were sterilized by steam in needles. Rabbits were implanted and were then euthanatized 30 days later. Muscle tissues were excised and the implant sites were examined macroscopically. A microscopic evaluation of representative implant sites from each rabbit was conducted to further define any tissue response.

Under the conditions of this study, the macroscopic reaction was not significant as compared to the negative and sponsor control implant material. The implanted test article met the USP requirements. Microscopically, the test article was classified as a nonirritant as compared to the USP reference and the sponsor control materials.

Study and Supervisory
Personnel:

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Anita Euresste, BS, ALAT
Study Director, Toxicology

3-7-95
Date Completed

/ync
mmg
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TU014-830, TH035-800
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-1-

Lab No. 94T 18398 00

INTRODUCTION

The test article identified below was evaluated for biocompatibility in accordance with the guidelines of the current USP. The purpose of the study was to evaluate the potential for a local irritant or toxic response to material implanted in direct contact with living muscle tissue. The animals were implanted on December 13, 1994, and muscles were explanted on January 12, 1995.

The study, initiated by protocol signature on December 6, 1994, was conducted in accordance with the provisions of the Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Certificate of Quality Assurance Inspections was issued in conjunction with this report.

MATERIALS

The test and control samples provided by the sponsor were identified and handled as follows:

Test Article:	Calcium Stearate on Ti6AL-4V-Wire-Test
Identification No:	Code RDM# 13-2-0355-00074-Test
Stability Testing:	In progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Sponsor Control Article:	Ti6AL4V-Wire-Pristine
Identification No:	Code RDM# 13-2-0355-00074-Control
Stability Testing:	In progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Storage Conditions:	Controlled room temperature
Negative Control Article:	USP plastic 1 mm diameter negative control reference strips purchased by NAMSA from the offices of the US Pharmacopeial Convention and cut into 10 mm sections.
Preparation:	The test and sponsor control articles were received from the sponsor pre-cut and sterile in approximate 10 mm x 1 mm lengths. A minimum of six 16 ga needles (4 test, 2 sponsor control) were steam sterilized for each rabbit. The test and sponsor control article were aseptically loaded at implantation. In addition, a minimum of two USP strips of similar size were loaded into the same size needles as used for the test article for each rabbit. USP control strips were sterilized by steam.



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Lab No. 94T 18398 00

METHODS

Animal Management:

Four healthy naive rabbits (2 male, 2 female) of the New Zealand White variety were obtained from an approved supplier traceable in NAMSA records. The animals were acclimated to the laboratory as specified in NAMSA SOP. Animals weighed 2.9 to 3.4 kg at the time of implantation; no particular rabbit gender or age was prescribed for this test in the USP. Animals were observed regularly for general health status.

Rabbits, identified by ear tag or tattoo, were individually housed in suspended cages and received a commercially pelleted rabbit feed on a daily basis; tap water was freely available. No diet or water analysis was performed since there were no contaminants suspected that could interfere with this study. Animal husbandry and environmental conditions conformed to current NAMSA SOP's which are based on the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23.

Experimental Procedure:

The fur was clipped from the area over the paravertebral muscles and the rabbits were sedated with acepromazine maleate (0.2 ml/kg dose). After the muscle area was wiped clean with 70% alcohol, each paravertebral muscle was infiltrated with 1% lidocaine to achieve local anesthesia on both sides of the vertebral column.

The test articles were dropped into needles pre-placed into the muscle at a 45° angle. A stylet was placed in the needle and the needle was withdrawn, leaving the sample in the paravertebral muscle. A minimum of four test article sections were implanted in each rabbit.

A minimum of two of the sponsor control articles were implanted in the opposite muscle in the same manner as the test article. A minimum of two needles containing the USP control strips were inserted into this same muscle at a 45° angle. A stylet was placed in the needle and the needle was withdrawn, leaving the sample in the muscle. Rabbits were returned to their respective cages following the procedure.

At 30 days, the rabbits were euthanatized with a sodium pentobarbital based drug. The paravertebral muscles were dissected free and fixed in 10% neutral buffered formalin (NBF) to facilitate cutting. After fixation, the muscles were methodically cut to locate the implanted test articles and the implanted controls in each rabbit. Capsule formation or other signs of irritation were scored and recorded as follows:

- 0 No capsule, no adverse reaction (other than minimal hemorrhage)
- 1 Up to 0.5 mm capsule or reaction area
- 2 0.6 to 1.0 mm capsule or reaction area
- 3 1.1 to 2.0 mm capsule or reaction area
- 4 >2.0 mm capsule or reaction area

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TU014-001, TH035-001

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Lab No. 94T 18398 00

Representative tissue implant sites (test and controls) from each rabbit were excised, allowing a sufficient area around the site for proper histological preparation. These sections were routinely embedded, cut, and stained in hematoxylin and eosin (H&E) for evaluation by a board certified pathologist.

Mean macroscopic scores for test implants were compared to mean scores of the control sites. Calculations were rounded off to the nearest 0.1. A Reaction Index difference of 0.0 to 0.5 in scores (test vs. each control) was regarded as "not significant," 0.6 to 1.0 as a "trace," 1.1 to 2.0 as "slight," 2.1 to 3.0 as "moderate" and ≥ 3.1 as "marked." The requirements of the USP test were met if the difference between test and negative control score mean (macroscopic) was not greater than 1.0. The requirements of the test were not met if the difference between the test and negative control score for two (or more) implant sites exceeded "1" for any animal implanted.

A microscopic evaluation of representative implant sites from each rabbit was conducted to further define any tissue response. The histopathology evaluation was conducted by a board certified pathologist. The microscopic irritant response was graded as nonirritant, slight, moderate, or severe.

RESULTS

Macroscopic:

The findings for the macroscopic evaluation are shown below:

Rabbit/ Weight	Sample	Test	USP Control	Sponsor Control
83439♀ 3.6 kg	1	0	0	0
	2	0	0	0
	3	0		
	4	0		
83443♀ 3.6 kg	1	0	0	0
	2	0	0	0
	3	0		
	4	0		
83395♂ 3.9 kg	1	0	0	0
	2	0	0	0
	3	0		
	4	0		
83396♂ 4.1 kg	1	0	0	0
	2	0	0	0
	3	0		
	4	0		

Mean: 0.0 0.0 0.0

♂ = Male
♀ = Female

Macroscopically, there was no difference between the reactions of the test and either of the control materials. This resulted in a macroscopic reaction classification of "not significant" tissue contact irritation.



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Lab No. 94T 18398 00

Microscopic:

The microscopic reaction was a nonirritant as compared to the USP negative control implant material and a nonirritant as compared to the sponsor provided control implant material (see report attached).

CONCLUSION

Under the conditions of this study, the macroscopic reaction was not significant as compared to the negative and sponsor control implant material. The implanted test article met the USP requirements. Microscopically, the test article was classified as a nonirritant as compared to the USP reference and the sponsor control materials.

RECORD STORAGE

All raw data, paraffin blocks, and tissue slides pertaining to this study and a copy of the final report are to be stored in the designated archive files at North American Science Associates, Inc. (NAmsA®), 2261 Tracy Road, Northwood, Ohio 43619-1397.



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GLP
ID NO. Code RDM#
13-2-0355-00074
-Test

Page 1 of 2

MUSCLE IMPLANTATION TEST IN RABBITS MICROSCOPIC EXAMINATION OF IMPLANT SITES

Test Article: Calcium Stearate on Ti6AL-4V-Wire-Test

Days Implanted: 30

Rabbit Number:	TEST				USP NEGATIVE CONTROL PLASTIC			
	83396	83395	83443	83439	83396	83395	83443	83439
Inflammation								
Polymorphonuclear	0	1	0	0	1	0	0	0
Lymphocytes	1	1	1	1	1	1	1	1
Plasma Cells	0	0	0	0	0	0	0	0
Macrophages	1	1	1	1	1	1	1	1
Giant Cells	0	1	0	0	0	0	1	1
Necrosis	0	0	0	0	0	0	0	0
SUB TOTAL (X2)	4	8	4	4	6	4	6	6
Fibroplasia	0	0	0	0	0	0	0	0
Fibrosis	2	2	2	2	2	2	2	2
Fatty Infiltrate	1	0	0	1	0	0	0	0
SUB TOTAL	3	2	2	3	2	2	2	2
TOTAL	30				30			
AVERAGE*	TEST	7.5	(-)	CONTROL	7.5	=	0.0	

*Used to determine Irritant Ranking Score shown below as the Conclusion. A negative difference was recorded as zero.

Traumatic Necrosis	0	0	0	0	0	0	0	0
Foreign Debris	0	0	1	0	0	0	0	0
No. Sites Examined	4	3	4	4	2	2	2	1

Comments: Animal numbers 83396 and 83395 are males; 83443 and 83439 are females.
83443T - Foreign body in one site contaminant - giant cell reaction not scored.

Conclusion: Under the conditions of this study, the test article was considered a
☒ Nonirritant (0.0-2.9), ☐ Slight Irritant (3.0-8.9), ☐ Moderate Irritant (9.0-15.0),
☐ Severe Irritant (≥ 15.1) to the tissue as compared to the USP Negative Control Plastic.

Daniel J. Hanson, M.D.
Diplomate
American Board of Pathology

Imms Histologist

Date

Pathologist

Date



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13-2-0355-00074
-Test

Page 2 of 2

MUSCLE IMPLANTATION TEST IN RABBITS MICROSCOPIC EXAMINATION OF IMPLANT SITES

Test Article: Calcium Stearate on Ti6AL-4V-Wire-Test

Days Implanted: 30

Rabbit Number:	TEST				REFERENCE CONTROL			
	83396	83395	83443	83439	83396	83395	83443	83439
Inflammation								
Polymorphonuclear	0	1	0	0	0	1	0	1
Lymphocytes	1	1	1	1	1	1	1	1
Plasma Cells	0	0	0	0	0	0	0	0
Macrophages	1	1	1	1	1	1	1	1
Giant Cells	0	1	0	0	0	1	0	0
Necrosis	0	0	0	0	0	0	0	0
SUB TOTAL (X2)	4	8	4	4	4	8	4	4
Fibroplasia	0	0	0	0	0	0	1	0
Fibrosis	2	2	2	2	2	2	2	2
Fatty Infiltrate	1	0	0	1	0	0	1	0
SUB TOTAL	3	2	2	3	2	2	4	2
TOTAL		30				30		
AVERAGE*	TEST	7.5	(-)	CONTROL	7.5	=	0.0	

*Used to determine Irritant Ranking Score shown below as the Conclusion. A negative difference was recorded as zero.

Traumatic Necrosis	0	0	0	0	0	0	0	0
Foreign Debris	0	0	1	0	0	0	0	0
No. Sites Examined	4	3	4	4	2	2	2	2

Comments: Animal numbers 83396 and 83395 are males; 83443 and 83439 are females.

Conclusion: Under the conditions of this study, the test article was considered a
X Nonirritant (0.0-2.9), Slight Irritant (3.0-8.9), Moderate Irritant (9.0-15.0),
 Severe Irritant (≥ 15.1) to the tissue as compared to the Reference Control.

Daniel J. Hanson, M.D.
Diplomate
American Board of Pathology

ImS Histologist

Date 3-3-95

Pathologist

Date 3/5/95

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Lab No. 94T 17135 00

TU014-807/S
TH035-800

P.O. No. A40065
GLP

STUDY TITLE:

MUSCLE IMPLANTATION STUDY
IN THE RABBIT WITH HISTOPATHOLOGY
(7 DAYS)

TEST ARTICLE:

CALCIUM STEARATE ON Ti6Al-4V WIRE

IDENTIFICATION NO.:

CODE RDM # 13-2-0355-00074 TEST

SPONSOR:

MARY K. REINHARD, D.V.M.
ETHICON ENDO-SURGERY
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CERTIFICATE OF QUALITY ASSURANCE INSPECTIONS

Ethicon Endo-Surgery

Representing Lab No(s). 94T-17135-00

Study: Implantation Test - 7 Day

Study Director: K. A. Herkowski

Phase Inspected	Date	By	Reports to Management	Date
Extract Preparation & Blood Draw Implantation	11-15-94	DWB		
Termination & Fixation	11-22-94	DWB		
Scoring & Histology Preparation	11-23-94	MLW		
			Periodic Status Report 12-12-94	
			Periodic Status Report 1-12-95	
			Periodic Status Report 2-15-95	
			Periodic Status Report 3-1-95	
Final Report Review	3-30-95	RTW		

This study has been conducted in accordance with the provisions of the Good Laboratory Practice Regulations (21 CFR, Part 58).

R. J. J. J. J.
Quality Assurance Unit

Date of Release March 30, 1995

TU014-807/S
TH035-800

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Lab No. 94T 17135 00

SUMMARY

The test article, Calcium Stearate on Ti6Al-4V Wire, Code RDM # 13-2-0355-00074 TEST, and a reference control article provided by the sponsor were implanted in living muscle tissue of the rabbit. The muscle tissue was evaluated for evidence of irritation. The systemic toxicity of the test article was also evaluated.

Sterile test and reference control implant samples were aseptically loaded into needles. Four rabbits were implanted with the test article. An additional group of four rabbits were implanted with the control article. Rabbits were then euthanatized 7 days later. Prior to implantation and at termination, blood specimens were collected for hematology and clinical chemistry analyses. At termination, the muscle implant sites were examined macroscopically. A gross necropsy was also conducted and designated tissues were excised. A microscopic evaluation of representative implant sites from each rabbit was conducted to further define any tissue response.

Under the conditions of this study, the macroscopic reaction of the implanted test article was not significant as compared to the reference control implant material. Microscopically, the test article was classified as a nonirritant as compared to the reference control article. There was no evidence of systemic toxicity from either of the implant materials. Daily clinical observations, body weights, necropsy findings, hematology and clinical chemistry values were within normal limits and were similar between test and control treatment groups.

Study and Supervisory
Personnel:

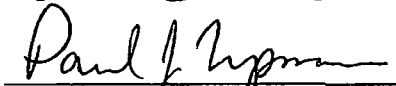
Paul A. Bell, HT(ASCP)
Christina M. Tolles
Merle J. Heineke, BS, LAT
Mary Jo Shepherd, DVM
Don R. Pohl, BS
Gregory L. Cox

Steven A. Schaaf
Sterling E. Calkins, BS
Tina M. Cox, BS, LAT
Suzanne Wallace
Marsha Ettl, RN, LAT
Jeffrey J. Etue

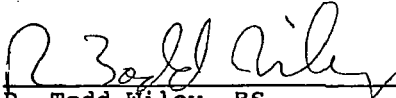
Approved by:


Gina M. Johnson, BA, LAT
Supervisor, GLP Study Directors

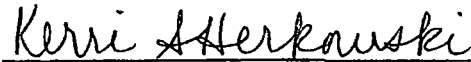
Approved by:

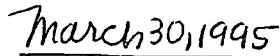

Paul J. Upman, BA, LAT
Senior Scientist

Inspected by QA:

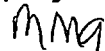

R. Todd Wiley, BS
Supervisor, GLP Compliance

Study Director:


Kerri A. Herkowski, BS
Study Director, Toxicology


March 30, 1995
Date Completed

/lmg



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Lab No. 94T 17135 00

INTRODUCTION

The test and control articles identified below were evaluated for biocompatibility. The purpose of the study was to evaluate the potential for a local irritant or toxic response to material implanted in direct contact with living muscle tissue. The systemic toxicity of the articles was also evaluated. The animals were implanted on November 15, 1994, and muscles were explanted on November 22, 1994.

The study, initiated by protocol signature on November 9, 1994, was conducted in accordance with the provisions of the Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Certificate of Quality Assurance Inspections was issued in conjunction with this report.

MATERIALS

The samples provided by the sponsor were identified and handled as follows:

Test Article:	Calcium Stearate on Ti6Al-4V Wire
Identification No:	Code RDM # 13-2-0355-00074 TEST
Stability Testing:	In progress (per sponsor)
Expiration Date:	Stable for the duration of intended testing (per sponsor)
Reference Control Article:	Ti6Al-4V Wire
Identification No:	Code RDM# 13-2-0355-00074 CONTROL
Stability Testing:	In progress (per sponsor)
Expiration Date:	Stable for the duration of intended testing (per sponsor)
Storage Conditions:	All samples were stored at room temperature
Preparation:	A minimum of six sections each of the test and control article per rabbit, each approximately 1 mm x 10 mm, were aseptically loaded into 16 ga needles.
Sample Disposition:	Any remaining sample was returned to the sponsor.

METHODS

Animal Management:

Eight healthy naive rabbits (four male, four female) of the New Zealand White variety were obtained from an approved supplier traceable in NAMSA records. The animals were acclimated to the laboratory for 7 days. Animals weighed at least 2.5 kg at the time of implantation; no particular age was prescribed for this test. Animals were observed regularly for general health status.

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Rabbits, identified by ear tag or tattoo, were individually housed in suspended cages and received a commercially pelleted rabbit feed on a daily basis (see Laboratory Observations for exceptions); tap water was freely available. No diet or water analysis was performed since there were no contaminants suspected that could interfere with this study. Animal husbandry and environmental conditions conformed to current NAMSA SOP's which are based on the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23.

Experimental Procedure:

The fur was clipped from the area over the paravertebral muscles and the rabbits were sedated with acepromazine maleate (0.2 ml/kg dose). Blood samples were collected via the central ear artery (see LABORATORY OBSERVATIONS). After the muscle area was wiped clean with 70% alcohol, each paravertebral muscle was infiltrated with 1% lidocaine to achieve local anesthesia on both sides of the vertebral column.

Needles containing test article were inserted into the muscle at a 45° angle. A stylet was placed in the needle and the needle was withdrawn, leaving the sample in the paravertebral muscle. Six test article sections were implanted, three per paravertebral muscle, in each test rabbit (two males, two females). Six reference control articles were similarly implanted in each designated control animal (two males, two females). Rabbits were returned to their respective cages following the procedure.

Terminal Procedure:

After 7 days, blood samples were collected via the central ear artery. After blood collection the rabbits were anesthetized with an intramuscular injection (hind leg muscle) of ketamine hydrochloride and xylazine (34 mg/kg + 5 mg/kg) at a dose of 0.6 ml/kg. If necessary, the animals were placed on halothane/oxygen inhalation for continued anesthesia. The rabbits were then euthanatized by exsanguination while anesthetized. The paravertebral muscles were dissected free and fixed in 10% neutral buffered formalin (NBF) to facilitate cutting. A gross visceral necropsy was conducted on each animal. Representative sections of the following organs and tissues were harvested and placed in NBF: adrenal gland, aorta, bone marrow, femur cross section, cecum, colon, corpus uteri, duodenum, epididymis, esophagus, eye, heart, ileum, jejunum, kidney, liver, lung, lymph node, mammary gland, sciatic nerve, seminal vesicle, skin, brain at three levels, spleen, stomach, testis, thymic region, thyroid/parathyroid, trachea, urinary bladder, vagina, prostate, pituitary, pancreas, and ovary.

After fixation, the muscles were methodically cut to locate the test and control implant sites. Capsule formation or other signs of irritation were scored and recorded as follows:

- 0 No capsule, no adverse reaction (other than minimal hemorrhage)
- 1 Up to 0.5 mm capsule or reaction area
- 2 0.6 to 1.0 mm capsule or reaction area
- 3 1.1 to 2.0 mm capsule or reaction area
- 4 >2.0 mm capsule or reaction area



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Located sites from each rabbit were excised, allowing a sufficient area around the site for proper histological preparation. These sections and all of the tissues indicated above were routinely embedded, cut, and stained in hematoxylin and eosin (H&E). The test and control muscle tissue sites were evaluated by a board certified pathologist. The remaining tissue slides were sent to the sponsor for evaluation. The sponsor has assumed responsibility for this data.

Laboratory Observations:

1. Individual body weights were recorded prior to implantation and at termination.
2. Animals were observed daily for general health and evidence of systemic toxicity.
3. Prior to implantation and just prior to termination, blood samples were collected for hematology and clinical chemistry analyses. Animals were deprived of food up to 24 hours prior to each blood draw.
 - a. Hematology analyses included hematocrit, hemoglobin, erythrocyte count, total and differential leukocyte count (see DEVIATION).
 - b. Clinical chemistry tests (diagnostic multi-chem profile) included: alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, chloride, creatinine, γ -glutamyltranspeptidase, glucose, ornithine carbamyl transferase, phosphorus, potassium, total protein, sodium, and blood urea nitrogen.

Hematology and clinical chemistry analyses were conducted by Roche Bioveterinary Services (Division of Roche Biomedical Laboratories, Inc.).

Evaluations and Statistics:

Average macroscopic scores for test implants were compared to average scores of the control sites. Calculations were rounded off to the nearest 0.1. A difference of 0.0 to 0.5 in scores (test minus control) was regarded as "not significant," 0.6 to 1.0 as a "trace," 1.1 to 2.0 as "slight," 2.1 to 3.0 as "moderate" and ≥ 3.1 as "marked." Numerical differences ≥ 1.0 were considered significant.

A microscopic evaluation of the implant sites from each rabbit was conducted to further define any tissue response. The histopathology evaluation was conducted by a board certified pathologist. Cellular changes in the muscle tissue implant sites were scored on a 0-4 basis based on severity of the change. Any resulting irritant response indicated by a difference between test and control values were graded as nonirritant, slight, moderate, or severe.

Statistical analysis of the hematology and clinical chemistry data was not warranted.

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DEVIATION

Per protocol, the hematology analyses should have included a measure of clotting time (including prothrombin time, thromboplastin time, and platelet count). However, this was not conducted. This omission did not affect the outcome of the study.

RESULTS AND DISCUSSION

Individual observations appear in Table I.

Clinical Observations: All animals appeared clinically normal throughout the duration of the study. No behavioral changes or signs of toxicity were observed.

Body Weight: Individual weight gain and group mean body weights for both male and female rabbits were considered clinically acceptable.

Necropsy: There were no macroscopic changes in the viscera at necropsy that could be attributed to the test or control article.

Clinical Chemistry and Hematology: The hematology and clinical chemistry values were similar between treatment groups and similar between pretreatment and termination blood draws. No statistical analysis was deemed necessary. Average clinical chemistry and hematology values appear in Table II and III.

Macroscopic Observations: There were no macroscopic findings ("0" scores) for any of the test and control sites.

Microscopic Observations: The microscopic reactions around the test and reference sites were nearly identical; neither would be considered an irritant to the muscle tissue (see report attached).

CONCLUSION

Under the conditions of this study, neither the macroscopic or microscopic reaction to the test article was significant as compared to the reference control implant material. There was no evidence of systemic toxicity from either of the implant materials. Daily clinical observations, body weights, necropsy findings, hematology and clinical chemistry values were within normal limits and were similar between test and control treatment groups.

RECORD STORAGE

All raw data, paraffin blocks, muscle tissue slides and wet tissue remnants pertaining to this study and a copy of the final report are to be stored in the designated archive files at North American Science Associates, Inc. (NAMSA®), 2261 Tracy Road, Northwood, Ohio 43619-1397. All other tissue slides were forwarded to the sponsor. The sponsor has assumed responsibility for the retention of these slides.



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TABLE ISUMMARY OF OBSERVATIONS

Rabbit Number	Sex/Group	Weight (kg)		CLINICAL OBSERVATIONS (DAYS 0-7)	NECROPSY (DAY 7)
		Day 0	Day 7		
82700	M/C	2.6	2.9	Appeared normal	MN
82704	M/C	2.5	2.7	Appeared normal	MN
82691	F/C	2.5	2.8	Appeared normal	MN
82693	F/C	2.8	3.0	Appeared normal	MN
82697	M/T	3.0	3.1	Appeared normal	MN
82698	M/T	3.0	3.1	Appeared normal	MN
82689	F/T	2.5	2.6	Appeared normal	MN
82688	F/T	2.7	2.9	Appeared normal	MN

M = Male C = Control
 F = Female T = Test
 MN = Macroscopically Normal



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TABLE II**AVERAGE HEMATOLOGY VALUES****Pretreatment:**

	PARAMETERS						
	WBC	RBC	HGB	MCV	MCH	HCT	MCHC
Control	4.6	5.8	12.7	66	21.6	38.2	33.1
Test	5.3	5.6	12.4	68	22.1	37.6	32.9

	PARAMETERS						
	POLY	BANDS	LYMPH	META	MONO	EOS	BASO
Control	63	0	30	0	3	2	3
Test	56	0	38	0	1	1	4

Termination:

	PARAMETERS						
	WBC	RBC	HGB	MCV	MCH	HCT	MCHC
Control	6.9	5.7	11.9	66	21.0	37.2	31.9
Test	8.3	5.4	11.6	69	21.6	36.7	31.6

	PARAMETERS						
	POLY	BANDS	LYMPH	META	MONO	EOS	BASO
Control	39	0	52	0	2	3	5
Test	30	0	62	0	2	2	4



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TABLE III

AVERAGE CLINICAL CHEMISTRY VALUES**Pretreatment:**

	CA	P	Na	K	CL	SGOT	Bilinbin	ORN	SGPT
Control	11.1	6.8	143	3.1	103	14	0.2	2.7	14
Test	11.0	6.7	145	3.5	106	13	0.1	3.6	30

	ALK.PHOS	Total PRO	ALB	GLO	BUN	CREAT	GLU	GGT
Control	206	4.9	3.3	1.6	15	1.1	130	13
Test	207	5.0	3.5	1.5	17	1.1	148	8

Termination:

	CA	P	Na	K	CL	SGOT	Bilinbin	ORN	SGPT
Control	13.6	7.7	144	4.3	100	17	0.2	6.2	18
Test	14.0	7.3	142	4.7	97	17	0.2	7.2	36

	ALK.PHOS	Total PRO	ALB	GLO	BUN	CREAT	GLU	GGT
Control	214	5.3	3.4	1.9	14	1.1	142	15
Test	202	5.2	3.4	1.8	14	1.1	151	10

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Lab No. 94T 17135 00

APPENDIX IHEMATOLOGY VALUES FOR INDIVIDUAL RABBITS
(PRETREAT)

Rabbit Number	Sex/Group	WBC $\times 10^3/\text{mm}^3$	RBC $\times 10^6/\text{mm}^3$	HGB (g/dL)	HCT (%)	MCV (μ^3)	MCH (μug)
82700	M/C	4.5	6.50	13.9	41.7	64	21.3
82704	M/C	4.2	5.81	12.1	36.5	63	20.8
82691	F/C	6.7	5.65	12.4	37.8	67	21.9
82693	F/C	3.1	5.42	12.2	36.8	68	22.5
82697	M/T	4.8	5.32	11.3	34.5	65	21.2
82698	M/T	4.0	5.65	12.4	37.8	67	21.9
82689	F/T	8.0	6.01	13.3	40.7	68	22.1
82688	F/T	4.2	5.36	12.5	37.5	70	23.3

Rabbit Number	Sex/Group	MCHC (%)	POLY (%)	BANDS (%)	LYMPH (%)	MONO (%)	EOS (%)	META (%)	BASO (%)
82700	M/C	33.3	62	0	33	1	1	0	3
82704	M/C	33.1	59	0	31	2	3	0	5
82691	F/C	32.8	63	0	28	6	2	0	1
82693	F/C	33.1	68	0	28	1	2	0	1
82697	M/T	32.7	55	0	40	0	1	0	4
82698	M/T	32.8	51	0	44	0	2	0	3
82689	F/T	32.6	63	0	26	4	1	0	6
82688	F/T	33.3	53	0	42	1	1	0	3

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F = FemaleC = Control
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APPENDIX IICLINICAL CHEMISTRY VALUES FOR INDIVIDUAL RABBITS
(PRETREAT)

Rabbit Number	Sex/ Group	Ca (mg/dL)	P (mg/dL)	Na (mEq/L)	K (mEq/L)	CL (mEq/L)	SGOT (AST) (IU/L)	Bilirubin (mg/dL)	ORN (μ M/DL)
82700	M/C	11.5	5.5	145	3.1	108	10	0.1	2.7
82704	M/C	10.5	7.7	145	2.9	103	13	0.1	2.3
82691	F/C	10.8	7.1	140	2.9	102	18	0.2	3.2
82693	F/C	11.4	7.0	142	3.3	100	15	0.2	2.7
82697	M/T	11.2	6.6	146	3.6	107	11	0.1	3.6
82698	M/T	10.5	6.6	144	3.5	108	17	0.1	4.0
82688	F/T	11.4	6.5	144	3.4	106	12	0.1	3.4
82689	F/T	11.0	7.2	144	3.4	103	13	0.2	3.5

Rabbit Number	Sex/ Group	SGPT (IU/L)	Alk Phos. (IU/L)	Total PRO (g/dL)	ALB (g/dL)	GLO (g/dL)	BUN (mg/dL)	CREAT (mg/dL)	GLU (mg/dL)	GGT (IU/L)
82700	M/C	19	202	5.1	3.4	1.7	17	1.2	113	13
82704	M/C	15	169	4.7	3.3	1.4	10	1.0	122	13
82691	F/C	13	217	4.6	3.0	1.6	12	1.1	159	16
82693	F/C	10	234	5.0	3.5	1.5	21	1.1	126	9
82697	M/T	38	173	4.8	3.4	1.4	11	0.8	159	6
82698	M/T	30	160	4.8	3.4	1.4	18	1.1	152	6
82688	F/T	31	192	5.2	3.6	1.6	17	1.1	135	8
82689	F/T	20	302	5.0	3.4	1.6	22	1.4	144	12

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APPENDIX IIIHEMATOLOGY VALUES FOR INDIVIDUAL RABBITS
(TERMINATION)

Rabbit Number	Sex/ Group	WBC $\times 10^3/\text{mm}^3$	RBC $\times 10^6/\text{mm}^3$	HGB (g/dL)	HCT (%)	MCV (μ^3)	MCH (μg)
82700	M/C	6.4	6.97	13.9	44.7	64	19.9
82704	M/C	7.6	6.14	12.5	39.1	64	20.3
82691	F/C	8.3	3.98	8.7	26.6	67	21.8
82693	F/C	5.2	5.58	12.3	38.4	69	22.0
82697	M/T	6.9	5.45	11.1	35.8	66	20.3
82698	M/T	5.8	5.48	11.9	38.4	70	21.7
82689	F/T	12.0	5.26	11.7	35.5	67	22.2
82688	F/T	8.3	5.26	11.7	37.1	71	22.2

Rabbit Number	Sex/ Group	MCHC (%)	POLY (%)	BANDS (%)	LYMPH (%)	MONO (%)	EOS (%)	META (%)	BASO (%)
82700	M/C	31.0	38	0	55	1	3	0	3
82704	M/C	31.9	24	0	62	4	3	0	7
82691	F/C	32.7	43	0	51	1	3	0	2
82693	F/C	32.0	50	0	40	1	1	0	8
82697	M/T	31.0	38	0	55	3	2	0	2
82698	M/T	30.9	25	0	64	0	5	0	6
82689	F/T	32.9	32	0	60	1	1	0	6
82688	F/T	31.5	26	0	69	2	1	0	2

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F = FemaleC = Control
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APPENDIX IVCLINICAL CHEMISTRY VALUES FOR INDIVIDUAL RABBITS
(TERMINATION)

Rabbit Number	Sex/Group	Ca (mg/dL)	P (mg/dL)	Na (mEq/L)	K (mEq/L)	CL (mEq/L)	SGOT (AST) (IU/L)	Bilirubin (mg/dL)	ORN (μ M/DL)
82700	M/C	12.9	6.6	144	4.1	100	14	0.2	5.3
82704	M/C	14.6	9.3	148	4.3	102	24	0.2	6.3
82691	F/C	13.5	7.8	142	4.4	99	19	0.2	7.5
82693	F/C	13.2	7.1	142	4.2	100	12	0.2	5.8
82697	M/T	13.8	7.7	142	5.1	96	14	0.2	6.7
82698	M/T	13.6	8.1	144	4.3	96	22	0.2	4.5
82689	F/T	14.3	6.4	142	4.6	98	14	0.2	9.5
82688	F/T	14.2	6.9	141	4.6	96	16	0.2	8.1

Rabbit Number	Sex/Group	SGPT (IU/L)	Alk Phos. (IU/L)	Total PRO (g/dL)	ALB (g/dL)	GLO (g/dL)	BUN (mg/dL)	CREAT (mg/dL)	GLU (mg/dL)	GGT (IU/L)
82700	M/C	23	206	5.3	3.3	2.0	15	1.2	135	17
82704	M/C	26	191	5.5	3.6	1.9	13	1.2	152	14
82691	F/C	22	224	5.3	3.2	2.1	14	1.0	151	17
82693	F/C	1	233	5.1	3.5	1.6	15	1.1	131	13
82697	M/T	50	182	5.0	3.3	1.7	14	1.1	178	9
82698	M/T	36	143	5.0	3.3	1.7	14	1.3	146	9
82689	F/T	23	281	5.5	3.5	2.0	15	1.2	142	15
82688	F/T	33	202	5.4	3.6	1.8	14	0.9	138	8

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Control

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MUSCLE IMPLANTATION TEST IN RABBITS MICROSCOPIC EXAMINATION OF IMPLANT SITES

Control Article: Ti6Al-4V Wire

Days Implanted: 7

Rabbit Number:	REFERENCE CONTROL			
	82700	82704	82691	82693
Inflammation				
Polymorphonuclear	<u>0</u>	<u>1</u>	<u>0</u>	<u>0</u>
Lymphocytes	<u>1</u>	<u>1</u>	<u>1</u>	<u>1</u>
Plasma Cells	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
Macrophages	<u>1</u>	<u>1</u>	<u>1</u>	<u>1</u>
Giant Cells	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
Necrosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
SUB TOTAL (X2)	<u>4</u>	<u>6</u>	<u>4</u>	<u>4</u>
Fibroplasia	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
Fibrosis	<u>2</u>	<u>2</u>	<u>2</u>	<u>2</u>
Fatty Infiltrate	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
SUB TOTAL	<u>2</u>	<u>2</u>	<u>2</u>	<u>2</u>

AVERAGE CONTROL*

6.5

*Used to determine Irritant Ranking Score shown below as the Conclusion. A negative difference was recorded as zero.

Traumatic Necrosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
Foreign Debris	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
No. Sites Examined	<u>4</u>	<u>4</u>	<u>4</u>	<u>4</u>

Comments:

Conclusion: See page 2.

Daniel J. Hanson, M.D.
Diplomate
American Board of Pathology

Img Histologist

Date 1/28/94

Pathologist

Date 12/1/94



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13-2-0355-00074
Test

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MUSCLE IMPLANTATION TEST IN RABBITS MICROSCOPIC EXAMINATION OF IMPLANT SITES

Test Article: Calcium Stearate on Ti6Al-4V Wire

Days Implanted: 7

	TEST ARTICLE			
Rabbit Number:	82697	82698	82689	82688
Inflammation	0	0	0	1
Polymorphonuclear	1	1	1	1
Lymphocytes	0	0	0	0
Plasma Cells	1	1	1	1
Macrophages	0	0	0	0
Giant Cells	0	0	0	0
Necrosis	0	0	0	0
SUB TOTAL (X2)	4	4	4	6
Fibroplasia	0	0	0	0
Fibrosis	2	2	2	2
Fatty Infiltrate	0	0	0	0
SUB TOTAL	2	2	2	2

AVERAGE TEST*

6.5

AVERAGE TEST 6.5 (-) AVERAGE CONTROL 6.5 = 0.0

*Used to determine Irritant Ranking Score shown below as the Conclusion. A negative difference was recorded as zero.

Traumatic Necrosis	0	0	0	0
Foreign Debris	0	0	0	0
No. Sites Examined	4	4	4	4

Comments:

Conclusion: Under the conditions of this study, the test article was considered a
☒ Nonirritant (0.0-2.9), ☐ Slight Irritant (3.0-8.9), ☐ Moderate Irritant (9.0-15.0),
☐ Severe Irritant (≥ 15.1) to the tissue as compared to the reference control article.

Daniel J. Hanson, M.D.
Diplomate
American Board of Pathology

Img Histologist

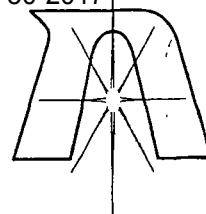
Date 11-28-94

Pathologist

Date 12/1/94

Northview Laboratories, Inc.

1880 Holste Road ♦ Northbrook, IL 60062 ♦ 708/564-8181 ♦ Fax 708/564-8269



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Dr. Mary K. Reinhard
Team Leader of Biocompatibility/Pathology/GLP
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati OH 45242

NV REPORT NUMBER

V4J033G

STUDY COMPLETION DATE

November 7, 1994

TEST ARTICLE

Calcium Stearate on Ti-6Al-4V Wire

Lot Number: 1048FW

RDM Number: 13-2-0355-00074

TEST PERFORMED

CYTOTOXICITY - ELUTION TEST

STUDY DIRECTOR

Kate Ford
Laboratory Supervisor
Sterility Assurance Laboratory Services
Northview Laboratories, Inc.

A Northview Biosciences Company



Atlantic, Midwest, and Pacific

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STUDY DATES

Study Initiation Date: 10/21/94
Study Completion Date: 11/07/94
Date On Test: 10/25/94
Date Off Test: 10/28/94
Study Authorization: Signed Protocol
Date Sample Received: 10/19/94
Purchase Order: A24442

TEST ARTICLE IDENTIFICATION

Calcium Stearate on Ti-6Al-4V Wire
32.5 grams inside a clear Baxter Tower Sterileze® Gas Sterilization Pouch
Sterilization Data: Cobalt & ETO 10/6/94 & 10/9/94

RDM Number: 13-2-0355-00074
Lot Number: 1048FW

TEST PERFORMED

Cytotoxicity - Elution

OBJECTIVE

This study was designed to determine the *in-vitro* biological reactivity of mammalian cell cultures to specific extracts prepared from the test samples in physiological media.

PROTOCOL

This test was conducted according to Protocol Number V4J033G which incorporates by reference Northview SOP 15B-01 and is on file at Northview Laboratories, Inc. This protocol satisfies the requirements of:

USP XXII <87>, Supplement 5, page 2702

DEVIATIONS FROM PROTOCOL

There were no deviations.

DATA DISPOSITION

Raw data and final report from these studies are archived at Northview Laboratories, Inc., 1880 Holste Road, Northbrook, IL 60062, under Northview Report Number V4J033G. The remaining test samples were returned to the client. It is the responsibility of the client to maintain test samples in accordance with GLP regulations.

PROCEDURE

1. See NVL SOP 15B-01 *Cytotoxicity - Elution Test*

Amount Tested: 4.0 grams

This sample was submitted as sterile.

2. Test Supplies:

Cell Line Identification: L-929 (ATCC CCL-1)
 Cell Preparation Date: 10/24/94
 MEM Lot Number: MEM264
 Positive Control Number: 41694PC
 Negative Control Number: 41694NC
 Incubator Code: VII

4.0 grams of test material was immersed in 20.0 mL of serum supplemented culture medium and incubated for 24 hours at $37^{\circ} \pm 1^{\circ}\text{C}$ with 4%-6% CO_2 . Two positive controls and two negative controls were each extracted in 10 mL of medium under the same extraction conditions.

Tissue culture dishes containing a monolayer of L-929 mouse fibroblast cells were exposed to the test sample, positive control and negative control extracts. All cell cultures were incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with 4 - 6% CO_2 . The cells were examined microscopically at 24 and 48 hours for cytotoxic response.

Scoring:

All dishes were scored at each examination period using the following USP XXII Table:

Reactivity Grades for Elution Test

<u>Grade</u>	<u>Reactivity</u>	<u>Conditions of all Cultures</u>
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present.
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; extensive cells lysis and empty areas between cells.
3	Moderate	Not more than 70% of the cells layers contain rounded cells and/or lysed.
4	Severe	Nearly complete destruction of the cell layers.

The average of the 48 hour results of the three test dishes from each sample was used to determine the cytotoxic response.

Interpretation: The sample meets the requirements of the test if the cell culture treated with the sample extract does not score greater than a Mild Reactivity (Grade 2).



RESULTS

<u>Test Item</u>	<u>Reactivity</u>
Calcium Stearate on Ti-6Al-4V Wire RDM Number: 13-2-0355-00074 Lot Number: 1048FW	Slight
Postive Control	Severe
Negative Control	None

CONCLUSION

This sample meets the requirements of USP XXII for this cytotoxicity test.



**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

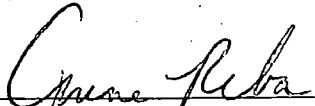
Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number V4J033G were conducted by the Quality Assurance Unit on the following dates:

<u>NV Protocol</u>	<u>Phase of Study</u>	<u>Date</u>
15B-01	Sample Preparation and Extraction	10/25/94
15B-01	Extract transfer to cell layers	10/26/94
15B-01	24 hour scoring	10/27/94
15B-01	48 hour scoring	10/28/94

QAU inspection findings are routinely reviewed by the management of Northview Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number V4J033G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.



Anne Riba
Quality Assurance Manager

11-7-94
Date

NORTHVIEW LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Janette Valignota, Microbiologist
2. Alison Kondratiuk, Microbiologist
3. Kate Ford, Study Director

STATEMENT OF COMPLIANCE

This study, Northview Report Number V4J033G, has been conducted in accordance with applicable Good Laboratory Practice regulations.

Kate Ford

Study Director

11-7-94

Date

Anne Liba

Quality Assurance Unit

11-7-94

Date

Morton Spalding Jr

Management

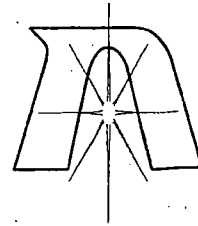
11-7-94

Date

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Northview Laboratories, Inc.

1880 Holste Road ✧ Northbrook, IL 60062 ✧ 708/564-8181 ✧ Fax 708/564-8269



SPONSOR

Dr. Mary K. Reinhard
Team Leader of Biocompatibility/Pathology/GLP
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati OH 45242

NV REPORT NUMBER

C4J138G

STUDY COMPLETION DATE

October 31, 1994

TEST ARTICLE

Calcium Stearate on Ti-6Al-4V Wire

Lot Number: 1048FW

RDM Number: 13-2-0355-00074

TEST PERFORMED

HEMOLYSIS - EXTRACTION METHOD

STUDY DIRECTOR

Elaine Gruber
Laboratory Manager
Analytical Laboratory Services
Northview Laboratories, Inc.

A Northview Biosciences Company

Atlantic, Midwest, and Pacific

F.D.A. REGISTRATION No. 14-18028

Reports are submitted to clients on a confidential basis. No reference to the work, the results, or to Northview Laboratories in any form of advertising, news release, or other public announcement may be made without our written authorization. Test results are applicable only to the samples being tested within the limits of the testing procedures identified and are not necessarily indicative of the characteristics of any other samples from the same or other lots. Northview Laboratories, Inc. shall not be liable under any circumstances for any amount in excess of the cost of the test performed.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS.GOV@fda.hhs.gov or 301-796-8188



STUDY DATES

Study Initiation Date: 10/21/94
Study Completion Date: 10/31/94
Date On Test: 10/21/94
Date Off Test: 10/24/94
Study Authorization: Signed Protocol
Date Sample Received: 10/19/94
Purchase Order: A24442

TEST ARTICLE IDENTIFICATION

Calcium Stearate on Ti-6Al-4V Wire
32.5 grams inside a clear Baxter Tower Sterileze® Gas Sterilization Pouch
Sterilization Data: Cobalt & ETO 10/6/94 & 10/9/94

RDM Number: 13-2-0355-00074
Lot Number: 1048FW

TEST PERFORMED

Hemolysis - Extraction Method

OBJECTIVE

This study was designed to determine if the composition of the sample has the ability to cause hemolysis *in vitro*.

PROTOCOL

This test was conducted according to Protocol Number C4J138G which incorporates by reference Northview SOP 12F-11 and is on file at Northview Laboratories, Inc. This protocol satisfies the requirements of:

1. American National Standards/American Dental Association Document No. 41 for Recommended Standard Practices for Biological Evaluation of Dental Materials. Approved June 11, 1979.
2. Textbook of Microbiology, Bourdon, 3rd Edition, Revised. The Macmillan Company, New York (1952).
3. USP XXII, page 1498, Extraction Guidelines.

DEVIATIONS FROM PROTOCOL

There were no deviations.



DATA DISPOSITION

Raw data and final report from these studies are archived at Northview Laboratories, Inc., 1880 Holste Road, Northbrook, IL 60062, under Northview Report Number C4J138G. The remaining test samples were returned to the client. It is the responsibility of the client to maintain test samples in accordance with GLP regulations.

PROCEDURE

1. See NVL SOP 12F-11 *Hemolysis - Spectrophotometric Method*

2. Test Supplies:

Negative Control: 0.9% Sodium Chloride for Injection, USP
McGaw, Inc. Lot Number J4C466, Exp. Date: 9/96

Positive Control: Water for Irrigation, USP
McGaw, Inc. Lot Number J3K338, Exp. Date: 8/96

Rabbit Blood Source: Crane Laboratories
Lot Number 1240, Exp. Date: 11/2/94 (Rabbit Blood citrated)

Sample portions of 4.02 gram and 4.00 gram of test material were transferred into separate test tubes. 20.0 mL of 0.9% Sodium Chloride for Injection, USP was added to each tube and 20 mL to two additional test tubes as negative controls. 20 mL of Water for Irrigation, USP was added to two tubes as positive controls. All tubes were extracted at 50°C for 72 hours. Following extraction, 10 mL of extract was taken from each tube and 0.2 mL of blood was added. The tubes were capped and gently inverted to mix. Following incubation of the test tubes at 37°C ± 2°C for 60 minutes, the contents of each tube was decanted into a centrifuge tube and centrifuged at 2000 RPM for 10 minutes. The supernatant was transferred to a cuvette using a pasteur pipet and the absorbance of the supernatant measured at 545 nm.

The percent hemolysis was calculated using the following formula, where A is absorbance.

$$\% \text{ Hemolysis} = \frac{A(\text{test sample}) - A(\text{negative control})}{A(\text{positive control}) - A(\text{negative control})} \times 100$$

RESULTS

<u>Test Item</u>	<u>Results</u>
Calcium Stearate on Ti-6Al-4V Wire	Trial 1: 1.62% Hemolysis
Lot Number: 1048FW	Trial 2: 1.94% Hemolysis
RDM Number: 13-2-0355-00074	Average: 1.78% Hemolysis

CONCLUSION

A test sample with 5% or less hemolysis is considered non-hemolytic.



**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

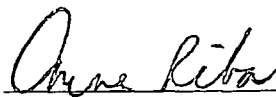
Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number C4J138G were conducted by the Quality Assurance Unit on the following dates:

<u>NV Protocol</u>	<u>Phase of Study</u>	<u>Date</u>
12F-11	Sample Preparation	10/21/94
12F-11	Extract Transfer/Addition of Blood	10/24/94

QAU inspection findings are routinely reviewed by the management of Northview Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number C4J138G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.



Anne Riba
Quality Assurance Manager

10-31-94

Date

Northview Laboratories, Inc.
A Northview Biosciences Company



Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS.GOV@fda.hhs.gov or 301-796-8188


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NORTHVIEW LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Marita Ocampo, Analyst
2. Rose-Marie Tobin, Supervisor
3. Elaine Gruber, Study Director

STATEMENT OF COMPLIANCE

This study, Northview Report Number C4J138G, has been conducted in accordance with applicable Good Laboratory Practice regulations.

Elaine Gruber

Study Director

10/31/94

Date

Anne Rita

Quality Assurance Unit

10-31-94

Date

Martin Halling

Management

10-31-94

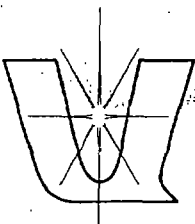
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SPONSOR

Dr. Mary K. Reinhard
Team Leader of Biocompatibility/Pathology/GLP
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati OH 45242

NV REPORT NUMBER

V4J032G

STUDY COMPLETION DATE

October 31, 1994

TEST ARTICLE

Calcium Stearate on Ti-6Al-4V Wire

Lot Number: 1048FW
RDM Number: 13-2-0355-00074

TEST PERFORMED

CYTOTOXICITY - AGAR DIFFUSION

STUDY DIRECTOR

Kate Ford
Laboratory Supervisor
Sterility Assurance Laboratory Services
Northview Laboratories, Inc.

A Northview Biosciences Company

Atlantic, Midwest, and Pacific

STUDY DATES

Study Initiation Date: 10/21/94
Study Completion Date: 10/31/94
Date On Test: 10/25/94
Date Off Test: 10/27/94
Study Authorization: Signed Protocol
Date Sample Received: 10/19/94
Purchase Order: A24442

TEST ARTICLE IDENTIFICATION

Calcium Stearate on Ti-6Al-4V Wire
32.5 grams inside a clear Baxter Tower Sterileze® Gas Sterilization Pouch
Sterilization Data: Cobalt & ETO 10/6/94 & 10/9/94

RDM Number: 13-2-0355-00074
Lot Number: 1048FW

TEST PERFORMED

Cytotoxicity - Agar Diffusion

OBJECTIVE

This study was designed to determine the *in-vitro* biological reactivity of mammalian cell cultures to specific portions of the test sample.

PROTOCOL

This test was conducted according to Protocol Number V4J032G which incorporates by reference Northview SOP 15B-02 and is on file at Northview Laboratories, Inc. This protocol satisfies the requirements of:

USP XXII <87>, Supplement 5, page 2702

DEVIATIONS FROM PROTOCOL

There were no deviations.

DATA DISPOSITION

Raw data and final report from these studies are archived at Northview Laboratories, Inc., 1880 Holste Road, Northbrook, IL 60062, under Northview Report Number V4J032G. The remaining test samples were returned to the client. It is the responsibility of the client to maintain test samples in accordance with GLP regulations.



PROCEDURE

1. See NVL SOP 15B-02 *Cytotoxicity - Agar Diffusion*

Amount Tested: 1 cm², in duplicate

This sample was submitted as sterile.

2. Test Supplies:

Cell Line Identification: L-929 (ATCC CCL-1)
 Cell Preparation Date: 10/21/94
 Medium Batch Number: A323, A324
 Positive Control Number: 41694PC
 Negative Control Number: 41694NC
 Incubator Code: VII

Plates were prepared with a solidified agar layer over a confluent monolayer of L-929 mouse fibroblast cells. Duplicate test samples of approximately 1 cm² each were placed on the agar layer of separate cell culture dishes. Two positive controls and two negative controls were prepared in the same manner as the test samples.

All cultures were incubated for 24 hours at 37°C ± 1°C with 4 - 6% CO₂. After incubation, the cells were stained, re-incubated for a minimum of 4 hours, and then examined microscopically for cytotoxic response.

Scoring:

All dishes were scored at each examination period using the following USP XXII Table:

Reactivity Grades for Agar Diffusion Test

<u>Grade</u>	<u>Reactivity</u>	<u>Conditions of all Cultures</u>
0	None	No detectable zone around or under specimen.
1	Slight	Some malformed or degenerated cells under specimen.
2	Mild	Zone limited to area under specimen.
3	Moderate	Zone extends 0.5 to 1.0 cm beyond specimen.
4	Severe	Zone extends greater than 1.0 cm beyond specimen but does not involve entire dish.

The average of the results of the two test dishes from each sample was used to determine the cytotoxic response.

Interpretation: The sample meets the requirements of the test if the cell culture treated with the sample does not score greater than a Mild Reactivity (Grade 2).

Northview Laboratories, Inc.

A Northview Biosciences Company

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@FDA.HHS.GOV or 301-796-8188



RESULTS

<u>Test Item</u>	<u>Reactivity</u>
Calcium Stearate on Ti-6Al-4V Wire RDM Number: 13-2-0355-00074 Lot Number: 1048FW	Mild
Postive Control	Severe
Negative Control	None

CONCLUSION

This sample meets the requirements of USP XXII for this cytotoxicity test.



**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

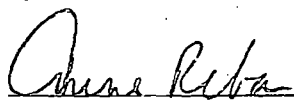
Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number V4J032G were conducted by the Quality Assurance Unit on the following dates:

<u>NV Protocol</u>	<u>Phase of Study</u>	<u>Date</u>
15B-02	Sample placement on agar	10/25/94
15B-02	Sample removal and cell staining	10/26/94
15B-02	Scoring	10/27/94

QAU inspection findings are routinely reviewed by the management of Northview Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number V4J032G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.



Anne Riba
Quality Assurance Manager

10-31-94
Date



NORTHVIEW LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Janette Valignota, Microbiologist
2. Alison Kondratiuk, Microbiologist
3. Kate Ford, Study Director

STATEMENT OF COMPLIANCE

This study, Northview Report Number V4J032G, has been conducted in accordance with applicable Good Laboratory Practice regulations.

Kate Ford

Study Director

10-31-94

Date

Alison Kondratiuk

Quality Assurance Unit

10-31-94

Date

Martin Spalding Jr.

Management

10-31-94

Date

:md

Northview Laboratories, Inc.

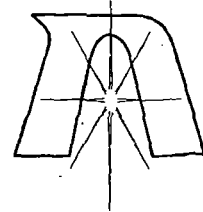
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Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS.GOV@fda.hhs.gov or 301-796-8188



Northview Pacific Laboratories, Inc.

2800 Seventh Street ♦ Berkeley, CA 94710 ♦ 510/548-8440 ♦ Fax 510/548-5425



SPONSOR

Mary Reinhard, D.V.M.
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

NV REPORT NUMBER

X4J126G

STUDY COMPLETION DATE

November 1, 1994

TEST ARTICLE IDENTIFICATION

Calcium Stearate on Ti-6Al-4V Wire

Code Identification: RDM #13-2-0355-00074

Lot Number: 1048FW

Sterilization Data: Cobalt & ETO 10/6/94 & 10/9/94

TEST PERFORMED

USP Rabbit Pyrogen Test

STUDY DIRECTOR

Robert Mosbauer
Laboratory Technician
In Vivo Laboratory Services
Northview Pacific Laboratories, Inc.

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STUDY DATES

Study Initiation Date: October 21, 1994
Study Completion Date: November 1, 1994
Date On Test: October 26, 1994
Date Off Test: October 26, 1994
Study Authorization: Signed Protocol
Date Sample Received: October 18, 1994
Purchase Order: A24442

TEST ARTICLE IDENTIFICATION

Calcium Stearate on Ti-6Al-4V Wire
1 plastic pouch containing approximately 800 brown cylinders 1 cm long and 0.1 cm in diameter
Lot Number: 1048FW
Code Identification: RDM #13-2-0355-00074
Sterilization Data: Cobalt & ETO 10/6/94 and 10/9/94
Storage Condition: Room Temperature

PROTOCOL

This test was conducted according to Protocol Number X4J126G which incorporates by reference Northview SOP No. 16E-02 and is on file at Northview Pacific Laboratories, Inc. This protocol satisfies the requirements of the current USP and Ethicon Endo-Surgery.

DEVIATIONS FROM PROTOCOL

There were no deviations.

DATA DISPOSITION

Raw data from this study is archived at Northview Pacific Laboratories, Inc., 2800 Seventh Street, Berkeley, CA 94710, under Northview Report Number X4J126G.

SUMMARY OF RESULTS

This test material met the USP XXII requirements for the absence pyrogens.



USP RABBIT PYROGEN TEST

INTRODUCTION

This test is designed to limit, to an acceptable level, the possibility of febrile reactions in patients following administration of the test material by injection.

Three New Zealand albino rabbits, weighing at least 2.0 kg each, were injected intravenously with the test material. The doses range in volume from approximately 1.0 mL/kg up to 10.0 mL/kg.

MATERIALS AND METHODS

Test System

Species	Rabbit
Strain	New Zealand, Albino
Source	Elkhorn Rabbitry, Watsonville, CA.
Number	Three
Sex	Female
Weight	At least 2.0 kg
Age	Adult
Housing	Individually; 17 - 21C and 55 ± 15% relative humidity
Feed	Purina rabbit chow (approximately 200 grams per day) and water <i>ad libitum</i>
Identification	Ear tag
Quarantine	Seven days
Photoperiod	Diurnal (12 hours on - 12 hours off)

Justification For Test System

Rabbits are the species required by the current version of the United States Pharmacopoeia.



**Table 1
Supplies**

Reagents/Equipment	Lot Number	Manufacturer	Expiration Date
Sodium Chloride for Injection (SCI), USP	J4D629	McGaw	4/96

**Table 2
Study Design**

Number of Rabbits	Route of Administration	Final Dose Volume (mL/kg)	Time Frequency of Temperature Readings (in hours)
3	Intravenous	10	0, 1.0, 1.5, 2.0, 2.5, 3.0

Sample Preparation And Dosing Procedure

Sample Preparation - Four hundred pieces of the test material were washed with 100 mL of SCI.

Dosing Procedure - Each rabbit was injected intravenously (through the marginal ear vein) with 10 mL/kg of the rinse solution.

Observation And Scoring

Sham Test - All rabbits used for pyrogen tests passed a sham pyrogen test conducted not more than seven days prior to the pyrogen test. (A sham pyrogen test consisted of the same procedure as the pyrogen test with the exception of the injection.)

Weights - All of the rabbits were weighed prior to administration of the test solution.

Food and Water - Food and water were withheld during the test period.

Housing - The rabbits were restrained in light fitting neck stocks that allowed the rabbits to assume a natural resting posture during the test period.



Control Temperature - The control temperature (T_0) of each rabbit was determined within thirty minutes prior to injection of the test solution. Only rabbits whose control temperature did not exceed 39.8C and did not vary more than 1C from the other test rabbits were used.

Temperature Readings - Rectal temperatures were recorded every thirty minutes from one to three hours following injection of the test solution.

Interpretation And Analysis

Any temperature decrease was considered a zero rise in temperature. If no rabbit showed an individual rise in temperature of 0.5C or more above its respective control temperature, the test material meets the requirements for the absence of pyrogens.

If these requirements are not met, the test may be continued using five additional rabbits. If not more than three of the eight rabbits show individual rises in temperature of 0.5C or more, and if the sum of the eight individual maximum temperature rises does not exceed 3.3C, the material under examination meets the requirements for the absence of pyrogens.

RESULTS AND DISCUSSION

No rabbit showed an individual rise in temperature of 0.5C or more.

CONCLUSION

This test material met the USP XXII requirements for the absence of pyrogens.

Table 3
Record of Temperatures (C)

Rabbit Number	Sex	Weight (kg)	Dose (mL)	T-0	T-60	T-90	T-120	T-150	T-180	Temperature Rise
11573	F	2.3	23	39.4	39.3	39.5	39.5	39.4	39.5	0.1
11555	F	2.4	24	39.4	39.4	39.3	39.3	39.4	39.3	0.0
11578	F	2.2	22	39.6	39.6	39.5	39.4	39.4	39.4	0.0

REFERENCES

NV SOP 16E-02

USP XXII [1990] p. 1515, p. 1627 (Biologics) CFR, 610.13

USP XXII, Supplement 8, p. 3350, (Effective May 15, 1993)



**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number X4J126G were conducted by the Quality Assurance Unit on the following dates:

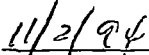
NV SOP No.	Phase of Study	Date
16E-02	T-90 Temperature Reading	10/26/94

QAU inspection findings are routinely reviewed by the management of Northview Pacific Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number X4J126G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.


Matthew Kanter, Quality Assurance Manager


Date



NORTHVIEW PACIFIC LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Timothy V. Doherty, D.V.M., Director *In Vivo* Laboratory Services
2. Roger O'Meara, Assistant Manager, *In Vivo* Laboratory Services
3. Robert Noonan, Ph.D., Senior Scientist, *In Vivo* Laboratory Services
4. Lisa Gearin, Technician, *In Vivo* Laboratory Services
5. Robert Mosbauer, Technician, *In Vivo* Laboratory Services
6. Edwin T. Pangilinan, M.S., Technician, *In Vivo* Laboratory Services
7. James Ruiz, Technician, *In Vivo* Laboratory Services
8. Patrice Pitot, Technician, *In Vivo* Laboratory Services
9. Heather McCracken, Technician, *In Vivo* Laboratory Services
10. Elizabeth Bashnick, Technician, *In Vivo* Laboratory Services
11. Diana L. Corey, Technician, *In Vivo* Laboratory Services

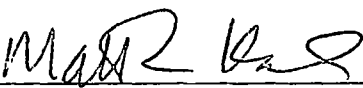
STATEMENT OF COMPLIANCE

This study, Northview Report Number X4J126G, has been conducted in accordance with applicable Good Laboratory Practice regulations.



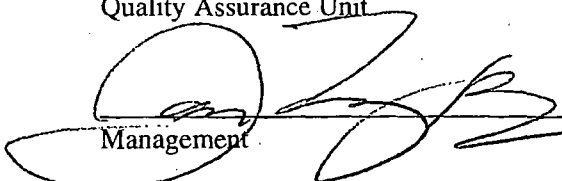
Study Director

11/02/94
Date



Quality Assurance Unit

11/2/94
Date



Management

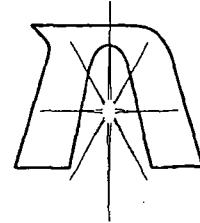
11/2/94
Date

:bw



Northview Pacific Laboratories, Inc.

2800 Seventh Street ✧ Berkeley, CA 94710 ✧ 510/548-8440 ✧ Fax 510/548-5425



SPONSOR

Mary K. Reinhard, D.V.M.
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

NV REPORT NUMBER

U4J010G

STUDY COMPLETION DATE

November 9, 1994

TEST ARTICLE IDENTIFICATION

Calcium Stearate on Ti-6Al-4V Wire

Lot Number: 1048FW

RDM Number: 13-2-0355-00074

TEST PERFORMED

Ames

Northview Pacific Laboratories, Inc.



STUDY DATES

Testing Facility: Nelson Laboratories, Inc.
P.O. Box 17557
4535 South 2300 East
Salt Lake City, UT 84117

Study Completion Date: November 9, 1994
Date Sample Received: October 18, 1984
Purchase Order: A24442

Study Authorization: Signed Protocol

Test Procedure: See Attached

CONCLUSION

The results are summarized in Tables 1 - 8. The tester strains were sensitive to selected marker mutagens both with and without S-9 activation using a rat liver homogenate. The tester strains were also tested for proper strain performance. These tests included UV light sensitivity to verify *uvrB* mutation, crystal violet sensitivity to verify increased cell wall permeability and corresponding loss of cell coat material, and ampicillin resistance to verify presence or absence of the R-factor plasmid.

None of the 5 tester strains produced two-fold increases in the number of revertants. The spot tests showed no zone of increased reversion or of inhibition.

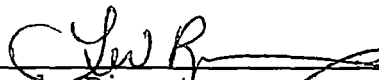
In summary, the extract tested against the five strains did not meet the criteria for a potential mutagen.



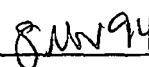


I CERTIFY THAT THIS STUDY WAS PERFORMED IN ACCORDANCE
WITH THE U.S. FDA GOOD LABORATORY PRACTICES.
(GLP REGULATIONS)

LABORATORY NO. 69288



Study Director
Nelson Laboratories, Inc.



Date

SOP/QAU/018B-9/0493

P.O. Box 17557 Salt Lake City, UT 84117-0557 • 6280 South Redwood Road Salt Lake City, UT 84123-6600
Telephone 801-963-2600 • Fax 801-963-2630 • Operator 800-826-2088 • Auto Attendant 800-963-6280

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS.GOV@fda.hhs.gov or 301-796-8188



QAU AUDIT STATEMENT

SALMONELLA TYPHIMURIUM REVERSE MUTATION ASSAY
(AMES TEST)

Study Director:

Final Report Dated:

Teri A. Roming, M.S. RM(AAM)

07 Nov 94

1. The test was conducted in accordance with USFDA Good Laboratory Practices Regulations [21 CFR Part 58]. All laboratory results which pertain to this study are recorded in Data File Number 69288.
2. In accordance with the Good Laboratory Practice regulations, this study was inspected by the Quality Assurance Unit on: 31 Oct 94. The findings of the inspection(s) were reported to management and to the study director on: 31 Oct 94.
3. The Quality Assurance Unit has reviewed this report and has determined that the methods and standard operating procedures are accurately described, and that the reported results accurately reflect the raw data.

M. Kaye Markel
M. Kaye Markel
Quality Assurance Unit

08 Nov. 94
Date

SOP/QAU/018B-10/0493

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SALMONELLA TYPHIMURIUM REVERSE MUTATION ASSAY
AMES TEST

LABORATORY NUMBER: 69288
PROTOCOL NUMBER: 942931-1 (As Per SOP/CTX/014)
SAMPLE SOURCE: Northview Pacific Laboratories,
Inc
SAMPLE IDENTIFICATION: Calcium Stearate on Ti-6Al-4V
Wire Lot Number: 1048FW
RDM #13-2-0355-00074
Reference U4J010G
P.O. #NVP 129
START DATE: 25 Oct 94
COMPLETION DATE: 07 Nov 94
REPORT DATE: 07 Nov 94

INTRODUCTION:

The Ames test employs several strains of *Salmonella typhimurium* which have been selected based on their sensitivity to mutation because of: 1) an increased cell wall permeability, due to mutation which causes partial loss of the lipopolysaccharide barrier that coats the bacterial surface and results in increased permeability to large molecules (rfa mutation); 2) a mutation in the bacterial cell system to excise and repair defects in the DNA, resulting in the inability to repair damaged or mutated sections (uvrB mutation); and 3) R-factor plasmids (some strains) and a multicopy plasmid (some strains) which contain error-prone DNA repair systems.

The *Salmonella* reverse mutation (Ames) tests are performed by mixing the test substance or an extract of the test substance and the test organism together in a soft agar solution, which contains only small amounts of histidine. The histidine permits the inoculated test organism to undergo a limited number of divisions, but is insufficient to permit normal growth. The tester strains require histidine for growth, due to a mutation in the gene which controls production of histidine. If, however, the strain undergoes a reverse mutation, (spontaneous or induced by the test substance or a positive control material), the organism no longer requires histidine to grow and can produce a visible colony or revertant. Only mutations to the test organism in the region of the histidine gene will cause the test organism to undergo a reverse mutation to an organism which then no longer requires histidine. The tester strains are selected to detect various types of mutagens. The tester strains employed were TA97A, TA98, TA100, TA102 and TA1535.

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The test extract was evaluated against the five strains listed. The tests were performed both with and without S-9 activation. The S-9 activation system is designed to simulate mammalian liver enzyme systems and is used to detect substances which undergo metabolic activation from non-mutagenic forms.

The test extract was evaluated in plate incorporation assays and in spot tests with and without S-9 activation.

PROCEDURE:

Strain Verification: Test strains were checked for presence of the desired properties prior to all tests. These tests included verification of the following:

- Presence of uvrB mutation
- Presence or absence of R-factor plasmid
- Presence of rfa mutation
- Requirement for histidine

The uvrB mutation was verified by demonstrating UV sensitivity (lack of repair system). The R-factor was checked by determining sensitivity or resistance to ampicillin (0.08% in 0.02 NaOH). The presence of the rfa mutation was verified by demonstrating sensitivity to crystal violet (0.1% in water) on nutrient agar plates. The histidine requirement was assured by plating onto minimal glucose agar plates spread with 0.1 mL of 0.5 mM biotin and both with and without 0.1 mL of 0.5 mM histidine.

Sample Preparation: 4.0 g of the sample was extracted in 20 mL of 0.9% saline at 70°C for 24 hours.

Metabolic Activation System: The S-9 activation system was used to screen for the presences of mutagens from byproducts of the test sample. Rat liver S-9, Lot #38277, liver homogenate was obtained from Organon Teknika, Durham, NC. The homogenate was kept frozen at <-70°C and has been well characterized in our laboratory.

The material is prepared from 8-10 week old Sprague Dawley male rats. The technique for preparation of rat liver microsomal enzymes (S-9) was identical to that described by Ames. The animals were injected intraperitoneally with Aroclor

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1254 in corn oil at a dose of 500 mg/kg five days prior to sacrifice. The S-9 was prepared as a KCl homogenate and stored at -70°C until use. All procedures were carried out at $0-4^{\circ}\text{C}$. Immediately before use, the S-9 preparation was mixed with 0.5 mL of S-9 base containing 20 μL S-9 preparation to each 2.0 mL aliquot agar overlay. When working with soft agar, temperatures never exceeded 47°C , and plates were poured within 2 minutes of the addition of S-9.

Plate Incorporation Tests: Minimal agar plates containing histidine-deficient Vogel Bonner Medium E (VBME) fortified with 2% glucose were prepared. Aliquots of top agar were melted and maintained at $45^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Each 100 mL aliquot of top agar was fortified with approximately 10 mL of 0.5 mM biotin and 0.5 mM histidine immediately before use. Five to ten mL of a thirteen-hour nutrient broth culture of the appropriate bacterial tester strain was added to the top agar, and 2.0 mL aliquots of this mixture were transferred to sterile, disposable capped tubes. One-tenth mL of the sample extract was added to 2 mL aliquots of top agar. The sample extract was tested with and without the S-9 activation system.

Spot Tests: The test material was also tested in spot tests on plates with and without the S-9 activation system. Two mL aliquots of the top agar mixture containing histidine-biotin solution and the broth culture of the appropriate tester strain were added to agar plates. The plates were allowed to harden then 10 μL spots were added to the plates. All strains were tested with the extract using 10 μL spots. The plates were placed into the incubator within an hour of plating.

Positive Control Materials: The following positive control materials were used: Sodium Azide, 4-nitro-0-phenylene-diamine (NPD), and 2 aminofluorene (2AF). The controls were tested with and without the S-9 activation system. The controls were tested with all of the strains using incorporation and spot tests.

Test Evaluation:

Test criteria: 1) Tested extract, spot test and plate incorporation. 2) Tested against tester strains TA97A, TA98, TA100, TA102 and TA1535. 3) Tested in the presence and the absence of liver microsomal enzymes preparation.

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Criteria for a mutagen: 1) A two-fold increase over the spontaneous reversion rate. 2) Demonstration of a dose-response curve when dilutions are tested.

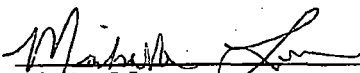
Criteria for a non-mutagen: 1) A less than two-fold increase over spontaneous reversion rate. 2) No dose response curve when dilutions are tested.

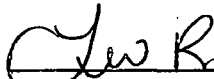
CONCLUSION:

The results are summarized in Tables 1-8. The tester strains were sensitive to selected marker mutagens both with and without S-9 activation using a rat liver homogenate. The tester strains were also tested for proper strain performance. These tests included UV light sensitivity to verify uvrB mutation, crystal violet sensitivity to verify increased cell wall permeability and corresponding loss of cell coat material, and ampicillin resistance to verify presence or absence of the R-factor plasmid.

None of the 5 tester strains produced two-fold increases in the number of revertants. The spot tests showed no zone of increased reversion or of inhibition.

In summary, the extract tested against the five strains did not meet the criteria for a potential mutagen.


Michelle A. Lee, B.S.
Study Director


Teri A. Roming, M.S. RM(AAM)
Biocompatibility Section Leader

jww

Northview Pacific Laboratories, Inc.
 Salmonella Reverse Mutation Assay
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TABLE 1. TA97A Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	160	105	129	80	119
S-9 Control	105	159	156	158	145

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	137	141	127	134	135
Sample with S-9	126	116	127	119	122

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TABLE 2. TA98 Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	37	28	25	44	34
S-9 Control	33	37	32	37	35

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	30	25	23	40	30
Sample with S-9	29	38	31	41	35

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TABLE 3. TA100 Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	160	162	135	161	155
S-9 Control	169	183	191	194	184

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	165	149	140	147	150
Sample with S-9	141	115	127	128	128

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TABLE 4. TA102 Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	248	266	192	201	227
S-9 Control	273	247	260	250	258

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	192	222	176	229	205
Sample with S-9	295	312	308	263	295

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TABLE 5. TA1535 Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	23	33	26	27	27
S-9 Control	19	22	18	24	21

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	21	25	20	19	21
Sample with S-9	19	13	22	26	20

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TABLE 6. Spot Test Results

SAMPLE EXTRACT	ACTIVATION	97A	98	100	102	1535
	None	-	-	-	-	-
	S-9	-	-	-	-	-

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TABLE 7. Strain Verification Results

PARAMETER	STRAINS				
	TA97A	TA98	TA100	TA102	TA1535
uvrB	+	+	+	-	+
R-factor	+	+	+	+	-
rfa	+	+	+	+	+
Histidine Requirement	+	+	+	+	+

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TABLE 8. Positive Controls

CONTROL ID	ACTIVATION	97A	98	100	102	1535
Sodium Azide (Expected Result)	No S-9	-	-	+	-	+
	No S-9	(-)	(-)	(+)	(-)	(+)
NPD (Expected Result)	No S-9	+	+	+	-	-
	No S-9	(+)	(+)	(+)	(-)	(-)
2AF (Expected Result)	S-9	+	+	+	-	-
	S-9	(+)	(+)	(+)	(-)	(-)

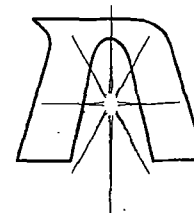
Positive controls were analyzed by incorporation assays and spot tests.



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SPONSOR

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Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

NV REPORT NUMBER

X4J127G

STUDY COMPLETION DATE

November 30, 1994

TEST ARTICLE IDENTIFICATION

Calcium Stearate on Ti-6Al-4V Wire

RDM #13-2-0355-00074

Lot Number: 1048FW

TEST PERFORMED

Guinea Pig Maximization Test

STUDY DIRECTOR

Robert A. Noonan, Ph.D.
Senior Scientist
In Vivo Laboratory Services
Northview Pacific Laboratories, Inc.



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Guinea Pig Maximization Test

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STUDY DATES

Study Initiation Date: October 20, 1994
Study Completion Date: November 30, 1994
Date On Test: October 25, 1994
Date Off Test: November 18, 1994
Study Authorization: Signed Protocol
Date Sample Received: October 18, 1994

TEST ARTICLE IDENTIFICATION

Calcium Stearate on Ti-6Al-4V Wire
1 plastic bag containing brown plastic rods, each approximately 0.1 cm in diameter and 1.0 cm long.
Code Identification: RDM #13-2-0355-00074
Lot Number: 1048FW
Storage Condition: Room temperature

CONTROL ARTICLE IDENTIFICATION

1-Chloro-2,4-Dinitrobenzene
Amber glass jar containing 100 g of yellow granular powder
Lot Number: 100H0738 - for CSO Positive Control, Exp. Date 01/01/95
Lot Number: 41H08112 - for SCI Positive Control, Exp. Date 02/24/97

PROTOCOL

This test was conducted according to Protocol Number X4J127G which incorporates by reference Northview SOP No. 16G-13 and is on file at Northview Pacific Laboratories, Inc. This protocol satisfies the requirements of Ethicon Endo-Surgery.

DEVIATIONS FROM PROTOCOL

Due to an insufficient number of animals in the weight range specified by protocol, 300 - 500 g, one of the naive positive control animal was used despite having an initial weight of 505 g. This animal, #20421, was from the same batch as the SCI positive control group and was the same age. Therefore it was equivalent.

In the judgement of the study director, this deviation did not affect the integrity of the study.

DATA DISPOSITION

Raw data from this study is archived at Northview Pacific Laboratories, Inc., 2800 Seventh Street, Berkeley, CA 94710, under Northview Report Number X4J127G.

SUMMARY OF RESULTS

None of the test group or negative control animals exhibited scores higher than 1. A response of 1 is not considered to reflect significant sensitization. According to the criteria for this test, the test material had a weak (Grade I) potential for sensitization. (See Table 7.)



GUINEA PIG MAXIMIZATION TEST

INTRODUCTION

This test is designed to determine to what extent a test material has the potential for acting as a contact sensitizer in guinea pigs.

Sixty Hartley albino guinea pigs were used: including fifteen test group animals and five negative controls for the 0.9% Sodium Chloride for Injection (SCI) extraction; and fifteen test group animals and five negative controls for the cottonseed oil (CSO) extraction. The results were compared to positive control groups: these included five positive controls and five naive positive controls prepared in deionized water, in addition to five positive controls and five naive positive controls prepared in cottonseed oil. The positive control tests were done as part of NV Study No. HSTPOSG, a historical study run in January, 1994 (CSO controls) and June, 1994 (SCI controls).

In this test, an extract of the test material was employed. As a negative control a vehicle blank was incubated under the same conditions as the test material. As a positive control a solution of 1-Chloro-2, 4-Dinitrobenzene (DNCB) was employed.

Groups of guinea pigs were exposed to the test material extracts and the control solutions in an induction phase lasting nine days. In this phase, the materials were first injected intradermally (ID). For the ID injections the solutions were given undiluted as well as emulsified in Freund's Complete Adjuvant (FCA). Seven days after the ID injections, the extracts, vehicle blank and positive control solution were mixed with petrolatum (1:20 w/w) and administered topically over the same area in which the ID injections were given. The topical exposure lasted 48 hours.

Fourteen days after the topical administration, the animals were challenged. For the challenge phase, extracts and control solutions were administered on Hill Top Chambers, which were applied to untreated sites, and remained on the animals for 24 hours. The dosing sites were scored for erythema and edema 24 and 48 hours after removal of the chambers.

Based on the erythema and edema scores observed, the test material was classified as to its allergic potential.

MATERIAL AND METHODS

Test System

Species	Guinea Pig
Strain	Hartley, Albino
Source	Charles River Laboratories, Portage, MI
Age	Young adult
Initial Weight	300 to 500 grams
Housing	In suspended cages; 18 - 26°C, 55 ± 15% relative humidity
Feed	Purina Guinea Pig Chow and water supplied in bottles <i>ad libitum</i>
Photoperiod	Diurnal (12 hours on - 12 hours off)
Quarantine Period	Seven days



Justification For Test System

Guinea pigs are the preferred species for skin sensitization tests. They are required for the maximization test (see Magnusson and Kligman, *J. Invest. Dermatol.* 52:268. [1969]). The Hartley albino guinea pig is specified for use in the maximization test by ASTM Standard F720-81.

Table 1
Supplies

Reagents/Equipment		Lot Number	Manufacturer	Expiration Date
Sodium Chloride for Injection, USP (SCI)	For Induction For Challenge	J4D649 J4H500	McGaw McGaw	4/1/96 6/1/96
Cottonseed Oil (CSO)		IA284	Spectrum	9/29/98
Freund's Complete Adjuvant (FCA)		063H-8800	Sigma	6/14/98
Petrolatum		115699/43799	VWR	5/1/98
Ethanol		93H06	McCormick Dist Co.	8/1/97
1-chloro-2, 4-dinitrobenzene (DNCB)		100H0738 ¹ 41H08112 ²	Sigma Sigma	1/1/95 2/24/97
10% Sodium Lauryl Sulfate (SLS)		83H0841	Sigma	10/31/98
Sterile Water for Injection (WFI)		J4H546	McGaw	12/1/96
Hill Top Chambers™		06MBA-2002	Hill Top Research	N/A
Whatman #3 Filter Paper		N/A	Whatman Lab. Div.	N/A
Blenderm™ Surgical Tape		Jun92-432	3M	N/A
Gauze - Sof Kling Conforming Bandage		1994-07	Johnson & Johnson	N/A
Anchor™ Masking Tape (½ in)		N/A	Anchor Continental, Inc.	N/A
Dental Dam™ elastic bandage		11994	Hygenic Corp.	N/A

1. For CSO Positive Controls
2. For SCI Positive Controls



Table 2
Study Design

Group	Number of Animals	INDUCTION PHASE		CHALLENGE PHASE
		1st Induction (Day 0) Route	2nd Induction (Day 7) Route	(Day 21) Route
SCI Test	15	Intradermal	Topical	Topical
CSO Test	15	Intradermal	Topical	Topical
SCI Positive Control	5	Intradermal	Topical	Topical
CSO Positive Control	5	Intradermal	Topical	Topical
SCI Naive Positive Control (for challenge)	5	N/A	N/A	Topical
CSO Naive Positive Control (for challenge)	5	N/A	N/A	Topical
SCI Negative Control	5	Intradermal	Topical	Topical
CSO Negative Control	5	Intradermal	Topical	Topical

SAMPLE PREPARATION AND DOSING PROCEDURE

Sample Extraction - The test material was extracted in two vehicles, SCI and CSO. For each extraction, four grams of test material were placed in a dry, sterile, glass, screw capped tube. The tube was filled with 20 mL of extraction vehicle. An additional sterile tube was filled with each vehicle to serve as a blank. The extracts and blanks were incubated for 72 hours at 50°C.

Animal Assignment - The animals were divided into eight groups; fifteen SCI test animals, fifteen CSO test animals, five SCI positive controls, five CSO positive controls, five naive SCI positive controls, five naive CSO positive controls, five SCI negative controls and five CSO negative controls.

Induction Phase - Intradermal Injection - The induction phase consisted of an intradermal injection of the test material followed a week later by a topical application. At least 24 hours prior to each application, the hair on the dorsocranial thorax was removed by clipping. At least one hour prior to dosing, the clipped areas were shaved.

The procedure for intradermal injections of test material extracted in SCI is illustrated in Table 3.

Three pairs of 0.1 ml intradermal injections were administered to the shaved areas on the test animals. The first pair of injections (cranial) consisted of an emulsion of Freund's Complete Adjuvant in an equal volume of WFI. The second pair (middle) was the test material extract. The third pair (caudal) consisted of an emulsion of the test material extract in an equal volume of FCA.

The five positive control animals were treated in a similar manner to the test animals, except that the test material extract was replaced in the second and third pair of injections with an equal amount of a 0.1% solution of a known sensitizer, 1-chloro-2,4-dinitrobenzene (DNCB) in 9.5% ethanol.



The five negative control animals were treated in a similar manner to the test animals, except that the test material extract was replaced in the second and third pair of injections with an equal amount of vehicle extract (blank).

Table 3
Induction Phase - Intradermal Injections (For SCI vehicle extraction)

Site Location	No. of Sites	TEST GROUP		POSITIVE CONTROL		NEGATIVE CONTROL	
		Volume/ Site	Dose Material	Volume/ Site	Dose Material	Volume/ Site	Dose Material
Cranial	2	0.1 ml	1:1 (FCA:WFI)	0.1 ml	1:1 (FCA:WFI)	0.1 ml	1:1 (FCA:WFI)
Middle	2	0.1 ml	Test Extract	0.1 ml	DNCB	0.1 ml	Blank
Caudal	2	0.1 ml	1:1 (FCA:Test Extract)	0.1 ml	1:1 (FCA:DNCB)	0.1 ml	1:1 (FCA:Blank)

The procedure for intradermal injections of test material extracted in CSO is illustrated in Table 4. Three pairs of 0.1 mL intradermal injections were administered to the shaved areas on test animals, the first pair of injections (cranial) consisted of an emulsion of FCA in CSO. The second pair of injections (center) was of CSO test material extract in an equal volume of WFI. The third pair of injections (caudal) consisted of an emulsion of CSO test material extract in an equal volume of FCA.

The five positive control animals were treated in a similar manner to the test animals, except that the test material extract was replaced in the second and third pair of injections with an equal amount of a 0.1% solution of a known sensitizer, 1-chloro-2,4-dinitrobenzene (DNCB) in ETOH/CSO (1:9 v/v).

The five negative control animals were treated in a similar manner to the test animals, except that the test material extract was replaced in the second and third pair of injections with an equal amount of vehicle control extract.



Table 4
Induction Phase - Intradermal Injections (For CSO vehicle extraction)

Site Location	No. of Sites	TEST GROUP		POSITIVE CONTROL		NEGATIVE CONTROL	
		Volume/ Site	Dose Material	Volume/ Site	Dose Material	Volume/ Site	Dose Material
Cranial	2	0.1 ml	1:1 (FCA:CSO)	0.1 ml	1:1 (FCA:CSO)	0.1 ml	1:1 (FCA:CSO)
Middle	2	0.1 ml	1:1 (Test Extract:WFI)	0.1 ml	1:1 (DNCB:WFI)	0.1 ml	1:1 (Blank:WFI)
Caudal	2	0.1 ml	1:1 (FCA:Test Extract)	0.1 ml	1:1 (FCA:DNCB)	0.1 ml	1:1 (FCA:Blank)

Induction Phase - Topical Dosing - Six days after performing the intradermal injections, the previously treated areas were clipped and shaved. The test sites were evaluated for evidence of irritation. Since the test material did not cause irritation, the dosing sites on the test and negative control group animals were pretreated with 10% SLS in petrolatum. This SLS mixture was massaged into the skin using a glass rod. Twenty-four hours later (seven days after the intradermal injections) the animals were reshaved.

For the topical application phase of the induction, 1.0 g of an extract of the test material, prepared in the same manner as for the intradermal injections, was mixed with 19.0 g of petrolatum (1:19 by weight).

The dosing patches were prepared by spreading a thick even layer of an extract of the test material, mixed with petrolatum (1:19 by weight) on 4.25 cm diameter disks of Whatman #3 filter paper. These were applied to the shaved areas on the test animals and held in place with surgical tape. The trunks of the animals were wrapped with gauze, which was held in place with ½ inch masking tape. They were then wrapped with an elastic bandage so that complete occlusion was obtained.

The positive and negative control animals were treated in a similar manner to the test group animals except that their patches contained 1:19 mixtures of 0.1% DNCB in petrolatum and a vehicle extract (blank) in petrolatum, respectively.

After forty-eight hours, the animals were unwrapped and the patches removed.



Table 5
Challenge Phase

Group	No. of Sites	Volume /Site	Material
SCI Test Extract	1	0.4 mL	Test Extract
SCI Positive Control	1	0.4 mL	0.025% DNCB
SCI Naive Positive Control	1	0.4 mL	0.025% DNCB
SCI Negative Control	1	0.4 mL	Blank Extract
CSO Test Extract	1	0.4 mL	Test Extract
CSO Positive Control	1	0.4 mL	0.05% DNCB
CSO Naive Positive Control	1	0.4 mL	0.05% DNCB
CSO Negative Control	1	0.4 mL	Blank Extract

Challenge Phase - The challenge phase dosing procedure is summarized in Table 5. Thirteen days after the Topical Induction application, a 5 x 5 cm area on the right side of each animal was clipped. On the next day (fourteen days after the topical application), the animals were shaved and Hill Top Chambers™ containing 0.4 mL volumes of the test extracts, vehicle control extracts, or the positive control solutions were applied to the shaved areas. For the challenge phase a positive control solution consisting of either 0.025% DNCB in 9.5% aqueous ethanol, or 0.05% DNCB in CSO/ethanol (9:1 v/v) was used. Two groups of five previously untreated animals were dosed with the positive control solution, in two vehicles, to serve as naive positive controls.

The animals were wrapped, as described for the topical application, so that complete occlusion was obtained. The patches remained in place for 24 hours. Then, the animals were unwrapped and the dosing sites for the positive controls, naive positive controls, and the CSO test group and its control were gently cleansed with 70% aqueous ethanol to remove chemical residues. The dosing sites for the SCI test group and the SCI negative control group were allowed to air dry.

OBSERVATION AND SCORING

Clinical Observations - All of the animals were observed for toxic signs immediately after dosing and daily until the end of the study.

Weights - All of the animals were weighed prior to the initiation and at the end of the study.

Scoring - Skin responses at the challenge sites were scored 24 and 48 hours after removal of the patches according to the criteria in Table 6.



INTERPRETATION AND ANALYSIS

Scores of 2 or greater are considered significant. Test group animals with scores of 2 or greater in either the 24 or 48 hour observation period may be considered sensitized. Magnusson and Kligman do not regard a score of 1 as representing significant sensitization. However, the allergic potential of the substance is considered proven only when the skin reactions to the test material clearly outweigh those of the negative control group to the vehicle blank.

Based upon the number of animals sensitized, a rating of the material's potential for sensitization is given according to the grading scale shown in Table 7.

RESULTS AND DISCUSSION

Clinical Observations - On Day 23, three CSO test group animals in one cage, (#22676, #22679 and #22682) appeared thin and dehydrated. Their water bottle was replaced. On Day 24, animal #22682 remained thin. The other two appeared normal. Animal #22682 was kept under observation for three more days. On Day 27, it was re-weighed. Its weight increased from 356.7 grams, measured on Day 24, to 400.3 grams on Day 28. Other than appearing small, the animal was alert and appeared healthy.

All other test and control animals appeared healthy. No toxic signs were observed over the duration of the study.

Scoring Results - The 24 and 48 hour scores for the primary challenge are shown in Tables 8 - 14.

SCI Test Group - At the 24 hour observation, three of fifteen animals exhibited scattered mild redness (score of 1). At the 48 hour observation, one of ten animals exhibited a score of 1. (See Table 8.)

SCI Negative Control Group - At both the 24 hour and 48 hour observations, two of five animals exhibited scores of 1. (See Table 9.)

SCI Positive Control Group - At the 24 hour observation, two of five animals exhibited intense redness and swelling (score of 3). Three exhibited moderate redness (score of 2). At the 48 hour observation, four exhibited scores of 1. (See Table 10.)

SCI Naive Positive Control Group - At the 24 hour observation, four of five animals exhibited a score of 1. At the 48 hour observation, two exhibited scores of 1. (See Table 10.)

CSO Test Group - At the 24 hour observation, eleven of fifteen animals exhibited scores of 1. Ten exhibited scores of 1 at the 48 hour observation. (See Table 11.)

CSO Negative Control Group - At both the 24 hour and 48 hour observations, two of five animals exhibited scores of 1. (See Table 12.)

CSO Positive Control Group - At the 24 hour observation, four of five animals exhibited scores of 3. One exhibited a score of 2. At the 48 hour observation, two exhibited scores of 3, and three, scores of 2. (See Table 13.)

CSO Naive Positive Control Group - At the 24 hour observation, four of five animals exhibited scores of 1. One exhibited a score of 1 at the 48 hour observation. (See Table 13.)

Weights - The pre-test and post-test body weights are shown in Tables 14 -23. All of the animals on this study exhibited weight gain over the course of the study.



CONCLUSION

None of the test group or negative control animals exhibited scores higher than 1. A response of 1 is not considered to reflect significant sensitization. By the criteria of this test, the test material had a weak (Grade I) potential for sensitization. (See Table 7.)

Under similar treatment conditions, all positive control animals, for both extraction conditions, exhibited sensitization responses to the challenge dose, while none of the naive positive controls exhibited scores higher than 1. This establishes that the treatment conditions were able to induce a response from a known sensitizer.

Table 6
Scoring Key

Reaction	Score
No Reaction	0
Scattered Mild Redness	1
Moderate and Diffuse Redness	2
Intense Redness and Swelling	3

Table 7
Classification of Potential for Sensitization

% Sensitized	Grade	Classification
0 - 8	I	Weak
9 - 28	II	Mild
29 - 64	III	Moderate
65 - 80	IV	Strong
81 - 100	V	Extreme



Table 8
Test Scores After Challenge Phase
Test Group (Extraction in SCI)

Animal Number	24 Hour Score	48 Hour Score
22652	0	0
22654	0	0
22656	0	0
22659	0	0
22660	0	0
22661	0	0
22663	0	0
22666	1	0
22667	1	0
22669	0	0
22672	1	1
22674	0	0
22675	0	0
22678	0	0
22680	0	0

Table 9
Test Scores After Challenge Phase
Negative Control Group (Extraction in SCI)

Animal Number	24 Hour Score	48 Hour Score
22653	0	0
22658	0	0
22668	1	1
22673	1	1
22677	0	0



Table 10
Test Scores After Challenge Phase
Positive Control Group (0.05% DNCB in ETOH/H₂O, 1:9 v/v)

Animal Number	24 Hour Score	48 Hour Score
Positive Control Group		
20417	2	2
20419	2	3
20424	3	3
20444	3	3
20654	2	3
Naive Positive Control Group		
20421	1	1
20422	1	0
20431	0	0
20649	1	1
20653	1	0

Table 11
Test Scores After Challenge Phase
Test Group (Extraction in CSO)

Animal Number	24 Hour Score	48 Hour Score
22651	1	0
22655	1	1
22657	0	0
22662	0	0
22665	1	1
22671	1	1
22676	1	1
22679	1	1
22682	1	1
22683	1	1
22685	1	1
22686	1	1
22696	1	1
22701	0	0
22702	0	0



Table 12
Test Scores After Challenge Phase
Negative Control Group (CSO Blank)

Animal Number	24 Hour Score	48 Hour Score
22664	1	1
22670	0	0
22681	0	0
22697	1	1
22703	0	0

Table 13
Test Scores After Challenge Phase
Positive Control Group (0.05% DNCB in ETOH/CSO, 1:9 v/v)

Animal Number	24 Hour Score	48 Hour Score
Positive Control Group		
18850	3	3
18854	3	3
18878	2	2
18882	3	2
18900	3	2
Naive Positive Control Group		
19111	1	0
19112	1	0
19113	1	0
19114	1	1
19115	0	0



Table 14
Animal Weights
Test Group (SCI)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
22652	F	342.1	469.1
22654	F	310.1	432.3
22656	F	317.9	445.0
22659	F	318.5	432.2
22660	F	347.2	444.2
22661	F	334.4	433.4
22663	F	353.6	466.9
22666	F	342.1	433.1
22667	F	345.1	428.9
22669	F	352.2	481.2
22672	F	328.4	443.7
22674	F	328.2	443.6
22675	F	327.2	457.7
22678	F	313.9	440.5
22680	F	326.9	441.6

Table 15
Animal Weights
Positive Control Group (SCI)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
20417	M	469.4	617.0
20419	M	457.0	510.0
20424	M	497.4	613.4
20444	M	490.1	652.3
20654	M	334.1	511.9

Table 16
Animal Weights
Naive Positive Control Group For Challenge Phase (SCI)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
20421	M	505.0	607.6
20422	M	475.4	601.7
20431	M	494.0	638.9
20649	M	314.0	508.5
20653	M	340.0	526.9



Table 17
Animal Weights
Negative Control Group (SCI)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
22653	F	332.3	486.1
22658	F	327.4	476.7
22668	F	337.6	450.9
22673	F	351.1	466.2
22677	F	338.2	497.3

Table 18
Animal Weights
Test Group (CSO Extraction)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
22651	F	329.2	500.8
22655	F	334.6	483.2
22657	F	336.3	431.6
22662	F	325.8	438.3
22665	F	344.8	469.8
22671	F	336.1	458.9
22676	F	326.8	412.2
22679	F	347.2	466.6
22682	F	323.8	356.7 ⁽¹⁾
			400.3 ⁽²⁾
22683	F	336.9	466.0
22685	F	336.6	403.2
22686	F	363.1	470.2
22696	F	310.4	420.3
22701	F	353.8	476.4
22702	F	330.5	426.6

(1) Weight determined on Day 24.

(2) Weight determined on Day 27.



Table 19
Animal Weights
Positive Control Group (CSO Extraction)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
18850	M	382.5	603.9
18854	M	391.0	621.0
18878	M	369.6	608.2
18882	M	395.4	633.2
18900	M	355.7	555.1

Table 20
Animal Weights
Naive Positive Control Group (CSO Extraction)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
19111	M	378.5	439.2
19112	M	364.5	432.9
19113	M	393.0	492.0
19114	M	356.0	414.1
19115	M	378.9	443.0



Table 21
Animal Weights
Negative Control Group (CSO Extraction)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
22664	F	335.2	425.9
22670	F	355.7	460.7
22681	F	316.5	427.0
22697	F	338.1	475.0
22703	F	337.9	458.0

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3. Standard Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test. ASTM Standard F720-81 (Reapproved 1986)
4. Guinea Pig Maximization Test. Wahlberg, J.E. and Boman, A., 1985, Curr. Probl. Dermatol. 14: 59-106



**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

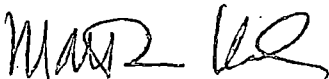
Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number X4J127G were conducted by the Quality Assurance Unit on the following dates:

NV SOP No.	Phase of Study	Date
16G-13	Topical Phase, Unwrapping	11/3/94
	Challenge Phase, Dosing	11/15/94

QAU inspection findings are routinely reviewed by the management of Northview Pacific Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number X4J127G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.


Matthew Kanter, Manager, Quality Assurance

11/30/94
Date



NORTHVIEW PACIFIC LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

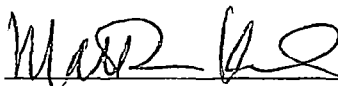
1. Timothy V. Doherty, D.V.M., Director *In Vivo* Laboratory Services
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9. Heather McCracken, Technician, *In Vivo* Laboratory Services
10. Elizabeth Bashnick, Technician, *In Vivo* Laboratory Services
11. Diana L. Corey, Technician, *In Vivo* Laboratory Services

STATEMENT OF COMPLIANCE

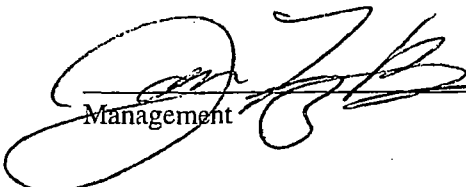
This study, Northview Report Number X4J127G, has been conducted in accordance with applicable Good Laboratory Practice regulations.


Study Director

11/30/94
Date


Quality Assurance Unit

11/30/94
Date


Management

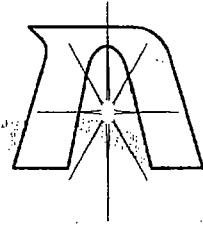
12/1/94
Date

:bw



Northview Pacific Laboratories, Inc.

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SPONSOR

Mary K. Reinhard, D.V.M.
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

NV REPORT NUMBER

X4J128G

STUDY COMPLETION DATE

November 15, 1994

TEST ARTICLE IDENTIFICATION

Calcium Stearate on Ti-6Al-4V Wire

Code Identification: RDM#13-2-0355-00074

Lot Number: 1048FW

TEST PERFORMED

USP Systemic Injection Test

USP Intracutaneous Test

STUDY DIRECTOR

Edwin T. Pangillinan, M.S.
In Vivo Laboratory Services
Northview Pacific Laboratories, Inc.



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STUDY DATES

Study Initiation Date: October 19, 1994
Study Completion Date: November 15, 1994
Date On Test: October 24, 1994
Date Off Test: October 31, 1994
Study Authorization: Signed Protocol
Date Sample Received: September 27, 1994
Purchase Order: A24442

TEST ARTICLE IDENTIFICATION

Calcium Stearate on Ti-6Al-4V Wire
1 sterile bag containing 32.5 g of metallic black wire approximately 1.1 cm x 0.1 cm
Code Identification: RDM #13-2-0355-00074
Lot Number: 1048FW
Sterilization: Cobalt & ETO, 10/6/94 & 10/9/94
Storage Condition: Room Temperature

CONTROL ARTICLE IDENTIFICATION

USP Negative Control Plastic RS Filaments
50 - 10 x 1 mm white plastic filaments in a glass vial
Batch Number: G-7
Storage Condition: Room Temperature

PROTOCOL

This test was conducted according to Protocol Number X4J128G which incorporates by reference Northview SOP Nos. 16E-03 and 16E-05, and is on file at Northview Pacific Laboratories, Inc. This protocol satisfies the requirements of Ethicon Endo-Surgery.

DEVIATIONS FROM PROTOCOL

There were no deviations.

DATA DISPOSITION

Raw data from these studies are archived at Northview Pacific Laboratories, Inc., 2800 Seventh Street, Berkeley, CA 94710, under Northview Report Number X4J128G.

SUMMARY OF RESULTS

This test material met the requirements of the USP Systemic Injection Test and the USP Intracutaneous Test when extracted under the conditions listed in the Procedure section.



USP SYSTEMIC INJECTION TEST

INTRODUCTION

This test evaluates systemic responses to extracts of the test material following injection into mice.

Ten Swiss Webster albino mice (five per sample and five per corresponding blank) are used for each extract. Each animal is injected with 50 mL/kg (or 10 g/kg in the case of PEG 400) of the extract or blank. The animals are observed immediately after and at 4, 24, 48, and 72 hours following injection.

MATERIALS AND METHODS

Test System

Species	Mouse
Strain	Swiss-Webster
Source	Simonsen Laboratories, Inc., Gilroy, CA
Sex	Male
Age	Young adult
Number Used	Ten per extract (5 test and 5 blank)
Initial Weight	17 to 23 grams
Housing	Five per cage; 18-26C, 55 $\pm$ 15% relative humidity
Feed and Water	Purina Rodent Chow and water <i>ad libitum</i> supplied in bottles
Identification	Cage labeling and tail marking
Photoperiod	Diurnal (12 hours on - 12 hours off)
Quarantine Period	Four days

Justification For Test System

The mouse is the species required by the current version of the United States Pharmacopoeia.



**Table 1
Supplies**

Reagents	Lot Number	Manufacturer	Expiration Date
Sodium Chloride for Injection (SCI), USP	J4D629	McGaw	4/96
Cottonseed Oil (CSO)	IA284	Spectrum Chemicals	9/98
95% Ethyl Alcohol, USP	93H06	McCormick	7/98
Polyethylene Glycol 400 (PEG 400)	IH269	Spectrum Chemicals	6/95

**Table 2
Study Design**

Extracting Medium	Number of Animals	Route of Administration	Dose	Injection Rate (μ L/sec)
SCI	10	IV	50 mL/kg	100
CSO	10	IP	50 mL/kg	-
1:20 Alcohol in SCI (A/S)	10	IV	50 mL/kg	100
PEG 400	10	IP	10 g/kg	-

IV - Intravenous

IP - Intraperitoneal

Sample Preparation And Dosing Procedure

Sample Preparation - For each of the extracts, a dry sterile glass tube with a screw cap was filled with 20 mL of the extracting medium. Pieces of the test material weighing 4 g were then added to the tube. An additional sterile tube was filled with 20 mL of the same medium to serve as a blank. The samples and blanks were extracted at 50C for 72 hours.

Prior to injection, each gram of the PEG 400 sample extract and blank was diluted with 4.1 volumes of SCI to obtain a solution having a concentration of approximately 200 mg of PEG 400 per mL.

Dosing Procedure - Each extract was vigorously agitated prior to withdrawal of injection doses to ensure even distribution of extracted matter.

Five mice were each injected with 50 mL/kg (in the case of the PEG 400, 10 g/kg) of the SCI sample extract. Five additional mice were each injected with 50 mL/kg of the corresponding SCI blank. This procedure was repeated for each extracting medium.



Observation

Clinical Observations - All of the animals were observed for toxic signs immediately after dosing and at 4, 24, 48, and 72 hours after dosing.

Weights - All of the animals were weighed prior to dosing and at the 72 hour observation.

Interpretation And Analysis

The test material meets the USP requirements if none of the animals treated with the sample extract show a significantly greater biological reactivity than those treated with the blank.

The test material does not meet the USP requirements if two or more mice die, if abnormal behavior such as convulsions or prostration occurs in two or more mice, or if a body weight loss greater than two grams occurs in three or more mice.

RESULTS AND DISCUSSION

Clinical Observations - No toxic signs were observed during the 72 hour observation period.

Weights - All of the animals gained weight during the test (see Tables 4 & 5).

CONCLUSION

This test material met the requirements of the USP Systemic Injection Test using the extracting media and conditions listed above.

Table 3
Mortalities And Toxic Signs

Extracting Medium	Mortalities		Other Toxic Signs*		Results
	Sample	Blank	Sample	Blank	
SCI	0/5	0/5	0/5	0/5	Pass
CSO	0/5	0/5	0/5	0/5	Pass
1:20 A/S	0/5	0/5	0/5	0/5	Pass
PEG 400	0/5	0/5	0/5	0/5	Pass

* Details are described above



Table 4
Dose Volumes and Animal Weights, SCI and CSO Samples and Blanks

Extracting Medium	Animal Number	Dose (mL)	Pre-Test Weight (g)	Post-Test Weight (g)	Weight Change (g)
SCI Sample	#1	1.0	19	22	+ 3
	#2	1.1	21	26	+ 5
	#3	1.1	22	26	+ 4
	#4	1.1	21	26	+ 5
	#5	1.1	22	25	+ 3
SCI Blank	#1	1.2	23	26	+ 3
	#2	1.2	23	27	+ 4
	#3	1.0	20	23	+ 3
	#4	1.1	22	26	+ 4
	#5	1.1	21	24	+ 3
CSO Sample	#1	1.1	21	24	+ 3
	#2	1.1	21	26	+ 5
	#3	1.0	20	26	+ 6
	#4	1.1	22	26	+ 4
	#5	1.1	21	24	+ 3
CSO Blank	#1	1.1	22	26	+ 4
	#2	1.0	20	26	+ 6
	#3	1.1	21	24	+ 3
	#4	1.1	21	23	+ 2
	#5	1.1	21	25	+ 4



Table 5
Dose Volumes and Animal Weights, 1:20 A/S and PEG 400 Samples and Blanks

Extracting Medium	Animal Number	Dose (mL)	Pre-Test Weight (g)	Post-Test Weight (g)	Weight Change (g)
1:20 A/S Sample	#1	1.2	23	27	+ 4
	#2	1.2	23	27	+ 4
	#3	1.1	21	24	+ 3
	#4	1.1	22	26	+ 4
	#5	1.1	21	26	+ 5
1:20 A/S Blank	#1	1.1	21	24	+ 3
	#2	1.1	22	26	+ 4
	#3	1.2	23	26	+ 3
	#4	1.0	20	26	+ 6
	#5	1.1	21	24	+ 3
PEG 400 Sample	#1	1.1	22	25	+ 3
	#2	1.1	21	25	+ 4
	#3	1.0	20	26	+ 6
	#4	1.0	19	25	+ 6
	#5	1.2	23	27	+ 4
PEG 400 Blank	#1	1.2	23	26	+ 3
	#2	1.0	20	24	+ 4
	#3	1.2	23	26	+ 3
	#4	1.1	22	25	+ 3
	#5	1.1	22	27	+ 5

REFERENCES

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USP XXII [1990] p. 1499

USP XXII Supplement 5 [Nov. 15, 1991] p. 2704



USP INTRACUTANEOUS TEST

INTRODUCTION

This test evaluates local responses to extracts of the test material following intracutaneous injections into rabbits.

On the day of the test the fur on the back of each rabbit is removed on both sides of the spinal column. A 0.2 mL portion of one of the test extracts is injected intracutaneously at each of five sites along one side of the spinal column of each of two rabbits. A 0.2 mL portion of the corresponding blank is injected intracutaneously at five sites along the other side of the spinal column of each of two rabbits. The injection sites are observed for erythema and edema, and scored at 24, 48 and 72 hours.

MATERIALS AND METHODS

Test System

Species	Rabbit
Strain	New Zealand White
Source	Elkhorn Rabbitry, Watsonville, CA
Number Used	Four
Initial Weight	At least 2.0 kg
Age	Adult
Sex	Male
Housing	Individually at 17-21C and 55 $\pm$ 15% relative humidity
Feed and Water	Purina Rabbit Chow (approximately 200 grams per day) and water <i>ad libitum</i> .
Identification	Ear tags
Photoperiod	Diurnal (12 hours on - 12 hours off)
Quarantine Period	Seven days

Justification For Test System

The rabbit is the species required by the current version of the United States Pharmacopoeia.



**Table 6
Supplies**

Reagents	Lot Number	Manufacturer	Expiration Date
Sodium Chloride for Injection (SCI), USP	J4D629	McGaw	4/96
Cottonseed Oil (CSO)	IA284	Spectrum Chemicals	9/98
95% Ethyl Alcohol, USP	93H06	McCormick	7/98
Polyethylene Glycol 400 (PEG 400)	IH269	Spectrum Chemicals	6/95

**Table 7
Study Design**

Extracting Medium	Number of Animals ¹	Route of Administration ²	Dose/ Site	Number of Sites/ Animal
SCI	2	IC	0.2 mL	5 sample, 5 blank
CSO	2	IC	0.2 mL	5 sample, 5 blank
1:20 Alcohol in SCI (A/S)	2	IC	0.2 mL	5 sample, 5 blank
PEG 400	2	IC	0.2 mL	5 sample, 5 blank

1. Two animals received the SCI and CSO preparations (twenty sites per animal), and two animals received the A/S and PEG 400 preparations (twenty sites per animal). Four animals were used in this study (see Dosing Procedure, p. 11).

2. IC - Intracutaneous Injection

Sample Preparation And Dosing Procedure

Sample Preparation - For each of the extracts, a dry sterile glass tube with a screw cap was filled with 20 mL of the extracting medium. Pieces of the test material weighing 4 g were then added to the tube. An additional sterile tube was filled with 20 mL of the same medium to serve as a blank. The samples and blanks were extracted at 50C for 72 hours.

Prior to injection, each gram of the PEG 400 sample extract and blank was diluted with 7.4 volumes of SCI to obtain a solution having a concentration of approximately 120 mg of PEG 400 per mL.



Dosing Procedure - On the day of the test, the fur on the back of each animal was removed with electric clippers. Each extract was vigorously agitated prior to withdrawal of injection doses to ensure even distribution of extracted matter.

On the right cranial portion of one rabbit's back, five 0.2 mL intracutaneous injections of the SCI sample extract were administered. The left cranial side was similarly treated with the corresponding blank. On the caudal portion of the rabbit's back, the injection procedure was repeated using the CSO extract and blank. A second rabbit was then injected with the same extracts and blanks. A second pair of rabbits was used for the remaining extracts and blanks.

Observation And Scoring

Clinical Observations - All of the animals were observed daily for signs of ill health.

Scoring - The injection sites were examined and scored for any tissue reactions, such as erythema/eschar formation and edema, at 24, 48, and 72 hours after injection according to Table 8.

Interpretation And Analysis

The average erythema and edema scores for the sample extracts and blanks are determined at each scoring interval (24, 48, and 72 hours) for each rabbit.

After the 72 hour scoring, all erythema and edema scores are totalled separately for each sample extract and blank. To determine the overall mean score, each of the totals are divided by 12 (2 animals x 3 scoring periods x 2 scoring categories).

The test material meets the USP requirements if the difference between the mean score for the sample extract and the mean score for the corresponding blank is 1.0 or less.

RESULTS AND DISCUSSION

Clinical Observations - The animals remained healthy throughout the test period.

Scoring - The difference between the mean scores for each of the sample extracts and corresponding blanks was 1.0 or less (see Tables 9 - 12).

CONCLUSION

This test material met the requirements of the USP Intracutaneous Test using the extracting media and conditions described above.



Table 8
Evaluation Of Skin Reactions

Erythema And Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet-redness) to slight eschar formation (injuries in depth)	4

Edema Formation ¹	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised about 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

¹ Excludes non-inflammatory (mechanical) edema from the blank or extraction fluid.



Table 9
Skin Reaction Scores
Sodium Chloride For Injection Sample And Blank

Rabbit #11525, M	Time	Reaction	Blank Sites					Sample Sites					Average Readings	
			1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema		0	0	0	0	0	0	0	0	0	0	0	0
	Edema		0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema		0	0	0	0	1	0	0	0	0	0	0.2	0
	Edema		0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema		0	0	0	0	0	0	0	0	0	0	0	0
	Edema		0	0	0	0	0	0	0	0	0	0	0	0
<hr/>														
Rabbit #11528, M	Time	Reaction	Blank Sites					Sample Sites					Average Readings	
			1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema		0	0	0	0	0	0	0	0	0	0	0	0
	Edema		0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema		0	0	0	0	0	0	0	0	0	0	0	0
	Edema		0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema		0	0	0	0	0	0	0	0	0	0	0	0
	Edema		0	0	0	0	0	0	0	0	0	0	0	0
<hr/>													<hr/>	
Overall Mean:													0	0
<hr/>														



Table 10
Skin Reaction Scores
Cottonseed Oil Sample And Blank

Rabbit #11525, M		Blank Sites					Sample Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema	1	1	1	1	1	1	1	1	1	1	1.0	1.0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	1	1	1	1	1	2	2	2	2	2	1.0	2.0
	Edema	0	0	0	0	0	0	0	0	1	1	0	0.4
72 hr	Erythema	1	1	1	1	1	2	2	2	2	2	1.0	2.0
	Edema	0	0	0	0	0	0	0	0	0	1	0	0.2
Rabbit #11528, M		Blank Sites					Sample Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema	1	1	1	1	0	1	1	1	1	1	0.8	1.0
	Edema	1	1	1	0	0	0	0	0	0	0	0.6	0
48 hr	Erythema	2	2	2	2	2	2	2	2	2	2	2.0	2.0
	Edema	1	0	1	1	1	0	0	1	1	1	0.8	0.6
72 hr	Erythema	2	2	2	2	2	2	2	2	2	2	2.0	2.0
	Edema	1	1	1	1	1	1	1	1	1	1	1.0	1.0
Overall Mean:												0.9	1.0



Table 11
Skin Reaction Scores
1 In 20 Solution Of Alcohol In SCI Sample And Blank

Rabbit #11529, F		Blank Sites					Sample Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
Rabbit #11536, F		Blank Sites					Sample Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
Overall Mean:												0	0



Table 12
Skin Reaction Scores
Polyethylene Glycol 400 Sample And Blank

Rabbit #11529, F		Blank Sites					Sample Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
Rabbit #11536, F		Blank Sites					Sample Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	0	0	0	0	0	1	1	1	1	0	0.8
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	1	1	1	0	0.6
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
Overall Mean:												0	0.1

REFERENCES

NV SOP 16E-05

USP XXII [1990], p. 1499

USP XXII Supplement 5 [Nov. 15, 1991], p. 2704-2705



**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number X4J128G were conducted by the Quality Assurance Unit on the following dates:

NV SOP No.	Phase of Study	Date
16E-03	48 Hour Observation	10/26/94
16E-05	48 Hour Scoring	10/26/94

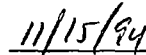
QAU inspection findings are routinely reviewed by the management of Northview Pacific Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number X4J128G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.



Matthew Kanter, Quality Assurance Unit



Date



NORTHVIEW PACIFIC LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Timothy V. Doherty, D.V.M., Director *In Vivo* Laboratory Services
2. Roger O'Meara, Assistant Manager, *In Vivo* Laboratory Services
3. Robert Noonan, Ph.D., Senior Scientist, *In Vivo* Laboratory Services
4. Lisa Gearin, Technician, *In Vivo* Laboratory Services
5. Robert Mosbauer, Technician, *In Vivo* Laboratory Services
6. Edwin T. Pangilinan, M.S., Technician, *In Vivo* Laboratory Services
7. James Ruiz, Technician, *In Vivo* Laboratory Services
8. Patrice Pitot, Technician, *In Vivo* Laboratory Services
9. Heather McCracken, Technician, *In Vivo* Laboratory Services
10. Lisa Bashnick, Technician, *In Vivo* Laboratory Services

STATEMENT OF COMPLIANCE

This study, Northview Report Number X4J128G, has been conducted in accordance with applicable Good Laboratory Practice regulations.

Edwin T. Pangilinan
Study Director

11/15/94
Date

MARZ
Quality Assurance Unit

11/15/94
Date

T. V. Doherty
Management

11/15/94
Date

:bw

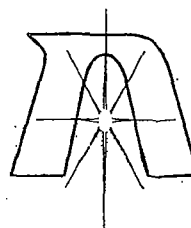


Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Part Name	Subject Device Material
Flex Joint	Polycarbonate (Styron)

Northview Laboratories, Inc.

1880 Holste Road ♦ Northbrook, IL 60062 ♦ 708/564-8181 ♦ Fax 708/564-8269



SPONSOR

Dr. Mary Reinhard
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati OH 45242

NV REPORT NUMBER

V4E026G

STUDY COMPLETION DATE

May 24, 1994

TEST ARTICLE

Calibre 2081-15 Polycarbonate, Blue Tint

Lot Number: TB920707B20
RDM Number: 13-2-0060-00508

TEST PERFORMED

CYTOTOXICITY - ELUTION TEST

STUDY DIRECTOR

Vikki Bilowich
Laboratory Manager
Sterility Assurance Laboratory Services
Northview Laboratories, Inc.

A Northview Biosciences Company



Atlantic, Midwest, and Pacific

F.D.A. REGISTRATION No. 14-18028

Reports are submitted to clients on a confidential basis. No reference to the results of a Northview Laboratories performance, whether positive, negative, or otherwise, shall be made in any public or private communication. The results of a Northview Laboratories performance are not to be used in any way to determine the quality of any other samples from the same or other lots. Northview Laboratories, Inc. shall not be liable under any circumstances for any amount in excess of the cost of the test performed.



PROCEDURE

1. See NVL SOP 15B-01 *Cytotoxicity - Elution Test*

Amount Tested: 69 cm²

2. Test Supplies:

Cell Line Identification: L-929 (ATCC CCL-1)
Cell Preparation Date: 5/9/94
MEM Lot Number: 202
Positive Control Number: 12293PC
Negative Control Number: 12293NC
Incubator Code: VII
Caliper I.D.: 23

69 cm² of test material was immersed in 23 mL of serum supplemented culture medium and incubated for 24 hours at 37° ± 1°C with 4%-6% CO₂. Two positive controls and two negative controls were each extracted in 10 mL of medium under the same extraction conditions.

Tissue culture dishes containing a monolayer of L-929 mouse fibroblast cells were exposed to the test sample, positive control and negative control extracts. All cell cultures were incubated at 37°C ± 1°C with 4 - 6% CO₂. The cells were examined microscopically at 24 and 48 hours for cytotoxic response.

Scoring:

All dishes were scored at each examination period using the following USP XXII Table:

Reactivity Grades for Elution Test

<u>Grade</u>	<u>Reactivity</u>	<u>Conditions of all Cultures</u>
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present.
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; extensive cells lysis and empty areas between cells.
3	Moderate	Not more than 70% of the cells layers contain rounded cells and/or lysed.
4	Severe	Nearly complete destruction of the cell layers.

The average of the 48 hour results of the three test dishes from each sample was used to determine the cytotoxic response.

Interpretation: The sample meets the requirements of the test if the cell culture treated with the sample extract does not score greater than a Mild Reactivity (Grade 2).



NV Report Number V4E026G

Page 4 of 6

RESULTS

<u>Test Item</u>	<u>Reactivity</u>
Calibre 2081-15 Polycarbonate, Blue Tint	None
Postive Control	Severe
Negative Control	None

CONCLUSION

This sample meets the requirements of USP XXII for this cytotoxicity test.



**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

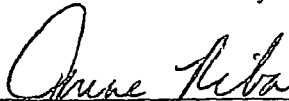
Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number V4E026G were conducted by the Quality Assurance Unit on the following dates:

<u>NV Protocol</u>	<u>Phase of Study</u>	<u>Date</u>
15B-01	Sample Preparation and Extraction	05/10/94
15B-01	Extract transfer to cell layers	05/11/94
15B-01	24 hour reading	05/12/94
15B-01	48 hour reading	05/13/94

QAU inspection findings are routinely reviewed by the management of Northview Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number V4E026G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.



Quality Assurance Unit

5-24-94
Date



NORTHVIEW LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

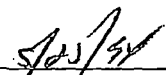
1. Janette Valignota, Technician
2. Alison Kondratiuk, Technician
3. Vikki Bilowich, Study Director
4. Anne Riba, Quality Assurance Manager

STATEMENT OF COMPLIANCE

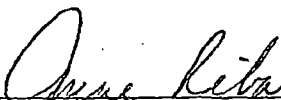
This study, Northview Report Number V4E026G, has been conducted in accordance with applicable Good Laboratory Practice regulations.



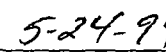
Study Director



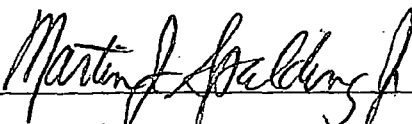
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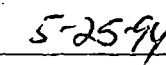
Quality Assurance Unit



Date



Management



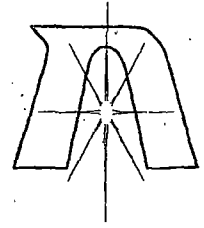
Date

:mc



Northview Laboratories, Inc.

1880 Holste Road ✧ Northbrook, IL 60062 ✧ 708/564-8181 ✧ Fax 708/564-8269



SPONSOR

Dr. Mary Reinhard
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati OH 45242

NV REPORT NUMBER

V4E025G

STUDY COMPLETION DATE

May 31, 1994

TEST ARTICLE

Calibre 2081-15 Polycarbonate, Blue Tint

Lot Number: TB920707B20

RDM Number: 13-2-0060-00508

TEST PERFORMED

CYTOTOXICITY - AGAR DIFFUSION

STUDY DIRECTOR

Vikki Bilowich
Laboratory Manager
Sterility Assurance Laboratory Services
Northview Laboratories, Inc.

A Northview Biosciences Company



Atlantic, Midwest, and Pacific

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Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOI@STATUS.GOV or fda.hhs.gov or 301-796-8188
made without our written authorization. Test results are applicable only to the samples being tested within the limits of the testing procedures described and are not necessarily indicative of the characteristics of any other samples from the same or other lots. Northview Laboratories, Inc. shall not be liable under any circumstances for any amount in excess of the cost of the test performed.



Printed on
recycled paper

STUDY DATES

Study Initiation Date: 05/06/94
Study Completion Date: 05/31/94
Date On Test: 05/11/94
Date Off Test: 05/13/94
Study Authorization: Signed Protocol
Date Sample Received: 04/28/94
Purchase Order: A2442

TEST ARTICLE IDENTIFICATION

Calibre 2081-15 Polycarbonate, Blue Tint
2 green colored squares in a tyvek pouch

Lot Number: TB920707B20
RDM Number: 13-2-0060-00508

TEST PERFORMED

Cytotoxicity - Agar Diffusion

OBJECTIVE

This study was designed to determine the *in-vitro* biological reactivity of mammalian cell cultures to specific portions of the test sample.

PROTOCOL

This test was conducted according to Protocol Number V4E025G which incorporates by reference Northview SOP 15B-02 and is on file at Northview Laboratories, Inc. This protocol satisfies the requirements of:

USP XXII <87>, Supplement 5, page 2702

DEVIATIONS FROM PROTOCOL

There were no deviations.

DATA DISPOSITION

Raw data and final report from these studies are archived at Northview Laboratories, Inc., 1880 Holste Road, Northbrook, IL 60062, under Northview Report Number V4E025G. The remaining test sample was returned to the client. It is the responsibility of the client to maintain test samples in accordance with GLP regulations.



PROCEDURE

1. See NVL SOP 15B-02 *Cytotoxicity - Agar Diffusion*

Amount Tested: 1 cm², in duplicate

This sample was submitted as sterile.

2. Test Supplies:

Cell Line Identification: L-929 (ATCC CCL-1)
 Cell Preparation Date: 5/6/94
 Medium Batch Number: A269, A270
 Positive Control Number: 12293PC
 Negative Control Number: 12293NC
 Incubator Code: VII

Plates were prepared with a solidified agar layer over a confluent monolayer of L-929 mouse fibroblast cells. Duplicate test samples of approximately 1 cm² each were placed on the agar layer of separate cell culture dishes. Two positive controls and two negative controls were prepared in the same manner as the test samples.

All cultures were incubated for 24 hours at 37°C ± 1°C with 4 - 6% CO₂. After incubation, the cells were stained, re-incubated for a minimum of 4 hours, and then examined microscopically for cytotoxic response.

Scoring:

All dishes were scored at each examination period using the following USP XXII Table:

Reactivity Grades for Agar Diffusion Test

<u>Grade</u>	<u>Reactivity</u>	<u>Conditions of all Cultures</u>
0	None	No detectable zone around or under specimen.
1	Slight	Some malformed or degenerated cells under specimen.
2	Mild	Zone limited to area under specimen.
3	Moderate	Zone extends 0.5 to 1.0 cm beyond specimen.
4	Severe	Zone extends greater than 1.0 cm beyond specimen but does not involve entire dish.

The average of the results of the two test dishes from each sample was used to determine the cytotoxic response.

Interpretation: The sample meets the requirements of the test if the cell culture treated with the sample does not score greater than a Mild Reactivity (Grade 2).



RESULTS

<u>Test Item</u>	<u>Reactivity</u>
Calibre 2081-15 Polycarbonate, Blue Tint Lot Number: TB920707B20	None
Positive Control	Severe
Negative Control	None

CONCLUSION

This sample meets the requirements of USP XXII for this cytotoxicity test.



**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

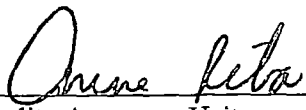
Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number V4E025G were conducted by the Quality Assurance Unit on the following dates:

<u>NV Protocol</u>	<u>Phase of Study</u>	<u>Date</u>
15B-02	Sample placement on agar	05/11/94
15B-02	Sample removal and cell staining	05/12/94
15B-02	Scoring	05/13/94

QAU inspection findings are routinely reviewed by the management of Northview Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number V4E025G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.



Quality Assurance Unit

5-31-94
Date

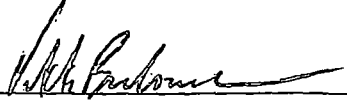
NORTHVIEW LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

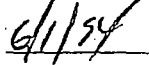
1. Janette Valignota, Microbiologist
2. Alison Kondratiuk, Microbiologist
3. Vikki Bilowich, Study Director
4. Anne Riba, Quality Assurance Manager

STATEMENT OF COMPLIANCE

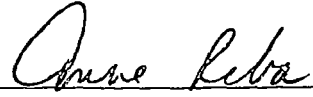
This study, Northview Report Number V4E025G, has been conducted in accordance with applicable Good Laboratory Practice regulations.



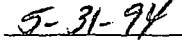
Study Director



Date



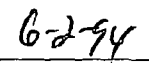
Quality Assurance Unit



Date



Management



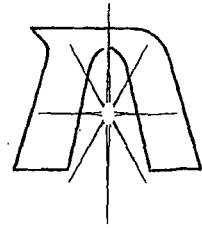
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Northview Laboratories, Inc.

1880 Holste Road ✧ Northbrook, IL 60062 ✧ 708/564-8181 ✧ Fax 708/564-8269



SPONSOR

Dr. Mary Reinhard
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati OH 45242

NV REPORT NUMBER

C4E026G

STUDY COMPLETION DATE

June 1, 1994

TEST ARTICLE

Calibre 2081-15 Polycarbonate, Blue Tint

Lot Number: TB920707B20

RDM Number: 13-2-0060-00508

TEST PERFORMED

HEMOLYSIS - EXTRACTION METHOD

STUDY DIRECTOR

Elaine Gruber
Laboratory Manager
Analytical Laboratory Services
Northview Laboratories, Inc.

A Northview Biosciences Company



Atlantic, Midwest, and Pacific

F.D.A. REGISTRATION No. 14-18028

Questions? Contact FDA/CDRH/CEPDR at CDRH-FOI@FDA.GOV or FDA-Info@FDA.GOV or 301-796-8188
Made without our written authorization. Test results are applicable only to the samples being tested within the limits of the testing procedures certified and are not necessarily indicative of the characteristics of any other samples from the same or other lots. Northview Laboratories, Inc. shall not be liable under any circumstances for any amount in excess of the cost of the test performed.



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STUDY DATES

Study Initiation Date: 05/09/94
Study Completion Date: 06/01/94
Date On Test: 05/13/94
Date Off Test: 05/16/94
Study Authorization: Signed Protocol
Date Sample Received: 04/28/94
Purchase Order: A2442

TEST ARTICLE IDENTIFICATION

Calibre 2018-15 Polycarbonate, Blue Tint
15 pieces (60 cm²) green colored rectangles in a tyvek pouch
Sterilization Data: Cobalt & ETO 4/6/94 & 4/11/94

Lot Number: TB920707B20
RDM Number: 13-2-0060-00508

TEST PERFORMED

Hemolysis - Extraction Method

OBJECTIVE

This study was designed to determine if the composition of the sample has the ability to cause hemolysis *in vitro*.

PROTOCOL

This test was conducted according to Protocol Number C4E026G which incorporates by reference Northview SOP 12F-11 and is on file at Northview Laboratories, Inc. This protocol satisfies the requirements of:

1. American National Standards/American Dental Association Document No. 41 for Recommended Standard Practices for Biological Evaluation of Dental Materials. Approved June 11, 1979.
2. Textbook of Microbiology, Bourdon, 3rd Edition, Revised. The Macmillan Company, New York (1952).
3. USP XXII, page 1498, Extraction Guidelines.

DEVIATIONS FROM PROTOCOL

There were no deviations.



DATA DISPOSITION

Raw data and final report from these studies are archived at Northview Laboratories, Inc., 1880 Holste Road, Northbrook, IL 60062, under Northview Report Number C4E026G. The remaining test sample was returned to the client. It is the responsibility of the client to maintain test samples in accordance with GLP regulations.

PROCEDURE

1. See NVL SOP 12F-11 *Hemolysis - Spectrophotometric Method*

2. Test Supplies:

Negative Control: 0.9% Sodium Chloride for Injection, USP
McGaw, Inc. Lot Number J3S573, Exp. Date: 6/96

Positive Control: Water for Irrigation, USP
McGaw, Inc. Lot Number J3L329, Exp. Date: 6/96

Rabbit Blood Source: Crane Laboratories
Lot Number 1120, Exp. Date: 6/1/94 (Rabbit Blood citrated)

Duplicate portions of 60 cm² of test material were transferred into separate test tubes. 20 mL of 0.9% Sodium Chloride for Injection, USP was added to each tube and 10 mL to two additional test tubes as negative controls. 10 mL of Water for Irrigation, USP was added to two tubes as positive controls. All tubes were extracted at 50°C for 72 hours. Following extraction, 10 mL of extract was taken from each tube and 0.2 mL of blood was added. The tubes were capped and gently inverted to mix. Following incubation of the test tubes at 37°C ± 2°C for 60 minutes, the contents of each tube was decanted into a centrifuge tube and centrifuged at 2000 RPM for 10 minutes. The supernatant was transferred to a cuvette using a pasteur pipet and the absorbance of the supernatant measured at 545 nm.

The percent hemolysis was calculated using the following formula, where A is absorbance.

$$\% \text{ Hemolysis} = \frac{A(\text{test sample}) - A(\text{negative control})}{A(\text{positive control}) - A(\text{negative control})} \times 100$$

RESULTS

<u>Test Item</u>	<u>Results</u>
Calibre 2081-15 Polycarbonate, Blue Tint Lot Number: TB920707B20	< 1% Hemolysis

CONCLUSION

A test sample with 5% or less hemolysis is considered non-hemolytic.



NV Report Number C4E026G

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**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

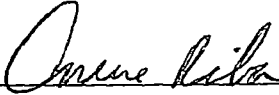
Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number C4E026G were conducted by the Quality Assurance Unit on the following dates:

<u>NV Protocol</u>	<u>Phase of Study</u>	<u>Date</u>
12F-11	Sample Preparation	05/13/94
12F-11	Addition of Blood	05/16/94
12F-11	Absorbance Readings	05/16/94

QAU inspection findings are routinely reviewed by the management of Northview Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number C4E026G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.



Quality Assurance Unit

6-1-94
Date



NORTHVIEW LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Marita Ocampo, Analyst
2. Rose-Marie Tobin, Analyst
3. Elaine Gruber, Study Director
4. Anne Riba, Quality Assurance Manager

STATEMENT OF COMPLIANCE

This study, Northview Report Number C4E026G, has been conducted in accordance with applicable Good Laboratory Practice regulations.

Elaine Gruber

Study Director

6-1-94

Date

Anne Riba

Quality Assurance Unit

6-1-94

Date

Morton J. Spalding

Management

6-1-94

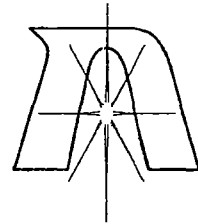
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Northview Laboratories, Inc.

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SPONSOR

Dr. Mary Reinhard
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati OH 45242

NV REPORT NUMBER

C4E027G

STUDY COMPLETION DATE

June 1, 1994

TEST ARTICLE

Calibre 2081-15 Polycarbonate, Blue Tint

Lot Number: TB920707B20
RDM Number: 13-2-0060-00508

TEST PERFORMED

USP PHYSICOCHEMICAL TESTS - PLASTICS

STUDY DIRECTOR

Elaine Gruber
Laboratory Manager
Analytical Laboratory Services
Northview Laboratories, Inc.

STUDY DATES

Study Initiation Date: 05/09/95
Study Completion Date: 06/01/94
Date On Test: 05/16/94
Date Off Test: 05/18/94
Study Authorization: Signed Protocol
Date Sample Received: 04/28/94
Purchase Order: A2442

TEST ARTICLE IDENTIFICATION

Calibre 2081-15 Polycarbonate, Blue Tint
139 pieces (720 cm²) (124 green colored cylinders and 15 green colored squares) in 3 tyvek pouches
Sterilization Data: Cobalt & ETO 4/6/94 & 4/11/94

Lot Number: TB920707B20
RDM Number: 13-2-0060-00508

TEST PERFORMED

USP Physicochemical Tests - Plastics

OBJECTIVE

This study was designed to determine physical and chemical properties of plastics and their extracts based on the extraction of the plastic material.

PROTOCOL

This test was conducted according to Protocol Number C4E027G which incorporates by reference USP XXII <661>, pages 1572 - 1573, on file at Northview Laboratories, Inc.

DEVIATIONS FROM PROTOCOL

There were no deviations.

DATA DISPOSITION

Raw data and final report from these studies are archived at Northview Laboratories, Inc., 1880 Holste Road, Northbrook, IL 60062, under Northview Report Number C4E027G. The remaining test sample was returned to the client. It is the responsibility of the client to maintain test samples in accordance with GLP regulations.



PROCEDURE

1. See USP XXII <661>, pages 1572 - 1573, *Physicochemical Test - Plastics*
2. Test Supplies:
 - A. Graduated Cylinder, 250 mL
 - B. Erlenmeyer Flasks, 250 mL, screw-capped with PTFE liners
 - C. USP Purified Water
 - D. Water Bath, 70°C
 - E. Silica crucibles
 - F. Pipets, burettes
 - G. Steam bath
 - H. Oven, 105°C
 - I. Color comparison tubes, Nessler, tall form, 50 mL
 - J. Hydrogen Sulfide TS
 - K. 1N Acetic Acid
 - L. 6N Ammonium Hydroxide
 - M. Short range pH paper
 - N. Standard lead solution
 - O. pH meter
 - P. 0.010N Hydrochloric Acid
 - Q. 0.010N Sodium Hydroxide

3. Procedure

- A. Preparation of Sample

The sample was tested as received. Following an initial rinsing with Purified Water, the sample was covered with Purified Water and maintained at 70°C for 24 hours. Portions of this extract were used to perform the indicated testing.

- B. Test Panel

- Non-volatile Residue

A designated volume of the extract was taken to dryness at 105°C along with a comparable volume of Purified Water as a blank. The difference in residue weights is computed.

- Residue on Ignition

Not performed, Non-volatile Residue results < 5 mg. If the Non-volatile Residue result exceeds 15 mg, the sample and blank residues are further heated to 800°C. Residues are again weighed and a sample to blank difference is computed.

- Heavy Metals

A volume of extract and a volume of diluted standard lead solution were brought to a pH between 3.0 - 4.0 and treated with hydrogen sulfide TS in separate Nessler color comparison tubes. Any brown color produced in the sample tube ten minutes later was compared to the brown color produced in the standard tube.



B. Test Panel (continued)Buffering Capacity

A volume of sample extract was brought to pH 7.0 using a pH meter and dilute acid or alkali. Purified water used as a blank was also titrated to pH 7.0. The total volumes and type of titrant used for each was recorded and the difference in volume computed according to the procedure.

RESULTS

<u>Test</u>	<u>USP Limits</u>	<u>Results</u>	<u>Status</u>
Non-Volatile Residue	≤ 15 mg*	< 15 mg	Conforms
Residue on Ignition	≤ 5 mg*	< 5 mg	Conforms
Heavy Metals	≤ 1 ppm in extract	< 1 ppm in extract	Conforms
Buffering Capacity	≤ 10.0 mL*	< 10.0 mL	Conforms

*Representing difference between sample and blank

CONCLUSION

The test sample conforms to USP XXII specifications for the Physicochemical Tests - Plastics.



**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number C4E027G were conducted by the Quality Assurance Unit on the following dates:

<u>NV Protocol</u>	<u>Phase of Study</u>	<u>Date</u>
USP XXII <661>	Sample Preparation	05/16/94
USP XXII <661>	Heavy Metals	05/18/94
USP XXII <661>	Non-volatile Residue	05/17/94
USP XXII <661>	Buffering Capacity	05/17/94

QAU inspection findings are routinely reviewed by the management of Northview Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number C4E027G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.



Quality Assurance Unit

6-1-94
Date



NORTHVIEW LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Gayla Vokoun, Analyst
2. Rose-Marie Tobin, Analyst
3. Elaine Gruber, Study Director
4. Anne Riba, Quality Assurance Manager

STATEMENT OF COMPLIANCE

This study, Northview Report Number C4E027G, has been conducted in accordance with applicable Good Laboratory Practice regulations.

Elaine Gruber

Study Director

6-1-94

Date

Anne Riba

Quality Assurance Unit

6-1-94

Date

Martin J. Spalding

Management

6-1-94

Date

:mc



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STUDY DATES

Study Initiation Date: May 4, 1994
Study Completion Date: June 1, 1994
Date On Test: May 5, 1994
Date Off Test: May 20, 1994
Study Authorization: Signed Protocol
Date Sample Received: April 25, 1994
Purchase Order: A2442

TEST ARTICLE IDENTIFICATION

Calibre 2081-15 Polycarbonate, Blue Tint
81 smoke-colored, polycarbonate cylinders each measuring 2.5 cm in length with a circumference of 1.57 cm, contained in a sealed sterile pouch. Two separate pouches each contained 30 pieces of 1 cm x 1 cm slabs, and 1 x 10 mm strips for the implantation test.
Code Identification: RDM #13-2-0060-00508
Lot Number: TB920707B20
Sterilization: Cobalt & ETO, 4/6/94 & 4/11/94
Storage Condition: Room Temperature

CONTROL ARTICLE IDENTIFICATION

Name: USP Negative Control Plastic RS Filaments
Physical Description: 10 x 1 mm white plastic filaments
Quantity/Container Type: 50 filaments in a glass vial
Expiration Date: N/A
Lot Number: Batch G-6
Storage Conditions: Room Temperature

PROTOCOL

This test was conducted according to Protocol Number X4D273G which incorporates by reference Northview SOP Nos. 16E-03, 16E-05, and 16E-06, and is on file at Northview Pacific Laboratories, Inc. This protocol satisfies the requirements of Ethicon Endo-Surgery.

DEVIATIONS FROM PROTOCOL

There were no deviations.

DATA DISPOSITION

Raw data from these studies is archived at Northview Pacific Laboratories, Inc., 2800 Seventh Street, Berkeley, CA 94710, under Northview Report Number X4D273G.

SUMMARY OF RESULTS

This test material met the requirements of the USP Systemic Injection Test, USP Intracutaneous Test, and USP Implantation Test (7 Day).



This test evaluates systemic responses to extracts of the test material following injection into mice.

Ten Swiss Webster albino mice (five per sample and five per corresponding blank) are used for each extract. Each animal is injected with 50 mL/kg (or 10 g/kg in the case of PEG 400) of the extract or blank. The animals are observed immediately after and at 4, 24, 48, and 72 hours following injection.

MATERIALS AND METHODS

Species	Mouse
Strain	Swiss-Webster
Source	Simonsen Laboratories, Inc., Gilroy, CA
Sex	Male
Age	Young adult
Number Used	Ten per extract (5 test and 5 blank)
Initial Weight	17 to 23 grams
Housing	Five per cage; 18-26C, 55 ± 15% relative humidity
Feed and Water	Purina Rodent Chow and water <i>ad libitum</i> supplied in bottles
Identification	Cage labeling and tail marking
Photoperiod	Diurnal (12 hours on - 12 hours off)
Quarantine Period	Four days

The mouse is the species required by the current version of the United States Pharmacopoeia.

**Table 1
Supplies**

Reagents/Equipment	Lot Number	Manufacturer	Expiration Date
Sodium Chloride for Injection (SCI), USP	J4A410	McGaw	1/96
Cottonseed Oil (CSO)	IA284	Spectrum Chemicals	7/97
95% Ethyl Alcohol, USP	93H06	McCormick	8/97
Polyethylene Glycol 400 (PEG 400)	2183402141	Kodak	N/A

**Table 2
Study Design**

Extracting Medium	Number of Animals	Route of Administration	Dose	Injection Rate (μ L/sec)
SCI	10	I.V.	50 mL/kg	100
CSO	10	I.P.	50 mL/kg	-
1:20 Alcohol in SCI (A/S)	10	I.V.	50 mL/kg	100
PEG 400	10	I.P.	10 g/kg	-

I.V. - Intravenous

I.P. - Intraperitoneal

Sample Preparation And Dosing Procedure

Sample Preparation - For each of the extracts, a dry sterile glass tube with a screw cap was filled with 20 mL of the extracting medium. Pieces of the test material measuring 60 cm² total surface area were then added to the tube. An additional sterile tube was filled with 20 mL of the same medium to serve as a blank. The samples and blanks were extracted at 50C for 72 hours.

Prior to injection, each gram of the PEG 400 sample extract and blank was diluted with 4.1 volumes of SCI to obtain a solution having a concentration of approximately 200 mg of PEG 400 per mL.

Dosing Procedure - Each extract was vigorously agitated prior to withdrawal of injection doses to ensure even distribution of extracted matter.

Five mice were each injected with 50 mL/kg (in the case of the PEG 400, 10 g/kg) of the SCI extract. Five additional mice were each injected with 50 mL/kg of the corresponding SCI blank. This procedure was repeated for each extracting medium.

Observation

Clinical Observations - All of the animals were observed for toxic signs immediately after dosing and at 4, 24, 48, and 72 hours after dosing.

Weights - All of the animals were weighed prior to dosing and at the 72 hour observation.

Interpretation And Analysis

The test material meets the USP requirements if none of the animals treated with the sample extract show a significantly greater biological reactivity than those treated with the blank.

The test material does not meet the USP requirements if two or more mice die, if abnormal behavior such as convulsions or prostration occurs in two or more mice, or if a body weight loss greater than two grams occurs in three or more mice.

RESULTS AND DISCUSSION

Clinical Observations - No toxic signs were observed during the 72 hour observation period.

Weights - All of the animals gained weight during the test (see Tables 4 & 5).

CONCLUSION

This test material met the requirements of the USP Systemic Injection Test using the extracting medium and conditions listed above.

Table 3
Mortalities And Toxic Signs

Extracting Medium	Mortalities		Other Toxic Signs*		Results
	Sample	Blank	Sample	Blank	
SCI	0/5	0/5	0/5	0/5	Pass
CSO	0/5	0/5	0/5	0/5	Pass
1:20 A/S	0/5	0/5	0/5	0/5	Pass
PEG 400	0/5	0/5	0/5	0/5	Pass

* Details are described above



Table 4
Dose Volumes and Animal Weights

Extracting Medium	Animal Number	Dose (mL)	Pre-Test Weight (g)	Post-Test Weight (g)	Weight Change (g)
SCI Sample	#1	0.9	17	22	+ 5
	#2	1.0	20	25	+ 5
	#3	1.1	21	26	+ 5
	#4	1.0	19	23	+ 4
	#5	1.1	21	26	+ 5
SCI Blank	#1	1.0	19	25	+ 6
	#2	1.0	19	23	+ 4
	#3	1.0	19	25	+ 6
	#4	1.0	20	25	+ 5
	#5	0.9	18	23	+ 5
CSO Sample	#1	1.0	19	23	+ 4
	#2	1.0	20	23	+ 3
	#3	0.9	17	21	+ 4
	#4	1.0	19	23	+ 4
	#5	1.0	20	24	+ 4
CSO Blank	#1	0.9	18	23	+ 5
	#2	1.1	21	24	+ 3
	#3	1.0	19	24	+ 5
	#4	1.1	21	25	+ 4
	#5	1.0	19	24	+ 5



Table 5
Dose Volumes and Animal Weights

Extracting Medium	Animal Number	Dose (mL)	Pre-Test Weight (g)	Post-Test Weight (g)	Weight Change (g)
1:20 A/S Sample	#1	1.0	19	24	+ 5
	#2	0.9	18	23	+ 5
	#3	1.1	21	25	+ 4
	#4	1.0	20	25	+ 5
	#5	1.0	20	25	+ 5
1:20 A/S Blank	#1	1.0	20	25	+ 5
	#2	1.1	21	25	+ 4
	#3	1.0	20	24	+ 4
	#4	1.1	21	25	+ 4
	#5	1.0	20	25	+ 5
PEG 400 Sample	#1	1.1	21	24	+ 3
	#2	0.9	18	23	+ 5
	#3	1.0	20	25	+ 5
	#4	1.0	19	24	+ 5
	#5	1.0	20	24	+ 4
PEG 400 Blank	#1	1.1	21	24	+ 3
	#2	1.0	20	24	+ 4
	#3	1.1	21	26	+ 5
	#4	1.0	19	25	+ 6
	#5	1.1	21	26	+ 5

REFERENCES

NV SOP 16E-03

USP XXII [1990] p. 1499

USP XXII Supplement 5 [Nov. 15, 1991] p. 2704

USP INTRACUTANEOUS TEST

INTRODUCTION

This test evaluates local responses to extracts of the test material following intracutaneous injections into rabbits.

On the day of the test the fur on the back of each rabbit is removed on both sides of the spinal column. A 0.2 mL portion of one of the test extracts is injected intracutaneously at each of five sites along one side of the spinal column of each of two rabbits. A 0.2 mL portion of the corresponding blank is injected intracutaneously at five sites along the other side of the spinal column of each of two rabbits. The injection sites are observed for erythema and edema, and scored at 24, 48 and 72 hours.

MATERIALS AND METHODS

Test System

Species	Rabbit
Strain	New Zealand White
Source	Elkhorn Rabbitry, Watsonville, CA
Number Used	Four total
Initial Weight	At least 2.0 kg
Age	Adult
Sex	Male
Housing	Individually at 17-21C and 55 ± 15% relative humidity
Feed and Water	Purina Rabbit Chow (approximately 200 grams per day) and water <i>ad libitum</i> .
Identification	Ear tags
Photoperiod	Diurnal (12 hours on - 12 hours off)
Quarantine Period	Seven days

Justification For Test System

The rabbit is the species required by the current version of the United States Pharmacopoeia.

**Table 6
Supplies**

Reagents/Equipment	Lot Number	Manufacturer	Expiration Date
Sodium Chloride for Injection (SCI), USP	J4A410	McGaw	1/96
Cottonseed Oil (CSO)	IA284	Spectrum Chemicals	7/97
95% Ethyl Alcohol, USP	93H06	McCormick	8/97
Polyethylene Glycol 400 (PEG 400)	2183402141	Kodak	N/A

**Table 7
Study Design**

Extracting Medium	Number of Animals ¹	Route of Administration ²	Dose/ Site	Number of Sites/ Animal
SCI	2	I.C.	0.2 mL	5 sample, 5 blank
CSO	2	I.C.	0.2 mL	5 sample, 5 blank
1:20 Alcohol in SCI (A/S)	2	I.C.	0.2 mL	5 sample, 5 blank
PEG 400	2	I.C.	0.2 mL	5 sample, 5 blank

1. Two animals received the SCI and CSO preparations (twenty sites per animal), and two animals received the A/S and PEG 400 preparations (twenty sites per animal). Four animals were used on this study (see Dosing Procedure, p. 11).

2. I.C. - Intracutaneous Injection

Sample Preparation And Dosing Procedure

Sample Preparation - For each of the extracts, a dry sterile glass tube with a screw cap was filled with 20 mL of the extracting medium. Pieces of the test material measuring 60 cm² total surface area were then added to the tube. An additional sterile tube was filled with 20 mL of the same medium to serve as a blank. The samples and blanks were extracted at 50C for 72 hours.

Prior to injection, each gram of the PEG 400 sample extract and blank was diluted with 7.4 mL of SCI to obtain a solution having a concentration of approximately 120 mg of PEG 400 per mL.

Dosing Procedure - On the day of the test, the fur on the back of each animal was removed with electric clippers. Each extract was vigorously agitated prior to withdrawal of injection doses to ensure even distribution of extracted matter.

On the right cranial portion of one rabbit's back, five 0.2 mL intracutaneous injections of one test extract were administered. The left cranial side was similarly treated with the corresponding blank. On the caudal portion of the rabbit's back, the injection procedure was repeated using a second extract and blank. A second rabbit was then injected with the same extracts and blanks. A second pair of rabbits was used for the remaining extracts and blanks.

Observation And Scoring

Clinical Observations - All of the animals were observed daily for signs of ill health.

Scoring - The injection sites were examined and scored for any tissue reactions, such as erythema/eschar formation and edema, at 24, 48, and 72 hours after injection according to Table 8.

Interpretation And Analysis

The average erythema and edema scores for the test extracts and blanks are determined at each scoring interval (24, 48, and 72 hours) for each rabbit.

After the 72 hour scoring, all erythema and edema scores are totalled separately for each test extract and blank. To determine the overall mean score, each of the totals are divided by 12 (2 animals x 3 scoring periods x 2 scoring categories).

The test material meets the USP requirements if the difference between the mean score for the test extract and the mean score for the corresponding blank is 1.0 or less.

RESULTS AND DISCUSSION

Clinical Observations - The animals remained healthy throughout the test period.

Scoring - The difference between the mean scores for each of the test extracts and corresponding blanks was 1.0 or less (see Tables 9 - 12).

CONCLUSION

This test material met the requirements of the USP Intracutaneous Test using the extracting medium and conditions described above.

Table 8
Evaluation Of Skin Reactions

Erythema And Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet-redness) to slight eschar formation (injuries in depth)	4
Edema Formation ¹	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised about 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

¹ Excludes non-inflammatory (mechanical) edema from the blank or extraction fluid.



NV Report Number X4D273G

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Table 9
Skin Reaction Scores
Sodium Chloride For Injection Extract And Blank

Rabbit # 10846 M		Blank Sites					Test Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Test
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
<hr/>													
Rabbit # 10847 M		Blank Sites					Test Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Test
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
Overall Mean:												0	0

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Table 10
Skin Reaction Scores
Coltseed Oil Extract And Blank

Rabbit # 10846 M		Blank Sites					Test Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Test
24 hr	Erythema	1	1	1	1	1	1	1	1	1	1	1.0	1.0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	1	1	1	1	1	0	0	0	0	0	1.0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
<hr/>													
Rabbit # 10847 M		Blank Sites					Test Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Test
24 hr	Erythema	1	1	1	1	1	1	1	1	1	1	1.0	1.0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	1	1	1	1	1	1	1	1	1	1	1.0	1.0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
Overall Mean:												0.3	0.3

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Table 11
Skin Reaction Scores
1 In 20 Solution Of Alcohol In SCI Extract And Blank

Rabbit # 10888 M		Blank Sites					Test Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Test
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
<hr/>													
Rabbit # 10889 M		Blank Sites					Test Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Test
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
Overall Mean:												0	0

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Table 12
Skin Reaction Scores
Polyethylene Glycol 400 Extract And Blank

Rabbit # 10888 M		Blank Sites					Test Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Test
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
<hr/>													
Rabbit # 10889 M		Blank Sites					Test Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Test
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
Overall Mean:												0	0

REFERENCES

NV SOP 16E-05

USP XXII [1990], p. 1499

USP XXII Supplement 5 [Nov. 15, 1991], p. 2704-2705



USP IMPLANTATION TEST

INTRODUCTION

This study is designed to evaluate the potential for local irritancy of plastic materials and other polymeric materials in direct contact with living tissue.

The test material is implanted into the paravertebral muscles of two rabbits. At the end of the implant period, the implant sites are harvested and evaluated for evidence of hemorrhage, necrosis, discoloration, infection and encapsulation.

MATERIALS AND METHODS

Test System

Species	Rabbit
Strain	New Zealand White
Source	Elkhorn Rabbitry, Watsonville, CA.
Number	Two
Initial Weight	At least 2.5 kg
Sex	Female
Age	Adult
Housing	Individually at 17 - 21C and 55 $\pm$ 15% relative humidity
Feed and Water	Purina Rabbit Chow (approximately 200 grams per day) and water <i>ad libitum</i>
Identification	Ear tags
Photoperiod	Diurnal (12 hours on - 12 hours off)
Quarantine Period	Seven days

Justification For Test System

The rabbit is the species required by the current version of the United States Pharmacopoeia.



Table 13
Supplies

Reagents/Equipment	Lot Number	Manufacturer	Expiration Date
Ketamine hydrochloride	440190	Aveco Co.	1/96
Sodium pentobarbital	F4A006	Anpro	1/96
Beuthanasia D solution	J3RR02	Schering-Plough	8/95
USP Negative Control Plastic RS	Batch G-6	USPC, Inc.	N/A
Hypodermic Needle (15 gauge)	100486	Monoject	N/A

Sample Preparation And Implant Procedure

Sample Preparation - Test samples were provided by the sponsor pre-cut into 1 x 10 mm strips. Control samples were cut into strips measuring not less than 1 x 10 mm.

Equipment - All materials and implements used on this study were sterilized prior to use or purchased sterile. Aseptic techniques were followed.

Implant Procedures - Prior to testing, the animal's fur on both sides of the spinal column was removed with electric clippers. Each animal was then anesthetized with ketamine hydrochloride (45 mg/kg of body weight) and sodium pentobarbital (15 mg/kg of body weight).

Four test strips were aseptically implanted into the paravertebral muscle with a sterile hypodermic needle fitted with a sterile trocar stylet. The test strips were implanted 2.5 to 5 cm from the midline, parallel to the spinal column and about 2.5 cm apart from each other.

The two control strips were implanted in the same way on the other side of the spinal column.

The animals were observed until they had recovered from the effects of the anesthesia, and then were gently placed back in their cages.

Observation And Scoring

Clinical Observations - Once daily, all animals were observed for mortality, signs of ill health, and at the implant sites for reaction to treatment (e.g., infection, etc.).

Euthanization - On Day 7 of the study, the animals were euthanized with a lethal injection of Beuthanasia D solution.

Postmortem - Samples (test and control) and their surrounding tissue were recovered and examined for gross evidence of hemorrhage, necrosis, discoloration, infection and encapsulation.

Encapsulation was scored according to Table 14. If present, encapsulation was measured by recording the width of the capsule (from the periphery of the space occupied by the implant to the periphery of the capsule) rounded to the nearest 0.1 mm.



Interpretation And Analysis

The average mean scores for encapsulation for both the sample and control sites are calculated. The test material meets the USP requirements if the difference between these scores does not exceed 1.0, or if the difference between the sample and control mean scores for more than one of the four implant sites does not exceed 1 for any implanted animal.

RESULTS AND DISCUSSION

Clinical Observations - All animals remained healthy throughout the study period. No evidence of infection was found at the incision sites.

Macroscopic Examination - No encapsulation was observed at the test or control sites upon sample recovery. All implantation sites were recovered from inside the muscle tissue.

CONCLUSION

This test material met the requirements of the USP Implantation Test when implanted for 7 Days.

Table 14
Evaluation of Encapsulation

Capsule Width	Score
None	0
up to 0.5 mm	1
0.6 - 1.0 mm	2
1.1 - 2.0 mm	3
> 2.0 mm	4



Table 15
Implantation Results

Rabbit Number	Sex	Sites	Encapsulation Score	Gross Examination
10859	F	T1	0	No reactions observed
		T2	0	No reactions observed
		T3	0	No reactions observed
		T4	0	No reactions observed
		Sample Mean:	0	
		C1	0	No reactions observed
		C2	0	No reactions observed
		Control Mean:	0	
10860	F	T1	0	No reactions observed
		T2	0	No reactions observed
		T3	0	No reactions observed
		T4	0	No reactions observed
		Sample Mean:	0	
		C1	0	No reactions observed
		C2	0	No reactions observed
		Control Mean:	0	

T = Test, C = Control

REFERENCES

NV SOP 16E-06

USP XXII [1990], p. 1499

USP XXII Supplement 5 [Nov. 15, 1991], p. 2705



**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number X4D273G were conducted by the Quality Assurance Unit on the following dates:

NV SOP No.	Phase of Study	Date
16E-03	72 Hour Weighing	05/20/94
16E-05	72 Hour Scoring	05/20/94
16E-06	Sample Recovery	05/12/94

QAU inspection findings are routinely reviewed by the management of Northview Pacific Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number X4D273G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.



Quality Assurance Unit

6/1/94

Date



NORTHVIEW PACIFIC LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Timothy V. Doherty, D.V.M., Director *In Vivo* Laboratory Services
2. Roger O'Meara, Assistant Manager, *In Vivo* Laboratory Services
3. Robert Noonan, Ph.D., Senior Scientist, *In Vivo* Laboratory Services
4. Benjamin Davis, Technician, *In Vivo* Laboratory Services
5. Lisa Gearin, Technician, *In Vivo* Laboratory Services
6. Robert Mosbauer, Technician, *In Vivo* Laboratory Services
7. Edwin T. Pangilinan, M.S., Technician, *In Vivo* Laboratory Services
8. James Ruiz, Technician, *In Vivo* Laboratory Services
9. Patrice Pitot, Technician, *In Vivo* Laboratory Services
10. Matthew I. Kanter, Manager, Quality Assurance

STATEMENT OF COMPLIANCE

This study, Northview Report Number X4D273G, has been conducted in accordance with applicable Good Laboratory Practice regulations.

Edwin T. Pangilinan
Study Director

6/1/94
Date

Matthew I. Kanter
Quality Assurance Unit

6/1/94
Date

[Signature]
Management

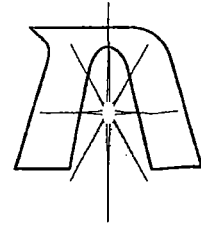
6/1/94
Date

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Northview Pacific Laboratories, Inc.

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SPONSOR

Mary K. Reinhard D.V.M.
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

NV REPORT NUMBER

X4D275G

STUDY COMPLETION DATE

July 5, 1994

TEST ARTICLE IDENTIFICATION

Calibre 2081-15 Polycarbonate, Blue Tint

Code Identification: RDM#13-2-0060-00508

Lot Number: TB920707B20

Sterilization Data: Cobalt & ETO 4/6/94 & 4/11/94

TEST PERFORMED

Guinea Pig Maximization Test

STUDY DIRECTOR

Dr. Robert A. Noonan
Senior Scientist
In Vivo Laboratory Services
Northview Pacific Laboratories, Inc.



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STUDY DATES

Study Initiation Date: May 12, 1994
Study Completion Date: July 5, 1994
Date On Test: May 17, 1994
Date Off Test: June 10, 1994
Study Authorization: Signed Protocol
Date Sample Received: May 5, 1994

TEST ARTICLE IDENTIFICATION

Calibre 2081-15 Polycarbonate, Blue Tint
Amber plastic cylinders (60 to 100) approximately 2.5 cm long and 0.5 cm in diameter and 27 amber squares 1 x 1 cm in sterile envelopes.
Code Identification: RDM#13-2-0060-00508
Lot Number: TB920707B20
Sterilization Data: Cobalt & ETO 4/6/94 & 4/11/94
Storage Condition: Room Temperature

CONTROL ARTICLE IDENTIFICATION

1-Chloro-2, 4-Dinitrobenzene
100 g of yellow granular powder in a amber glass jar
Lot Number: 100H0738
Expiration Date: Jan. 1, 1995
Storage Condition: Room Temperature

PROTOCOL

This test was conducted according to Protocol Number X4D275G which incorporates by reference Northview SOP No. 16G-13 and is on file at Northview Pacific Laboratories, Inc. This protocol satisfies the requirements of Ethicon Endo-Surgery.

DEVIATIONS FROM PROTOCOL

For the first extraction, five cylinders (each with a surface area of 4.44 cm²), were extracted in 7.4 mL vehicle. For the second, four cylinders were extracted in 5.9 mL vehicle. For the third, seven cylinders were extracted in 10.4 mL. The protocol specifies extraction of 60cm² in 20 mL vehicle.

There was insufficient test material to extract 60 cm² of surface area for each extraction. The amounts of surface area used were proportional to 60 cm² in 20 mL vehicle. Therefore, the extractions were equivalent to the amount specified in the protocol.

In the judgement of the study director, this deviation did not affect the integrity of the study.



DATA DISPOSITION

Raw data from this study is archived at Northview Pacific Laboratories, Inc., 2800 Seventh Street, Berkeley, CA 94710, under Northview Report Number X4D275G.

SUMMARY OF RESULTS

None of the test group or negative control animals exhibited scores higher than 1. A response of 1 is not considered to reflect significant sensitization. According to the criteria for this test, the test material had a weak (Grade I) potential for sensitization. (See Table 7.)



GUINEA PIG MAXIMIZATION TEST

INTRODUCTION

This test is designed to determine to what extent a test material has the potential for acting as a contact sensitizer in guinea pigs.

Fifty-five Hartley albino guinea pigs were used: including ten test group animals and five negative controls for the 0.9% Sodium Chloride for Injection (SCI) extraction; and ten test group animals and five negative controls for the cottonseed oil (CSO) extraction. The results were compared to positive control groups: these included five positive controls and two groups of five naive positive controls (one for challenge and one for rechallenge) prepared in deionized water, in addition to five positive controls and five naive positive controls prepared in cottonseed oil. The positive control tests were done as part of NV Study No. HSTPOSG, a historical study run in January 1994.

In this test an extract of the test material was employed. As a negative control a vehicle blank was incubated under the same conditions as the test material. As a positive control a solution of 1-Chloro-2, 4-Dinitrobenzene (DNCB) was employed.

Groups of guinea pigs were exposed to the test material extracts and the control solutions in an induction phase lasting nine days. In this phase, the materials were first injected intradermally (ID). For the ID injections the solutions were given undiluted as well as emulsified in Freund's Complete Adjuvant (FCA). Seven days after the ID injections, the extracts, vehicle blank and positive control solution were mixed with petrolatum (1:20 w/w) and administered topically over the same area in which the ID injections were given. The topical exposure lasted 48 hours.

Fourteen days after the topical administration, the animals were challenged. For the challenge phase, extracts and control solutions were administered on Hill Top Chambers, which were applied to untreated sites, and remained on the animals for 24 hours. The dosing sites were scored for erythema and edema 24 and 48 hours after removal of the chambers.

Based on the erythema and edema scores observed, the test material was classified as to its allergic potential.

MATERIAL AND METHODS

Test System

Species	Guinea Pig
Strain	Hartley, Albino
Source	Charles River Laboratories, Wilmington, MA
Age	Young adult
Initial Weight	300 to 500 grams
Housing	Individually; 18 - 26C, 55 ± 15% relative humidity
Feed	Purina Guinea Pig Chow and water supplied in bottles <i>ad libitum</i>
Photoperiod	Diurnal (12 hours on - 12 hours off)
Quarantine Period	Seven days



Justification For Test System

Guinea pigs are the preferred species for skin sensitization tests. They are required for the maximization test (see Magnusson and Kligman, *J. Invest. Dermatol.* 52:268. [1969]). The Hartley albino guinea pig is specified for use in the maximization test by ASTM Standard F720-81.

Table 1
Supplies

Reagents/Equipment	Lot Number	Manufacturer	Expiration Date
Sodium Chloride for Injection, USP (SCI)	For Induction - J4A410 For Challenge - J3P619	McGaw McGaw	1/1/96 11/1/95
Cottonseed Oil (CSO)	IA284	Spectrum	6/23/98
Freund's Complete Adjuvant (FCA)	063H-8800	Sigma	11/23/97
Petrolatum	113948	VWR	6/1/97
Ethanol	93H06	McCormick Dist Co.	8/1/97
1-chloro-2, 4-dinitrobenzene (DNCB)	100H0738	Sigma	1/1/95
10% Sodium Lauryl Sulfate (SLS)	83H0841	Sigma	4/12/98
Sterile Water for Injection (WFI)	C246421	Baxter	11/1/94
Hill Top Chambers™	06MBA-2002	Hill Top Research	6/1/97
Whatman #3 Filter Paper	N/A	Whatman Lab. Div.	N/A
Blenderm™ Surgical Tape	Jun92-432	3M	N/A
Gauze - Soft Kling Conforming Bandage	1994-02	Johnson & Johnson	N/A
Anchor™ Masking Tape (½ inch)	N/A	Anchor Continental, Inc.	N/A
Dental Dam™ elastic bandage	11994	Hygenic Corp.	N/A

Table 2
Study Design

Group	Number of Animals	INDUCTION PHASE		CHALLENGE PHASE
		1st Induction (Day 0) Route	2nd Induction (Day 7) Route	(Day 21) Route
SCI Test	10	Intradermal	Topical	Topical
CSO Test	10	Intradermal	Topical	Topical
SCI Positive Control	5	Intradermal	Topical	Topical
CSO Positive Control	5	Intradermal	Topical	Topical
SCI Naive Positive Control (for challenge)	5	N/A	N/A	Topical
SCI Naive Positive Control (for rechallenge)	5	N/A	N/A	Topical
CSO Naive Positive Control (for challenge)	5	N/A	N/A	Topical
SCI Negative Control	5	Intradermal	Topical	Topical
CSO Negative Control	5	Intradermal	Topical	Topical

SAMPLE PREPARATION AND DOSING PROCEDURE

Sample Extraction - The test material was extracted in two vehicles, SCI and CSO. For the first extraction, five cylinders, each with an approximate surface area of 4.44 cm² were cut up and placed in dry, sterile, glass, screw capped tubes filled with 7.4 mL of extraction vehicle. An additional sterile tube was filled with each vehicle to serve as a blank. The extracts and blanks were incubated for 72 hours at 50C. For the second extraction, four cylinders were extracted in 5.9 mL of vehicle. For the third extraction, seven cylinders were extracted in 10.4 mL of vehicle.

Animal Assignment - The animals were divided into nine groups; ten SCI test animals, ten CSO test animals, five SCI positive controls, five CSO positive controls, two groups of five naive SCI positive controls (for challenge and rechallenge), one group of five naive CSO positive controls, one group of five SCI negative controls and one group of five CSO negative controls.

Induction Phase - Intradermal Injection - The induction phase consisted of an intradermal injection of the test material followed a week later by a topical application. At least 24 hours prior to each application, the hair on the dorsocranial thorax was removed by clipping. On the day of dosing, at least one hour prior to dosing, the clipped areas were shaved.

The procedure for intradermal injections of test material extracted in SCI is illustrated in Table 3.

Three pairs of 0.1 ml intradermal injections were administered to the shaved areas on the test animals. The first pair of injections (cranial) consisted of an emulsion of Freund's Complete Adjuvant in an equal volume of WFI. The second pair (middle) was the test material extract. The third pair (caudal) consisted of an emulsion of the test material extract in an equal volume of FCA.



The five positive control animals were treated in a similar manner to the test animals, except that the test material extract was replaced in the second and third pair of injections with an equal amount of a 0.1% solution of a known sensitizer, 1-chloro-2,4-dinitrobenzene (DNCB) in 9.5% ethanol.

The five negative control animals were treated in a similar manner to the test animals, except that the test material extract was replaced in the second and third pair of injections with an equal amount of vehicle extract (blank).

Table 3
Induction Phase - Intradermal Injections (For SCI vehicle extraction)

Site Location	No. of Sites	TEST GROUP		POSITIVE CONTROL		NEGATIVE CONTROL	
		Volume/ Site	Dose Material	Volume/ Site	Dose Material	Volume/ Site	Dose Material
Cranial	2	0.1 ml	1:1 (FCA:WFI)	0.1 ml	1:1 (FCA:WFI)	0.1 ml	1:1 (FCA:WFI)
Middle	2	0.1 ml	Test Extract	0.1 ml	DNCB	0.1 ml	Blank
Caudal	2	0.1 ml	1:1 (FCA:Test Extract)	0.1 ml	1:1 (FCA:DNCB)	0.1 ml	1:1 (FCA:Blank)

The procedure for intradermal injections of test material extracted in CSO is illustrated in Table 4. Three pairs of 0.1 mL intradermal injections were administered to the shaved area of test animals, the first pair of injections (cranial) consisted of an emulsion of Freund's Complete Adjuvant (FCA) in CSO. The second pair of injections (center) was of CSO test material extract in an equal volume of WFI. The third pair of injections (caudal) consisted of an emulsion of CSO test material extract in an equal volume of FCA.

The five positive control animals were treated in a similar manner to the test animals, except that the test material extract was replaced in the second and third pair of injections with an equal amount of a 0.1% solution of a known sensitizer, 1-chloro-2,4-dinitrobenzene (DNCB) in ETOH/CSO (1:9 v/v).

The five negative control animals were treated in a similar manner to the test animals, except that the test material extract was replaced in the second and third pair of injections with an equal amount of vehicle control extract.



Table 4
Induction Phase - Intradermal Injections (For CSO vehicle extraction)

Site Location	No. of Sites	TEST GROUP		POSITIVE CONTROL		NEGATIVE CONTROL	
		Volume/ Site	Dose Material	Volume/ Site	Dose Material	Volume/ Site	Dose Material
Cranial	2	0.1 ml	1:1 (FCA:CSO)	0.1 ml	1:1 (FCA:CSO)	0.1 ml	1:1 (FCA:CSO)
Middle	2	0.1 ml	1:1 (Test Extract:WFI)	0.1 ml	1:1 (DNCB:WFI)	0.1 ml	1:1 (Blank:WFI)
Caudal	2	0.1 ml	1:1 (FCA:Test Extract)	0.1 ml	1:1 (FCA:DNCB)	0.1 ml	1:1 (FCA:Blank)

Induction Phase - Topical Dosing - Six days after performing the intradermal injections, the previously treated areas were clipped and shaved. The test sites were evaluated for evidence of irritation. Since the test material did not cause irritation, the dosing sites on the test and negative control group animals were pretreated with 10% SLS in petrolatum. This SLS mixture was massaged into the skin using a glass rod. Twenty-four hours later (seven days after the intradermal injections) the animals were reshaved.

For the topical application phase of the induction, 1.0 g of an extract of the test material, prepared in the same manner as for the intradermal injections, was mixed with 19.0 g of petrolatum (1:19 by weight).

The dosing patches were prepared by spreading a thick even layer of an extract of the test material, mixed with petrolatum (1:19 by weight) on 4.25 cm diameter disks of Whatman #3 filter paper. These were applied to the shaved areas on the test animals and held in place with surgical tape. The trunks of the animals were wrapped with gauze, which was held in place with ½ inch masking tape. They were then wrapped with an elastic bandage so that complete occlusion was obtained.

The positive and negative control animals were treated in a similar manner to the test group animals except that their patches contained 1:19 mixtures of 0.1% DNCB in petrolatum and a vehicle extract (blank) in petrolatum, respectively.

Forty-eight hours after dosing, the animals were unwrapped and the patches removed.



Table 5
Challenge Phase

Group	No. of Sites	Volume /Site	Material
SCI Test Extract	1	0.4 mL	Test Extract
SCI Positive Control	1	0.4 mL	0.05% DNCB
SCI Naive Positive Control	1	0.4 mL	0.05% DNCB
SCI Negative Control	1	0.4 mL	Blank Extract
CSO Test Extract	1	0.4 mL	Test Extract
CSO Positive Control	1	0.4 mL	0.05% DNCB
CSO Naive Positive Control	1	0.4 mL	0.05% DNCB
CSO Negative Control	1	0.4 mL	Blank Extract

Challenge Phase - The challenge phase dosing procedure is summarized in Table 5. Thirteen days after the topical induction application, a 5 x 5 cm area on the right side of each animal was clipped. On the next day (fourteen days after the topical application), the animals were shaved and Hill Top Chambers™ (approximate diameter 25 mm) containing 0.4 mL volumes of the test extracts, vehicle control extracts, or the positive control solutions were applied to the shaved areas. For the challenge phase a positive control solution consisting of 0.05% DNCB in either 9.5% aqueous ethanol or in CSO/ethanol (9:1 v/v) was used. Two groups of five previously untreated animals were dosed with the positive control solution, in two vehicles, to serve as naive positive controls.

The animals were wrapped, as described for the topical application, so that complete occlusion was obtained. The patches remained in place for 24 hours. Then, the animals were unwrapped and the test sites gently cleansed with 70% aqueous ethanol to remove chemical residues.

Because of the presence of irritation in the SCI naive positive control group, the SCI positive controls were rechallenged ten days after the primary challenge. A new group of five SCI naive positive control animals was used. The animals were treated in the same manner as in primary challenge, except that they were dosed at the previously unexposed site, and the dose concentration was reduced to 0.025% DNCB.

OBSERVATION AND SCORING

Clinical Observations - All of the animals were observed for toxic signs immediately after dosing and daily until the end of the study.

Weights - All of the animals were weighed prior to the initiation and at the end of the study.

Scoring - Skin responses at the challenge sites were scored 24 and 48 hours after removal of the patches according to the criteria in Table 6.



INTERPRETATION AND ANALYSIS

Scores of 2 or greater are considered significant. Test group animals with scores of 2 or greater in either the 24 or 48 hour observation period may be considered sensitized. Magnusson and Kligman do not regard a score of 1 as representing significant sensitization. However, the allergic potential of the substance is considered proven only when the skin reactions to the test material clearly outweigh those of the negative control group to the vehicle blank.

Based upon the number of animals sensitized, a rating of the material's potential for sensitization is given according to the grading scale shown in Table 7.

RESULTS AND DISCUSSION

Clinical Observations - All of the test and control animals appeared healthy. No toxic symptoms were observed over the duration of the study.

Scoring Results - The 24 and 48 hour scores for the primary challenge are shown in Tables 8 - 14.

SCI Test Group - At both the 24 and 48 hour observations, two of the ten animals exhibited scattered mild redness (Score of 1). (See Table 8.)

SCI Negative Control Group - At the 24 hour observation, two of five animals exhibited scores of 1. At the 48 hour observation, one exhibited a score of 1. (See Table 9.)

SCI Positive Control Group - All five of the animals exhibited intense redness and swelling (scores of 3) at both the 24 hour and 48 hour observations. (See Table 10.)

SCI Positive Naive Control Group - One of five animals exhibited a score of 3 at both the 24 and 48 hour observations. Four others exhibited scores of 1. (See Table 10.)

Because of the presence of a score of 3 in the positive naive control group, responses seen in the positive control group could not be evaluated. Therefore, a rechallenge was performed. For the rechallenge the concentration of DNCB was reduced to 0.025%. A new group of five naive animals were employed as naive controls. The 24 and 48 hour scores for the rechallenged positive controls are shown in Table 11.

Rechallenge SCI Positive Control Group - After the rechallenge, three of the five positive controls exhibited scores of 3 at the 24 hour observation. Two others exhibited scores of 2. At the 48 hour observation, four exhibited scores of 3 and one a score of 2.

Rechallenge Naive Positive Control Group - One naive positive control exhibited a score of 1 at the 24 hour observation. None exhibited responses at the 48 hour observation.

CSO Test Group - At the 24 hour observation, three of ten animals exhibited scores of 1. Two exhibited scores of 1 at the 48 hour observation. (See Table 12.)

CSO Negative Control Group - Three of five animals exhibited scores of 1 at the 24 hour observation. One exhibited a score of 1 at the 48 hour observation. (See Table 13.)

CSO Positive Control Group - At the 24 hour observation, four of five animals exhibited scores of 3. One exhibited a score of 2. At the 48 hour observation, two exhibited scores of 3, and three, scores of 2. (See Table 14.)

CSO Naive Positive Control Group - At the 24 hour observation, four animals exhibited scores of 1. One exhibited a score of 1 at the 48 hour observation. (See Table 14.)

Weights - All of the animals on this study exhibited normal weight gain. (See Tables 15 - 23.)

CONCLUSION

None of the test group or negative control animals exhibited scores higher than 1. A response of 1 is not considered to reflect significant sensitization. By the criteria of this test, the test material had a weak (Grade I) potential for sensitization. (See Table 2.)

Under similar treatment conditions, all five positive control animals exhibited sensitization responses to the challenge dose, while none of the naive positive controls exhibited scores higher than 1. This establishes that the treatment conditions were able to induce a response from a known sensitizer.

Table 6
Scoring Key

Reaction	Score
No Reaction	0
Scattered Mild Redness	1
Moderate and Diffuse Redness	2
Intense Redness and Swelling	3

Table 7
Classification of Potential for Sensitization

% Sensitized	Grade	Classification
0 - 8	I	Weak
9 - 28	II	Mild
29 - 64	III	Moderate
65 - 80	IV	Strong
81 - 100	V	Extreme



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Table 8
Test Scores After Challenge Phase
Test Group (Extraction in SCI)

Animal Number	24 Hour Score	48 Hour Score
20224	0	0
20239	0	0
20240	0	0
20243	0	0
20308	0	0
20314	0	0
20320	0	0
20321	0	0
20326	1	1
20335	1	1

Table 9
Test Scores After Challenge Phase
Negative Control Group (Extraction in SCI)

Animal Number	24 Hour Score	48 Hour Score
20233	0	0
20313	1	0
20315	0	0
20323	0	0
20324	1	1



Table 10
Test Scores After Challenge Phase
Positive Control Group (0.05% DNCB in ETOH/H₂O, 1:9 v/v)

Animal Number	24 Hour Score	48 Hour Score
Positive Control Group		
18853	3	3
18859	3	3
18870	3	3
18901	3	3
18906	3	3
Positive Naive Control Group		
19106	3	3
19107	1	1
19108	1	0
19109	1	1
19110	1	1

Table 11
Test Scores After Rechallenge Phase
Positive Control Group (0.025% DNCB in ETOH/H₂O, 1:9 v/v)

Animal Number	24 Hour Score	48 Hour Score
Positive Control Group		
18853	3	3
18859	3	3
18870	2	2
18901	2	3
18906	3	3
Positive Naive Control Group		
19211	0	0
19212	0	0
19214	0	0
19278	1	0
19279	0	0



Table 12
Test Scores After Challenge Phase
Test Group (Extraction in CSO)

Animal Number	24 Hour Score	48 Hour Score
20222	0	0
20223	1	1
20228	1	1
20230	0	0
20232	0	0
20237	0	0
20251	1	0
20319	0	0
20328	0	0
20330	0	0

Table 13
Test Scores After Challenge Phase
Negative Control Group (CSO Blank)

Animal Number	24 Hour Score	48 Hour Score
20238	0	0
20244	1	0
20246	0	0
20311	1	1
20317	1	0



Table 14
Test Scores After Challenge Phase
Positive Control Group (0.05% DNCB in ETOH/CSO, 1:9 v/v)

Animal Number	24 Hour Score	48 Hour Score
Positive Control Group		
18850	3	3
18854	3	3
18878	2	2
18882	3	2
18900	3	2
Positive Naive Control Group		
19111	1	0
19112	1	0
19113	1	0
19114	1	1
19115	0	0

Table 15
Animal Weights
Test Group (SCI)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
20224	M	392.8	502.7
20239	M	406.2	500.8
20240	M	380.5	474.5
20243	M	415.2	531.1
20308	M	409.1	499.4
20314	M	432.0	524.9
20320	M	359.0	498.4
20321	M	401.0	488.6
20326	M	423.0	564.0
20335	M	426.4	518.5



Table 16
Animal Weights
Positive Control Group (SCI)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
18853	M	362.0	588.7
18859	M	405.6	670.1
18870	M	374.9	640.4
18901	M	405.8	613.3
18906	M	392.8	551.5

Table 17
Animal Weights
Positive Naive Control Group For Challenge Phase (SCI)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
19106	M	345.8	411.4
19107	M	324.1	390.1
19108	M	305.8	351.9
19109	M	393.4	486.3
19110	M	367.2	438.0

Table 18
Animal Weights
Positive Naive Control Group For Rechallenge Phase (SCI)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
19211	M	440.9	489.9
19212	M	441.0	486.0
19214	M	421.3	457.8
19278	M	346.0	374.0
19279	M	371.7	418.9

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Table 19
Animal Weights
Negative Control Group (SCI)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
20233	M	440.0	530.3
20313	M	410.0	488.4
20315	M	423.0	497.9
20323	M	378.0	465.3
20324	M	401.0	504.4

Table 20
Animal Weights
Test Group (CSO Extraction)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
20222	M	378.7	458.2
20223	M	385.4	490.5
20228	M	424.2	521.8
20230	M	433.6	529.2
20232	M	435.6	549.2
20237	M	426.9	528.2
20251	M	492.5	573.9
20319	M	393.0	488.6
20328	M	399.0	487.0
20330	M	408.4	483.7



Table 21
Animal Weights
Positive Control Group (CSO Extraction)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
18850	M	382.6	603.9
18854	M	391.0	621.0
18878	M	369.6	608.2
18882	M	395.4	633.2
18900	M	355.7	555.1

Table 22
Animal Weights
Positive Naive Control Group (CSO Extraction)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
19111	M	378.5	439.2
19112	M	364.5	432.9
19113	M	393.0	492.0
19114	M	356.0	414.1
19115	M	378.9	443.0



Table 23
Animal Weights
Negative Control Group (CSO Extraction)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
20238	M	391.9	493.0
20244	M	434.9	495.3
20246	M	414.1	512.3
20311	M	415.4	526.0
20317	M	398.0	512.3

REFERENCES

1. Northview SOP No. 16G-13
2. The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test. Magnusson B. and Kligman, A.M., 1969, J. Invest. Dermatol. 52:268-276
3. Standard Practice for Testing Guinea Pigs for Contact Allergens Guinea Pig Maximization Test. ASTM Standard F720-81 (Reapproved 1986)
4. Guinea Pig Maximization Test. Wahlberg, J.E. and Boman, A., 1985, Curr. Probl. Dermatol. 14: 59-106

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QUALITY ASSURANCE UNIT GLP INSPECTION & AUDIT SUMMARY

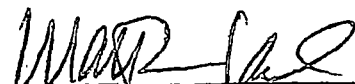
Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number X4D275G were conducted by the Quality Assurance Unit on the following dates:

NV SOP No.	Phase of Study	Date
16G-13	<u>Induction</u> Intradermal Dosing	05/17/94
	<u>Challenge</u> 24 Hour Scoring	06/09/94

QAU inspection findings are routinely reviewed by the management of Northview Pacific Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number X4D275G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.


Quality Assurance Unit

7/8/94
Date



NV Report Number X4D275G

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NORTHVIEW PACIFIC LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

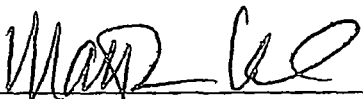
1. Timothy V. Doherty, D.V.M., Director *In Vivo* Laboratory Services
2. Roger O'Meara, Assistant Manager, *In Vivo* Laboratory Services
3. Robert Noonan, Ph.D., Senior Scientist, *In Vivo* Laboratory Services
4. Benjamin Davis, Technician, *In Vivo* Laboratory Services
5. Lisa Gearin, Technician, *In Vivo* Laboratory Services
6. Robert Mosbauer, Technician, *In Vivo* Laboratory Services
7. Edwin T. Pangilinan, M.S., Technician, *In Vivo* Laboratory Services
8. James Ruiz, Technician, *In Vivo* Laboratory Services
9. Patrice Pitot, Technician, *In Vivo* Laboratory Services
10. Matthew I. Kanter, Manager, Quality Assurance

STATEMENT OF COMPLIANCE

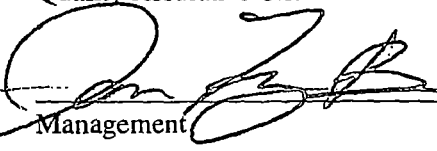
This study, Northview Report Number X4D275G, has been conducted in accordance with applicable Good Laboratory Practice regulations.


Study Director

7/6/94
Date


Quality Assurance Unit

7/8/94
Date


Management

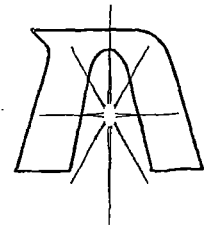
7/8/94
Date

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Northview Pacific Laboratories, Inc.

2800 Seventh Street ✧ Berkeley, CA 94710 ✧ 510/548-9440 ✧ Fax 510/548-5425



REISSUED REPORT

SPONSOR

Ms. Mary Reinhard D.V.M.
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

NV REPORT NUMBER

X4D274G

STUDY COMPLETION DATE

August 11, 1994

TEST ARTICLE IDENTIFICATION

Calibre 2081-15 Polycarbonate, Blue Tint

TEST PERFORMED

USP Rabbit Pyrogen Test

STUDY DIRECTOR

Robert Mosbauer
Laboratory Technician
In Vivo Laboratory Services
Northview Pacific Laboratories, Inc.

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STUDY DATES

Study Initiation Date: May 4, 1994
Study Completion Date: August 11, 1994
Date On Test: May 23, 1994
Date Off Test: May 23, 1994
Study Authorization: Signed Protocol
Date Sample Received: April 25, 1994

TEST ARTICLE IDENTIFICATION

Calibre 2081-15 Polycarbonate, Blue Tint
Smoked colored cylinders 2.5 cm long and 0.5 cm in diameter
Lot Number: TB920707B20
Sample Code: RDM# 13-2-0060-00508
Sterilization Dates: Cobalt 4/6/94 and ETO 4/11/94

PROTOCOL

This test was conducted according to Protocol Number X4D274G which incorporates by reference Northview SOP No. 16E-02 and is on file at Northview Pacific Laboratories, Inc. This protocol satisfies the requirements of the current USP and Ethicon Endo-Surgery.

DEVIATIONS FROM PROTOCOL

There were no deviations.

DATA DISPOSITION

Raw data from this study is archived at Northview Pacific Laboratories, Inc., 2800 Seventh Street, Berkeley, CA 94710, under Northview Report Number X4D274G.

SUMMARY OF RESULTS

This test material met the USP XXII requirements for the absence of pyrogens.



USP RABBIT PYROGEN TEST

INTRODUCTION

This test is designed to limit, to an acceptable level, the possibility of febrile reactions in patients following administration of the test material by injection.

Three New Zealand albino rabbits, weighing at least 2.0 kg each, were injected intravenously with the test material. The doses range in volume from approximately 1.0 mL/kg up to 10.0 mL/kg.

MATERIALS AND METHODS

Test System

Species	Rabbit
Strain	New Zealand, Albino
Source	Elkhorn Rabbitry, Watsonville, CA.
Number	Three
Sex	Female
Weight	At least 2.0 kg
Age	Adult
Housing	Individually; 17 - 21C and 55 $\pm$ 15% relative humidity
Feed	Purina rabbit chow (approximately 200 grams per day) and water <i>ad libitum</i>
Identification	Ear tag
Quarantine	Seven days
Photoperiod	Diurnal (12 hours on - 12 hours off)

Justification For Test System

Rabbits are the species required by the current version of the United States Pharmacopeia.

**Table 1
Supplies**

Reagents/Equipment	Lot Number	Manufacturer	Expiration Date
Sodium Chloride for Injection (SCI), USP	J4A410	McGaw	1/96

**Table 2
Study Design**

Number of Rabbits	Route of Administration	Final Dose Volume (mL/kg)	Time Frequency of Temperature Readings (in hours)
3	Intravenous	10	0, 1.0, 1.5, 2.0, 2.5, 3.0

Sample Preparation And Dosing Procedure

Sample Preparation - Ten pieces of the test material were soaked in 400 mL of SCI, USP for 50 minutes at room temperature.

Dosing Procedure - Each rabbit was injected intravenously (through the marginal ear vein) with 10 mL of the test material rinse per kg of body weight per rabbit.

Observation And Scoring

Sham Test - All rabbits used for pyrogen tests passed a sham pyrogen test conducted not more than seven days prior to the pyrogen test. (A sham pyrogen test consisted of the same procedure as the pyrogen test with the exception of the injection.)

Weights - All of the rabbits were weighed prior to administration of the test material.

Food and Water - Food and water were withheld during the test period.

Housing - The rabbits were restrained in light fitting neck stocks that allowed the rabbits to assume a natural resting posture during the test period.

Control Temperature - The control temperature (T_0) of each rabbit was determined within thirty minutes prior to injection of the test material. Only rabbits whose control temperature did not exceed 39.8C and did not vary more than 1C from the other test rabbits were used.

Temperature Readings - Rectal temperatures were recorded every thirty minutes from one to three hours following injection of the test material.

Interpretation And Analysis

Any temperature decrease was considered a zero rise in temperature. If no rabbit showed an individual rise in temperature of 0.5C or more above its respective control temperature, the test material meets the requirements for the absence of pyrogens.

If these requirements are not met, the test may be continued using five additional rabbits. If not more than three of the eight rabbits show individual rises in temperature of 0.5C or more, and if the sum of the eight individual maximum temperature rises does not exceed 3.3C, the material under examination meets the requirements for the absence of pyrogens.

RESULTS AND DISCUSSION

No rabbit showed an individual rise in temperature of 0.5C or more.

CONCLUSION

This test material met the USP XXII requirements for the absence of pyrogens.

Table 3
Record of Temperatures (C)

Rabbit Number	Sex	Weight (kg)	Dose (mL)	T-0	T-60	T-90	T-120	T-150	T-180	Temperature Rise
10939	F	2.7	27	39.2	39.1	39.0	39.4	39.1	39.6	0.4
10927	F	2.7	27	39.0	38.9	39.0	39.1	39.0	39.1	0.1
10818	F	2.8	28	39.0	39.0	38.9	39.0	38.9	39.3	0.3

REFERENCES

NV SOP 16E-02

USP XXII [1990] p. 1515, p. 1627 (Biologics) CFR, 610.13

USP XXII, Supplement 8, p. 3350, (Effective May 15, 1993)



NV Report Number X4D274G

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**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number X4D274G were conducted by the Quality Assurance Unit on the following dates:

NV SOP No.	Phase of Study	Date
16E-02	T=180 Minute Temperature Reading	05/23/94

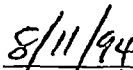
QAU inspection findings are routinely reviewed by the management of Northview Pacific Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number X4D274G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.



Quality Assurance Unit



Date



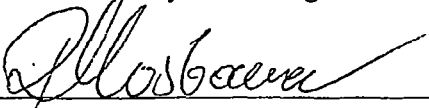
NORTHVIEW PACIFIC LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Timothy V. Doherty, D.V.M., Director *In Vivo* Laboratory Services
2. Roger O'Meara, Assistant Manager, *In Vivo* Laboratory Services
3. Robert Noonan, Ph.D., Senior Scientist, *In Vivo* Laboratory Services
4. Benjamin Davis, Technician, *In Vivo* Laboratory Services
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7. Edwin Pangilinan, Technician, *In Vivo* Laboratory Services
8. James Ruiz, Technician, *In Vivo* Laboratory Services
9. Lisa Gearin, Technician, *In Vivo* Laboratory Services
10. Matthew I. Kanter, Manager, Quality Assurance

STATEMENT OF COMPLIANCE

This study, Northview Report Number X4D274G, has been conducted in accordance with applicable Good Laboratory Practice regulations.


Study Director

8/11/94
Date


Quality Assurance Unit

8/11/94
Date


Management

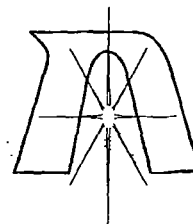
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Northview Pacific Laboratories, Inc.

2800 Seventh Street ♦ Berkeley, CA 94710 ♦ 510/548-8440 ♦ Fax 510/548-5425



SPONSOR

Mary Reinhard D.V.M.
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

NV REPORT NUMBER

U4D005G

STUDY COMPLETION DATE

May 27, 1994

TEST ARTICLE IDENTIFICATION

Calibre 2081-15 Polycarbonate, Blue Tint

Lot Number: TB920707B20

Sample Code: RDM #13-2-0060-00508

Sterilization Date: Cobalt and ETO 4/6/94 and 4/11/94

TEST PERFORMED

Ames

Northview Pacific Laboratories, Inc.

NV Report Number U4D005G

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STUDY DATES

Testing Facility: Nelson Laboratories, Inc.
P.O. Box 17557
4535 South 2300 East
Salt Lake City, UT 84117

Study Completion Date: May 18, 1994
Date Sample Received: April 25, 1994
Purchase Order: A2442

Study Authorization: Signed Protocol

Test Procedure: See Attached

CONCLUSION

The results are summarized in Tables 1-8. The tester strains were sensitive to selected marker mutagens both with and without S-9 activation using the rat liver homogenate. The tester strains were also tested for proper strain performance. These tests included UV light sensitivity to verify *uvrB* mutation, crystal violet sensitivity to verify increased cell wall permeability and corresponding loss of cell coat material, and ampicillin resistance to verify presence or absence of the R-factor plasmid.

None of the 5 tester strains produced two-fold increases in the number of revertants. The spot tests showed no zone of increased reversion or of inhibition.

In summary, the extract tested against the five strains did not meet the criteria for a potential mutagen.

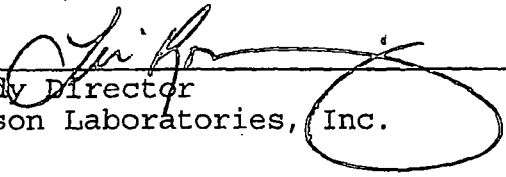




I CERTIFY THAT THIS STUDY WAS PERFORMED IN ACCORDANCE
WITH THE U.S. FDA GOOD LABORATORY PRACTICES.

(GLP REGULATIONS)

LABORATORY NO. 62300


Study Director
Nelson Laboratories, Inc.

19 May 94
Date

SOP/QU/018B-9/0493



QAU AUDIT STATEMENT

SALMONELLA TYPHIMURIUM REVERSE MUTATION ASSAY
AMES TEST

Study Director:

Final Report Dated:

Teri A. Roming, M.S. RM(AAM)

18 May 94

1. The test was conducted in accordance with USFDA Good Laboratory Practices Regulations [21 CFR Part 58]. All laboratory results which pertain to this study are recorded in Data File Number 62300.
2. In accordance with the Good Laboratory Practice regulations, this study was inspected by the Quality Assurance Unit on: 17 May 94. The findings of the inspection(s) were reported to management and to the study director on: 17 May 94.
3. The Quality Assurance Unit has reviewed this report and has determined that the methods and standard operating procedures are accurately described, and that the reported results accurately reflect the raw data.

M. Kaye Markel
M. Kaye Markel
Quality Assurance Unit

24 MAY 94
Date

SOP/QAU/018B-10/0493



SALMONELLA TYPHIMURIUM REVERSE MUTATION ASSAY
AMES TEST

LABORATORY NUMBER: 62300
PROTOCOL NUMBER: 941181-1 (As Per SOP/CTX/014)
SAMPLE SOURCE: Northview Pacific Laboratories, Inc.
SAMPLE IDENTIFICATION: Calibre 2081-15 Polycarbonate, Blue Tint
Sterilization Data: Cobalt and EtO 4/6/94 and 4/11/94
Code Identification: RDM #13-2-0060-00508
Lot #TB920707B20
Reference #U4D005G
P.O. #NVP 115
START DATE: 05 May 94
COMPLETION DATE: 18 May 94
REPORT DATE: 18 May 94

INTRODUCTION:

The Ames test employs several strains of *Salmonella typhimurium* which have been selected based on their sensitivity to mutation because of: 1) an increased cell wall permeability, due to mutation which causes partial loss of the lipopolysaccharide barrier that coats the bacterial surface and results in increased permeability to large molecules (rfa mutation); 2) a mutation in the bacterial cell system to excise and repair defects in the DNA, resulting in the inability to repair damaged or mutated sections (uvrB mutation); and 3) R-factor plasmids (some strains) and a multicopy plasmid (some strains) which contain error-prone DNA repair systems.

The *Salmonella* reverse mutation (Ames) tests are performed by mixing the test substance or an extract of the test substance and the test organism together in a soft agar solution, which contains only small amounts of histidine. The histidine permits the inoculated test organism to undergo a limited number of divisions, but is insufficient to permit normal growth. The tester strains require histidine for growth, due to a mutation in the gene which controls production of histidine. If, however, the strain undergoes a reverse mutation, (spontaneous or induced by the test substance or a positive control material), the organism no longer requires

Northview Pacific Laboratories, Inc.
Salmonella Reverse Mutation Assay
Lab Number 62300
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histidine to grow and can produce a visible colony or revertant. Only mutations to the test organism in the region of the histidine gene will cause the test organism to undergo a reverse mutation to an organism which then no longer requires histidine. The tester strains are selected to detect various types of mutagens. The tester strains employed were TA97A, TA98, TA100, TA102 and TA1535.

The test extract was evaluated against the five strains listed. The tests were performed both with and without S-9 activation. The S-9 activation system is designed to simulate mammalian liver enzyme systems and is used to detect substances which undergo metabolic activation from non-mutagenic forms.

The test extract was evaluated in plate incorporation assays and in spot tests with and without S-9 activation.

PROCEDURE:

Strain Verification: Test strains were checked for presence of the desired properties prior to all tests. These tests included verification of the following:

- Presence of uvrB mutation
- Presence or absence of R-factor plasmid
- Presence of rfa mutation
- Requirement for histidine

The uvrB mutation was verified by demonstrating UV sensitivity (lack of repair system). The R-factor was checked by determining sensitivity or resistance to ampicillin (0.08% in 0.02 NaOH). The presence of the rfa mutation was verified by demonstrating sensitivity to crystal violet (0.1% in water) on nutrient agar plates. The histidine requirement was assured by plating onto minimal glucose agar plates spread with 0.1 mL of 0.5 mM biotin and both with and without 0.1 mL of 0.5 mM histidine.

Sample Preparation: 70 cm² of the sample was extracted in 23.3 mL of 0.9% saline at 70°C for 24 hours.

Metabolic Activation System: The S-9 activation system was used to screen for the presences of mutagens from byproducts of the test sample. Rat liver S-9, Lot #38275, liver homogenate was obtained from Organon Teknika, Durham, NC. The homogenate was kept frozen at -70°C and has been well characterized in our laboratory.

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The material is prepared from 8-10 week old Sprague Dawley male rats. The technique for preparation of rat liver microsomal enzymes (S-9) was identical to that described by Ames. The animals were injected intraperitoneally with Aroclor 1254 in corn oil at a dose of 500 mg/kg five days prior to sacrifice. The S-9 was prepared as a KCl homogenate and stored at -70°C until use. All procedures were carried out at 0-4°C. Immediately before use, the S-9 preparation was mixed with 0.5 mL of S-9 base containing 20 μ L S-9 preparation to each 2.0 mL aliquot agar overlay. When working with soft agar, temperatures never exceeded 47°C, and plates were poured within 2 minutes of the addition of S-9.

Plate Incorporation Tests: Minimal agar plates containing histidine-deficient Vogel Bonner Medium E (VBME) fortified with 2% glucose were prepared. Aliquots of top agar were melted and maintained at 45°C $\pm$ 2°C. Each 100 mL aliquot of top agar was fortified with approximately 10 mL of 0.5 mM biotin and 0.5 mM histidine immediately before use. Five to ten mL of a thirteen-hour nutrient broth culture of the appropriate bacterial tester strain was added to the top agar, and 2.0 mL aliquots of this mixture were transferred to sterile, disposable capped tubes. One-tenth mL of the sample extract was added to 2 mL aliquots of top agar. The sample extract was tested with and without the S-9 activation system.

Spot Tests: The test material was also tested in spot tests on plates with and without the S-9 activation system. Two mL aliquots of the top agar mixture containing histidine-biotin solution and the broth culture of the appropriate tester strain were added to agar plates. The plates were allowed to harden then 10 μ L spots were added to the plates. All strains were tested with the extract using 10 μ L spots. The plates were placed into the incubator within an hour of plating.

Positive Control Materials: The following positive control materials were used: Sodium Azide, 4-nitro-0-phenylene-diamine (NPD), and 2 aminofluorene (2AF). The controls were tested with and without the S-9 activation system. The controls were tested with all of the strains using incorporation and spot tests.

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Test Evaluation:

Test criteria: 1) Tested extract, spot test and plate incorporation. 2) Tested against tester strains TA97A, TA98, TA100, TA102 and TA1535. 3) Tested in the presence and the absence of liver microsomal enzymes preparation.

Criteria for a mutagen: 1) A two-fold increase over the spontaneous reversion rate. 2) Demonstration of a dose-response curve when dilutions are tested.

Criteria for a non-mutagen: 1) A less than two-fold increase over spontaneous reversion rate. 2) No dose response curve when dilutions are tested.

CONCLUSION:

The results are summarized in Tables 1-8. The tester strains were sensitive to selected marker mutagens both with and without S-9 activation using a rat liver homogenate. The tester strains were also tested for proper strain performance. These tests included UV light sensitivity to verify uvrB mutation, crystal violet sensitivity to verify increased cell wall permeability and corresponding loss of cell coat material, and ampicillin resistance to verify presence or absence of the R-factor plasmid.

None of the 5 tester strains produced two-fold increases in the number of revertants. The spot tests showed no zone of increased reversion or of inhibition.

In summary, the extract tested against the five strains did not meet the criteria for a potential mutagen.


Jeffery R. Nelson
Microbiology Technician


Teri Roming, M.S. RM(AAM)
Study Director

Northview Pacific Laboratories, Inc.
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TABLE 1. TA97A Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	195	107	161	117	145
S-9 Control	175	180	197	165	179

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	118	104	187	179	147
Sample with S-9	163	144	155	150	153

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TABLE 2. TA98 Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	32	30	25	28	29
S-9 Control	42	40	22	29	33

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	26	21	21	22	23
Sample with S-9	41	37	41	39	40

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TABLE 3. TA100 Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	106	100	104	123	108
S-9 Control	150	109	120	117	124

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	131	112	135	120	125
Sample with S-9	118	129	131	117	124

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TABLE 4. TA102 Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	253	215	217	220	226
S-9 Control	227	233	212	196	217

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	246	229	221	214	228
Sample with S-9	252	255	270	250	257

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TABLE 5. TA1535 Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	24	26	31	20	25
S-9 Control	19	26	20	27	23

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	20	29	31	26	27
Sample with S-9	18	25	15	21	20

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TABLE 6. Spot Test Results

SAMPLE EXTRACT	ACTIVATION	97A	98	100	102	1535
	None	-	-	-	-	-
	S-9	-	-	-	-	-

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TABLE 7. Strain Verification Results

PARAMETER	STRAINS				
	TA97A	TA98	TA100	TA102	TA1535
uvrB	+	+	+	-	+
R-factor	+	+	+	+	-
rfa	+	+	+	+	+
Histidine Requirement	+	+	+	+	+

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TABLE 8. Positive Controls

CONTROL ID	ACTIVATION	97A	98	100	102	1535
Sodium Azide (Expected Result)	No S-9	-	-	+	-	+
	No S-9	(-)	(-)	(+)	(-)	(+)
NPD (Expected Result)	No S-9	+	+	+	-	-
	No S-9	(+)	(+)	(+)	(-)	(-)
2AF (Expected Result)	S-9	+	+	+	-	-
	S-9	(+)	(+)	(+)	(-)	(-)

Positive controls were analyzed by incorporation assays and spot tests.

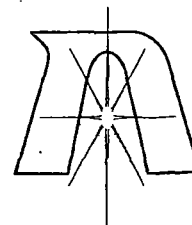
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Northview Laboratories, Inc.

1880 Holste Road ♦ Northbrook, IL 60062 ♦ 708/564-8181 ♦ Fax 708/564-8269



REPORT OF ANALYSIS

CLIENT 1733-1

REPORT DATE: 7/12/94

Dr. Mary Reinhard
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati OH 45242

NVL REPORT NUMBER: T4E008

YOUR P.O. NUMBER: A2442

SAMPLE IDENTIFICATION

Calibre 2081-15 Polycarbonate, Blue Tint
2 pieces
Lot Number: TB920707B20
RDM Number: 13-2-0060-00508
Date Received: 04/28/94

ANALYSIS

INFRARED SPECTROSCOPY - FTIR SPECTRA

RESULTS

An FTIR spectrum of the sample described above was recorded upon receipt and again 50 days later, using a microscope accessory.

The spectra generally appear to be concordant and free from noticeable change; therefore, the sample may be considered to be stable over this time period as judged by this technique.

Copies of the spectra are attached to this report, as well as a copy of the subcontracting laboratory's report to us.

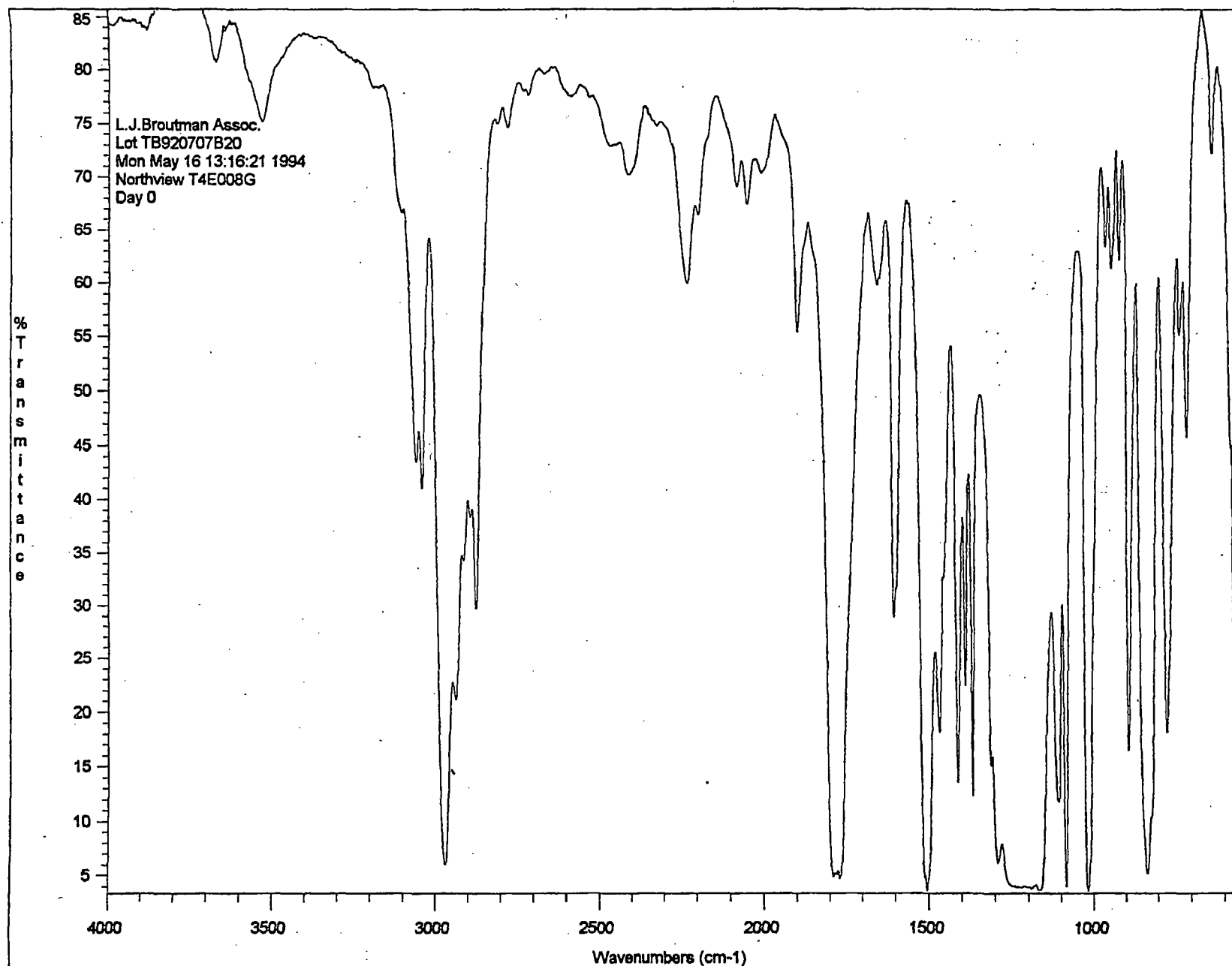
PROCEDURE REFERENCE

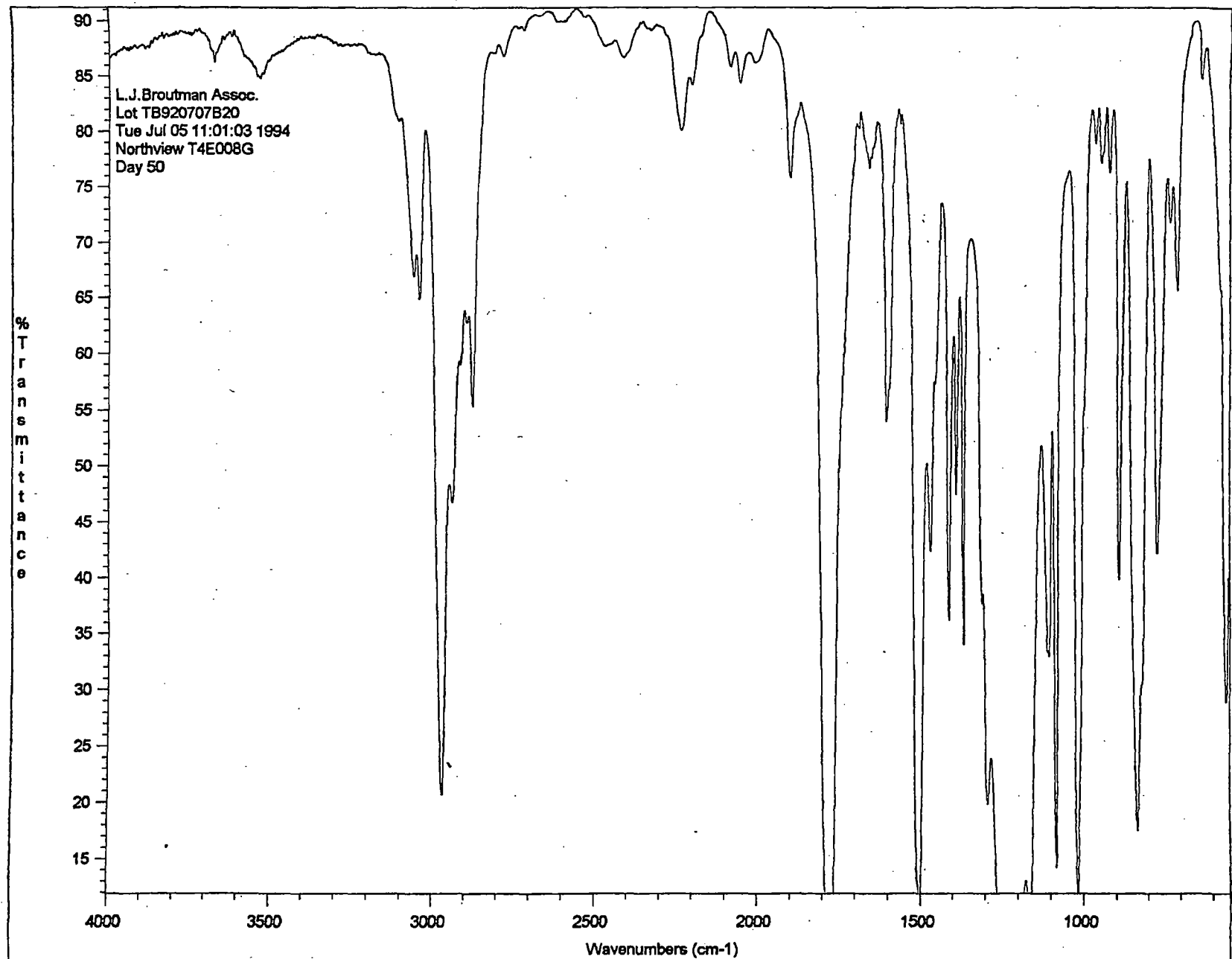
FTIR Spectroscopy

:mc

NORTHVIEW LABORATORIES, INC.

Paul Adlaf 7/12/94
Paul Adlaf, Ph. D., Director
Analytical Laboratory Services





Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Part Name	Subject Device Material
Inner Sleeve Insulator and Rod	<p>PolyEtherEtherKetone (PEEK)</p> <p>Please note that we have updated our database to reflect that:</p> <p>The Thrombogenicity test and the Prothrombin time has been replaced with Partial Thromboplastin Time (PTT).</p> <p>The implantation study is a class VI test report which includes implantation, systemic toxicity, and irritation. (There is an additional systemic toxicity test and the irritation test contained within the Class VI test report).</p> <p>There are 2 sensitization reports: one is extracted in sodium chloride, the other is in cottonseed oil (CSO).</p>

CONFIDENTIAL

07/23/97

10:13

CORDIS - SECURITY

NO. 069

P002/002

Cordis**Quality Assurance Laboratories****COPY****Laboratory Report
Chemical and Physical Quality Assurance**

Part/Material Description: <i>Tacher SDS w/PEEK Balloon (Gamma Ste.)</i>		ISR No. <i>30681</i>
Cordis Part No / Catalog No:	Cordis Lot No:	Date Received: <i>1/10/97</i>
Requestor:	Dept. No:	Hours Charged:

<u>Test</u>	<u>Method</u>	<u>Limits</u>	<u>Results</u>
Aqueous Extraction and Analysis of Extract (U.S. Pharmacopeia)	QCOP 21-108 Rev. 11		
	Section 5.4.1	Nonvolatile Residue (≤ 15 mg difference)	<u>1.0</u>
	Section 5.4.2	Residue on Ignition (≤ 5 mg difference)	<u>≤ 1.0</u>
	Section 5.4.3	Heavy Metals (≤ 1 ppm)	<u>< 1</u>
	Section 5.4.4	Buffering Capacity (≤ 10 mL difference)	<u>0.0</u>

Accept ✓

Reject _____

Written By: <i>T. Valdez</i>	Date: <i>2/4/97</i>	Approved By: <i>[Signature]</i>	Date: <i>2/5/97</i>
Notebook Reference: <i>92093-4: 91798-15</i>	Other Reference: <i>File # 1572</i>		

COPY

TOXIKON FINAL REPORT: 97G-0375

UNACTIVATED PARTIAL THROMBOPLASTIN TIME ASSAY-ISO

Author

Vasudev P. Anand, Ph.D.

FINAL REPORT DATE:

March 18, 1997

MANAGEMENT OF THE STUDY

Performing Laboratory

Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

SPONSOR

Cordis Corporation
14201 N.W. 60th Ave.
Miami, FL 33102

Toxikon Corporation
Unactivated Partial Thromboplastin Time Assay-ISO
Project: 97G-0375

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Toxikon Corporation

~~Unactivated Partial Thromboplastin Time Assay-ISO~~

Project: 97G-0375

STUDY SUMMARY

The test article, File # 1572 Tacker w/ Peek Balloon, was tested for its effects on the clotting time of human plasma by comparing its Unactivated Partial Thromboplastin Time (UPTT) with that of two negative controls: plasma exposed to USP Negative Control Plastic RS and untreated plasma. The UPTT of the plasma exposed to test article was not significantly different than the UPTT's of untreated plasma and plasma exposed to USP Negative Control Plastic RS. Based on the criteria of the protocol, the test article meets the requirements of the UPTT test, with a mean UPTT of 124% of untreated control, i.e., >100% (non-thrombogenic).

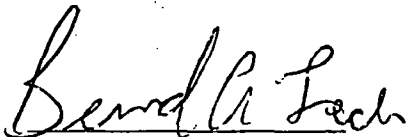
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Unactivated Partial Thromboplastin Time Assay-ISO
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QUALITY ASSURANCE STATEMENT

Based on a review of this study report, protocol and Standard Operating Procedures utilized, and inspection of study events listed below, this test conformed to the current FDA Good Laboratory Practice (GLP) regulations, 21 CFR, Part 58.

The Sponsor was responsible for all test article purity, stability and characterization data as specified in the current version of 21 CFR, Parts 58.105 and 58.113. The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to Toxikon Management.

INSPECTIONS	DATE OF INSPECTION	DATE REPORTED MANAGEMENT	DATE REPORTED STUDY DIRECTOR
SCORING	03/12/97	03/12/97	03/12/97
RAW DATA	03/18/97	03/18/97	03/18/97
FINAL REPORT	03/18/97	03/18/97	03/18/97


Bernard A. Lech, M.S.
Quality Assurance Officer

3/18/97
Date

Toxikon Corporation
Unactivated Partial Thromboplastin Time Assay-ISO
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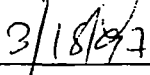
STUDY DIRECTOR SIGNATURE AND VERIFICATION DATES

This study meets with the technical requirements of the protocol. The study also meets with the current version of the requirements of the Good Laboratory Practice Regulations, 21 CFR, Part 58.

Study Director: Vasudev P. Anand, Ph.D.

Company: Toxikon Corporation

Signature:

Date:

Study Supervisor: Mark Turner, B.S.

VERIFICATION DATES:

The study dates were as follows:

Protocol Effective Date:	02/25/97
Test Article Receipt:	03/03/97
Project Log Date:	03/03/97
Technical Initiation:	03/12/97
Technical Completion:	03/12/97
Final Report Date:	03/18/97

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1.0 PURPOSE

The purpose of this study was to measure the effect of a test article on the PTT, i.e., activation of the intrinsic coagulation pathway. An increased PTT correlates with a non-activating (anti-thrombogenic) potential for the test article. A decreased PTT indicates activation of the coagulation pathway (thrombogenic potential). This protocol is Sponsor-specific and is confidential.

2.0 REFERENCES

The study was conducted based on the following references:

2.1 Biological Evaluation of Medical Devices-Part 4: Selection of Tests for Interactions with Blood, ANSI/AAMI/ISO 10993-4:1993.

2.2 Lenahan, J.L. and Smith, K. Hemostasis (1979).

2.3 Extraction procedures, if applicable, will be based on the standard titled Biological Evaluation of Medical Devices-Part 12: Sample Preparation and Reference Materials, ISO/CD (1993) 10993-12.

3.0 COMPLIANCE

The present study conformed to all applicable laws and regulations. Specific regulatory requirements include the current Good Laboratory Practice for Nonclinical Laboratory Studies, FDA, 21 CFR, Part 58.

4.0 IDENTIFICATION OF THE TEST AND CONTROL ARTICLES

4.1 The following information was supplied by the Sponsor wherever applicable; it does not apply to confidential information. The Sponsor was responsible for all test substance characterization data as specified in the GLP regulations. Test and control articles (exclusive of extracts) that are mixed with carriers require verification of concentration, homogeneity and stability. Samples of test and control article mixtures will be returned to the Sponsor for characterization, wherever applicable.

Test Article: File # 1572 Tacker w/ Peek Balloon

CAS/Code #: SR# 12-96-231

Lot/Batch #: SR# 96-0278

Physical State: Solid

Color: Black Catheter w/ White Hub

Density: Not Supplied by Sponsor (N/S)

Stability: N/S

Toxikon Corporation
Unactivated Partial Thromboplastin Time Assay-ISO
Project: 97G-0375

Solubility: N/S
Expiration Date: N/S
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2 Negative Control Article (Toxikon Supplied)
Control Article Name: USP Negative Control Plastic RS
QC #: CSC-96-03-002-VIV
Physical State: Solid
Color: White
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.3 Positive Control Article (Toxikon Supplied)
Control Article Name: Glass Tube
QC #: CSC-96-02-002-CC
Physical State: Solid
Color: Clear
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

5.0 IDENTIFICATION OF THE TEST SYSTEM

5.1 The test system was human plasma. The plasma was obtained from blood outside sources and did not require any preparation.

6.0 JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

The test article was administered *in vitro*, directly to the test system, per sponsor specification. This was the optimal route of administration available in this test system.

7.0 EXPERIMENTAL DESIGN

7.1 Plasma Preparation:

Citrated human plasma was obtained from outside sources and did not require any preparation. The sodium citrate removed calcium which is a required factor in coagulation. The plasma was examined to ensure that it was free of hemolysis and clots.

7.2 Negative Controls: One negative control article consisted of plasma in the absence of test article. The other negative control article consisted of plasma exposed to USP Negative Control Plastic RS.

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Unactivated Partial Thromboplastin Time Assay-ISO
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7.3 Positive Controls: Positive control consisted of plasma exposed to glass tubing.

7.4 Replication: The test and negative and positive controls were tested in triplicate.

7.5 Pieces of control articles were placed in polypropylene centrifuge tubes in triplicate. Tubes were capped and incubated for 15 minutes at 37°C in an agitating (60 rpm) water bath. The test article was exposed to plasma per Sponsor Specification as described in Section 8.0.

7.6 After incubation, calcium chloride (100 µL) was added to each cuvette and the length of time required for the formation of a clot was measured via LED photo-optic detection. Two readings were made for each of the triplicate samples (i.e., six readings total per test article and control article). The mean PTT with standard deviation was determined.

7.7. Step 7.6. was repeated for each of the other cuvettes at the end of their 3 minute incubation.

8.0 DOSAGE

8.1 As specified by the Sponsor, only the portions of the test article contacting blood was exposed to plasma at a ratio of 4 cm²/mL, after segmentation of the test article to maximize exposure of test article to plasma. The negative control article, USP Plastic RS, and the positive control article, Glass rods, were exposed to plasma at a ratio of 4 cm²/ mL.

9.0 EVALUATION CRITERIA

The mean PTT for test and control articles was evaluated for differences by Analysis of Variance (ANOVA) including *post-hoc* pairwise comparisons by the Newman-Keuls test. The coefficient of variation for the control and test article replicates must be within 20%. The mean PTT for the positive control should be statistically significantly different from both the negative controls ($p \leq 0.05$). The mean PTT for the negative control(s) was normalized to 100%.

The test article passed the PTT test if the mean PTT was: >100% (non-thrombogenic); 75-100% (minimally thrombogenic); and 50-75% (mildly thrombogenic) relative to the negative control. If the mean PTT was 25-50% (moderately thrombogenic, a RETEST was performed. The test article FAILS the PTT test if the mean PTT is < 25% (thrombogenic).

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 Unactivated Partial Thromboplastin Time Assay-ISO
 Project: 97G-0375

10.0 RESULTS

The UPTT for the plasma exposed to positive control (glass tubing) was significantly different ($p < 0.05$) from that of untreated plasma and plasma exposed to negative control article (USP Negative Control Plastic RS), thus validating the proper functioning of the assay. The UPTT of the plasma exposed to test article, File # 1572 Tacker w/ Peek Balloon, was not significantly different from that of both negative controls ($p < 0.05$). The results of the test are summarized in Table's I and II.

TABLE I
Unactivated Partial Thromboplastin Times (Seconds)

Replicates	Untreated Plasma	Negative Control Plasma	Positive Control Plasma	Test Article
1	200.0	283.1	38.3	299.6
2	181.5	263.8	38.3	246.0
3	276.8	306.9	38.8	273.6
Mean	219.4	284.6	38.5	273.1
S.D.	50.5	21.6	0.3	26.8

na: not applicable

TABLE II
Statistical Analysis by Neuman-Keuls Test

Test Article vs. Untreated Plasma	No Significant Difference
Test Article vs. USP Negative Control Plastic	No Significant Difference
Positive Control vs. Untreated Plasma	Significant Difference
Positive Control vs. USP Negative Control Plastic	Significant Difference

Toxikon Corporation
Unactivated Partial Thromboplastin Time Assay-ISO
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11.0 CONCLUSION

The test article, File # 1572 Tacker w/ Peek Balloon was tested for its effects on the clotting time of human plasma by comparing its Unactivated Partial Thromboplastin Time (UPTT) with that of untreated plasma and plasma exposed to negative control article (USP Negative Control Plastic RS). The average UPTT of the plasma exposed to the test article was not significantly different than that of untreated plasma and plasma exposed to negative control article. Based on the criteria of the study, the test article meets the requirements of the UPTT test, with a mean UPTT of 124% of untreated control, i.e., >100% (non-thrombogenic).

12.0 RECORDS

- 12.1 Original raw data is archived at Toxikon Corporation.
- 12.2 A copy of the final report and any amendments is archived at Toxikon Corporation.
- 12.3 The original final report and a copy of any protocol amendments or deviations is forwarded to the Sponsor.
- 12.4 All unused test article shall be discarded, per sponsor request.
- 12.5 Final reports shall not be reproduced except in full, without the written authorization/approval from Toxikon.

13.0 CONFIDENTIALITY AGREEMENT

Statements of confidentiality were agreed upon prior to study initiation.

K122797/s1
V.2

1572

TOXIKON FINAL REPORT: 97G-0386
***IN VITRO* HEMOCOMPATIBILITY ASSAY-ISO**

COPY

Author
Vasudev P. Anand, Ph.D.

FINAL REPORT DATE
March 28, 1997

MANAGEMENT OF THE STUDY

Performing Laboratory
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

Sponsor
Cordis Corporation
14201 N.W. 60th Avenue
Miami, FL 33102

Toxikon Corporation
In Vitro Hemocompatibility Assay-ISO
Project Number: 97G-0386

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Table 1: Hemocompatibility Parameters

Table 2: Summary of Hemocompatibility Test Results

Toxikon Corporation
In Vitro Hemocompatibility Assay-ISO
Project Number: 97G-0386

STUDY SUMMARY

The test article, File # 1572 Tacker w/ Peek Balloon, was evaluated for its potential to adversely affect selected hematological parameters of human blood in comparison with two controls: untreated blood and blood exposed to negative control article (USP plastic RS). The hematological parameters evaluated were Complete Blood Count, Hematocrit, Erythrocyte indices, and Platelet count. The test article did not cause significantly adverse effects on any of the hematological parameters tested. Based on the evaluation criteria of the study protocol, the test article passes the test for *in vitro* hemocompatibility, under the experimental conditions employed.

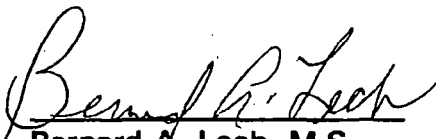
Toxikon Corporation
In-Vitro Hemocompatibility Assay-ISO
Project Number: 97G-0386

QUALITY ASSURANCE STATEMENT

Based on a review of this study report, protocol and Standard Operating Procedures utilized, and inspection of study events listed below, this test conformed to the current FDA Good Laboratory Practice (GLP) regulations, 21 CFR, Part 58.

The Sponsor was responsible for all test article purity, stability and characterization data as specified in the current version of 21 CFR, Parts 58.105 and 58.113. The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to Toxikon's Management.

INSPECTIONS	DATE OF INSPECTION	DATE REPORTED MANAGEMENT	DATE REPORTED STUDY DIRECTOR
SCORING	03/12/97	03/12/97	03/12/97
RAW DATA	03/28/97	03/28/97	03/28/97
FINAL REPORT	03/28/97	03/28/97	03/28/97


Bernard A. Lech, M.S.
Quality Assurance Officer

3/28/97
Date

Toxikon Corporation
In Vitro Hemocompatibility Assay-ISO
Project Number: 97G-0386

STUDY DIRECTOR SIGNATURE AND VERIFICATION DATES

This study meets with the technical requirements of the protocol. The study also meets with the requirements of the Good Laboratory Practice Regulations, 21 CFR, Part 58.

Study Director: Vasudev P. Anand, Ph.D.

Company: Toxikon Corporation

Signature: _____

Date: _____

3/28/97

Study Supervisor: Shirley Orfao, B.S.

VERIFICATION DATES:

The study dates were as follows:

Protocol Effective Date:	02/25/97
Test Article Receipt:	03/03/97
Project Log Date:	03/04/97
Technical Initiation:	03/12/97
Technical Completion:	03/12/97
Final Report Date:	03/28/97

Toxikon Corporation
In Vitro Hemocompatibility Assay-ISO
Project Number: 97G-0386

1.0 PURPOSE

In vitro hemocompatibility testing was undertaken to show that a test article did not adversely affect the cellular components of human blood. This procedure is Sponsor-specific and is confidential.

2.0 REFERENCES

The study was conducted based on the following references:

2.1 Biological Evaluation of Medical Devices-Part 4: Selection of Tests for Interactions with Blood, ANSI/AAMI/ISO 10993-4:1993; and EN 30993-4 (1994).

2.2 Extraction procedures, if applicable, were based on the standard titled Biological Evaluation of Medical Devices-Part 12: Sample Preparation and Reference Materials, ISO 10993-12; 1993 (Draft).

2.3 Sponsor Specified Procedure

3.0 COMPLIANCE

The study conformed to all applicable laws and regulations. Specific regulatory requirements included the current FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies.

4.0 IDENTIFICATION OF THE TEST AND CONTROL ARTICLES

The following information was supplied by the Sponsor wherever applicable; it did not apply to confidential information. The Sponsor was responsible for all test substance characterization data as specified in the GLP regulations. Test and control articles (exclusive of extracts) that are mixed with carriers require verification of concentration, homogeneity and stability. Samples of test and control article mixtures will be returned to the sponsor for characterization (if applicable).

4.1 Test Article Name: File # 1572 Tacker w/ Peek Balloon

CAS/Code #: SR# 12-96-231

Lot/Batch #: SSG# 96-0278

Physical State: Solid

Color: Black Catheter w/ White Hub

Density: N/S

Stability: N/S

Solubility: N/S

Toxikon Corporation
In Vitro Hemocompatibility Assay-ISO
Project Number: 97G-0386

Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2 Control Article (Toxikon Supplied)
Control Article Name: USP Negative Control Plastic RS
Q.C.#: CSC9603002VIV
Physical State: Solid
Color: Opaque
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

5.0 IDENTIFICATION OF THE TEST SYSTEM

The use of human blood as a test system for the determination of activity of a test article on the cellular component of blood when in direct contact is a Sponsor-specified procedure.

6.0 JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

The test article was administered *in vitro*, directly to the test system, per sponsor specification. This was the only route of administration available in this test system. The use of human blood as a test system is recommended by the ISO 10993-4 guidelines for the determination of activity of a test article on the components of blood.

7.0 EXPERIMENTAL DESIGN

7.1 Blood Collection: Fresh, whole human blood containing EDTA was used for Complete Blood Counts (CBC), Hematocrit, Erythrocyte indices and Platelet count. The blood was obtained from donors who had not taken aspirin, Tylenol, Motrin or the birth control pill within 10 days of blood collection. Blood from the same donor, collected at the same time, was used for the test.

Note: Blood only came in contact with plastic labware and silicone-coated glass blood collection tubes. Uncoated glass tubes or pipets were avoided since they may activate the coagulation system.

7.2 Prior to conducting the assay, the functioning of the instrumentation was validated per Toxikon Standard Operating Procedures for the In vitro Hemocompatibility and Coulter Counter MD II 16 Parameter Hematology System. The assay was conducted only after verification that values obtained with the manufacturer-supplied controls were within the stated specifications.

Toxikon Corporation
~~In Vitro Hemocompatibility Assay-ISO~~
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7.3 Negative Controls: Whole blood in the absence of test article was one negative control. The other negative control consisted of blood exposed to USP Negative Control Plastic RS.

7.4 Replication: The test and negative control articles were tested in triplicate for complete blood cell counts.

7.5 Using a plastic pipet, well mixed blood was added per vial. The vials were capped and incubated in a $37 \pm 2^\circ\text{C}$ water bath for 60 ± 2 minutes with gentle agitation. The test article was exposed to blood as described in section 8.0 per sponsor specification.

7.6 For blood cell counts, the blood was carefully removed from the vials and the complete blood counts (CBC), including hematocrit, erythrocyte indices, and platelet count were determined per Toxikon Standard Operating Procedure for the Coulter Counter MD II 16 Parameter Hematology System.

7.7 All blood contaminated material was disposed of in the biohazard container.

8.0 DOSAGE

As specified by the Sponsor, only the portions of the test article contacting blood was exposed to blood at a ratio of $4 \text{ cm}^2/\text{mL}$, after segmentation of the test article to maximize exposure of test article surfaces to blood. The negative control article, USP Plastic RS was exposed to blood at a ratio of $4 \text{ cm}^2/\text{mL}$.

9.0 EVALUATION CRITERIA

The mean and standard deviation of the results for each test sample and negative control results were calculated. The test means were compared to the negative controls. Data analysis was verified by a method such as Analysis of Variance (ANOVA), e.g., see R.J. Tallarida and R.B. Murray (Manual of Pharmacologic Calculations with Computer Programs, Springer-Verlag, New York, 1986). The Newman-Keul's test was used to verify *post-hoc* pairwise comparison. Differences between the means of the test and control groups were considered statistically significant if the probability of the differences being due to chance is equal to or less than 5% ($p \leq 0.05$). For each of the hematological parameters, the test article would have failed if the differences were statistically significant from both the negative controls. Statistical and Biological significance was considered in the final evaluation of results.

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10.0 RESULTS

The test article, File # 1572 Tacker w/ PEEK Balloon, was evaluated for its potential to adversely affect selected hematological parameters of human blood in comparison with two controls: untreated blood and blood exposed to negative control article (USP plastic RS). The hematological parameters evaluated were Complete Blood Count, Hematocrit, Erythrocyte indices, and Platelet count. The test article did not cause significantly adverse effects on any of the hematological parameters tested. The results are summarized in Tables 1 and 2.

11.0 CONCLUSION

The test article, File # 1572 Tacker w/ PEEK Balloon, was evaluated for its potential to adversely affect selected hematological parameters of human blood in comparison with two controls: untreated blood and blood exposed to negative control article (USP plastic RS). The hematological parameters evaluated were Complete Blood Count, Hematocrit, Erythrocyte indices, and Platelet count. The test article did not cause significantly adverse effects on any of the hematological parameters tested. Based on the evaluation criteria of the study protocol, the test article passes the test for *in vitro* hemocompatibility, under the experimental conditions employed.

12.0 RECORDS

- 12.1 Original raw data is archived at Toxikon Corporation.
- 12.2 A copy of the final report and any amendments is archived at Toxikon Corporation.
- 12.3 The original final report and a copy of any protocol amendments or deviations is forwarded to the Sponsor.
- 12.4 All unused test article shall be discarded by Toxikon, per Sponsor's request.
- 12.5 Final reports will not be reproduced except in full, without written authorization/approval from Toxikon.

13.0 CONFIDENTIALITY AGREEMENT

Statements of confidentiality were as agreed upon prior to study initiation.

TABLE 1: HEMOCOMPATIBILITY PARAMETERS
97G-0386

Group .	Sample No.	WBC($10^3/\mu\text{l}$)	RBC($10^6/\mu\text{l}$)	HgB(g/dl)	Hct(%)	MCV(fl)	MCH(pg)	MCHC(g/dl)	Platelets ($10^3/\mu\text{l}$)
Test Article	1	8.30	5.16	16.60	47.10	91.30	32.20	35.30	294.00
	2	8.50	5.24	16.80	48.00	91.50	32.10	35.10	304.00
	3	8.40	5.18	16.70	47.30	91.50	32.20	35.20	299.00
mean		8.40	5.19	16.70	47.47	91.43	32.17	35.20	299.00
$\pm$ S.D.		0.10	0.04	0.10	0.47	0.12	0.06	0.10	5.00
Group	Sample No.	WBC($10^3/\mu\text{l}$)	RBC($10^6/\mu\text{l}$)	HgB(g/dl)	Hct(%)	MCV(fl)	MCH(pg)	MCHC(g/dl)	Platelets ($10^3/\mu\text{l}$)
Untreated Control	1	8.20	5.17	16.50	47.70	92.30	32.00	34.70	299.00
	2	8.20	5.17	16.60	47.10	91.10	32.20	35.30	312.00
	3	8.00	5.08	16.60	46.40	91.30	32.60	35.70	301.00
mean		8.13	5.14	16.57	47.07	91.57	32.27	35.23	304.00
$\pm$ S.D.		0.12	0.05	0.06	0.65	0.64	0.31	0.50	7.00
Group.	Sample No.	WBC($10^3/\mu\text{l}$)	RBC($10^6/\mu\text{l}$)	HgB(g/dl)	Hct(%)	MCV(fl)	MCH(pg)	MCHC(g/dl)	Platelets ($10^3/\mu\text{l}$)
USP Negative Control	1	8.00	5.16	16.50	47.00	90.90	32.00	35.20	300.00
	2	8.20	5.16	16.70	47.30	91.60	32.40	35.40	316.00
	3	8.10	5.14	16.50	46.40	90.30	32.00	35.50	309.00
mean		8.10	5.15	16.57	46.90	90.93	32.13	35.37	308.33
$\pm$ S.D.		0.10	0.01	0.12	0.46	0.65	0.23	0.15	8.02

LEGEND

WBC
 RBC
 HgB
 Hct
 S.D

White Blood Cell Count
 Red Blood Cell Count
 Hemoglobin
 Hematocrit
 Standard Deviation

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MCV
 MCH
 MCHC
 Platelets

Mean Corpuscular Volume
 Mean Corpuscular Hemoglobin
 Mean Corpuscular Hemoglobin Concentration
 Platelet Count

Toxikon Corporation
In Vitro Hemocompatibility Assay-ISO
Project Number: 97G-0386

TABLE 2: SUMMARY OF HEMOCOMPATIBILITY TEST RESULTS

Parameter	Test Article vs Untreated Blood	Test Article vs USP Negative Control Plastic	Results
WBC	-	-	Pass
RBC	-	-	Pass
HgB	-	-	Pass
Hct	-	-	Pass
MCV	-	-	Pass
MCH	-	-	Pass
MCHC	-	-	Pass
Platelets	-	-	Pass

- No Adverse Effect

@ Significantly and Adversely Increased

* Significantly and Adversely Decreased

Statistically but not Biologically Significant

MAR-24-97 MON 10:08

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FAX NO. 8035335694

P. 01
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RECEIVED
MAR 19 1997



ZEUS

Medical Products Division

Fax Memorandum

To: ETHICON ENDO-SURGERY

FROM : JOE COX

Attn: DALE SHULZE

Date: 3/19/97

Fax #513-438-3394

Page: 1 of 10

Re: PEEK CLASS VI

DALE,

HERE IS THE USP CLASS VI TEST FOR BIOCOMPATIBILITY FOR THE PEEK MATERIAL.
IF YOU HAVE ANY FURTHER QUESTIONS OR NEED INFORMATION, PLEASE FEEL FREE TO
GIVE ME A CALL.

JOE

The information contained within this facsimile transmission is intended to be restricted to only the personal and confidential use of the addressee specified hereabove. This transmission is a confidential client communication and may also for other reasons be confidential or privileged. If you have received or otherwise obtained this transmitted information in error, then any review, retransmission, dissemination, copying, distribution by other means, or copying of any of the information herein, either in whole or in part, is strictly prohibited. If you have received this transmission in error, please notify us immediately by telephone and return the complete original transmission to us by mail. Thank You.

ZEUS, INC.

620 MAGNOLIA ST., P.O. BOX 2167, ORANGEBURG, SOUTH CAROLINA 29118-2167
TOLL FREE: 800-526-3642 • PHONE: 803-531-2174 • FAX: 803-533-5694 • www.zeusinc.com

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P.02



**LEBERCO
TESTING/INC**

123 Hawthorne Street
Roselle Park, NJ 07204-0206
(908) 245-1933 • Fax (908) 245-6253
(800) 523-LABS

June 15, 1995

SUBMITTED TO: Zeus Industries, Inc.
48 Orlando Dr.
Raritan, NJ 08869

ASSAY NUMBER: 958339, 958340, 958341

DATE RECEIVED: 5/17/95

TEST MATERIAL: P.E.E.K. 381G

Assay Number	Test
958339	Systemic Injection
958340	Implantation Test
958341	Intracutaneous Test

METHOD OF ASSAY: Biological Reactivity, In Vivo,
USP 23
(Type VI-121° Plastic Classification)

MAR-24-97 MON 10:09

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P. 03

LEBERCO TESTING/INC

SYSTEMIC INJECTION

958339

958340

958341

958339A - Sample in Sodium Chloride Injection

Dose: 50 ml/kg

Animal #/ Sex	IV Dose (ml)	Initial Weight (g)	Observations					Final Weight (g)
			Immed.	Four Hr	Day 1	Day 2	Day 3	
1M	1.00	19.9	An	An	An	An	An	25.4
2M	1.00	20.0	An	An	An	An	An	24.5
3M	1.10	21.1	An	An	An	An	An	27.2
4M	1.10	21.8	An	An	An	An	An	26.1
5M	1.10	21.9	An	An	An	An	An	26.9

958339B - Sample in Sodium Chloride Injection and Ethyl Alcohol

Dose: 50 ml/kg

Animal #/ Sex	IV Dose (ml)	Initial Weight (g)	Observations					Final Weight (g)
			Immed.	Four Hr	Day 1	Day 2	Day 3	
1F	0.97	19.3	An	An	An	An	An	22.0
2F	0.94	18.7	An	An	An	An	An	21.8
3F	1.10	21.1	An	An	An	An	An	24.0
4F	0.92	18.3	An	An	An	An	An	21.6
5F	0.98	19.6	An	An	An	An	An	22.5

958339C - Sample in Polyethylene Glycol 400 (dilution)

Dose: 10 g/kg

Animal #/ Sex	IP Dose (ml)	Initial Weight (g)	Observations					Final Weight (g)
			Immed.	Four Hr	Day 1	Day 2	Day 3	
1M	0.21	21.9	An	An	An	An	An	25.5
2M	0.21	22.1	An	An	An	An	An	25.7
3M	0.21	22.2	An	An	An	An	An	26.3
4M	0.21	22.1	An	An	An	An	An	27.4
5M	0.21	22.3	An	An	An	An	An	26.3

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LEBERCO TESTING/INC**SYSTEMIC INJECTION**

958339

958340

958361

958339D - Sample in Sesame Oil
Dose: 50 ml/kg

Animal #/ Sex	IP Dose (ml)	Initial Weight (g)	Observations					Final Weight (g)
			Immed.	Four Hr	Day 1	Day 2	Day 3	
1F	0.96	19.2	An	An	An	An	An	21.6
2F	0.97	19.4	An	An	An	An	An	22.8
3F	0.93	18.6	An	An	An	An	An	20.9
4F	0.95	19.0	An	An	An	An	An	21.7
5F	1.00	20.2	An	An	An	An	An	21.9

958339E - Polyethylene Glycol 400 (blank dilution)
Dose: 10 g/kg

Animal #/ Sex	IP Dose (ml)	Initial Weight (g)	Observations					Final Weight (g)
			Immed.	Four Hr	Day 1	Day 2	Day 3	
1M	0.21	22.0	An	An	An	An	An	25.6
2M	0.21	22.1	An	An	An	An	An	26.3
3M	0.20	21.0	An	An	An	An	An	24.3
4M	0.22	22.4	An	An	An	An	An	26.2
5M	0.22	22.4	An	An	An	An	An	26.0

958339F - Sesame Oil (blank)
Dose: 50 ml/kg

Animal #/ Sex	IP Dose (ml)	Initial Weight (g)	Observations					Final Weight (g)
			Immed.	Four Hr	Day 1	Day 2	Day 3	
1F	0.99	19.7	An	An	An	An	An	22.0
2F	0.96	19.2	An	An	An	An	An	21.4
3F	0.94	18.8	An	An	An	An	An	21.1
4F	0.96	19.2	An	An	An	An	An	22.1
5F	1.00	19.9	An	An	An	An	An	22.6

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LEBERCO TESTING/INC958339
958340
958341

SYSTEMIC INJECTION

958339G - Sodium Chloride Injection and Ethyl Alcohol (blank)
Dose: 50 ml/kg

Animal #/ Sex	IV Dose (ml)	Initial Weight (g)	Observations					Final Weight (g)
			Immed.	Four Hr	Day 1	Day 2	Day 3	
1F	0.97	19.3	An	An	An	An	An	22.5
2F	0.90	18.0	An	An	An	An	An	22.6
3F	0.96	19.1	An	An	An	An	An	20.6
4F	0.91	18.1	An	An	An	An	An	21.7
5F	0.97	19.3	An	An	An	An	An	21.7

958339H - Sodium Chloride Injection (blank)
Dose: 50 ml/kg

Animal #/ Sex	IV Dose (ml)	Initial Weight (g)	Observations					Final Weight (g)
			Immed.	Four Hr	Day 1	Day 2	Day 3	
1M	1.00	20.5	An	An	An	An	An	22.2
2M	1.10	21.7	An	An	An	An	An	22.6
3M	1.10	21.2	An	An	An	An	An	22.4
4M	1.00	20.7	An	An	An	An	An	21.8
5M	1.10	21.4	An	An	An	An	An	23.6

An = Appears Normal

MAR-24-97 MON 10:11

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P.06

LEBERCO TESTING/INC958339
958340
958341**IMPLANTATION TEST**

Assay #: 958340

The fur of two rabbits was clipped on both sides of the spinal column. Each animal was then anesthetized with sodium pentobarbital for injection. Four strips of the test sample were implanted into the paravertebral muscle on one side of the spinal column. Two strips of USP Negative Control Plastic RS were implanted into the opposite muscle of each animal. The implants remained in place for 7 days. On day 7, the animals were sacrificed and the tissue surrounding the implants was examined macroscopically for any hemorrhage, film or encapsulation.

At necropsy, all reference standards and implants were found. One test material strip on animal #14279M was partially encapsulated, width = 1mm. Animal #14299F also had a partially encapsulated test strip, width = 0.3mm. All other implant sites appeared normal.

Animal # Sex	Body Wt (Kg)	SCORES			
		Sample	Avg.	Control	Avg.
14279M	2.85	2	0.50	0	0
14299F	2.86	1	0.25	0	0

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~~LEBERCO TESTING/INC~~958339
958340
958341

INTRACUTANEOUS TEST

Dose = 0.20ml/each of 5 sites (test material solution);
0.20ml/each of 5 sites (blank solution).

958341A - Sample In Sodium Chloride Injection
& Blank

Animal #/ Sex	Test Site	Observations					
		24 Hr		48 Hr		72 Hr	
		T	B	T	B	T	B
14320M	1	0/0	0/0	0/0	0/0	0/0	0/0
	2	0/0	0/0	0/0	0/0	0/0	0/0
	3	0/0	0/0	0/0	0/0	0/0	0/0
	4	0/0	0/0	0/0	0/0	0/0	0/0
	5	0/0	0/0	0/0	0/0	0/0	0/0
Avg. Score		0	0	0	0	0	0
14342F	1	0/0	0/0	0/0	0/0	0/0	0/0
	2	0/0	0/0	0/0	0/0	0/0	0/0
	3	0/0	0/0	0/0	0/0	0/0	0/0
	4	0/0	0/0	0/0	0/0	0/0	0/0
	5	0/0	0/0	0/0	0/0	0/0	0/0
Avg. Score		0	0	0	0	0	0

72 Hour Overall Mean Score: T = 0, B = 0, D = 0

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FAX NO. 8035335694

P.08

LEBERCO TESTING/INC958339
958340
958341**INTRACUTANEOUS TEST**958341B - Sample in Sodium Chloride Injection & Ethyl Alcohol
& Blank

Animal #/ Sex	Test Site	Observations					
		24 Hr		48 Hr		72 Hr	
		T	B	T	B	T	B
14321M	1	0/0	0/0	0/0	0/0	0/0	0/0
	2	0/0	0/0	0/0	0/0	0/0	0/0
	3	0/0	0/0	0/0	0/0	0/0	0/0
	4	0/0	0/0	0/0	0/0	0/0	0/0
	5	0/0	0/0	0/0	0/0	0/0	0/0
Avg. Score		0	0	0	0	0	0
14343F	1	0/0	0/0	0/0	0/0	0/0	0/0
	2	0/0	0/0	0/0	0/0	0/0	0/0
	3	0/0	0/0	0/0	0/0	0/0	0/0
	4	0/0	0/0	0/0	0/0	0/0	0/0
	5	0/0	0/0	0/0	0/0	0/0	0/0
Avg. Score		0	0	0	0	0	0

72 Hour Overall Mean Score: T = 0, B = 0, D = 0

958341C - Sample In Polyethylene Glycol 400 Extract (diluted)
& Blank (diluted)

Animal #/ Sex	Test Site	Observations					
		24 Hr		48 Hr		72 Hr	
		T	B	T	B	T	B
14334M	1	0/0	0/0	0/0	0/0	0/0	0/0
	2	0/0	0/0	0/0	0/0	0/0	0/0
	3	0/0	0/0	0/0	0/0	0/0	0/0
	4	0/0	0/0	0/0	0/0	0/0	0/0
	5	0/0	0/0	0/0	0/0	0/0	0/0
Avg. Score		0	0	0	0	0	0
14356F	1	0/0	0/0	0/0	0/0	0/0	0/0
	2	0/0	0/0	0/0	0/0	0/0	0/0
	3	0/0	0/0	0/0	0/0	0/0	0/0
	4	0/0	0/0	0/0	0/0	0/0	0/0
	5	0/0	0/0	0/0	0/0	0/0	0/0
Avg. Score		0	0	0	0	0	0

72 Hour Overall Mean Score: T = 0, B = 0, D = 0

MAR-24-97 MON 10:12

ZEUS

FAX NO. 8035335694

P. 09

LEBERCO TESTING/ING958339
958340
958341**INTRACUTANEOUS TEST**958341D - Sample In Sesame Oil
& Blank

Animal #/ Sex	Test Site	Observations					
		24 Hr		48 Hr		72 Hr	
		T	B	T	B	T	B
14357F	1	1/0	1/0	1/0	1/0	1/0	1/0
	2	1/0	1/0	1/0	1/0	0/0	0/0
	3	1/0	1/0	1/0	1/0	1/0	0/0
	4	1/0	1/0	1/0	1/0	0/0	0/0
	5	1/0	1/0	1/0	1/0	1/0	1/0
Avg. Score		0.5	0.5	0.5	0.5	0.3	0.2
14319M	1	1/1	1/1	1/0	1/0	1/0	1/0
	2	1/1	1/1	1/0	1/0	1/0	1/0
	3	1/1	1/0	1/1	1/0	1/1	1/0
	4	1/1	1/1	1/0	1/0	1/0	1/0
	5	1/1	1/1	1/0	1/0	1/0	1/0
Avg. Score		1.0	0.9	0.6	0.5	0.6	0.5
72 Hour Overall Mean Score:		T = 2.91, B = 2.58, D = 0.37					

Key to Abbreviations:

T = Test Material

B = Blank

D = Difference between T and B at 72 hours
Erythema/Edema

MAR-24-97 MON 10:13

ZEUS

FAX NO. 8035335694

P. 10

LEBERCO TESTING/INC

958339
958340
958341

DISCUSSION:

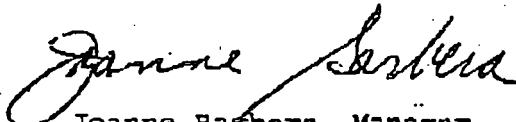
Systemic Injection - None of the animals had a weight loss during the three day test. All animals appeared normal.

Intracutaneous Test - The difference between the test materials and the blanks for the 72 Hour Overall Mean Score for all samples was less than 1.0.

Implantation Test - All implanted test materials were found. One test site in each of the animals was encapsulated, all other test sites and reference standard sites appeared normal. The difference between the control and sample mean scores for any one animal did not exceed 1.0.

CONCLUSION: The test material meets the requirements of the USP <88> Biological Reactivity Tests, In Vivo which includes the systemic injection, the intracutaneous test and the implantation test. The test material would be classified as a Class VI-121⁰ plastic.

Work Performed Under
the Supervision of:


Joanne Barbera, Manager
Toxicology

LEBERCO TESTING, INC.



Edwin C. Rothstein, Ph.D.
Director

or
William Gilman, M.S.
Associate Director



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LABORATORIES, INC.

REPORT TITLE

**IN VITRO RABBIT BLOOD HEMOLYSIS DETERMINATION
USING THE DIRECT CONTACT METHOD**

PRODUCT IDENTITY

Tacker SDS w/PEEK balloon File # 1572
Lot SR# 12-96-231

PROJECT NUMBER

3243

PROTOCOL NUMBER

HMD100

PREPARED BY

Isis Koenders
Study Director

FINAL REPORT

January 22, 1997

PERFORMING LABORATORY

ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

SPONSOR

Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014

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JAN 24 1997



Cordis Corporation

Project No. 3243

Page 2 of 5

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Laboratory Practice (GLP) regulations set forth in 21 CFR part 58. The studies not performed by or under the direction of ViroMed Laboratories, Inc. are exempt from this Good Laboratory Practice statement and include: characterization of the compound(s)/test substance.

Study Director: Isis Koenders
Isis Koenders

Date: 1-22-97

Submitter: _____

Date: _____

Sponsor: [Signature]

Date: 2/1/97

QUALITY ASSURANCE UNIT SUMMARY

Study: In Vitro Rabbit Blood Hemolysis - Direct Contact Method

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice regulations (21 CFR Part 58) and in accordance with standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to management and the Study Director.

<u>Phase Inspected</u>	<u>Date</u>
Project Set-Up	1/15/97
Final Data Reading	1/15/97
Final Report	1/20/97
Study Director Review Date	1/22/97
Management Review Date	1/22/97

Documentation of the above Quality Assurance audits have been reviewed.

Quality Assurance Director: [Signature]
Joyce A. Nelson

Date: 22 Jan, 1997

Study Director: Isis Koenders

Date: 1-22-97

Professional personnel involved in study:

Bonita L. Baskin, Ph.D.	-	Laboratory Director
Isis Koenders	-	Study Director
Erika Reubin, BA	-	Research Assistant I

In Vitro RABBIT BLOOD HEMOLYSIS DETERMINATION - DIRECT CONTACT METHOD

OBJECTIVE

The objective of this study is to assess the in vitro hemolytic activity of the test article after direct contact with rabbit blood.

SAMPLE NAME AND CODE: Tacker SDS w/PEEK balloon File # 1572, Lot SR# 12-96-231

SUBSTANCE CHARACTERIZATION

The identity, strength, stability, purity, and chemical composition are the responsibility of the Sponsor.

DATA RETENTION

A certified copy of the original final report and all raw data pertinent to the study will be stored at ViroMed Laboratories, Inc., 6101 Blue Circle Drive Minneapolis, MN 55343. All test substance used in this assay was expended in testing.

SPONSOR: Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014

TEST FACILITY: ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

INITIATION DATE: 1/10/97
SAMPLES RECEIVED: 1/10/97
EXPERIMENTAL START DATE: 1/15/97
COMPLETION DATE: 1/22/97

STATISTICAL METHODS: None used.

TEST SYSTEM JUSTIFICATION

The use of rabbit blood to measure the hemolytic activity of a test article is recommended in DHEW Publication, "Evaluation of Hemodialyzers and Dialysis Membranes", NIH 77-1294, p. 213, 1977. No alternative (non-animal) test methods are described in the guidelines.

EXPERIMENTAL DESIGN

Triplicate samples of the test article were prepared on the day of testing, weighed, and placed in tubes. Saline for injection was added at a ratio of 10ml/5g of test article. Duplicate positive and negative controls, consisting of 0.1% sodium carbonate and saline for injection, respectively, were prepared for incubation along with the test article samples. All test article and control samples were incubated at $37 \pm 2^{\circ}\text{C}$ for 30 minutes. After this initial incubation, proportional aliquots of a dilute suspension of oxalated rabbit blood were added to each tube at a ratio of 0.2ml/10ml of saline. All tubes were gently mixed by inversion and then incubated at $37 \pm 2^{\circ}\text{C}$ for a period of one hour. At the end of the incubation period, the tubes were centrifuged at 1500g for 10 minutes. The supernatant was then removed and transferred to labeled cuvettes. The absorbance of samples and controls were read at 545 nm using a standard laboratory spectrophotometer. The percent hemolysis was calculated using the following formula:

$$\% \text{ Hemolysis} = \frac{(\text{Ave. Absorb. Test Article}) - (\text{Ave. Absorb. Neg. Cont.})}{(\text{Ave. Absorb. Pos. Cont.}) - (\text{Ave. Absorb. Neg. Cont.})} \times 100$$

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Cordis Corporation

Project No. 3243

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Controls

Positive Control: 0.1% Sodium Carbonate - Lot 010697

Negative Control: Saline for Injection - Lot C319665

New Zealand White Rabbit Blood: Lots 1475, 873, 562

DESCRIPTION	WEIGHT	SALINE VOLUME	BLOOD VOLUME
TEST ARTICLE Tacker SDS w/PEEK balloon File # 1572, SR# 12-96-231	#1) 2.77 grams	5.5 ml	0.11 ml
	#2) 2.78 grams	5.6 ml	0.11 ml
	#3) 2.76 grams	5.5 ml	0.11 ml
POSITIVE CONTROL	---	10 ml	0.2 ml
NEGATIVE CONTROL	---	10 ml	0.2 ml

RESULTS

The percent hemolysis is determined using the average absorbance values of the three test article samples and the duplicate controls. All data points obtained are displayed in Table 1.

TABLE 1

TUBE IDENTIFICATION	ABSORBANCE	AVERAGE ABSORBANCE
TEST ARTICLE: Tacker SDS w/PEEK balloon File # 1572 Lot SR# 12-96-231	#1) .030	.028
	#2) .025	
	#3) .030	
POSITIVE CONTROL	#1) .84	.845
	#2) .85	
NEGATIVE CONTROL	#1) .045	.046
	#2) .047	

% HEMOLYSIS OF THE TEST ARTICLE = - 2.25%
--

In this experimental design, a test article yielding a hemolysis value of 5.0% or less is considered non-hemolytic. Values greater than 5.0% are considered hemolytic. Products exhibiting consistent results between 3% and 5% hemolysis upon repeat testing, may have low level hemolytic activity.

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Cordis Corporation

Project No. 3243

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ANALYSIS AND CONCLUSION

The test article, Tacker SDS w/PEEK balloon File # 1572 (Lot SR# 12-96-231) is considered **NON-HEMOLYTIC** under the test conditions employed.

REFERENCES

American Society for Testing and Materials, "Standard Practice for Assessment of Hemolytic Properties of Materials F 756 - 87", in Annual Book of ASTM Standards (1992), Vol. 13.01, Medical Devices, p. 273.

DHEW Publication, "Evaluation of Hemodialyzers and Dialysis Membranes" NIH 77-1294, p. 213, 1977.

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COPY

AMES MUTAGENICITY TEST

REPORT TITLE

Ames Mutagenicity Testing of Cordis Corporation,
Tacker with PEEK Balloon File# 1572 Extracted in Sodium Chloride

PRODUCT IDENTITY

Cordis Corporation
Tacker with PEEK Balloon File# 1572
Lot # NB# 91968 p15

PROJECT NUMBER

3275

PROTOCOL NUMBER

AM100

PREPARED BY

Neal T. Wetherall, Ph.D.
Scientific Director

STUDY COMPLETION ON

February 26, 1997

PERFORMING LABORATORY

ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

SPONSOR

Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014

RECEIVED

MAR 04 1997

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with current FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies.

The studies not performed by or under the direction of ViroMed Laboratories, Inc. are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the test article.

Submitter: _____ Date: _____
Sponsor: [Signature] Date: 3/1/97
Study Director: [Signature] Date: 26 Feb. 1997
Neal T. Wetherall, Ph.D.

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Salmonella Mutagenicity Plate Incorporation Assay (Ames Assay)

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to management and the Study Director.

Phase Inspected:

Project Set-up	Date: 1/30/97
In Process	Date: 1/31/97
Final Data Reading	Date: 2/3/97
Final Report	Date: 2/26/97
Study Director Review	Date: 2/26/97
Management Review	Date: 2/26/97

Documentation of the above Quality Assurance audits have been reviewed.

Quality Assurance Director: [Signature] Date: 26 Feb. 1997
Joyce A. Nelson

Study Director: [Signature] Date: 26 Feb. 1997
Neal T. Wetherall, Ph.D.

Professional Personnel Involved:

Bonita L. Baskin, Ph.D.	Laboratory Director
Neal T. Wetherall, Ph.D.	Scientific Director
Xuebin Zhang, Ph.D.	Senior Genotoxicologist
Lisa Stokvis, B.S.	Research Assistant I



Cordis Corporation
Project # 3275
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PROJECT NUMBER: 3275

REPORT DATE: 2/26/97

SPONSOR: Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014

DATA RETENTION

A certified copy of the original final report and all raw data pertinent to this study will be stored at ViroMed Laboratories, Inc., 6101 Blue Circle Drive, Minneapolis, MN 55343. The test substance used in this assay was expended in testing.

SAMPLE IDENTIFICATION

Cordis Corporation, Tacker with PEEK Balloon File# 1572, Lot # NB# 91968 p15.

CHARACTERIZATION

The identity, strength, stability, purity, and chemical composition are the responsibility of the Sponsor.

PURPOSE

The purpose of this study was to evaluate the mutagenic potential of the test article (or its metabolites) by measuring its ability to induce back mutations at selected loci of several strains of *Salmonella typhimurium* in the presence and absence of microsomal enzymes. This test system has been shown to detect diverse groups of chemical mutagens (McCann *et al.*, 1975; McCann and Ames, 1976). The ability to induce mutation is indicative of a chemical's genotoxic potential.

TEST FACILITY: ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

DATE SAMPLE RECEIVED: 1/21/97
STUDY INITIATION DATE: 1/21/97
EXPERIMENTAL START DATE: 1/29/97
STUDY COMPLETION DATE: 2/26/97



Cordis Corporation
Project # 3275
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METHOD

This study was conducted in accordance with the plate incorporation methodology originally described by Ames *et al.* (1975) and updated by Maron and Ames (1983). This methodology has been shown to detect diverse classes of chemical mutagens which produce heritable DNA mutations of a type which are associated with adverse effects.

EXPERIMENTAL SUMMARY

This *Salmonella typhimurium* Reverse Mutation Assay (Ames Assay) evaluated the potential of a Sodium Chloride extract of the test article to induce histidine (his) reversion (his- to his+) caused by base changes or frameshift mutations in the genome of this organism. This plate incorporation assay was conducted with four strains of *Salmonella typhimurium*, (TA97a, TA98, TA100, TA102) in the presence and absence of an exogenous mammalian activation system (S9). The Range Finding Study was not required for this assay. Based on the criteria and conditions of the study protocol, the test article is considered non-mutagenic.

TEST MATERIAL AND PREPARATION

STERILIZATION

The test article was submitted in sterile form by the Sponsor.

TEST ARTICLE PREPARATION AND EXTRACTION

The test article was sampled according to the sponsors directions to obtain a representative sample of the finished device in accordance with USP 23 recommendations. The test article was prepared at a ratio equivalent to 120 cm² of test article to 20 mls of extraction vehicle, and extracted under the following conditions:

Extract vehicle: Sodium Chloride, Lot #C319665, exp. 9/97

Surface area of test article	ml vehicle	Time	Temp
126 cm ²	21	24 hours	70°C

After 24 hours, the extract fluid was decanted into a sterile dry glass vessel. The extract was maintained at room temperature and used within 24 hours of preparation.

TEST SYSTEM

JUSTIFICATION OF TEST SYSTEM

The Reverse Mutation Assay using *Salmonella typhimurium* has historically been used to detect mutation in a gene of a histidine requiring strain to produce a histidine independent strain of the organism. This test is recommended in Ames et al, Mutation Res., 31:347-364, 1975.

IDENTIFICATION OF THE TEST SYSTEM

The *Salmonella typhimurium* strains used in this assay, TA97a, TA98, TA100, TA102, were received from Carol Wehr, The Ames Laboratory, Department of Biochemistry, University of California, Berkeley, CA 94720.

JUSTIFICATION OF TEST ARTICLE ADMINISTRATION ROUTE

The test article was administered *in vitro*, through a solvent compatible with the test system.

EXPERIMENTAL DESIGN

METHOD

Master plates were prepared from frozen working stocks. For the assay, a single colony was transferred into Oxoid nutrient broth and incubated, with shaking, for 14 hours 45 minutes at $37 \pm 2^\circ\text{C}$. This overnight culture was used for the mutagenicity test and for genotypic confirmation. The culture density was confirmed by measuring the optical density at 650 nm. A top agar consisting of 0.6% Difco agar in 0.5% NaCl was melted. A solution of 0.5 mM L-histidine / 0.5 mM biotin was added to the melted top agar at a ratio of 10 ml per 100 ml. This solution was aliquoted, 2 ml per 12 mm X 75 mm tube and held at 47°C . The test article extract, in a 0.1 ml volume, 0.1 ml of the overnight culture and 0.5 ml of phosphate buffered saline (PBS) was added and the mixture briefly vortexed and poured, at room temperature, onto a minimal glucose agar plate (1.5% Difco agar, 2% glucose, in Vogel-Bonner medium E). The plates were allowed to harden and then incubated 48-72 hours at $37 \pm 2^\circ\text{C}$. Metabolic activation was provided by adding 0.5 ml of the S9 mix in place of the PBS. Revertant colonies were counted on the test plates and control plates after 48-72 hours. The presence of a bacterial lawn was confirmed on all plates.

EXOGENOUS METABOLIC ACTIVATION

Aroclor 1254-induced rat liver S9 microsomal enzyme was used as the metabolic activation system. Immediately prior to use, the S9 was thawed and mixed with a cofactor pool to contain 4% S9, 5 mM glucose 6-phosphate, 4 mM β -nicotine-adenine dinucleotide phosphate, 8 mM MgCl_2 and 33 mM KCl in a 100 mM phosphate buffer at pH 7.4.



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Project # 3275
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DOSE LEVELS AND REPLICATES

The test article extract was tested at five dose levels (neat, 1:2, 1:4, 1:8, 1:10) along with appropriate vehicle and positive controls. All dose levels of test article extract and vehicle controls were analyzed using tester strains TA97a, TA98, TA100 and TA102 with and without S9 microsomal enzymes. All dose levels of test article extract, vehicle controls and positive controls were plated in triplicate.

GENOTYPIC CHARACTERIZATION OF THE TEST STRAINS

Frozen working stocks of test strains were confirmed for genotypic markers and acceptable spontaneous reversion rates and ranges. Spontaneous reversion rates were within published ranges and historical in-house ranges. All working stocks demonstrated a requirement for histidine (Appendix B). The following confirmations were made at the time of the mutagenicity assay:

- 1) Sensitivity to crystal violet due to the *rfa* wall mutation strains TA100, TA98, TA97a, and TA102.
- 2) Sensitivity to ultraviolet light due to the deletion of the *uvrB* gene strains TA100, TA98, and TA97a.
- 3) Resistance to ampicillin due to the presence of the pKM101 plasmid strains TA100, TA98, TA97a, and TA102.
- 4) Resistance to tetracycline due to the presence of the pAQ1 plasmid strain TA102.
- 5) Spontaneous reversion rates for each strain were compared to historical ranges using the solvent/vehicle controls.

CONTROL SUBSTANCES

Control substances were prepared and used in the mutagenicity assay as described in published reports.

ASSAY POSITIVE CONTROLS

ICR-191 Acridine for TA97a at 1.0 µg/plate, Lot# 1CR101596
2-nitrofluorene for TA98 at 1.0 µg/plate, Lot# NF123096
Sodium azide for TA100 at 1.5 µg/plate, Lot# SA022895B
Cumene hydroperoxide for TA102 at 100 µg/plate, Lot# CH022895
2-aminoanthracene for all strains with S9 activation at 10 µg/plate, Lot# 85H2508

VEHICLE (NEGATIVE) CONTROL

Tester strains were plated with Sodium Chloride at the corresponding maximum concentration, with and without S9. This served as the negative control article and provided information regarding background lawn and revertant colony formation.



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Project # 3275
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CRITERIA AND RESULTS

REVERSE MUTATION ASSAY

Each separate bacterial strain, with and without S9 was considered a separate experiment with its own concurrent positive and vehicle controls. The results are presented here by bacterial strain. The positive control article assays consisted of direct-acting mutagens and mutagens requiring metabolic transformation. The negative control article reversion rates for each strain were within the expected range from published and laboratory historical data. A two fold or greater increase in reversion rates was observed for all strains with the appropriate positive control. All plates were scored, and the mean number of revertant colonies per plate for each tester strain at each concentration of the test article extract was recorded. A positive mutagen result for any strain would demonstrate at least a two-fold, concentration dependent, increase in the number of revertant colonies per plate over the negative control values. The data results are presented in tabular form in Appendix A, Tables 1-4.

DOSE-RESPONSE

Dose related increases in numbers of revertant colonies is an important criterion in establishing mutagenicity of a test article. Positive mutagens will exhibit an increased number of revertant colonies with increased concentration of the test article. Five dose levels of the test article extract were utilized in this assay and a dose-related response was not observed.

ANALYSIS AND CONCLUSION

The *Salmonella typhimurium* Reverse Mutation Assay (Ames Assay) evaluates the potential of a test article to induce histidine reversion, caused by base changes or frameshift mutations in the genome of the test organism. This plate incorporation method was conducted using strains TA97a, TA98, TA100, and TA102 of *Salmonella typhimurium*, in the presence and absence of S9 exogenous mammalian activation. Based on the criteria of the assay, the test article, Cordis Corporation, Tacker with PEEK Balloon File# 1572, Lot # NB# 91968 p15, is considered non-mutagenic.

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Cordis Corporation
Project # 3275
Page 8 of 8

TECHNICAL REFERENCES

- 1) Ames, B.N., McCann, J. and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutation Research* 31:347-367.
- 2) McCann, J. and B.N. Ames (1976) Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals: discussion, *Proc. Natl. Aca. Sci. USA*, 83:950-954.
- 3) McCann, J., E. Choi, E. Yamasaki and B.N. Ames (1975) Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals, *Proc. Natl. Acad. Sci. USA*, 72:5135-5139.
- 4) Levin, D., Hollstein, M., Christman, M., Schwiers, E., B.N. Ames (1982) A new *Salmonella* tester strain (TA102) with A-T base pairs at the site of mutation detects oxidative mutagens, *Proc. Natl. Aca. Sci. USA*, 79:7445-7449.
- 5) Maron, D. M. and B.N. Ames (1983) Revised Methods for the *Salmonella* Mutagenicity Test, *Mutation Research*, 113:173-215.

STATISTICAL METHODS:

Arithmetic Mean and Standard Deviation.

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Cordis Corporation
Project # 3275
Appendix A
Page 1 of 2

AMES ASSAY DATA

Bacterial strains tested: TA97a, TA98, TA100, TA102

Test substance: Cordis Corporation, Tacker with PEEK Balloon File# 1572

Lot number: LOT# NB# 91968 p15

Dilution: Neat, 1:2, 1:4, 1:8, 1:10

Diluent: NaCl

TABLE 1: COLONY COUNT DATA FOR TEST ARTICLE EXTRACT, VEHICLE, AND POSITIVE CONTROLS

TESTER STRAIN TA97a with S9

Dilution	Rep 1	Rep 2	Rep 3	Mean	Std dev
Neat	172	158	166	165	7.02
Dil. 1:2	168	158	198	175	20.82
Dil. 1:4	179	130	146	152	24.99
Dil. 1:8	172	156	181	170	12.66
Dil. 1:10	154	157	139	150	9.64

TESTER STRAIN TA97a without S9

Dilution	Rep 1	Rep 2	Rep 3	Mean	Std dev
Neat	128	131	149	136	11.36
Dil. 1:2	150	137	155	147	9.29
Dil. 1:4	143	141	142	142	1.00
Dil. 1:8	156	133	151	147	12.10
Dil. 1:10	169	135	171	158	20.23

TESTER STRAIN TA97a with S9

Control	Rep 1	Rep 2	Rep 3	Mean	Std dev
NaCl	158	167	179	168	10.54
2-aminoanthracene*	1096	1336	1435	1289	174.32

*Positive control value is two fold or greater than the mean vehicle control for the strain.

TESTER STRAIN TA97a without S9

Control	Rep 1	Rep 2	Rep 3	Mean	Std dev
NaCl	173	151	138	154	17.69
ICR-191 Acridine**	1140	1122	1156	1139	17.01

**Positive control value is two fold or greater than the mean vehicle control for the strain.

TABLE 2: COLONY COUNT DATA FOR TEST ARTICLE EXTRACT, VEHICLE, AND POSITIVE CONTROLS

TESTER STRAIN TA98 with S9

Dilution	Rep 1	Rep 2	Rep 3	Mean	Std dev
Neat	45	45	54	48	5.20
Dil. 1:2	41	50	33	41	8.50
Dil. 1:4	27	40	54	40	13.50
Dil. 1:8	51	63	55	56	6.11
Dil. 1:10	66	44	60	57	11.37

TESTER STRAIN TA98 without S9

Dilution	Rep 1	Rep 2	Rep 3	Mean	Std dev
Neat	46	31	33	37	8.14
Dil. 1:2	40	37	40	39	1.73
Dil. 1:4	33	27	38	33	5.51
Dil. 1:8	61	40	39	47	12.42
Dil. 1:10	45	36	49	43	6.66

TESTER STRAIN TA98 with S9

Control	Rep 1	Rep 2	Rep 3	Mean	Std dev
NaCl	60	57	63	60	3.00
2-aminoanthracene*	1544	1562	1660	1589	62.43

*Positive control value is two fold or greater than the mean vehicle control for the strain.

TESTER STRAIN TA98 without S9

Control	Rep 1	Rep 2	Rep 3	Mean	Std dev
NaCl	48	53	60	54	6.03
2-nitrofluorene**	724	937	1505	1055	403.72

**Positive control value is two fold or greater than the mean vehicle control for the strain.



Cordis Corporation
Project # 3275
Appendix A
Page 2 of 2

AMES ASSAY DATA

Bacterial strains tested: TA97a, TA98, TA100, TA102

Test substance: Cordis Corporation, Tacker with PEEK Balloon File# 1572

Lot number: LOT# NB# 91968 p15

Dilution: Neat, 1:2, 1:4, 1:8, 1:10

Diluent: NaCl

TABLE 3: COLONY COUNT DATA FOR TEST ARTICLE EXTRACT, VEHICLE, AND POSITIVE CONTROLS

TESTER STRAIN TA100 with S9

Dilution	Rep 1	Rep 2	Rep 3	Mean	Std dev
Neat	179	134	157	157	22.50
Dil. 1:2	197	205	191	198	7.02
Dil. 1:4	154	167	158	160	6.66
Dil. 1:8	183	185	98	155	49.66
Dil. 1:10	127	86	82	98	24.91

TESTER STRAIN TA100 without S9

Dilution	Rep 1	Rep 2	Rep 3	Mean	Std dev
Neat	157	169	155	160	7.57
Dil. 1:2	171	191	164	175	14.01
Dil. 1:4	166	183	188	179	11.53
Dil. 1:8	47	178	58	94	72.67
Dil. 1:10	44	44	43	44	0.58

TESTER STRAIN TA100 with S9

Control	Rep 1	Rep 2	Rep 3	Mean	Std dev
NaCl	175	154	174	168	11.85
2-aminoanthracene*	1502	1498	1425	1475	43.35

*Positive control value is two fold or greater than the mean vehicle control for the strain.

TESTER STRAIN TA100 without S9

Control	Rep 1	Rep 2	Rep 3	Mean	Std dev
NaCl	180	179	173	177	3.79
Sodium azide**	1413	1268	1167	1283	123.65

**Positive control value is two fold or greater than the mean vehicle control for the strain.

TABLE 4: COLONY COUNT DATA FOR TEST ARTICLE EXTRACT, VEHICLE, AND POSITIVE CONTROLS

TESTER STRAIN TA102 with S9

Dilution	Rep 1	Rep 2	Rep 3	Mean	Std dev
Neat	217	195	206	206	11.00
Dil. 1:2	191	167	275	211	56.71
Dil. 1:4	218	197	172	196	23.03
Dil. 1:8	127	139	118	128	10.54
Dil. 1:10	144	181	164	163	18.52

TESTER STRAIN TA102 with S9

Control	Rep 1	Rep 2	Rep 3	Mean	Std dev
NaCl	216	190	190	199	15.01
2-aminoanthracene*	1571	1662	1561	1598	55.65

*Positive control value is two fold or greater than the mean vehicle control for the strain.

TESTER STRAIN TA102 without S9

Dilution	Rep 1	Rep 2	Rep 3	Mean	Std dev
Neat	196	197	184	192	7.23
Dil. 1:2	224	215	204	214	10.02
Dil. 1:4	222	216	230	223	7.02
Dil. 1:8	189	151	199	180	25.32
Dil. 1:10	145	154	153	151	4.93

TESTER STRAIN TA102 without S9

Control	Rep 1	Rep 2	Rep 3	Mean	Std dev
NaCl	214	211	195	207	10.21
Cumene hydroperoxide**	1085	1200	1163	1149	58.71

**Positive control value is two fold or greater than the mean vehicle control for the strain.

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Cordis Corporation
Project #: 3275
Appendix B

GENOTYPIC CHARACTERIZATION

rfa mutation (Crystal Violet sensitivity)

Tester Strain	Results
TA97a	No growth (positive for <i>rfa</i> mutation)
TA98	No growth (positive for <i>rfa</i> mutation)
TA100	No growth (positive for <i>rfa</i> mutation)
TA102	No growth (positive for <i>rfa</i> mutation)

uvrB gene deletion (UV light sensitivity)

Tester Strain	Results UV exposure/ no UV exposure
TA97a	No growth (positive for uvrB mutation)/growth
TA98	No growth (positive for uvrB mutation)/growth
TA100	No growth (positive for uvrB mutation)/growth
TA102	Growth/ growth

pKM101 plasmid Ampicillin resistance

Tester Strain	Results
TA97a	Growth (positive for pKM101 plasmid)
TA98	Growth (positive for pKM101 plasmid)
TA100	Growth (positive for pKM101 plasmid)
TA102	Growth (positive for pKM101 plasmid)

pAQ1 plasmid Tetracycline resistance

Tester Strain	Results
TA102	Growth (positive for pAQ1 plasmid)
TA97a	No growth
TA98	No growth
TA100	No growth

Spontaneous Reversion rates Historic

Tester Strain	Average Ranges*	Ranges**
TA97a	90 - 180	98-253
TA98	30 - 50	11-64
TA100	120 - 200	139-324
TA102	240 - 320	326-640

* Published Standards see reference 5

**ViroMed Historical Ranges.



Cordis Corporation

Project # 3253

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GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with current FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies.

The studies not performed by or under the direction of ViroMed Laboratories, Inc. are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the test article.

Submitter: _____

Date: _____

Sponsor: _____

Date: _____

Study Director: David L. HodgeDate: 2-11-97

David L. Hodge

QUALITY ASSURANCE UNIT SUMMARY

STUDY: USP Intracutaneous Test Method.

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to management and the Study Director.

Phase Inspected:

Project Set-up Date: 1/24/97

Final Report Date: 2/11/97

Study Director Review Date: 2/11/97

Management Review Date: 2/11/97

Documentation of the above Quality Assurance audits have been reviewed.

Quality Assurance Director: Joyce A. NelsonDate: 11 Feb., 1997

Joyce A. Nelson

Study Director: David L. HodgeDate: 2-11-97

David L. Hodge

Professional Personnel Involved:

Bonita L. Baskin, Ph.D.

Laboratory Director

David L. Hodge, BA

Director of In-Life Studies

Terry Haugen, BS

Research Assistant I

Siamak Amiralaii, BA

Research Assistant I

Jill Aurand, BA

Research Assistant I

Ryan Bonham

Research Assistant I



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LABORATORIES, INC.

Cordis Corpo
Project #
Page 3



PROJECT NUMBER: 3253

REPORT DATE: 2/11/97

SPONSOR: Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014

DATA RETENTION: A certified copy of the original final report and all raw data pertinent to this study will be stored at ViroMed Laboratories, Inc., 6101 Blue Circle Drive, Minneapolis, MN 55343. The test substance used in this assay was expended in testing.

SAMPLE IDENTIFICATION: Cordis Corporation
Tacker with PEEK balloon (File # 1572)
Lot # NB # 92000 (10-11)

CHARACTERIZATION: The identity, strength, stability, purity, and chemical composition are the responsibility of the Sponsor.

PURPOSE: The purpose of this test was to determine if any chemicals that may leach or be extracted from the test article were capable of causing local irritation in the dermal tissues of the rabbit using the standard USP Intracutaneous Test procedure.

TEST FACILITY: ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

DATE SAMPLE RECEIVED: 1/17/97
STUDY INITIATION DATE: 1/20/97
EXPERIMENTAL START DATE: 1/20/97
STUDY COMPLETION DATE: 2/11/97
IACUC APPROVAL DATE: 4/18/96

METHOD: This study was conducted in accordance with the USP Intracutaneous Test Procedure, United States Pharmacopeia 23 (1995), Pages 1701-1702.



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Project # 3253

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EXPERIMENTAL SUMMARY: Each rabbit received five sequential 0.2 ml intracutaneous injections along either side of the dorsal mid-line with the test article extract on one side and the concurrent vehicle control on the other. The vehicles used were 0.9% Normal Saline and Cotton Seed Oil. The irritation reaction of the extracts was compared to vehicle controls and recorded over a 72 hour period according to the standard USP Irritation Scoring System. According to USP 23 test criteria, if the difference between the average scores for the extract of the test article and the vehicle control is less than or equal to 1.0, the test article is considered non-irritating. The difference in the mean test and control scores of the 0.9% Normal Saline and Cotton Seed Oil extract dermal observations was less than 1.0, indicating that the requirements of the USP Intracutaneous test have been met by the Cordis Corporation, Tacker with PEEK balloon (File # 1572), Lot # NB# 92000 (10-11).

TEST MATERIAL AND PREPARATION

STERILIZATION: The test article was submitted in sterile form by the Sponsor.

TEST SAMPLE PREPARATION: A representative sample of the test article was cut into pieces, placed into test tubes and prepared at a ratio of 60 cm² to 20 ml of extraction vehicle.

<u>EXTRACT VEHICLE</u>	<u>VEHICLE AMOUNT</u>	<u>TEST ARTICLE AMOUNT</u>
0.9% Normal Saline (NaCl)	18.3 ml	54.90 cm ²
Cottonseed Oil (CSO)	18.3 ml	54.90 cm ²

Vehicle Materials:

0.9% USP Normal Saline, lot # C327718 supplied by Baxter
USP Cottonseed Oil, lot # A007854101 supplied by Acros

EXTRACTION: The sample preparation and control solution (vehicle without test article) were extracted for 24 hours at 70° C.

The extracts were cooled, shaken well and decanted into a sterile, dry glass vessel. The extracts were used within 24 hours of preparation.

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TEST SYSTEM

SPECIES/STRAIN/SEX: Albino Rabbits, New Zealand White Strain, Specific Pathogen Free, Female.

SOURCE: Charles River Canada, St. Constant, Canada

BODY WEIGHT RANGE: All animals weighed in excess of the 1.50 kg minimum USP weight limit at study initiation.

ANIMAL IDENTIFICATION: Individually numbered ear tags and ear markings with a permanent marker.

HUSBANDRY

RECEIPT: The animals were received on 10/23/96 and 11/27/96. Each animal was examined for signs of disease and injury prior to entry into the research area.

HOUSING: One rabbit per suspended stainless steel cage.

ENVIRONMENT: Animal rooms were maintained at $68 \pm 3^{\circ}$ F and 30 to 70% relative humidity with a 12 hour light/dark cycle. Room air exchange was maintained between 12 and 15 air changes per hour. The laboratory and animal rooms were maintained as limited-access facilities.

DIET: Animals were supplied with certified commercial feed, Purina Rabbit Chow. No known contaminants present in the feed are expected to interfere with the test results.

WATER: Potable water.

TERMINATION: Animals were euthanized by lethal injection with a sodium pentobarbital based solution, Euthasol.

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Cordis Corporation

Project # 3253

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COMPLIANCE: The care, housing and handling of the animals was in compliance with AAALAC, "Guide for the Care and Use of Laboratory Animals", DHHS Pub. No. (NIH) 85-23, Revised 1985; (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals", and USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1,2 and 3, Animal Welfare, Final Rule 1989.

SELECTION OF ANIMALS: Animals were randomly placed in cages upon receipt, and were placed on study as available at the time of study initiation. Any animals considered unsuitable due to poor health or outlying body weight were excluded from the study.

ANIMAL PREPARATION: Each rabbit was weighed and the weight recorded at the initiation of the study. The fur of the animals was clipped on both sides of the spinal column to expose a sufficient sized area for injection. Loose fur was removed and the skin was swabbed with 70% isopropyl alcohol solution and air dried.

TEST ARTICLE ADMINISTRATION: The two test article extracts and the two vehicle controls were each injected into two rabbits. Each rabbit received five sequential 0.2 ml intracutaneous injections of the test article extract on the right side of the vertebral column and similarly with the control vehicle on the left side. The second test and control extract injections were parallel and distal to the first injection sites. (Table I)

TABLE I : Injection Sites on Rabbits

HEAD					
	Ext#2	Ext #1	Ext #1	Ext #2	
	1	1	1	1	
0.2 ml INJ.	2	2	2	2	0.2 ml INJ.
CONTROL	3	3	3	3	Test Article
	4	4	4	4	Extract
	5	5	5	5	

OBSERVATIONS AND SCORING: The appearance of each injection site was noted at 24, 48 and 72 hours post injection. The tissue reactions were rated for gross evidence of erythema, edema and necrosis using diluted alcohol to lightly swab the skin. Table II was used to score the reactions.



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TABLE II : Evaluation of Dermal Reactions

<u>Skin Reactions</u>	<u>Score</u>
<u>Erythema & Eschar Formation</u>	
No Erythema	0
Very Slight Erythema (barely perceptible)	1
Well-Defined Erythema	2
Moderate to Severe Erythema	3
Severe Erythema (beet red) to Slight Eschar Formation (injuries in depth)	4
<u>Edema Formation</u>	
No Edema	0
Very Slight Edema (barely perceptible)	1
Slight Edema (edges of area well defined)	2
Moderate Edema (raised approximately 1 millimeter)	3
Severe Edema (raised more than 1 mm and extracting beyond the area of exposure)	4

The animals were observed once each day for abnormal clinical signs.

CALCULATION AND RESULTS: None of the animals on study showed abnormal clinical signs during the 72 hour test period.

The average erythema and edema scores for the five test article extract and vehicle control injection sites were calculated for each observation period for each rabbit. The average erythema and edema scores were totaled separately for test and control. Each total score was divided by 60 (2 animals x 3 observation periods x 2 scoring categories x 5 injection sites) to determine the overall mean score for each test extract versus each corresponding control. The results are presented in Data Tables A and B for each test group.

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Cordis Corporation

Project # 3253

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DATA TABLE A: Intracutaneous Irritation Scores for the 0.9% Normal Saline Test Group

Extract 0.9% NaCl, Test Article Extract							
Animal #	717			Animal #	1349		
Obser	Erythema	Edema		Obser	Erythema	Edema	
24 hr	0	0		24 hr	0	0	
48 hr	0	0		48 hr	0	0	
72 hr	0	0		72 hr	0	0	
Animal Total =			0	Animal Total =			0
			Grand Total =				0
			Test article average score =				0

Extract 0.9% NaCl, Control Vehicle							
Animal #	717			Animal #	1349		
Obser	Erythema	Edema		Obser	Erythema	Edema	
24 hr	0	0		24 hr	0	0	
48 hr	0	0		48 hr	0	0	
72 hr	0	0		72 hr	0	0	
Animal Total =			0	Animal Total =			0
			Grand Total =				0
			Vehicle control average score =				0

Test article extract average score - vehicle average score =							0
--	--	--	--	--	--	--	---

DATA TABLE B: Intracutaneous Irritation Scores for the Cottonseed Oil Test Group

Extract Cottonseed Oil, Test article Extract							
Animal #	717			Animal #	1349		
Obser	Erythema	Edema		Obser	Erythema	Edema	
24 hr	0	0		24 hr	0	0	
48 hr	0	0		48 hr	0	0	
72 hr	0	0		72 hr	0	0	
Animal Total =			0	Animal Total =			0
			Grand Total =				0
			Test article average score =				0
Extract Cottonseed Oil, Control Vehicle							
Animal #	717			Animal #	1349		
Obser	Erythema	Edema		Obser	Erythema	Edema	
24 hr	0	0		24 hr	0	0	
48 hr	0	0		48 hr	0	0	
72 hr	0	0		72 hr	0	0	
Animal Total =			0	Animal Total =			0
			Grand Total =				0
			Vehicle control average score =				0
Test article extract average score - vehicle average score =							0

No erythema or edema was observed for the test article extracts or the vehicle control injection sites.

Questions? Contact FDA/CDRH/OCE/DID at CDRH.FOISTATUS.GOV@fda.hhs.gov or 301-796-8188

6101 Blue Circle Drive • Minneapolis, MN 55343-9108 • (800) 582-0077 • (612) 931-0077 • Fax: (612) 939-4215

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Cordis Corporation

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INTERPRETATION OF RESULTS: According to USP 23, the requirements of the test are met if the difference between the test article and the control mean score is 1.0 or less. No apparent irritation was observed in association with the 0.9% Normal Saline and Cottonseed Oil test article extract. Under the test conditions of this protocol, the difference in test article scores and corresponding vehicle control scores was less than 1.0.

ANALYSIS AND CONCLUSION: The test was considered valid as the vehicle control injection sites were within acceptable parameters. The difference in the mean test and control scores of the 0.9% Normal Saline and Cottonseed Oil extract dermal observations was less than 1.0, indicating that the requirements of the USP Intracutaneous test have been met by the test article. Under the test conditions of this protocol, Cordis Corporation, Tacker with PEEK balloon (File # 1572), Lot # NB # 92000 (10-11) would be considered a non-irritant.

DEVIATIONS: There was one deviation to the approved protocol; it is stated that the animals will be identified by ear tag and two rabbits were identified by ear mark. Ear marking with a permanent marker is allowable per our identification SOP and all animals are identified by a cage card which indicates what test the animal is currently assigned. This deviation did not affect the results or conclusions of this study.

STATISTICAL METHODS: None.

TECHNICAL REFERENCES: USP 23 (1995), pp. 1701-1702.

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COPY

USP SYSTEMIC INJECTION TEST RESULTS

REPORT TITLE

Acute Systemic Toxicity Testing of Cordis Corporation, Tacker SDS with PEEK
Balloon Using the USP Systemic Injection Test

PRODUCT IDENTITY

Cordis Corporation
Tacker SDS with PEEK Balloon, Lot # File 1572

PROJECT NUMBER

3167

PROTOCOL NUMBER

ST100

PREPARED BY

David L. Hodge
Director of In-Life Studies

STUDY COMPLETION ON

January 17, 1997

PERFORMING LABORATORY

ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

SPONSOR

Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014

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LABORATORIES, INC.

Cordis Corporation

Project # 3167

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GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with current FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies.

The studies not performed by or under the direction of ViroMed Laboratories, Inc. are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the test article.

Submitter: _____

Date: _____

Sponsor: _____

Date: _____

Study Director: David L. HodgeDate: 1-17-97

David L. Hodge

QUALITY ASSURANCE UNIT SUMMARY

STUDY: USP Systemic Injection Test Method.

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to management and the Study Director.

Phase Inspected:

Project Set-up Date: 1/6/97

Final Report Date: 1/17/97

Study Director Review Date: 1/17/97

Management Review Date: 1/17/97

Documentation of the above Quality Assurance audits have been reviewed.

Quality Assurance Director: Joyce A. NelsonDate: 17 Jan. 1997

Joyce A. Nelson

Study Director: David L. HodgeDate: 1-17-97

David L. Hodge

Professional Personnel Involved:

Bonita L. Baskin, Ph.D.

Laboratory Director

David L. Hodge, BA

Director of In-Life Studies

Terry Haugen, BS

Laboratory Aide I

Siamak Amiralaie, BA

Research Assistant I

Thomas Cunningham, BA

Research Assistant I

Erika Reubin, BA

Research Assistant I

Ryan Bonham

Research Assistant I

PROJECT NUMBER: 3167**REPORT DATE:** 1/17/97

SPONSOR: Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014

DATA RETENTION: A certified copy of the original final report and all raw data pertinent to this study will be stored at ViroMed Laboratories, Inc., 6101 Blue Circle Drive, Minneapolis, MN 55343. The test substance used in this assay was expended in testing.

SAMPLE IDENTIFICATION: Cordis Corporation, Tacker SDS with PEEK
Balloon, Lot # File 1572

CHARACTERIZATION: The identity, strength, stability, purity, and chemical composition are the responsibility of the Sponsor.

PURPOSE: The purpose of this test was to screen test article extracts for potential toxic effects as a result of a single-dose systemic injection in mice using the standard USP Systemic Injection Test Procedure.

TEST FACILITY: ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

DATE SAMPLE RECEIVED: 12/20/96
STUDY INITIATION DATE: 12/20/96
EXPERIMENTAL START DATE: 12/23/96
STUDY COMPLETION DATE: 1/17/97
IACUC APPROVAL DATE: 4/18/96

METHOD: This study was conducted in accordance with the USP Systemic Injection Test Procedure, United States Pharmacopeia 23 (1995), Pages 1701-1702.

EXPERIMENTAL SUMMARY: For the safety evaluation of the test article, mice were injected systemically with extracts of the test article in standard USP solutions (Normal Saline, Cotton Seed Oil). The animals were observed for signs of toxicity immediately after injection and at 4, 24, 48 and 72 hours post-injection. The requirements of the test are met if none of the animals treated with the test article extract have a significantly greater adverse reaction than the animals treated with the vehicle control. None of the test article extract treated animals were observed with adverse clinical signs at any of the observation periods and none of the animals lost in excess of 2g of body weight over the course of the study. Therefore the requirements of the USP Systemic Injection Test have been met by the Cordis Corporation, Tacker SDS with PEEK Balloon, Lot # File 1572.



TEST MATERIAL AND PREPARATION

STERILIZATION: The test article was submitted in sterile form by the Sponsor.

TEST SAMPLE PREPARATION: A representative sample of the test article was cut into pieces, placed into test tubes and prepared at a ratio of 60 cm² to 20 ml of extraction vehicle.

EXTRACT VEHICLE	VEHICLE AMOUNT	TEST ARTICLE AMOUNT
0.9% Normal Saline (NaCl)	15.78 ml	47.35 cm ²
Cottonseed Oil (CSO)	15.78 ml	47.35 cm ²

Vehicle Materials:

0.9% USP Normal Saline, lot # C327718 supplied by Baxter
USP Cottonseed Oil, lot # A007854101 supplied by Acros

EXTRACTION: The sample preparation and control solution (vehicle without test article) were extracted for 24 hours at 70° C.

The extracts were cooled, shaken well and decanted into sterile, dry glass vessels. The extracts were used within 24 hours of preparation.

TEST SYSTEM

SPECIES/STRAIN/SEX: Albino Swiss Mouse (*Mus musculus*), naive, Female.

SOURCE: Charles River Portage, Portage, Michigan

ANIMAL NUMBER AND WEIGHTS:

0.9% Normal Saline Test Group:	Mouse # 41 - 45
0.9% Normal Saline Control Group:	Mouse # 11 - 15
Cottonseed Oil Test Group:	Mouse # 51 - 55
Cottonseed Oil Control Group:	Mouse # 31 - 35

All animals weights were within the 17 - 23 g USP weight limit at the start of the study.

ANIMAL IDENTIFICATION: Individually identified by ear punch.

HUSBANDRY

RECEIPT: The animals were received on 12/30/96 and examined for signs of disease and injury prior to entry into the research area.



HOUSING: Animals were housed in groups of 5 in polycarbonate microisolator cages with contact bedding.

ENVIRONMENT: Animal rooms were maintained at $68 \pm 3^{\circ}$ F and 30 - 70% relative humidity with a 12 hour light/dark cycle. Room air exchange was maintained between 12 and 15 air changes per hour. The laboratory and animal rooms were maintained as limited-access facilities.

DIET: Animals were supplied with certified Teklad Rodent Diet. No known contaminants present in the feed are expected to interfere with the test results.

WATER: Potable water.

TERMINATION: Animals were euthanized by CO₂ asphyxiation.

COMPLIANCE: The care, housing and handling of the animals was in compliance with AAALAC, "Guide for the Care and Use of Laboratory Animals", DHHS Pub. No. (NIH) 85-23, Revised 1985; (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals", and USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1,2 and 3, Animal Welfare, Final Rule 1989.

SELECTION OF ANIMALS: Animals were randomly placed in cages upon receipt, and were placed on study as available at the time of study initiation. Any animals considered unsuitable due to poor health or outlying body weight were excluded from the study.

TEST ARTICLE ADMINISTRATION: Groups of 5 animals were injected with either the test article extract or the corresponding control article extract as indicated in the table below:

Extract or Control	Route	Dose/Kg	Injection Rate
Normal Saline	Intravenous	50 ml	0.1 ml/sec
CSO	Intraperitoneal	50 ml	-

OBSERVATIONS

BODY WEIGHTS: Body weight recordings, to the nearest 0.1g, were made on the day of dosing and at 72 hours post-injection.

CLINICAL SIGNS: Observations for mortality and signs of pharmacological and/or toxicological effects were made immediately post-injection and at 4, 24, 48 and 72 hours post-injection.

INTERPRETATION OF RESULTS: According to USP 23, the test is considered negative if none of the animals injected with the test article show a significantly greater biological reaction than the animals treated with the control article. A significant biological reaction is interpreted as death in two or more mice or other toxic signs such as convulsions, prostration, or body weight loss greater than 2 grams in three or more mice.

If any animal treated with a test article shows only slight signs of biological reaction, and not more than one animal shows gross signs of biological reaction or dies, a repeat test should be conducted using groups of 10 mice. On the repeat test, all 10 animals must not show a significantly greater biological reaction than the animals treated with the control article.

RESULTS: None of the animals on study were observed with abnormal clinical signs indicative of toxicity during the 72 hour test period. All were alive at the end of the 72 hour test duration and body weight loss was less than 2 grams over the course of the study. See Data Table A.

DATA TABLE A: Mortality, Clinical Signs and Weight Loss Incidence.

Extract	Fatalities		Toxicity Clinical Signs		Animals with > 2 g Body Weight Loss	
	Test	Control	Test	Control	Test	Control
NaCl	0/5	0/5	0/5	0/5	0/5	0/5
CSO	0/5	0/5	0/5	0/5	0/5	0/5

ANALYSIS AND CONCLUSION: The vehicle control treated animals had no signs of toxicity at any of the observation periods and no animals lost weight in excess of 2 g, indicating a valid test. None of the test article extract treated animals were observed with clinical signs consistent with toxicity at any of the observation periods. Body weight changes were within acceptable parameters over the course of the study these findings indicate that the requirements of the USP Systemic Injection test have been met by Cordis Corporation, Tacker SDS with PEEK Balloon, Lot # File 1572.

DEVIATIONS: None.

STATISTICAL METHODS: None.

TECHNICAL REFERENCES: USP 23 (1995), pp. 1701-1702.

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VITOMED

LABORATORIES, INC.

IN VITRO CYTOTOXICITY TEST

MEM Elution Method Using L-929 Mouse
Fibroblast Cells

COPY

PRODUCT IDENTITY

Tacker SDS w/PEEK balloon File # 1572, Lot SR# 12-96-230

PROJECT NUMBER

3242

PROTOCOL NUMBER

MEM929

PREPARED BY

Isis Koenders
Study Director

FINAL REPORT

January 22, 1997

PERFORMING LABORATORY

ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

RECEIVED
FEB 04 1997

SPONSOR

Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014

IN VITRO CYTOTOXICITY
MEM ELUTION ASSAY**OBJECTIVE**

The objective of this study is to evaluate the ability of the test article extract to elicit a cytotoxic response in cultured mouse fibroblast cells. This assay employs L-929 cells.

TEST ARTICLE CHARACTERIZATION

The identity, strength, stability, purity, and chemical composition are the responsibility of the Sponsor.

DATA RETENTION

A certified copy of this report, and raw data pertinent to this study will be stored at ViroMed Laboratories, Inc., 6101 Blue Circle Drive, Minneapolis, MN 55343. The test substance used in this assay was expended in testing.

SPONSOR: Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014

TEST FACILITY: ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

EXTRACTION MEDIA

Eagles minimal essential medium (E-MEM) supplemented with 5% (v/v) fetal bovine serum (FBS) heat-inactivated at 56°C for 30 minutes, 10mM Hepes, 10 µg/ml gentamicin, 100 units/ml penicillin, 10µg/ml vancomycin, and 2.5 µg/ml fungizone was used as the test article extraction media.

POSITIVE CONTROL: 100 µM CdCl₂, Lot VML 091296

INTERMEDIATE CONTROL: Black Rubber Stopper, Lot VML 120393

NEGATIVE CONTROL: Dram Vial Caps, Lot VML 111096

CELL CONTROL: 5% MEM, Lot 620113-6

TEST CELL CULTURE

L-929 mouse fibroblast cells, Lot# I0110 were originally acquired from the American Type Culture Collection, Strain # CCL1. Cultures were grown and used as monolayers in disposable tissue culture labware at 37 ± 1°C in a humidified atmosphere of 6 ± 1% CO₂.

DATE SAMPLE RECEIVED: 1/10/97

INITIATION DATE: 1/10/97

TEST START DATE: 1/13/97

COMPLETION DATE: 1/22/97



TEST ARTICLE DESCRIPTION: Tacker SDS w/PEEK balloon File # 1572,
Lot SR# 12-96-230

MEDIA EXTRACTION

A ratio equivalent to 60 cm² test article and 20 ml extraction vehicle was used.

EXPERIMENTAL DESIGN

A monolayer of L-929 mouse fibroblast cells was grown to confluency and exposed to an extract of the test article. The extract was prepared by placing the test article in E-MEM and fetal bovine serum (5%) and extracting at 37 ± 1°C and 6 ± 1% CO₂ for 24 hours. A positive, an intermediate and a negative control were run in parallel with the test article. Test article and control extracts were plated in duplicate immediately following extraction. All cultures were incubated for 48 hours at 37 ± 1°C in a humidified atmosphere of 6 ± 1% CO₂ in air. At the end of each 24 hour incubation period, cultures were evaluated for cytotoxic effects by microscopic examination.

RESULTS

TABLE 1

Test Article	Sample Extract		Cytotoxicity Score	
	Size	(ml)	24 Hours	48 Hours
Tacker SDS w/PEEK balloon File # 1572, Lot SR# 12-96-230	42 cm ²	14	0/0	0/0
Positive Control	100µM CdCl ₂	1	4/4	4/4
Intermediate Control	4g	20	2/2	2/2
Negative Control	4g	20	0/0	0/0
Cell Control	-	1	0/0	0/0

TABLE 2 - TEST SCORING

Grade	Reactivity	Conditions of all Cultures
0	None	Discrete intracytoplasmic granules; no cell lysis.
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present.
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; extensive cell lysis and empty areas between cells.
3	Moderate	Not more than 70% of the cell layers contain rounded cells and/or are lysed.
4	Severe	Nearly complete destruction of the cell layers.

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Cordis Corporation
Project No. 3242
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STATISTICAL METHODS: None used.

ANALYSIS AND CONCLUSION

The controls responded appropriately under the conditions of this test. The test sample, Tacker SDS w/PEEK balloon File # 1572, Lot SR# 12-96-230 was scored "0" at 24 and 48 hours and is considered **NON-TOXIC** under the conditions of this test.

REFERENCES

American Society for Testing and Materials, "Standard Practice for Extraction of Medical Plastics" in Annual Book of ASTM Standards (1992), Vol. 13.01, Medical Devices, p. 172.

Eagle, H. (1959) Science 130:432.

U.S. Pharmacopeia - XXIII (1995).

Wilsnack, R.E., Meyer, F.J., and Smith, J.G. (1973) Biomater., Med. Dev., Art. Org. 1:543.

The use of the ViroMed name, logo or any other representation of ViroMed, other than distribution of this report in its entirety, without the written approval of ViroMed is prohibited. In addition, ViroMed may not be referred to in any form of promotional materials, press releases, advertising or similar materials (whether by print, broadcast, communication or electronic means) without the express written permission of ViroMed Laboratories, Inc.

GUINEA PIG DELAYED CONTACT SENSITIZATION TEST
(Maximization Method for Biomaterial Extracts)

REPORT TITLE

Sensitization Testing of Cordis Corporation,
Tacker SDS with PEEK Balloon Extracted in Cottonseed Oil
Using the Guinea Pig Maximization Test
(Magnusson-Kligman)

PRODUCT IDENTITY

Cordis Corporation
Tacker SDS with PEEK Balloon
Lot # File 1572

PROJECT NUMBER

3166

PROTOCOL NUMBER

GM100

PREPARED BY

David L. Hodge
Director of In-Life Studies

STUDY COMPLETION ON

February 10, 1997

PERFORMING LABORATORY

ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

SPONSOR

Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014



Cordis Corporation
Project # 3166
Page 2 of 12

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with current FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies.

The studies not performed by or under the direction of ViroMed Laboratories, Inc. are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the test article.

Submitter: _____ Date: 2/10/97
Sponsor: [Signature] Date: 2/10/97
Study Director: David L. Hodge Date: 2-10-97

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Guinea Pig Maximization Test (Magnusson - Kligman)

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to management and the Study Director.

Phase Inspected:

Project Set-up	Date: 12/31/96
Final Report	Date: 2/10/97
Study Director Review	Date: 2/10/97
Management Review	Date: 2/10/97

Documentation of the above Quality Assurance audits have been reviewed.

Quality Assurance Director: [Signature]

Joyce A. Nelson

Date: 10 Feb, 1997

Study Director: [Signature]

David L. Hodge

Date: 2-10-97

Professional Personnel Involved:

Bonita L. Baskin, Ph.D.	Laboratory Director
David L. Hodge, BA	Director of In-Life Studies
Terry Haugen, BS	Laboratory Aide I
Siamak Amirali, BA	Research Assistant I
Thomas Cunningham, BA	Research Assistant I
Erika Reubin, BA	Research Assistant I
Ryan Bonham	Research Assistant I



Cordis Corporation

Project # 3166

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PROJECT NUMBER: 3166

REPORT DATE: 2/10/97

SPONSOR: Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014

DATA RETENTION: A certified copy of the original final report and all raw data pertinent to this study will be stored at ViroMed Laboratories, Inc., 6101 Blue Circle Drive, Minneapolis, MN 55343. The test substance used in this assay was expended in testing.

SAMPLE IDENTIFICATION: Cordis Corporation, Tacker SDS with PEEK Balloon, Lot # File 1572

CHARACTERIZATION: The identity, strength, stability, purity, and chemical composition are the responsibility of the Sponsor.

PURPOSE: This test was designed to evaluate the allergenic potential or sensitizing capacity of a test article. This test was used as a procedure for the screening of contact allergens in guinea pigs and extrapolating the results to humans, but does not establish the actual risk of sensitization in humans.

TEST FACILITY: ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

DATE SAMPLE RECEIVED: 12/20/96
STUDY INITIATION DATE: 12/20/96
EXPERIMENTAL START DATE: 12/23/96
STUDY COMPLETION DATE: 2/10/97
IACUC APPROVAL DATE: 5/28/96

METHOD: This study was based upon the procedures described in Dermatoxicology, F.N. Marzulli and H.I. Maibach, editors, 4th edition, 1991, pages 374-378, Hemisphere Publishing Corporation, New York. Magnusson, B. and Kligman, A.M. 1969, The identification of contact allergens by animal assay, The guinea pig maximization test. J. Invest. Dermatol. 52:268-276.; Magnusson, B. and Kligman, A.M. 1970. Allergic contact dermatitis in the guinea pig, Identification of contact allergens. Springfield, Ill.: Thomas (Publ.), Extraction procedures were based upon the standards set by USP 23 (1995), pages 1701-2.



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Project # 3166
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EXPERIMENTAL METHODS SUMMARY: In selecting a new material for human contact in medical applications, it is important to ensure that the material will not stimulate the immune system to produce an allergic response. The response generally is due to leachable substances in the test material. Therefore, USP material extracts are used for animal administrations. The extraction is performed according to the standard USP 23 Extraction Method in the sponsor designated vehicle. The use of Freund's complete adjuvant and sodium lauryl sulfate enhance the potential of weak sensitizing agents.

Thirteen female Duncan-Hartley strain guinea pigs (10 test animals, 3 primary irritant control animals) between 300 and 500 g were assigned to the study. Two guinea pigs received with the same shipment as the test animals were assigned separately as positive control animals. A 3 by 5 centimeter area over the intrascapular region is clipped free of fur on the test animals and positive control animals. The primary irritant control animals did not receive induction treatment and were only used in the challenge phase of the test. Three pairs of intradermal injections were administered within the clipped area according to the following:

Test Animal Group:

- (1) 0.1 ± 0.01 ml FCA (Difco) 1:1 with Normal Saline
- (2) 0.1 ± 0.01 ml test article extract
- (3) 0.1 ± 0.01 ml test article extract 1:1 with FCA

Positive Control Group:

- (1) 0.1 ± 0.01 ml FCA 1:1 with Normal Saline
- (2) 0.1 ± 0.01 ml 0.1% Dinitrochlorobenzene (DNCB) in 95% Ethanol (EtOH)
- (3) 0.1 ± 0.01 ml 0.1% DNCB in 95% EtOH 1:1 with FCA

On day 7, the area of the injection sites was treated with 0.5 g of 10% (w/w) sodium lauryl sulfate (SLS) prepared by mixing solid SLS with petrolatum. The SLS was removed 24 hours after application. After removal of the SLS, a topical application of 0.3 ml of fresh test article extract (test animals) or 0.1% DNCB solution in 95% ethanol positive control (positive control animals) was applied topically over the injection sites in a patch which was occlusively wrapped. The patch was removed after 48 hours. Twelve to fourteen days after topical induction, the fur was removed from both flanks of all animals with an electric clipper. The test animals and the primary irritant control animals were challenged on the right flank with a Hill Top Chamber saturated with fresh test article extract and on the left flank with vehicle control in the same manner. The positive control animals were challenged in the same manner using 0.05% DNCB solution in acetone in place of the test article extract. The challenge sites were observed for signs of erythema and edema at 24, 48, and 72 hours after challenge patch removal. The response was scored according to a standard scale.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS.GOV@fda.hhs.gov or 301-796-8188

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Project # 3166
Page 5 of 12

The responses were compared within the test group and between the test group and control conditions. Control conditions include:

- 1) The vehicle control reaction on the left flank of the test animals.
- 2) The test article extract, and vehicle control reaction on the primary irritant control animals.
- 3) The positive control animals' reaction to 0.05% DNCB.

In the final analysis of data, consideration was given to the overall pattern, intensity, duration, and character of reactions of the test article extract as compared with the control conditions. An effect interpreted as "irritation" is generally observed at day 1 after patch removal, but diminishes thereafter, and is also concurrently present as a transient response in the control animals. Closed patches typically show maximal sensitization response 2 to 3 days after patch removal in test conditions, but not in the control conditions. Only when the response to the challenge with the test article extract clearly outweighs the reaction from those of any control condition is the material judged to have allergic potential.

The test results were interpreted based upon the percentage of animals exhibiting a sensitization response.

TEST MATERIAL AND PREPARATION

STERILIZATION: The test article was received in sterile form from the Sponsor.

TEST SAMPLE PREPARATION: A representative sample of the test article was cut into pieces, placed into test tubes and prepared at a ratio of 60 cm² to 20 ml of extraction vehicle.

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EXTRACTION: Fresh extract of the test article was made for each of the two induction steps and the challenge step at a ratio equivalent to 60 cm² of test article to 20 ml of extraction vehicle and conditions listed below :

Intradermal Injection Induction:

Extract Vehicle: Cotton Seed Oil (CSO), Lot # A007854101 (Acros)

Surface area of Test Article	ml of Vehicle	Vehicle	Time	Temp (°C)
47.35 cm ²	15.80	CSO	24 hrs	70 ± 2

Patch Induction:

Extract Vehicle: Cotton Seed Oil (CSO), Lot # A007854101 (Acros)

Surface area of Test Article	ml of Vehicle	Vehicle	Time	Temp (°C)
47.35 cm ²	15.80	CSO	24 hr	70 ± 2

Challenge:

Extract Vehicle: Cotton Seed Oil (CSO), Lot # A007854101 (Acros)

Surface area of Test Article	ml of Vehicle	Vehicle	Time	Temp (°C)
47.35 cm ²	15.78	CSO	24 hrs	70 ± 2

The extracts were cooled, shaken well and decanted into sterile, dry glass vessels. The extract was used within 24 hours of preparation.

PREPARATION OF FREUND'S COMPLETE ADJUVANT (FCA) MIXTURES FOR INJECTION: The FCA mixtures were prepared under aseptic conditions and ratios according to the following :

Test Article

Injection #	FCA (ml)	Test Article Extract (ml)	Extract Vehicle (ml)
1)	1.2	0.0	1.2
2)	0.0	2.0	0.0
3)	1.2	1.2	0.0

0.1% DNCB/Ethanol Positive Control

Injection #	FCA (ml)	0.1% DNCB (ml)	Extract Vehicle (ml)
1)	0.3	0.0	0.3
2)	0.0	0.4	0.0
3)	0.3	0.3	0.0

Materials:

FCA	Lot # 80678LA, Difco
DNCB (0.1%)	Lot # 06929JZ, Aldrich
Ethanol (95%)	Lot # 952753, Fisher

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOI@STATUS.GOV or 301-796-8188

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Cordis Corporation
Project # 3166
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TEST SYSTEM

SPECIES/STRAIN AND SEX: Duncan-Hartley Strain, Albino Guinea Pig.
(Specific Pathogen Free), Female

SOURCE: Charles River Kingston, Kingston, New York

ANIMAL BODY WEIGHT RANGE: All animals weighed between 300 and 500g at the initiation of the study and were within the required range for the test.

ANIMAL IDENTIFICATION: Individually numbered ear tags.

HUSBANDRY

RECEIPT: Animals were received on 12/23/96. Each animal was examined for signs of disease and injury prior to entry into the research area.

HOUSING: Animals were housed in suspended stainless steel cages with separation of the test, positive control and irritant control animals. Housing density was in compliance with AAALAC standards.

ENVIRONMENT: Animal rooms were maintained at $68 \pm 3^{\circ}$ F and 30 - 70% relative humidity with a 12 hour light/dark cycle. Rooms were maintained at 12-14 air changes per hour. The laboratory and animal rooms were maintained as limited - access facilities.

DIET: Animals were supplied with certified Purina Guinea Pig Chow. No known contaminants present in the feed are expected to interfere with the test results.

WATER: Potable Water.

TERMINATION: Animals were euthanized by CO₂ asphyxiation.

COMPLIANCE: The care, housing and handling of the animals was in compliance with AAALAC, "Guide for the Care and Use of Laboratory Animals", DHHS Pub. No. (NIH) 85-23, Revised 1985; (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals", and USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1,2 and 3, Animal Welfare, Final Rule 1989.



Cordis Corporation
Project # 3166
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SELECTION OF ANIMALS: Animals were randomly placed in cages upon receipt, and were placed on study as available at the time of study initiation. Any animals considered unsuitable due to poor health or outlying body weight were excluded from the study.

ANIMAL PREPARATION: The application sites were prepared by clipping the skin of the test site free of fur with a #40 blade. On day 0 and on day 7, an approximately 5 x 7 cm area over the shoulder region was prepared. The day before challenge, an approximately 4 x 4 cm area of the flanks was prepared by removing the fur with an electric clipper.

TEST ARTICLE ADMINISTRATION

Induction/Intradermal Application (Day 0): Three pairs of intradermal injections were made with one row of three injections on each side of the dorsal mid-line. The injection sites (6) were just within the boundaries of a 2 x 4 cm patch. Injections with FCA are made slightly deeper to minimize tissue reactions. The injection doses were made according to the following:

Experimental Group:

- (1) 0.1 ± 0.01 ml FCA (Difco) 1:1 with Normal Saline
- (2) 0.1 ± 0.01 ml test article
- (3) 0.1 ± 0.01 ml test article 1:1 with FCA

Positive Control Group:

- (1) 0.1 ± 0.01 ml FCA 1:1 95% EtOH
- (2) 0.1 ± 0.01 ml 0.1% DNCB in 95% EtOH
- (3) 0.1 ± 0.01 ml 0.1% DNCB in 95% EtOH 1:1 with FCA

Topical Application (Day 7): On day 7 the area of the injection sites was clipped free of fur again and treated with 0.5 to 1 g of 10% (w/w) sodium lauryl sulfate (SLS) prepared by mixing solid SLS with petrolatum. The day following the SLS treatment, the remaining SLS residue was gently wiped from the area with gauze.

Experimental Group: The test article extract was applied to a 2 x 4 cm piece of filter paper (Whatman 3) to saturation (0.3 ml) and applied after SLS removal. The patch was secured to the site with non-permeable tape and the trunk wrapped with Vetwrap™ and Transpore™ tape. This preparation was left in place for 48 hours. Freshly prepared extract was used for this administration.

Positive Control Group: The animals were exposed to 0.1% DNCB solution in 95% ethanol in the same manner as the experimental group.

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Project # 3166

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Challenge Application: On day 13, after the topical induction phase, the challenge procedure was initiated on the 10 experimental animals and the 3 irritant control animals. A nonwoven cotton disk, backed by a flexible chamber (Hill Top Chamber) and a semi occlusive tape bandage, was saturated with 0.3 ml of freshly prepared test article extract and applied to the fur clipped right flank of each animal. In addition, the vehicle control was patched in the same manner to the left flank of each animal. The positive control animals were exposed in the same manner using 0.05% DNCB solution in acetone in place of the test article extract. The trunk of each animal was wrapped for 24 hours with Vetwrap™ and Transpore™ tape. The following day after challenge exposure, the patches were removed and the area cleaned gently with gauze.

OBSERVATIONS AND SCORING: Daily challenge observation scores were recorded 1, 2 and 3 days after patch removal in accordance with the following scheme:

- 0 = No reaction
- 0.5 = Background response (very faint erythema usually not confluent or pinpoint erythema not considered significant.)
- 1 = Scattered reaction
- 2 = Moderate and diffuse or confluent reaction
- 3 = Intense reddening and swelling

Note: Erythema is defined as redness and edema is defined as a swelling at the challenge site.

Daily animal health observations were recorded throughout the study period.

INTERPRETATION OF RESULTS: The responses were compared within the test group and between the test group and control group conditions. Control group conditions include:

- 1) The vehicle control solution reaction on the left flank of the test animal.
- 2) The test extract, and control solution reaction on the primary irritant control animals.
- 3) The positive control animals' reaction to 0.05% DNCB.

In the final analysis of data, consideration was given to the overall pattern, intensity, duration, and character of reactions of the test article as compared with the control conditions. An effect interpreted as "irritation" is generally observed at 1 day after patch removal, but diminishes thereafter, and is also concurrently present as a transient response in the control animals. Closed patches typically show maximal sensitization response 2 to 3 days after patch removal in test conditions, but not in the control conditions. Only when the challenge response to the test article extract clearly outweighs the reaction from those of any control condition will the material be judged to be sensitizing.



Cordis Corporation
Project # 3166
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Background or artifactual reactions from fur clipping or patch edge or nonspecific FCA effects were not considered as evidence of sensitization. The treatment with FCA and occlusive dressings may lower the threshold level for skin irritation.

Using the scoring system of Kligman (TABLE 1), the allergic potential of a test article was classified as follows:

TABLE 1

<u>Sensitization Rate (%)</u>	<u>Grade</u>	<u>Class</u>
0-10 %	I	Weak
11-30 %	II	Mild
31-60 %	III	Moderate
61-80 %	IV	Strong
81-100 %	V	Extreme

The test results are interpreted based upon the percentage of animals exhibiting sensitization. In the Magnusson-Kligman model, weak sensitizers are not regarded as significant.

RESULTS: None of the animals in the study showed abnormal clinical signs during the test period. The individual daily challenge observations are listed in Table A for the Positive Control Group, Table B for the Irritant Control Group and Table C for the Test Article Extract Group.

Table A: Positive Control Group Daily Challenge Observations

Animal #	Experimental Group	24 hour Control	24 hour DNCB	48 hour Control	48 hour DNCB	72 hour Control	72 hour DNCB	Results
263	Positive Control	0	2	0	2	0	2	Positive
264	Positive Control	0	1	0	1	0	1	Positive

The positive control animals exhibited an extreme sensitization response to the positive control article (0.05% DNCB in Acetone).



Cordis Corporation
Project # 3166
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Table B: Irritant Control Daily Challenge Observations

Animal #	Experimental Group	24 hour Control	24 hour Test Article	48 hour Control	48 hour Test Article	72 hour Control	72 hour Test Article	Results
276	Irritant Control	0	0	0	0	0	0	Negative
277	Irritant Control	0	0	0	0	0	0	Negative
278	Irritant Control	0	0	0	0	0	0	Negative

The test article extract did not have a primary irritation response in the irritant control animals.

Table C: Test Article Extract Daily Challenge Observations

Animal #	Experimental Group	24 hour Control	24 hour Test Article	48 hour Control	48 hour Test Article	72 hour Control	72 hour Test Article	Results
266	Test	0	0	0	0	0	0	Negative
267	Test	0	0	0	0	0	0	Negative
268	Test	0	0	0	0	0	0	Negative
269	Test	0	0	0	0	0	0	Negative
270	Test	0	0	0	0	0	0	Negative
271	Test	0	0	0	0	0	0	Negative
272	Test	0	0	0	0	0	0	Negative
273	Test	0	0	0	0	0	0	Negative
274	Test	0	0	0	0	0	0	Negative
275	Test	0	0	0	0	0	0	Negative

None of the test animals challenged with the test article extract were observed with a challenge response greater than 0. A negative sensitization response was interpreted for all test animals.

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Cordis Corporation

Project # 3166

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ANALYSIS AND CONCLUSION: The three test control conditions, as listed below, were met for this test, thus indicating a valid test.

<u>Control Condition</u>	<u>Results</u>
1) Vehicle Control	The extraction vehicle did not have a primary irritation response nor did it act as a sensitizer alone.
2) Test Article Extract Primary Irritation	The test article extract was not a primary irritant.
3) Positive Control (Animals from the same shipment)	DNCB was classified as a Grade V extreme sensitizer.

The classification of materials for sensitization according to the Magnusson-Kligman Model is based on the percent incidence of positive test animals in the test group. The cotton seed oil extract of the test material had a 0% sensitization response under valid test conditions. Cordis Corporation, Tacker SDS with PEEK Balloon, Lot # File 1572 is therefore classified as a Grade I, weak sensitizer. In the Magnusson-Kligman model, weak sensitizers are not regarded as significant.

DEVIATIONS: None.

STATISTICAL METHODS: None.

TECHNICAL REFERENCES:

Marzulli FN, Maibach HI, editors: Dermatoxicology, 4th edition, pp 374-378.
Hemisphere Publishing Corporation, New York, 1991

Magnusson B, Kligman AM: The identification of contact allergens by animal assay, The guinea pig maximization test. J. Invest. Dermatol 52:268-276., 1969.

Magnusson B, Kligman AM: Allergic contact dermatitis in the guinea pig, Identification of contact allergens. Thomas (Publ.), Springfield, Ill. 1970.

Extraction procedures were based upon the standards set by USP 23 (1995), pages 1701-2.

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USP RABBIT PYROGEN TEST RESULTS**REPORT TITLE**

Pyrogen Testing of Cordis Corporation, Tacker SDS w/PEEK balloon File # 1572
Using the USP Rabbit Pyrogen Test

PRODUCT IDENTITY

Cordis Corporation
Tacker SDS w/PEEK balloon File # 1572
Lot # SR# 12-96-230

PROJECT NUMBER

3240

PROTOCOL NUMBER

RP100

PREPARED BY

David L. Hodge
Director of In-Life Studies

STUDY COMPLETION ON

February 6, 1997

PERFORMING LABORATORY

ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

SPONSOR

Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014

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Cordis Corporation

Project # 3240

Page 2 of 7

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with current FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies.

The studies not performed by, or under the direction of ViroMed Laboratories, Inc., are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the test article.

Submitter: _____

Date: _____

Sponsor: _____

Date: _____

Study Director: David L. HodgeDate: 2-6-97

David L. Hodge

QUALITY ASSURANCE UNIT SUMMARY**STUDY: USP Rabbit Pyrogen Test Method.**

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance with standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to management and the Study Director.

Phase Inspected:

Project Set-up

Date: 1/22/97

Final Report

Date: 2/6/97

Study Director Review

Date: 2/6/97

Management Review

Date: 2/6/97

Documentation of the above Quality Assurance audits have been reviewed.

Quality Assurance Director: Joyce A. NelsonDate: 6 Feb, 1997

Joyce A. Nelson

Study Director: David L. HodgeDate: 2-6-97

David L. Hodge

Professional Personnel Involved:

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Research Assistant I

PROJECT NUMBER: 3240

REPORT DATE: 2/6/97

SPONSOR: Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014

DATA RETENTION: A certified copy of the original final report and all raw data pertinent to this study will be stored at ViroMed Laboratories, Inc., 6101 Blue Circle Drive, Minneapolis, MN 55343. The test substance used in this assay was expended in testing.

SAMPLE IDENTIFICATION: Cordis Corporation, Tacker SDS w/PEEK balloon
File # 1572, Lot #SR# 12-96-230

CHARACTERIZATION: The identity, strength, stability, purity, and chemical composition are the responsibility of the Sponsor.

PURPOSE: The purpose of this test was to determine if the test material (or saline washing of the test article) causes a febrile response when administered intravenously in rabbits using the standard USP Rabbit Pyrogen Test procedure. This test is used to evaluate test articles for the presence of materials mediated pyrogens.

TEST FACILITY: ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

DATE SAMPLE RECEIVED: 1/10/97
STUDY INITIATION DATE: 1/10/97
EXPERIMENTAL START DATE: 1/13/97
STUDY COMPLETION DATE: 2/6/97
IACUC APPROVAL DATE: 4/12/96

METHOD: This study was conducted in accordance with the USP Rabbit Pyrogen Test Procedure, United States Pharmacopeia 23 (1995), Page 1718.

EXPERIMENTAL SUMMARY: The test article or saline washings of the test article were injected into the marginal ear vein of each of three animals. Rectal temperatures were recorded for each animal prior to injection and at 30 minute intervals between 1 and 3 hours after injection. The test article is negative if none of the animals show an individual temperature increase of 0.5°C or more at any reading period above its respective baseline temperature. If any rabbit shows an individual temperature rise of 0.5° C or more, the test is continued using five other rabbits. If not more than three of the eight rabbits show individual rises in temperature of 0.5° C or more and if the sum of the eight individual maximum temperature rises does not exceed 3.3° C, the test article under examination meets the USP 23 requirements for the absence of pyrogens.

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TEST MATERIAL AND PREPARATION

STERILIZATION: The test article was submitted in sterile form by the Sponsor.

TEST SAMPLE PREPARATION: The 10 devices were soaked for 40 minutes in 400 ml of 0.9% saline for injection solution (40ml/device) at a temperature of $37 \pm 1^\circ \text{C}$, with swirling every 10 minutes. The eluent was used as the test solution for animal administration.

Vehicle Materials:

0.9% USP Normal Saline, lot # C327718 supplied by Baxter Exp. Date: 12/97,

TEST SYSTEM

SPECIES/STRAIN/SEX: Albino Rabbits, New Zealand White Strain, Specific Pathogen Free, Female.

SOURCE: Charles River Canada, St. Constant, Canada

ANIMAL NUMBER AND WEIGHTS:

Animal # 794, 3.0 Kg
Animal # 798, 2.9 Kg
Animal # 2526, 4.6 Kg

All animals weighed equal to or greater than the 1.50 kg minimum USP weight limit.

ANIMAL IDENTIFICATION: Individually numbered ear tags or ear marks with a permanent marker. Rabbits #794 and #798 had ear marks.

HUSBANDRY

RECEIPT: The animals were received on 10/30/96 and 1/15/97. Each animal was examined for signs of disease and injury prior to entry into the research area.

HOUSING: One rabbit per suspended stainless steel cage.

ENVIRONMENT: Animal rooms were maintained at $68 \pm 3^\circ \text{F}$ and 30 to 70% relative humidity with a 12 hour light/dark cycle. Room air exchange was maintained between 12 and 15 air changes per hour. The laboratory and animal rooms were maintained as limited-access facilities.

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DIET: Animals were supplied with certified commercial feed, Purina Rabbit Chow. No known contaminants present in the feed are expected to interfere with the test results.

WATER: Potable water.

COMPLIANCE: The care, housing and handling of the animals was in compliance with AAALAC, "Guide for the Care and Use of Laboratory Animals", DHHS Pub. No. (NIH) 85-23, Revised 1985; (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals", and USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1,2 and 3, Animal Welfare, Final Rule 1989.

SELECTION OF ANIMALS: Animals were randomly placed in cages upon receipt, and were placed on study as available at the time of study initiation. Any animals considered unsuitable due to poor health or outlying body weight were excluded from the study. All animals included in the study were sham tested within 7 days prior to testing. Only animals with baseline temperatures within a 1° C range were used for the test. No animal with a temperature exceeding 39.8° C was used for this test.

EQUIPMENT AND EQUIPMENT PREPARATION: A Kaye Digi Strip Automated temperature recorder was used in this study. The recorder was calibrated prior to use.

TEST ANIMAL PREPARATION: PRETEST (SHAM TEST): Prior to using the rabbits in the pyrogen test, they were conditioned not more than 7 days before use, in a Sham Test. This included all the steps and documentation as directed in the pyrogen test, except injection.

SAMPLE PREPARATION AND DOSING: All samples were warmed to 37°C (36-38°). The test article eluent was administered at 10 ml/ kg of body weight.

TEST ARTICLE ADMINISTRATION: The animals were injected within 30 minutes of recording the baseline temperature. The test sample was injected slowly and steadily intravenously into the marginal ear vein of each animal using a sterile pyrogen-free syringe of appropriate size with a sterile pyrogen-free 25 gauge butterfly catheter needle. Each injection was completed within 10 minutes of initiation.

OBSERVATIONS: The temperature was recorded at 30-minute intervals between 1 and 3 hours subsequent to the injection. Any restlessness or struggling of the rabbits that were injected was recorded.



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INTERPRETATION OF RESULTS: Temperature decreases were considered as zero temperature rise. If no rabbit shows an individual rise in temperature of 0.5°C or more above its respective control temperature, the product meets the requirements for the absence of pyrogens. If any rabbit shows an individual temperature rise of 0.5°C or more, the test is continued using five other rabbits. If not more than three of the eight rabbits show individual rises in temperature of 0.5°C or more and if the sum of the eight individual maximum temperature rises does not exceed 3.3°C, the material under examination meets the requirements for the absence of pyrogens.

REUSING ANIMALS: No rabbit used for this pyrogen test had been used for pyrogen testing within the last 48 hours, nor within 2 weeks following a maximum rise of its temperature of 0.5°C or more while being subjected to the pyrogen test, or following its having been given a test article that was judged pyrogenic.

RESULTS: Data Table A contains the animal weight, dose, baseline temperature and test temperature results.

DATA TABLE A

Animal #	794	798	2526
Weight (kg)	3.0	2.9	4.6
Dose (ml)	30.0	29.0	46.0
BaselineTemp (C)	39.6	39.1	39.8
1.0 hr Temp (C)	39.3	39.2	39.5
1.5 hr Temp (C)	39.3	39.1	39.5
2.0 hr Temp (C)	39.4	39.1	39.4
2.5 hr Temp (C)	39.3	39.1	39.4
3.0 hr Temp (C)	39.3	39.0	39.4
Max Temp Rise (C)	0.0	0.1	0.1
Max Temp (C) ^a	39.4	39.2	39.5

a = post injection

During the 3 hour observation period, none of the rabbits administered the test article eluent exhibited a temperature rise $\geq 0.5^{\circ}\text{C}$.

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ANALYSIS AND CONCLUSION: The baseline temperatures for all the rabbits in this study were within 1° C at the initiation of the test and no animals exhibited a temperature above 39.8° C. These conditions indicate a valid test. None of the animals exhibited a temperature rise greater than 0.4° C and therefore the test article response is below the USP 23 limit of $\geq 0.5^{\circ}$ C. These results indicate that the Cordis Corporation, Tacker SDS w/PEEK balloon File # 1572, Lot # SR# 12-96-230 met the USP 23 requirements for the absence of pyrogens.

DEVIATIONS: There was one deviation to the approved protocol; it is stated that the animals will be identified by ear tag and two rabbits were identified by ear mark. Ear marking with a permanent marker is allowable per our identification SOP and all animals are identified by a cage card which indicates what test the animal is currently assigned. This deviation did not affect the results or conclusions of this study.

STATISTICAL METHODS: None.

TECHNICAL REFERENCES: USP 23 (1995), p. 1718

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USP INTRACUTANEOUS TEST RESULTS

COPY

REPORT TITLE

Irritation Testing of Cordis Corporation, Tacker with PEEK balloon (File # 1572)
Using the USP Intracutaneous Test

PRODUCT IDENTITY

Cordis Corporation
Tacker with PEEK balloon (File # 1572)
Lot # NB # 92000 (10-11)

PROJECT NUMBER

3253

PROTOCOL NUMBER

IC100

PREPARED BY

David L. Hodge
Director of In-Life Studies

STUDY COMPLETION ON

February 11, 1997

PERFORMING LABORATORY

ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

SPONSOR

Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014



**GUINEA PIG DELAYED CONTACT SENSITIZATION TEST
(Maximization Method for Biomaterial Extracts)**

REPORT TITLE

Sensitization Testing of Cordis Corporation,
Tacker SDS with PEEK Balloon Extracted in Sodium Chloride
Using the Guinea Pig Maximization Test
(Magnusson-Kligman)

PRODUCT IDENTITY

Cordis Corporation
Tacker SDS with PEEK Balloon
Lot # File 1572

PROJECT NUMBER

3165

PROTOCOL NUMBER

GM100

PREPARED BY

David L. Hodge
Director of In-Life Studies

STUDY COMPLETION ON

February 11, 1997

PERFORMING LABORATORY

ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

SPONSOR

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14201 NW 60th Avenue
Miami Lakes, FL 33014

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GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with current FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies.

The studies not performed by or under the direction of ViroMed Laboratories, Inc. are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the test article.

Submitter: _____

Date: _____

Sponsor: _____

Date: _____

Study Director: David L. HodgeDate: 2-11-97

David L. Hodge

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Guinea Pig Maximization Test (Magnusson - Kligman)

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to management and the Study Director.

Phase Inspected:

Project Set-up Date: 12/31/96

Final Report Date: 2/11/97

Study Director Review Date: 2/11/97

Management Review Date: 2/11/97

Documentation of the above Quality Assurance audits have been reviewed.

Quality Assurance Director: Joyce A. NelsonDate: 11 Feb, 1997

Joyce A. Nelson

Study Director: David L. HodgeDate: 2-11-97

David L. Hodge

Professional Personnel Involved:

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PROJECT NUMBER: 3165

REPORT DATE: 2/11/97

SPONSOR: Cordis Corporation
14201 NW 60th Avenue
Miami Lakes, FL 33014

DATA RETENTION: A certified copy of the original final report and all raw data pertinent to this study will be stored at ViroMed Laboratories, Inc., 6101 Blue Circle Drive, Minneapolis, MN 55343. The test substance used in this assay was expended in testing.

SAMPLE IDENTIFICATION: Cordis Corporation, Tacker SDS with PEEK Balloon, Lot # File 1572

CHARACTERIZATION: The identity, strength, stability, purity, and chemical composition are the responsibility of the Sponsor.

PURPOSE: This test was designed to evaluate the allergenic potential or sensitizing capacity of a test article. This test was used as a procedure for the screening of contact allergens in guinea pigs and extrapolating the results to humans, but does not establish the actual risk of sensitization in humans.

TEST FACILITY: ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

DATE SAMPLE RECEIVED: 12/20/96
STUDY INITIATION DATE: 12/20/96
EXPERIMENTAL START DATE: 12/23/96
STUDY COMPLETION DATE: 2/11/97
IACUC APPROVAL DATE: 5/28/96

METHOD: This study was based upon the procedures described in Dermatoxicology, F.N. Marzulli and H.I. Maibach, editors, 4th edition, 1991, pages 374-378, Hemisphere Publishing Corporation, New York. Magnusson, B. and Kligman, A.M. 1969, The identification of contact allergens by animal assay, The guinea pig maximization test. J. Invest. Dermatol. 52:268-276.; Magnusson, B. and Kligman, A.M. 1970. Allergic contact dermatitis in the guinea pig, Identification of contact allergens. Springfield, Ill.: Thomas (Publ.), Extraction procedures were based upon the standards set by USP 23 (1995), pages 1701-2.

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EXPERIMENTAL METHODS SUMMARY: In selecting a new material for human contact in medical applications, it is important to ensure that the material will not stimulate the immune system to produce an allergic response. The response generally is due to leachable substances in the test material. Therefore, USP material extracts are used for animal administrations. The extraction is performed according to the standard USP 23 Extraction Method in the sponsor designated vehicle. The use of Freund's complete adjuvant and sodium lauryl sulfate enhance the potential of weak sensitizing agents.

Thirteen female Duncan-Hartley strain guinea pigs (10 test animals, 3 primary irritant control animals) between 300 and 500 g were assigned to the study. Two guinea pigs received with the same shipment as the test animals were assigned separately as positive control animals. A 3 by 5 centimeter area over the intrascapular region is clipped free of fur on the test animals and positive control animals. The primary irritant control animals did not receive induction treatment and were only used in the challenge phase of the test. Three pairs of intradermal injections were administered within the clipped area according to the following:

Test Animal Group:

- (1) 0.1 ± 0.01 ml FCA (Difco) 1:1 with Normal Saline
- (2) 0.1 ± 0.01 ml test article extract
- (3) 0.1 ± 0.01 ml test article extract 1:1 with FCA

Positive Control Group:

- (1) 0.1 ± 0.01 ml FCA 1:1 with Normal Saline
- (2) 0.1 ± 0.01 ml 0.1% Dinitrochlorobenzene (DNCB) in 95% Ethanol (EtOH)
- (3) 0.1 ± 0.01 ml 0.1% DNCB in 95% EtOH 1:1 with FCA

On day 7, the area of the injection sites was treated with 0.5 g of 10% (w/w) sodium lauryl sulfate (SLS) prepared by mixing solid SLS with petrolatum. The SLS was removed 24 hours after application. After removal of the SLS, a topical application of 0.3 ml of fresh test article extract (test animals) or 0.1% DNCB solution in 95% ethanol positive control (positive control animals) was applied topically over the injection sites in a patch which was occlusively wrapped. The patch was removed after 48 hours. Twelve to fourteen days after topical induction, the fur was removed from both flanks of all animals with an electric clipper. The test animals and the primary irritant control animals were challenged on the right flank with a Hill Top Chamber saturated with fresh test article extract and on the left flank with vehicle control in the same manner. The positive control animals were challenged in the same manner using 0.05% DNCB solution in acetone in place of the test article extract. The challenge sites were observed for signs of erythema and edema at 24, 48, and 72 hours after challenge patch removal. The response was scored according to a standard scale.



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The responses were compared within the test group and between the test group and control conditions. Control conditions include:

- 1) The vehicle control reaction on the left flank of the test animals.
- 2) The test article extract, and vehicle control reaction on the primary irritant control animals.
- 3) The positive control animals' reaction to 0.05% DNCB.

In the final analysis of data, consideration was given to the overall pattern, intensity, duration, and character of reactions of the test article extract as compared with the control conditions. An effect interpreted as "irritation" is generally observed at day 1 after patch removal, but diminishes thereafter, and is also concurrently present as a transient response in the control animals. Closed patches typically show maximal sensitization response 2 to 3 days after patch removal in test conditions, but not in the control conditions. Only when the response to the challenge with the test article extract clearly outweighs the reaction from those of any control condition is the material judged to have allergic potential.

The test results were interpreted based upon the percentage of animals exhibiting a sensitization response.

TEST MATERIAL AND PREPARATION

STERILIZATION: The test article was received in sterile form from the Sponsor.

TEST SAMPLE PREPARATION: A representative sample of the test article was cut into pieces, placed into test tubes and prepared at a ratio of 60 cm² to 20 ml of extraction vehicle.

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EXTRACTION: Fresh extract of the test article was made for each of the two induction steps and the challenge step at a ratio equivalent to 60 cm² of test article to 20 ml of extraction vehicle and conditions listed below :

Intradermal Injection Induction:

Extract Vehicle: Sodium Chloride (NaCl), Lot # C327718 (Baxter)

Surface area of Test Article	ml of Vehicle	Vehicle	Time	Temp (°C)
47.35 cm ²	15.80	NaCl	24 hrs	70 ± 2

Patch Induction:

Extract Vehicle: Sodium Chloride (NaCl), Lot # C327718 (Baxter)

Surface area of Test Article	ml of Vehicle	Vehicle	Time	Temp (°C)
47.35 cm ²	15.80	NaCl	24 hr	70 ± 2

Challenge:

Extract Vehicle: Sodium Chloride (NaCl), Lot # C327718 (Baxter)

Surface area of Test Article	ml of Vehicle	Vehicle	Time	Temp (°C)
47.35 cm ²	15.78	NaCl	24 hrs	70 ± 2

The extracts were cooled, shaken well and decanted into sterile, dry glass vessels. The extract was used within 24 hours of preparation.

PREPARATION OF FREUND'S COMPLETE ADJUVANT (FCA) MIXTURES FOR INJECTION: The FCA mixtures were prepared under aseptic conditions and ratios according to the following :

Test Article

Injection #	FCA (ml)	Test Article Extract (ml)	Extract Vehicle (ml)
1)	1.2	0.0	1.2
2)	0.0	2.0	0.0
3)	1.2	1.2	0.0

0.1% DNCB/Ethanol Positive Control

Injection #	FCA (ml)	0.1% DNCB (ml)	Extract Vehicle (ml)
1)	0.3	0.0	0.3
2)	0.0	0.4	0.0
3)	0.3	0.3	0.0

Materials:

FCA	Lot # 80678LA, Difco
DNCB (0.1%)	Lot # 06929JZ, Aldrich
Ethanol (95%)	Lot # 952753, Fisher

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TEST SYSTEM

SPECIES/STRAIN AND SEX: Duncan-Hartley Strain, Albino Guinea Pig.
(Specific Pathogen Free), Female

SOURCE: Charles River Kingston, Kingston, New York

ANIMAL BODY WEIGHT RANGE: All animals weighed between 300 and 500g at the initiation of the study and were within the required range for the test.

ANIMAL IDENTIFICATION: Individually numbered ear tags.

HUSBANDRY

RECEIPT: Animals were received on 12/23/96. Each animal was examined for signs of disease and injury prior to entry into the research area.

HOUSING: Animals were housed in suspended stainless steel cages with separation of the test, positive control and irritant control animals. Housing density was in compliance with AAALAC standards.

ENVIRONMENT: Animal rooms were maintained at $68 \pm 3^{\circ}$ F and 30 - 70% relative humidity with a 12 hour light/dark cycle. Rooms were maintained at 12-14 air changes per hour. The laboratory and animal rooms were maintained as limited - access facilities.

DIET: Animals were supplied with certified Purina Guinea Pig Chow. No known contaminants present in the feed are expected to interfere with the test results.

WATER: Potable Water.

TERMINATION: Animals were euthanized by CO₂ asphyxiation.

COMPLIANCE: The care, housing and handling of the animals was in compliance with AAALAC, "Guide for the Care and Use of Laboratory Animals", DHHS Pub. No. (NIH) 85-23, Revised 1985; (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals", and USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1,2 and 3, Animal Welfare, Final Rule 1989.



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SELECTION OF ANIMALS: Animals were randomly placed in cages upon receipt, and were placed on study as available at the time of study initiation. Any animals considered unsuitable due to poor health or outlying body weight were excluded from the study.

ANIMAL PREPARATION: The application sites were prepared by clipping the skin of the test site free of fur with a #40 blade. On day 0 and on day 7, an approximately 5 x 7 cm area over the shoulder region was prepared. The day before challenge, an approximately 4 x 4 cm area of the flanks was prepared by removing the fur with an electric clipper.

TEST ARTICLE ADMINISTRATION

Induction/Intradermal Application (Day 0): Three pairs of intradermal injections were made with one row of three injections on each side of the dorsal mid-line. The injection sites (6) were just within the boundaries of a 2 x 4 cm patch. Injections with FCA are made slightly deeper to minimize tissue reactions. The injection doses were made according to the following:

Experimental Group:

- (1) 0.1 ± 0.01 ml FCA (Difco) 1:1 with Normal Saline
- (2) 0.1 ± 0.01 ml test article
- (3) 0.1 ± 0.01 ml test article 1:1 with FCA

Positive Control Group:

- (1) 0.1 ± 0.01 ml FCA 1:1 95% EtOH
- (2) 0.1 ± 0.01 ml 0.1% DNCB in 95% EtOH
- (3) 0.1 ± 0.01 ml 0.1% DNCB in 95% EtOH 1:1 with FCA

Topical Application (Day 7): On day 7 the area of the injection sites was clipped free of fur again and treated with 0.5 to 1 g of 10% (w/w) sodium lauryl sulfate (SLS) prepared by mixing solid SLS with petrolatum. The day following the SLS treatment, the remaining SLS residue was gently wiped from the area with gauze.

Experimental Group: The test article extract was applied to a 2 x 4 cm piece of filter paper (Whatman 3) to saturation (0.3 ml) and applied after SLS removal. The patch was secured to the site with non-permeable tape and the trunk wrapped with Vetwrap™ and Transpore™ tape. This preparation was left in place for 48 hours. Freshly prepared extract was used for this administration.

Positive Control Group: The animals were exposed to 0.1% DNCB solution in 95% ethanol in the same manner as the experimental group.



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Challenge Application: On day 13, after the topical induction phase, the challenge procedure was initiated on the 10 experimental animals and the 3 irritant control animals. A nonwoven cotton disk, backed by a flexible chamber (Hill Top Chamber) and a semi occlusive tape bandage, was saturated with 0.3 ml of freshly prepared test article extract and applied to the fur clipped right flank of each animal. In addition, the vehicle control was patched in the same manner to the left flank of each animal. The positive control animals were exposed in the same manner using 0.05% DNCB solution in acetone in place of the test article extract. The trunk of each animal was wrapped for 24 hours with Vetwrap™ and Transpore™ tape. The following day after challenge exposure, the patches were removed and the area cleaned gently with gauze.

OBSERVATIONS AND SCORING: Daily challenge observation scores were recorded 1, 2 and 3 days after patch removal in accordance with the following scheme:

- 0 = No reaction
- 0.5 = Background response (very faint erythema usually not confluent or pinpoint erythema not considered significant.)
- 1 = Scattered reaction
- 2 = Moderate and diffuse or confluent reaction
- 3 = Intense reddening and swelling

Note: Erythema is defined as redness and edema is defined as a swelling at the challenge site.

Daily animal health observations were recorded throughout the study period.

INTERPRETATION OF RESULTS: The responses were compared within the test group and between the test group and control group conditions. Control group conditions include:

- 1) The vehicle control solution reaction on the left flank of the test animal.
- 2) The test extract, and control solution reaction on the primary irritant control animals.
- 3) The positive control animals' reaction to 0.05% DNCB.

In the final analysis of data, consideration was given to the overall pattern, intensity, duration, and character of reactions of the test article as compared with the control conditions. An effect interpreted as "irritation" is generally observed at 1 day after patch removal, but diminishes thereafter, and is also concurrently present as a transient response in the control animals. Closed patches typically show maximal sensitization response 2 to 3 days after patch removal in test conditions, but not in the control conditions. Only when the challenge response to the test article extract clearly outweighs the reaction from those of any control condition will the material be judged to be sensitizing.

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Background or artifactual reactions from fur clipping or patch edge or nonspecific FCA effects were not considered as evidence of sensitization. The treatment with FCA and occlusive dressings may lower the threshold level for skin irritation.

Using the scoring system of Kligman (TABLE 1), the allergic potential of a test article was classified as follows:

TABLE 1

<u>Sensitization Rate (%)</u>	<u>Grade</u>	<u>Class</u>
0-10 %	I	Weak
11-30 %	II	Mild
31-60 %	III	Moderate
61-80 %	IV	Strong
81-100 %	V	Extreme

The test results are interpreted based upon the percentage of animals exhibiting sensitization. In the Magnusson-Kligman model, weak sensitizers are not regarded as significant.

RESULTS: None of the animals in the study showed abnormal clinical signs during the test period. The individual daily challenge observations are listed in Table A for the Positive Control Group, Table B for the Irritant Control Group and Table C for the Test Article Extract Group.

Table A: Positive Control Group Daily Challenge Observations

Animal #	Experimental Group	24 hour Control	24 hour DNCB	48 hour Control	48 hour DNCB	72 hour Control	72 hour DNCB	Results
263	Positive Control	0	2	0	2	0	2	Positive
264	Positive Control	0	1	0	1	0	1	Positive

The positive control animals exhibited an extreme sensitization response to the positive control article (0.05% DNCB in Acetone).

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Table B: Irritant Control Daily Challenge Observations

Animal #	Experimental Group	24 hour Control	24 hour Test Article	48 hour Control	48 hour Test Article	72 hour Control	72 hour Test Article	Results
289	Irritant Control	0	0	0	0	0	0	Negative
290	Irritant Control	0	0	0	0	0	0	Negative
291	Irritant Control	0	0	0	0	0	0	Negative

The test article extract did not have a primary irritation response in the irritant control animals.

Table C: Test Article Extract Daily Challenge Observations

Animal #	Experimental Group	24 hour Control	24 hour Test Article	48 hour Control	48 hour Test Article	72 hour Control	72 hour Test Article	Results
279	Test	0	0	0	0	0	0	Negative
280	Test	0	0	0	0	0	0	Negative
281	Test	0	0	0	0	0	0	Negative
282	Test	0	0	0	0	0	0	Negative
283	Test	0	0	0	0	0	0	Negative
284	Test	0	0	0	0	0	0	Negative
285	Test	0	0	0	0	0	0	Negative
286	Test	0	0	0	0	0	0	Negative
287	Test	0	0	0	0	0	0	Negative
288	Test	0	0	0	0	0	0	Negative

None of the test animals challenged with the test article extract were observed with a challenge response greater than 0. A negative sensitization response was interpreted for all test animals.

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 Project # 3165
 Page 12 of 12

ANALYSIS AND CONCLUSION: The three test control conditions, as listed below, were met for this test, thus indicating a valid test.

<u>Control Condition</u>	<u>Results</u>
1) Vehicle Control	The extraction vehicle did not have a primary irritation response nor did it act as a sensitizer alone.
2) Test Article Extract Primary Irritation	The test article extract was not a primary irritant.
3) Positive Control (Animals from the same shipment)	DNCB was classified as a Grade V extreme sensitizer.

The classification of materials for sensitization according to the Magnusson-Kligman Model is based on the percent incidence of positive test animals in the test group. The sodium chloride extract of the test material had a 0% sensitization response under valid test conditions. Cordis Corporation, Tacker SDS with PEEK Balloon, Lot # File 1572 is therefore classified as a Grade I, weak sensitizer. In the Magnusson-Kligman model, weak sensitizers are not regarded as significant.

DEVIATIONS: None.

STATISTICAL METHODS: None.

TECHNICAL REFERENCES:

- Marzulli FN, Maibach HI, editors: Dermatotoxicology, 4th edition, pp 374-378. Hemisphere Publishing Corporation, New York, 1991
- Magnusson B, Kligman AM: The identification of contact allergens by animal assay, The guinea pig maximization test. J. Invest. Dermatol 52:268-276., 1969.
- Magnusson B, Kligman AM: Allergic contact dermatitis in the guinea pig, Identification of contact allergens. Thomas (Publ.), Springfield, Ill. 1970.
- Extraction procedures were based upon the standards set by USP 23 (1995), pages 1701-2.

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Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Part Name	Subject Device Material
PTC Clip, PTC Clip Plating	304 Stainless Steel, Nickel - Tin Plating

FDA CDRH DMC

NOV 13 2012

Received

NAMSACDRH FOIA
REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455**STUDY TITLE**Determination of Extractable Species From a
Test Article by Infrared Spectroscopy**TEST ARTICLE NAME**

Eagle 3mm Jaw and Shaft Sub assembly

SPONSORBrittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242**TEST ARTICLE IDENTIFICATION**

Built according to PRC049736

TEST ARTICLE PHYSICAL DESCRIPTIONsilver metal jaw attached to black shaft with
white printing**TEST ARTICLE RECEIVED**

August 15, 2011

SUMMARY/RESULTS

The test article was characterized for extractable species as required by ISO 10993-18, Biological Evaluation of Medical Devices - Part 18: Chemical Characterization of Materials by first extracting the test article in Purified Water. The resulting test extract was then evaporated to a residue and then the residue was analyzed by infrared spectroscopy to identify the species present in the test extract.

There were no major bands of interest detected in the test article residue. The lack of species present as represented by the infrared spectrum, can be used to aid in the assessment of the test article by ISO 10993, Part 18.

The test article spectrum is located in Appendix 1.

METHOD

The test article was cut approximately 4 cm from the end of the jaw. The cut piece with the metal jaw plus a little of the shaft with the pad print ink was included in the testing. A 9.4 gram portion (3 pieces) of the test article was extracted at 50°C for 72 hours in 47 mL of Purified Water. The method and results of this extraction were reported under Lab Number 11T_44631_01. A small portion of the Purified Water test extract was transferred to a clean vial and placed in a 105°C drying oven to evaporate the extracting solvent. A small amount of chloroform was added to the vial to dissolve the residue. The resulting solution was placed on a clean KBr disc and the chloroform was allowed to evaporate. The residue was analyzed by infrared spectroscopy using the instrument conditions listed below.

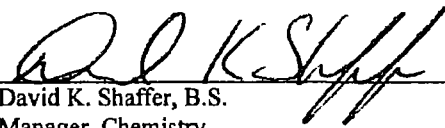
Instrument	Fourier Transform Infrared Spectrophotometer
Wavenumber Range—Collected	4400 cm ⁻¹ – 350 cm ⁻¹
Wavenumber Range—Displayed	4000 cm ⁻¹ – 400 cm ⁻¹
Scan number	8
Resolution	4 cm ⁻¹

REFERENCES

International Organization for Standardization (ISO) 10993-18, Biological Evaluation of Medical Devices - Part 18: Chemical Characterization of Materials (2005).

European Pharmacopoeia (Ph. Eur.) 7th Edition, Section 2.2.24. Absorption Spectrophotometry, Infrared (2011).

United States Pharmacopeia 34, National Formulary 29 (USP), General Chapter <197>, Spectrophotometric Identification Tests, Infrared Absorption (2011).

APPROVAL

David K. Shaffer, B.S.
Manager, Chemistry
9-1-11
Date

Results apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.

P.O. No.:

992733667

Lab Number:

11T_44631_01

C0022_100

Page 1 of 2

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOI@STATUS.GOV or 301-796-8188

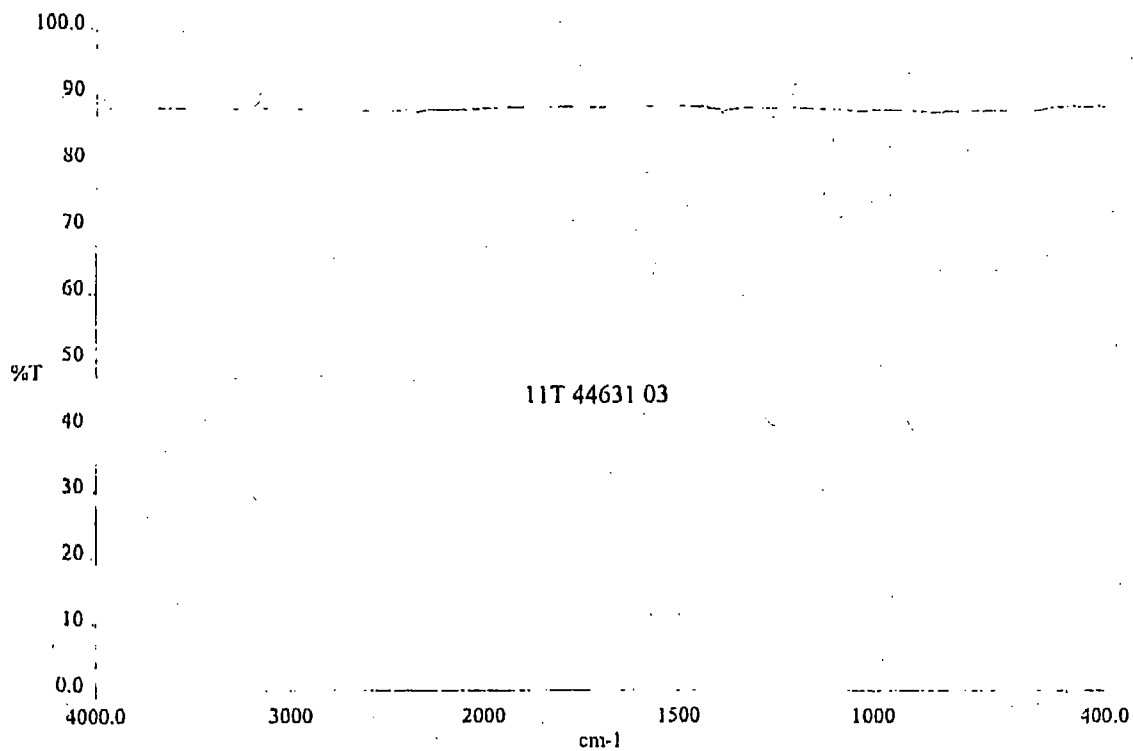
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REPORT

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Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

APPENDIX 1



NAMSA

REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

STUDY TITLE

Determination of Extractable Species From a
Test Article by Infrared Spectroscopy

TEST ARTICLE NAME

Eagle 3mm Jaw and Shaft Sub assembly

SPONSOR

Brittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

TEST ARTICLE IDENTIFICATION

Built according to PRC049736

TEST ARTICLE PHYSICAL DESCRIPTION

silver metal jaw attached to black shaft with
white printing

TEST ARTICLE RECEIVED

August 15, 2011

SUMMARY/RESULTS

The test article was characterized for extractable species as required by ISO 10993-18, Biological Evaluation of Medical Devices - Part 18: Chemical Characterization of Materials by first extracting the test article in isopropyl alcohol. The resulting test extract was then evaporated to a residue and then the residue was analyzed by infrared spectroscopy to identify the species present in the test extract.

The infrared spectrum of the test article residue most closely matches that of polydimethylsiloxane. The identified species, as represented by the infrared spectrum, can be used to aid in the assessment of the test article by ISO 10993, Part 18.

The test article and reference spectra are located in Appendix 1.

METHOD

The test article was cut approximately 4 cm from the end of the jaw. The cut piece with the metal jaw plus a little of the shaft with the pad print ink was included in the testing. A 9.5 gram portion (3 pieces) of the test article was extracted at 50°C for 72 hours in 48 mL of isopropyl alcohol. The method and results of this extraction were reported under Lab Number 11T_44631_02. A small portion of the isopropyl alcohol test extract was transferred to a clean vial and placed in a 105°C drying oven to evaporate the extracting solvent. A small amount of chloroform was added to the vial to dissolve the residue. The resulting solution was placed on a clean and the chloroform was allowed to evaporate. The residue was analyzed by infrared spectroscopy using the instrument conditions listed below.

Instrument	Fourier Transform Infrared Spectrophotometer
Wavenumber Range—Collected	4400 cm ⁻¹ –350 cm ⁻¹
Wavenumber Range—Displayed	4000 cm ⁻¹ –400 cm ⁻¹
Scan number	8
Resolution	4 cm ⁻¹

REFERENCES

International Organization for Standardization (ISO) 10993-18, Biological Evaluation of Medical Devices - Part 18: Chemical Characterization of Materials (2005).

European Pharmacopoeia (Ph. Eur.) 7th Edition, Section 2.2.24. Absorption Spectrophotometry, Infrared (2011).

United States Pharmacopeia 34, National Formulary 29 (USP), General Chapter <197>, Spectrophotometric Identification Tests, Infrared Absorption (2011).

APPROVAL

David K. Shaffer, B.S.
Manager, Chemistry

Date

9-1-11

Results apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.

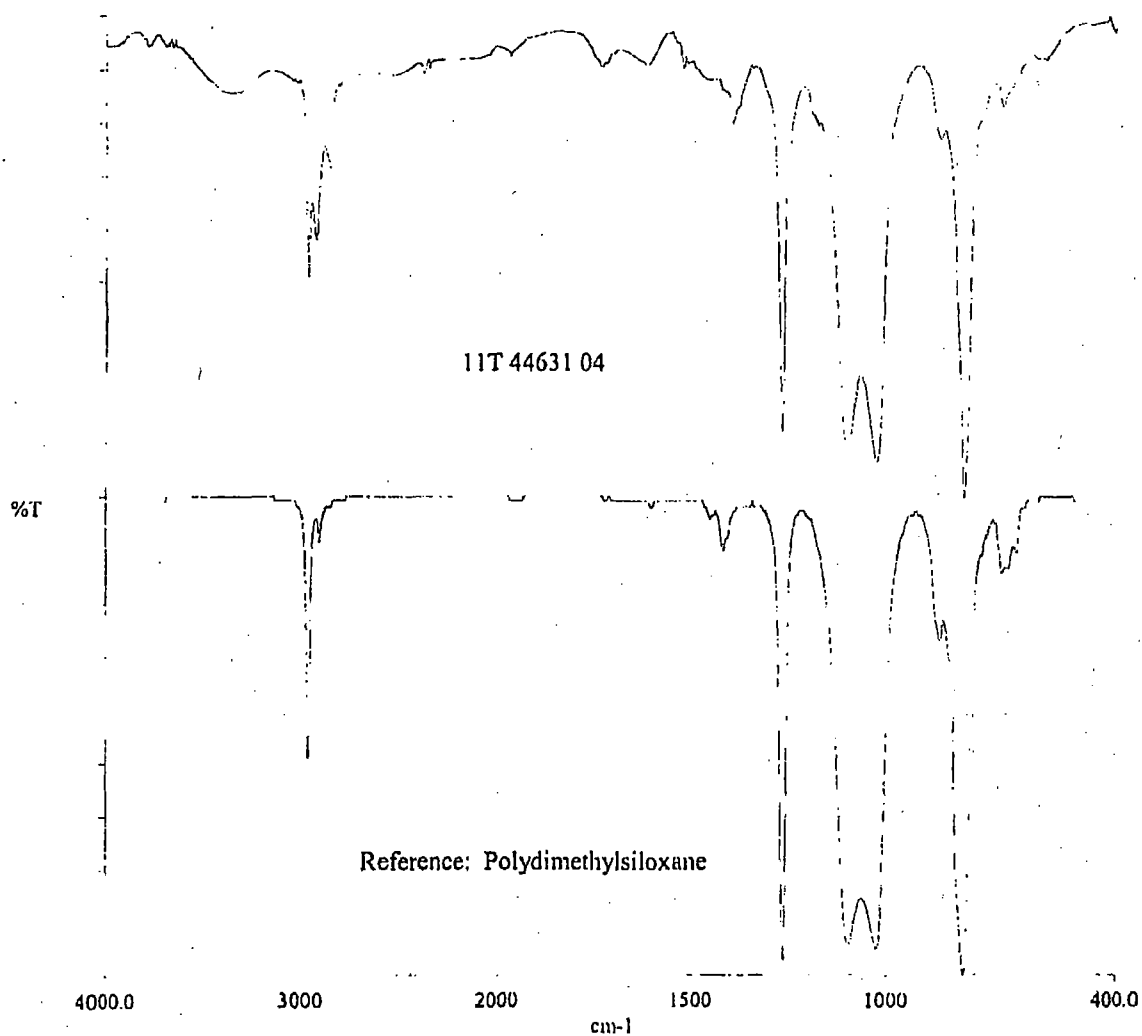
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COMPLIANCE
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Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

APPENDIX 1



NAMSACERTIFICATE
REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455**STUDY TITLE**Determination of Trace Metals/Elements From
Materials Using Inductively Coupled Plasma
Spectroscopy – Extractable Metal Content**SPONSOR**Brittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242**TEST ARTICLE NAME**

Eagle 3mm Jaw and Shaft Sub assembly

TEST ARTICLE IDENTIFICATION

Built according to PRC049736

TEST ARTICLE PHYSICAL DESCRIPTIONsilver metal jaw attached to black shaft with
white printing**TEST ARTICLE RECEIVED**

August 15, 2011

RESULTS

Metal/Element Analyzed	Dilution Factor	Instrument Detection Limit (ppm)	Sample Detection Limit (ppm)	Sample Concentration (ppm)
Aluminum	1	0.050	0.050	BDL
Antimony	1	0.050	0.050	BDL
Arsenic	1	0.050	0.050	BDL
Barium	1	0.050	0.050	BDL
Beryllium	1	0.050	0.050	BDL
Calcium	1	0.050	0.050	BDL
Cadmium	1	0.050	0.050	BDL
Cobalt	1	0.050	0.050	BDL
Chromium	1	0.050	0.050	BDL
Copper	1	0.050	0.050	BDL
Iron	1	0.050	0.050	BDL
Lead	1	0.050	0.050	BDL
Magnesium	1	0.050	0.050	BDL
Manganese	1	0.050	0.050	BDL
Molybdenum	1	0.050	0.050	BDL
Nickel	1	0.050	0.050	BDL
Potassium	1	0.050	0.050	BDL
Selenium	1	0.050	0.050	BDL
Strontium	1	0.050	0.050	BDL
Silver	1	0.013	0.013	BDL
Titanium	1	0.050	0.050	BDL
Tin	1	0.050	0.050	BDL
Vanadium	1	0.050	0.050	BDL
Zinc	1	0.050	0.050	BDL
Zirconium	1	0.050	0.050	BDL

ppm = µg/mL

BDL = Below Detection Limit

CONCLUSION

There were no elements detected in the isopropyl alcohol test extract.

P.O. No.:

992732667

Lab Number:

11T-44631-06

C0087_000

Page 1 of 2

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOI@STATUS.GOV or 301-796-8188

NAMSA

REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

METHOD

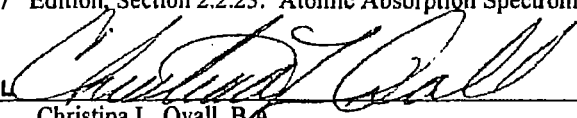
A 6 mL portion of the isopropyl alcohol test extract prepared under Lab Number 11T_44631_02 and a 6 mL portion of the control extract were placed in appropriate glassware and evaporated to dryness. The residue was reconstituted using 6 mL of 5% nitric acid as the solvent and tested for all trace metals/elements of interest by Inductively Coupled Plasma (ICP) Spectroscopy.

REFERENCES

European Pharmacopoeia (Ph. Eur.) 7th Edition, Section 2.2.22: Atomic Emission Spectrometry (2011).

European Pharmacopoeia (Ph. Eur.) 7th Edition, Section 2.2.23: Atomic Absorption Spectrometry (2011).

APPROVAL


Christina L. Oval, B.A.
Supervisor, Chemistry

8-31-11
Date

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices and certified to ISO 13485:2003.

NAMSA**CONFIDENTIAL
REPORT**

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455**STUDY TITLE**Determination of Trace Metals/Elements From
Materials Using Inductively Coupled Plasma
Spectroscopy – Extractable Metal Content**SPONSOR**Brittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242**TEST ARTICLE NAME**

Eagle 3mm Jaw and Shaft Sub assembly

TEST ARTICLE IDENTIFICATION

Built according to PRC049736

TEST ARTICLE PHYSICAL DESCRIPTIONsilver metal jaw attached to black shaft with white
printing**TEST ARTICLE RECEIVED**

August 15, 2011

RESULTS

Metal/Element Analyzed	Dilution Factor	Instrument Detection Limit (ppm)	Sample Detection Limit (ppm)	Sample Concentration (ppm)
Aluminum	1	0.050	0.050	BDL
Antimony	1	0.050	0.050	BDL
Arsenic	1	0.050	0.050	BDL
Barium	1	0.050	0.050	BDL
Beryllium	1	0.050	0.050	BDL
Calcium	1	0.050	0.050	1.4
Cadmium	1	0.050	0.050	BDL
Cobalt	1	0.050	0.050	BDL
Chromium	1	0.050	0.050	BDL
Copper	1	0.050	0.050	BDL
Iron	1	0.050	0.050	BDL
Lead	1	0.050	0.050	BDL
Magnesium	1	0.050	0.050	0.36
Manganese	1	0.050	0.050	BDL
Molybdenum	1	0.050	0.050	2.5
Nickel	1	0.050	0.050	BDL
Potassium	1	0.050	0.050	0.22
Selenium	1	0.050	0.050	BDL
Strontium	1	0.050	0.050	BDL
Silver	1	0.013	0.013	BDL
Titanium	1	0.050	0.050	BDL
Tin	1	0.050	0.050	BDL
Vanadium	1	0.050	0.050	BDL
Zinc	1	0.050	0.050	BDL
Zirconium	1	0.050	0.050	BDL

ppm = µg/mL

BDL = Below Detection Limit

CONCLUSION

Calcium, magnesium, molybdenum and potassium were detected in the Purified Water test extract.

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REPORT

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Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455**METHOD**

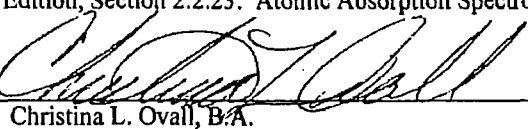
A portion of the Purified Water test extract prepared under Lab Number 11T_44631_01 and the control extract were tested for all trace metals/elements of interest by Inductively Coupled Plasma (ICP) Spectroscopy.

REFERENCES

European Pharmacopoeia (Ph. Eur.) 7th Edition, Section 2.2.22: Atomic Emission Spectrometry (2011).

European Pharmacopoeia (Ph. Eur.) 7th Edition, Section 2.2.23: Atomic Absorption Spectrometry (2011).

APPROVAL


Christina L. Oval, B.A.
Supervisor, Chemistry8-31-11
Date

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices and certified to ISO 13485:2003.

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REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455**STUDY TITLE**Physicochemical Testing Using an Alternative
Extracting Solvent - Non-Volatile Residue**TEST ARTICLE NAME**

Eagle 3mm Jaw and Shaft Sub assembly

TEST ARTICLE IDENTIFICATION

Built according to PRC049736

TEST ARTICLE PHYSICAL DESCRIPTIONsilver metal jaw attached to black shaft with
white printing**TEST ARTICLE RECEIVED**

August 15, 2011

SPONSORBrittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242**RESULTS**

Assay Results	
Non-Volatile Residue	9 mg

Condition of Test Article Extract: clear and colorless

Condition of Control Blank: clear and colorless

METHOD

The test article was cut approximately 4 cm from the end of the jaw. The cut piece with the metal jaw plus a little of the shaft with the pad print ink was included in the testing. A 9.5 gram portion (3 pieces) of the test article was extracted at 50°C for 72 hours in 48 mL of isopropyl alcohol. Non-volatile residue was determined on a 10.0 mL portion of the eluate.

COMMENT

Limits for physicochemical tests using lipophilic solvents have not yet been established. Test articles that are low in lipophilic soluble extractables are generally preferred for use in test articles contacting blood and other tissues.

REFERENCE

United States Pharmacopeia 34, National Formulary 29 (USP), General Chapter <661>, Containers - Plastics (2011).

APPROVAL

David K. Shaffer, B.S.
Manager, Chemistry8-24-11
Date

Results apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.

NAMSACONFIDENTIAL
REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455**STUDY TITLE**Determination of the Physicochemical
Attributes of a Polymeric Material per USP
<661> Containers - Plastics, Physicochemical
Tests - Non-Volatile Residue**SPONSOR**Brittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242**TEST ARTICLE NAME**

Eagle 3mm Jaw and Shaft Sub assembly

TEST ARTICLE IDENTIFICATION

Built according to PRC049736

TEST ARTICLE PHYSICAL DESCRIPTIONsilver metal jaw attached to black shaft with
white printing**TEST ARTICLE RECEIVED**

August 15, 2011

RESULTS

	Assay Results ¹	Limits Based on Area
Non-Volatile Residue	2 mg	≤15 mg

¹Extraction conditions were based upon weight rather than USP specified surface area. Test article extract results may be compared to USP area limits to determine significance.

Date Extract Prepared: August 16, 2011

Date Test Concluded: August 22, 2011

Condition of Test Article Extract: clear and colorless

Condition of Control: clear and colorless

CONCLUSION

Under the condition of this test, no significant extractables originated from the test article.

METHOD

The test article was cut approximately 4 cm from the end of the jaw. The cut piece with the metal jaw plus a little of the shaft with the pad print ink was included in the testing. A 9.4 gram portion (3 pieces) of the test article was extracted at 50°C for 72 hours in 47 mL of Purified Water. A control blank of Purified Water was similarly prepared without the test article. Non-volatile residue was determined in the test article extract as outlined in the current USP.

REFERENCE

United States Pharmacopeia 34, National Formulary 29 (USP), General Chapter <661>, Containers - Plastics (2011).

APPROVALDavid K. Shaffer, B.S.
Manager, Chemistry8-24-11
Date

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.

NAMSA

CONFIDENTIAL

REVISED REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455**STUDY TITLE**Determination of Extractable Semi-Volatile
Organic Compounds by Gas
Chromatography/Mass Spectrometry (GC/MS)**SPONSOR**Brittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242**TEST ARTICLE NAME**

Eagle 3mm Jaw and Shaft Sub assembly

TEST ARTICLE IDENTIFICATION

Built according to PRC049736

TEST ARTICLE PHYSICAL DESCRIPTIONsilver metal jaw attached to black shaft with
white printing**TEST ARTICLE RECEIVED**

August 15, 2011

RESULTS

The results for the isopropyl alcohol test extract are listed in the table below.

Approximate Retention Time (minutes)	Quality Match	Tentatively Identified Compound(s)	CAS Number	Estimated Molecular Weight	Average Estimated Concentration (µg/mL)
22.66	72	1-Phenyl-4-(3,5-dimethylphenyl)buta-1,3-diene	NA	234	3.14
24.01	74	Benzene, 1,1'-(1,5-hexadiene-1,6-diyl)bis-	4439-45-6	234	3.00

NA – Not Available

CONCLUSION

There were two semi-volatile organic compounds detected in the isopropyl alcohol test extract.

METHOD

The test article was cut approximately 4 cm from the end of the jaw. The cut piece with the metal jaw plus a little of the shaft with the pad print ink was included in the testing. A 9.5 gram portion (3 pieces) of the test article was extracted at 50°C for 72 hours in 48 mL of isopropyl alcohol. The method and results of this extraction were reported under Lab Number 11T_44631_02. The isopropyl alcohol test extract and its associated control were analyzed by GC/MS using the following instrument conditions.

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REVISED REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

Instrument: Shimadzu QP 2010 GC/MS			
Column	HP-5MS J+W Scientific 30m length x 0.25mm i.d. x 0.25µm d _f		
Column Oven Temp.	40.0°C	Injection Temp.	250.0°C
Injection Mode	Splitless	Sampling Time	1.00 min
Pressure	63.9 kPa	Total Flow	26.9 mL/min
Column Flow	1.20 mL/min	Purge Flow	1.7 mL/min
Ion Source Temp.	230.0°C	Carrier Gas	Helium
Solvent Cut Time	2.50 min	Interface Temp.	300.0°C
Detector Gain	0.00 kV	Detector Gain Mode	Relative
Injection Volume	1 µL	Threshold	10
Oven Temperature Program			
Rate(°C/min)	Temperature(°C)		Hold time(min)
-	40.0		4.00
10.0	280.0		4.00
45.0	310.0		10.00

Standards of phenanthrene at concentrations of 2.0, 25 and 50 ppm were prepared and injected in triplicate. A linear regression was performed using the resulting peak areas. A library scan of the test article extract was performed to tentatively identify any peaks not attributed to the blank, with the results listed in the Results Section.

REFERENCES

International Organization for Standardization (ISO) 10993-18, Biological Evaluation of Medical Devices-Part 18: Chemical Characterization of Materials (2005).

This report was revised to add a CAS number. The original report was signed on 8/25/11 by David Shaffer.

APPROVAL

David K. Shaffer
David K. Shaffer, B.S.
Manager, Chemistry

Date

11-1-11

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.

NAMSA**REPORT**

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

STUDY TITLE

Determination of Extractable Semi-Volatile
Organic Compounds by Gas
Chromatography/Mass Spectrometry (GC/MS)

SPONSOR

Brittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

TEST ARTICLE NAME

Eagle 3mm Jaw and Shaft Sub assembly

TEST ARTICLE IDENTIFICATION

Built according to PRC049736

TEST ARTICLE PHYSICAL DESCRIPTION

silver metal jaw attached to black shaft with
white printing

TEST ARTICLE RECEIVED

August 15, 2011

RESULTS

There were no semi-volatile compounds greater than 2.0 ppm for the Purified Water test and control extracts.

METHOD

The test article was cut approximately 4 cm from the end of the jaw. The cut piece with the metal jaw plus a little of the shaft with the pad print ink was included in the testing. A 9.4 gram portion (3 pieces) of the test article was extracted at 50°C for 72 hours in 47 mL of Purified Water. The method and results of this extraction were reported under Lab Number 11T_44631_01. A 3.0 mL portion of the Purified Water test extract was solvent exchanged with 3.0 mL of dichloromethane. A 3.0 mL portion of the Purified Water control was solvent exchanged with 3.0 mL of dichloromethane. The resulting solutions were analyzed by GC/MS for semi-volatiles using the following instrument conditions.

Instrument: Shimadzu QP 2010 GC/MS			
Column	HP-5MS J+W Scientific 30m length x 0.25mm i.d. x 0.25µm d _f		
Column Oven Temp.	40.0°C	Injection Temp.	250.0°C
Injection Mode	Splitless	Sampling Time	1.00 min
Pressure	63.9 kPa	Total Flow	26.9 mL/min
Column Flow	1.20 mL/min	Purge Flow	1.7 mL/min
Ion Source Temp.	230.0°C	Carrier Gas	Helium
Solvent Cut Time	2.50 min	Interface Temp.	300.0°C
Detector Gain	0.00 kV	Detector Gain Mode	Relative
Injection Volume	1 µL	Threshold	10
Oven Temperature Program			
Rate(°C/min)	Temperature(°C)		Hold time(min)
-	40.0		4.00
10.0	280.0		4.00
45.0	310.0		10.00

Standards of phenanthrene at concentrations of 2.0, 25 and 50 ppm were prepared and injected in triplicate. A linear regression was performed using the resulting peak areas. A library scan of the test article extract was performed to tentatively identify any peaks not attributed to the blank, with the results listed in the Results Section.



REFERENCES

U.S. Pharmacopeia, Section 88, current revision.

WuXi AppTec SOP: ALS-0260, Sample Extraction Procedures

WuXi AppTec Reference Library Contents, Form ALS-4650-1

WuXi AppTec SOP: ILS-0018, Environmental Conditions in the Animal Facility

WuXi AppTec SOP: ILS-0092, Receiving Shipments of Animals

WuXi AppTec SOP: ILS-0100, ISO/USP/Japanese Intracutaneous Reactivity Test

WuXi AppTec SOP: ILS-0112, Animal Identification

WuXi AppTec SOP: ILS-0230, Euthanasia Procedures

WuXi AppTec SOP: ILS-0233, Proper Handling of Sick and Moribund Animals

WuXi AppTec SOP: TRG-0300, Preparation of Biomaterials for Extraction



FINAL STUDY REPORT

STUDY TITLE: ISO Guinea Pig Maximization Sensitization Test

PROTOCOL NUMBER: 900850-25

TEST ARTICLE IDENTIFICATION:

Trade Name: ENSEAL G2 MIM I-Blades
Generic Name: MIM 17-4 Stainless Steel with TiN Coating
R-spec: R2003-02-H, SPE002436-A
Engr. Study: PRC055882
Color: Gold
Reference Number: EE2725
Lot # PRC055882

PERFORMING LABORATORY:

WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR:

Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

STUDY NUMBER: 167722

CLIENT MNEMONIC: ETH02

RESULT SUMMARY:

The test article **did not** elicit a sensitization response under the conditions of this assay.



QUALITY ASSURANCE UNIT SUMMARY

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the quality and integrity of the study.

<u>Phase Inspected</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Induction I	06/01/12	06/05/12	07/05/12
Final Report	07/05/12	07/05/12	07/05/12

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: _____

Scott W. Sherman
Scott W. Sherman

Date: 7/5/12

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: _____

Katai Nkhata
Katai Nkhata

Date: 07/05/12

Professional Personnel Involved:

Teri Tanquist, BS
Christine Olson, BS
Roxanne Miller, AA, CVT
Katai Nkhata, MA
Jean Mesarich, AA

Vice President of Process Improvement and Operations
Director of Study Operations
Associate Director, In-Life Studies
Study Director
Client Relations Manager

**PURPOSE**

This test was designed to evaluate the allergenic potential or sensitizing capacity of a test article. The test was used as a procedure for the screening of contact allergens in guinea pigs and extrapolating the results to humans, but it does not establish the actual risk of sensitization.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

DATE SAMPLE RECEIVED: 03/26/12
STUDY INITIATION DATE: 05/08/12
STUDY COMPLETION DATE: 07/05/12

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: ENSEAL G2 MIM I-Blades Generic Name: MIM 17-4 Stainless Steel with TiN coating R-spec: R2003-02-H, SPE002436-A Engr. Study: PRC055882 Color: Gold Reference Number: EE2725
Lot/Batch #:	PRC055882
Sterilization Method:	Gamma 60 kGy
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Device
Physical Description:	According to the Sponsor, the test article consisted of 1.5" gold rectangular rods.

CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.



EXPERIMENTAL DESIGN

Experimental Summary

In selecting a new material for human contact in medical applications, it is important to ensure that the material will not stimulate the immune system to produce an allergic reaction. The reaction generally is due to substances that may leach out of a material. Therefore, this practice provides for the use of material extracts. The use of Freund's complete adjuvant (FCA) and Sodium Lauryl Sulfate (SLS) tend to enhance the potential of weak sensitizing agents. While this test does not ensure that test materials are completely non-allergenic, it is the most sensitive animal test in common use today.

Eleven test guinea pigs (per extract) were injected with the test article extract and FCA, and six guinea pigs (per extract) were injected with the corresponding control blank and FCA. On Day 6, the dorsal site was re-shaved and SLS in mineral oil was applied. The day after the SLS application, the test animals were topically patched with the appropriate test extract and the control animals were patched with the corresponding control blank. The patches were removed after 48 ± 2 hours of exposure. Following an approximate two-week rest period, the animals were topically patched with the appropriate test extract and corresponding control blank. The patches were removed after 24 ± 2 hours of exposure. The dermal patch sites were observed for erythema and edema 24 ± 2 and 48 ± 2 hours after patch removal. Each animal was assessed for a sensitization response based upon the dermal scores. The test results were based upon the percentage of animals exhibiting a sensitization response.

Justification For Selection Of The Test System

The albino guinea pig has historically been used in skin sensitization tests and is generally accepted as the most appropriate animal model for human allergic contact dermatitis. The guidelines have no alternative (non-animal) methods.

Institutional Animal Care and Use Committee (IACUC)

The protocol and any amendments or procedures involving the care or use of animals on this study were reviewed and approved by WuXi AppTec's IACUC prior to the initiation of such procedures.

IACUC Protocol / Effective Date: 98-02F / May, 2012.

AMENDMENTS:

1. Section 9.5.3 in the Protocol was amended to change the volume of 10% SLS to 0.5 mL to 1.0 mL.
2. Section 7.3 in the Protocol was amended to reflect the new IACUC Protocol revision and effective date.

DEVIATIONS: None.

IDENTIFICATION OF THE TEST SYSTEM

Species/Strain: Albino guinea pigs (*Cavia porcellus*), Hartley strain (specific pathogen free).

Source: Charles River Laboratories.

Sex: Male.

Weight Range: All animals weighed between 356.7 g and 439.1 g upon assignment to the test.

Age: All animals were approximately 6 weeks of age at the start of the study.

Animal Identification: The animals were identified per WuXi AppTec SOP: ILS-0112.

HUSBANDRY

Receipt: Animals were received on 05/23/12 according to WuXi AppTec SOP: ILS-0092. Each animal was examined for signs of disease and injury. The animals were acclimated for a minimum of 5 days under the same conditions as the actual test.



Housing: Animals were housed in solid bottom cages with contact bedding and up to two guinea pigs per cage. Housing density complied with NIH and AAALAC International recommendations. The test and negative control animals were housed separately.

Environment: The environmental conditions in the animal rooms were set to be maintained according to WuXi AppTec SOP: ILS-0018. The temperature and photo-period were set to meet the AAALAC International recommendations for these species. The laboratory and animal rooms were maintained as limited-access facilities.

Diet: Animals were supplied with certified commercial guinea pig feed, *ad libitum*. No known contaminants present in the feed were expected to interfere with the test results.

Water: Potable water was obtained from the St. Paul municipal water supply. No known contaminants present in the water were expected to interfere with the test results.

TEST MATERIAL PREPARATION

The test article remained intact, was placed into test tubes and extracted at a ratio 4 g to 20 mL of extraction vehicle.

TABLE 1: TEST ARTICLE RECORD

TEST PHASE AND VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	NUMBER OF TEST ARTICLE DEVICES USED
First Induction Injection Normal Saline (NS)	2.0	10.0	2
First Induction Injection Cottonseed Oil (CSO)	2.0	10.0	2
Second Induction Patch NS	2.0	10.0	2
Second Induction Patch CSO	2.0	10.0	2
Challenge Patch NS	2.0	10.0	2
Challenge Patch CSO	2.0	10.0	2

TEST ARTICLE EXTRACTION

The extraction mixtures and corresponding control blanks were incubated for 72 ± 2 hours at 50 ± 2 °C. At the start of the extraction, the solutions appeared clear and free of particulates. The extracts were manually agitated. At the end of the extraction period, the vessels were shaken well and the liquid aseptically decanted into a sterile glass vessel. The test article was observed after all extractions to be intact with no macroscopically observable degradation and the extracts were clear. After decanting, the extracts were not filtered prior to use. The extracts were maintained at room temperature and used within 24 hours of preparation. See Tables 1-3.

TABLE 2: EXTRACTION RECORD

TEST PHASE AND VEHICLE	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END
First Induction Injection NS	50.2 °C	05/29/12 7:52 am	50.2 °C	06/01/12 6:14 am
First Induction Injection CSO	50.2 °C	05/29/12 7:52 am	50.2 °C	06/01/12 6:14 am
Second Induction Patch NS	50.2 °C	06/05/12 8:05 am	50.2 °C	06/08/12 6:21 am
Second Induction Patch CSO	50.2 °C	06/05/12 8:05 am	50.2 °C	06/08/12 6:21 am
Challenge Patch NS	50.2 °C	06/21/12 7:32 am	50.2 °C	06/24/12 6:05 am
Challenge Patch CSO	50.2 °C	06/21/12 7:32 am	50.2 °C	06/24/12 6:05 am



TABLE 3: VEHICLE RECORD

VEHICLE IDENTIFICATION:	TEST PHASE	LOT #	SUPPLIED BY:	EXPIRATION
0.9% Normal Saline (NS)	First, Second & Challenge	J2B510	Braun	08/2014
Cottonseed Oil NF (CSO)	First & Second	ZN1009B	Spectrum	06/15/12
Cottonseed Oil NF (CSO)	Challenge	2AL080B	Spectrum	12/08/12

TABLE 4: ADDITIONAL TEST REAGENT RECORD

ADDITIONAL TEST REAGENTS:	LOT #	SUPPLIED BY:	EXPIRATION
FCA	1117270	Difco	05/03/14
10% SLS in Mineral Oil	TDSL-0016	WuXi AppTec	11/09/12
0.9% Sterile Saline	J1K443	Braun	02/2014

SELECTION OF ANIMALS

Animals were randomly placed in cages upon receipt and were assigned on test as available. Animals considered unsuitable due to poor health or outlying body weight were excluded from the study.

ANIMAL PREPARATION

The application sites were prepared by clipping the fur of the test site using an electric clipper with an appropriate blade. Prior to the induction phases, an approximate 5 x 7 cm area over the shoulder region was shaved. Prior to challenge, an approximate 4 x 4 cm area of the right and left flank was shaved.

TEST ARTICLE ADMINISTRATION

First Induction / Intradermal Injection: Three syringes were prepared for the test animals and three for the negative control animals as indicated in Table 5. The prepared syringes were injected in pairs on each side of the dorsal mid-line. The six injection sites were within the boundaries of a 2 x 4 cm area.

TABLE 5: FIRST INDUCTION SYRINGE PREPARATION AND DOSE VOLUME

PREPARATION	VOLUME INJECTED PER SITE	SYRINGE CONTENTS	RATIO (v/v)
TEST GROUP			
Syringe 1	0.1 mL	FCA + 0.9% Sterile Saline	1:1
Syringe 2	0.1 mL	Test Extract	NA
Syringe 3	0.1 mL	FCA + 0.9% Sterile Saline (1:1) + Test Extract	1:1
CONTROL GROUP			
Syringe 1	0.1 mL	FCA + 0.9% Sterile Saline	1:1
Syringe 2	0.1 mL	Control Vehicle	NA
Syringe 3	0.1 mL	FCA + 0.9% Sterile Saline (1:1) + Control Vehicle	1:1

Second Induction / Topical Application: On Day 6, the injection site area was clipped free of fur and treated with 0.5 mL to 1.0 mL of 10% (w/w) SLS prepared by mixing solid SLS with mineral oil. The day following the SLS treatment, the remaining SLS residue was gently wiped from the area with gauze.

On Day 7, the test article extracts (0.3 mL) were applied to a 2 x 4 cm piece of filter paper (Whatman) to saturation and applied after SLS removal. The patch was secured to the site with non-permeable tape and the trunk wrapped with elastic bandage and Transpore[®] tape. The control animals received a similar patch with the control vehicles. Freshly prepared extracts were used for this administration. This preparation was removed after 48 ± 2 hours of application.

Challenge Patch / Topical Application: Fourteen days after completion of the topical induction phase, the challenge procedure was initiated on the twenty-two test animals and the twelve negative control animals. A filter paper patch was saturated with 0.3 mL of freshly prepared test article extract and applied to the fur clipped right flank of each test animal. A filter paper patch was saturated with 0.3 mL of freshly prepared control vehicle and applied to the fur clipped left flank of each test animal.



The negative control animals were challenged in an identical fashion with similarly prepared patches. The left side of each animal was patched with a filter paper patch saturated with 0.3 mL of control vehicle. The right side was patched with a filter paper patch saturated with 0.3 mL of the prepared test article extract applied to the fur clipped flank. The trunk of each animal was wrapped for 24 ± 2 hours with an expandable wrapping material and secured with tape.

OBSERVATIONS AND SCORING

The following day (24 ± 2 hours) after challenge exposure, the patches were removed and the site was wiped gently with a 70% isopropyl alcohol soaked gauze sponge prior to each scoring period. The challenge sites were observed for irritation and sensitization reaction, as indicated by erythema and edema. Daily challenge observation scores were recorded 24 ± 2 and 48 ± 2 hours after patch removal in accordance with the classification system for skin reactions in Table 6. Daily animal health observations were recorded throughout the study period.

TABLE 6: DERMAL OBSERVATION SCORING (AS PER MAGNUSSON AND KLIGMAN SCALE)

PATCH TEST REACTION	GRADING SCALE
No visible change – No erythema and edema	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and/or swelling	3

Note: Erythema is defined as redness and edema is defined as a swelling at the challenge site. Any other adverse changes at the skin sites were recorded and reported.

TERMINATION

Animals were euthanized by CO₂ asphyxiation following completion of this test.

EVALUATION CRITERIA

- Grades of '1' or greater in the test group generally indicate sensitization, provided grades of less than '1' are observed on the control animals. If grades of '1' or greater are noted on control animals, then the reactions of the test animals which exceed the most severe control reaction are presumed to be due to sensitization.
- Occasionally, the test group has a greater number of animals showing a response than the controls, although the intensity of the reaction is not greater than that observed on the controls. In these instances, a re-challenge may be necessary to define the response clearly. A re-challenge shall be carried out 1-2 weeks after the first challenge using the same animals. The method used shall be as described for the first challenge, with the exception that testing will be performed on a naïve site.
- The outcome of the test will be presented as the frequency of positive challenge results in test and control animals.

ASSAY VALIDITY

Final evaluation of the validity of the assay and test article results was based upon the following criteria and scientific judgment:

A test was considered invalid and will be repeated if:

- Less than ten test animals or less than five control animals survived the duration of the study.
- Any animals developed frank infection of the injection sites.



METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS: Not applicable.

STATISTICAL METHODS: None used.

RECORD RETENTION: An exact copy of the original final report and all raw data pertinent to this study will be stored by WuXi AppTec, Inc. It was the responsibility of the Sponsor to retain a sample of the test article.

COMPLIANCE

Animal Welfare

WuXi AppTec, Inc. maintains the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accreditation. All applicable portions of this study conformed to the following regulations and guidelines regarding animal care and welfare:

- NIH guidelines as reported in the "Guide for the Care and Use of Laboratory Animals," National Research Council of the National Academies, eighth edition, 2011;
- (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Health Research Extension Act of 1985 (Public Law 99-158), Revised 1986;
- USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1, 2, and 3, *Animal Welfare*, Final Rule 1989; and
- WuXi AppTec Policy on Humane Care.

International Standards

ISO 10993-10: 2010. Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Skin Sensitization, pp. 18-26.

ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

TEST ARTICLE DISPOSITION: Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

Clinical Observations: None of the animals in the study showed abnormal clinical signs during the test period.

Main Test Results: None of the negative control animals challenged with the control vehicles were observed with a sensitization response greater than '0'. None of the test animals challenged with the test article extracts were observed with a sensitization response greater than '0'. A negative sensitization incidence was interpreted for all test animals. See Table 7 for individual animal scores.



TABLE 7: DAILY CHALLENGE OBSERVATIONS

NORMAL SALINE (NS)					
ANIMAL #	24 HOURS SCORE		48 HOURS SCORE		RESULTS (+) OR (-)
	TEST GROUP				
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
2841	0	0	0	0	-
2842	0	0	0	0	-
2843	0	0	0	0	-
2844	0	0	0	0	-
2845	0	0	0	0	-
2846	0	0	0	0	-
2847	0	0	0	0	-
2848	0	0	0	0	-
2849	0	0	0	0	-
2850	0	0	0	0	-
2851	0	0	0	0	-
ANIMAL #	NEGATIVE CONTROL GROUP				RESULTS (+) OR (-)
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
2835	0	0	0	0	-
2836	0	0	0	0	-
2837	0	0	0	0	-
2838	0	0	0	0	-
2839	0	0	0	0	-
2840	0	0	0	0	-

COTTONSEED OIL (CSO)					
ANIMAL #	24 HOURS SCORE		48 HOURS SCORE		RESULTS (+) OR (-)
	TEST GROUP				
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
2859	0	0	0	0	-
2860	0	0	0	0	-
2861	0	0	0	0	-
2862	0	0	0	0	-
2863	0	0	0	0	-
2864	0	0	0	0	-
2865	0	0	0	0	-
2866	0	0	0	0	-
2867	0	0	0	0	-
2868	0	0	0	0	-
2869	0	0	0	0	-
ANIMAL #	NEGATIVE CONTROL GROUP				RESULTS (+) OR (-)
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
2853	0	0	0	0	-
2854	0	0	0	0	-
2855	0	0	0	0	-
2856	0	0	0	0	-
2857	0	0	0	0	-
2858	0	0	0	0	-

Positive Control: WuXi AppTec completes positive controls every 3 months. A positive control was completed on 03/08/12 (see Table 8 for individual animal scores). The methods for the positive control assay are similar to the methods described above in the "Experimental Summary." Guinea pigs utilized for positive control studies are of the Hartley strain and are supplied by the same vendor as animals used for general testing (Charles River Laboratories). For the Induction I and Induction II phases, a known sensitizer, 0.3% dinitrochlorobenzene (DNCB) in ethanol, was used. For the challenge phase, 0.15% DNCB in acetone was used. The negative control animals were exposed to the appropriate vehicle (ethanol was used for the Inductions I and II and acetone was used for the challenge).

The positive control data presented here is from a re-challenge experiment, conducted one week after the initial challenge. The sensitization rate for the test animals at 24 hour following the re-challenge was identical to what had been observed at the first challenge (91%) with scores of 1-2 for the test animals, with the exception of one animal (Table 8).

Three animals in the negative control group were observed with a sensitization response after the re-challenge (scores of 1 and 2, see Table 8), but this was not unexpected since this was a re-challenge in which the controls had their first exposure to the DNCB at the challenge phase a week earlier. This observation suggests that these three control animals mounted a primary immune response in the intervening one week upon initial DNCB exposure and developed the immunological memory necessary for the delayed hypersensitivity response upon re-exposure to the DNCB. This response was however, limited to the 24 hour time point and was not sustained at 48 hours like in the majority of the test animals.

The sensitization rate for the test animals at 48 hours following the re-challenge was 82% with two animals not showing signs of skin redness/sensitization. The sensitization rate for the control animals following the re-challenge at 48 hours was 0% (Table 8).



A sustained sensitization response was demonstrated in the test group at 48 hours, with a majority of the animals exhibiting discrete or patchy erythema (scores of 1) or moderate and confluent erythema (a score of 2). This represented an incidence of 82% and a severity index of approximately 1 for the sensitization reaction in the test group. By contrast, all of the animals in the negative control group exhibited no erythema or edema at 48 hours when treated with the 0.15% w/v mixture of DNCB in acetone (an incidence of 0% and a severity index of 0). Based on the incidence and severity index, the scores of 1 or 2 in the positive control test group at 48 hours are considered to be sensitization reactions. Therefore this test methodology demonstrated dermal sensitization in guinea pigs using DNCB, a known sensitizer.

TABLE 8: POSITIVE (DNCB) AND NEGATIVE CONTROL DAILY RE-CHALLENGE OBSERVATIONS

POSITIVE CONTROL GROUP					
ANIMAL #	24 HOUR SCORE		48 HOUR SCORE		RESULTS (+) OR (-)
	CONTROL VEHICLE	DNCB SOLUTION	CONTROL VEHICLE	DNCB SOLUTION	
0428	0	1	0	1	+
0429	0	1	0	1	+
0430	0	1	0	1	+
0431	0	1	0	0	-
0432	0	0	0	0	-
0433	0	1	0	1	+
0434	0	2	0	2	+
0435	0	1	0	1	+
0436	0	1	0	1	+
0437	0	1	0	1	+
0438	0	1	0	1	+
Total of Scores	0	11	0	10	
Severity (Total / 11)	0/11	11/11	0/11	10/11	
Incidence	0%	91%	0%	82%	
NEGATIVE CONTROL GROUP					
ANIMAL #	24 HOUR SCORE		48 HOUR SCORE		RESULTS (+) OR (-)
	CONTROL VEHICLE	DNCB SOLUTION	CONTROL VEHICLE	DNCB SOLUTION	
0422	0	2 ^a	0	0	-
0423	0	1 ^a	0	0	-
0424	0	0	0	0	-
0425	0	0	0	0	-
0426	0	0	0	0	-
0427	0	1 ^a	0	0	-
Total of Scores	0	4	0	0	
Severity (Total / 6)	0/6	4/6	0/6	0/6	
Incidence	0%	50%	0%	0%	

DNCB= dinitrochlorobenzene ^aErythema observed on three control animals because this was a re-challenge

ANALYSIS AND CONCLUSION

None of the negative control animals challenged with the control vehicle were observed with a sensitization response greater than '0'. None of the animals challenged with the test article extracts were observed with a sensitization response greater than '0'. The normal saline extract of the test material had a sensitization response of '0' under valid test conditions. The cottonseed oil extract of the test material had a sensitization response of '0' under valid test conditions. Under the conditions of this protocol, the test article **did not** elicit a sensitization response.



REFERENCES

ASTM Designation: F720-81 (Reapproved 2002) Standard Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test. Section 13, Volume 13.01, pp. 268-270.

Guidance for Industry (Draft Guidance), 2008: Coronary Drug - Eluting Stents - Nonclinical and Clinical Studies: Companion Document, pg. 12. US Department of Health and Human Services, FDA. March, 2008.

Magnusson, B. and Kligman, A.M. "Allergic Contact Dermatitis in the Guinea Pig, Identification of Contact Allergens." Springfield, Ill: Thomas. 1970.

Magnusson, B. and Kligman, A.M. "The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test." *J. Invest. Dermatol* 52:268-276. 1969.

WuXi AppTec Reference Library Contents, Form ALS-4650-1.

WuXi AppTec SOP: ILS-0018, Environmental Conditions in the Animal Facility.

WuXi AppTec SOP: ILS-0092, Receiving Shipments of Animals.

WuXi AppTec SOP: ILS-0112, Animal Identification.

WuXi AppTec SOP: ILS-0155, ISO Guinea Pig Maximization Sensitization Test.

WuXi AppTec SOP: ILS-0233, Proper Handling of Sick, Injured, and/or Moribund Animals.

WuXi AppTec SOP: ILS-0165, ISO Guinea Pig Positive Control Validation for Maximization Sensitization Test.



Diet: Animals were supplied with a certified commercial rodent diet. No known contaminants present in the feed were expected to interfere with the test results.

Water: Animals were supplied with potable water obtained from the St. Paul municipal water supply. No known contaminants present in the water were expected to interfere with the results.

TEST MATERIAL PREPARATION

The test article was extracted intact, placed into test tubes and prepared at a ratio of 4 g to 20 mL of extraction vehicle.

TABLE 1: TEST ARTICLE RECORD

EXTRACT VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	NUMBER OF TEST ARTICLES USED PER EXTRACT
0.9% Normal Saline (NS)	2.0	10.0	2
Cottonseed Oil (CSO)	2.0	10.0	2

Test Article Extraction: The extraction mixtures and corresponding control blanks were incubated for 72 ± 2 hours at 50 ± 2 °C. At the start of the extraction, the solutions appeared clear and free of particulates. The extracts were agitated during the course of the extraction period. At the end of the extraction period, the vessels were shaken well and the liquid aseptically decanted into a sterile glass vessel. The test article was observed after all extractions to be intact with no macroscopically observable degradation and the extracts were clear. After decanting, the extracts were not filtered prior to use. The extracts were maintained at room temperature and used within 24 hours of preparation. See Tables 1-3.

TABLE 2: EXTRACTION RECORD

VEHICLE	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END
NS	50.2 °C	05/18/12 8:23 am	50.2 °C	05/21/12 6:31 am
CSO	50.2 °C	05/18/12 8:23 am	50.2 °C	05/21/12 6:31 am

TABLE 3: VEHICLE RECORD

VEHICLE IDENTIFICATION:	LOT #	SUPPLIED BY:	EXPIRATION
NS	J2B510	Braun	08/2014
CSO	ZN1009B	Spectrum	06/15/12

SELECTION OF ANIMALS

Animals were randomly placed in cages upon receipt and were placed on study as available. Animals considered unsuitable due to poor health or outlying body weight were excluded from the study.

TEST ARTICLE ADMINISTRATION

Groups of five animals were injected with either the test article extract or the corresponding control vehicle as indicated in the table below:

EXTRACT OR CONTROL	ROUTE	DOSE/KG	INJECTION RATE
NS	Intravenous	50 mL	~0.1 mL/sec
CSO	Intraperitoneal	50 mL	Not Applicable



OBSERVATIONS

Body Weights: Body weight recordings, to the nearest 0.1 g, were made on the day of dosing and at 24 ± 2 , 48 ± 2 and 72 ± 2 hours post-injection. See Data Table 5 for individual weight results.

Clinical Signs: Observations for mortality and signs of pharmacological and/or toxicological effects were made immediately post-injection and at 4 ± 0.75 , 24 ± 2 , 48 ± 2 , and 72 ± 2 hours post-injection.

TERMINATION

Following the final observations the animals were euthanized by CO₂ asphyxiation.

EVALUATION CRITERIA

According to ISO guidelines, the test is considered negative if none of the animals injected with the test article extract show a significantly greater biological reaction than the animals treated with the control vehicle extract. Death in two or more mice or other toxic signs such as convulsions, prostration, or body weight loss greater than 10% in three or more mice are interpreted as significant biological reactions.

ASSAY VALIDITY

Final evaluation of the validity of the assay and test article results was based upon the following criteria and scientific judgment:

A control failure is defined as death in two or more control animals showing signs of toxicity such as convulsions or prostration or weight loss of more than 10% of body weight in three or more mice.

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS: Not applicable.

STATISTICAL METHODS: Descriptive statistics are presented in Data Table 5.

RECORD RETENTION: An exact copy of the original final report and all raw data pertinent to this study will be stored by WuXi AppTec, Inc. It was the responsibility of the Sponsor to retain a sample of the test article.

COMPLIANCE

Animal Welfare

WuXi AppTec, Inc. maintains the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accreditation. All applicable portions of the study will also conform to the following regulations and guidelines regarding animal care and welfare:

- NIH guidelines as reported in the "Guide for the Care and Use of Laboratory Animals," National Research Council of the National Academies, eighth edition, 2011;
- (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Health Research Extension Act of 1985 (Public Law 99-158), Revised 1986;
- USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1, 2, and 3, *Animal Welfare*, Final Rule 1989; and
- WuXi AppTec Policy on Humane Care.

International Standards

The study was also in compliance with the following International Standards:

ISO 10993-11: 2006 Biological Evaluation of Medical Devices Part 11: Tests for Systemic Toxicity.

ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.



TEST ARTICLE DISPOSITION: Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

None of the animals on study were observed with abnormal clinical signs indicative of toxicity during the 72 hour test period. All were alive at the end of the 72 hour test duration and body weight loss was within acceptable parameters over the course of the study. See Table 4.

TABLE 4: MORTALITY, CLINICAL SIGNS AND WEIGHT LOSS INCIDENCE

EXTRACT	FATALITIES		TOXICITY CLINICAL SIGNS		ANIMALS WITH >10% BODY WEIGHT LOSS	
	TEST	CONTROL	TEST	CONTROL	TEST	CONTROL
NS	0/5	0/5	0/5	0/5	0/5	0/5
CSO	0/5	0/5	0/5	0/5	0/5	0/5

TABLE 5: ANIMAL WEIGHTS (g) AND STANDARD DEVIATION CALCULATIONS

GROUP	ANIMAL #	INITIAL	24 HRS	48 HRS	72 HRS	BW CHANGE
Test NS	1	24.6	24.1	24.0	24.4	-0.2
	2	22.5	22.1	22.3	22.9	0.4
	3	25.1	24.7	24.5	24.5	-0.6
	4	24.1	24.0	24.2	24.9	0.8
	5	24.3	24.2	24.4	25.5	1.2
Average Body Weight		24.1	23.8	23.9	24.4	0.3
Standard Deviation		1.0	1.0	0.9	1.0	0.7
Control NS	21	24.3	24.1	24.0	24.9	0.6
	22	24.1	24.0	24.1	24.6	0.5
	23	23.5	23.5	23.3	24.0	0.5
	24	21.4	21.5	21.7	22.4	1.0
	25	21.9	21.4	21.4	23.0	1.1
Average Body Weight		23.0	22.9	22.9	23.8	0.7
Standard Deviation		1.3	1.3	1.3	1.1	0.3
Test CSO	11	26.2	24.1	25.4	26.1	-0.1
	12	23.7	22.5	24.6	24.3	0.6
	13	23.8	22.7	24.2	23.6	-0.2
	14	21.9	21.0	21.8	21.8	-0.1
	15	25.7	24.4	25.8	26.3	0.6
Average Body Weight		24.3	22.9	24.4	24.4	0.2
Standard Deviation		1.7	1.4	1.6	1.9	0.4
Control CSO	31	22.0	20.9	21.0	22.2	0.2
	32	23.8	22.8	23.2	24.5	0.7
	33	23.7	22.9	23.8	23.9	0.2
	34	26.5	24.7	24.4	25.1	-1.4
	35	24.1	23.2	23.3	24.4	0.3
Average Body Weight		24.0	22.9	23.1	24.0	0.0
Standard Deviation		1.6	1.4	1.3	1.1	0.8

ANALYSIS AND CONCLUSION

The vehicle control treated animals had no signs of toxicity at any of the observation periods and no animals lost weight in excess of 10%, indicating a valid test. None of the test article extract treated animals were observed with clinical signs consistent with toxicity at any of the observation periods. Body weight changes were within acceptable parameters over the course of the study. These findings indicate that the requirements of the ISO Acute Systemic Injection Test have been met by the test article.



REFERENCES

U.S. Pharmacopeia, Section 88, current revision.

WuXi AppTec Reference Library Contents, Form ALS-4650-1

WuXi AppTec SOP ALS-0260, Sample Extraction Procedures

WuXi AppTec SOP: ILS-0018, Environmental Conditions in the Animal Facility

WuXi AppTec SOP: ILS-0092, Receiving Shipments of Animals

WuXi AppTec SOP: ILS-0112, Animal Identification

WuXi AppTec SOP: ILS-0115, ISO/USP/Japanese Acute Systemic Injection Test

WuXi AppTec SOP: ILS-0233, Proper Handling of Sick, Injured, and/or Moribund Animals



FINAL STUDY REPORT

STUDY TITLE: ISO Intracutaneous Reactivity Test

PROTOCOL NUMBER: 9107015-2

TEST ARTICLE IDENTIFICATION: Trade Name: ENSEAL G2 MIM I-Blades
Generic Name: MIM 17-4 Stainless Steel with Tin coating
R-spec: R2003-02-H, SPE002436-A
Engr. Study: PRC055882, Color: Gold
Reference Number: EE2725 Lot # PRC055882

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery, Inc.
4545 Creek Road
Cincinnati, OH 45242-2803

STUDY NUMBER: 167723

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: The requirements of the ISO Intracutaneous Reactivity Test **have been met** by the test article.

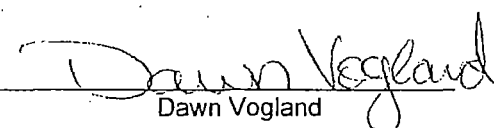


QUALITY ASSURANCE UNIT SUMMARY

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the quality and integrity of the study.

<u>Phase Inspected</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Scoring	05/23/12	05/23/12	06/07/12
Final Report	06/07/12	06/07/12	06/07/12

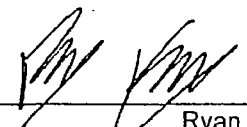
The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor:  Date: 6/7/12
Dawn Vogland

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58.

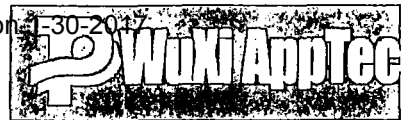
The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director:  Date: 6/7/12
Ryan Koslofsky

Professional Personnel Involved:

Teri Tanquist, BS
Christine Olson, BS
Roxanne Miller, AA, CVT
Ryan Koslofsky, MS
Jean Mesarich, AA

Vice President of Process Improvement and Operations
Study Operations Director
Associate Director, In-Life Studies
Study Director
Client Relations Manager



PURPOSE

The purpose of this test was to determine if any chemicals that may leach or be extracted from the test article were capable of causing local irritation in the dermal tissues of rabbits.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

DATE SAMPLE RECEIVED: 03/26/12
STUDY INITIATION DATE: 05/08/12
STUDY COMPLETION DATE: 06/07/12

TEST ARTICLE IDENTIFICATION :

Test Article Name:	Trade Name: ENSEAL G2 MIM I-Blades, Generic Name: MIM 17-4 Stainless Steel with Tin coating, R-spec: R2003-02-H, SPE002436-A, Engr. Study: PRC055882, Color: Gold, Reference Number: EE2725
Lot/Batch #:	PRC055882
Sterilization Method:	Gamma 60kGy
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Devices
Physical Description:	According to the Sponsor, the test article consisted of 1.5" gold rectangular rods

CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

EXPERIMENTAL DESIGN

Experimental Summary

The study was conducted in accordance with ISO 10993-10: 2010 Standard, Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Skin Sensitization, Pages 11-14. Each rabbit received five sequential 0.2 mL intracutaneous injections along either side of the dorsal mid-line with the test article extract on one side and the concurrent vehicle control on the other. The irritation reaction of the test extracts were compared to vehicle controls and recorded over a 72-hour period according to the standard ISO irritation scoring system.



Justification For Selection Of The Test System

This test method and species have historically been used to assess the potential of the material under test to produce intradermal irritation to help determine biocompatibility of materials used in medical devices. The animal species, number and route of test article administration will be as recommended in ISO 10993-10.

Institutional Animal Care and Use Committee (IACUC)

The protocol and any amendments or procedures involving the care or use of animals on this study were reviewed and approved by WuXi AppTec's IACUC prior to the initiation of such procedures.

IACUC Protocol / Effective Date: 07-122B / June, 2010

AMENDMENTS/DEVIATIONS: None.

TEST SYSTEM

Species/Strain: All of the animals used in this testing were albino rabbits (*Oryctolagus cuniculus*) / New Zealand White strain.

Source: The animals were obtained from Bakkom Rabbitry, a previously approved vendor of commercial laboratory animals.

Sex: Male/Female. Females used were nulliparous and non-pregnant.

Weight Range: Animals weighed between 2.5 – 2.9 kg at the experimental start of the study.

Age: The rabbits were young adults.

Number: A total of three animals were used for this study.

Animal Identification: Individual animals were identified per WuXi AppTec SOP: ILS-0112.

HUSBANDRY

Receipt: Animals were received on 05/08/12 according to WuXi AppTec SOP: ILS-0092. The animals were acclimated for a minimum of 5 days under the same conditions as the actual test.

Housing: Animals were individually housed in stainless steel caging. Housing dimensions complied with NIH and AAALAC International guidelines for this species.

Environment: The environmental conditions in the animal rooms were maintained according to WuXi AppTec SOP: ILS-0018. The temperature and photo-period were set to meet the AAALAC International recommendations for these species. The laboratory and animal rooms were maintained as limited-access facilities.

Diet: Animals were supplied with certified commercial feed, *ad libitum*. There were no known contaminants present in the feed expected to interfere with the test results. Feed analysis results are available and archived by WuXi AppTec.

Water: Potable water was supplied from the municipal water supply, *ad libitum*. There were no known contaminants present in the water expected to interfere with the test results. Periodic analysis of the water is conducted and the results are archived by WuXi AppTec.

TEST AND CONTROL MATERIAL PREPARATION

The test article was cut for extraction, placed into test tubes and prepared at a ratio of 4 g to 20 mL of extraction vehicle.



TABLE 1: TEST ARTICLE RECORD

EXTRACT VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	NUMBER OF TEST ARTICLE DEVICES USED PER VESSEL
0.9% Normal Saline (NS)	1.5	7.5	1.5
Cottonseed Oil (CSO)	1.5	7.5	1.5

Test Article Extraction: The extraction mixtures and corresponding control blanks were incubated for 72 ± 2 hours at 50 ± 2 °C. At the start of the extraction, the solutions appeared clear and free of particulates. The extracts were agitated during the course of the extraction period. At the end of the extraction period, the vessels were shaken well and the liquid aseptically decanted into a sterile glass vessel. The test article was observed after all extractions to be intact with no macroscopically observable degradation and the extracts were clear. After decanting, the extracts were not filtered prior to use. The extracts were maintained at room temperature and used within 24 hours of preparation. See Tables 1-3.

TABLE 2: EXTRACTION RECORD

Vehicle	Extraction Temperature (In)	Date/Time of Extraction Start	Extraction Temperature (Out)	Date/Time of Extraction End
NS	50.2 °C	05/18/12 8:23 am	50.2 °C	05/21/12 6:31 am
CSO	50.2 °C	05/18/12 8:23 am	50.2 °C	05/21/12 6:31 am

TABLE 3: VEHICLE RECORD

Vehicle Identification:	Lot #	Supplied By:	Expiration
NS	J2B510	Braun	08/2014
CSO	ZN1009B	Spectrum	06/15/12

SELECTION OF ANIMALS

Animals were randomly placed in cages upon receipt, and were placed on study as available. Any animals considered unsuitable due to poor health, abraded skin or outlying body weight were excluded from the study.

ANIMAL PREPARATION

Each animal was weighed and the weight recorded prior to test injection. The fur of the animals was clipped on both sides of the spinal column to expose a sufficient sized area for injection.

TEST ARTICLE ADMINISTRATION

The two test article extracts and the two vehicle controls were each injected into three rabbits. Each rabbit received five sequential 0.2 mL intracutaneous injections of the test article extract on the right side of the vertebral column and similarly the control vehicle on the left side. The second test and control extract injections were parallel and distal to the first injection sites. (See Figure 1.)

	CONTROL		HEAD	TEST		
	Control Vehicle #2	Control Vehicle #1		Test Extract #1	Test Extract #2	
	1	1		1	1	
0.2 mL Control	2	2		2	2	
Vehicle Injected	3	3		3	3	
	4	4		4	4	
	5	5		5	5	
			TAIL			

0.2 mL Test
Article Extract
Injected

FIGURE 1: INJECTION SITES ON RABBITS

**OBSERVATIONS AND SCORING**

The animals were observed daily for abnormal clinical signs. The appearance of each injection site was noted at 24 ± 2 , 48 ± 2 and 72 ± 2 hours post injection. The tissue reactions were rated for gross evidence of erythema and edema. The skin was lightly swabbed with dilute alcohol to enhance the appearance of any erythema or edema. The intradermal injection of CSO frequently elicits an inflammatory response. CSO erythema scores ≤ 2 are considered normal. A well-defined positive response is characterized by a score greater than 2. Table 4 was used to score the reactions.

TABLE 4: DERMAL OBSERVATION SCORING

ERYTHEMA	EDEMA
0 = No erythema	0 = No edema
1 = Very slight erythema (barely perceptible)	1 = Very slight edema (barely perceptible)
2 = Well defined erythema	2 = Well defined edema (edges of area well-defined by definite raising)
3 = Moderate to severe erythema	3 = Moderate edema (raised ~1 mm)
4 = Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4 = Severe edema (raised > 1 mm and extending beyond area)

TERMINATION

All animals were euthanized according to WuXi AppTec SOP: ILS-0230 with a sodium pentobarbital based solution after the 72-hour observations were recorded.

EVALUATION CRITERIA

According to ISO 10993:10 test criteria, if the difference between the average scores for the extract of the test article and the vehicle control is less than or equal to 1.0, the test article is considered non-irritating.

ASSAY VALIDITY

Final evaluation of the validity of the assay and test article results was based upon scientific judgment.

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS: See calculations section.

STATISTICAL METHODS: None used.

RECORD RETENTION: An exact copy of the original final report and all raw data pertinent to this study will be stored by WuXi AppTec, Inc. It was the responsibility of the Sponsor to retain a sample of the test article.



COMPLIANCE

Animal Welfare

WuXi AppTec, Inc. maintains the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accreditation. All applicable portions of the study will also conform to the following regulations and guidelines regarding animal care and welfare:

- NIH guidelines as reported in the "Guide for the Care and Use of Laboratory Animals," National Research Council of the National Academies, eighth edition, 2011;
- (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Health Research Extension Act of 1985 (Public Law 99-158), Revised 1986.
- USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1, 2, and 3, Animal Welfare, Final Rule 1989;
- WuXi AppTec Policy on Humane Care.

The study was also performed in compliance with the following International Standards:

ISO 10993-10: 2010 Standard, Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Skin Sensitization, Pages 11-14.

ISO 10993-12: 2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

TEST ARTICLE DISPOSITION: Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

Clinical Observations

None of the animals on study showed abnormal clinical signs during the 72 hour test period.

Dermal Observations

Moderate to severe erythema (score of 3) was observed on rabbit # 26982 at CSO Test injection sites 1 and 3 at the 48 hour observation period.

Moderate to severe erythema (score of 3) was observed on rabbit # 26982 at CSO Control injection sites 1, 2, and 3, and CSO Test injection sites 1, 2, 3, and 5 at the 72 hour observation period.

These observations should not have a direct impact on the study interpretation as the criteria for a passing study is based on the difference between the mean test and control scores and not the scores of the individual rabbits.

There were no other significant dermal reactions observed at the injected test and control sites on the rabbits at the 24, 48 and 72 hour observation periods.

CALCULATIONS

After the 72 ± 2 hour observation period, all erythema grades plus edema grades 24 ± 2 , 48 ± 2 , and 72 ± 2 hour were totaled separately for each test sample or control for each individual animal. To calculate the score of a test sample or control on each individual animal, each of the totals was divided by 15 (three scoring time points x five test or control sample injection sites). To determine the overall mean score for each test sample and each corresponding control, the scores were added for the three animals and divided by three. The final test sample score was obtained by subtracting the score of the control from the test sample score. The results are presented in Tables 5 and 6.



TABLE 5: DERMAL OBSERVATIONS – 0.9% NORMAL SALINE

Rabbit # 26982	Control Scores						Test Scores					
	24 Hour ER ED		48 Hour ER ED		72 Hour ER ED		24 Hour ER ED		48 Hour ER ED		72 Hour ER ED	
Site 1	0	0	0	0	0	0	0	0	0	0	0	0
Site 2	0	0	0	0	0	0	0	0	0	0	0	0
Site 3	0	0	0	0	0	0	0	0	0	0	0	0
Site 4	0	0	0	0	0	0	0	0	0	0	0	0
Site 5	0	0	0	0	0	0	0	0	0	0	0	0
Total	0		0		0		0		0		0	

Rabbit # 26983	Control Scores						Test Scores					
	24 Hour ER ED		48 Hour ER ED		72 Hour ER ED		24 Hour ER ED		48 Hour ER ED		72 Hour ER ED	
Site 1	0	0	0	0	0	0	0	0	0	0	0	0
Site 2	0	0	0	0	0	0	0	0	0	0	0	0
Site 3	0	0	0	0	0	0	0	0	0	0	0	0
Site 4	0	0	0	0	0	0	0	0	0	0	0	0
Site 5	0	0	0	0	0	0	0	0	0	0	0	0
Total	0		0		0		0		0		0	

Rabbit # 26987	Control Scores						Test Scores					
	24 Hour ER ED		48 Hour ER ED		72 Hour ER ED		24 Hour ER ED		48 Hour ER ED		72 Hour ER ED	
Site 1	0	0	0	0	0	0	0	0	0	0	0	0
Site 2	0	0	0	0	0	0	0	0	0	0	0	0
Site 3	1	0	0	0	0	0	0	0	0	0	0	0
Site 4	0	0	0	0	0	0	0	0	0	0	0	0
Site 5	0	0	0	0	0	0	0	0	0	0	0	0
Total	1		0		0		0		0		0	

Rabbit #	Control Scores (Total ER & ED)						Test Scores (Total ER & ED)					
26982	0		0		0		0		0		0	
26983	0		0		0		0		0		0	
26987	1		0		0		0		0		0	

Rabbit #	Total / 15						Total / 15					
26982	0						0					
26983	0						0					
26987	0.1						0					
Average (Total / 3)	0 / 3 = 0						0 / 3 = 0					

Comparative Results (Average Test – Average Control)				0 – 0 = 0			
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ER=Erythema ED=Edema

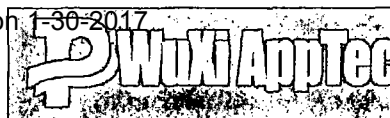


TABLE 6: DERMAL OBSERVATIONS – COTTONSEED OIL

Rabbit # 26982	Control Scores						Test Scores					
	24 Hour ER ED		48 Hour ER ED		72 Hour ER ED		24 Hour ER ED		48 Hour ER ED		72 Hour ER ED	
Site 1	2	0	2	0	3	0	2	0	3	0	3	0
Site 2	2	0	2	0	3	1	2	0	2	0	3	0
Site 3	2	0	2	0	3	0	2	0	3	0	3	0
Site 4	2	0	2	0	2	0	2	0	2	0	2	1
Site 5	2	1	2	0	2	1	2	0	2	0	3	0
Total	11		10		15		10		12		15	

Rabbit # 26983	Control Scores						Test Scores					
	24 Hour ER ED		48 Hour ER ED		72 Hour ER ED		24 Hour ER ED		48 Hour ER ED		72 Hour ER ED	
Site 1	1	0	2	0	1	0	2	1	2	0	1	0
Site 2	1	1	2	0	1	0	2	1	2	0	1	0
Site 3	1	0	1	0	1	0	1	1	1	0	1	0
Site 4	1	0	1	1	1	0	1	0	1	1	1	0
Site 5	1	0	1	1	1	1	1	0	2	0	1	0
Total	6		9		6		10		9		5	

Rabbit # 26987	Control Scores						Test Scores					
	24 Hour ER ED		48 Hour ER ED		72 Hour ER ED		24 Hour ER ED		48 Hour ER ED		72 Hour ER ED	
Site 1	2	1	2	0	1	0	2	0	1	0	1	0
Site 2	1	1	1	0	1	0	2	1	2	0	1	0
Site 3	1	0	1	0	1	0	2	1	2	1	2	0
Site 4	1	0	1	0	1	0	2	0	2	0	2	0
Site 5	1	0	1	0	1	0	2	0	1	0	1	0
Total	8		6		5		12		9		7	

Rabbit #	Control Scores (Total ER & ED)						Test Scores (Total ER & ED)					
26982	11						10					
26983	6						10					
26987	8						12					

Rabbit #	Total / 15						Total / 15					
26982	2.4						2.5					
26983	1.4						1.6					
26987	1.3						1.9					
Average (Total / 3)	5.1 / 3 = 1.7						6.0 / 3 = 2.0					

Comparative Results (Average Test – Average Control)				2.0 – 1.7 = 0.3			
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ER=Erythema ED=Edema

ANALYSIS AND CONCLUSION

The test was considered valid based upon scientific judgment. The differences in the mean test and control scores of the extract dermal observations were **less than 1.0**, indicating that the requirements of the ISO Intracutaneous Reactivity Test **have been met** by the test article.



EXPERIMENTAL DESIGN

Experimental Summary

Test articles and controls were prepared for direct testing. Rabbit blood was diluted to achieve an approximate concentration of 10 mg/mL hemoglobin. Diluted blood was then added to the test and control materials. These mixtures were incubated for at least three hours at 37 ± 2 °C.

After this incubation, the mixtures were transferred to centrifuge tubes and centrifuged to remove cellular materials. After centrifugation, supernatants were observed. A cyanmethemoglobin reagent was then added to the supernatant and these samples were read at 540 nm on a spectrophotometer. The hemolytic index was calculated based on these results.

Justification For Selection Of The Test System

Rabbit blood has been used extensively for testing the hemolytic potential of medical device materials. In this case, the use of rabbit blood is specified in ASTM Method F-756-08.

Amendments / Deviations: None

IDENTIFICATION OF THE TEST SYSTEM

New Zealand White rabbit blood: 22417, 21094, 21092

The animals appeared healthy. Age, weight, sex are not factors for this test.

Source of Supply: WuXi AppTec approved vendor

TEST MATERIAL PREPARATION

The test article was cut for testing. A ratio of 4 g / 20 mL was used per the Sponsor's request.

TABLE 1: TEST ARTICLE RECORD

SAMPLE	AMOUNT OF TEST ARTICLE USED PER REPLICATE
Test Article	1 1/3

BLOOD COLLECTION AND PREPARATION

Three healthy, mature New Zealand White rabbits of either sex, from a certified commercial vendor, were selected as donors for this study. Five to seven milliliters of blood was collected from each rabbit using appropriate venipuncture, employing evacuated tubes containing 3.2% sodium citrate. The time and date of blood collection and rabbit ear tag number was recorded directly on the vial, and the blood was used within 96 hours of collection. Blood was stored under refrigeration until time of use. Just prior to use, the blood was pooled and tested to ensure that the plasma free hemoglobin level was less than 2 mg/mL. The whole blood was then diluted with an appropriate volume of phosphate buffered saline (PBS, calcium and magnesium free) to obtain a total blood hemoglobin concentration of 10 ± 1 mg/mL.

STANDARD CURVE

A hemoglobin standard (concentration 2.0 mg/mL), obtained from STANBIO, was used to create the standard curve. A modified National Commission for Clinical Laboratory Studies cyanmethemoglobin reagent (NCCLS CMR) (0.14 g potassium monophosphate, 0.05 g potassium cyanide, 0.2 g potassium ferricyanide, and 0.5 mL BRIJ 35 – pH 7.0-7.4) was used to dilute the stock solution to 1.0, 0.72, 0.6, 0.3, 0.12, 0.06 and 0.03 mg/mL.

After a five minute room temperature incubation, the preparations were scanned with a Beckman spectrophotometer at 540 nm. A standard curve was established by the reading from the spectrophotometer. The slope used for analysis is derived by the curve when the concentration is on the y-axis and the absorbance is on the x-axis. In the prepared curve, the axes are switched due to the spectrophotometer plotting template; therefore, the reciprocal of this slope was calculated to determine the actual value (F), which is used to calculate the unknown concentration.

TEST PROCEDURE

Phosphate buffered saline (PBS, calcium and magnesium free) was added to the test article replicates at a ratio of 20 mL to 4 g. See Table 2 for the ratios used for the controls. Next, diluted blood was added to



the test and control solutions at a ratio of 1.0 mL blood to 7.0 mL PBS. These mixtures were incubated for three hours at 37 ± 2 °C. After this incubation, the liquid was removed from the samples and transferred to centrifuge tubes. The tubes were centrifuged at 2400 rpm for 15 minutes to remove cellular material.

A cyanmethemoglobin reagent (NCCLS CMR reagent) was added to each of the sample supernatants at a 1:1 ratio. 1.0 mL of NCCLS CMR reagent was combined with 1.0 mL supernatant and incubated for 5 minutes at room temperature. After 5 minute incubation, the samples were read at a wavelength of 540 nm on a spectrophotometer. The hemolytic index was then calculated based on these results. See Table 6 for the condition of the test sample and control supernatant.

TABLE 2: TEST ARTICLE AND CONTROL PREPARATIONS

DESCRIPTION	SIZE	PBS VOLUME (mL)	DILUTED RABBIT BLOOD VOLUME (mL)
Test Article	# 1) 1.4 g	7.0	1.0
	# 2) 1.4 g	7.0	1.0
	# 3) 1.4 g	7.0	1.0
Blank Control	# 1) NA	7.0	1.0
	# 2) NA	7.0	1.0
	# 3) NA	7.0	1.0
Negative Control	# 1) 42.0 cm ²	7.0	1.0
	# 2) 42.0 cm ²	7.0	1.0
	# 3) 42.0 cm ²	7.0	1.0
Positive Control	# 1) 42.0 cm ²	7.0	1.0
	# 2) 42.0 cm ²	7.0	1.0
	# 3) 42.0 cm ²	7.0	1.0

NA = Not Applicable

TABLE 3: REAGENT/CONTROL RECORD

REAGENT / CONTROL IDENTIFICATION	CLASS	LOT #	SUPPLIED BY:	EXPIRATION
Hemoglobin	Standard	27011	STANBIO	09/2013
NCCLS CMR	Reagent	TDS1134	WuXi AppTec	08/16/12
Phosphate Buffered Saline (PBS, calcium and magnesium free)	Blank/Vehicle	0000270426	Lonza	11/28/13
Nitrile	Positive Control	WXAT073010	Kimberly-Clark	07/30/15
High Density Polyethylene (HDPE)	Negative Control	C-091	Halano Research Institute, FDSC	02/2016

TABLE 4: BLOOD INCUBATION

VEHICLE	INCUBATION TEMPERATURE (IN)	DATE/TIME OF INCUBATION START	INCUBATION TEMPERATURE (OUT)	DATE/TIME OF INCUBATION END
PBS (Ca and Mg free)	37.0 °C	04/19/12 10:53 am	37.0 °C	04/19/12 1:53 pm

TEST EVALUATION

TABLE 5: HEMOLYTIC GRADE SYSTEM

BLANK CORRECTED HEMOLYTIC INDEX ABOVE THE NEGATIVE CONTROL	CLASSIFICATION	INTERPRETATION
0-2%	Non - hemolytic	Pass
2.1-5.0%	Slightly hemolytic	Pass
5.1% or greater	Hemolytic	Fail

ASSAY VALIDITY

Final evaluation of the validity of the assay was based on the following criteria and scientific judgment. The blood must have a plasma free hemoglobin value less than 2 mg/mL. The negative control should



have a hemolytic index less than or equal to 2% and the positive control must have a hemolytic index greater than 5%.

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS

The following equations were used as applicable to analyze data.

Plasma Free Hemoglobin (PFH), reported in mg/mL.

$$PFH = (A^{PFH}) \times F \times 2$$

A^{PFH} = absorbance of untreated, centrifuged blood sample

$F = 1 / \text{slope of calibration curve}$

Total Blood Hemoglobin Concentration (TBHC)

$$TBHC = (A^C) \times F \times 251$$

$$\text{Verified TBHC} = (A^T) \times F \times 16$$

A^C = average absorbance of untreated blood; $F = 1 / \text{slope of calibration curve}$

A^T = average absorbance of blood dilution

Sample Hemoglobin Concentration

$$S = (A^S) \times F \times 2$$

A^S = average absorbance of sample or control; $F = 1 / \text{slope of calibration curve}$

Blank Hemoglobin Concentration

$$B = (A^B) \times F \times 2$$

A^B = average absorbance of blank; $F = 1 / \text{slope of calibration curve}$

% Hemolytic Index (% HI)

$$\% HI = \left(\frac{\text{Concentration of Hemoglobin released in supernatant}}{\text{Total Hemoglobin Concentration in tube}} \right) \times 100$$

Blank Corrected % Hemolytic Index (% BCHI)

$$\% BCHI = \% HI (\text{test group}) - \% HI (\text{blank})$$

% Hemolysis (% H)

$$\% H = (S/W) \times 100$$

S = Sample Hemoglobin Concentration; W = Total Hemoglobin / 8

Blank Corrected % Hemolysis

$$\% BCH = \frac{A^S - A^B}{A^T - A^B} \times 100$$

A^S = average absorbance of sample; A^B = average absorbance of blank

A^T = average absorbance for total hemoglobin



Blank Corrected Hemolytic Index Above the Negative Control

$$BCHI^{AN} = BCHI^T - BCHI^N$$

$BCHI^{AN}$ = blank corrected hemolytic index above the negative control

$BCHI^T$ = average blank corrected hemolytic index of the test sample

$BCHI^N$ = average blank corrected hemolytic index of the negative control

STATISTICAL METHODS: None used.

RECORD RETENTION

A certified copy of this report and data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

COMPLIANCE

International Standards

ASTM Guideline F 619-03. Standard Practice for Extraction of Medical Plastics. 2010. Annual Book of ASTM Standards, Volume 13.01: 182-185.

ASTM Guideline F-756-08. Standard Practice for Assessment of Hemolytic Properties of Materials. 2010. Annual Book of ASTM Standards, Volume 13.01: 314-318.

ISO 10993-4:2002 and Amendment 1, 2006. Standard Biological Evaluation of Medical Devices, Part 4 – Selection of Tests for Interaction with Blood.

ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

TEST ARTICLE DISPOSITION: Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

This assay met the criteria for a valid assay. The blood used in this assay had a plasma free hemoglobin level of 0.578 mg/mL. The total hemoglobin content of the blood was 272.26 mg/mL prior to dilution. After dilution the hemoglobin level was confirmed to be 9.892 mg/mL.

The negative control replicates returned a blank corrected hemolytic index of 0.1%. The positive control replicates returned a blank corrected hemolytic index of 8.9%. The Hemolytic Grade system is presented in Table 5. The test results are presented in Table 7.

After centrifugation, the condition of the control and test article supernatants were observed (See Table 6).

TABLE 6: CONDITION OF SAMPLE SUPERNATANT AFTER CENTRIFUGATION

SAMPLE/CONTROL	APPEARANCE OF THE SUPERNATANT
Test Article	Colorless and free of particulate
Blank Control	Colorless and free of particulate
Negative Control	Colorless and free of particulate
Positive Control	Clear dark red and free of particulate

The test article returned a hemolytic index value less than that found in the negative control and fell within the non-hemolytic range.



TABLE 7: RESULTS

GROUP	ABSORBANCE	AVERAGE ABSORBANCE	AVERAGE BLANK CORRECTED %HEMOLYSIS	AVERAGE BLANK CORRECTED %HEMOLYTIC INDEX	AVERAGE BLANK CORRECTED %HEMOLYTIC INDEX ABOVE THE NEGATIVE CONTROL
Test Article	0.002	0.003	0.2	0.0	0.0*
	0.003				
	0.003				
Blank Control	0.003	0.002	0.0	0.0	NA
	0.002				
	0.002				
Negative Control	0.003	0.003	0.6	0.1	NA
	0.003				
	0.004				
Positive Control	0.122	0.122	72.3	8.9	NA
	0.118				
	0.125				

*Negative result reported as zero (0).

NA = Not Applicable

CONCLUSION

Based on the criteria set forth in the protocol, this assay was valid. Therefore, it was capable of correctly assessing the hemolytic potential of test articles. In this case, the test article, is considered **non-hemolytic** under the test conditions employed and **passes** this test.

REFERENCES

International Committee for Standardization in Haematology. *J. Clin. Pathol.* 1978, 31:139-423.

Malinauskas, R.A., "Plasma Hemoglobin Measurement Techniques for the *In Vitro* Evaluation of Blood Damage Caused by Medical Devices." *Artificial Organs.* 1997; 21:1255-1267.

Moore, G.L., Ledford, M.E., Merydith, A. A., "Micromodification of the Drabkin Hemoglobin Assay for Measuring Plasma Hemoglobin in the Range of 5-2000 mg/dL." *Biochem. Med.* 1981; 26:167-173.

WuXi AppTec Reference Library Contents, Form ALS-4650-1

WuXi AppTec SOP: MED-8910, ASTM Hemolysis Assay



FINAL STUDY REPORT

STUDY TITLE: ISO Acute Systemic Injection Test

PROTOCOL NUMBER: 901770-21

TEST ARTICLE IDENTIFICATION: Trade Name: ENSEAL G2 MIM I-Blades, Generic Name: MIM 17-4 Stainless Steel with TiN coating, R-spec: R2003-02-H, SPE002436-A, Engr. Study: PRC055882, Color: Gold, Reference Number: EE2725
Lot # PRC055882

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery, Inc.
4545 Creek Road
Cincinnati, OH 45242

STUDY NUMBER: 167724

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: The requirements of the ISO Acute Systemic Injection Test **have been met** by the test article.



QUALITY ASSURANCE UNIT SUMMARY

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the quality and integrity of the study.

<u>Phase Inspected</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Ending	05/24/12	05/25/12	06/05/12
Final Report	06/04/12	06/04/12	06/05/12

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: Kim Steele Date: 6/5/12
Kim Steele

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: Michelle Dietzel Date: 6/5/12
Michelle Dietzel

Professional Personnel Involved:

Teri Tanquist, BS	Vice President of Process Improvement and Operations
Christine Olson, BS	Director of Study Operations
Roxanne Miller, AA, CVT	Associate Director, In-Life Studies
Michelle Dietzel, BS	Senior Study Director
Jean Mesarich, AA	Client Relations Manager



PURPOSE: The purpose of this test was to screen test article extracts or solutions for potential toxic effects as a result of a single-dose systemic injection in mice.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

DATE TEST ARTICLE RECEIVED: 03/26/12
STUDY INITIATION DATE: 05/08/12
STUDY COMPLETION DATE: 06/05/12

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: ENSEAL G2 MIM I-Blades, Generic Name: MIM 17-4 Stainless Steel with TiN coating, R-spec: R2003-02-H, SPE002436-A, Engr. Study: PRC055882, Color: Gold, Reference Number: EE2725
Lot/Batch #:	PRC055882
Sterilization Method:	Gamma 60 kGy
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Device
Physical Description:	According to the Sponsor, the test article consisted of 1.5" gold rectangular rods.

CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

EXPERIMENTAL DESIGN

Experimental Summary

Animals were treated by intravenous or intraperitoneal routes to screen solutions or test article extracts for potential toxic effects as a result of a single-dose systemic injection. The animal species, number, and route of test article administration were as recommended in ISO 10993-11.



For the safety evaluation of the test article, mice were injected systemically with extracts of the test article in standard solutions (normal saline and cottonseed oil). The animals were observed for signs of toxicity immediately after injection and at 4, 24, 48, and 72 hours post-injection. The requirements of the test are met if none of the animals treated with the test article extract have a significantly greater adverse reaction than the animals treated with the vehicle control.

Justification For Selection Of The Test System

Mice were used in this study because they have historically been used in systemic safety evaluation studies and the guidelines have no alternative (non-animal) methods. Animals were treated by intravenous and intraperitoneal routes. The animal species, number, and route of test article administration were as recommended in ISO 10993-11.

Institutional Animal Care and Use Committee (IACUC)

The protocol and any amendments or procedures involving the care or use of animals on this study were reviewed and approved by the WuXi AppTec IACUC prior to the initiation of such procedures.

IACUC Protocol / Effective Date: 98-03E / June, 2010

AMENDMENTS / DEVIATIONS: None.

IDENTIFICATION OF TEST SYSTEM

Species/Strain: Albino Swiss mice (*Mus musculus*), CFW, naive

Source: Charles River Laboratories

Sex: Female. Females used were nulliparous and not pregnant.

Weight Range: All animal weights were within $\pm 20\%$ of the mean body weight (21.4 g - 26.5 g) at the start of the study.

Age: All animals were approximately 6 weeks old at the start of the study.

Animal Identification: The animals were identified per WuXi AppTec SOP: ILS-0112.

Animal Numbers:	Mouse #
0.9% Normal Saline Test Group:	1 - 5
0.9% Normal Saline Control Group:	21 - 25
Cottonseed Oil Test Group:	11 - 15
Cottonseed Oil Control Group:	31 - 35

HUSBANDRY

Receipt: Animals were received on 05/09/12 according to WuXi AppTec SOP: ILS-0092. Each animal was examined for signs of disease and injury. The animals were acclimated for a minimum of 5 days under the same conditions as the actual test.

Housing: Animals were housed in groups of five in polycarbonate cages with contact bedding. Housing density complied with AAALAC International recommendations and NIH guidelines. The test and control animals were housed separately.

Environment: The environmental conditions in the animal rooms were maintained according to WuXi AppTec SOP: ILS-0018. The temperature and photo-period were set to meet the AAALAC International recommendations for these species. The laboratory and animal rooms were maintained as limited-access facilities.



PURPOSE

The purpose of this procedure was evaluate to the cytotoxic response of a specified mammalian culture cell line when exposed to an extract of the test article. This assay utilized the sensitivity of low-cell density to evaluate the cytotoxicity of medical devices.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

TEST ARTICLE RECEIVED: 03/26/12
INITIATION DATE: 03/27/12
COMPLETION DATE: 05/07/12
AMENDED REPORT DATE: 05/08/12

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: ENSEAL G2 MIM I-Blades, Generic Name: MIM 17-4 Stainless Steel with TiN coating, R-spec: R2003-02-H, SPE002436-A, Engr. Study: PRC055882, Color: Gold, Sterilization: 60 kGy Gamma, Reference Number: EE2725
Lot #:	PRC055882
Sterilization Method:	60 kGy Gamma
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Devices
Physical Description:	According to the Sponsor, the test article consisted of 1.5" gold rectangular rods.

CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

EXPERIMENTAL DESIGN

Experimental Summary

Test articles and controls were prepared and extracted for 24 ± 2 hours at 37 ± 1 °C. Appropriate dilutions of the extracts were prepared using V79 Plant Media. After removing spent medium, 2.0 mL of the extract was added to the plates. The cells were incubated at 37 ± 1 °C for 6-7 days before fixation, staining, and counting.



Justification For Selection Of The Test System

The extract colony assay is one of several *in vitro* mammalian cell tests which have been used to screen materials for their potential cytotoxicity. The V79 cell line has a history of use in assays of this type.

AMENDMENT: Sections 7.1 and 7.2 of the protocol were modified to update the cell line to V79 cells.

Sections 4.2.3, 7.1, 9.1 and 9.2.4 of the protocol were modified to update the extraction media to V79 Plant Media (EMEM + 5% FBS, NEAA, SP).

Section 8.0 of the protocol was modified to the following:

Cultures of V79 cells will be obtained from Health Protection Agency Culture Collections (HPACC #189072). Cell lines that are mycoplasma-free are purchased from the vendor and are kept frozen in the lab until use. Cultures are grown and used as monolayers in disposable tissue culture labware at $37 \pm 1^\circ\text{C}$ in a humidified atmosphere of $5 \pm 1\%\text{CO}_2$ in air at WuXi AppTec. The V79 Plant Media used for growth of cells is Eagle's minimal essential medium (EMEM) supplemented with 5% (v/v) fetal bovine serum (FBS). The medium is also supplemented with the following: 2 mM L-glutamine, 0.05 mM non-essential amino acids (NEAA), 1 mM sodium pyruvate (SP), 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 u/mL penicillin, and 1% 2.50 $\mu\text{g/mL}$ amphotericin B (Fungizone). WuXi AppTec has a long-standing history using antibiotics in culture medium. The use of antibiotics in culture media has not shown adverse effects when used for this assay; this is exhibited in the varying degrees of toxicity seen in the assay controls.

Section 9.3 of the protocol was updated to the following:

The test article and controls will be extracted in the appropriate volume of V79 Plant Media (EMEM + 5% FBS, NEAA, SP) in a sterile vessel for 24 ± 2 hours at $37 \pm 1^\circ\text{C}$ in a humidified atmosphere of $5 \pm 1\%\text{CO}_2$. At the end of the extraction period, the extracts will be diluted with V79 Plant Media, as shown below:

Extract	Final Extract Concentrations (v/v)						
	2.0%	1.5%	1.0%	0.75%	0.50%	0.25%	0.125%
Positive ZDEC							
Positive ZDBC	100%		56.2%		31.6%		17.7%
Negative Control	100%						
Test Article	100%	75%	50%	25%	12.5%	6.25%	3.125%
Cell Control	100%						

A V79 cell suspension will be diluted using V79 Plant Media to a final concentration of 33.3 cells/mL. A volume of 3 mL (approximately 100 cells) of diluted cell suspension will be seeded into each well of a 6 well plate (well size, 35 mm) and incubated for 24 ± 2 hours at $37 \pm 1^\circ\text{C}$ in a humidified atmosphere of $5 \pm 1\%\text{CO}_2$. After incubation, the maintenance culture medium will be decanted from test culture wells and replaced with 2 mL of each dilution from above. The test article and control extracts and cell control media will be added to the culture plates in triplicate wells. The plates will then be incubated at $37 \pm 1^\circ\text{C}$ in a humidified atmosphere of $5 \pm 1\%\text{CO}_2$ in air for 6-7 days. At the end of the incubation period, the extracts will be removed from the plates and the wells will be rinsed with 2 mL of phosphate buffered saline without magnesium or calcium (PBS (-)). The PBS will be decanted and the colonies will be fixed by placing 1 mL of methanol into each well for 2-10 minutes. Once the cells are fixed, they will be stained for 2-3 hours with 1 mL of Giemsa stain solution that has been diluted 1:20 with PBS(-) (pH 6.5-7.5). At the end of the staining period, the stain will be decanted and 2 mL of PBS(-) will be added to each well. The colonies will then be counted after the removal of PBS(-).

The ZDBC positive control failed to meet the validity criteria using L-929 cells. According to the JMHLW guidelines, V79 cells are suitable for testing. The V79 cells have been validated and produced valid ZDBC results. Therefore, testing was completed using this cell line and methodology.



DEVIATIONS: None.

IDENTIFICATION OF TEST SYSTEM

Cultures of V79 cells were obtained from Health Protection Agency Culture Collections (HPACC #189072). Cell lines that are mycoplasma-free were purchased from the vendor and were kept frozen in the lab until use. Cultures were grown and used as monolayers in disposable tissue culture labware at $37 \pm 1^\circ\text{C}$ in a humidified atmosphere of $5 \pm 1\%$ CO_2 in air at WuXi AppTec. The V79 Plant Media used for growth of cells is Eagle's minimal essential medium (EMEM) supplemented with 5% (v/v) fetal bovine serum (FBS). The medium is also supplemented with the following: 2 mM L-glutamine, 0.05 mM non-essential amino acids (NEAA), 1 mM sodium pyruvate (SP), 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 units/mL penicillin, and 1% 2.50 $\mu\text{g}/\text{mL}$ amphotericin B (Fungizone). WuXi AppTec Inc. has a long-standing history using antibiotics in culture medium. The use of antibiotics in culture media has not shown adverse effects when used for this assay; this is exhibited in the varying degrees of toxicity seen in the assay controls.

TEST ARTICLE PREPARATION

The test article was cut for extraction, placed into test tubes, and prepared at a ratio of 2 g to 20 mL of extraction vehicle.

TABLE 1: TEST ARTICLE RECORD

VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	NUMBER OF TEST ARTICLE DEVICES USED
V79 Plant Media	3.1	31.0	3

CONTROLS PREPARATION

A negative control (high density polyethylene film) was prepared at a ratio of 2 grams / 20 mL. A moderately positive control (polyurethane film containing 0.1% zinc diethyldithiocarbamate (ZDEC)) was prepared at a ratio of 2 grams / 20 mL. A weak positive control (polyurethane film containing 0.25% zinc dibutyldithiocarbamate (ZDBC)) was prepared at a ratio of 2 grams / 20 mL. The cell control consisted V79 plant elution media and was incubated in parallel with test article and controls.

EXTRACTION MEDIA

The V79 Plant Media used for growth of cells is Eagle's minimal essential medium (EMEM) supplemented with 5% (v/v) fetal bovine serum (FBS). The medium is also supplemented with the following: 2 mM L-glutamine, 0.05 mM non-essential amino acids (NEAA), 1 mM sodium pyruvate (SP), 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 units/mL penicillin, and 1% 2.50 $\mu\text{g}/\text{mL}$ amphotericin B (Fungizone). The pH was confirmed to be 7.29.

TEST ARTICLE EXTRACTION

The extraction mixture and controls were incubated for 24 ± 2 hours at $37 \pm 1^\circ\text{C}$ in a humidified atmosphere of $5 \pm 1\%$ CO_2 . At the start of the extraction, the solutions appeared clear and free of particulates. At the end of the extraction period, the vessels were shaken well and the liquid aseptically decanted. The test article was observed after extraction to be intact with no macroscopically observable degradation and the extract was clear. After decanting the extract was not filtered and was used immediately. See Tables 1-3.

TABLE 2: EXTRACTION RECORD

VEHICLE	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END
V79 Plant Media	37.0 $^\circ\text{C}$	04/10/12 2:51 pm	37.0 $^\circ\text{C}$	04/11/12 1:28 pm



TABLE 3: CONTROL / CELL LINE RECORD

CONTROL IDENTIFICATION:	CLASS	LOT #	SUPPLIED BY:	EXPIRATION
ZDEC	Positive Control	A-091	Hatano	10/2014
ZDBC	Positive Control	B-102	Hatano	05/2015
High Density Polyethylene (HDPE)	Negative Control	C-091	Hatano	02/2016
V79 Plant Media	Medium	VP041012	WXAT	05/10/12
V79	Cell Line	VP041012	HPACC	NA

NA = Not applicable

EXPERIMENTAL PROCEDURE

The test article was extracted in the appropriate volume of V79 Plant media in a sterile vessel for 24 ± 2 hours at 37 ± 1°C in a humidified atmosphere of 5 ± 1% CO₂ in air.

TABLE 4: DILUTION RATIOS

EXTRACT	% NEAT EXTRACTS						
Positive ZDEC	2.0%	1.5%	1.0%	0.75%	0.50%	0.25%	0.125%
Positive ZDBC	100%		56.2%		31.6%		17.7%
Negative Control	100%						
Test Article	100%	75%	50%	25%	12.5%	6.25%	3.125%
Cell Control	100%						

After incubation, the maintenance culture medium was decanted from test culture wells and replaced with 2 mL of each dilution from Table 4. The test article and control extracts and cell control media were added to the culture plates in triplicate wells. The plates were then incubated at 37 ± 1 °C in a humidified atmosphere of 5 ± 1% CO₂ in air for 6-7 days.

At the end of the incubation period, the extracts were removed from the plates and the wells were rinsed with 2 mL of phosphate buffered saline without magnesium or calcium (PBS (-)). The PBS was decanted and the colonies were fixed by placing 1 mL of methanol into each well for 2-10 minutes. Once the cells were fixed, they were stained for 2-3 hours with 1 mL of Giemsa stain solution that had been diluted 1:20 with PBS(-) (pH 6.5-7.5). At the end of the staining period, the stain was decanted and 2 mL of PBS(-) was added to each well. The colonies will were then counted after the removal of PBS(-).

EVALUATION CRITERIA

The relative colony formation rates (% RCF) were plotted against the % dilutions of the extract on a semi-logarithmic graph using Microsoft Excel. The resulting inhibition curve of each graph was used to determine the % dilution that inhibited colony formation to 50%. This is the IC₅₀ value. Sigma Stat was used to perform a t-test comparing the cell control results with the average colony count from the negative control % dilutions.

VALIDITY

Final evaluation of the validity of the assay and test article results was based upon the criteria listed below and scientific judgment.

The t-test results must show that statistically significant inhibition of colony formation was not found in the negative control results.

IC₅₀ values of the positive ZDEC and ZDBC controls must not exceed the following values:

Positive ZDEC: 7% and Positive ZDBC: 80%



METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS

The colonies were counted and the triplicate wells averaged for each % dilution. These averages were divided by the average number of colonies obtained in the cell control wells. This percentage is the Relative Colony Formation for each % dilution.

STATISTICAL METHODS

A t-test was performed using Sigma Stat and a difference of $p < 0.05$ was considered significant.

RECORD RETENTION

A certified copy of this report and data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

TEST ARTICLE DISPOSITION: Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

Raw data and calculated results, including averages and relative colony formation are presented in Table 5. The test article extract was in contact with the test system for seven days.

A dramatic, dose dependant decrease in relative colony formation was noted as a result of increasing concentrations in the positive control preparations (ZDEC and ZDBC), indicating a sensitive assay (Table 5). Additionally, the relative colony formation (% RCF) in preparations treated with the negative control showed high degrees of survival. ZDEC induced a maximum IC_{50} of 0.60% while ZDBC returned an IC_{50} of 42.74%. Both met the criteria for a valid assay. Accordingly, this assay was determined to be valid.

None of the standard dilutions of the test article induced biologically relevant decreases in colony formation. Further, no dose response trends were noted.



TABLE 5: Results

DOSE GROUP	% OF NEAT EXTRACT	WELL 1	WELL 2	WELL 3	AVERAGE	% RCF**	IC ₅₀
Test Article	100	29	43	25	32	97	NA*
	75	26	25	31	27	82	
	50	19	26	23	23	70	
	25	21	29	30	27	82	
	12.5	39	27	32	33	100	
	6.25	37	30	40	36	109	
	3.125	39	30	29	33	100	
ZDEC	2.0	0	0	0	0	0	0.60
	1.5	0	0	0	0	0	
	1.0	0	0	0	0	0	
	0.75	26	19	18	21	64	
	0.50	32	27	24	28	85	
	0.25	33	32	28	31	94	
	0.125	28	34	29	30	91	
ZDBC	100	0	0	0	0	0	42.74
	56.2	18	8	11	12	36	
	31.6	32	36	19	29	88	
	17.7	26	27	25	26	79	
Negative Control	100	33	33	30	32	97	NA
Cell Control	100	34	29	35	33	100	NA

* This value could not be calculated as a 50% value was not achieved.

** Relative Colony Formation = Average colony formation divided by average colony formulation of cell control.

NA = Not applicable

ANALYSIS AND CONCLUSION

Negative and positive controls were run concurrently with the test article to provide ranges of cytotoxicity. Each of the positive controls induced relative colony formation well within acceptable limits. No significant differences in colony formation were found between the negative control treated wells and the cell control. The results from the control cultures indicate that the assay retained normal sensitivity. Therefore, the criteria for a valid assay were met.

None of the standard dilutions of the test article induced biologically relevant decreases in colony formation. Further, no dose response trends were noted. Therefore, an IC₅₀ could not be calculated for the test article. These results would be indicative of a **non-cytotoxic** material.

REFERENCES

Guidelines for Basic Biological Tests of Medical Materials and Devices – Part I Cytotoxicity Test (Colony Assay). Japanese Ministry of Health and Welfare, 2003.

ISO 10993-5: 2009 Biological Evaluation of Medical Devices, Part 5: Tests for *In Vitro* Cytotoxicity.

WuXi AppTec SOP: MED-8710, Extract Colony Assay Cytotoxicity Test



AMENDMENT TO GLP FINAL REPORT (05/08/12)

Modification(s) to Report:

After review of the data package, the original final report dated 05/07/12 was amended on 05/08/12 to include one change. The change was included in the body of the report as appropriate. The changes had no impact on the scientific interpretations or conclusions of the study.

1. The incorrect study director signed the final report. The report was amended in order for the study director, Jamie Herzan, to sign the report. This change was reflected on page 2 of the report.

Study Director

Jamie Herzan

Date

5-8-12



FINAL STUDY REPORT

STUDY TITLE: ISO MEM Endpoint Dilution Assay
Using L-929 Mouse Fibroblast Cells

PROTOCOL NUMBER: 140350-15

TEST ARTICLE IDENTIFICATION: Trade Name: ENSEAL G2 MIM I-Blades, Generic
Name: MIM 17-4 Stainless Steel with TiN coating,
R-spec: R2003-02-H, SPE002436-A, Engr. Study:
PRC055882, Color: Gold, Reference Number:
EE2725
Lot # PRC055882

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

STUDY NUMBER: 167721

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: The test article is considered **non-cytotoxic** at the
Neat dilution under the conditions of this test.



QUALITY ASSURANCE UNIT SUMMARY

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR part 58) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Each inspection was performed to assure the quality and integrity of the study.

<u>Phase Inspected</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Inoculation of Extract	03/30/12	04/06/12	04/16/12
Final Report	04/16/12	04/16/12	04/16/12

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor:

Dawn Vogland
Dawn Vogland

Date: 4-16-12

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director:

Jamie Herzan
Jamie Herzan

Date: 4-16-12

Professional personnel involved:

Lisa Olson, BS
Teri Tanquist, BS
Jean Kringstad, BS
Jamie Herzan, BS
Jean Mesarich, AA

Vice President, Testing and Service Development
Vice President of Process Improvement and Operations
Director, *In Vitro* Testing Services
Study Director
Client Relations Manager



PURPOSE

The purpose of this study was to evaluate a serially diluted extract of a test article for cytotoxicity to mammalian cells in culture by a method compliant with the requirements specified in ISO 10993-5:2009.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

TEST ARTICLE RECEIVED: 03/26/12
INITIATION DATE: 03/27/12
COMPLETION DATE: 04/16/12

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: ENSEAL G2 MIM I-Blades, Generic Name: MIM 17-4 Stainless Steel with TiN coating, R-spec: R2003-02-H, SPE002436-A, Engr. Study: PRC055882, Color: Gold, Reference Number: EE2725
Lot #:	PRC055882
Sterilization Method:	Gamma 60 kGy
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Device
Physical Description:	According to the Sponsor, the test article consisted of 1.5" gold rectangular rods.

CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.



EXPERIMENTAL DESIGN

Experimental Summary

The test article was incubated in the appropriate volume of E-MEM + 5% FBS in a sterile vessel for 24 ± 2 hours at 37 ± 1 °C. At the end of the extraction period, the extract was decanted from the test article. A series of eight dilutions of the extract were prepared in E-MEM + 5% FBS in a two-fold dilution. After the dilutions were prepared, the maintenance culture media was removed from test culture wells and replaced with 1 ml of test media dilutions in triplicate wells. The positive control media, test article extract, and control extracts were added at the same time to the culture plate in triplicate wells. The plates were incubated for 72 hours at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air. The cultures were evaluated for cytotoxic effects by microscopic examination at 24, 48 and 72 ± 4 hours of incubation and the results scored and recorded.

Justification For Selection Of The Test System

The MEM Endpoint Dilution Assay is one of several *in vitro* mammalian cell tests which have been used to screen materials for their potential cytotoxicity. The L-929 cell line has a history of use in assays of this type.

AMENDMENTS/DEVIATIONS: None.

IDENTIFICATION OF THE TEST SYSTEM

Cultures of L-929 cells (mouse fibroblast) were obtained from American Type Culture Collection (ATCC # CCL-1). Mycoplasma-free cell lines which were purchased from the vendor and kept frozen in the lab until used. To maintain the sensitivity, they are only sub-cultured for up to 15 passages and then discarded.

Cultures were grown and used as monolayers in disposable tissue culture labware at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air at WuXi AppTec, Inc. The media used for growth of cells is Eagle's minimal essential medium (E-MEM) supplemented with 5% (v/v) fetal bovine serum (FBS). The medium is also supplemented with the following: 2 mM L-glutamine, 10 mM HEPES, 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 units/mL penicillin, and 1% 2.50 ug/mL amphotericin B (Fungizone). WuXi AppTec Inc. has a long-standing history using antibiotics in culture medium. The use of antibiotics in culture media has not shown adverse affects when used for this assay, exhibited by the varying degrees of toxicity seen in the assay controls.

TEST MATERIAL PREPARATION

The test article was extracted intact, placed into an extraction vessel, and prepared at a ratio of 4 g to 20 mL of extraction vehicle.

TABLE 1: TEST ARTICLE RECORD

VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	QUANTITY OF TEST ARTICLE USED
E-MEM + 5% FBS	2.0	10.0	2

EXTRACTION MEDIA

Eagle's minimal essential medium (E-MEM), supplemented with 5% (v/v) fetal bovine serum (FBS), was used to extract the test article. The medium was also supplemented with the following: 2 mM L-glutamine, 10 mM HEPES, 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 units/mL penicillin, and 1% 2.50 µg/mL amphotericin B (Fungizone). The pH was confirmed to be 7.26.



CONTROL ARTICLE PREPARATION

A negative, intermediate, positive, and cell control were run in parallel with the test article. A negative control (high density polyethylene (HDPE)) known to be non-toxic under the test conditions was prepared at a ratio of 120 cm² to 20 mL of extraction vehicle. An intermediate control (black rubber) known to be mildly toxic under the test conditions was prepared and extracted at a ratio of 4 g to 20 mL of extraction vehicle. A positive control (100 µM CdCl₂) known to be toxic under the test conditions was prepared by diluting cadmium chloride stock powder with 100 mL of sterile water to create a 10 mM solution. The solution was then prepared at a ratio of 0.1 mL to 9.9 mL of extraction vehicle. A cell control (E-MEM + 5% FBS) was incubated in parallel with the test sample and controls.

TABLE 2: CONTROL / CELL LINE RECORD

CONTROL IDENTIFICATION:	CLASS	LOT #	SUPPLIED BY:	EXPIRATION
100 µM CdCl ₂	Positive Control	TDSI1074	WXAT	09/20/12
Black Rubber Stopper	Intermediate Control	WXAT010710	Fisher	02/28/13
High Density Polyethylene (HDPE)	Negative Control	C-091	Halano	02/2016
E-MEM + 5% FBS	Medium	TDSI1142B	WXAT	04/11/12
L-929	Cell Line	L032612	ATCC	NA

NA = Not applicable

TEST ARTICLE EXTRACTION

The extraction mixture and controls were incubated for 24 ± 2 hours at 37 ± 1 °C. At the start of the extraction, the solutions appeared clear and free of particulates. The extracts were agitated during the course of the extraction period. At the end of the extraction period, the vessels were well shaken. The test article was observed after extraction to be intact with no macroscopically observable degradation and the extract was clear. The extract was not filtered and was used immediately. See Tables 1-3.

TABLE 3: EXTRACTION RECORD

VEHICLE	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END
E-MEM + 5% FBS	37.0 °C	03/29/12 3:30 pm	37.0 °C	03/30/12 1:40 pm

EXPERIMENTAL PROCEDURE

L-929 cells were plated at 2.5 x 10⁴ cells/mL at least 24 hours before use. Prior to inoculation, the cell cultures were examined to ensure they had formed a nearly confluent monolayer and that they appeared uniform and viable.

The test article and controls were extracted with the appropriate amount of E-MEM + 5% FBS for 24 ± 2 hours at 37 ± 1 °C. At the end of the extraction period, the vessels were well shaken and the extraction media was used immediately. A series of eight dilutions of the extract were prepared in E MEM in a two-fold dilution. One mL aliquots of the test article and control extract and the series of dilutions were plated in triplicate onto the cell line.

The cell cultures were incubated in a humidified atmosphere for 72 hours. The monolayers were evaluated for cytotoxic effects at 24, 48, and 72 hours. At each incubation period, the cell cultures were observed for signs of cytopathic effect (CPE) including lysis, crenation, plaques, and excessive rounding of cells.

TEST EVALUATION

Criteria for evaluating cytotoxicity will include morphologic changes in cells, such as granulation, crenation, or rounding, and loss of viable cells from the monolayer by lysis or detachment. The validity of the test requires that negative control cultures maintain a healthy normal appearance throughout the duration of the test. Degrees of toxicity were scored as described in Table 4.



TABLE 4: SCORING

GRADE	REACTIVITY	CONDITIONS OF ALL CULTURES
0	None	Discrete intracytoplasmic granules; no cell lysis, no reduction of cell growth
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cells layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers

According to the current ISO guidelines, test articles scoring '0', '1', or '2' are considered NON-CYTOTOXIC. Test articles scoring '3' or '4' are considered CYTOTOXIC.

VALIDITY CRITERIA

Validity of the assay and the test article result was based upon the criteria listed below and scientific judgment.

The negative and cell control should display no cytotoxic reaction. The intermediate control should display a mild to moderate cytotoxic reaction, a score of '2' or '3'. The positive control should display a moderate to severe cytotoxic reaction, a score of '3' or '4'.

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS: Not applicable.

STATISTICAL METHODS: None used.

RECORD RETENTION

A certified copy of this report and data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

COMPLIANCE

This study was performed in compliance with the following International Standards:

ISO 10993-5:2009 Biological Evaluation of Medical Devices, Part 5: Tests for *In Vitro* Cytotoxicity.

ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

TEST ARTICLE DISPOSITION

Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

The negative and cell controls exhibited healthy growth throughout the experiment. The monolayers exposed to CdCl₂ exhibited a toxic response while the black rubber intermediate control induced a mild level of toxicity. Therefore, the test system was responding normally and met the criteria for a valid assay. See Table 5.



When scoring lysis, each sample was rated relative to the amount of lysis displayed by the negative control. The negative and cell control were considered '0', the cells displayed at least 70% viability. The intermediate control displayed a mild cytotoxic reaction resulting in a score of '2'. The positive control displayed a severe cytotoxic reaction, resulting in a score of '4'. Therefore, the test system was responding normally and met the criteria for a valid assay. See Table 5.

In this assay, the test article did not induce cytotoxicity. No abnormal events such as pH change or debris were noted.

TABLE 5: RESULTS

TEST ARTICLE AND CONTROLS	SAMPLE EXTRACT		CYTOTOXICITY SCORES		
	SIZE	(mL)	24 HOURS	48 HOURS	72 HOURS
Test Article – Neat	2.0 g	10.0	0/0/0	0/0/0	0/0/0
Test Article – 1:2	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:4	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:8	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:16	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:32	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:64	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:128	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:256	NA	NA	0/0/0	0/0/0	0/0/0
Positive Control	0.1 mL	9.9	4/4/4	4/4/4	4/4/4
Intermediate Control	1.9 g	9.5	2/2/2	2/2/2	2/2/2
Negative Control	30 cm ²	5.0	0/0/0	0/0/0	0/0/0
Cell Control	NA	10.0	0/0/0	0/0/0	0/0/0

NA = Not Applicable

ANALYSIS AND CONCLUSION

The test article, at the Neat, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256 dilutions scored "0" at the 24, 48 and 72 ± 4 hours observation periods. Therefore, the test article is considered **non-cytotoxic** at the Neat dilution.

REFERENCES

U.S. Pharmacopeia, Section 87, current revision.

WuXi AppTec Reference Library Contents, Form ALS-4650-1

WuXi AppTec SOP: MED-8750, Minimum Essential Medium (MEM) Elution Assay



FINAL STUDY REPORT

STUDY TITLE: ASTM Hemolysis Assay - Direct Contact Method

PROTOCOL NUMBER: 150300-12

TEST ARTICLE IDENTIFICATION:
Trade Name: ENSEAL G2 MIM I-Blades
Generic Name: MIM 17-4 Stainless Steel with TiN coating
R-spec: R2003-02-H, SPE002436-A
Engr. Study: PRC055882
Color: Gold
Reference Number: EE2725
Lot # PRC055882

PERFORMING LABORATORY:
WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR:
Ethicon Endo-Surgery, Inc.
4545 Creek Road
Cincinnati, OH 45242-2803

STUDY NUMBER: 167725

CLIENT MNEMONIC: ETH02

RESULT SUMMARY:
The test article is considered **non-hemolytic** under the test conditions employed and **passes** this test.



QUALITY ASSURANCE UNIT SUMMARY

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR part 58) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Each inspection was performed to assure the quality and integrity of the study.

<u>Phase Inspected</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Reading Absorbances	04/19/12	04/27/12	05/08/12
Final Report	05/07/12	05/07/12	05/08/12

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: _____

Sheri Handy
Sheri Handy

Date: 5/8/12

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: _____

Joshua Kamrud
Joshua Kamrud

Date: 5-8-12

Professional personnel involved:

Lisa Olson, BS	Vice President of Testing and Service Development
Teri Tanquist, BS	Vice President of Process Improvement and Operations
Jean Kringstad, BS	Director, In Vitro Testing Services
Joshua Kamrud, BS	Study Director
Jean Mesarich, AA	Client Relations Manager



PURPOSE

The purpose of this study was to evaluate the hemolytic potential of test articles according to the ASTM guideline (F-756-08).

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SAMPLES RECEIVED: 03/26/12, 04/16/12
INITIATION DATE: 04/10/12
COMPLETION DATE: 05/08/12

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: ENSEAL G2 MIM I-Blades, Generic Name: MIM 17-4 Stainless Steel with TiN coating, R-spec: R2003-02-H, SPE002436-A, Engr. Study: PRC055882 Color: Gold Reference Number: EE2725
Lot #:	PRC055882
Sterilization Method:	Gamma 60 kGy
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Device
Physical Description:	According to the Sponsor, the test articles were 1.5 inch gold rectangular rods

CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing.

Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

NAMSA

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

STUDY TITLE

Determination of Extractable Species From a
Test Article by Infrared Spectroscopy

SPONSOR

Brittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

TEST ARTICLE NAME

ENSEAL G2 MIM I-Blades

TEST ARTICLE IDENTIFICATION

EE2725

TEST ARTICLE PHYSICAL DESCRIPTION

1.5" gold rectangular rods

TEST ARTICLE RECEIVED

March 26, 2012

PURPOSE

The purpose of this study was to prepare an infrared spectrum of a material for the purpose of identifying the material or examining it for impurities.

SUMMARY/RESULTS

The test article was characterized for extractable species as required by ISO 10993-18, Biological Evaluation of Medical Devices - Part 18: Chemical Characterization of Materials by first extracting the test article in hexane. The resulting test extract was then evaporated to a residue and then the residue was analyzed by infrared spectroscopy to identify the species present in the test extract.

There were no major bands of interest detected in the test article residue. The lack of species present as represented by the infrared spectrum, can be used to aid in the assessment of the test article by ISO 10993, Part 18.

The test article spectrum is located in Appendix 1.

METHOD

A 20.4 gram portion (20 pieces) of the test article was extracted at 50°C for 72 hours in 102 mL of hexane. The method and results of this extraction were reported under Lab Number 12T_29166_02. An approximate 1-2 mL portion of the hexane test extract was transferred to a clean vial and placed in a 105°C drying oven to evaporate the extracting solvent. A small amount of chloroform was added to the vial to dissolve the residue. The resulting solution was placed on a clean potassium bromide crystal and the chloroform was allowed to evaporate. The residue was analyzed by transmission infrared spectroscopy using the instrument conditions listed below.

Instrument	Fourier Transform Infrared Spectrophotometer
Wavenumber Range—Collected	4400 cm ⁻¹ – 350 cm ⁻¹
Scan number	8
Resolution	4 cm ⁻¹

NAMSA

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Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

REFERENCES

European Pharmacopoeia (Ph. Eur.) 7th Edition, Section 2.2.24. Absorption Spectrophotometry, Infrared (2011).

International Organization for Standardization (ISO) 10993-18, Biological evaluation of medical devices - Part 18: Chemical characterization of materials (2005).

United States Pharmacopeia 34, National Formulary 29 (USP), General Chapter <197>, Spectrophotometric Identification Tests, Infrared Absorption (2011).

APPROVAL


Christina L. Oval, B.A.
Supervisor, Chemistry

4-9-12
Date

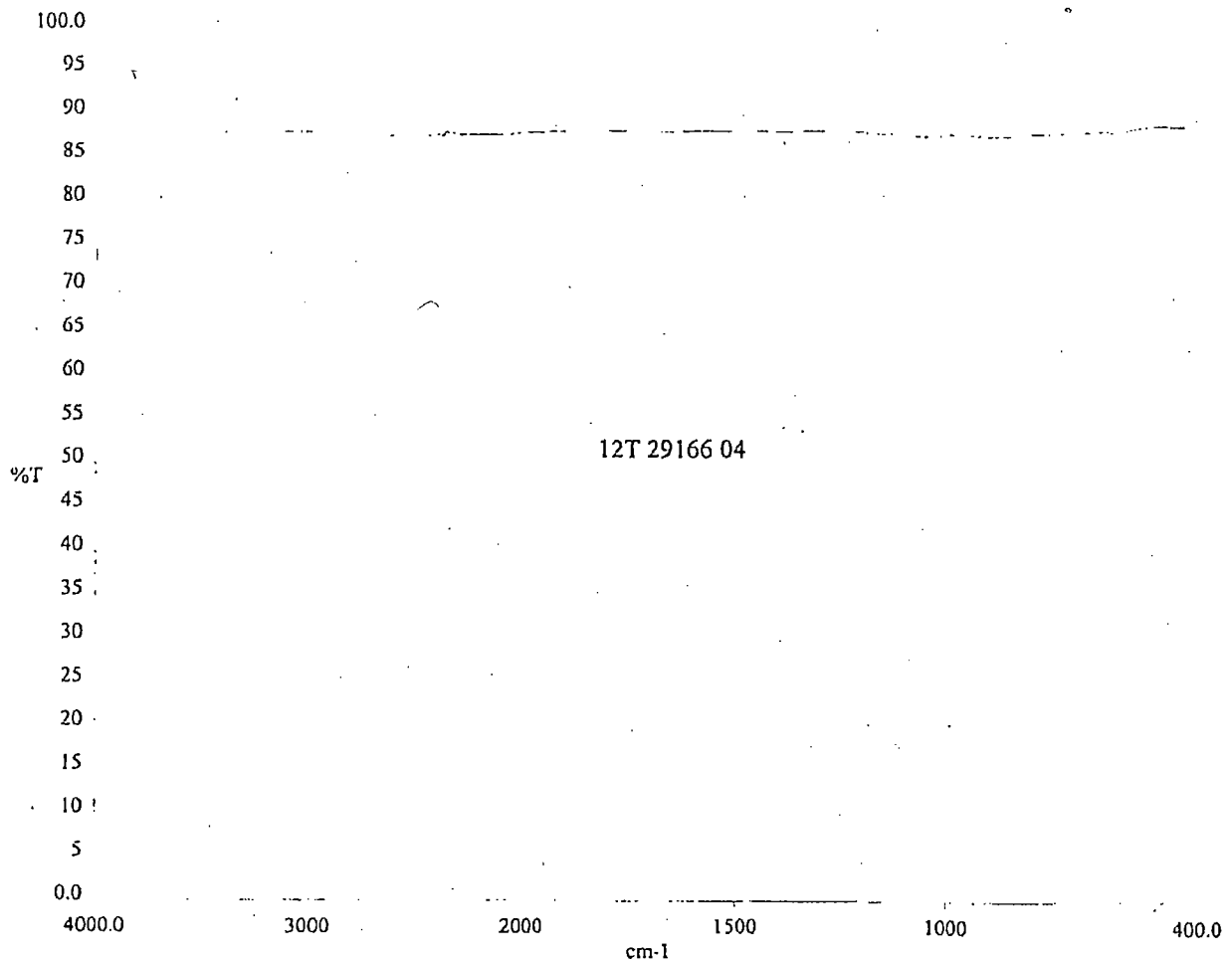
Results apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.



PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

APPENDIX 1





PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

STUDY TITLE

Determination of Extractable Species From a
Test Article by Infrared Spectroscopy

TEST ARTICLE NAME

ENSEAL G2 MIM I-Blades

TEST ARTICLE IDENTIFICATION

EE2725

TEST ARTICLE PHYSICAL DESCRIPTION

1.5" gold rectangular rods

TEST ARTICLE RECEIVED

March 26, 2012

SPONSOR

Brittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

PURPOSE

The purpose of this study was to prepare an infrared spectrum of a material for the purpose of identifying the material or examining it for impurities.

SUMMARY/RESULTS

The test article was characterized for extractable species as required by ISO 10993-18, Biological Evaluation of Medical Devices - Part 18: Chemical Characterization of Materials by first extracting the test article in purified water. The resulting test extract was then evaporated to a residue and then the residue was analyzed by infrared spectroscopy to identify the species present in the test extract.

There were no major bands of interest detected in the test article residue. The lack of species present as represented by the infrared spectrum, can be used to aid in the assessment of the test article by ISO 10993, Part 18.

The test article spectrum is located in Appendix 1.

METHOD

A 20.4 gram portion (20 pieces) of the test article was rinsed twice with a sufficient volume of purified water to cover the test article, and then extracted at 50°C for 72 hours in 102 mL of purified water. The method and results of this extraction were reported under Lab Number 12T_29166_01. An approximate 1-2 mL portion of the purified water test extract was transferred to a clean vial and placed in a 105°C drying oven to evaporate the extracting solvent. A small amount of chloroform was added to the vial to dissolve the residue. The resulting solution was placed on a clean potassium bromide crystal and the chloroform was allowed to evaporate. The residue was analyzed by transmission infrared spectroscopy using the instrument conditions listed below.

Instrument	Fourier Transform Infrared Spectrophotometer
Wavenumber Range—Collected	4400 cm ⁻¹ – 350 cm ⁻¹
Scan number	8
Resolution	4 cm ⁻¹



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REFERENCES

European Pharmacopoeia (Ph. Eur.) 7th Edition, Section 2.2.24. Absorption Spectrophotometry, Infrared (2011).

International Organization for Standardization (ISO) 10993-18, Biological evaluation of medical devices - Part 18: Chemical characterization of materials (2005).

United States Pharmacopeia 34, National Formulary 29 (USP), General Chapter <197>, Spectrophotometric Identification Tests, Infrared Absorption (2011).

APPROVAL

Christina L. Ovall, B.A.
Supervisor, Chemistry

4-9-12

Date

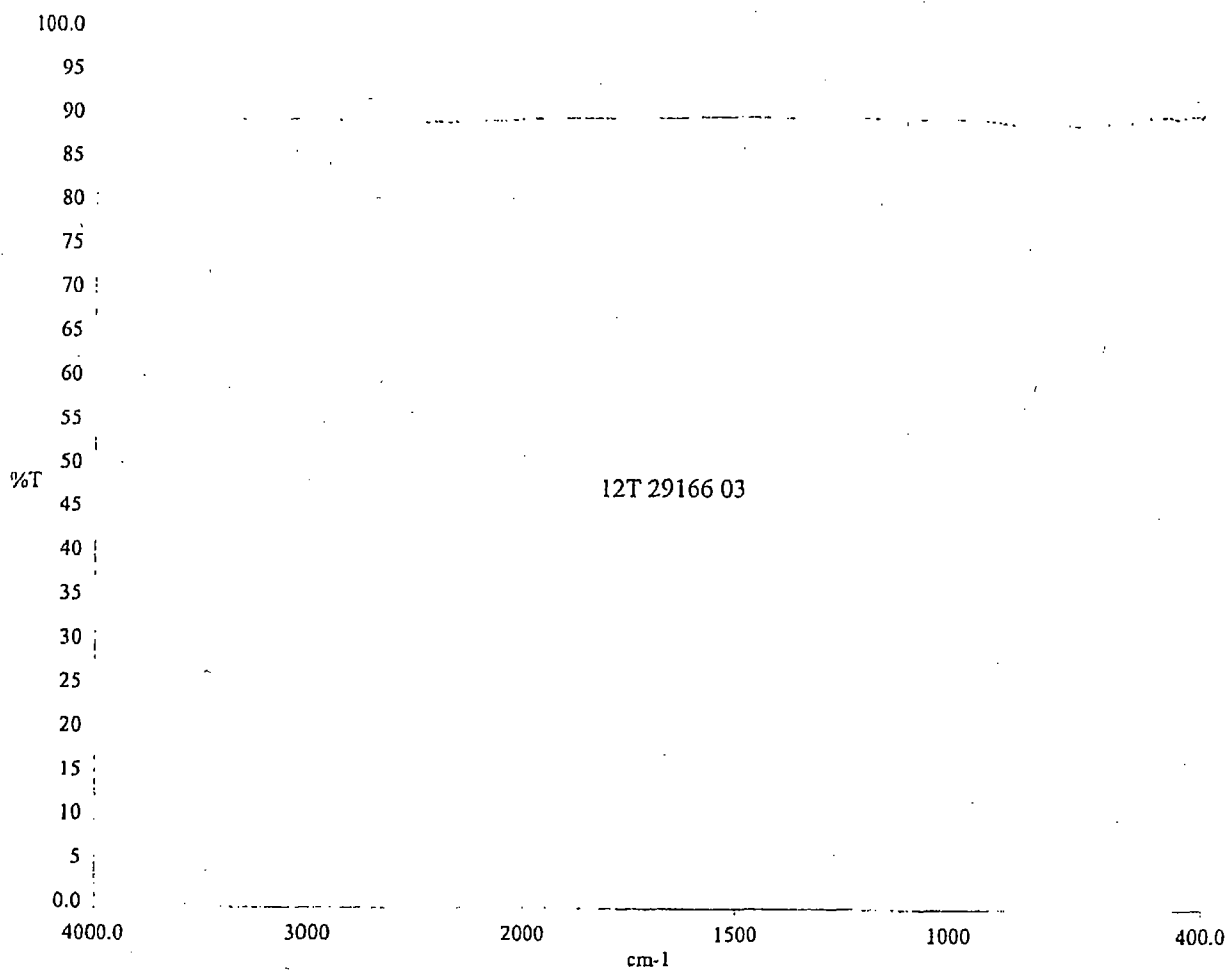
Results apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.



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APPENDIX 1



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419.666.9455

STUDY TITLE

Determination of the Physicochemical
Attributes of a Polymeric Material per USP
<661> Containers - Plastics, Physicochemical
Tests - Non-Volatile Residue

SPONSOR

Brittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

TEST ARTICLE NAME

ENSEAL G2 MIM I-Blades

TEST ARTICLE IDENTIFICATION

EE2725

TEST ARTICLE PHYSICAL DESCRIPTION

1.5" gold rectangular rods

TEST ARTICLE RECEIVED

March 26, 2012

PURPOSE

The purpose of this study was to describe the physicochemical attributes as part of the overall characterization of the test article.

RESULTS

	Assay Results ¹	Limits Based on Area
Non-Volatile Residue	<1 mg	≤15 mg

¹Extraction conditions were based upon weight rather than USP specified surface area. Test article extract results may be compared to USP area limits to determine significance.

Condition of Extracts	
Test Article	Clear and colorless
Control Blank	Clear and colorless

Date Extract Prepared: March 30, 2012

Date Test Concluded: April 3, 2012

CONCLUSION

No significant extractables originated from the test article.

METHOD

A 20.4 gram portion (20 pieces) of the test article was rinsed twice with a sufficient volume of purified water to cover the test article, and then extracted at 50°C for 72 hours in 102 mL of purified water. A control blank of purified water was similarly prepared without the test article. Non-volatile residue was determined on the test article extract as outlined in the current USP.

REFERENCE

United States Pharmacopeia 34, National Formulary 29 (USP), General Chapter <661>, Containers - Plastics (2011).

APPROVAL

Stephen T. Pisle
Stephen T. Pisle, B.S.
Technical Support Specialist

4/4/12
Date

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2003.

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Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

STUDY TITLE

Physicochemical Testing Using an Alternative
Extracting Solvent - Non-Volatile Residue

TEST ARTICLE NAME

ENSEAL G2 MIM I-Blades

TEST ARTICLE IDENTIFICATION

EE2725

SPONSOR

Brittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

TEST ARTICLE PHYSICAL DESCRIPTION

1.5" gold rectangular rods

TEST ARTICLE RECEIVED

March 26, 2012

PURPOSE

The purpose of this study was to describe the physicochemical attributes as part of the overall characterization of the test article.

RESULTS

Assay Results	
Non-Volatile Residue	<1 mg

Condition of Test Article Extract: clear and colorless

Condition of Control Blank: clear and colorless

Date Extract Prepared: March 30, 2012

Date Test Concluded: April 3, 2012

METHOD

A 20.4 gram portion (20 pieces) of the test article was extracted at 50°C for 72 hours in 102 mL of hexane. Non-volatile residue was determined on a 50.0 mL portion of the eluate.

COMMENT

Limits for physicochemical tests using lipophilic solvents have not yet been established. Test articles that are low in lipophilic soluble extractables are generally preferred for use in test articles contacting blood and other tissues.

REFERENCE

United States Pharmacopeia 34, National Formulary 29 (USP), General Chapter <661>, Containers - Plastics (2011).

APPROVAL

Stephen T. Pisle
Stephen T. Pisle, B.S.
Technical Support Specialist

4/4/12
Date

Results apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.



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Test Facility
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419.666.9455

STUDY TITLE

Determination Extractable Elements By
Inductively Coupled Plasma Mass Spectroscopy

SPONSOR

Brittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

TEST ARTICLE NAME

ENSEAL G2 MIM I-Blades

TEST ARTICLE IDENTIFICATION

EE2725

TEST ARTICLE PHYSICAL DESCRIPTION

1.5" gold rectangular rods

TEST ARTICLE RECEIVED

March 26, 2012

PURPOSE

The purpose of this study was to analyze an aqueous extract for trace elements using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS).

RESULTS

Element	Quantitation Limit (ng/mL)	Dilution Factor	Sample Concentration (ng/mL)	Sample Concentration (ng/g)
Beryllium (Be)	10	1	<10	<50.0
Magnesium (Mg)	10	1	<10	<50.0
Aluminum (Al)	10	1	<10	<50.0
Titanium (Ti)	10	1	<10	<50.0
Vanadium (V)	10	1	<10	<50.0
Chromium (Cr)	10	1	<10	<50.0
Manganese (Mn)	10	1	<10	<50.0
Cobalt (Co)	10	1	<10	<50.0
Nickel (Ni)	10	1	<10	<50.0
Copper (Cu)	10	1	<10	<50.0
Zinc (Zn)	10	1	<10	<50.0
Gallium (Ga)	10	1	<10	<50.0
Arsenic (As)	10	1	<10	<50.0
Selenium (Se)	10	1	<10	<50.0
Rubidium (Rb)	10	1	<10	<50.0
Strontium (Sr)	10	1	<10	<50.0
Zirconium (Zr)	10	1	<10	<50.0
Niobium (Nb)	10	1	<10	<50.0
Molybdenum (Mo)	10	1	<10	<50.0
Ruthenium (Ru)	10	1	<10	<50.0
Cadmium (Cd)	10	1	<10	<50.0
Tin (Sn)	10	1	<10	<50.0
Antimony (Sb)	10	1	<10	<50.0
Tellurium (Te)	10	1	<10	<50.0
Cesium (Cs)	10	1	<10	<50.0
Cerium (Ce)	10	1	<10	<50.0
Praseodymium (Pr)	10	1	<10	<50.0
Neodymium (Nd)	10	1	<10	<50.0



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Element	Quantitation Limit (ng/mL)	Dilution Factor	Sample Concentration (ng/mL)	Sample Concentration (ng/g)
Samarium (Sm)	10	1	<10	<50.0
Europium (Eu)	10	1	<10	<50.0
Gadolinium (Gd)	10	1	<10	<50.0
Lanthanum (La)	10	1	<10	<50.0
Dysprosium (Dy)	10	1	<10	<50.0
Erbium (Er)	10	1	<10	<50.0
Ytterbium (Yb)	10	1	<10	<50.0
Thorium (Th)	10	1	<10	<50.0
Uranium (U)	10	1	<10	<50.0
Hafnium (Hf)	10	1	<10	<50.0
Tantalum (Ta)	10	1	<10	<50.0
Tungsten (W)	10	1	<10	<50.0
Rhenium (Re)	10	1	<10	<50.0
Osmium (Os)	10	1	<10	<50.0
Iridium (Ir)	10	1	<10	<50.0
Platinum (Pt)	10	1	<10	<50.0
Thallium (Tl)	10	1	<10	<50.0
Lead (Pb)	10	1	<10	<50.0

CONCLUSION

No extractable elements were detected in the purified water test extract.

METHOD

A 20.4 gram portion (20 pieces) of the test article was rinsed twice with a sufficient volume of purified water to cover the test article, and then extracted at 50°C for 72 hours in 102 mL of purified water. The method and results of this extraction were reported under Lab Number 12T_29166_01.

A 5 mL portion of the purified water test extract and the control extract was placed in a clean sample tube and 145 µL of Ultra High Purity HNO₃ (69%) was added and analyzed for elements by ICP-MS.

REFERENCE

International Organization for Standardization (ISO) 10993-18, Biological evaluation of medical devices - Part 18: Chemical characterization of materials (2005).

APPROVAL

Christina L. Ovali
Christina L. Ovali, B.A.
Supervisor, Chemistry

4-13-12
Date

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices and certified to ISO 13485:2003.

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Test Facility
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419.666.9455

STUDY TITLE

Determination of Extractable Semi-Volatile
Organic Compounds by Gas
Chromatography/Mass Spectrometry (GC/MS)

SPONSOR

Brittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

TEST ARTICLE NAME

ENSEAL G2 MIM I-Blades

TEST ARTICLE IDENTIFICATION

EE2725

TEST ARTICLE PHYSICAL DESCRIPTION

1.5" gold rectangular rods

TEST ARTICLE RECEIVED

March 26, 2012

PURPOSE

The purpose of this study was to screen an extract for extractable semi-volatile organic compounds by Gas Chromatography/Mass Spectrometry (GC/MS).

RESULTS

There were no semi-volatile compounds greater than the quantitation limit of 1.0 µg/mL for the purified water test and control extracts.

METHOD

A 20.4 gram portion (20 pieces) of the test article was rinsed twice with a sufficient volume of purified water to cover the test article, and then extracted at 50°C for 72 hours in 102 mL of purified water. The method and results of this extraction were reported under Lab Number 12T_29166_01. A 4.0 mL portion of the purified water test extract was solvent exchanged with 4.0 mL of dichloromethane. A 4.0 mL portion of the purified water control was solvent exchanged with 4.0 mL of dichloromethane. The resulting solutions were analyzed by GC/MS for semi-volatiles using the following instrument conditions.

Instrument: Shimadzu QP 2010 GC/MS

GC Method			
Column	(5%-phenyl)-methylpolysiloxane, 30 m x 0.25 mm i.d., 0.25 µm d _f		
Carrier Gas	Helium	Injection Temperature	250.0°C
Column Flow	1.20 mL/min	Injection Mode	Splitless
Injection Volume	1 µL	Interface Temperature	300.0°C
Oven Temperature Program			
Rate (°C/min)		Temperature (°C)	Hold Time (min)
-		40.0	4.00
10.0		280.0	4.00
Post Run Oven Program			
		310.0	10.00
MS Method			
Ion Source Temperature	230°C		
Ionization Mode	Electron Ionization		
Spectral Range	45 – 550 amu		
Detector type	Quadrupole		

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Standards of phenanthrene at concentrations of 1.0, 25 and 50 µg/mL were prepared and injected in triplicate. A linear regression was performed using the resulting responses of phenanthrene at these concentrations. Compounds present in the extracts were tentatively identified using a combination of items, which may have included, but was not limited to, comparison of retention time, comparison or mass spectra, structural elucidation using the resulting mass spectra, library search results, and the GC/MS software.

REFERENCES

International Organization for Standardization (ISO) 10993-18, Biological Evaluation of Medical Devices-Part 18: Chemical Characterization of Materials (2005).

APPROVAL

Steph T. Pisle
Stephen T. Pisle, B.S.
Technical Support Specialist

4/12/12
Date

Results apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.



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Northwood, OH 43619
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STUDY TITLE

Determination of Extractable Semi-Volatile
Organic Compounds by Gas
Chromatography/Mass Spectrometry (GC/MS)

SPONSOR

Brittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

TEST ARTICLE NAME

ENSEAL G2 MIM I-Blades

TEST ARTICLE IDENTIFICATION

EE2725

TEST ARTICLE PHYSICAL DESCRIPTION

1.5" gold rectangular rods

TEST ARTICLE RECEIVED

March 26, 2012

PURPOSE

The purpose of this study was to screen an extract for extractable semi-volatile organic compounds by Gas Chromatography/Mass Spectrometry (GC/MS).

RESULTS

There were no semi-volatile compounds greater than the quantitation limit of 1.0 µg/mL for the hexane test and control extracts.

METHOD

A 20.4 gram portion (20 pieces) of the test article was extracted at 50°C for 72 hours in 102 mL of hexane. The method and results of this extraction were reported under Lab Number 12T_29166_02. The hexane test extract and its associated control were analyzed by GC/MS using the following instrument conditions.

Instrument: Shimadzu QP 2010 GC/MS

GC Method			
Column	(5%-phenyl)-methylpolysiloxane, 30 m x 0.25 mm i.d., 0.25 μm d _f		
Carrier Gas	Helium	Injection Temperature	250.0°C
Column Flow	1.20 mL/min	Injection Mode	Splitless
Injection Volume	1 μL	Interface Temperature	300.0°C
Oven Temperature Program			
Rate (°C/min)	Temperature (°C)		Hold Time (min)
-	40.0		4.00
10.0	280.0		4.00
Post Run Oven Program			
	310.0		10.00
MS Method			
Ion Source Temperature	230°C		
Ionization Mode	Electron Ionization		
Spectral Range	45 – 550 amu		
Detector type	Quadrupole		

Standards of phenanthrene at concentrations of 1.0, 25 and 50 µg/mL were prepared and injected in triplicate. A linear regression was performed using the resulting responses of phenanthrene at these concentrations. Compounds present in the extracts were tentatively identified using a combination of items, which may have included, but was not limited to, comparison of retention time, comparison or mass spectra, structural elucidation using the resulting mass spectra, library search results, and the GC/MS software.

NAMSA

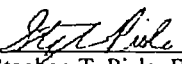
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Test Facility
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419.666.9455

REFERENCES

International Organization for Standardization (ISO) 10993-18, Biological Evaluation of Medical Devices-Part 18: Chemical Characterization of Materials (2005).

APPROVAL


Stephen T. Pisle, B.S.
Technical Support Specialist

4/12/12

Date

Results apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.



**AMENDED
FINAL STUDY REPORT**

STUDY TITLE: Japanese Cytotoxicity Test
Colony Microassay By Elution –
Expanded Dilution Series

PROTOCOL NUMBER: C11662-1

TEST ARTICLE IDENTIFICATION: Trade Name: ENSEAL G2 MIM I-Blades, Generic
Name: MIM 17-4 Stainless Steel with TiN coating,
R-spec: R2003-02-H, SPE002436-A, Engr. Study:
PRC055882, Color: Gold, Sterilization: 60 kGy
Gamma, Reference Number: EE2725
Lot # PRC055882

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

STUDY NUMBER: 167720

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: None of the standard dilutions of the test article
induced biologically relevant decreases in colony
formation. Further, no dose response trends were
noted. Therefore, an IC₅₀ could not be calculated for
the test article. These results would be indicative of
a **non-cytotoxic** material.



**AMENDED
FINAL STUDY REPORT**

STUDY TITLE: ISO Guinea Pig Maximization Sensitization Test

PROTOCOL NUMBER: 900850-24

TEST ARTICLE IDENTIFICATION: Trade Name: Eagle 3mm Jaw and Shaft
Built according to Assembly: PRC049736 Rev C
Sterilization: 110-130 kGy Gamma
Lot#: PRC049736
Ref# EE2702

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

STUDY NUMBER: 157892

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: The test article **did not** elicit a sensitization response under the conditions of this assay.

Protocol Number: 900850-24
Amended Report: October 26, 2011

Project No. 157892
Page 2 of 14



QUALITY ASSURANCE UNIT SUMMARY

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 – Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the quality and integrity of the study.

<u>Phase Inspected</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Induction I	09/16/11	09/16/11	10/17/11
Final Report	10/17/11	10/17/11	10/17/11
Amended Report	10/26/11	10/26/11	10/26/11

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: Jim Ritala Date: 10/26/11
Jim Ritala

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: Katai Nkhata Date: 10/26/11
Katai Nkhata

Professional Personnel Involved:

Teri Tanquist, BS
Christine Olson, BS
Roxanne Miller, AA, CVT
Katai Nkhata, MA
Jean Mesarich, AA

Vice President of Process Improvement and Operations
Study Operations Manager
Associate Director, In-Life Studies
Study Director
Client Relations Manager

Protocol Number: 900850-24
Amended Report: October 26, 2011

Project No. 157892
Page 3 of 14



PURPOSE

This test was designed to evaluate the allergenic potential or sensitizing capacity of a test article. The test was used as a procedure for the screening of contact allergens in guinea pigs and extrapolating the results to humans, but it does not establish the actual risk of sensitization.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

DATE SAMPLE RECEIVED: 08/15/11
STUDY INITIATION DATE: 09/06/11
STUDY COMPLETION DATE: 10/17/11
AMENDED REPORT DATE: 10/26/11

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: Eagle 3mm Jaw and Shaft Built according to Assembly: PRC049736 Rev C Sterilization: 110-130 kGy Gamma Lot#: PRC049736 Ref# EE2702
Lot #:	PRC049736
Sterilization Method:	110-130 kGy Gamma
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Device

TEST ARTICLE CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

EXPERIMENTAL DESIGN

Experimental Summary

In selecting a new material for human contact in medical applications, it is important to ensure that the material will not stimulate the immune system to produce an allergic reaction. The reaction generally is due to substances that may leach out of a material. Therefore, this practice provides for the use of material extracts. The use of Freund's complete adjuvant (FCA) and sodium lauryl sulfate (SLS) tend to enhance the potential of weak sensitizing agents. While this test does not ensure that test materials are completely non-allergenic, it is the most sensitive animal test in common use today.

Protocol Number: 900850-24
Amended Report: October 26, 2011

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Eleven test guinea pigs (per extract) were injected with the test article extract and FCA, and six guinea pigs (per extract) were injected with the corresponding control blank and FCA. On Day 6, the dorsal site was reshaved and SLS in mineral oil was applied. The day after the SLS application, the test animals were topically patched with the appropriate test extract and the control animals were patched with the corresponding control blank. The patches were removed after 48 ± 2 hours of exposure. Following an approximate two-week rest period, the animals were topically patched with the appropriate test extract and corresponding control blank. The patches were removed after 24 ± 2 hours of exposure. The dermal patch sites were observed for erythema and edema 24 ± 2 and 48 ± 2 hours after patch removal. Each animal was assessed for a sensitization response based upon the dermal scores. The test results were based upon the percentage of animals exhibiting a sensitization response.

Justification For Selection Of The Test System

The albino guinea pig has historically been used in skin sensitization tests and is generally accepted as the most appropriate animal model for human allergic contact dermatitis. The guidelines have no alternative (non-animal) methods.

Institutional Animal Care and Use Committee (IACUC)

The protocol and any amendments or procedures involving the care or use of animals on this study were reviewed and approved by WuXi AppTec's IACUC prior to the initiation of such procedures.

IACUC Protocol / Effective Date: 98-02E / May, 2009

AMENDMENT#1: According to the Sponsor request, the Test Article Identification was changed from: "Built according to Assembly: PRC049736" to "Built according to Assembly: PRC049736 Rev C" and also added "Ref # EE2703".

AMENDMENT#2: Per Sponsor request, the test article identification was changed from "sterilization 45-75 kGy Gamma" to "110-130 kGy Gamma" and the Reference number "Ref # EE2703" was changed to "Ref # EE2702".

DEVIATIONS: None.

IDENTIFICATION OF THE TEST SYSTEM

Species/Strain: Albino guinea pigs (*Cavia porcellus*), Hartley strain (specific pathogen free).

Source: Charles River Laboratories.

Sex: Male.

Weight Range: All animals weighed between 328.5 and 399.6 g upon assignment to the test.

Age: Healthy young adults were used.

Animal Identification: The animals were identified per WuXi AppTec SOP: ILS-0112.

HUSBANDRY

Receipt: Animals were received on 09/07/11 according to WuXi AppTec SOP: ILS-0092. Each animal was examined for signs of disease and injury. The animals were acclimated for a minimum of 5 days under the same conditions as the actual test.

Housing: Animals were housed in solid bottom cages with contact bedding and up to two guinea pigs per cage. Housing density complied with AAALAC International recommendations. The test and negative control animals were housed separately.

Protocol Number: 900850-24
Amended Report: October 26, 2011

Project No. 157892
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Environment: The environmental conditions in the animal rooms were set to be maintained according to WuXi AppTec SOP: ILS-0018. The temperature and photo-period were set to meet the AAALAC International recommendations for these species. The laboratory and animal rooms were maintained as limited-access facilities.

Diet: Animals were supplied with certified commercial guinea pig feed, *ad libitum*. No known contaminants present in the feed were expected to interfere with the test results.

Water: Potable water was obtained from the St. Paul municipal water supply. No known contaminants present in the water were expected to interfere with the test results.

TEST MATERIAL PREPARATION

According to the Sponsor, the test article consisted of a silver metal jaw attached to a black shaft with white printing. The test article was cut 4 cm from the jaw end and included part of the shaft with pad printing on it (see Appendix). For extraction, the test article was placed into test tubes and prepared at a ratio of 4 g to 20 mL of extraction vehicle.

TABLE 1: TEST ARTICLE RECORD

TEST PHASE AND VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	NUMBER OF TEST ARTICLE DEVICES USED PER EXTRACT
First Induction Injection Normal Saline (NS)	3.1	15.5	1
First Induction Injection Cottonseed Oil (CSO)	3.1	15.5	1
Second Induction Patch NS	3.2	16.0	1
Second Induction Patch CSO	3.2	16.0	1
Challenge Patch NS	3.2	16.0	1
Challenge Patch CSO	3.2	16.0	1

TEST ARTICLE EXTRACTION

The extraction mixtures and corresponding control blanks were incubated for 72 ± 2 hours at 50 ± 2 °C. At the start of the extraction, the solutions appeared clear and free of particulates. The extracts were manually agitated. At the end of the extraction period, the vessels were shaken well and the liquid aseptically decanted into a sterile glass vessel. Some rusting of test article components was observed in the NS extractions at inductions one and two, but not at the challenge phase. Brown and white flakes were observed in the NS extract at the first induction, but the extract was clear and slightly white. At induction two, white flakes were also observed in the NS extract and although slightly opaque, the extract appeared relatively clear. There were no particulates observed in the NS extract for the challenge phase, and all of the CSO extracts at all three test phases were also clear and free of particulates. After decanting, the extracts were not filtered, but allowed to settle prior to use. The extracts were maintained at room temperature and used within 24 hours of preparation. See Tables 1-3.

TABLE 2: EXTRACTION RECORD

TEST PHASE AND VEHICLE	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END
First Induction Injection NS	50.2 °C	09/13/11 6:48 am	50.2 °C	09/16/11 6:12 am
First Induction Injection CSO	50.2 °C	09/13/11 6:48 am	50.2 °C	09/16/11 6:12 am
Second Induction Patch NS	50.2 °C	09/20/11 6:46 am	50.2 °C	09/23/11 6:24 am
Second Induction Patch CSO	50.2 °C	09/20/11 6:46 am	50.2 °C	09/23/11 6:24 am
Challenge Patch NS	50.2 °C	10/06/11 6:50 am	50.2 °C	10/09/11 6:28 am
Challenge Patch CSO	50.2 °C	10/06/11 6:50 am	50.2 °C	10/09/11 6:28 am

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TABLE 3: VEHICLE RECORD

VEHICLE IDENTIFICATION:	TEST PHASE:	LOT #:	SUPPLIED BY:	EXPIRATION
0.9% Normal Saline (NS)	First, Second & Challenge	J1C616	Braun	09/2013
Cottonseed Oil NF (CSO)	First, Second & Challenge	ZN1009	Spectrum	01/02/12

TABLE 4: ADDITIONAL TEST REAGENT RECORD

ADDITIONAL TEST REAGENTS:	LOT #:	SUPPLIED BY:	EXPIRATION
FCA	0167744	Difco	06/14/13
10% SLS	104120	Fisher	03/12/12
0.9% Sterile Saline	J9J568	Braun	01/2012
Mineral Oil	110083	Fisher	03/12/12

SELECTION OF ANIMALS

Animals were randomly placed in cages upon receipt and were assigned on test as available. Animals considered unsuitable due to poor health or outlying body weight were excluded from the study.

ANIMAL PREPARATION

The application sites were prepared by clipping the fur of the test site using an electric clipper with an appropriate blade. Prior to the induction phases, an approximate 5 x 7 cm area over the shoulder region was shaved. Prior to challenge, an approximate 4 x 4 cm area of the right and left flank was shaved.

TEST ARTICLE ADMINISTRATION

First Induction / Intradermal Injection: Three syringes were prepared for the test animals and three for the negative control animals as indicated in Table 5. The prepared syringes were injected in pairs on each side of the dorsal mid-line. The six injection sites were within the boundaries of a 2 x 4 cm area.

TABLE 5: FIRST INDUCTION SYRINGE PREPARATION AND DOSE VOLUME

PREPARATION	VOLUME INJECTED PER SITE	SYRINGE CONTENTS	RATIO (V/V)
TEST GROUP			
Syringe 1	0.1 mL	FCA + 0.9% Sterile Saline	1:1
Syringe 2	0.1 mL	Test Extract	NA
Syringe 3	0.1 mL	FCA + 0.9% Sterile Saline (1:1) + Test Extract	1:1
CONTROL GROUP			
Syringe 1	0.1 mL	FCA + 0.9% Sterile Saline	1:1
Syringe 2	0.1 mL	Control Vehicle	NA
Syringe 3	0.1 mL	FCA + 0.9% Sterile Saline (1:1) + Control Vehicle	1:1

Second Induction / Topical Application: On Day 6, the injection site area was clipped free of fur and treated with 0.5 mL to 1.0 mL of 10% (w/w) SLS prepared by mixing solid SLS with mineral oil. The day following the SLS treatment, the remaining SLS residue was gently wiped from the area with gauze.

On Day 7, the test article extracts (0.3 mL) were applied to a 2 x 4 cm piece of filter paper (Whatman) to saturation and applied after SLS removal. The patch was secured to the site with non-permeable tape and the trunk wrapped with elastic bandage and Transpore™ tape. The control animals received a similar patch with the control vehicles. Freshly prepared extracts were used for this administration. This preparation was removed after 48 ± 2 hours of application.



Challenge Patch / Topical Application: Fourteen days after completion of the topical induction phase, the challenge procedure was initiated on the twenty-two test animals and the twelve negative control animals. A filter paper patch was saturated with 0.3 mL of freshly prepared test article extract and applied to the fur clipped right flank of each test animal. A filter paper patch was saturated with 0.3 mL of freshly prepared control vehicle and applied to the fur clipped left flank of each test animal.

The negative control animals were challenged in an identical fashion with similarly prepared patches. The left side of each animal was patched with a filter paper patch saturated with 0.3 mL of control vehicle. The right side was patched with a filter paper patch saturated with 0.3 mL of the prepared test article extract applied to the fur clipped flank. The trunk of each animal was wrapped for 24 ± 2 hours with an expandable wrapping material and secured with tape.

OBSERVATIONS AND SCORING

The following day (24 ± 2 hours) after challenge exposure, the patches were removed and the site was wiped gently with a 70% isopropyl alcohol soaked gauze sponge prior to each scoring period. The challenge sites were observed for irritation and sensitization reaction, as indicated by erythema and edema. Daily challenge observation scores were recorded 24 ± 2 and 48 ± 2 hours after patch removal in accordance with the classification system for skin reactions in Table 6. Daily animal health observations were recorded throughout the study period.

**TABLE 6: DERMAL OBSERVATION SCORING
(AS PER MAGNUSSON AND KLIGMAN SCALE)**

PATCH TEST REACTION	GRADING SCALE
No visible change – No erythema and edema	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and/or swelling	3

Note: Erythema is defined as redness and edema is defined as a swelling at the challenge site. Any other adverse changes at the skin sites were recorded and reported.

TERMINATION

Animals were euthanized by CO₂ asphyxiation following completion of this test.

EVALUATION CRITERIA

- Grades of '1' or greater in the test group generally indicate sensitization, provided grades of less than '1' are observed on the control animals. If grades of '1' or greater are noted on control animals, then the reactions of the test animals which exceed the most severe control reaction are presumed to be due to sensitization.
- Occasionally, the test group has a greater number of animals showing a response than the controls, although the intensity of the reaction is not greater than that observed on the controls. In these instances, a rechallenge may be necessary to define the response clearly. A rechallenge shall be carried out 1 - 2 weeks after the first challenge using the same animals. The method used shall be as described for the first challenge, with the exception that testing will be performed on a naïve site.
- The outcome of the test will be presented as the frequency of positive challenge results in test and control animals.



ASSAY VALIDITY

Final evaluation of the validity of the assay and test article results was based upon the following criteria and scientific judgment:

A test was considered invalid and will be repeated if:

- less than ten test animals or less than five control animals survived the duration of the study.
- any animals developed frank infection of the injection sites.

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS: Not applicable.

STATISTICAL METHODS: None used.

RECORD RETENTION: An exact copy of the original final report and all raw data pertinent to this study will be stored by WuXi AppTec, Inc. It was the responsibility of the Sponsor to retain a sample of the test article.

COMPLIANCE

Animal Welfare

WuXi AppTec, Inc. maintains the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accreditation. All applicable portions of this study conformed to the following regulations and guidelines regarding animal care and welfare:

- NIH guidelines as reported in the "Guide for the Care and Use of Laboratory Animals," National Research Council of the National Academies, eighth edition, 2011;
- (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Health Research Extension Act of 1985 (Public Law 99-158), Revised 1986;
- USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1, 2, and 3, *Animal Welfare*, Final Rule 1989; and
- WuXi AppTec Policy on Humane Care.

International Standards

ISO 10993-10: 2010. Biological Evaluation of Medical Devices, Part 10-Tests for Irritation and Skin Sensitization, pp. 18-26.

ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

TEST ARTICLE DISPOSITION: Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

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RESULTS

Clinical Observations: None of the animals in the study showed abnormal clinical signs during the test period.

Main Test Results: None of the negative control animals challenged with the control vehicles were observed with a sensitization response greater than '0'. None of the test animals challenged with the test article extracts were observed with a sensitization response greater than '0'. A negative sensitization incidence was interpreted for all test animals. See Table 7 for individual animal scores.

TABLE 7: DAILY CHALLENGE OBSERVATIONS

NORMAL SALINE (NS)					
ANIMAL #	24 HOURS SCORE		48 HOURS SCORE		RESULTS (+) OR (-)
	TEST GROUP				
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
1075	0	0	0	0	-
1076	0	0	0	0	-
1077	0	0	0	0	-
1078	0	0	0	0	-
1079	0	0	0	0	-
1080	0	0	0	0	-
1081	0	0	0	0	-
1082	0	0	0	0	-
1083	0	0	0	0	-
1084	0	0	0	0	-
1085	0	0	0	0	-
Negative Control Group					
Animal #	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	Results (+) or (-)
1069	0	0	0	0	-
1070	0	0	0	0	-
1071	0	0	0	0	-
1072	0	0	0	0	-
1073	0	0	0	0	-
1074	0	0	0	0	-

COTTONSEED OIL (CSO)					
ANIMAL #	24 HOURS SCORE		48 HOURS SCORE		RESULTS (+) OR (-)
	TEST GROUP				
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
1093	0	0	0	0	-
1094	0	0	0	0	-
1095	0	0	0	0	-
1096	0	0	0	0	-
1097	0	0	0	0	-
1098	0	0	0	0	-
1099	0	0	0	0	-
1100	0	0	0	0	-
1101	0	0	0	0	-
1102	0	0	0	0	-
1103	0	0	0	0	-
Negative Control Group					
Animal #	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	Results (+) or (-)
1087	0	0	0	0	-
1088	0	0	0	0	-
1089	0	0	0	0	-
1090	0	0	0	0	-
1091	0	0	0	0	-
1092	0	0	0	0	-

Positive Control: WuXi AppTec completes positive controls every 3 months. A positive control was completed on 06/13/11 (see Table 8 for individual animal scores). The methods for the positive control assay are similar to the methods described above in the "Experimental Method Summary." Guinea pigs utilized for positive control studies are of the Hartley strain and are supplied by the same vendor as animals used for general testing (Charles River Laboratories). For the Induction I and Induction II phases, 0.3% dinitrochlorobenzene (DNCB), a known sensitizer, in ethanol is used. For the challenge phase, 0.15% DNCB in acetone is used. The negative control animals are exposed to the appropriate vehicle (acetone is used for the challenge and ethanol is used for the Inductions I and II) only.

Animals in the positive control test group exhibited moderate and confluent erythema to intense erythema and swelling at the challenge sites (scores of 2-3 during 48 hour score) and all of the animals in the negative control group exhibited discrete or patchy erythema at the challenge sites (score of 1 during 48 hour score) when treated with the 0.15% w/v mixture of DNCB in acetone. Since the sensitization results are based on the scores of the test group subtracted by the scores of the negative control group, the score of 1 for the negative control animals are subtracted from that of the scores of 2-3, observed for the positive control test group. Therefore, all reactions in the positive control test group are considered to be sensitization reactions. Based on the results obtained, this test methodology demonstrated dermal sensitization in guinea pigs using DNCB, a known sensitizer.

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Table 8: Positive (DNCB) and Negative Control Daily Challenge Observations

ANIMAL #	24 HOURS	48 HOURS	RESULTS		
	SCORE	SCORE			
POSITIVE CONTROL GROUP					
	CONTROL VEHICLE	DNCB SOLUTION	CONTROL VEHICLE	DNCB SOLUTION	RESULTS (+) OR (-)
116914	0	1	0	2	+
116915	0	1	0	2	+
116916	0	3	0	3	+
116917	0	2	0	2	+
116918	0	2	0	2	+
78396	0	3	0	3	+
116013	0	2	0	2	+
115810	0	2	0	2	+
115828	0	2	0	2	+
115846	0	1	0	2	+
115864	0	1	0	2	+
NEGATIVE CONTROL GROUP					
	CONTROL VEHICLE	DNCB SOLUTION	CONTROL VEHICLE	DNCB SOLUTION	RESULTS (+) OR (-)
116908	0	1	0	1	-*
116909	0	1	0	1	-*
116910	0	1	0	1	-*
116911	0	1	0	1	-*
116912	0	1	0	1	-*
116913	0	1	0	1	-*

DNCB= dinitrochlorobenzene

*See positive control section

ANALYSIS AND CONCLUSION

None of the negative control animals challenged with the control vehicle were observed with a sensitization response greater than '0'. None of the animals challenged with the test article extracts were observed with a sensitization response greater than '0'. The normal saline extract of the test material had a sensitization response of '0' under valid test conditions. The cottonseed oil extract of the test material had a sensitization response of '0' under valid test conditions. Under the conditions of this protocol, the test article **did not** elicit a sensitization response.

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REFERENCES

ASTM Designation: F720-81 (Reapproved 2002) Standard Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test. Section 13, Volume 13.01, pp. 268-270.

Guidance for Industry (Draft Guidance), 2008: Coronary Drug - Eluting Stents - Nonclinical and Clinical Studies: Companion Document, pg. 12. US Department of Health and Human Services, FDA. March, 2008.

Magnusson, B. and Kligman, A.M. "Allergic Contact Dermatitis in the Guinea Pig, Identification of Contact Allergens." Springfield, Ill.: Thomas. 1970.

Magnusson, B. and Kligman, A.M. "The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test." *J. Invest. Dermatol.* 52:268-276. 1969.

WuXi AppTec Reference Library Contents, Form ALS-4650-1

WuXi AppTec SOP: ILS-0018, Environmental Conditions in the Animal Facility

WuXi AppTec SOP: ILS-0092, Receiving Shipments of Animals

WuXi AppTec SOP: ILS-0112, Animal Identification

WuXi AppTec SOP: ILS-0155, ISO Guinea Pig Maximization Sensitization Test

WuXi AppTec SOP: ILS-0233, Proper Handling of Sick, Injured, and/or Moribund Animals

WuXi AppTec SOP: ILS-0165, ISO Guinea Pig Positive Control Validation for Maximization Sensitization Test

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AMENDMENTS TO GLP FINAL REPORT (10/26/11)

Modification(s) to Report:

At the request of the Sponsor, the original final report dated 10/17/11 was amended on 10/26/11 to include two changes. The changes were included in the body of the report as appropriate. The changes had no impact on the scientific interpretations or conclusions of the study.

1. The Test Article Identification was changed to read: "Trade Name: Eagle 3mm Jaw and Shaft, Built according to Assembly: PRC049736 Rev C, Sterilization: 110-130 kGy Gamma, Lot#: PRC049736, Ref# EE2702" on pages 1 and 3 of the report.
2. For clarity and to reduce ambiguity, the following statement under test article extraction section: "Brown and white flakes were observed in the NS extract at the first induction, but the extract was clear with a slightly white color. At induction two, flakes were also observed (white in color) and the extract had an opaque color, but was otherwise clear in appearance (i.e. no turbidity)" was changed to "Brown and white flakes were observed in the NS extract at the first induction, but the extract was clear and slightly white. At induction two, white flakes were also observed in the NS extract and although slightly opaque, the extract appeared relatively clear".

Study Director

A handwritten signature in black ink, appearing to be 'J. Hill'.

Date

10/26/11

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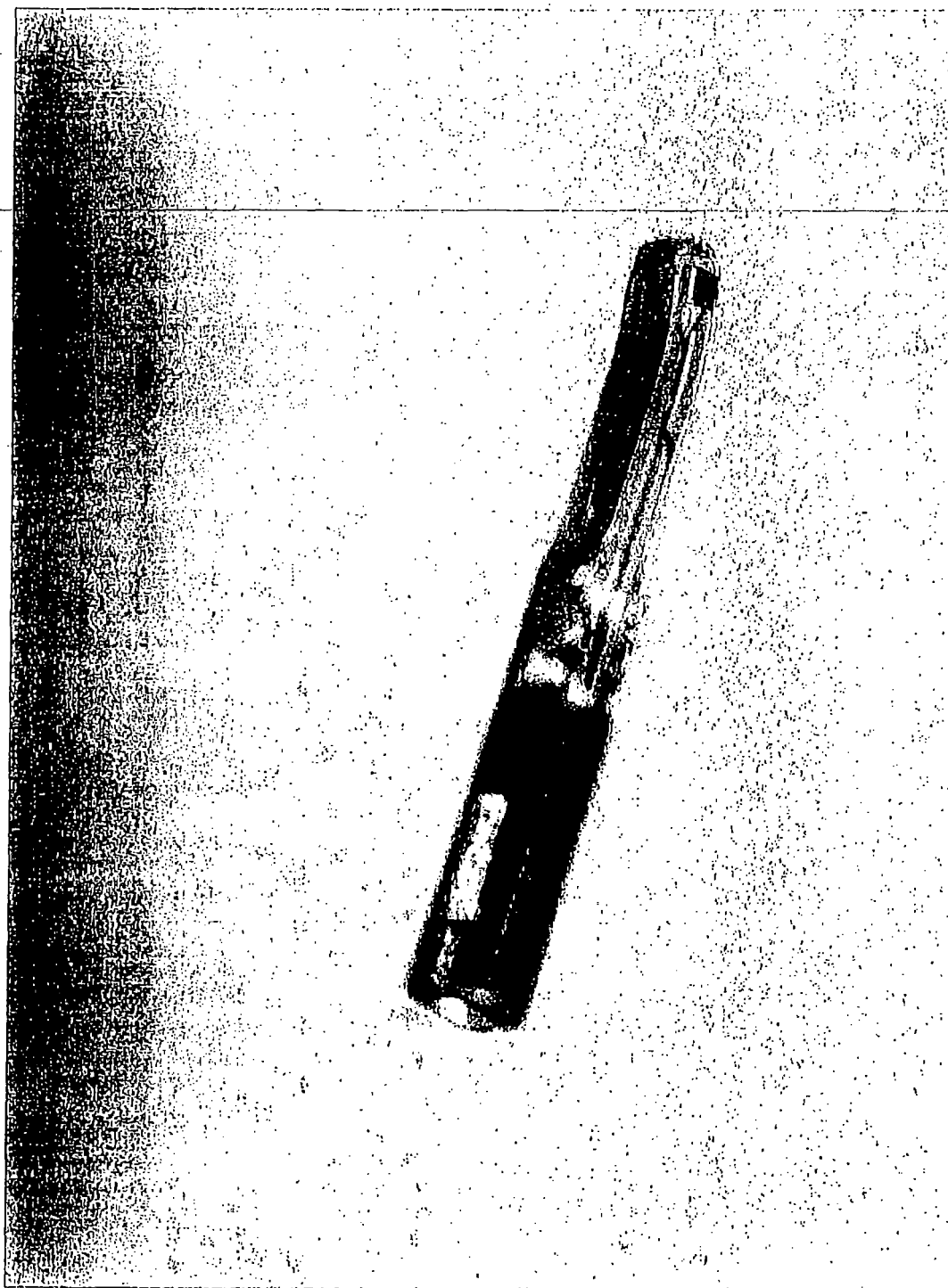
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APPENDIX

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Diet: Animals were supplied with a certified commercial rodent diet. No known contaminants present in the feed were expected to interfere with the test results.

Water: Animals were supplied with potable water obtained from the St. Paul municipal water supply. No known contaminants present in the water were expected to interfere with the results.

TEST MATERIAL PREPARATION

According to the Sponsor, the test article consisted of silver metal jaw attached to black shaft with white printing. The test article was cut for extraction, placed into test tubes and prepared at a ratio of 4 g to 20 mL of extraction vehicle. Per the request of the Sponsor, the samples were cut 4 cm from the jaw end. The sample tested included the jaw and part of the shaft with pad printing.

TABLE 1: TEST ARTICLE RECORD

EXTRACT VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	NUMBER OF TEST ARTICLES USED PER EXTRACT
0.9% Normal Saline (NS)	3.2	16.0	1
Cottonseed Oil (CSO)	3.2	16.0	1

Test Article Extraction: The extraction mixtures and corresponding control blanks were incubated for 72 ± 2 hours at 50 ± 2 °C. At the start of the extraction, the solutions appeared clear and free of particulates. The extracts were agitated during the course of the extraction period. At the end of the extraction period, the vessels were shaken well and the liquid aseptically decanted into a sterile glass vessel. The test article was observed after all extractions to be intact with no macroscopically observable degradation. The NS test extract appeared cloudy and contained white flakes. The particles were allowed to settle prior to drawing the extract into the syringe for dosing. The remaining extracts were clear. After decanting, the extracts were not filtered prior to use. The extracts were maintained at room temperature and used within 24 hours of preparation. See Tables 1-3.

TABLE 2: EXTRACTION RECORD

VEHICLE	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END
NS	50.2 °C	09/17/11 8:06 am	50.2 °C	09/20/11 6:15 am
CSO	50.2 °C	09/17/11 8:06 am	50.2 °C	09/20/11 6:15 am

TABLE 3: VEHICLE RECORD

VEHICLE IDENTIFICATION	LOT #	SUPPLIED BY	EXPIRATION
NS	J1C616	Braun	09/2013
CSO	ZN1009	Spectrum	01/02/12

SELECTION OF ANIMALS

Animals were randomly placed in cages upon receipt and were placed on study as available. Animals considered unsuitable due to poor health or outlying body weight were excluded from the study.

TEST ARTICLE ADMINISTRATION

Groups of five animals were injected with either the test article extract or the corresponding control vehicle as indicated in the table below:

EXTRACT OR CONTROL	ROUTE	DOSE/KG	INJECTION RATE
NS	Intravenous	50 mL	~0.1 mL/sec
CSO	Intraperitoneal	50 mL	Not Applicable



OBSERVATIONS

Body Weights: Body weight recordings, to the nearest 0.1 g, were made on the day of dosing and at 24 ± 2 , 48 ± 2 and 72 ± 2 hours post-injection. See Data Table B for individual weight results.

Clinical Signs: Observations for mortality and signs of pharmacological and/or toxicological effects were made immediately post-injection and at 4 ± 0.75 , 24 ± 2 , 48 ± 2 , and 72 ± 2 hours post-injection.

TERMINATION

Following the final observations the animals were euthanized by CO₂ asphyxiation.

EVALUATION CRITERIA

According to ISO guidelines, the test is considered negative if none of the animals injected with the test article extract show a significantly greater biological reaction than the animals treated with the control vehicle extract. Death in two or more mice or other toxic signs such as convulsions, prostration, or body weight loss greater than 10% in three or more mice are interpreted as significant biological reactions.

ASSAY VALIDITY

Final evaluation of the validity of the assay and test article results was based upon the following criteria and scientific judgment:

A control failure is defined as death in two or more control animals showing signs of toxicity such as convulsions or prostration or weight loss of more than 10% of body weight in three or more mice.

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS: Not applicable.

STATISTICAL METHODS: Descriptive statistics are presented in Data Table B.

RECORD RETENTION: An exact copy of the original final report and all raw data pertinent to this study will be stored by WuXi AppTec, Inc. It was the responsibility of the Sponsor to retain a sample of the test article.

COMPLIANCE

Animal Welfare

WuXi AppTec, Inc. maintains the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accreditation. All applicable portions of the study will also conform to the following regulations and guidelines regarding animal care and welfare:

- NIH guidelines as reported in the "Guide for the Care and Use of Laboratory Animals," National Research Council of the National Academies, eighth edition, 2011;
- (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Health Research Extension Act of 1985 (Public Law 99-158), Revised 1986;
- USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1, 2, and 3, *Animal Welfare*, Final Rule 1989; and
- WuXi AppTec Policy on Humane Care.

International Standards

The study was also in compliance with the following International Standards:

ISO 10993-11: 2006 Biological Evaluation of Medical Devices Part 11: Tests for Systemic Toxicity.

ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.



TEST ARTICLE DISPOSITION: Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

None of the animals on study were observed with abnormal clinical signs indicative of toxicity during the 72 hour test period. All were alive at the end of the 72 hour test duration and body weight loss was within acceptable parameters over the course of the study. See Data Table A.

DATA TABLE A: MORTALITY, CLINICAL SIGNS AND WEIGHT LOSS INCIDENCE

EXTRACT	FATALITIES		TOXICITY CLINICAL SIGNS		ANIMALS WITH >10% BODY WEIGHT LOSS	
	TEST	CONTROL	TEST	CONTROL	TEST	CONTROL
NS	0/5	0/5	0/5	0/5	0/5	0/5
CSO	0/5	0/5	0/5	0/5	0/5	0/5

ANALYSIS AND CONCLUSION

The vehicle control treated animals had no signs of toxicity at any of the observation periods and no animals lost weight in excess of 10%, indicating a valid test. None of the test article extract treated animals were observed with clinical signs consistent with toxicity at any of the observation periods. Body weight changes were within acceptable parameters over the course of the study. These findings indicate that the requirements of the ISO Acute Systemic Injection Test **have been met** by the test article.

**DATA TABLE B: ANIMAL WEIGHTS (g) AND
STANDARD DEVIATION CALCULATIONS**

GROUP	ANIMAL #	INITIAL	24 HRS	48 HRS	72 HRS	BW CHANGE
Test NS	1	23.5	23.7	24.1	24.1	0.6
	2	25.4	25.4	25.8	25.1	-0.3
	3	23.8	24.1	24.2	24.6	0.8
	4	24.3	25.3	25.1	24.6	0.3
	5	24.3	24.5	25.2	24.4	0.1
Average Body Weight		24.3	24.6	24.9	24.6	0.3
Standard Deviation		0.7	0.7	0.7	0.4	0.4
Control NS	21	24.6	25.5	24.7	25.9	1.3
	22	24.1	24.3	24.6	24.8	0.7
	23	24.1	25.0	25.1	24.9	0.8
	24	23.8	24.7	24.9	24.9	1.1
	25	23.4	24.8	24.1	24.4	1.0
Average Body Weight		24.0	24.9	24.7	25.0	1.0
Standard Deviation		0.4	0.4	0.4	0.6	0.2
Test CSO	11	25.0	25.4	26.5	25.5	0.5
	12	25.0	24.5	24.8	25.0	0.0
	13	23.9	24.6	24.7	24.1	0.2
	14	24.2	23.9	24.5	24.0	-0.2
	15	23.2	23.6	23.5	23.6	0.4
Average Body Weight		24.3	24.4	24.8	24.4	0.2
Standard Deviation		0.8	0.7	1.1	0.8	0.3
Control CSO	31	26.2	27.6	27.6	27.6	1.4
	32	24.0	24.3	24.5	25.9	1.9
	33	22.7	23.7	23.5	23.1	0.4
	34	23.3	23.6	24.2	24.0	0.7
	35	21.8	22.3	22.9	22.7	0.9
Average Body Weight		23.6	24.3	24.5	24.7	1.1
Standard Deviation		1.7	2.0	1.8	2.1	0.6



REFERENCES

U.S. Pharmacopeia, Section 88, current revision.

WuXi AppTec Reference Library Contents, Form ALS-4650-1

WuXi AppTec SOP ALS-0260, Sample Extraction Procedures

WuXi AppTec SOP: ILS-0018, Environmental Conditions in the Animal Facility

WuXi AppTec SOP: ILS-0092, Receiving Shipments of Animals

WuXi AppTec SOP: ILS-0112, Animal Identification

WuXi AppTec SOP: ILS-0115, ISO/USP/Japanese Acute Systemic Injection Test

WuXi AppTec SOP: ILS-0233, Proper Handling of Sick, Injured, and/or Moribund Animals



AMENDMENT TO GLP FINAL REPORT (10/27/11)

Modification(s) to Report:

At the request of the Sponsor, the original final report dated 09/29/11 was amended on 10/27/11 to include the following changes. The changes were included in the body of the report as appropriate. The change had no impact on the scientific interpretations or conclusions of the study.

1. The sterilization portion of the test article name along with the sterilization section of the test request form was changed to the following: Sterilization: 110-130 kGy Gamma on pages 1 and 3.
2. The amendment changing the sterilization was added to page 4.

Michelle Dietz
Study Director

10/27/11
Date



**AMENDED (2)
FINAL STUDY REPORT**

STUDY TITLE: ISO Intracutaneous Reactivity Test

PROTOCOL NUMBER: 9107015-2

TEST ARTICLE IDENTIFICATION: Trade Name: Eagle 3mm Jaw and Shaft
Built according to Assembly: PRC049736 Rev C
Sterilization: 110-130 kGy Gamma
Lot#: PRC049736
Ref# EE2702

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

STUDY NUMBER: 157893

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: The requirements of the ISO Intracutaneous Reactivity Test have been met by the test article.

Protocol Number: 9107015-2

Project No. 157893

Amended Report (2): October 31, 2011

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QUALITY ASSURANCE UNIT SUMMARY

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the quality and integrity of the study.

<u>Phase Inspected</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Test Article Preparation	09/07/11	09/07/11	10/03/11
Final Report	10/03/11	10/03/11	10/03/11
Amended Report	10/05/11	10/05/11	10/10/11
Amended Report (2)	10/27/11	10/27/11	11/1/11

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: Dawn Vogland Date: 11/1/11
Dawn Vogland

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: Brianna Carlson Date: 11/1/11
Brianna Carlson

Professional Personnel Involved:

Teri Tanquist, BS
Christine Olson, BS
Roxanne Miller, AA, CVT
Brianna Carlson, BA
Jean Mesarich, AA

Vice President of Process Improvement and Operations
Study Operations Manager
Associate Director, In-Life Studies
Study Director
Client Relations Manager

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**PURPOSE**

The purpose of this test was to determine if any chemicals that may leach or be extracted from the test article were capable of causing local irritation in the dermal tissues of rabbits.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

DATE SAMPLE RECEIVED: 08/15/11
STUDY INITIATION DATE: 09/06/11
STUDY COMPLETION DATE: 10/03/11
AMENDED REPORT DATE: 10/10/11
AMENDED REPORT DATE (2): 11/1/11

TEST ARTICLE IDENTIFICATION :

Test Article Name:	Trade Name: Eagle 3mm Jaw and Shaft Built according to Assembly: PRC049736 Rev C Sterilization: 110-130 kGy Gamma Lot#: PRC049736 Ref# EE2702
Lot #:	PRC049736
Sterilization Method:	Gamma 110-130 kGy
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Device

CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

EXPERIMENTAL DESIGN**Experimental Summary**

The study was conducted in accordance with ISO 10993-10: 2010 Standard, Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Skin Sensitization, Pages 11-14. Each rabbit received five sequential 0.2 mL intracutaneous injections along either side of the dorsal mid-line with the test article extract on one side and the concurrent vehicle control on the other. The irritation reaction of the test extracts were compared to vehicle controls and recorded over a 72-hour period according to the standard ISO irritation scoring system.

Justification For Selection Of The Test System

This test method and species have historically been used to assess the potential of the material under test to produce intradermal irritation to help determine biocompatibility of materials used in medical devices. The animal species, number and route of test article administration will be as recommended in ISO 10993-10.

Institutional Animal Care and Use Committee (IACUC)

The protocol and any amendments or procedures involving the care or use of animals on this study were reviewed and approved by WuXi AppTec's IACUC prior to the initiation of such procedures.

IACUC Protocol / Effective Date: 07-122B / June, 2010

AMENDMENTS 1: The test article identification was amended to include a reference number, Ref# EE2702.

AMENDMENTS 2: The sterilization method was changed to "Gamma 110-130 kGy" and the test article identification was changed to "Trade Name: Eagle 3mm Jaw and Shaft Built according to Assembly: PRC049736 Rev C Sterilization: 110-130 kGy Gamma Lot PRC049736 Ref# EE2702"

DEVIATIONS: None.

TEST SYSTEM

Species/Strain: Albino rabbits (*Oryctolagus cuniculus*) / New Zealand White strain

Source: Bakkom Rabbitry

Sex: Female. Females used were nulliparous and non-pregnant.

Weight Range: Each rabbit weighed at least 2.0 kg.

Age: The rabbits were young adults.

Number: A total of three animals were used for this study.

Animal Identification: Individual animals were identified per WuXi AppTec SOP: ILS-0112.

HUSBANDRY

Receipt: Animals were received on 09/06/11 according to WuXi AppTec SOP: ILS-0092. The animals were acclimated for a minimum of 5 days under the same conditions as the actual test.

Housing: Animals were individually housed in stainless steel caging. Housing dimensions complied with NIH and AAALAC International guidelines for this species.

Environment: The environmental conditions in the animal rooms were maintained according to WuXi AppTec SOP: ILS-0018. The temperature and photo-period were set to meet the AAALAC International recommendations for these species. The laboratory and animal rooms were maintained as limited-access facilities.

Diet: Animals were supplied with certified commercial feed, *ad libitum*. There were no known contaminants present in the feed expected to interfere with the test results. Feed analysis results are available and archived by WuXi AppTec.



Water: Potable water was supplied from the St. Paul municipal water supply, *ad libitum*. There were no known contaminants present in the water expected to interfere with the test results. Periodic analysis of the water is conducted and the results are archived by WuXi AppTec.

TEST AND CONTROL MATERIAL PREPARATION

According to the Sponsor the test article consisted of a silver metal jaw attached to a black shaft with white printing. The test article was cut for extraction, placed into test tubes and prepared at a ratio of 4 g to 20 mL of extraction vehicle. Per Sponsor instruction, samples were cut at 4 cm from the end. The sample was 4 cm long and contained the jaw and part of the shaft that has pad printing on it.

TABLE 1: TEST ARTICLE RECORD

EXTRACT VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	NUMBER OF TEST ARTICLE DEVICES USED PER EXTRACT
0.9% Normal Saline (NS)	3.2	16.0	1
Cottonseed Oil (CSO)	3.2	16.0	1

Test Article Extraction: The extraction mixtures and corresponding control blanks were incubated for 72 ± 2 hours at 50 ± 2 °C. At the start of the extraction, the solutions appeared clear and free of particulates. The extracts were agitated during the course of the extraction period. At the end of the extraction period, the vessels were shaken well and the liquid aseptically decanted into a sterile glass vessel. The test article was observed after all extractions to be intact with no macroscopically observable degradation. There were white flake-like particulates observed throughout the NS test extract. The remaining extracts appeared clear and free of particulates. The particles were allowed to settle prior to drawing the extract into the syringe for dosing. After decanting, the extracts were not filtered prior to use. The extracts were maintained at room temperature and used within 24 hours of preparation. See Tables 1-3.

TABLE 2: EXTRACTION RECORD

VEHICLE	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END
NS	50.2 °C	09/09/11 7:33 am	50.2 °C	09/12/11 6:25 am
CSO	50.2 °C	09/09/11 7:33 am	50.2 °C	09/12/11 6:25 am

TABLE 3: VEHICLE RECORD

VEHICLE IDENTIFICATION:	LOT #	SUPPLIED BY:	EXPIRATION
NS	J1C616	Braun	09/2013
CSO	ZN1009	Spectrum	01/02/12

SELECTION OF ANIMALS

Animals were randomly placed in cages upon receipt, and were placed on study as available. Any animals considered unsuitable due to poor health, abraded skin or outlying body weight were excluded from the study.

ANIMAL PREPARATION

Each animal was weighed and the weight recorded prior to test injection. The fur of the animals was clipped on both sides of the spinal column to expose a sufficient sized area for injection.



TEST ARTICLE ADMINISTRATION

The two test article extracts and the two vehicle controls were each injected into three rabbits. Each rabbit received five sequential 0.2 mL intracutaneous injections of the test article extract on the right side of the vertebral column and similarly the control vehicle on the left side. The second test and control extract injections were parallel and distal to the first injection sites. (See Figure 1.)

FIGURE 1: INJECTION SITES ON RABBITS

	CONTROL		HEAD	TEST		
	Control Vehicle #2	Control Vehicle #1		Test Extract #1	Test Extract #2	
	1	1		1	1	
0.2 mL Control	2	2		2	2	0.2 mL Test
Vehicle Injected	3	3		3	3	Article Extract
	4	4		4	4	Injected
	5	5		5	5	
			TAIL			

OBSERVATIONS AND SCORING

The animals were observed daily for abnormal clinical signs. The appearance of each injection site was noted at 24 ± 2 , 48 ± 2 and 72 ± 2 hours post injection. The tissue reactions were rated for gross evidence of erythema and edema. The skin was lightly swabbed with dilute alcohol to enhance the appearance of any erythema or edema. The intradermal injection of CSO frequently elicits an inflammatory response. CSO erythema scores ≤ 2 are considered normal. A well-defined positive response is characterized by a score greater than 2. Table 4 was used to score the reactions.

TABLE 4: DERMAL OBSERVATION SCORING

ERYTHEMA	EDEMA
0 = No erythema	0 = No edema
1 = Very slight erythema (barely perceptible)	1 = Very slight edema (barely perceptible)
2 = Well defined erythema	2 = Well defined edema (edges of area well-defined by definite raising)
3 = Moderate to severe erythema	3 = Moderate edema (raised ~1 mm)
4 = Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4 = Severe edema (raised > 1 mm and extending beyond area)

TERMINATION

All animals were euthanized according to WuXi AppTec SOP: ILS-0230 with a sodium pentobarbital based solution after the 72-hour observations were recorded.

EVALUATION CRITERIA

According to ISO 10993:10 test criteria, if the difference between the average scores for the extract of the test article and the vehicle control is less than or equal to 1.0, the test article is considered non-irritating.

ASSAY VALIDITY

Final evaluation of the validity of the assay and test article results was based upon scientific judgment.

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS: See calculations section.

STATISTICAL METHODS: None used.



RECORD RETENTION: An exact copy of the original final report and all raw data pertinent to this study will be stored by WuXi AppTec, Inc. It was the responsibility of the Sponsor to retain a sample of the test article.

COMPLIANCE

Animal Welfare

WuXi AppTec, Inc. maintains the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accreditation. All applicable portions of the study will also conform to the following regulations and guidelines regarding animal care and welfare:

- NIH guidelines as reported in the "Guide for the Care and Use of Laboratory Animals," National Research Council of the National Academies, eighth edition, 2011;
- (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Health Research Extension Act of 1985 (Public Law 99-158), Revised 1986.
- USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1, 2, and 3, Animal Welfare, Final Rule 1989.
- WuXi AppTec Policy on Humane Care.

The study was also performed in compliance with the following International Standards:

ISO 10993-10: 2010 Standard, Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Skin Sensitization, Pages 11-14.

ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

TEST ARTICLE DISPOSITION: Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

Clinical Observations

None of the animals on study showed abnormal clinical signs during the 72 hour test period.

Dermal Observations

At the 48 hour observation period, a score of '3' was noted at site 1 of CSO control for animal 22453. This observation does not impact the scientific interpretation of the study, as it was limited to one site on one animal. The mean control value for CSO was less than 2.0, indicating the assay is valid despite this observation.

There were no other significant dermal reactions observed at the injected test and control sites on the rabbits at the 24, 48 and 72 hour observation periods.

CALCULATIONS

After the 72 $\pm$ 2 hour observation period, all erythema grades plus edema grades 24 $\pm$ 2, 48 $\pm$ 2, and 72 $\pm$ 2 hour were totaled separately for each test sample or control for each individual animal. To calculate the score of a test sample or control on each individual animal, each of the totals was divided by 15 (three scoring time points x five test or control sample injection sites). To determine the overall mean score for each test sample and each corresponding control, the scores were added for the three animals and divided by three. The final test sample score was obtained by subtracting the score of the control from the test sample score. The results are presented in Tables 5 and 6.



TABLE 5: DERMAL OBSERVATIONS – 0.9% NORMAL SALINE

Rabbit # 22452	Control Scores						Test Scores					
	24 Hour ER ED		48 Hour ER ED		72 Hour ER ED		24 hour ER ED		48 hour ER ED		72 hour ER ED	
Site 1	0	0	0	0	0	0	0	0	0	0	0	0
Site 2	0	0	0	0	0	0	0	0	0	0	0	0
Site 3	0	0	0	0	0	0	0	0	0	0	0	0
Site 4	0	0	0	0	0	0	0	0	0	0	0	0
Site 5	0	0	0	0	0	0	0	0	0	0	0	0
Total	0		0		0		0		0		0	

Rabbit # 22453	Control Scores						Test Scores					
	24 Hour ER ED		48 Hour ER ED		72 Hour ER ED		24 hour ER ED		48 hour ER ED		72 hour ER ED	
Site 1	0	0	0	0	0	0	0	0	0	0	0	0
Site 2	0	0	0	0	0	0	0	0	0	0	0	0
Site 3	0	0	0	0	0	0	0	0	0	0	0	0
Site 4	0	0	0	0	0	0	0	0	0	0	0	0
Site 5	0	0	0	0	0	0	0	0	0	0	0	0
Total	0		0		0		0		0		0	

Rabbit # 22454	Control Scores						Test Scores					
	24 Hour ER ED		48 Hour ER ED		72 Hour ER ED		24 hour ER ED		48 hour ER ED		72 hour ER ED	
Site 1	0	0	0	0	0	0	0	0	0	0	0	0
Site 2	0	0	0	0	0	0	0	0	0	0	0	0
Site 3	0	0	0	0	0	0	0	0	0	0	0	0
Site 4	0	0	0	0	0	0	0	0	0	0	0	0
Site 5	0	0	0	0	0	0	0	0	0	0	0	0
Total	0		0		0		0		0		0	

Rabbit	Control scores (Total ER & ED)			Test scores (Total ER & ED)		
22452	0			0		
22453	0			0		
22454	0			0		

Rabbit	Total / 15	Total /15
22452	0	0
22453	0	0
22454	0	0
Average (Total/3)	0/3 = 0	0/3 = 0

Comparative Results (Average Test – Average Control)	0 – 0 = 0
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ER=Erythema ED=Edema



TABLE 6: DERMAL OBSERVATIONS – COTTONSEED OIL

Rabbit # 22452	Control Scores						Test Scores					
	24 Hour ER ED		48 Hour ER ED		72 Hour ER ED		24 hour ER ED		48 hour ER ED		72 hour ER ED	
Site 1	1	0	1	0	1	0	1	0	1	0	1	0
Site 2	1	0	1	0	1	0	1	0	1	0	1	0
Site 3	1	0	1	0	1	0	1	0	1	0	1	0
Site 4	1	0	1	0	1	0	1	0	1	0	1	0
Site 5	1	0	1	0	1	0	1	0	1	0	1	0
Total	5		5		5		5		5		5	
Rabbit # 22453	Control Scores						Test Scores					
	24 Hour ER ED		48 Hour ER ED		72 Hour ER ED		24 hour ER ED		48 hour ER ED		72 hour ER ED	
Site 1	1	0	3**	0	2	0	1	0	1	0	1	0
Site 2	1	0	2	1	2	0	1	0	1	0	1	0
Site 3	1	0	2	1	2	0	1	0	2	0	1	0
Site 4	1	0	2	1	2	0	1	0	2	0	3	0
Site 5	1	0	2	0	2	1	1	0	2	0	3	1
Total	5		14		11		5		8		10	
Rabbit # 22454	Control Scores						Test Scores					
	24 Hour ER ED		48 Hour ER ED		72 Hour ER ED		24 hour ER ED		48 hour ER ED		72 hour ER ED	
Site 1	1	0	1	0	1	0	1	0	1	0	1	0
Site 2	1	0	1	0	1	0	1	0	1	0	1	0
Site 3	1	0	1	0	1	0	1	0	1	1	1	0
Site 4	1	0	1	0	1	0	1	0	1	1	1	0
Site 5	1	0	1	0	1	0	1	0	1	1	1	0
Total	5		5		5		5		8		5	
Rabbit	Control scores (Total ER & ED)						Test scores (Total ER & ED)					
22452	5		5		5		5		5		5	
22453	5		14		11		5		8		10	
22454	5		5		5		5		8		5	
Rabbit	Total / 15						Total /15					
22452	1.0						1.0					
22453	2.0						1.5					
22454	1.0						1.2					
Average (Total/3)	4.0/3 = 1.3						3.7/3 = 1.2					
Comparative Results (Average Test – Average Control)			1.2 – 1.3 = 0*									

ER=Erythema ED=Edema

*Negative values are interpreted as zero (0).

See Dermal Observation section.

ANALYSIS AND CONCLUSION

The test was considered valid based upon scientific judgment. The differences in the mean test and control scores of the extract dermal observations were **less than 1.0**, indicating that the requirements of the ISO Intracutaneous Reactivity Test **have been met** by the test article.



REFERENCES

U.S. Pharmacopeia, Section 88, current revision.

WuXi AppTec SOP: ALS-0260, Sample Extraction Procedures

WuXi AppTec Reference Library Contents, Form ALS-4650-1

WuXi AppTec SOP: ILS-0018, Environmental Conditions in the Animal Facility

WuXi AppTec SOP: ILS-0092, Receiving Shipments of Animals

WuXi AppTec SOP: ILS-0100, ISO/USP/Japanese Intracutaneous Reactivity Test

WuXi AppTec SOP: ILS-0112, Animal Identification

WuXi AppTec SOP: ILS-0230, Euthanasia Procedures

WuXi AppTec SOP: ILS-0233, Proper Handling of Sick and Moribund Animals

WuXi AppTec SOP: TRG-0300, Preparation of Biomaterials for Extraction



AMENDMENT TO GLP FINAL REPORT (10/10/11)

Modification(s) to Report:

At the request of the Sponsor, the original final report dated 10/03/11 was amended on 10/10/11 to include one change. The change was included in the body of the report as appropriate. The change had no impact on the scientific interpretations or conclusions of the study.

1. The Dermal Observations section on page 7 of the final report was updated for clarity. Also the corresponding table, Table 6 on page 9 was updated to reflect the change.

AMENDMENT (2) TO GLP FINAL REPORT (10/31/11)

Modification(s) to Report:

At the request of the Sponsor, the final report dated 10/03/11 and amended report dated 10/10/11, amended on 10/31/11 to include two changes. These amendment were included in the body of the report as appropriate. These changes had no impact on the scientific interpretations or conclusions of the study.

1. The correct wording for the test article identification was as follows:
Trade Name: Eagle 3mm Jaw and Shaft
Built according to Assembly: PRC049736 Rev C
Sterilization: 110-130 kGy Gamma
Lot# PRC049736, Ref.# EE2702
2. The sterilization method was changed to "Gamma 110-130 kGy".
3. The amendment section on page 4 was changed to include the above amendment.

Study Director

A handwritten signature of the Study Director, appearing to be 'JES'.

Date

A handwritten signature for the date, appearing to be '11/1/11'.

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Justification For Selection Of The Test System

Rabbit blood has been used extensively for testing the hemolytic potential of medical device materials. In this case, the use of rabbit blood is specified in ASTM Method F-756-08.

Amendments:

- 1) The study directorship was reassigned to Tamara Charbonneau.
- 2) At the request of the Sponsor, the test article identification was updated to read as follows:
Trade Name: Eagle 3mm Jaw and Shaft
Built according to Assembly: PRC049736 Rev C
Sterilization: 45-75 kGy Gamma
Lot # PRC049736
Ref# EE2702
- 3) At the request of the Sponsor, the test article identification and sterilization method were updated to read "Sterilization: 110-130 kGy Gamma."

Deviations: None

IDENTIFICATION OF THE TEST SYSTEM

New Zealand White rabbit blood: 21095, 21093, 21094

The animals appeared healthy. Age, weight, sex are not factors for this test.

Source of Supply: WuXi AppTec approved vendor

TEST MATERIAL PREPARATION

According to the Sponsor, the test article consisted of a silver metal jaw attached to a black shaft with white printing. At the request of the Sponsor, the sample was cut 4 cm from the jaw end. The jaw and part of the shaft containing the pad printing were tested. A ratio of 4 g / 20 mL was used per the Sponsor's request.

TABLE 1: TEST ARTICLE RECORD

SAMPLE	NUMBER OF DEVICES USED PER REPLICATE
Test Article	1

BLOOD COLLECTION AND PREPARATION

Three healthy, mature New Zealand White rabbits of either sex, from a certified commercial vendor, were selected as donors for this study. Five to seven milliliters of blood was collected from each rabbit using appropriate venipuncture, employing evacuated tubes containing 3.2% sodium citrate. The time and date of blood collection and rabbit ear tag number was recorded directly on the vial, and the blood was used within 96 hours of collection. Blood was stored under refrigeration until time of use. Just prior to use, the blood was pooled and tested to ensure that the plasma free hemoglobin level was less than 2 mg/mL. The total blood hemoglobin concentration was determined and then diluted with an appropriate volume of phosphate buffered saline (PBS, calcium and magnesium free) to obtain a total blood hemoglobin concentration of 10 ± 1 mg/mL.

STANDARD CURVE

A hemoglobin standard (concentration 2.0 mg/mL), obtained from STANBIO, was used to create the standard curve. A modified National Committee for Clinical Laboratory Studies cyanmethemoglobin reagent (NCCLS CMR) (0.14 g potassium monophosphate, 0.05 g potassium cyanide, 0.2 g potassium ferricyanide, and 0.5 mc BRIJ 35 - pH 7.0-7.4) was used to dilute the stock solution to 1.0, 0.72, 0.6, 0.3, 0.12, 0.06 and 0.03 mg/mL.

After a five minute room temperature incubation, the preparations were scanned with a Beckman spectrophotometer at 540 nm. A standard curve was established by the reading from the spectrophotometer. The slope used for analysis is derived by the curve when the concentration is on the y-axis and the absorbance is on the x-axis. In the prepared curve, the axes are switched due to the

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spectrophotometer plotting template; therefore, the reciprocal of this slope was calculated to determine the actual value (F), which is used to calculate the unknown concentration.

TEST PROCEDURE

Phosphate buffered saline (PBS, calcium and magnesium free) was added to the test article replicates at a ratio of 20 mL to 4 g. See Table 2 for the ratios used for the controls. Next, diluted blood was added to the test and control solutions at a ratio of 1.0 mL blood to 7.0 mL PBS. These mixtures were incubated for three hours at 37 ± 2 °C. After this incubation, the liquid was removed from the samples and transferred to centrifuge tubes. The tubes were centrifuged at 2400 rpm for 15 minutes to remove cellular material.

A cyanmethemoglobin reagent (NCCLS CMR reagent) was added to each of the sample supernatants at a 1:1 ratio. 1.0 mL of NCCLS CMR reagent was combined with 1.0 mL supernatant and incubated for 5 minutes at room temperature. After 5 minute incubation, the samples were read at a wavelength of 540 nm on a spectrophotometer. The hemolytic index was then calculated based on these results. See Table 6 for the condition of the test sample and control supernatant.

TABLE 2: TEST ARTICLE AND CONTROL PREPARATIONS

DESCRIPTION	SIZE	PBS VOLUME (mL)	DILUTED RABBIT BLOOD VOLUME (mL)
Test Article	# 1) 3.2 g	16.0	2.3
	# 2) 3.2 g	16.0	2.3
	# 3) 3.2 g	16.0	2.3
Blank Control	# 1) NA	7.0	1.0
	# 2) NA	7.0	1.0
	# 3) NA	7.0	1.0
Negative Control	# 1) 42.0 cm ²	7.0	1.0
	# 2) 42.0 cm ²	7.0	1.0
	# 3) 42.0 cm ²	7.0	1.0
Positive Control	# 1) 42.0 cm ²	7.0	1.0
	# 2) 42.0 cm ²	7.0	1.0
	# 3) 42.0 cm ²	7.0	1.0

NA = Not Applicable

TABLE 3: REAGENT/CONTROL RECORD

REAGENT / CONTROL IDENTIFICATION	CLASS	LOT #	SUPPLIED BY:	EXPIRATION
Hemoglobin	Standard	06111	STANBIO	02/2013
NCCLS CMR	Reagent	TDS1044	WuXi AppTec	01/19/12
Phosphate Buffered Saline (PBS, calcium and magnesium free)	Blank/Vehicle	0000242307	Lonza	06/13/13
Nitrile	Positive Control	WXAT073010	Kimberly-Clark	07/30/15
High Density Polyethylene (HDPE)	Negative Control	C-091	Hatano Research Institute, FDSC	02/2016

TABLE 4: BLOOD INCUBATION

VEHICLE	INCUBATION TEMPERATURE (IN)	DATE/TIME OF INCUBATION START	INCUBATION TEMPERATURE (OUT)	DATE/TIME OF INCUBATION END
PBS	37.0 °C	09/08/11 10:50 am	37.0 °C	09/08/11 1:50 pm

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TEST EVALUATION

TABLE 5: HEMOLYTIC GRADE SYSTEM

BLANK CORRECTED HEMOLYTIC INDEX ABOVE THE NEGATIVE CONTROL	CLASSIFICATION	INTERPRETATION
0-2%	Non - hemolytic	Pass
2.1-5.0%	Slightly hemolytic	Pass
5.1% or greater	Hemolytic	Fail

ASSAY VALIDITY

Final evaluation of the validity of the assay was based on the following criteria and scientific judgment. The blood must have a plasma free hemoglobin value less than 2 mg/mL. The negative control should have a hemolytic index less than or equal to 2% and the positive control must have a hemolytic index greater than 5%.

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS

The following equations were used as applicable to analyze data.

Plasma Free Hemoglobin (PFH), reported in mg/mL.

$$PFH = (A^{PFH}) \times F \times 2$$

A^{PFH} = absorbance of untreated, centrifuged blood sample

$F = 1 / \text{slope of calibration curve}$

Total Blood Hemoglobin Concentration (TBHC)

$$TBHC = (A^C) \times F \times 251$$

$$\text{Verified TBHC} = (A^T) \times F \times 16$$

A^C = average absorbance of untreated blood; $F = 1 / \text{slope of calibration curve}$

A^T = average absorbance of blood dilution

Sample Hemoglobin Concentration

$$S = (A^S) \times F \times 2$$

A^S = average absorbance of sample or control; $F = 1 / \text{slope of calibration curve}$

Blank Hemoglobin Concentration

$$B = (A^B) \times F \times 2$$

A^B = average absorbance of blank; $F = 1 / \text{slope of calibration curve}$

% Hemolytic Index (% HI)

$$\% HI = \frac{\text{Concentration of Hemoglobin released in supernatant} \times 100}{\text{Total Hemoglobin Concentration in tube}}$$

Blank Corrected % Hemolytic Index (% BCHI)

$$\% BCHI = \% HI (\text{test group}) - \% HI (\text{blank})$$

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% Hemolysis (% H)

$$\% H = (S/W) \times 100$$

S = Sample Hemolysis Concentration; W = Total Hemoglobin / 8

Blank Corrected % Hemolysis

$$\% BCH = \frac{A^S - A^B}{A^T - A^B} \times 100$$

A^S = average absorbance of sample; A^B = average absorbance of blank
 A^T = average absorbance for total hemoglobin

Blank Corrected Hemolytic Index Above the Negative Control

$$BCHI^{AN} = BCHI^T - BCHI^N$$

$BCHI^{AN}$ = blank corrected hemolytic index above the negative control
 $BCHI^T$ = average blank corrected hemolytic index of the test sample
 $BCHI^N$ = average blank corrected hemolytic index of the negative control

STATISTICAL METHODS: None used.

RECORD RETENTION

A certified copy of this report and data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

COMPLIANCE

International Standards

ASTM Guideline F 619-03. Standard Practice for Extraction of Medical Plastics. 2010. Annual Book of ASTM Standards, Volume 13.01: 182-185.

ASTM Guideline F-756-08. Standard Practice for Assessment of Hemolytic Properties of Materials. 2010. Annual Book of ASTM Standards, Volume 13.01: 314-318.

ISO 10993-4:2002 and Amendment 1, 2006. Standard Biological Evaluation of Medical Devices, Part 4 – Selection of Tests for Interaction with Blood.

ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

TEST ARTICLE DISPOSITION

Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

This assay met the criteria for a valid assay. The blood used in this assay had a plasma free hemoglobin level of 0.460 mg/mL. The total hemoglobin content of the blood was 310.15 mg/mL prior to dilution. After dilution the hemoglobin level was confirmed to be 9.483 mg/mL.

The negative control replicates returned a blank corrected hemolytic index of 0.1%. The positive control replicates returned a blank corrected hemolytic index of 9.7%. The Hemolytic Grade system is presented in Table 5. The test results are presented in Table 7.

After centrifugation, the condition of the control and test article supernatants were observed (See Table 6).

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TABLE 6: CONDITION OF SAMPLE SUPERNATANT AFTER CENTRIFUGATION

SAMPLE/CONTROL	APPEARANCE OF THE SUPERNATANT
Test Article	Colorless and free of particulate
Blank Control	Colorless and free of particulate
Negative Control	Colorless and free of particulate
Positive Control	Clear dark red and free of particulate

The test article returned a hemolytic index value less than that found in the negative control and fell within the non-hemolytic range.

TABLE 7: RESULTS

GROUP	ABSORBANCE	AVERAGE ABSORBANCE	AVERAGE BLANK CORRECTED %HEMOLYSIS	AVERAGE BLANK CORRECTED %HEMOLYTIC INDEX	AVERAGE BLANK CORRECTED %HEMOLYTIC INDEX ABOVE THE NEGATIVE CONTROL
Test Article	0.001	0.002	0.0*	0.0*	0.0
	0.002				
	0.002				
Blank Control	0.002	0.003	0.0	0.0	NA
	0.003				
	0.003				
Negative Control	0.003	0.003	0.4	0.1	NA
	0.004				
	0.003				
Positive Control	0.125	0.131	79.3	9.7	NA
	0.145				
	0.124				

*Negative result reported as zero (0).
NA = Not Applicable

CONCLUSION

Based on the criteria set forth in the protocol, this assay was valid. Therefore, it was capable of correctly assessing the hemolytic potential of test articles. In this case, the test article is considered **non-hemolytic** under the test conditions employed and **passes** this test.

REFERENCES

International Committee for Standardization in Haematology. *J. Clin. Pathol*, 1978, 31:139-423.

Malinauskas, R.A., "Plasma Hemoglobin Measurement Techniques for the *In Vitro* Evaluation of Blood Damage Caused by Medical Devices." *Artificial Organs*. 1997; 21:1255-1267.

Moore, G.L., Ledford, M.E., Merydith, A. A., "Micromodification of the Drabkin Hemoglobin Assay for Measuring Plasma Hemoglobin in the Range of 5-2000 mg/dL." *Biochem. Med*. 1981; 26:167-173.

WuXi AppTec Reference Library Contents, Form ALS-4650-1

WuXi AppTec SOP: MED-8910, ASTM Hemolysis Assay

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AMENDMENT TO GLP FINAL REPORT (10/27/11)

Modification(s) to Report:

At the request of the Sponsor, the original final report dated 09/26/11 was amended on 10/27/11 to include the following changes. The changes were included in the body of the report as appropriate. These changes had no impact on the scientific interpretations or conclusions of the study.

1. At the request of the Sponsor, the Test Article identification and sterilization method was updated to read "Sterilization: 110-130 kGy Gamma" on pages 1 and 3.
2. An amendment for the identification and sterilization change was added to page 4 of the report.

Tamara Chelmonow
Study Director

10/27/11
Date



**AMENDED
FINAL STUDY REPORT**

STUDY TITLE: ISO Acute Systemic Injection Test

PROTOCOL NUMBER: 901770-21

TEST ARTICLE IDENTIFICATION: Trade Name: Eagle 3mm Jaw and Shaft
Built according to Assembly: PRC049736 Rev C
Sterilization: 110-130 kGy Gamma
Ref# EE2702
Lot # PRC049736

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242-2803

STUDY NUMBER: 157894

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: The requirements of the ISO Acute Systemic Injection Test **have been met** by the test article.

**QUALITY ASSURANCE UNIT SUMMARY**

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the quality and integrity of the study.

<u>Phase Inspected</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Ending	09/23/11	09/23/11	09/29/11
Final Report	09/28/11	09/28/11	09/29/11
Amended Report	10/26/11	10/26/11	10/27/11

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: Kim Steele Date: 10/27/11
Kim Steele

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: Michelle Dietzel Date: 10/27/11
Michelle Dietzel

Professional Personnel Involved:

Teri Tanquist, BS

Christine Olson, BS

Roxanne Miller, AA, CVT

Michelle Dietzel, BS

Jean Mesarich, AA

Vice President of Process Improvement and Operations

Study Operations Manager

Associate Director, In-Life Studies

Senior Study Director

Client Relations Manager



PURPOSE: The purpose of this test was to screen test article extracts or solutions for potential toxic effects as a result of a single-dose systemic injection in mice.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

DATE TEST ARTICLE RECEIVED: 08/15/11
STUDY INITIATION DATE: 09/06/11
STUDY COMPLETION DATE: 09/29/11
AMENDED REPORT DATE: 10/27/11

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: Eagle 3mm Jaw and Shaft Built according to Assembly: PRC049736 Rev C Sterilization: 110-130 kGy Gamma Ref# EE2702
Lot/Batch #:	PRC049736
Sterilization Method:	Gamma 110-130 kGy
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Device

CHARACTERIZATION: The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

EXPERIMENTAL DESIGN

Experimental Summary

Animals were treated by intravenous or intraperitoneal routes to screen solutions or test article extracts for potential toxic effects as a result of a single-dose systemic injection. The animal species, number, and route of test article administration were as recommended in ISO 10993-11.

For the safety evaluation of the test article, mice were injected systemically with extracts of the test article in standard solutions (normal saline and cottonseed oil). The animals were observed for signs of toxicity immediately after injection and at 4, 24, 48, and 72 hours post-injection. The requirements of the test are met if none of the animals treated with the test article extract have a significantly greater adverse reaction than the animals treated with the vehicle control.

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**Justification For Selection Of The Test System**

Mice were used in this study because they have historically been used in systemic safety evaluation studies and the guidelines have no alternative (non-animal) methods. Animals were treated by intravenous and intraperitoneal routes. The animal species, number, and route of test article administration were as recommended in ISO 10993-11.

Institutional Animal Care and Use Committee (IACUC)

The protocol and any amendments or procedures involving the care or use of animals on this study were reviewed and approved by the WuXi AppTec IACUC prior to the initiation of such procedures.

IACUC Protocol / Effective Date: 98-03E / June, 2010

AMENDMENTS:

1. The test article name was changed to "Trade Name: Eagle 3mm Jaw and Shaft, Built according to Assembly: PRC049736 Rev C, Sterilization: 45-75 kGy Gamma, Ref# EE2702".
2. The sterilization portion of the test article name along with the sterilization section of the test request form was changed to "Sterilization: 110-130 kGy Gamma".

DEVIATIONS: None.

IDENTIFICATION OF TEST SYSTEM

Species/Strain: Albino Swiss mice (*Mus musculus*), CFW, naive

Source: Charles River Laboratories

Sex: Female. Any females used were nulliparous and not pregnant.

Weight Range: All animal weights were within $\pm 20\%$ of the mean body weight (21.8g – 26.2g) at the start of the study.

Age: All animals were approximately 6 weeks old at the start of the study.

Animal Identification: The animals were identified per WuXi AppTec SOP: ILS-0112.

Animal Numbers:**Mouse #**

0.9% Normal Saline Test Group:	1 - 5
0.9% Normal Saline Control Group:	21 - 25
Cottonseed Oil Test Group:	11 - 15
Cottonseed Oil Control Group:	31 - 35

HUSBANDRY

Receipt: Animals were received on 09/07/11 according to WuXi AppTec SOP: ILS-0092. Each animal was examined for signs of disease and injury. The animals were acclimated for a minimum of 5 days under the same conditions as the actual test.

Housing: Animals were housed in groups of five in polycarbonate cages with contact bedding. Housing density complied with AAALAC International recommendations and NIH guidelines. The test and control animals were housed separately.

Environment: The environmental conditions in the animal rooms were maintained according to WuXi AppTec SOP: ILS-0018. The temperature and photo-period were set to meet the AAALAC International recommendations for these species. The laboratory and animal rooms were maintained as limited-access facilities.



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EXPERIMENTAL DESIGN

Experimental Summary

The test article was incubated in the appropriate volume of E-MEM in a sterile vessel for 24 ± 2 hours at 37 ± 1 °C. At the end of the extraction period, the extract was decanted from the test article. A series of eight dilutions of the extract were prepared in E-MEM in a two-fold dilution. After the dilutions were prepared, the maintenance culture media was removed from test culture wells and replaced with 1 ml of test media dilutions in triplicate wells. The positive control media, test article extract, and control extracts were added at the same time to the culture plate in triplicate wells. The plates were incubated for 72 hours at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air. The cultures were evaluated for cytotoxic effects by microscopic examination at 24, 48 and 72 ± 4 hours of incubation and the results scored and recorded.

Justification For Selection Of The Test System

The MEM Endpoint Dilution Assay is one of several *in vitro* mammalian cell tests which have been used to screen materials for their potential cytotoxicity. The L-929 cell line has a history of use in assays of this type.

AMENDMENTS

1. The test article identification was updated to read the following: "Trade Name: Eagle 3mm Jaw and Shaft, Built according to Assembly: PRC049736 Rev C, Sterilization: 45-75 kGy Gamma, Lot # PRC049736, Ref# EE2702."
2. The Sterilization method was updated to read the following: "Gamma 110-130 kGy."
3. The test article identification was updated to read the following: "Trade Name: Eagle 3mm Jaw and Shaft, Built according to Assembly: PRC049736 Rev C, Sterilization: 110-130 kGy Gamma, Lot # PRC049736, Ref# EE2702."

DEVIATIONS: None.

IDENTIFICATION OF THE TEST SYSTEM

Cultures of L-929 cells (mouse fibroblast) were obtained from American Type Culture Collection (ATCC # CCL-1). Mycoplasma-free cell lines which were purchased from the vendor and kept frozen in the lab until used. To maintain the sensitivity, they are only sub-cultured for up to 15 passages and then discarded.

Cultures were grown and used as monolayers in disposable tissue culture labware at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air at WuXi AppTec, Inc. The media used for growth of cells is Eagle's minimal essential medium (E-MEM) supplemented with 5% (v/v) fetal bovine serum (FBS). The medium is also supplemented with the following: 2 mM L-glutamine, 10 mM HEPES, 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 u/mL penicillin, and 1% 2.50 ug/mL amphotericin B (Fungizone). WuXi AppTec Inc. has a long-standing history using antibiotics in culture medium. The use of antibiotics in culture media has not shown adverse affects when used for this assay; this is exhibited by the varying degrees of toxicity seen in the assay controls.

TEST MATERIAL PREPARATION

According to the Sponsor, the test article consisted of silver metal jaw attached to black shaft with white printing. The test article was cut for extraction, placed into an extraction vessel, and prepared at a ratio of 4g to 20 mL of extraction vehicle. As per Sponsor request, the test article was cut 4 cm from the jaw end. The test article that was included for testing was 4 cm long and consisted of the jaw and part of the shaft that contained pad printing.



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TABLE 1: TEST ARTICLE RECORD

VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	QUANTITY OF TEST ARTICLE USED
E-MEM + 5% FBS	3.2	16.0	1

EXTRACTION MEDIA

Eagle's minimal essential medium (E-MEM), supplemented with 5% (v/v) fetal bovine serum (FBS), was used to extract the test article. The medium was also supplemented with the following: 2 mM L-glutamine, 10 mM HEPES, 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 µg/mL penicillin, and 1% 2.50 µg/mL amphotericin B (Fungizone). The pH was confirmed to be 7.24. The pH of the EMEM + 5% FBS (lot TDSI1057, expiration date of 8/29/11), that was used to make dilutions was confirmed to be 7.25.

CONTROL ARTICLE PREPARATION

A negative, intermediate, positive, and cell control were run in parallel with the test article. A negative control (high density polyethylene (HDPE)) known to be non-toxic under the test conditions was prepared at a ratio of 120 cm² to 20 mL of extraction vehicle. An intermediate control (black rubber) known to be mildly toxic under the test conditions was prepared and extracted at a ratio of 4 g to 20 mL of extraction vehicle. A positive control (100 µM CdCl₂) known to be toxic under the test conditions was prepared by diluting cadmium chloride stock powder with 100 mL of sterile water to create a 10 mM solution. The solution was then prepared at a ratio of 0.1 mL to 9.9 mL of extraction vehicle. A cell control (E-MEM + 5% FBS) was incubated in parallel with the test sample and controls.

TABLE 2: CONTROL / CELL LINE RECORD

CONTROL IDENTIFICATION	CLASS	LOT #	SUPPLIED BY	EXPIRATION
100 µM CdCl ₂	Positive control	TDSI1038	WXAT	06/24/12
Black Rubber Stopper	Intermediate control	WXAT010710	Fisher	06/17/12
High Density Polyethylene (HDPE)	Negative Control	C-091	Hatano	02/2016
E-MEM + 5% FBS	Medium	TDSI1052	WXAT	08/29/11
E-MEM + 5% FBS	Medium (Dilutions)	TDSI1057	WXAT	08/29/11
L-929	Cell Line	L081211	ATCC	N/A

N/A= Not Applicable

TEST ARTICLE EXTRACTION

The extraction mixture and controls were then incubated for 24 ± 2 hours at 37 ± 1 °C. At the start of the extraction, the solutions appeared clear and free of particulates. The extracts were agitated during the course of the extraction period. At the end of the extraction period, the vessels were well shaken and the liquid was aseptically decanted. The test article was observed after extraction to be intact with no macroscopically observable degradation and the extract was clear. After decanting, the extract was not filtered and was used immediately. See Tables 1-3.

TABLE 3: EXTRACTION RECORD

VEHICLE	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END
E-MEM + 5% FBS	37.0 °C	08/15/11 2:24 pm	37.0 °C	08/16/11 2:24 pm

EXPERIMENTAL PROCEDURE

L-929 cells were plated at 2.5 x 10⁴ cells/mL at least 24 hours before use. Prior to inoculation, the cell cultures were examined to ensure they had formed a nearly confluent monolayer and that they appeared uniform and viable.



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The test article and controls were extracted with the appropriate amount of E-MEM + 5% FBS for 24 ± 2 hours at 37 ± 1 °C. At the end of the extraction period, the vessels were well shaken and the extraction media was used immediately. A series of eight dilutions of the extract were prepared in E MEM in a two-fold dilution. One mL aliquots of the test article and control extract and the series of dilutions were plated in triplicate onto the cell line.

The cell cultures were incubated in a humidified atmosphere for 72 hours. The monolayers were evaluated for cytotoxic effects at 24, 48, and 72 hours. At each incubation period, the cell cultures were observed for signs of cytopathic effect (CPE) including lysis, crenation, plaques, and excessive rounding of cells.

TEST EVALUATION

Criteria for evaluating cytotoxicity will include morphologic changes in cells, such as granulation, crenation, or rounding, and loss of viable cells from the monolayer by lysis or detachment. The validity of the test requires that negative control cultures maintain a healthy normal appearance throughout the duration of the test. Degrees of toxicity were scored as described in Table 4.

TABLE 4: SCORING

GRADE	REACTIVITY	CONDITIONS OF ALL CULTURES
0	None	Discrete intracytoplasmic granules; no cell lysis, no reduction of cell growth
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cells layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers

According to the current ISO guidelines, test articles scoring '0', '1', or '2' are considered NON-CYTOTOXIC. Test articles scoring '3' or '4' are considered CYTOTOXIC.

VALIDITY CRITERIA

Validity of the assay and the test article result was based upon the criteria listed below and scientific judgment.

The negative and cell control should display no cytotoxic reaction. The intermediate control should display a mild to moderate cytotoxic reaction, a score of '2' or '3'. The positive control should display a moderate to strong cytotoxic reaction, a score of '3' or '4'.

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS: Not applicable.

STATISTICAL METHODS: None used.

RECORD RETENTION

A certified copy of this report and data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

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COMPLIANCE

This study was performed in compliance with the following International Standards:

ISO 10993-5:2009 Biological Evaluation of Medical Devices, Part 5: Tests for *In Vitro* Cytotoxicity.

ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

TEST ARTICLE DISPOSITION

Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

The negative and cell cultures exhibited healthy growth throughout the experiment. The monolayers exposed to CdCl_2 exhibited a toxic response while the black rubber intermediate control induced a mild level of toxicity. Therefore, the test system was responding normally and met the criteria for a valid assay. See Table 5.

When scoring lysis, each sample was rated relative to the amount of lysis displayed by the negative control. The negative and cell control were considered '0', the cells displayed at least 70% viability. The intermediate control displayed a mild cytotoxic reaction resulting in a score of '2'. The positive control displayed a strong cytotoxic reaction, resulting in a score of '4'. Therefore, the test system was responding normally and met the criteria for a valid assay. See Table 5.

In this assay, the test article **did not** induce substantial cytotoxicity. No abnormal events such as pH change or debris were noted.

TABLE 5: RESULTS

TEST ARTICLE AND CONTROLS	SAMPLE EXTRACT		CYTOTOXICITY SCORES		
	SIZE	(mL)	24 HOURS	48 HOURS	72 HOURS
Test Article	3.2 g	16.0	1/1/1	1/1/1	1/1/1
Test Article – 1:2	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:4	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:8	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:16	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:32	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:64	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:128	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:256	NA	NA	0/0/0	0/0/0	0/0/0
Positive Control	0.1 mL	9.9	4/4/4	4/4/4	4/4/4
Intermediate Control	1.9 g	9.5	2/2/2	2/2/2	2/2/2
Negative Control	30 cm ²	5.0	0/0/0	0/0/0	0/0/0
Cell Control	NA	10.0	0/0/0	0/0/0	0/0/0

NA = Not Applicable

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ANALYSIS AND CONCLUSION

The neat concentration of the test article scored '1' at 24, 48 and 72 $\pm$ 4 hours. The test article, at the 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256 dilutions scored '0' at 24, 48 and 72 $\pm$ 4 hours. Therefore, the test article is considered **non-cytotoxic at the neat concentration**.

REFERENCES

U.S. Pharmacopeia, Section 87, current revision.

WuXi AppTec Reference Library Contents, Form ALS-4650-1

WuXi AppTec SOP: MED-8750, Minimum Essential Medium (MEM) Elution Assay

**AMENDMENT TO GLP FINAL REPORT (09/13/11)****Modification(s) to Report:**

At the request of the Sponsor, the original final report dated 08/26/11 was amended on 09/13/11 to include one change. The change was included in the body of the report as appropriate. The change had no impact on the scientific interpretations or conclusions of the study.

1. On pages 1 and 3 of the report, the Test Article Identification was updated to read the following: "Trade Name: Eagle 3mm Jaw and Shaft, Built according to Assembly: PRC049736 Rev C, Sterilization: 45-75 kGy Gamma, Lot # PRC049736, Ref# EE2702."

AMENDMENT (2) TO GLP FINAL REPORT (10/27/11)**Modification(s) to Report:**

At the request of the Sponsor, the final report dated 08/26/11 and amended report dated 09/13/11, were reviewed for completeness and correctness. It was found that three amendments to the amended report were required. The amendments are listed below and were included in the body of the report as appropriate. The changes had no impact on the scientific interpretations or conclusions of the study.

1. The Test Article Identification was changed to read: "Trade Name: Eagle 3mm Jaw and Shaft, Built according to Assembly: PRC049736 Rev C, Sterilization: 110-130 kGy Gamma, Lot#: PRC049736, Ref# EE2702" on pages 1 and 3 of the report.
2. As per Sponsor request, the sterilization method was updated to read, "Gamma 110-130 kGy" on page 3 of the report.
3. An amendment for the test article identification and sterilization method change was added to page 4 of the report.

Study Director

Date

10/27/11



**AMENDED
FINAL STUDY REPORT**

STUDY TITLE: ASTM HEMOLYSIS ASSAY -
DIRECT CONTACT METHOD

PROTOCOL NUMBER: 150300-12

TEST ARTICLE IDENTIFICATION: Trade Name: Eagle 3mm Jaw and Shaft
Built according to Assembly: PRC049736 Rev C
Sterilization: 110-130 kGy Gamma
Lot#: PRC049736
Ref# EE2702

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery, Inc.
4545 Creek Road
Cincinnati, OH 45242-2803

STUDY NUMBER: 157896

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: The test article is considered **non-hemolytic** under
the test conditions employed and **passes** this test.

Protocol Number: 150300-12
Amended Report: October 27, 2011

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QUALITY ASSURANCE UNIT SUMMARY

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR part 58) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Each inspection was performed to assure the quality and integrity of the study.

<u>Phase Inspected</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Addition of Blood	09/08/11	09/09/11	09/26/11
Final Report	09/26/11	09/26/11	09/26/11
Amended Report	10/27/11	10/27/11	10/27/11

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: Kim Steele

Kim Steele

Date: 10/27/11

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: Tamara Charbonneau

Tamara Charbonneau

Date: 10/27/11

Professional personnel involved:

Lisa Olson, BS	Vice President of Testing and Service Development
Teri Tanquist, BS	Vice President of Process Improvement and Operations
Jean Kringstad, BS	Director, In Vitro Testing Services
Tamara Charbonneau, BS	Study Director, Supervisor In Vitro Testing Laboratory
Jean Mesarich, AA	Client Relations Manager

Protocol Number: 150300-12
Amended Report: October 27, 2011

Project No. 157896
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PURPOSE

The purpose of this study was to evaluate the hemolytic potential of test articles according to the ASTM guideline (F-756-08).

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SAMPLES RECEIVED: 08/15/11
INITIATION DATE: 09/06/11
COMPLETION DATE: 09/26/11
AMENDED REPORT DATE: 10/27/11

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: Eagle 3mm Jaw and Shaft Built according to Assembly: PRC049736 Rev C Sterilization: 110-130 kGy Gamma Lot#: PRC049736 Ref# EE2702
Lot/Batch #:	PRC049736
Sterilization Method:	110-130 kGy Gamma
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Device

TEST ARTICLE CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

EXPERIMENTAL DESIGN

Experimental Summary

Test articles and controls were prepared for direct testing. Rabbit blood was diluted to achieve an approximate concentration of 10 mg/mL hemoglobin. Diluted blood was then added to the test and control materials. These mixtures were incubated for at least three hours at 37 ± 2 °C. After this incubation, the mixtures were transferred to centrifuge tubes and centrifuged to remove cellular materials. After centrifugation, supernatants were observed. A cyanmethemoglobin reagent was then added to the supernatant and these samples were read at 540 nm on a spectrophotometer. The hemolytic index was calculated based on these results.

NAMSA

CONFIDENTIAL
REPORT

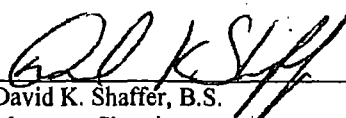
PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

REFERENCES

International Organization for Standardization (ISO) 10993-18, Biological Evaluation of Medical Devices-Part 18: Chemical Characterization of Materials (2005).

APPROVAL


David K. Shaffer, B.S.
Manager, Chemistry

8-25-11
Date

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.



**AMENDED
FINAL STUDY REPORT**

STUDY TITLE: JAPANESE CYTOTOXICITY TEST COLONY
MICROASSAY BY ELUTION – EXPANDED
DILUTION SERIES

PROTOCOL NUMBER: C11662-1

TEST ARTICLE IDENTIFICATION: Trade Name: Eagle 3mm Jaw and Shaft
Built according to Assembly: PRC049736 Rev C
Sterilization: 110-130 kGy Gamma
Lot#: PRC049736
Ref# EE2702

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

STUDY NUMBER: 157895

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: None of the standard dilutions of the test article induced biologically relevant decreases in colony formation. Therefore, an IC_{50} could not be calculated for the test article. These results would be indicative of a **non-cytotoxic** material.

**QUALITY ASSURANCE UNIT SUMMARY**

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR part 58) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Each inspection was performed to assure the quality and integrity of the study.

<u>Critical Phase</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Scoring	08/26/11	08/26/11	09/02/11
Final Report	09/01/11	09/01/11	09/02/11
Amended Report	09/09/11	09/09/11	09/09/11
Amended Report (2)	10/26/11	10/26/11	10/27/11

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor:

Kim Steele

Date:

10/27/11

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58:

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director:

Sarah Olson

Date:

10/27/11

Professional Personnel Involved:

Lisa Olson, BS
Teri Tanquist, BS
Jean Kringstad, BS
Sarah Olson, BA
Jean Mesarich, AA

Vice President, Testing and Service Development
Vice President of Process Improvement and Operations
Director, *In Vitro* Testing Services
Study Director
Client Relations Manager

**PURPOSE**

The purpose of this procedure was to evaluate the cytotoxic response of a specified mammalian culture cell line when exposed to an extract of the test article. This assay utilized the sensitivity of low cell density to evaluate the cytotoxicity of medical devices.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

TEST ARTICLE RECEIVED: 08/15/11
INITIATION DATE: 08/16/11
COMPLETION DATE: 09/02/11
AMENDED REPORT DATE: 09/09/11
AMENDED REPORT DATE (2): 10/27/11

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: Eagle 3mm Jaw and Shaft Built according to Assembly: PRC049736 Rev C Sterilization: 110-130 kGy Gamma Lot#: PRC049736 Ref# EE2702
Lot #:	PRC049736
Sterilization Method:	Gamma 110-130 kGy
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Device

TEST ARTICLE CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

EXPERIMENTAL DESIGN**Experimental Overview**

Test articles and controls were prepared and extracted for 24-25 hours at 37 ± 1 °C. Appropriate dilutions of the extracts were prepared using E-MEM +10% FBS. After removing spent medium, 2.0 ml of the extract was added to the plates. The cells were incubated at 37 ± 1 °C for 7-9 days before fixation, staining, and counting.

**Justification For Selection Of The Test System**

The extract colony assay is one of several *in vitro* mammalian cell tests which have been used to screen materials for their potential cytotoxicity. The L-929 cell line has a history of use in assays of this type.

AMENDMENTS

1. The Test Article Identification was changed to read: "Trade Name: Eagle 3mm Jaw and Shaft, Built according to Assembly: PRC049736 Rev C, Sterilization: 110-130 kGy Gamma, Lot#: PRC049736, Ref# EE2702."
2. The Sterilization method was updated to read: "Gamma 110-130 kGy."

DEVIATIONS: None.

IDENTIFICATION OF TEST SYSTEM

Cultures of L-929 cells (mouse fibroblast) were obtained from American Type Culture Collection (ATCC # CCL-1). Cultures were grown and used as monolayers in disposable tissue culture labware at $37 \pm 1^\circ\text{C}$ in a humidified atmosphere of $5 \pm 1\%$ CO_2 in air at WuXi AppTec, Inc.

TEST ARTICLE PREPARATION

According to the Sponsor, the test article consisted of a silver metal jaw attached to a black shaft with white printing. The test article was cut for extraction, placed into an extraction vessel and prepared at a ratio of 2 g to 20 mL of extraction vehicle. As per Sponsor request, the test article was cut 4 cm from the jaw end. The test article that was included for testing was 4 cm long and consisted of the jaw and part of the shaft that contained pad printing.

TABLE 1: TEST ARTICLE RECORD

VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	NUMBER OF TEST ARTICLE DEVICES USED PER TEST
E-MEM + 10% FBS	3.1	31.0	1

CONTROLS PREPARATION

A negative control (high density polyethylene film) was prepared at a ratio of 2 grams / 20 mL. A moderately positive control (polyurethane film containing 0.1% zinc diethyldithiocarbamate (ZDEC)) was prepared at a ratio of 2 grams / 20 mL. A weak positive control (polyurethane film containing 0.25% zinc dibutyldithiocarbamate (ZDBC)) was prepared at a ratio of 2 grams / 20 mL. The cell control consisted of E-MEM + 10% FBS elution media that was incubated in parallel with test article and controls.

EXTRACTION MEDIA

Eagle's minimal essential medium (E-MEM), supplemented with 10% (v/v) fetal bovine serum (FBS), was used to extract the test article. The medium was also supplemented with the following: L-glutamine, HEPES, gentamicin, penicillin, vancomycin and amphotericin B (Fungizone). The pH was confirmed to be 7.21.

TEST ARTICLE EXTRACTION

The extraction mixture and controls were then incubated for 24-25 hours at $37 \pm 1^\circ\text{C}$ in a humidified atmosphere of $5 \pm 1\%$ CO_2 . At the start of the extraction, the solutions appeared clear and free of particulates. At the end of the extraction period, the vessels were shaken well and the liquid aseptically decanted. The test article was observed after extraction to be intact with no macroscopically observable degradation and the extract was clear. After decanting the extract was not filtered and was used immediately. See Tables 1-3.



TABLE 2: EXTRACTION RECORD

VEHICLE	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END
E-MEM + 10% FBS	37.0 °C	08/17/11 7:40 am	37.0 °C	08/18/11 8:10 am

TABLE 3: CONTROL / CELL LINE RECORD

CONTROL IDENTIFICATION:	CLASS	LOT #	SUPPLIED BY:	EXPIRATION
ZDEC	Positive control	A-091	Hatano	10/2014
ZDBC	Positive control	B-101K	Hatano	04/2015
High Density Polyethylene (HDPE)	Negative Control	C-091	Hatano	02/2016
E-MEM + 10% FBS	Medium	TDS11055	WXAT	08/29/11
L-929	Cell Line	L081211	ATCC	N/A

N/A= Not Applicable

EXPERIMENTAL PROCEDURE

The test article was extracted in the appropriate volume of E-MEM with 10% FBS in a sterile vessel for 24 to 25 hours at $37 \pm 1^\circ\text{C}$ in a humidified atmosphere of $5 \pm 1\%$ CO_2 in air. At the end of the extraction period, the extracts were diluted with E-MEM + 10% FBS as shown in Table 4.

TABLE 4: DILUTION RATIOS

EXTRACT	% NEAT EXTRACTS						
Test Article	100	75	50	25	12.5	6.25	3.125
Positive ZDEC	2.0	1.5	1.0	0.75	0.50	0.25	0.125
Positive ZDBC	100	75		50		25	
Negative Control	100						
Cell Control	100						

The maintenance culture medium was decanted from test culture wells and replaced with 2 mL of each % dilution from above. The test article and control extracts and cell control media was added to the culture plates in triplicate wells. The plates was then incubated at $37 \pm 1^\circ\text{C}$ in a humidified atmosphere of $5 \pm 1\%$ CO_2 in air until adequate cell colony growth is observed (7-9 days). At the end of the incubation period, the extracts were removed from the plates and the wells were rinsed with 2 mL of DPBS (-) solution. The DPBS was decanted and the colonies were fixed by placing 1 mL of 10% formalin solution into each well for a minimum of 2-10 minutes. Once the cells were fixed they were stained for a minimum of 2-3 hours with 1 mL of Giemsa stain solution that was diluted 1:20 with PBS. At the end of the staining period, the stain was decanted and 2 mL of DPBS (-) was added to each well. The DPBS (-) was removed and then the colonies were counted.

EVALUATION CRITERIA

The relative colony formation rates (% RCF) were plotted against the % dilutions of the extract on a semi-logarithmic graph using Microsoft Excel. The resulting inhibition curve of each graph was used to determine the % dilution that inhibited colony formation to 50%. This is the IC_{50} value. This value derived from the concentration-response will be backed by at least three responses between 10% and 90% inhibition of the control.

Sigma Stat was used to perform a t-test comparing the cell control results with the average colony count from the negative control % dilutions.



VALIDITY

Final evaluation of the validity of the assay and test article results was based upon the criteria listed below and scientific judgment.

The t-test results must show that statistically significant inhibition of colony formation was not found in the negative control results.

IC₅₀ values of the positive ZDEC and ZDBC controls must not exceed the following values:

Positive ZDEC: 7% and Positive ZDBC: 80%

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS

The colonies were counted and the triplicate wells averaged for each % dilution. These averages were divided by the average number of colonies obtained in the cell control wells. This percentage is the Relative Colony Formation for each % dilution.

STATISTICAL METHODS

ANOVA (Analysis of Variance) was used and, when appropriate, Dunnett's post-hoc test in addition to the Student's paired t-test, performed using Sigma Stat. A difference is considered significant when $p < 0.05$.

RECORD RETENTION

A certified copy of this report and data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

TEST ARTICLE DISPOSITION

Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

Raw data and calculated results, including averages and relative colony formation are presented in Table 5. The test article extract was in contact with the test system for eight days.

A dramatic, dose dependant decrease in relative colony formation was noted as a result of increasing concentrations in the positive control preparations (ZDEC and ZDBC), indicating a sensitive assay (Table 5). Additionally, the relative colony formation (% RCF) in preparations treated with the negative control showed high degrees of survival. ZDEC induced a maximum IC₅₀ of 0.52% while ZDBC returned an IC₅₀ of 46.01%. Both met the criteria for a valid assay. Accordingly, this assay was determined to be valid.

None of the standard dilutions of the test article induced biologically relevant decreases in colony formation. Further, no dose response trends were noted.



TABLE 5: Results

DOSE GROUP	% OF NEAT EXTRACT	WELL 1	WELL 2	WELL 3	AVERAGE	% RCF**	IC ₅₀
Test Article	100	44	43	51	46	92	NA*
	75	49	45	56	50	100	
	50	48	50	48	49	98	
	25	50	51	49	50	100	
	12.5	43	51	60	51	102	
	6.25	62	52	47	54	108	
	3.125	53	58	65	59	118	
ZDEC	2.0	0	0	0	0	0	0.52%
	1.5	0	0	0	0	0	
	1.0	0	0	2	1	2	
	0.75	3	3	5	4	8	
	0.50	45	46	38	43	86	
	0.25	54	42	47	48	96	
	0.125	52	52	53	52	104	
ZDBC	100	0	0	0	0	0	46.01%
	75	0	0	0	0	0	
	50	34	31	33	33	66	
	25	43	41	43	42	84	
Negative Control	100	46	54	53	51	102	NA
Cell Control	100	46	54	50	50	100	NA

* This value could not be calculated as a 50% value was not achieved.

** Relative Colony Formation = Average colony formation divided by average colony formulation of cell control.

ANALYSIS AND CONCLUSION

Negative and positive controls were run concurrently with the test article to provide ranges of cytotoxicity. Each of the positive controls induced relative colony formation well within acceptable limits. No significant differences in colony formation were found between the negative control treated wells and the cell control. The results from the control cultures indicate that the assay retained normal sensitivity. Therefore, the criteria for a valid assay were met.

None of the standard dilutions of the test article induced biologically relevant decreases in colony formation. Further, no dose response trends were noted. Therefore, an IC₅₀ could not be calculated for the test article. These results would be indicative of a **non-cytotoxic** material.

REFERENCES

Guidelines for Basic Biological Tests of Medical Materials and Devices – Part I Cytotoxicity Test (Colony Assay). Japanese Ministry of Health and Welfare, 2003.

ISO 10993-5: 2009 Biological Evaluation of Medical Devices, Part 5: Tests for *In Vitro* Cytotoxicity.

WuXi AppTec SOP: MED-8710, Extract Colony Assay Cytotoxicity Test

**AMENDMENT TO GLP FINAL REPORT (09/09/11)****Modification(s) to Report:**

After review of the data package and per Sponsor request, the original final report dated 09/02/11 was amended on 09/09/11 to include one change. The change was included in the body of the report as appropriate. The change had no impact on the scientific interpretations or conclusions of the study.

1. The last sentence in the first paragraph of the Evaluation Criteria section on page 5 of the report was updated to read: "This value derived from the concentration-response will be backed by at least three responses between 10% and 90% inhibition of the control."

AMENDMENT (2) TO GLP FINAL REPORT (10/27/11)**Modification(s) to Report:**

At the request of the Sponsor, the final report dated 09/02/11 and amended report dated 09/09/11, were reviewed for completeness and correctness. It was found that three amendments to the amended report were required. The amendments are listed below and were included in the body of the report as appropriate. The changes had no impact on the scientific interpretations or conclusions of the study.

1. The Test Article Identification was changed to read: "Trade Name: Eagle 3mm Jaw and Shaft, Built according to Assembly: PRC049736 Rev C, Sterilization: 110-130 kGy Gamma, Lot#: PRC049736, Ref# EE2702" on pages 1 and 3 of the report.
2. The Sterilization method was to read: "Gamma 110-130 kGy" on page 3 of the report.
3. An amendment for the test article identification and sterilization method change was added to page 4 of the report.

Study Director

A handwritten signature in black ink, appearing to read 'Jacob Olson', written over a horizontal line.

Date

10/27/11



**AMENDED
FINAL STUDY REPORT**

STUDY TITLE: ISO MEM Endpoint Dilution Assay
Using L-929 Mouse Fibroblast Cells

PROTOCOL NUMBER: 140350-15

TEST ARTICLE IDENTIFICATION: Trade Name: Eagle 3mm Jaw and Shaft
Built according to Assembly: PRC049736 Rev C
Sterilization: 110-130 kGy Gamma
Lot#: PRC049736
Ref# EE2702

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242-2803

STUDY NUMBER: 157891

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: The test article is considered **non-cytotoxic** under the conditions of this test.

Protocol Number: 140350-15

Project No. 157891

Amended Report (2): October 27, 2011

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**QUALITY ASSURANCE UNIT SUMMARY**

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR part 58) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Each inspection was performed to assure the quality and integrity of the study.

<u>Phase Inspected</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Reading cells	08/19/11	08/19/11	08/26/11
Final Report	08/26/11	08/26/11	08/26/11
Amended Report	09/13/11	09/13/11	09/13/11
Amended Report (2)	10/27/11	10/27/11	10/27/11

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: _____

Dawn VoglandDate: 10/27/11**GOOD LABORATORY PRACTICE STATEMENT**

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: _____

Sarah OlsonDate: 10/27/11**Professional personnel involved:**

Lisa Olson, BS
Teri Tanquist, BS
Jean Kringstad, BS
Sarah Olson, BA
Jean Mesarich, AA

Vice President, Testing and Service Development
Vice President of Process Improvement and Operations
Director, *In Vitro* Testing Services
Study Director
Client Relations Manager



Protocol Number: 140350-15

Project No. 157891

Amended Report (2): October 27, 2011

Page 3 of 9

PURPOSE

The purpose of this study was to evaluate a serially diluted extract of a test article for cytotoxicity to mammalian cells in culture by a method compliant with the requirements specified in ISO 10993-5:2009.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

TEST ARTICLE RECEIVED: 08/15/11
INITIATION DATE: 08/15/11
COMPLETION DATE: 08/26/11
AMENDED REPORT DATE: 09/13/11
AMENDED REPORT DATE (2): 10/27/11

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: Eagle 3mm Jaw and Shaft Built according to Assembly: PRC049736 Rev C Sterilization: 110-130 kGy Gamma Lot#: PRC049736 Ref# EE2702
Lot #:	PRC049736
Sterilization Method:	Gamma 110-130 kGy
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Device

TEST ARTICLE CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Part Name	Subject Device Material
I-BLADE Plating	Titanium Nitride



EXPERIMENTAL DESIGN

Experimental Summary

The test article was incubated in the appropriate volume of E-MEM + 5% FBS in a sterile vessel for 24 ± 2 hours at 37 ± 1 °C. At the end of the extraction period, the extract was decanted from the test article. A series of eight dilutions of the extract were prepared in E-MEM + 5% FBS in a two-fold dilution. After the dilutions were prepared, the maintenance culture media was removed from test culture wells and replaced with 1.0 ml of test media dilutions in triplicate wells. The positive control media, test article extract, and control extracts were added at the same time to the culture plate in triplicate wells. The plates were incubated for 72 hours at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air. The cultures were evaluated for cytotoxic effects by microscopic examination at 24, 48 and 72 ± 4 hours of incubation and the results scored and recorded.

Justification For Selection Of The Test System

The MEM Endpoint Dilution Assay is one of several *in vitro* mammalian cell tests which have been used to screen materials for their potential cytotoxicity. The L-929 cell line has a history of use in assays of this type.

AMENDMENTS/DEVIATIONS: None.

IDENTIFICATION OF THE TEST SYSTEM

Cultures of L-929 cells (mouse fibroblast) were obtained from American Type Culture Collection (ATCC # CCL-1). Mycoplasma-free cell lines which were purchased from the vendor and kept frozen in the lab until used. To maintain the sensitivity, they are only sub-cultured for up to 15 passages and then discarded.

Cultures were grown and used as monolayers in disposable tissue culture labware at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air at WuXi AppTec, Inc. The media used for growth of cells is Eagle's minimal essential medium (E-MEM) supplemented with 5% (v/v) fetal bovine serum (FBS). The medium is also supplemented with the following: 2 mM L-glutamine, 10 mM HEPES, 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 units/mL penicillin, and 1% 2.50 ug/mL amphotericin B (Fungizone). WuXi AppTec Inc. has a long-standing history using antibiotics in culture medium. The use of antibiotics in culture media has not shown adverse affects when used for this assay, exhibited by the varying degrees of toxicity seen in the assay controls.

TEST MATERIAL PREPARATION

The test article was extracted intact, placed into an extraction vessel, and prepared at a ratio of 4 g to 20 mL of extraction vehicle.

TABLE 1: TEST ARTICLE RECORD

VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	QUANTITY OF TEST ARTICLE USED
E-MEM + 5% FBS	1.7	8.5	1.7 g

EXTRACTION MEDIA

Eagle's minimal essential medium (E-MEM), supplemented with 5% (v/v) fetal bovine serum (FBS), was used to extract the test article. The medium was also supplemented with the following: 2 mM L-glutamine, 10 mM HEPES, 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 units/mL penicillin, and 1% 2.50 ug/mL amphotericin B (Fungizone). The pH was confirmed to be 7.30.



CONTROL ARTICLE PREPARATION

A negative, intermediate, positive, and cell control were run in parallel with the test article. A negative control (high density polyethylene (HDPE)) known to be non-toxic under the test conditions was prepared at a ratio of 120 cm² to 20 mL of extraction vehicle. An intermediate control (black rubber) known to be mildly toxic under the test conditions was prepared and extracted at a ratio of 4 g to 20 mL of extraction vehicle. A positive control (100 µM CdCl₂) known to be toxic under the test conditions was prepared by diluting cadmium chloride stock powder with 100 mL of sterile water to create a 10 mM solution. The solution was then prepared at a ratio of 0.1 mL to 9.9 mL of extraction vehicle. A cell control (E-MEM + 5% FBS) was incubated in parallel with the test sample and controls.

TABLE 2: CONTROL / CELL LINE RECORD

CONTROL IDENTIFICATION:	CLASS	LOT #	SUPPLIED BY:	EXPIRATION
100 µM CdCl ₂	Positive Control	TDSI1074	WXAT	09/20/12
Black Rubber Stopper	Intermediate Control	WXAT010710	Fisher	02/28/13
High Density Polyethylene (HDPE)	Negative Control	C-091	Hatano	02/2016
E-MEM + 5% FBS	Medium	TDSI1173	WXAT	06/18/12
L-929	Cell Line	L061112	ATCC	NA

NA = Not applicable

TEST ARTICLE EXTRACTION

The extraction mixture and controls were incubated for 24 ± 2 hours at 37 ± 1 °C. At the start of the extraction, the solutions appeared clear and free of particulates. The extracts were agitated during the course of the extraction period. At the end of the extraction period, the vessels were well shaken. The test article was observed after extraction to be intact with no macroscopically observable degradation and the extract was clear. The extract was not filtered and was used immediately. See Tables 1-3.

TABLE 3: EXTRACTION RECORD

VEHICLE	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END
E-MEM + 5% FBS	37.0 °C	06/14/12 2:30 pm	37.0 °C	06/15/12 1:50 pm

EXPERIMENTAL PROCEDURE

L-929 cells were plated at 2.5 x 10⁴ cells/mL at least 24 hours before use. Prior to inoculation, the cell cultures were examined to ensure they had formed a nearly confluent monolayer and that they appeared uniform and viable.

The test article and controls were extracted with the appropriate amount of E-MEM + 5% FBS for 24 ± 2 hours at 37 ± 1 °C. At the end of the extraction period, the vessels were well shaken and the extraction media was used immediately. A series of eight dilutions of the extract were prepared in E-MEM in a two-fold dilution. One mL aliquots of the test article and control extract and the series of dilutions were plated in triplicate onto the cell line.

The cell cultures were incubated in a humidified atmosphere for 72 hours. The monolayers were evaluated for cytotoxic effects at 24, 48, and 72 hours. At each incubation period, the cell cultures were observed for signs of cytopathic effect (CPE) including lysis, crenation, plaques, and excessive rounding of cells.

TEST EVALUATION

Criteria for evaluating cytotoxicity included morphologic changes in cells, such as granulation, crenation, or rounding, and loss of viable cells from the monolayer by lysis or detachment. The validity of the test required that negative control cultures maintain a healthy normal appearance throughout the duration of the test. Degrees of toxicity were scored as described in Table 4.



TABLE 4: SCORING

GRADE	REACTIVITY	CONDITIONS OF ALL CULTURES
0	None	Discrete intracytoplasmic granules; no cell lysis, no reduction of cell growth
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cells layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers

According to the current ISO guidelines, test articles scoring '0', '1', or '2' are considered NON-CYTOTOXIC. Test articles scoring '3' or '4' are considered CYTOTOXIC.

VALIDITY CRITERIA

Validity of the assay and the test article result was based upon the criteria listed below and scientific judgment.

The negative and cell control should display no cytotoxic reaction. The intermediate control should display a mild to moderate cytotoxic reaction, a score of '2' or '3'. The positive control should display a moderate to severe cytotoxic reaction, a score of '3' or '4'.

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS: Not applicable.

STATISTICAL METHODS: None used.

RECORD RETENTION

A certified copy of this report and data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

COMPLIANCE

This study was performed in compliance with the following International Standards:

ISO 10993-5:2009 Biological Evaluation of Medical Devices, Part 5: Tests for *In Vitro* Cytotoxicity.

ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

TEST ARTICLE DISPOSITION

Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

The negative and cell controls exhibited healthy growth throughout the experiment. The monolayers exposed to CdCl_2 exhibited a toxic response while the black rubber intermediate control induced a mild level of toxicity. Therefore, the test system was responding normally and met the criteria for a valid assay. See Table 5.



When scoring lysis, each sample was rated relative to the amount of lysis displayed by the negative control. The negative and cell control were considered '0', the cells displayed at least 70% viability. The intermediate control displayed a mild cytotoxic reaction resulting in a score of '2'. The positive control displayed a severe cytotoxic reaction, resulting in a score of '4'. Therefore, the test system was responding normally and met the criteria for a valid assay. See Table 5.

In this assay, the test article **did not** induce cytotoxicity. No abnormal events such as pH change or debris were noted.

TABLE 5: RESULTS

TEST ARTICLE AND CONTROLS	SAMPLE EXTRACT		CYTOTOXICITY SCORES		
	SIZE	(mL)	24 HOURS	48 HOURS	72 HOURS
Test Article - Neat	1.7 g	8.5	0/0/0	0/0/0	0/0/0
Test Article - 1:2	NA	NA	0/0/0	0/0/0	0/0/0
Test Article - 1:4	NA	NA	0/0/0	0/0/0	0/0/0
Test Article - 1:8	NA	NA	0/0/0	0/0/0	0/0/0
Test Article - 1:16	NA	NA	0/0/0	0/0/0	0/0/0
Test Article - 1:32	NA	NA	0/0/0	0/0/0	0/0/0
Test Article - 1:64	NA	NA	0/0/0	0/0/0	0/0/0
Test Article - 1:128	NA	NA	0/0/0	0/0/0	0/0/0
Test Article - 1:256	NA	NA	0/0/0	0/0/0	0/0/0
Positive Control	0.1 mL	9.9	4/4/4	4/4/4	4/4/4
Intermediate Control	1.9 g	9.5	2/2/2	2/2/2	2/2/2
Negative Control	30 cm ²	5.0	0/0/0	0/0/0	0/0/0
Cell Control	NA	10.0	0/0/0	0/0/0	0/0/0

NA = Not Applicable

ANALYSIS AND CONCLUSION

The test article at the neat concentration, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256 ratios scored '0' at 24, 48 and 72 ± 4 hours period. Therefore the test article is considered **non-cytotoxic** at the neat concentration.

REFERENCES

U.S. Pharmacopeia, Section 87, current revision.

WuXi AppTec Reference Library Contents, Form ALS-4650-1

WuXi AppTec SOP: MED-8750, Minimum Essential Medium (MEM) Elution Assay



FINAL STUDY REPORT

STUDY TITLE: ISO MEM Endpoint Dilution Assay
Using L-929 Mouse Fibroblast Cells

PROTOCOL NUMBER: 140350-15

TEST ARTICLE IDENTIFICATION: Trade Name: OnTop Plated PTC Clips, Generic
Name: Stainless Steel Clip with Nickel and Tin
Plating, R-spec: R2004-01-G, SPE002496,
SPE002497, Manufactured according to:
PRC057314, Sterilization: 110 kGy Gamma,
2xETO, Reference Number: EE2730
Lot # PRC057314

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

STUDY NUMBER: 171503

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: The test article is considered **non-cytotoxic** at the
neat concentration under the conditions of this test.



QUALITY ASSURANCE UNIT SUMMARY

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR part 58) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Each inspection was performed to assure the quality and integrity of the study.

<u>Phase Inspected</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Inoculation of Extract	06/15/12	06/18/12	06/28/12
Final Report	06/27/12	06/27/12	06/28/12

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor:  Date: 6-28-12
Mike Burngartner

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director:  Date: 6-28-12
Jaime Haueter

Professional personnel involved:

Lisa Olson, BS	Vice President, Testing and Service Development
Teri Tanquist, BS	Vice President of Process Improvement and Operations
Jean Kringstad, BS	Director, <i>In Vitro</i> Testing Services
Jaime Haueter, BS	Study Director
Jean Mesarich, AA	Client Relations Manager



PURPOSE

The purpose of this study was to evaluate a serially diluted extract of a test article for cytotoxicity to mammalian cells in culture by a method compliant with the requirements specified in ISO 10993-5:2009.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

TEST ARTICLE RECEIVED: 06/13/12
INITIATION DATE: 06/14/12
COMPLETION DATE: 06/28/12

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: OnTop Plated PTC Clips, Generic Name: Stainless Steel Clip with Nickel and Tin Plating, R-spec: R2004-01-G, SPE002496, SPE002497, Manufactured according to: PRC057314, Sterilization: 110 kGy Gamma, 2xETO, Reference Number: EE2730
Lot #:	PRC057314
Sterilization Method:	Gamma 110 kGy, 2xETO
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Device
Physical Description:	According to the Sponsor, the test article consisted of silver gray U-shaped metal pieces.

CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.



EXPERIMENTAL DESIGN

Experimental Summary

The test article was incubated in the appropriate volume of E-MEM + 5% FBS in a sterile vessel for 24 ± 2 hours at 37 ± 1 °C. At the end of the extraction period, the extract was decanted from the test article. A series of eight dilutions of the extract were prepared in E-MEM + 5% FBS in a two-fold dilution. After the dilutions were prepared, the maintenance culture media was removed from test culture wells and replaced with 1.0 ml of test media dilutions in triplicate wells. The positive control media, test article extract, and control extracts were added at the same time to the culture plate in triplicate wells. The plates were incubated for 72 hours at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air. The cultures were evaluated for cytotoxic effects by microscopic examination at 24, 48 and 72 ± 4 hours of incubation and the results scored and recorded.

Justification For Selection Of The Test System

The MEM Endpoint Dilution Assay is one of several *in vitro* mammalian cell tests which have been used to screen materials for their potential cytotoxicity. The L-929 cell line has a history of use in assays of this type.

AMENDMENTS/DEVIATIONS: None.

IDENTIFICATION OF THE TEST SYSTEM

Cultures of L-929 cells (mouse fibroblast) were obtained from American Type Culture Collection (ATCC # CCL-1). Mycoplasma-free cell lines which were purchased from the vendor and kept frozen in the lab until used. To maintain the sensitivity, they are only sub-cultured for up to 15 passages and then discarded.

Cultures were grown and used as monolayers in disposable tissue culture labware at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air at WuXi AppTec, Inc. The media used for growth of cells is Eagle's minimal essential medium (E-MEM) supplemented with 5% (v/v) fetal bovine serum (FBS). The medium is also supplemented with the following: 2 mM L-glutamine, 10 mM HEPES, 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 units/mL penicillin, and 1% 2.50 ug/mL amphotericin B (Fungizone). WuXi AppTec Inc. has a long-standing history using antibiotics in culture medium. The use of antibiotics in culture media has not shown adverse affects when used for this assay, exhibited by the varying degrees of toxicity seen in the assay controls.

TEST MATERIAL PREPARATION

The test article remained intact, and was placed into an extraction vessel and prepared at a ratio of 4g to 20 mL of extraction vehicle.

TABLE 1: TEST ARTICLE RECORD

VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	QUANTITY OF TEST ARTICLE USED
E-MEM + 5% FBS	1.7	8.5	1.7 g

EXTRACTION MEDIA

Eagle's minimal essential medium (E-MEM), supplemented with 5% (v/v) fetal bovine serum (FBS), was used to extract the test article. The medium was also supplemented with the following: 2 mM L-glutamine, 10 mM HEPES, 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 units/mL penicillin, and 1% 2.50 µg/mL amphotericin B (Fungizone). The pH was confirmed to be 7.30.

CONTROL ARTICLE PREPARATION

A negative, intermediate, positive, and cell control were run in parallel with the test article. A negative control (high density polyethylene (HDPE)) known to be non-toxic under the test conditions was prepared at a ratio of 120 cm² to 20 mL of extraction vehicle. An intermediate control (black rubber) known to be mildly toxic under the test conditions was prepared and extracted at a ratio of 4 g to 20 mL of extraction vehicle. A positive control (100 µM CdCl₂) known to be toxic under the test conditions was prepared by diluting cadmium chloride stock powder with 100 mL of sterile water to create a 10 mM solution. The solution was then prepared at a ratio of 0.1 mL to 9.9 mL of extraction vehicle. A cell control (E-MEM + 5% FBS) was incubated in parallel with the test sample and controls.

TABLE 2: CONTROL / CELL LINE RECORD

CONTROL IDENTIFICATION:	CLASS	LOT #	SUPPLIED BY	EXPIRATION
100 µM CdCl ₂	Positive Control	TDSI1074	WXAT	09/20/12
Black Rubber Stopper	Intermediate Control	WXAT010710	Fisher	02/28/13
High Density Polyethylene (HDPE)	Negative Control	C-091	Hatano	02/2016
E-MEM + 5% FBS	Medium	TDSI1173	WXAT	06/18/12
L-929	Cell Line	L061112	ATCC	NA

NA = Not applicable

TEST ARTICLE EXTRACTION

The extraction mixture and controls were incubated for 24 ± 2 hours at 37 ± 1 °C. At the start of the extraction, the solutions appeared clear and free of particulates. The extracts were agitated during the course of the extraction period. At the end of the extraction period, the vessels were well shaken. The test article was observed after extraction to be intact with no macroscopically observable degradation and the extract was clear. The extract was not filtered and was used immediately. See Tables 1-3.

TABLE 3: EXTRACTION RECORD

VEHICLE	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END
E-MEM + 5% FBS	37.0 °C	06/14/12 2:30 pm	37.0 °C	06/15/12 1:50 pm

EXPERIMENTAL PROCEDURE

L-929 cells were plated at 2.5 x 10⁴ cells/mL at least 24 hours before use. Prior to inoculation, the cell cultures were examined to ensure they had formed a nearly confluent monolayer and that they appeared uniform and viable.

The test article and controls were extracted with the appropriate amount of E-MEM + 5% FBS for 24 ± 2 hours at 37 ± 1 °C. At the end of the extraction period, the vessels were well shaken and the extraction media was used immediately. A series of eight dilutions of the extract were prepared in E MEM in a two-fold dilution. One mL aliquots of the test article and control extract and the series of dilutions were plated in triplicate onto the cell line.

The cell cultures were incubated in a humidified atmosphere for 72 hours. The monolayers were evaluated for cytotoxic effects at 24, 48, and 72 hours. At each incubation period, the cell cultures were observed for signs of cytopathic effect (CPE) including lysis, crenation, plaques, and excessive rounding of cells.

TEST EVALUATION

Criteria for evaluating cytotoxicity will include morphologic changes in cells, such as granulation, crenation, or rounding, and loss of viable cells from the monolayer by lysis or detachment. The validity of the test requires that negative control cultures maintain a healthy normal appearance throughout the duration of the test. Degrees of toxicity were scored as described in Table 4.



TABLE 4: SCORING

GRADE	REACTIVITY	CONDITIONS OF ALL CULTURES
0	None	Discrete intracytoplasmic granules; no cell lysis, no reduction of cell growth
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cells layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers

According to the current ISO guidelines, test articles scoring '0', '1', or '2' are considered NON-CYTOTOXIC. Test articles scoring '3' or '4' are considered CYTOTOXIC.

VALIDITY CRITERIA

Validity of the assay and the test article result was based upon the criteria listed below and scientific judgment.

The negative and cell control should display no cytotoxic reaction. The intermediate control should display a mild to moderate cytotoxic reaction, a score of '2' or '3'. The positive control should display a moderate to severe cytotoxic reaction, a score of '3' or '4'.

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS: Not applicable.

STATISTICAL METHODS: None used.

RECORD RETENTION

A certified copy of this report and data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

COMPLIANCE

This study was performed in compliance with the following International Standards:

ISO 10993-5:2009 Biological Evaluation of Medical Devices, Part 5: Tests for *In Vitro* Cytotoxicity.

ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

TEST ARTICLE DISPOSITION

Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

The negative and cell controls exhibited healthy growth throughout the experiment. The monolayers exposed to CdCl₂ exhibited a toxic response while the black rubber intermediate control induced a mild level of toxicity. Therefore, the test system was responding normally and met the criteria for a valid assay. See Table 5.



When scoring lysis, each sample was rated relative to the amount of lysis displayed by the negative control. The negative and cell control were considered '0', the cells displayed at least 70% viability. The intermediate control displayed a mild cytotoxic reaction resulting in a score of '2'. The positive control displayed a severe cytotoxic reaction, resulting in a score of '4'. Therefore, the test system was responding normally and met the criteria for a valid assay. See Table 5.

In this assay, the test article **did not** induce cytotoxicity. No abnormal events such as pH change or debris were noted.

TABLE 5: RESULTS

TEST ARTICLE AND CONTROLS	SAMPLE EXTRACT		CYTOTOXICITY SCORES		
	SIZE	(mL)	24 HOURS	48 HOURS	72 HOURS
Test Article	1.7 g	8.5	0/0/0	0/0/0	0/0/0
Test Article – 1:2	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:4	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:8	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:16	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:32	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:64	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:128	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:256	NA	NA	0/0/0	0/0/0	0/0/0
Positive Control	0.1 mL	9.9	4/4/4	4/4/4	4/4/4
Intermediate Control	1.9 g	9.5	2/2/2	2/2/2	2/2/2
Negative Control	30 cm ²	5.0	0/0/0	0/0/0	0/0/0
Cell Control	NA	10.0	0/0/0	0/0/0	0/0/0

NA = Not Applicable

ANALYSIS AND CONCLUSION

The test article at the neat concentration, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256 ratios scored '0' at 24, 48 and 72 ± 4 hours period. Therefore the test article is considered **non-cytotoxic** at the neat concentration.

REFERENCES

U.S. Pharmacopeia, Section 87, current revision.

WuXi AppTec Reference Library Contents, Form ALS-4650-1

WuXi AppTec SOP: MED-8750, Minimum Essential Medium (MEM) Elution Assay



PURPOSE

The purpose of this procedure was evaluate to the cytotoxic response of a specified mammalian culture cell line when exposed to an extract of the test article. This assay utilized the sensitivity of low-cell density to evaluate the cytotoxicity of medical devices.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

TEST ARTICLE RECEIVED: 06/13/12
INITIATION DATE: 06/25/12
COMPLETION DATE: 07/13/12

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: Aleph Plated PTC Clips, Generic Name: Stainless Steel Clip with Nickel and Tin Plating, R-spec: R2004-01-G, Nickel Plating, Tin Plating, Manufactured according to: PRC057314, Sterilization: 110 kGy Gamma, 2xETO, Reference Number: EE2730
Lot #:	PRC057314
Sterilization Method:	110 kGy Gamma, 2xETO
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Devices
Physical Description:	According to the Sponsor, the test article consisted of silver gray U-shaped metal pieces.

CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.



EXPERIMENTAL DESIGN

Experimental Summary

The extract colony assay evaluates the cytotoxic effects of a test article extract on V79 Chinese hamster fibroblast cells. The assay utilizes the sensitivity of low cell density to evaluate material/device cell toxicity. The assay was conducted by extracting a test article and controls in V79 plant media (EMEM + 5% FBS, NEAA, SP). Extracts were placed in contact with V79 cells seeded at a low cell density. The resulting cell colony growth was fixed, stained, and counted.

Test articles and controls were prepared and extracted for 24 ± 2 hours at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air. Appropriate dilutions of the extracts were prepared using V79 plant media (EMEM + 5% FBS, NEAA, SP). After removing spent medium, 2.0 ml of the extract was added to the plates. The cells were incubated at 37 ± 1 °C for 6-7 days before fixation, staining, and counting. The colony growth was then expressed as a percentage of the cell control. These percentages were plotted on a semi-logarithmic graph against the % dilution of the test article extract. The percent dilution that inhibited colony formation to 50% was determined from the resulting inhibition curve.

Justification For Selection Of The Test System

The extract colony assay is one of several *in vitro* mammalian cell tests which have been used to screen materials for their potential cytotoxicity. The V79 cell line has a history of use in assays of this type.

AMENDMENTS/DEVIATIONS: None.

IDENTIFICATION OF TEST SYSTEM

Cultures of V79 cells (Chinese hamster fibroblast) were obtained from Health Protection Agency Culture Collections (HPACC #86041102). Cultures were grown and used as monolayers in disposable tissue culture labware at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air at WuXi AppTec, Inc.

Cultures were grown and used as monolayers in disposable tissue culture labware at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air at WuXi AppTec, Inc. The media used for growth of cells is Eagle's minimal essential medium (E-MEM) supplemented with 5% (v/v) fetal bovine serum (FBS). The medium is also supplemented with the following: 2 mM L-glutamine, 0.5% non-essential amino acids (NEAA), 1% 100mM sodium pyruvate (SP), 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 u/mL penicillin, and 1% 2.50 ug/mL amphotericin B (Fungizone). WuXi AppTec Inc. has a long-standing history using antibiotics in culture medium. The use of antibiotics in culture media has not shown adverse effects when used for this assay; this is exhibited in the varying degrees of toxicity seen in the assay controls.

TEST ARTICLE PREPARATION

The test article was cut for extraction, placed into test tubes, and prepared at a ratio of 2 g to 20 mL of extraction vehicle.

TABLE 1: TEST ARTICLE RECORD

VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	QUANTITY OF TEST ARTICLE USED PER TEST
V79 Plant Media	2.3	23.0	2.3 g

CONTROLS PREPARATION

A negative control (high density polyethylene film) was prepared at a ratio of 2 grams / 20 mL.

A moderately positive control (polyurethane film containing 0.1% zinc diethyldithiocarbamate (ZDEC)) was prepared at a ratio of 2 grams / 20 mL.



A weak positive control (polyurethane film containing 0.25% zinc dibutyldithiocarbamate (ZDBC)) was prepared at a ratio of 2 grams / 20 mL.

Cell Control: V79 plant media (EMEM + 5% FBS, NEAA, SP) was incubated in parallel with test article and controls.

EXTRACTION MEDIA

Eagle's minimal essential medium (E-MEM), supplemented with 5% (v/v) fetal bovine serum (FBS), was used to extract the test article. The medium was also supplemented with the following: L-glutamine, NEAA, SP, gentamicin, penicillin, vancomycin and amphotericin B (Fungizone). The pH was confirmed to be 7.21.

TEST ARTICLE EXTRACTION

The extraction mixture and controls were incubated for 24 ± 2 hours at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂. At the start of the extraction, the solutions appeared clear and free of particulates. At the end of the extraction period, the vessels were shaken well and the liquid aseptically decanted. The test article was observed after extraction to be intact with no macroscopically observable degradation and the extract was clear. After decanting the extract was not filtered prior to use, and was used immediately. See Tables 1-3.

TABLE 2: EXTRACTION RECORD

VEHICLE	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END
V79 Plant Media	37.0 °C	06/25/12 4:28 pm	36.9 °C	06/26/12 2:34 pm

TABLE 3: CONTROL / CELL LINE RECORD

CONTROL IDENTIFICATION	CLASS	LOT #	SUPPLIED BY	EXPIRATION
ZDEC	Positive Control	A-091	Hatano	10/2014
ZDBC	Positive Control	B-102K	Hatano	05/2015
High Density Polyethylene (HDPE)	Negative Control	C-091	Hatano	02/2016
V79 Plant Media	Medium	VP061212A1	Lonza	07/12/12
V79	Cell Line	V062512	HPACC	NA

NA = Not Applicable

EXPERIMENTAL PROCEDURE

The test article and controls were extracted in the appropriate volume of V79 Plant Media in a sterile vessel for 24 ± 2 hours at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air. At the end of the extraction period, the extracts were diluted with V79 Plant Media as shown below:

Table 4: Dilution Scheme

EXTRACT	% OF NEAT						
Test Article	100	75	50	25	12.5	6.25	3.125
Positive ZDEC	2.0	1.5	1.0	0.75	0.50	0.25	0.125
Positive ZDBC	100	56.2		31.6		17.7	
Negative Control	100	NA		NA		NA	
Cell Control	100	NA		NA		NA	

NA = Not Applicable



A V79 cell suspension, with a concentration of 50 cells/mL, was diluted using V79 Plant Media to a final concentration of 33.3 cells/mL of medium. A volume of 3 mL (~100 cells) of diluted cell suspension was seeded into each well of a six-well plate (well size, 35 mm) and incubated for 24 ± 2 hours at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂. After incubation, the maintenance culture medium was decanted from test culture wells and replaced with 2 mL of each dilution from above. The test article and control extracts and cell control media were added to the culture plates in triplicate wells. The plates were then incubated at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air until adequate cell colony growth was observed (6-7 days).

At the end of the incubation period, the extracts were removed from the plates and the wells were rinsed with 2 mL of Dulbecco's phosphate buffered saline without magnesium or calcium (DPBS). The DPBS was decanted and the colonies were fixed by placing 1 mL of methanol solution into each well for 2-10 minutes. Once the cells were fixed, they were stained for 2-3 hours with 1 mL of Giemsa stain solution that had been diluted 1:20 with PBS (pH 6.4). At the end of the staining period, the stain was decanted and 2 mL of DPBS(-) was added to each well. The colonies were then counted after the removal of DPBS.

EVALUATION CRITERIA

The relative colony formation rates (%) were plotted against the % dilutions of the extract on a semi-logarithmic graph. The resulting inhibition curve of each graph was used to determine the % dilution that inhibited colony formation to 50%. This was the IC₅₀ value. Sigma Stat was used to perform a t-test comparing the cell control results with the average colony count from the negative control.

VALIDITY

Final evaluation of the validity of the assay and test article results was based upon the criteria listed below and scientific judgment.

The t-test results must show that statistically significant inhibition of colony formation was not found in the negative control results.

IC₅₀ values of the positive ZDEC and ZDBC controls must be less than the following values:

Positive ZDEC: 7% and Positive ZDBC: 80%

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS

The colonies were counted and the triplicate wells averaged for each % dilution. These averages were divided by the average number of colonies obtained in the cell control wells. This percentage is the Relative Colony Formation for each % dilution.

STATISTICAL METHODS

A t-test was performed using Sigma Stat and a difference of $p < 0.05$ was considered significant.

RECORD RETENTION

A certified copy of this report and data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

TEST ARTICLE DISPOSITION

Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

Raw data and calculated results, including averages and relative colony formation are presented in Table 5. The test article extract was in contact with the test system for seven days.



A dramatic, dose dependant decrease in relative colony formation was noted as a result of increasing concentrations in the positive control preparations (ZDEC and ZDBC), indicating a sensitive assay (Table 5). Additionally, the relative colony formation (% RCF) in preparations treated with the negative control showed high degrees of survival. ZDEC induced a maximum IC_{50} of 1.20% while ZDBC returned an IC_{50} of 61.89%. Both met the criteria for a valid assay. Accordingly, this assay was determined to be valid.

None of the standard dilutions of the test article induced biologically relevant decreases in colony formation. Further, no dose response trends were noted.

TABLE 5: Results

DOSE GROUP	% OF NEAT EXTRACT	WELL 1	WELL 2	WELL 3	AVERAGE	% RCF**	IC_{50}
Test Article	100	29	27	25	27	79	NA*
	75	29	34	26	30	88	
	50	27	33	30	30	88	
	25	27	32	34	31	91	
	12.5	30	38	34	34	100	
	6.25	42	36	34	37	109	
	3.125	40	38	34	37	109	
ZDEC	2.0	0	0	0	0	0	1.20%
	1.5	0	1	0	0	0	
	1.0	22	23	23	23	68	
	0.75	41	38	32	37	109	
	0.50	51	54	45	50	147	
	0.25	43	44	40	42	124	
	0.125	29	30	41	33	97	
ZDBC	100	0	0	0	0	0	61.89%
	56.2	24	35	30	30	88	
	31.6	31	27	35	31	91	
	17.7	30	42	40	37	109	
Negative Control	100	42	41	33	39	115	NA
Cell Control	100	31	37	35	34	100	NA

* This value could not be calculated as a 50% value was not achieved.

** Relative Colony Formation = Average colony formation divided by average colony formulation of cell control x 100.

ANALYSIS AND CONCLUSION

Negative and positive controls were run concurrently with the test article to provide ranges of cytotoxicity. Each of the positive controls induced relative colony formation well within acceptable limits. No significant differences in colony formation were found between the negative control treated wells and the cell control. The results from the control cultures indicate that the assay retained normal sensitivity. Therefore, the criteria for a valid assay were met.

None of the standard dilutions of the test article induced biologically relevant decreases in colony formation. Further, no dose response trends were noted. Therefore, an IC_{50} could not be calculated for the test article. These results would be indicative of a **non-cytotoxic** material.

REFERENCES

Guidelines for Basic Biological Tests of Medical Materials and Devices – Part I Cytotoxicity Test (Colony Assay). Japanese Ministry of Health and Welfare, 2012.

ISO 10993-5: 2009 Biological Evaluation of Medical Devices, Part 5: Tests for *In Vitro* Cytotoxicity.

WuXi AppTec SOP: MED-8710, Extract Colony Assay Cytotoxicity Test



FINAL STUDY REPORT

STUDY TITLE: ISO MEM Endpoint Dilution Assay
Using L-929 Mouse Fibroblast Cells

PROTOCOL NUMBER: 140350-15

TEST ARTICLE IDENTIFICATION: Trade Name: Aleph Plated PTC Clips, Generic
Name: Stainless Steel Clip with Nickel and Tin
Plating, R-spec: R2004-01-G, Nickel Plating, Tin
Plating, Manufactured according to: PRC057314,
Sterilization: 110 kGy Gamma, 2xETO, Reference
Number: EE2730
Lot # PRC057314

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

STUDY NUMBER: 171505

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: The test article is considered **non-cytotoxic** at the
neat concentration under the conditions of this test.



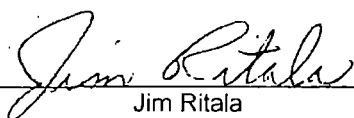
QUALITY ASSURANCE UNIT SUMMARY

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR Part 58) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Each inspection was performed to assure the quality and integrity of the study.

<u>Phase Inspected</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Inoculation of Extract	06/15/12	06/18/12	06/29/12
Final Report	06/28/12	06/28/12	06/29/12

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: _____


Jim Ritala

Date: _____

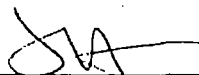
6/29/12

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: _____



Jaime Haueter

Date: _____

6-29-12

Professional personnel involved:

Lisa Olson, BS
Teri Tanquist, BS
Jean Kringstad, BS
Jaime Haueter, BS
Jean Mesarich, AA

Vice President, Testing and Service Development
Vice President of Process Improvement and Operations
Director, *In Vitro* Testing Services
Study Director
Client Relations Manager



PURPOSE

The purpose of this study was to evaluate a serially diluted extract of a test article for cytotoxicity to mammalian cells in culture by a method compliant with the requirements specified in ISO 10993-5:2009.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

TEST ARTICLE RECEIVED: 06/13/12
INITIATION DATE: 06/14/12
COMPLETION DATE: 06/29/12

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: Aleph Plated PTC Clips, Generic Name: Stainless Steel Clip with Nickel and Tin Plating, R-spec: R2004-01-G, Nickel Plating, Tin Plating, Manufactured according to: PRC057314, Sterilization: 110 kGy Gamma, 2xETO, Reference Number: EE2730
Lot #:	PRC057314
Sterilization Method:	Gamma 110 kGy, 2xETO
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Device
Physical Description:	According to the Sponsor, the test article consisted of Silver gray U-shaped metal pieces.

CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

NAMSA**REPORT**

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455**STUDY TITLE**Determination of Extractable Species From a
Test Article by Infrared Spectroscopy**TEST ARTICLE NAME**

OnTop Plated PTC Clip

TEST ARTICLE IDENTIFICATION

EE2730

SPONSORBrittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242**TEST ARTICLE PHYSICAL DESCRIPTION**

silver gray U-shaped metal pieces

TEST ARTICLE RECEIVED

June 13, 2012

PURPOSE

The purpose of this study was to prepare an infrared spectrum of a material for the purpose of identifying the material or examining it for impurities.

SUMMARY/RESULTS

The test article was characterized for extractable species as required by ISO 10993-18, Biological evaluation of medical devices - Part 18: Chemical characterization of materials by first extracting the test article in dichloromethane. The resulting test extract was then evaporated to a residue and then the residue was analyzed by infrared spectroscopy to identify the species present in the test extract.

There were no major bands of interest detected in the test article residue. The lack of species present as represented by the infrared spectrum, can be used to aid in the assessment of the test article by ISO 10993, Part 18.

The test article spectrum is located in Appendix I.

Date Test Concluded: June 27, 2012

METHOD

A 10.2 gram portion (249 pieces) of the test article was extracted at 37°C for 72 hours in 51 mL of dichloromethane. The method and results of this extraction were reported under Lab Number 12T_37839_01. An approximate 1-2 mL portion of the dichloromethane test extract was transferred to a clean vial and placed in a 105°C drying oven to evaporate the extracting solvent. A small amount of chloroform was added to the vial to dissolve the residue. The resulting solution was placed on a clean potassium bromide crystal and the chloroform was allowed to evaporate. The residue was analyzed by transmission infrared spectroscopy using the instrument conditions listed below.

Instrument	Fourier Transform Infrared Spectrophotometer
Wavenumber Range—Collected	4400 cm ⁻¹ – 350 cm ⁻¹
Scan number	8
Resolution	4 cm ⁻¹

NAMSA

REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

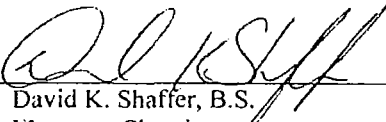
REFERENCES

European Pharmacopoeia (Ph. Eur.) 7th Edition, Section 2.2.24. Absorption Spectrophotometry, Infrared (2011).

International Organization for Standardization (ISO) 10993-18, Biological evaluation of medical devices - Part 18: Chemical characterization of materials (2005).

United States Pharmacopeia 35, National Formulary 30 (USP), General Chapter <197>, Spectrophotometric Identification Tests, Infrared Absorption (2012).

APPROVAL


David K. Shaffer, B.S.
Manager, Chemistry

Date

7-2-12

Results apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.

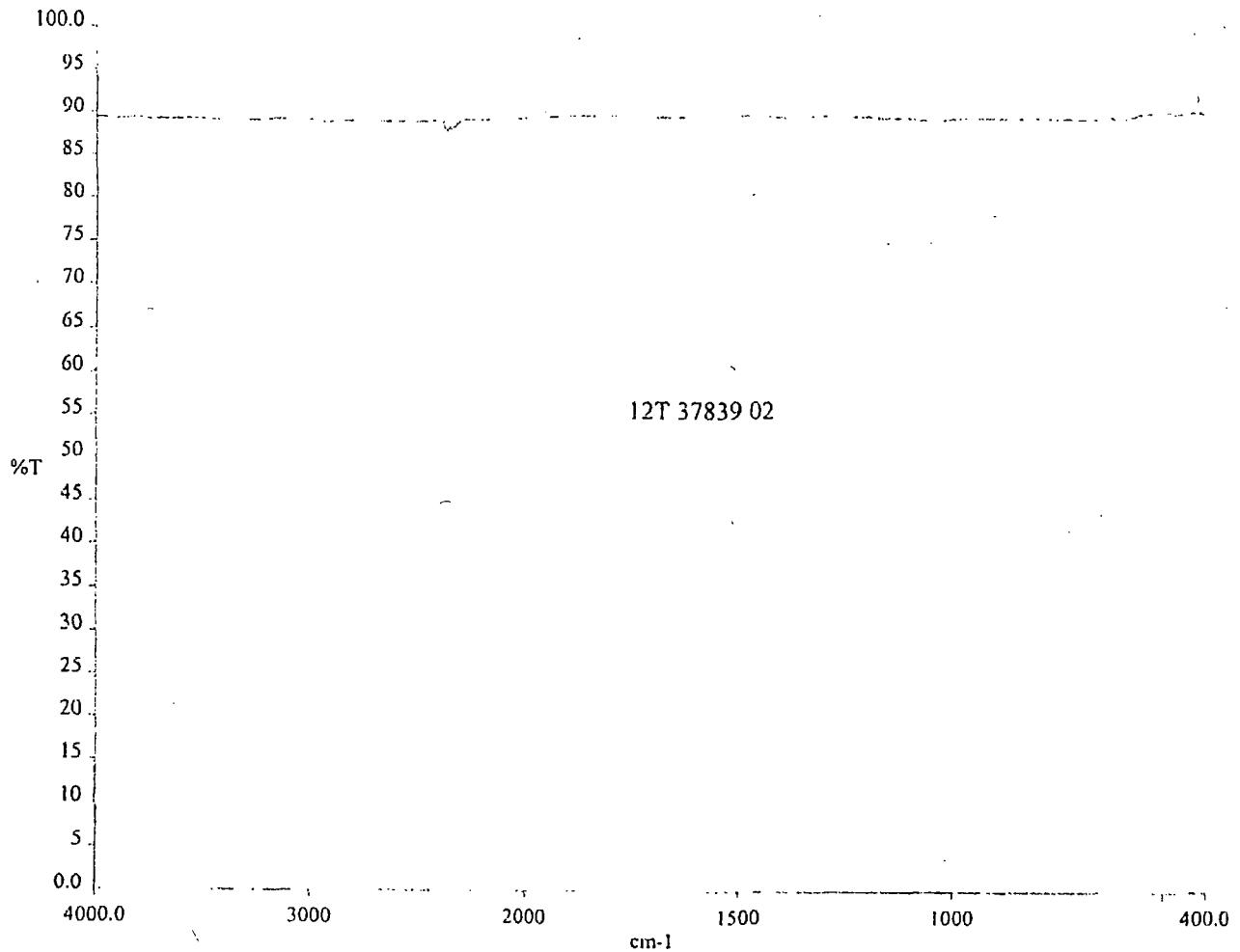


REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

APPENDIX 1





FINAL STUDY REPORT

STUDY TITLE: JAPANESE CYTOTOXICITY TEST
COLONY MICROASSAY BY ELUTION
EXPANDED DILUTION SERIES

PROTOCOL NUMBER: C11662-3

TEST ARTICLE IDENTIFICATION: Trade Name: OnTop Plated PTC Clips, Generic
Name: Stainless Steel Clip with Nickel and Tin
Plating, R-spec: R2004-01-G, SPE002496,
SPE002497, Manufactured according to:
PRC057314, Sterilization: 110 kGy Gamma,
2xETO, Reference Number: EE2730
Lot # PRC057314

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

STUDY NUMBER: 171502

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: None of the standard dilutions of the test article induced biologically relevant decreases in colony formation. Further, no dose response trends were noted. Therefore, an IC_{50} could not be calculated for the test article. These results would be indicative of a **non-cytotoxic** material.



QUALITY ASSURANCE UNIT SUMMARY

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR part 58) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Each inspection was performed to assure the quality and integrity of the study.

<u>Critical Phase</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Dilutions/Plating	06/26/12	06/27/12	07/13/12
Final Report	07/13/12	07/13/12	07/13/12

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor:

Sheri Handy
Sheri Handy

Date: 7/13/12

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director:

Jaime Haueter

Jaime Haueter

Date: 7-13-12

Professional Personnel Involved:

Lisa Olson, BS
Teri Tanquist, BS
Jean Kringstad, BS
Jaime Haueter, BS
Jean Mesarich, AA

Vice President, Testing and Service Development
Vice President of Process Improvement and Operations
Director, *In Vitro* Testing Services
Study Director
Client Relations Manager



PURPOSE

The purpose of this procedure was evaluate to the cytotoxic response of a specified mammalian culture cell line when exposed to an extract of the test article. This assay utilized the sensitivity of low-cell density to evaluate the cytotoxicity of medical devices.

TEST FACILITY: WuXi AppTec, Inc.
 2540 Executive Drive
 St. Paul, MN 55120

TEST ARTICLE RECEIVED: 06/13/12
INITIATION DATE: 06/25/12
COMPLETION DATE: 07/13/12

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: OnTop Plated PTC Clips, Generic Name: Stainless Steel Clip with Nickel and Tin Plating, R-spec: R2004-01-G, SPE002496, SPE002497, Manufactured according to: PRC057314, Sterilization: 110 kGy Gamma, 2xETO, Reference Number: EE2730
Lot #:	PRC057314
Sterilization Method:	110 kGy Gamma, 2xETO
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Devices
Physical Description:	According to the Sponsor, the test article consisted of silver gray U-shaped metal pieces.

CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.



EXPERIMENTAL DESIGN

Experimental Summary

The extract colony assay evaluates the cytotoxic effects of a test article extract on V79 Chinese hamster fibroblast cells. The assay utilizes the sensitivity of low cell density to evaluate material/device cell toxicity. The assay was conducted by extracting a test article and controls in V79 plant media (EMEM + 5% FBS, NEAA, SP). Extracts were placed in contact with V79 cells seeded at a low cell density. The resulting cell colony growth was fixed, stained, and counted.

Test articles and controls were prepared and extracted for 24 ± 2 hours at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air. Appropriate dilutions of the extracts were prepared using V79 plant media (EMEM + 5% FBS, NEAA, SP). After removing spent medium, 2.0 ml of the extract was added to the plates. The cells were incubated at 37 ± 1 °C for 6-7 days before fixation, staining, and counting. The colony growth was then expressed as a percentage of the cell control. These percentages were plotted on a semi-logarithmic graph against the % dilution of the test article extract. The percent dilution that inhibited colony formation to 50% was determined from the resulting inhibition curve.

Justification For Selection Of The Test System

The extract colony assay is one of several *in vitro* mammalian cell tests which have been used to screen materials for their potential cytotoxicity. The V79 cell line has a history of use in assays of this type.

AMENDMENTS/DEVIATIONS: None.

IDENTIFICATION OF TEST SYSTEM

Cultures of V79 cells (Chinese hamster fibroblast) were obtained from Health Protection Agency Culture Collections (HPACC #86041102). Cultures were grown and used as monolayers in disposable tissue culture labware at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air at WuXi AppTec, Inc.

Cultures were grown and used as monolayers in disposable tissue culture labware at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air at WuXi AppTec, Inc. The media used for growth of cells is Eagle's minimal essential medium (E-MEM) supplemented with 5% (v/v) fetal bovine serum (FBS). The medium is also supplemented with the following: 2 mM L-glutamine, 0.5% non-essential amino acids (NEAA), 1% 100mM sodium pyruvate (SP), 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 u/mL penicillin, and 1% 2.50 ug/mL amphotericin B (Fungizone). WuXi AppTec Inc. has a long-standing history using antibiotics in culture medium. The use of antibiotics in culture media has not shown adverse effects when used for this assay; this is exhibited in the varying degrees of toxicity seen in the assay controls.

TEST ARTICLE PREPARATION

The test article was cut for extraction, placed into test tubes, and prepared at a ratio of 2 g to 20 mL of extraction vehicle.

TABLE 1: TEST ARTICLE RECORD

VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	QUANTITY OF TEST ARTICLE USED PER TEST
V79 Plant Media	2.3	23.0	2.3 g

CONTROLS PREPARATION

A negative control (high density polyethylene film) was prepared at a ratio of 2 grams / 20 mL.

A moderately positive control (polyurethane film containing 0.1% zinc diethyldithiocarbamate (ZDEC)) was prepared at a ratio of 2 grams / 20 mL.



A weak positive control (polyurethane film containing 0.25% zinc dibutylidithiocarbamate (ZDBC)) was prepared at a ratio of 2 grams / 20 mL.

Cell Control: V79 plant media (EMEM + 5% FBS, NEAA, SP) was incubated in parallel with test article and controls.

EXTRACTION MEDIA

Eagle's minimal essential medium (E-MEM), supplemented with 5% (v/v) fetal bovine serum (FBS), was used to extract the test article. The medium was also supplemented with the following: L-glutamine, NEAA, SP, gentamicin, penicillin, vancomycin and amphotericin B (Fungizone). The pH was confirmed to be 7.21.

TEST ARTICLE EXTRACTION

The extraction mixture and controls were incubated for 24 ± 2 hours at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂. At the start of the extraction, the solutions appeared clear and free of particulates. At the end of the extraction period, the vessels were shaken well and the liquid aseptically decanted. The test article was observed after extraction to be intact with no macroscopically observable degradation and the extract was clear. After decanting the extract was not filtered prior to use, and was used immediately. See Tables 1-3.

TABLE 2: EXTRACTION RECORD

VEHICLE	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END
V79 Plant Media	37.0 °C	06/25/12 4:28 pm	36.9 °C	06/26/12 2:34 pm

TABLE 3: CONTROL / CELL LINE RECORD

CONTROL IDENTIFICATION	CLASS	LOT #	SUPPLIED BY	EXPIRATION
ZDEC	Positive Control	A-091	Hatano	10/2014
ZDBC	Positive Control	B-102K	Hatano	05/2015
High Density Polyethylene (HDPE)	Negative Control	C-091	Hatano	02/2016
V79 Plant Media	Medium	VP061212A1	Lonza	07/12/12
V79	Cell Line	V062512	HPACC	NA

NA = Not Applicable

EXPERIMENTAL PROCEDURE

The test article and controls were extracted in the appropriate volume of V79 Plant Media in a sterile vessel for 24 ± 2 hours at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air. At the end of the extraction period, the extracts were diluted with V79 Plant Media as shown below:

Table 4: Dilution Scheme

EXTRACT	% OF NEAT						
Test Article	100	75	50	25	12.5	6.25	3.125
Positive ZDEC	2.0	1.5	1.0	0.75	0.50	0.25	0.125
Positive ZDBC	100	56.2		31.6		17.7	
Negative Control	100	NA		NA		NA	
Cell Control	100	NA		NA		NA	

NA = Not Applicable



A V79 cell suspension, with a concentration of 50 cells/mL, was diluted using V79 Plant Media to a final concentration of 33.3 cells/mL of medium. A volume of 3 mL (~100 cells) of diluted cell suspension was seeded into each well of a six-well plate (well size, 35 mm) and incubated for 24 ± 2 hours at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂. After incubation, the maintenance culture medium was decanted from test culture wells and replaced with 2 mL of each dilution from above. The test article and control extracts and cell control media were added to the culture plates in triplicate wells. The plates were then incubated at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air until adequate cell colony growth was observed (6-7 days).

At the end of the incubation period, the extracts were removed from the plates and the wells were rinsed with 2 mL of Dulbecco's phosphate buffered saline without magnesium or calcium (DPBS). The DPBS was decanted and the colonies were fixed by placing 1 mL of methanol solution into each well for 2-10 minutes. Once the cells were fixed, they were stained for 2-3 hours with 1 mL of Giemsa stain solution that had been diluted 1:20 with PBS (pH 6.4). At the end of the staining period, the stain was decanted and 2 mL of DPBS(-) was added to each well. The colonies were then counted after the removal of DPBS.

EVALUATION CRITERIA

The relative colony formation rates (%) were plotted against the % dilutions of the extract on a semi-logarithmic graph. The resulting inhibition curve of each graph was used to determine the % dilution that inhibited colony formation to 50%. This was the IC₅₀ value. Sigma Stat was used to perform a t-test comparing the cell control results with the average colony count from the negative control.

VALIDITY

Final evaluation of the validity of the assay and test article results was based upon the criteria listed below and scientific judgment.

The t-test results must show that statistically significant inhibition of colony formation was not found in the negative control results.

IC₅₀ values of the positive ZDEC and ZDBC controls must be less than the following values:

Positive ZDEC: 7% and Positive ZDBC: 80%

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS

The colonies were counted and the triplicate wells averaged for each % dilution. These averages were divided by the average number of colonies obtained in the cell control wells. This percentage is the Relative Colony Formation for each % dilution.

STATISTICAL METHODS

A t-test was performed using Sigma Stat and a difference of $p < 0.05$ was considered significant.

RECORD RETENTION

A certified copy of this report and data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

TEST ARTICLE DISPOSITION

Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

Raw data and calculated results, including averages and relative colony formation are presented in Table 5. The test article extract was in contact with the test system for seven days.



A dramatic, dose dependant decrease in relative colony formation was noted as a result of increasing concentrations in the positive control preparations (ZDEC and ZDBC), indicating a sensitive assay (Table 5). Additionally, the relative colony formation (% RCF) in preparations treated with the negative control showed high degrees of survival. ZDEC induced a maximum IC₅₀ of 1.20% while ZDBC returned an IC₅₀ of 61.89%. Both met the criteria for a valid assay. Accordingly, this assay was determined to be valid.

None of the standard dilutions of the test article induced biologically relevant decreases in colony formation. Further, no dose response trends were noted.

TABLE 5: Results

DOSE GROUP	% OF NEAT EXTRACT	WELL 1	WELL 2	WELL 3	AVERAGE	% RCF**	IC ₅₀
Test Article	100	22	35	26	28	82	NA*
	75	25	29	39	31	91	
	50	41	38	42	40	118	
	25	38	42	38	39	115	
	12.5	34	46	35	38	112	
	6.25	36	45	40	40	118	
	3.125	50	51	41	47	138	
ZDEC	2.0	0	0	0	0	0	1.20%
	1.5	0	1	0	0	0	
	1.0	22	23	23	23	68	
	0.75	41	38	32	37	109	
	0.50	51	54	45	50	147	
	0.25	43	44	40	42	124	
	0.125	29	30	41	33	97	
ZDBC	100	0	0	0	0	0	61.89%
	56.2	24	35	30	30	88	
	31.6	31	27	35	31	91	
	17.7	30	42	40	37	109	
Negative Control	100	42	41	33	39	115	NA
Cell Control	100	31	37	35	34	100	NA

* This value could not be calculated as a 50% value was not achieved.

** Relative Colony Formation = Average colony formation divided by average colony formulation of cell control x 100.

ANALYSIS AND CONCLUSION

Negative and positive controls were run concurrently with the test article to provide ranges of cytotoxicity. Each of the positive controls induced relative colony formation well within acceptable limits. No significant differences in colony formation were found between the negative control treated wells and the cell control. The results from the control cultures indicate that the assay retained normal sensitivity. Therefore, the criteria for a valid assay were met.

None of the standard dilutions of the test article induced biologically relevant decreases in colony formation. Further, no dose response trends were noted. Therefore, an IC₅₀ could not be calculated for the test article. These results would be indicative of a **non-cytotoxic** material.

REFERENCES

Guidelines for Basic Biological Tests of Medical Materials and Devices – Part I Cytotoxicity Test (Colony Assay). Japanese Ministry of Health and Welfare, 2012.

ISO 10993-5: 2009 Biological Evaluation of Medical Devices, Part 5: Tests for *In Vitro* Cytotoxicity.

WuXi AppTec SOP: MED-8710, Extract Colony Assay Cytotoxicity Test



FINAL STUDY REPORT

STUDY TITLE: JAPANESE CYTOTOXICITY TEST
COLONY MICROASSAY BY ELUTION
EXPANDED DILUTION SERIES

PROTOCOL NUMBER: C11662-3

TEST ARTICLE IDENTIFICATION: Trade Name: Aleph Plated PTC Clips, Generic
Name: Stainless Steel Clip with Nickel and Tin
Plating, R-spec: R2004-01-G, Nickel Plating,
Tin Plating, Manufactured according to:
PRC057314, Sterilization: 110 kGy Gamma,
2xETO, Reference Number: EE2730
Lot # PRC057314

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

STUDY NUMBER: 171504

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: None of the standard dilutions of the test article
induced biologically relevant decreases in colony
formation. Further, no dose response trends were
noted. Therefore, an IC_{50} could not be calculated for
the test article. These results would be indicative of
a **non-cytotoxic** material.

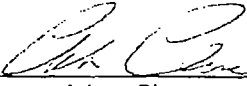


QUALITY ASSURANCE UNIT SUMMARY

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR part 58) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Each inspection was performed to assure the quality and integrity of the study.

<u>Critical Phase</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Dilutions/Plating	06/26/12	06/27/12	07/13/12
Final Report	07/12/12	07/13/12	07/13/12

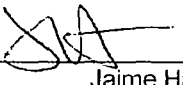
The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor:  Date: 7/13/12
 Adam Choe

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director:  Date: 7-13-12
 Jaime Haueter

Professional Personnel Involved:

Lisa Olson, BS	Vice President, Testing and Service Development
Teri Tanquist, BS	Vice President of Process Improvement and Operations
Jean Kringstad, BS	Director, <i>In Vitro</i> Testing Services
Jaime Haueter, BS	Study Director
Jean Mesarich, AA	Client Relations Manager

NAMSA

REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455**STUDY TITLE**Physicochemical Testing Using an
Alternative Extracting Solvent - Non-Volatile
Residue**TEST ARTICLE NAME**

OnTop Plated PTC Clip

SPONSORBrittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242**TEST ARTICLE IDENTIFICATION**

EE2730

TEST ARTICLE PHYSICAL DESCRIPTION

silver gray U-shaped metal pieces

TEST ARTICLE RECEIVED

June 13, 2012

PURPOSE

The purpose of this study was to describe the physicochemical attributes as part of the overall characterization of the test article.

RESULTS

Assay Results	
Non-Volatile Residue	<1 mg

Condition of Test Article Extract: slightly cloudy

Condition of Control Blank: clear and colorless

Date Extract Prepared: June 15, 2012

Date Test Concluded: June 21, 2012

METHOD

A 10.2 gram portion (249 pieces) of the test article was extracted at 37°C for 72 hours in 51 mL of dichloromethane. Non-volatile residue (NVR) was determined using a 25.0 mL portion of the eluate. NVR results have already been adjusted to compare with other results from similar testing, which are based on a 50.0 mL volume.

COMMENT

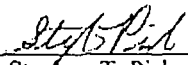
Limits for physicochemical tests using lipophilic solvents have not yet been established. Test articles that are low in lipophilic soluble extractables are generally preferred for use in test articles contacting blood and other tissues.

REFERENCES

United States Pharmacopeia 35, National Formulary 30 (USP), General Chapter <661>, Containers - Plastics (2012).

United States Pharmacopeia 35, National Formulary 30 (USP), General Chapter <731>, Loss on Drying (2012).

APPROVAL


Stephen T. Pisle, B.S.
Technical Support Specialist6-22-12
Date

Results apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.

P.O. No.:
992979234Lab Number:
12T_37839_01

C0066_001/S

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NAMSA**REPORT**

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455**STUDY TITLE**Physicochemical Testing Using an
Alternative Extracting Solvent - Non-Volatile
Residue**SPONSOR**Brittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242**TEST ARTICLE NAME**

Aleph Plated PTC Clip

TEST ARTICLE IDENTIFICATION

EE2730

TEST ARTICLE PHYSICAL DESCRIPTION

silver gray U-shaped metal pieces

TEST ARTICLE RECEIVED

June 13, 2012

PURPOSE

The purpose of this study was to describe the physicochemical attributes as part of the overall characterization of the test article.

RESULTS

Assay Results	
Non-Volatile Residue	<1 mg

Condition of Test Article Extract: slightly cloudy

Condition of Control Blank: clear and colorless

Date Extract Prepared: June 15, 2012

Date Test Concluded: June 21, 2012

METHOD

A 10.2 gram portion (251 pieces) of the test article was extracted at 37°C for 72 hours in 51 mL of dichloromethane. Non-volatile residue (NVR) was determined using a 25.0 mL portion of the eluate. NVR results have already been adjusted to compare with other results from similar testing, which are based on a 50.0 mL volume.

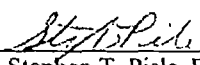
COMMENT

Limits for physicochemical tests using lipophilic solvents have not yet been established. Test articles that are low in lipophilic soluble extractables are generally preferred for use in test articles contacting blood and other tissues.

REFERENCES

United States Pharmacopeia 35, National Formulary 30 (USP), General Chapter <661>, Containers - Plastics (2012).

United States Pharmacopeia 35, National Formulary 30 (USP), General Chapter <731>, Loss on Drying (2012).

APPROVAL
Stephen T. Pisle, B.S.
Technical Support Specialist6-22-12
Date

Results apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.

P.O. No.:
992979234Lab Number:
12T_37838_02

C0066_001/S

Page 1 of 1

NAMSA

REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455**STUDY TITLE**Determination of Extractable Species From a
Test Article by Infrared Spectroscopy**TEST ARTICLE NAME**

Aleph Plated PTC Clip

TEST ARTICLE IDENTIFICATION

EE2730

TEST ARTICLE PHYSICAL DESCRIPTION

silver gray U-shaped metal pieces

TEST ARTICLE RECEIVED

June 13, 2012

SPONSORBrittany Olson
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242**PURPOSE**

The purpose of this study was to prepare an infrared spectrum of a material for the purpose of identifying the material or examining it for impurities.

SUMMARY/RESULTS

The test article was characterized for extractable species as required by ISO 10993-18, Biological evaluation of medical devices - Part 18: Chemical characterization of materials by first extracting the test article in dichloromethane. The resulting test extract was then evaporated to a residue and then the residue was analyzed by infrared spectroscopy to identify the species present in the test extract.

There were no major bands of interest detected in the test article residue. The lack of species present as represented by the infrared spectrum, can be used to aid in the assessment of the test article by ISO 10993, Part 18.

The test article spectrum is located in Appendix 1.

Date Test Concluded: June 27, 2012

METHOD

A 10.2 gram portion (251 pieces) of the test article was extracted at 37°C for 72 hours in 51 mL of dichloromethane. The method and results of this extraction were reported under Lab Number 12T_37838_02. An approximate 1-2 mL portion of the dichloromethane test extract was transferred to a clean vial and placed in a 105°C drying oven to evaporate the extracting solvent. A small amount of chloroform was added to the vial to dissolve the residue. The resulting solution was placed on a clean potassium bromide crystal and the chloroform was allowed to evaporate. The residue was analyzed by transmission infrared spectroscopy using the instrument conditions listed below.

Instrument	Fourier Transform Infrared Spectrophotometer
Wavenumber Range—Collected	4400 cm ⁻¹ – 350 cm ⁻¹
Scan number	8
Resolution	4 cm ⁻¹

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REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

REFERENCES

European Pharmacopoeia (Ph. Eur.) 7th Edition, Section 2.2.24. Absorption Spectrophotometry, Infrared (2011).

International Organization for Standardization (ISO) 10993-18, Biological evaluation of medical devices - Part 18: Chemical characterization of materials (2005).

United States Pharmacopeia 35, National Formulary 30 (USP), General Chapter <197>, Spectrophotometric Identification Tests, Infrared Absorption (2012).

APPROVAL


David K. Shaffer, B.S.
Manager, Chemistry7-2-12
Date

Results apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.

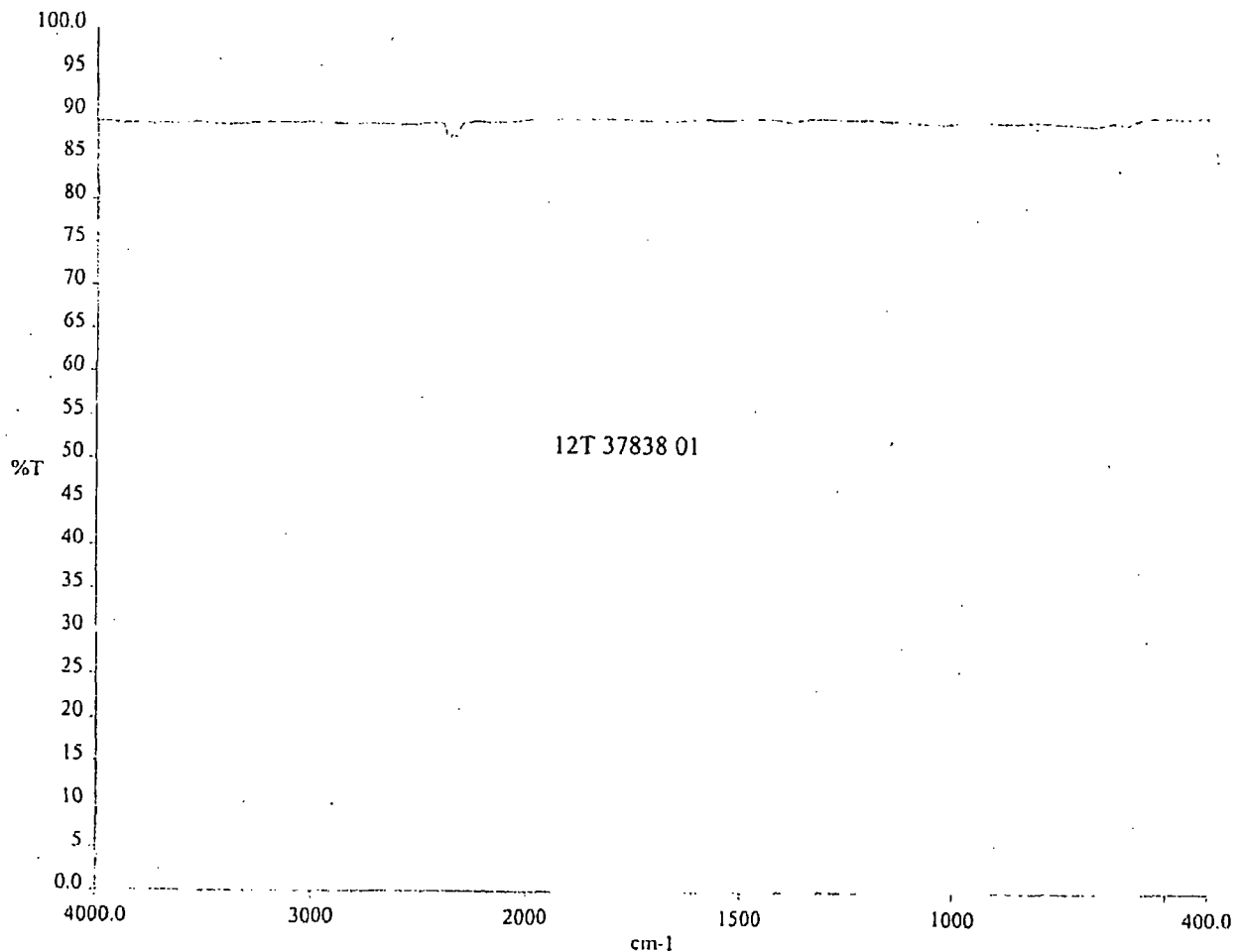
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Test Facility
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419.666.9455

APPENDIX 1



P.O. No.:
992979234

Lab Number:
12T_37838_01

C0022_100

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NV Report Number X4G247G

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Table 8
Test Scores After Challenge Phase
Test Group (Extraction in SCI)

Animal Number	24 Hour Score	48 Hour Score
21631	0	0
21636	0	0
21657	0	0
21666	0	0
21750	0	0
21752	0	0
21773	0	0
21760	1	0
21764	0	0
21765	0	0

Table 9
Test Scores After Challenge Phase
Negative Control Group (Extraction in SCI)

Animal Number	24 Hour Score	48 Hour Score
21661	0	0
21663	0	0
21755	0	0
21766	0	0
21768	0	0



NV Report Number X4G247G

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Table 10
Test Scores After Challenge Phase
Positive Control Group (0.05% DNCB in ETOH/H₂O, 1:9 v/v)

Animal Number	24 Hour Score	48 Hour Score
Positive Control Group		
20417	2	2
20419	2	3
20424	3	3
20444	3	3
20654	2	3
Naive Positive Control Group		
20421	1	1
20422	1	0
20431	0	0
20649	1	1
20653	1	0

Table 11
Test Scores After Challenge Phase
Test Group (Extraction in CSO)

Animal Number	24 Hour Score	48 Hour Score
21624	0	0
21630	0	0
21633	0	0
21749	1	1
21751	0	0
21753	1	1
21757	0	1
21759	1	0
21761	0	0
21762	0	0



Table 12
Test Scores After Challenge Phase
Negative Control Group (CS0 Blank)

Animal Number	24 Hour Score	48 Hour Score
21747	0	0
21748	0	0
21754	0	0
21763	0	0
21771	NA ¹	NA ¹

1. Animal died prior to challenge dosing.

Table 13
Test Scores After Challenge Phase
Positive Control Group (0.05% DNCB in ETOH/CS0, 1:9 v/v)

Animal Number	24 Hour Score	48 Hour Score
Positive Control Group		
18850	3	3
18854	3	3
18878	2	2
18882	3	2
18900	3	2
Naive Positive Control Group		
19111	1	0
19112	1	0
19113	1	0
19114	1	1
19115	0	0



NV Report Number X4G247G

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Table 14
Animal Weights
Test Group (SCI)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
21631	M	313.2	443.9
21636	M	331.6	446.5
21657	M	338.8	452.7
21666	M	331.4	461.4
21750	M	324.8	437.9
21752	M	324.0	441.4
21773	M	308.7	463.6
21760	M	303.4	407.2
21764	M	314.9	468.6
21765	M	332.0	478.4

Table 15
Animal Weights
Positive Control Group (SCI)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
20417	M	469.4	617.0
20419	M	457.0	510.0
20424	M	497.4	613.4
20444	M	490.1	652.3
20654	M	334.1	511.9

Table 16
Animal Weights
Naive Positive Control Group For Challenge Phase (SCI)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
20421	M	505.0	607.6
20422	M	475.4	601.7
20431	M	494.0	638.9
20649	M	314.0	508.5
20653	M	340.0	526.9



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Table 17
Animal Weights
Negative Control Group (SCI)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
21661	M	317.5	411.1
21663	M	338.8	473.9
21755	M	328.7	440.6
21766	M	302.5	406.6
21768	M	327.5	427.6

Table 18
Animal Weights
Test Group (CSO Extraction)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
21624	M	326.8	466.5
21630	M	323.5	449.9
21633	M	339.7	425.9
21749	M	315.8	488.2
21751	M	306.8	468.5
21753	M	309.0	429.6
21757	M	323.1	489.0
21759	M	323.4	479.7
21761	M	326.2	425.0
21762	M	320.2	457.8



NV Report Number: X4G247G

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Table 19
Animal Weights
Positive Control Group (CSO Extraction)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
18850	M	382.6	603.9
18854	M	391.0	621.0
18878	M	369.6	608.2
18882	M	395.4	633.2
18900	M	355.7	555.1

Table 20
Animal Weights
Naive Positive Control Group (CSO Extraction)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
19111	M	378.5	439.2
19112	M	364.5	432.9
19113	M	393.0	492.0
19114	M	356.0	414.1
19115	M	378.9	443.0



Table 21
Animal Weights
Negative Control Group (CSO Extraction)

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
21747	M	315.4	474.1
21748	M	332.6	424.8
21754	M	319.9	351.4
21763	M	318.8	492.2
21771	M	326.5	Found Dead

REFERENCES

1. Northview SOP No. 16G-13
2. The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test. Magnusson B. and Kilgman, A.M., 1969, J. Invest. Dermatol. 52:268-276
3. Standard Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test. ASTM Standard F720-81 (Reapproved 1986)
4. Guinea Pig Maximization Test. Wahlberg, J.E. and Boman, A., 1985, Curr. Probl. Dermatol. 14: 59-106



NV Report Number X4G247G

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**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

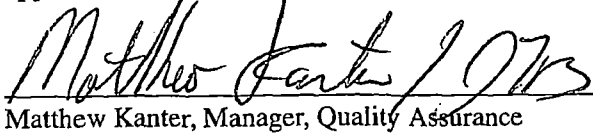
Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number X4G247G were conducted by the Quality Assurance Unit on the following dates:

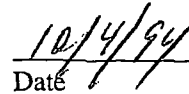
NV SOP No.	Phase of Study	Date
16G-13	Challenge - Dosing	09/15/94

QAU inspection findings are routinely reviewed by the management of Northview Pacific Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number X4G247G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.


Matthew Kanter, Manager, Quality Assurance


Date



NORTHVIEW PACIFIC LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Timothy V. Doherty, D.V.M., Director *In Vivo* Laboratory Services
2. Roger O'Meara, Assistant Manager, *In Vivo* Laboratory Services
3. Robert Noonan, Ph.D., Senior Scientist, *In Vivo* Laboratory Services
4. Benjamin Davis, Technician, *In Vivo* Laboratory Services
5. Lisa Gearin, Technician, *In Vivo* Laboratory Services
6. Robert Mosbauer, Technician, *In Vivo* Laboratory Services
7. Edwin T. Pangilinan, M.S., Technician, *In Vivo* Laboratory Services
8. James Ruiz, Technician, *In Vivo* Laboratory Services
9. Patrice Pitot, Technician, *In Vivo* Laboratory Services
10. Heather McCracken, Technician, *In Vivo* Laboratory Services

STATEMENT OF COMPLIANCE

This study, Northview Report Number X4G247G, has been conducted in accordance with applicable Good Laboratory Practice regulations.


Study Director

10/4/94
Date


Quality Assurance Unit

10/4/94
Date

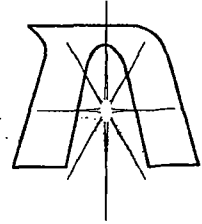

Management

10-4-94
Date



Northview Pacific Laboratories, Inc.

2800 Seventh Street ✧ Berkeley, CA 94710 ✧ 510/548-8440 ✧ Fax 510/548-5425



SPONSOR

Mary Reinhard, D.V.M.
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

NV REPORT NUMBER

X4G247G

STUDY COMPLETION DATE

October 3, 1994

TEST ARTICLE IDENTIFICATION

2061-15 Black (FC770001) &
2061-15 Charcoal Grey (FC780546) with
White Trans Tech Ink-PG2NT
Lot Number: IB1201QCN3 & IC0201QCM1

TEST PERFORMED

Maximization Test - Magnussen Kligman

STUDY DIRECTOR

Robert A. Noonan, Ph.D.
Senior Scientist
In Vivo Laboratory Services
Northview Pacific Laboratories, Inc.



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STUDY DATES

Study Initiation Date: August 10, 1994
Study Completion Date: October 3, 1994
Date On Test: August 25, 1994
Date Off Test: September 19, 1994
Study Authorization: Signed Protocol
Date Sample Received: July 27, 1994
Purchase Order: A2442

TEST ARTICLE IDENTIFICATION

2061-15 Black (FC770001) & 2061-15 Charcoal Grey (FC780546) with White Trans Tech Ink-PG2NT
50 black plastic squares in a sealed bag, each 2.5 cm x 2.5 cm with a white stripe across the middle
Code Identification: RDM #13-2-0060-00534
Lot Numbers: IB1201QCN3 & IC0201QCM1
Storage Condition: Room Temperature
Sterilization Data: Cobalt & ETO, 6/30/94 & 7/6/94
Stability Data: In Progress

CONTROL ARTICLE IDENTIFICATION

1-Chloro-2,4-Dinitrobenzene
Amber glass jar containing 100 g of yellow granular powder
Lot Number: 100H0738 - for CSO Positive Control, Exp. Date: 01/01/95
Lot Number: 41H08112 - for SCI Positive Control, Exp. Date: 02/24/97

PROTOCOL

This test was conducted according to Protocol Number X4G247G, which incorporates by reference Northview SOP No. 16G-13 and is on file at Northview Pacific Laboratories, Inc. This protocol satisfies the requirements of Ethicon Endo-Surgery.

DEVIATIONS FROM PROTOCOL

1. On Day 15, one negative control animal, #21771 was found dead in its cage. No necropsy was done. The protocol specified that all animals found dead during the study need to be necropsied.

The animal was a negative control and was not exposed to the test material. Even though the cause of death of this animal was not determined, the other animals in the test and negative control groups remained healthy for the duration of the study. The loss of this animal did not affect the final conclusion.

2. A naive positive control animal (#20421) had an initial weight of 505 grams. The protocol specifies use of animals weighing from 300 to 500 grams.

One animal, slightly heavier than the specified range, was used due to an insufficient number of animals available in the correct weight range. The animal was from the same batch as the SCI and positive control groups and was the same age. Therefore, it was equivalent.

In the judgement of the study director, these deviations did not affect the integrity of the study.



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GUINEA PIG MAXIMIZATION TEST

INTRODUCTION

This test is designed to determine to what extent a test material has the potential for acting as a contact sensitizer in guinea pigs.

Fifty Hartley albino guinea pigs were used: including ten test group animals and five negative controls for the 0.9% Sodium Chloride for Injection (SCI) extraction; and ten test group animals and five negative controls for the cottonseed oil (CSO) extraction. The results were compared to positive control groups: these included five positive controls and five naive positive controls prepared in deionized water, in addition to five positive controls and five naive positive controls prepared in cottonseed oil. The positive control tests were done as part of NV Study No. HSTPOSG, a historical study run in January 1994 (CSO controls) and June 1994 (SCI controls).

In this test, an extract of the test material was employed. As a negative control a vehicle blank was incubated under the same conditions as the test material. As a positive control a solution of 1-Chloro-2, 4-Dinitrobenzene (DNCB) was employed.

Groups of guinea pigs were exposed to the test material extracts and the control solutions in an induction phase lasting nine days. In this phase, the materials were first injected intradermally (ID). For the ID injections the solutions were given undiluted as well as emulsified in Freund's Complete Adjuvant (FCA). Seven days after the ID injections, the extracts, vehicle blank and positive control solution were mixed with petrolatum (1:20 w/w) and administered topically over the same area in which the ID injections were given. The topical exposure lasted 48 hours.

Fourteen days after the topical administration, the animals were challenged. For the challenge phase, extracts and control solutions were administered on Hill Top Chambers, which were applied to untreated sites, and remained on the animals for 24 hours. The dosing sites were scored for erythema and edema 24 and 48 hours after removal of the chambers.

Based on the erythema and edema scores observed, the test material was classified as to its allergic potential.

MATERIAL AND METHODS

Test System

Species	Guinea Pig
Strain	Hartley, Albino
Source	Charles River Laboratories, Portage, MI
Age	Young adult
Initial Weight	300 to 500 grams
Housing	Individually; 18 - 26°C, 55 ± 15% relative humidity
Feed	Purina Guinea Pig Chow and water supplied in bottles <i>ad libitum</i>
Photoperiod	Diurnal (12 hours on - 12 hours off)
Quarantine Period	Seven days



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Justification For Test System

Guinea pigs are the preferred species for skin sensitization tests. They are required for the maximization test (see Magnusson and Kligman, *J. Invest. Dermatol.* 52:268. [1969]). The Hartley albino guinea pig is specified for use in the maximization test by ASTM Standard F720-81.

Table 1
Supplies

Reagents/Equipment		Lot Number	Manufacturer	Expiration Date
Sodium Chloride for Injection, USP (SCI)	For Induction For Challenge	J4A410 J4D629	McGaw McGaw	1/1/96 4/1/96
Cottonseed Oil (CSO)		IA284	Spectrum	7/1/98
Freund's Complete Adjuvant (FCA)		063H-8800	Sigma	6/29/98
Petrolatum		115699/42086	VWR	8/5/98
Ethanol		93H06	McCormick Dist Co.	7/7/98
1-chloro-2, 4-dinitrobenzene (DNCB)		100H0738 ¹ 41H08112 ²	Sigma Sigma	1/1/95 2/24/97
10% Sodium Lauryl Sulfate (SLS)		83H0841	Sigma	2/24/98
Sterile Water for Injection (WFI)		C249029	Baxter	1/1/95
Hill Top Chambers™		06MBA-2002	Hill Top Research	N/A
Whatman #3 Filter Paper		N/A	Whatman Lab. Div.	N/A
Blenderm™ Surgical Tape		Jun92-432	3M	N/A
Gauze - Sof Kling Conforming Bandage		1994-02	Johnson & Johnson	N/A
Anchor™ Masking Tape (½ in)		N/A	Anchor Continental, Inc.	N/A
Dental Dam™ elastic bandage		11994	Hygenic Corp.	N/A

1. For CSO Positive Controls
2. For SCI Positive Controls



Table 2
Study Design

Group	Number of Animals	INDUCTION PHASE		CHALLENGE PHASE
		1st Induction (Day 0) Route	2nd Induction (Day 7) Route	(Day 21) Route
SCI Test	10	Intradermal	Topical	Topical
CSO Test	10	Intradermal	Topical	Topical
SCI Positive Control	5	Intradermal	Topical	Topical
CSO Positive Control	5	Intradermal	Topical	Topical
SCI Naive Positive Control (for challenge)	5	N/A	N/A	Topical
CSO Naive Positive Control (for challenge)	5	N/A	N/A	Topical
SCI Negative Control	5	Intradermal	Topical	Topical
CSO Negative Control	5	Intradermal	Topical	Topical

SAMPLE PREPARATION AND DOSING PROCEDURE

Sample Extraction - The test material was extracted in two vehicles, SCI and CSO. For each extraction, four and four-fifths pieces (each one inch square), with a combined surface area of 60 cm², were cut up and placed in a dry, sterile, glass, screw capped tube. The tube was filled with 20 mL of extraction vehicle. Additional sterile tubes were filled with each vehicle to serve as blanks. The extracts and blanks were incubated for 72 hours at 50°C.

Animal Assignment - The animals were divided into eight groups; ten SCI test animals, ten CSO test animals, five SCI positive controls, five CSO positive controls, five naive SCI positive controls, five naive CSO positive controls, five SCI negative controls and five CSO negative controls.

Induction Phase - Intradermal Injection - The induction phase consisted of an intradermal injection of the test material followed a week later by a topical application. At least 24 hours prior to each application, the hair on the dorsocranial thorax was removed by clipping. On the day of dosing, at least one hour prior to dosing, the clipped areas were shaved.

The procedure for intradermal injections of test material extracted in SCI is illustrated in Table 3.

Three pairs of 0.1 ml intradermal injections were administered to the shaved areas on the test animals. The first pair of injections (cranial) consisted of an emulsion of FCA in an equal volume of WFI. The second pair (middle) was the test material extract. The third pair (caudal) consisted of an emulsion of the test material extract in an equal volume of FCA.

The five positive control animals were treated in a similar manner to the test animals, except that the test material extract was replaced in the second and third pair of injections with an equal amount of a 0.1% solution of a known sensitizer, 1-chloro-2,4-dinitrobenzene (DNCB) in 9.5% ethanol.



The five negative control animals were treated in a manner similar to the test animals, except that the test material extract was replaced in the second and third pair of injections with an equal amount of vehicle extract (blank).

Table 3
Induction Phase - Intradermal Injections (For SCI vehicle extraction)

Site Location	No. of Sites	TEST GROUP		POSITIVE CONTROL		NEGATIVE CONTROL	
		Volume/ Site	Dose Material	Volume/ Site	Dose Material	Volume/ Site	Dose Material
Cranial	2	0.1 ml	1:1 (FCA:WFI)	0.1 ml	1:1 (FCA:WFI)	0.1 ml	1:1 (FCA:WFI)
Middle	2	0.1 ml	Test Extract	0.1 ml	DNCB	0.1 ml	Blank
Caudal	2	0.1 ml	1:1 (FCA:Test Extract)	0.1 ml	1:1 (FCA:DNCB)	0.1 ml	1:1 (FCA:Blank)

The procedure for intradermal injections of test material extracted in CSO is illustrated in Table 4. Three pairs of 0.1 mL intradermal injections were administered to the shaved area of test animals, the first pair of injections (cranial) consisted of an emulsion of FCA in CSO. The second pair of injections (center) was of a CSO test material extract in an equal volume of WFI. The third pair of injections (caudal) consisted of an emulsion of CSO test material extract in an equal volume of FCA.

The five positive control animals were treated in a similar manner to the test animals, except that the test material extract was replaced in the second and third pair of injections with an equal amount of a 0.1% solution of a known sensitizer, 1-chloro-2,4-dinitrobenzene (DNCB) in ETOH/CSO (1:9 v/v).

The five negative control animals were treated in a similar manner to the test animals, except that the test material extract was replaced in the second and third pair of injections with an equal amount of vehicle control extract.



Table 4
Induction Phase - Intradermal Injections (For CSO vehicle extraction)

Site Location	No. of Sites	TEST GROUP		POSITIVE CONTROL		NEGATIVE CONTROL	
		Volume/ Site	Dose Material	Volume/ Site	Dose Material	Volume/ Site	Dose Material
Cranial	2	0.1 ml	1:1 (FCA:CSO)	0.1 ml	1:1 (FCA:CSO)	0.1 ml	1:1 (FCA:CSO)
Middle	2	0.1 ml	1:1 (Test Extract:WFI)	0.1 ml	1:1 (DNCB:WFI)	0.1 ml	1:1 (Blank:WFI)
Caudal	2	0.1 ml	1:1 (FCA:Test Extract)	0.1 ml	1:1 (FCA:DNCB)	0.1 ml	1:1 (FCA:Blank)

Induction Phase - Topical Dosing - Six days after performing the intradermal injections, the previously treated areas were clipped and shaved. The test sites were evaluated for evidence of irritation. Since the test material did not cause irritation, the dosing sites on the test and negative control group animals were pretreated with 10% SLS in petrolatum. This SLS mixture was massaged into the skin using a glass rod. Twenty-four hours later (seven days after the intradermal injections) the animals were reshaved.

For the topical application phase of the induction, 1.0 g of an extract of the test material, prepared in the same manner as for the intradermal injections, was mixed with 19.0 g of petrolatum (1:19 by weight).

The dosing patches were prepared by spreading a thick even layer of an extract of the test material, mixed with petrolatum (1:19 by weight) on 4.25 cm diameter disks of Whatman #3 filter paper. These were applied to the shaved areas on the test animals and held in place with surgical tape. The trunks of the animals were wrapped with gauze, which was held in place with ½ inch masking tape. They were then wrapped with an elastic bandage so that complete occlusion was obtained.

The positive and negative control animals were treated in a similar manner to the test group animals except that their patches contained 1:19 mixtures of 0.1% DNCB in petrolatum and a vehicle extract (blank) in petrolatum, respectively.

After forty-eight hours, the animals were unwrapped and the patches removed.



Table 5
Challenge Phase

Group	No. of Sites	Volume /Site	Material
SCI Test Extract	1	0.4 mL	Test Extract
SCI Positive Control	1	0.4 mL	0.025% DNCB
SCI Naive Positive Control	1	0.4 mL	0.025% DNCB
SCI Negative Control	1	0.4 mL	Blank Extract
CSO Test Extract	1	0.4 mL	Test Extract
CSO Positive Control	1	0.4 mL	0.05% DNCB
CSO Naive Positive Control	1	0.4 mL	0.05% DNCB
CSO Negative Control	1	0.4 mL	Blank Extract

Challenge Phase - The challenge phase dosing procedure is summarized in Table 5. Thirteen days after the topical induction application, a 5 x 5 cm area on the right side of each animal was clipped. On the next day (fourteen days after the topical application), the animals were shaved and Hill Top Chambers™ containing 0.4 mL volumes of the test extract, vehicle control extracts, or the positive control solutions were applied to the shaved areas. For the challenge phase a positive control solution consisting of either 0.025% DNCB in 9.5% aqueous ethanol, or 0.05% DNCB in CSO/ethanol (9:1 v/v) was used. Two groups of five previously untreated animals were dosed with the positive control solution, in the two vehicles, to serve as naive positive controls.

The animals were wrapped, as described for the topical application, so that complete occlusion was obtained. The patches remained in place for 24 hours. Then, the animals were unwrapped and the dosing sites for the positive controls, naive positive controls, and the CSO test group and its control were gently cleansed with 70% aqueous ethanol to remove chemical residues. The dosing sites for the SCI test group and the negative control group were allowed to air dry.

OBSERVATION AND SCORING

Clinical Observations - All of the animals were observed for toxic signs immediately after dosing and daily until the end of the study.

Weights - All of the animals were weighed prior to the initiation and at the end of the study.

Scoring - Skin responses at the challenge sites were scored 24 and 48 hours after removal of the patches according to the criteria in Table 6.



INTERPRETATION AND ANALYSIS

Scores of 2 or greater are considered significant. Test group animals with scores of 2 or greater in either the 24 or 48 hour observation period may be considered sensitized. Magnusson and Kligman do not regard a score of 1 as representing significant sensitization. However, the allergic potential of the substance is considered proven only when the skin reactions to the test material clearly outweigh those of the negative control group to the vehicle blank.

Based upon the number of animals sensitized, a rating of the material's potential for sensitization is given according to the grading scale shown in Table 7.

RESULTS AND DISCUSSION

Clinical Observations - On Day 15, one CSO negative control animal, #21771, was found dead in its cage. No necropsy was done. The cause of death of this animal was not determined. (See Deviation #1).

The remaining test and control animals appeared healthy. No other toxic signs were observed over the duration of the study.

Scoring Results - The 24 and 48 hour scores for the primary challenge are shown in Tables 8 - 13.

SCI Test Group - At the 24 hour observation, one of the ten test group animals exhibited scattered mild redness (score of 1). At the 48 hour observation, there was no response. (See Table 8.)

SCI Negative Control Group - At both the 24 and 48 hour observations, no responses were exhibited. (See Table 9.)

SCI Positive Control Group - At the 24 hour observations, two of five animals exhibited intense redness and swelling (score of 3). Three exhibited moderate redness (score of 2). At the 48 hour observation, four exhibited scores of 3, and one, a score of 2. (See Table 10.)

SCI Naive Positive Control Group - At the 24 hour observation four of five animals exhibited a score of 1. At the 48 hour observation, two exhibited scores of 1. (See Table 10.)

CSO Test Group - At the 24 hour observation, three of ten animals exhibited scores of 1. Three exhibited scores of 1 at the 48 hour observation. (See Table 11.)

CSO Negative Control Group - At both the 24 and 48 hour observations, none of the four animals exhibited responses. (See Table 12.)

CSO Positive Control Group - At the 24 hour observation, four of five animals exhibited scores of 3. One exhibited a score of 2. At the 48 hour observation, two exhibited scores of 3, and three, scores of 2. (See Table 13.)

CSO Naive Positive Control Group - At the 24 hour observation, four of five animals exhibited scores of 1. One exhibited a score of 1 at the 48 hour observation. (See Table 13.)

Weights - The individual pre-test and post-test body weights are shown in Tables 14 - 21. All animals on this study exhibited weight gain over the course of the study.



CONCLUSION

None of the test group or negative control animals exhibited scores higher than 1. A response of 1 is not considered to reflect significant sensitization. According to the criteria for this test, the test material had a weak (Grade I) potential for sensitization. (See Table 7.)

Under similar treatment conditions, all positive control animals, for both extraction conditions, exhibited sensitization responses to the challenge dose, while none of the naive positive controls exhibited scores higher than 1. This establishes that the treatment conditions were able to induce a response from a known sensitizer.

Table 6
Scoring Key

Reaction	Score
No Reaction	0
Scattered Mild Redness	1
Moderate and Diffuse Redness	2
Intense Redness and Swelling	3

Table 7
Classification of Potential for Sensitization

% Sensitized	Grade	Classification
0 - 8	I	Weak
9 - 28	II	Mild
29 - 64	III	Moderate
65 - 80	IV	Strong
81 - 100	V	Extreme



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Table 11
Skin Reaction Scores
1 In 20 Solution Of Alcohol In SCI Extract And Blank

Rabbit # 11296 F		Blank Sites					Sample Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	1	0	0	0	0	0	1	0	1	0.2	0.4
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
<hr/>													
Rabbit # 11298 M		Blank Sites					Sample Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
Overall Mean:												0	0

Table 12
Skin Reaction Scores
Polyethylene Glycol 400 Extract And Blank

Rabbit # 11296 F		Blank Sites					Sample Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	0	0	0	0	1	0	0	0	0	0	0.2
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0

Rabbit # 11298 F		Blank Sites					Sample Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema	0	0	0	0	0	1	0	0	0	0	0	0.2
	Edema	0	1	0	1	0	0	0	0	0	0	0.4	0
48 hr	Erythema	0	1	0	0	0	0	0	0	0	0	0.2	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
Overall Mean:												0.1	0

REFERENCES

NV SOP 16E-05

USP XXII [1990], p. 1499

USP XXII Supplement 5 [Nov. 15, 1991], p. 2704-2705



USP IMPLANTATION TEST

INTRODUCTION

This study is designed to evaluate the potential for local irritancy of plastic materials and other polymeric materials in direct contact with living tissue.

The test material is implanted into the paravertebral muscles of two rabbits. At the end of the implant period, the implant sites are harvested and evaluated for evidence of hemorrhage, necrosis, discoloration, infection and encapsulation.

MATERIALS AND METHODS

Test System

Species	Rabbit
Strain	New Zealand White
Source	Elkhorn Rabbitry, Watsonville, CA
Number	Two
Initial Weight	At least 2.5 kg
Sex	Female
Age	Adult
Housing	Individually at 17 - 21C and 55 ± 15% relative humidity
Feed and Water	Purina Rabbit Chow (approximately 200 grams per day) and water <i>ad libitum</i>
Identification	Ear tags
Photoperiod	Diurnal (12 hours on - 12 hours off)
Quarantine Period	Seven days

Justification For Test System

The rabbit is the species required by the current version of the United States Pharmacopoeia.

**Table 13
Supplies**

Reagents/Equipment	Lot Number	Manufacturer	Expiration Date
Ketamine hydrochloride	440190	Aveco Co.	1/96
Sodium pentobarbital	F4A006	Anpro	1/96
Beuthanasia D solution	J3RR02	Schering-Plough	8/95
USP Negative Control Plastic RS	Batch G-7	USPC, Inc.	N/A
Hypodermic Needle (15 gauge)	231735	Monoject	N/A

Sample Preparation And Implant Procedure

Sample Preparation - Test samples were provided by the sponsor pre-cut into 1 x 10 mm strips. Control samples were cut into strips measuring not less than 1 x 10 mm.

Equipment - All materials and implements used on this study were sterilized prior to use or purchased sterile. Aseptic techniques were followed.

Implant Procedures - Prior to testing, the animal's fur on both sides of the spinal column was removed with electric clippers. Each animal was then anesthetized with ketamine hydrochloride (45 mg/kg of body weight) and sodium pentobarbital (15 mg/kg of body weight).

Four test strips were aseptically implanted into the paravertebral muscle with a sterile hypodermic needle fitted with a sterile trocar stylet. The test strips were implanted 2.5 to 5 cm from the midline, parallel to the spinal column and about 2.5 cm apart from each other.

The two control strips were implanted in the same way on the other side of the spinal column.

The animals were observed until they had recovered from the effects of the anesthesia, and then were gently placed back in their cages.

Observation And Scoring

Clinical Observations - Once daily, all animals were observed for mortality, signs of ill health, and at the implant sites for reaction to treatment (e.g., infection, etc.).

Euthanization - On Day 7 of the study, the animals were euthanized with a lethal injection of Beuthanasia D solution.

Postmortem - Samples (test and control) and their surrounding tissue were recovered and examined for gross evidence of hemorrhage, necrosis, discoloration, infection and encapsulation.

Encapsulation was scored according to Table 14. If present, encapsulation was measured by recording the width of the capsule (from the periphery of the space occupied by the implant to the periphery of the capsule) rounded to the nearest 0.1 mm.

At the request of the sponsor, histopathology evaluation was performed at California Veterinary Diagnostics, Sacramento, CA.

Interpretation And Analysis

The average mean scores for encapsulation for both the sample and control sites are calculated. The test material meets the USP requirements if the difference between these scores does not exceed 1.0, or if the difference between the sample and control mean scores for more than one of the four implant sites does not exceed 1 for any implanted animal.

RESULTS AND DISCUSSION

Clinical Observations - All animals remained healthy throughout the study period. No evidence of infection was found at the incision sites.

Macroscopic Examination - No encapsulation was observed at the test or control sites upon sample recovery. All implantation sites were recovered from inside the muscle tissue.

Histopathology Evaluation - There was mild irritation observed around the test sites compared to the control sites (see appendix).

CONCLUSION

This test material met the requirements of the USP Implantation Test when implanted for 7 Days.

Table 14
Evaluation of Encapsulation

Capsule Width	Score
None	0
up to 0.5 mm	1
0.6 - 1.0 mm	2
1.1 - 2.0 mm	3
> 2.0 mm	4



Table 15
Implantation Results

Rabbit Number	Sex	Sites	Encapsulation Score	Gross Examination
11225	F	T1	0	No reactions observed
		T2	0	No reactions observed
		T3	0	No reactions observed
		T4	0	No reactions observed
		Sample Mean:	0	
		C1	0	No reactions observed
		C2	0	No reactions observed
		Control Mean:	0	
11226	F	T1	0	No reactions observed
		T2	0	No reactions observed
		T3	0	No reactions observed
		T4	0	No reactions observed
		Sample Mean:	0	
		C1	0	No reactions observed
		C2	0	No reactions observed
		Control Mean:	0	

T = Test, C = Control

REFERENCES

NV SOP 16E-06

USP XXII [1990], p. 1499

USP XXII Supplement 5 [Nov. 15, 1991], p. 2705

CVD Accession No. X4004797

**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

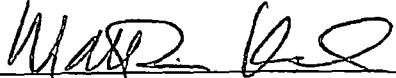
Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number X4G248G were conducted by the Quality Assurance Unit on the following dates:

NV SOP No.	Phase of Study	Date
16E-03	72 Hour Observation/Weighing	08/26/94
16E-05	48 Hour Scoring	08/26/94
16E-06	Day Six Health Observation	08/11/94

QAU inspection findings are routinely reviewed by the management of Northview Pacific Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number X4G248G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.


Matthew Kanter, Quality Assurance Unit

9/12/94
Date

NORTHVIEW PACIFIC LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Timothy V. Doherty, D.V.M., Director *In Vivo* Laboratory Services
2. Roger O'Meara, Assistant Manager, *In Vivo* Laboratory Services
3. Robert Noonan, Ph.D., Senior Scientist, *In Vivo* Laboratory Services
4. Benjamin Davis, Technician, *In Vivo* Laboratory Services
5. Lisa Gearin, Technician, *In Vivo* Laboratory Services
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8. James Ruiz, Technician, *In Vivo* Laboratory Services
9. Patrice Pitot, Technician, *In Vivo* Laboratory Services
10. Heather McCracken, Technician, *In Vivo* Laboratory Services

STATEMENT OF COMPLIANCE

This study, Northview Report Number X4G248G, has been conducted in accordance with applicable Good Laboratory Practice regulations.

Edwin T. Pangilinan
Study Director

9-7-94
Date

Mark Q. Q.
Quality Assurance Unit

9/12/94
Date

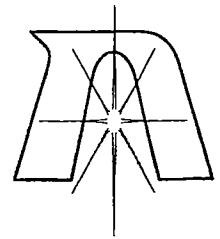
Timothy Doherty
Management

9/13/94
Date

:dc

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SPONSOR

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NV REPORT NUMBER

X4G246G

STUDY COMPLETION DATE

August 19, 1994

TEST ARTICLE IDENTIFICATION

2061-15 Black (FC770001) & 2061-15 Charcoal Grey
(FC780546) with White Trans Tech Ink-PG2NT

Code Identification: RDM #13-2-0060-00534

Lot Number: IB11201QCN3 & IC0201QCM1

Sterilization Data: Cobalt & ETO 6/30/94 & 7/6/94

TEST PERFORMED

USP Rabbit Pyrogen Test

STUDY DIRECTOR

Robert Mosbauer
Laboratory Technician
In Vivo Laboratory Services
Northview Pacific Laboratories, Inc.



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STUDY DATES

Study Initiation Date: August 2, 1994
Study Completion Date: August 19, 1994
Date On Test: August 10, 1994
Date Off Test: August 10, 1994
Study Authorization: Signed Protocol
Date Sample Received: July 28, 1994
Purchase Order: A2442

TEST ARTICLE IDENTIFICATION

2061-15 Black (FC770001) & 2061-15 Charcoal Grey (FC780546) with White Trans Tech Ink-PG2NT
Black with white strip cylinders 2.5 cm long 0.5 cm in diameter in one plastic pouch containing 50 cylinders.

Lot Number: IB1201QCN3 & IC0201QCM1
Code Identification: RDM #13-2-0060-00534
Sterilization Data: Cobalt & ETO 6/30/94 and 7/6/94
Storage Condition: Room Temperature

PROTOCOL

This test was conducted according to Protocol Number X4G246G which incorporates by reference Northview SOP No. 16E-02 and is on file at Northview Pacific Laboratories, Inc. This protocol satisfies the requirements of Ethicon Endo-Surgery.

DEVIATIONS FROM PROTOCOL

There were no deviations.

DATA DISPOSITION

Raw data from this study is archived at Northview Pacific Laboratories, Inc., 2800 Seventh Street, Berkeley, CA 94710, under Northview Report Number X4G246G.

SUMMARY OF RESULTS

This test material met the USP XXII requirements for the absence pyrogens.



USP RABBIT PYROGEN TEST

INTRODUCTION

This test is designed to limit, to an acceptable level, the possibility of febrile reactions in patients following administration of the test material by injection.

Three New Zealand albino rabbits, weighing at least 2.0 kg each, were injected intravenously with the test material. The doses range in volume from approximately 1.0 mL/kg up to 10.0 mL/kg.

MATERIALS AND METHODS

Test System

Species	Rabbit
Strain	New Zealand, Albino
Source	Elkhorn Rabbitry, Watsonville, CA.
Number	Three
Sex	Female
Weight	At least 2.0 kg
Age	Adult
Housing	Individually; 17 - 21C and 55 ± 15% relative humidity
Feed	Purina rabbit chow (approximately 200 grams per day) and water <i>ad libitum</i>
Identification	Ear tag
Quarantine	Seven days
Photoperiod	Diurnal (12 hours on - 12 hours off)

Justification For Test System

Rabbits are the species required by the current version of the United States Pharmacopeia.



**Table 1
Supplies**

Reagents/Equipment	Lot Number	Manufacturer	Expiration Date
Sodium Chloride for Injection (SCI), USP	J4D647	McGaw	4/96

**Table 2
Study Design**

Number of Rabbits	Route of Administration	Final Dose Volume (mL/kg)	Time Frequency of Temperature Readings (in hours)
3	Intravenous	10	0, 1.0, 1.5, 2.0, 2.5, 3.0

Sample Preparation And Dosing Procedure

Sample Preparation - Ten pieces of the test material were soaked in 400 mL of SCI, for 50 minutes at room temperature.

Dosing Procedure - Each rabbit was injected intravenously (through the marginal ear vein) with 10 mL/kg of the rinse solution.

Observation And Scoring

Sham Test - All rabbits used for pyrogen tests passed a sham pyrogen test conducted not more than seven days prior to the pyrogen test. (A sham pyrogen test consisted of the same procedure as the pyrogen test with the exception of the injection.)

Weights - All of the rabbits were weighed prior to administration of the test solution.

Food and Water - Food and water were withheld during the test period.

Housing - The rabbits were restrained in light fitting neck stocks that allowed the rabbits to assume a natural resting posture during the test period.



Control Temperature - The control temperature (T_0) of each rabbit was determined within thirty minutes prior to injection of the test solution. Only rabbits whose control temperature did not exceed 39.8C and did not vary more than 1C from the other test rabbits were used.

Temperature Readings - Rectal temperatures were recorded every thirty minutes from one to three hours following injection of the test solution.

Interpretation And Analysis

Any temperature decrease was considered a zero rise in temperature. If no rabbit showed an individual rise in temperature of 0.5C or more above its respective control temperature, the test material meets the requirements for the absence of pyrogens.

If these requirements are not met, the test may be continued using five additional rabbits. If not more than three of the eight rabbits show individual rises in temperature of 0.5C or more, and if the sum of the eight individual maximum temperature rises does not exceed 3.3C, the material under examination meets the requirements for the absence of pyrogens.

RESULTS AND DISCUSSION

No rabbit showed an individual rise in temperature of 0.5C or more.

CONCLUSION

This test material met the USP XXII requirements for the absence of pyrogens.

Table 3
Record of Temperatures (C)

Rabbit Number	Sex	Weight (kg)	Dose (mL)	T-0	T-60	T-90	T-120	T-150	T-180	Temperature Rise
11204	F	2.7	27	39.4	39.4	39.3	39.4	39.3	39.2	0.0
11195	F	2.4	24	39.1	39.4	39.4	39.3	39.3	39.2	0.3
11198	F	2.4	24	39.1	39.0	38.9	39.5	39.3	39.3	0.4

REFERENCES

NV SOP 16E-02

USP XXII [1990] p. 1515, p. 1627 (Biologics) CFR, 610.13

USP XXII, Supplement 8, p. 3350, (Effective May 15, 1993)



**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number X4G246G were conducted by the Quality Assurance Unit on the following dates:

NV SOP No.	Phase of Study	Date
16E-02	T=90 Minute Temperature Reading	08/10/94

QAU inspection findings are routinely reviewed by the management of Northview Pacific Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number X4G246G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.



Matthew Kanter, Quality Assurance Unit

8/22/94
Date



NORTHVIEW PACIFIC LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Timothy V. Doherty, D.V.M., Director *In Vivo* Laboratory Services
2. Roger O'Meara, Assistant Manager, *In Vivo* Laboratory Services
3. Robert Noonan, Ph.D., Senior Scientist, *In Vivo* Laboratory Services
4. Benjamin Davis, Technician, *In Vivo* Laboratory Services
5. Robert Mosbauer, Technician, *In Vivo* Laboratory Services
6. Edwin T. Pangilinan, M.S., Technician, *In Vivo* Laboratory Services
7. James Ruiz, Technician, *In Vivo* Laboratory Services
8. Patrice Pitot, Technician, *In Vivo* Laboratory Services
9. Lisa Gearin, Technician, *In Vivo* Laboratory Services

STATEMENT OF COMPLIANCE

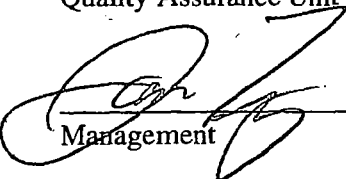
This study, Northview Report Number X4G246G, has been conducted in accordance with applicable Good Laboratory Practice regulations.


Study Director

8/19/94
Date


Quality Assurance Unit

8/22/94
Date


Management

8/22/94
Date

:dc



**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

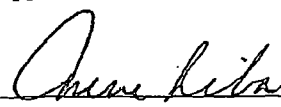
Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number V4H029G were conducted by the Quality Assurance Unit on the following dates:

<u>NV Protocol</u>	<u>Phase of Study</u>	<u>Date</u>
15B-01	Sample Preparation and Extraction	08/09/94
15B-01	Extract transfer to cell layers	08/10/94
15B-01	24 hour scoring	08/11/94
15B-01	48 hour scoring	08/12/94

QAU inspection findings are routinely reviewed by the management of Northview Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number V4H029G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.



Anne Riba
Quality Assurance Manager

8-22-94
Date



NORTHVIEW LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Alison Kondratiuk, Microbiologist
2. Cathy Burger, Technician
3. Kate Ford, Study Director

STATEMENT OF COMPLIANCE

This study, Northview Report Number V4H029G, has been conducted in accordance with applicable Good Laboratory Practice regulations.

Kate Ford
Study Director

8-22-94
Date

Anne Kuba
Quality Assurance Unit

8-22-94
Date

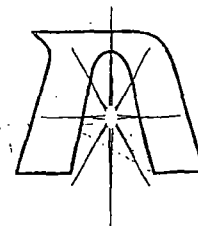
Martin Spalding
Management

8-22-94
Date

:mc

Northview Pacific Laboratories, Inc.

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SPONSOR

Mary K. Reinhard, D.V.M.
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

NV REPORT NUMBER

X4G248G

STUDY COMPLETION DATE

September 7, 1994

TEST ARTICLE IDENTIFICATION

2061-15 Black (FC770001) &
2061-15 Charcoal Grey (FC780546)
with White Trans Tech Ink-PG2NT
Code Identification: RDM #13-2-0060-00534
Lot Number: IB1201QCN3 & IC0201QCM1

TEST PERFORMED

USP Systemic Injection Test
USP Intracutaneous Test
USP Implantation Test (7 Day)

STUDY DIRECTOR

Edwin T. Pangilinan, M.S.
In Vivo Laboratory Services
Northview Pacific Laboratories, Inc.

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STUDY DATES

Study Initiation Date: August 10, 1994
Study Completion Date: September 7, 1994
Date On Test: August 5, 1994
Date Off Test: August 27, 1994
Study Authorization: Signed Protocol
Date Sample Received: July 27, 1994
Purchase Order: A2442

TEST ARTICLE IDENTIFICATION

2061-15 Black (FC770001) & 2061-15 Charcoal Grey (FC780546) with White Trans Tech Ink - PG2NT Black (with white solid strip) cylinders, 2.5 cm long and 0.5 cm in diameter contained in 2 sealed pouches containing 50 pieces each and 1 sealed pouch of implant strips approximately 30 pieces.
Code Identification: RDM #13-2-0060-00534
Lot Number: IB1201QCN3 & IC0201QCM1
Sterilization: Cobalt & ETO, 6/30/94 & 7/6/94
Storage Condition: Room Temperature

CONTROL ARTICLE IDENTIFICATION

USP Negative Control Plastic RS Filaments
10 x 1 mm white plastic filaments
50 filaments in a glass vial
Lot Number: Batch G-7
Storage Condition: Room Temperature

PROTOCOL

This test was conducted according to Protocol Number X4G248G which incorporates by reference Northview SOP Nos. 16E-03, 16E-05, and 16E-06, and are on file at Northview Pacific Laboratories, Inc. This protocol satisfies the requirements of Ethicon Endo-Surgery.

DEVIATIONS FROM PROTOCOL

There were no deviations.

DATA DISPOSITION

Raw data from these studies are archived at Northview Pacific Laboratories, Inc., 2800 Seventh Street, Berkeley, CA 94710, under Northview Report Number X4G248G.

SUMMARY OF RESULTS

This test material met the requirements of the USP Systemic Injection Test, USP Intracutaneous Test, and USP Implantation Test (7 Day).



USP SYSTEMIC INJECTION TEST

INTRODUCTION

This test evaluates systemic responses to extracts of the test material following injection into mice.

Ten Swiss Webster albino mice (five per sample and five per corresponding blank) are used for each extract. Each animal is injected with 50 mL/kg (or 10 g/kg in the case of PEG 400) of the extract or blank. The animals are observed immediately after and at 4, 24, 48, and 72 hours following injection.

MATERIALS AND METHODS

Test System

Species	Mouse
Strain	Swiss-Webster
Source	Simonsen Laboratories, Inc., Gilroy, CA
Sex	Male
Age	Young adult
Number Used	Ten per extract (5 test and 5 blank)
Initial Weight	17 to 23 grams
Housing	Five per cage; 18-26C, 55 $\pm$ 15% relative humidity
Feed and Water	Purina Rodent Chow and water <i>ad libitum</i> supplied in bottles
Identification	Cage labeling and tail marking
Photoperiod	Diurnal (12 hours on - 12 hours off)
Quarantine Period	Four days

Justification For Test System

The mouse is the species required by the current version of the United States Pharmacopoeia.



**Table 1
Supplies**

Reagents	Lot Number	Manufacturer	Expiration Date
Sodium Chloride for Injection (SCI), USP	J4D647	McGaw	4/96
Cottonseed Oil (CSO)	IA284	Spectrum Chemicals	8/98
95% Ethyl Alcohol, USP	93H06	McCormick	7/98
Polyethylene Glycol 400 (PEG 400)	2183402141	Kodak	N/A

**Table 2
Study Design**

Extracting Medium	Number of Animals	Route of Administration	Dose	Injection Rate (μ L/sec)
SCI	10	IV	50 mL/kg	100
CSO	10	IP	50 mL/kg	-
1:20 Alcohol in SCI (A/S)	10	IV	50 mL/kg	100
PEG 400	10	IP	10 g/kg	-

IV - Intravenous

IP - Intraperitoneal

Sample Preparation And Dosing Procedure

Sample Preparation - For each of the extracts, a dry sterile glass tube with a screw cap was filled with 20 mL of the extracting medium. Pieces of the test material measuring 60 cm² total surface area were then added to the tube. An additional sterile tube was filled with 20 mL of the same medium to serve as a blank. The samples and blanks were extracted at 50C for 72 hours.

Prior to injection, each gram of the PEG 400 sample extract and blank was diluted with 4.1 volumes of SCI to obtain a solution having a concentration of approximately 200 mg of PEG 400 per mL.

Dosing Procedure - Each extract was vigorously agitated prior to withdrawal of injection doses to ensure even distribution of extracted matter.

Five mice were each injected with 50 mL/kg (in the case of the PEG 400, 10 g/kg) of the SCI sample extract. Five additional mice were each injected with 50 mL/kg of the corresponding SCI blank. This procedure was repeated for each extracting medium.



Observation

Clinical Observations - All of the animals were observed for toxic signs immediately after dosing and at 4, 24, 48, and 72 hours after dosing.

Weights - All of the animals were weighed prior to dosing and at the 72 hour observation.

Interpretation And Analysis

The test material meets the USP requirements if none of the animals treated with the sample extract show a significantly greater biological reactivity than those treated with the blank.

The test material does not meet the USP requirements if two or more mice die, if abnormal behavior such as convulsions or prostration occurs in two or more mice, or if a body weight loss greater than two grams occurs in three or more mice.

RESULTS AND DISCUSSION

Clinical Observations - No toxic signs were observed during the 72 hour observation period.

Weights - All of the animals gained weight during the test (see Tables 4 & 5).

CONCLUSION

This test material met the requirements of the USP Systemic Injection Test using the extracting media and conditions listed above.

Table 3
Mortalities And Toxic Signs

Extracting Medium	Mortalities		Other Toxic Signs*		Results
	Sample	Blank	Sample	Blank	
SCI	0/5	0/5	0/5	0/5	Pass
CSO	0/5	0/5	0/5	0/5	Pass
1:20 A/S	0/5	0/5	0/5	0/5	Pass
PEG 400	0/5	0/5	0/5	0/5	Pass

* Details are described above



Table 4
Dose Volumes and Animal Weights, SCI and CSO Samples and Blanks

Extracting Medium	Animal Number	Dose (mL)	Pre-Test Weight (g)	Post-Test Weight (g)	Weight Change (g)
SCI Sample	#1	1.0	20	24	+ 4
	#2	1.0	19	24	+ 5
	#3	1.1	22	26	+ 4
	#4	1.0	20	23	+ 3
	#5	1.0	20	22	+ 2
SCI Blank	#1	1.1	22	25	+ 3
	#2	1.1	22	26	+ 4
	#3	1.1	22	24	+ 2
	#4	1.2	23	25	+ 2
	#5	1.2	23	28	+ 5
CSO Sample	#1	0.9	18	22	+ 4
	#2	1.0	20	25	+ 5
	#3	1.0	19	24	+ 5
	#4	1.0	20	24	+ 4
	#5	1.0	20	25	+ 5
CSO Blank	#1	1.1	22	25	+ 3
	#2	1.1	21	25	+ 4
	#3	1.1	22	26	+ 4
	#4	1.1	22	25	+ 3
	#5	1.1	21	24	+ 3



Table 5
Dose Volumes and Animal Weights, 1:20 A/S and PEG 400 Samples and Blanks

Extracting Medium	Animal Number	Dose (mL)	Pre-Test Weight (g)	Post-Test Weight (g)	Weight Change (g)
1:20 A/S Sample	#1	1.1	22	26	+ 4
	#2	1.1	21	25	+ 4
	#3	1.1	21	26	+ 5
	#4	1.0	19	23	+ 4
	#5	1.0	20	24	+ 4
1:20 A/S Blank	#1	1.0	19	24	+ 5
	#2	1.1	21	24	+ 3
	#3	1.0	20	25	+ 5
	#4	1.0	19	25	+ 6
	#5	1.1	22	24	+ 2
PEG 400 Sample	#1	1.0	19	24	+ 5
	#2	0.9	18	23	+ 5
	#3	0.9	18	24	+ 6
	#4	1.0	19	23	+ 4
	#5	1.1	21	25	+ 4
PEG 400 Blank	#1	1.0	19	24	+ 5
	#2	1.1	21	26	+ 5
	#3	1.1	22	26	+ 4
	#4	1.0	19	23	+ 4
	#5	1.0	19	24	+ 5

REFERENCES

NV SOP 16E-03

USP XXII [1990] p. 1499

USP XXII Supplement 5 [Nov. 15, 1991] p. 2704



USP INTRACUTANEOUS TEST

INTRODUCTION

This test evaluates local responses to extracts of the test material following intracutaneous injections into rabbits.

On the day of the test the fur on the back of each rabbit is removed on both sides of the spinal column. A 0.2 mL portion of one of the test extracts is injected intracutaneously at each of five sites along one side of the spinal column of each of two rabbits. A 0.2 mL portion of the corresponding blank is injected intracutaneously at five sites along the other side of the spinal column of each of two rabbits. The injection sites are observed for erythema and edema, and scored at 24, 48 and 72 hours.

MATERIALS AND METHODS

Test System

Species	Rabbit
Strain	New Zealand White
Source	Elkhorn Rabbitry, Watsonville, CA
Number Used	Four
Initial Weight	At least 2.0 kg
Age	Adult
Sex	Male
Housing	Individually at 17-21C and 55 $\pm$ 15% relative humidity
Feed and Water	Purina Rabbit Chow (approximately 200 grams per day) and water <i>ad libitum</i> .
Identification	Ear tags
Photoperiod	Diurnal (12 hours on - 12 hours off)
Quarantine Period	Seven days

Justification For Test System

The rabbit is the species required by the current version of the United States Pharmacopoeia.



**Table 6
Supplies**

Reagents	Lot Number	Manufacturer	Expiration Date
Sodium Chloride for Injection (SCI), USP	J4D647	McGaw	4/96
Cottonseed Oil (CSO)	IA284	Spectrum Chemicals	8/98
95% Ethyl Alcohol, USP	93H06	McCormick	7/98
Polyethylene Glycol 400 (PEG 400)	2183402141	Kodak	N/A

**Table 7
Study Design**

Extracting Medium	Number of Animals ¹	Route of Administration ²	Dose/ Site	Number of Sites/ Animal
SCI	2	IC	0.2 mL	5 sample, 5 blank
CSO	2	IC	0.2 mL	5 sample, 5 blank
1:20 Alcohol in SCI (A/S)	2	IC	0.2 mL	5 sample, 5 blank
PEG 400	2	IC	0.2 mL	5 sample, 5 blank

1. Two animals received the SCI and CSO preparations (twenty sites per animal), and two animals received the A/S and PEG 400 preparations (twenty sites per animal). Four animals were used on this study (see Dosing Procedure, p. 11).

2. IC - Intracutaneous Injection

Sample Preparation And Dosing Procedure

Sample Preparation - For each of the extracts, a dry sterile glass tube with a screw cap was filled with 20 mL of the extracting medium. Pieces of the test material measuring 60 cm² total surface area were then added to the tube. An additional sterile tube was filled with 20 mL of the same medium to serve as a blank. The samples and blanks were extracted at 50C for 72 hours.

Prior to injection, each gram of the PEG 400 sample extract and blank was diluted with 7.4 volumes of SCI to obtain a solution having a concentration of approximately 120 mg of PEG 400 per mL.



Dosing Procedure - On the day of the test, the fur on the back of each animal was removed with electric clippers. Each extract was vigorously agitated prior to withdrawal of injection doses to ensure even distribution of extracted matter.

On the right cranial portion of one rabbit's back, five 0.2 mL intracutaneous injections of the SCI sample extract were administered. The left cranial side was similarly treated with the corresponding blank. On the caudal portion of the rabbit's back, the injection procedure was repeated using the CSO extract and blank. A second rabbit was then injected with the same extracts and blanks. A second pair of rabbits was used for the remaining extracts and blanks.

Observation And Scoring

Clinical Observations - All of the animals were observed daily for signs of ill health.

Scoring - The injection sites were examined and scored for any tissue reactions, such as erythema/eschar formation and edema, at 24, 48, and 72 hours after injection according to Table 8.

Interpretation And Analysis

The average erythema and edema scores for the sample extracts and blanks are determined at each scoring interval (24, 48, and 72 hours) for each rabbit.

After the 72 hour scoring, all erythema and edema scores are totalled separately for each sample extract and blank. To determine the overall mean score, each of the totals are divided by 12 (2 animals x 3 scoring periods x 2 scoring categories).

The test material meets the USP requirements if the difference between the mean score for the sample extract and the mean score for the corresponding blank is 1.0 or less.

RESULTS AND DISCUSSION

Clinical Observations - The animals remained healthy throughout the test period.

Scoring - The difference between the mean scores for each of the sample extracts and corresponding blanks was 1.0 or less (see Tables 9 - 12).

CONCLUSION

This test material met the requirements of the USP Intracutaneous Test using the extracting media and conditions described above.



Table 8
Evaluation Of Skin Reactions

Erythema And Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet-redness) to slight eschar formation (injuries in depth)	4
Edema Formation ¹	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised about 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

¹ Excludes non-inflammatory (mechanical) edema from the blank or extraction fluid.



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Table 9
Skin Reaction Scores
Sodium Chloride For Injection Extract And Blank

Rabbit # 11285 F		Blank Sites					Sample Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
<hr/>													
Rabbit # 11288 F		Blank Sites					Sample Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
48 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
72 hr	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
Overall Mean:												0	0



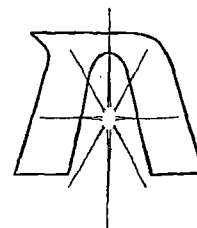
Table 10
Skin Reaction Scores
Cottonseed Oil Extract And Blank

Rabbit # 11285 F		Blank Sites					Sample Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema	2	2	2	2	2	2	2	2	2	2	2.0	2.0
	Edema	1	1	1	0	0	0	0	1	1	1	0.6	0.6
48 hr	Erythema	2	3	3	2	2	2	2	2	2	2	2.4	2.0
	Edema	1	2	2	1	1	0	0	1	1	1	1.4	0.6
72 hr	Erythema	2	2	2	2	2	2	2	2	2	2	2.0	2.0
	Edema	0	0	1	1	0	0	0	0	0	0	0.4	0
<hr/>													
Rabbit # 11288 F		Blank Sites					Sample Sites					Average Readings	
Time	Reaction	1	2	3	4	5	1	2	3	4	5	Blank	Sample
24 hr	Erythema	1	1	1	2	2	1	1	1	1	1	1.4	1.0
	Edema	1	1	1	1	2	1	1	1	1	1	1.2	1.0
48 hr	Erythema	1	1	1	2	2	1	1	1	1	1	1.4	1.0
	Edema	1	1	1	1	1	1	1	1	0	0	1.0	0.6
72 hr	Erythema	1	1	1	1	2	1	1	1	1	1	1.2	1.0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0
Overall Mean:												1.2	1.0



Northview Laboratories, Inc.

1880 Holste Road ♦ Northbrook, IL 60062 ♦ 708/564-8181 ♦ Fax 708/564-8269



SPONSOR

Dr. Mary K. Reinhard
Team Leader of Biocompatibility/Pathology/GLP
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati OH 45242

NV REPORT NUMBER

V4H023G

STUDY COMPLETION DATE

August 22, 1994

TEST ARTICLE

2061-15 Black (FC770001) &
2061-15 Charcoal Grey (FC780546) with
White Trans Tech Ink-PG2NT
Lot Number: IB1201QCN3 & IC0201QCM1

TEST PERFORMED

CYTOTOXICITY - AGAR DIFFUSION

STUDY DIRECTOR

Kate Ford
Laboratory Supervisor
Sterility Assurance Laboratory Services
Northview Laboratories, Inc.

A Northview Biosciences Company



Atlantic, Midwest, and Pacific

F.D.A. REGISTRATION No. 14-18028

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STUDY DATES

Study Initiation Date: 08/08/94
Study Completion Date: 08/22/94
Date On Test: 08/11/94
Date Off Test: 08/12/94
Study Authorization: Signed Protocol
Date Sample Received: 08/03/94
Purchase Order: A2442

TEST ARTICLE IDENTIFICATION

2061-15 Black (FC770001) & 2061-15 Charcoal Grey (FC780546) with White Trans Tech Ink-PG2NT
8 pieces in a clear sealed Baxter Tower Sterileze Gas Sterilization Pouch
Sterilization Data: Cobalt & ETO 6/30/94 & 7/6/94

RDM Number: 13-2-0060-00534
Lot Number: IB1201QCN3 & IC0201QCM1

TEST PERFORMED

Cytotoxicity - Agar Diffusion

OBJECTIVE

This study was designed to determine the *in-vitro* biological reactivity of mammalian cell cultures to specific portions of the test sample.

PROTOCOL

This test was conducted according to Protocol Number V4H023G which incorporates by reference Northview SOP 15B-02, Rev. 2C.00 and is on file at Northview Laboratories, Inc. This protocol satisfies the requirements of:

USP XXII <87>, Supplement 5, page 2702

DEVIATIONS FROM PROTOCOL

There were no deviations.

DATA DISPOSITION

Raw data and final report from these studies are archived at Northview Laboratories, Inc., 1880 Holste Road, Northbrook, IL 60062, under Northview Report Number V4H023G. There were no remaining test samples. It is the responsibility of the client to maintain test samples in accordance with GLP regulations.

PROCEDURE

1. See NVL SOP 15B-02 *Cytotoxicity - Agar Diffusion*

Amount Tested: 1 cm², in duplicate

This sample was submitted as sterile.

2. Test Supplies:

Cell Line Identification: L-929 (ATCC CCL-1)
Cell Preparation Date: 8/8/94
Medium Batch Number: A299, A300
Positive Control Number: 41694PC
Negative Control Number: 41694NC
Incubator Code: VI1

Plates were prepared with a solidified agar layer over a confluent monolayer of L-929 mouse fibroblast cells. Duplicate test samples of approximately 1 cm² each were placed on the agar layer of separate cell culture dishes. Two positive controls and two negative controls were prepared in the same manner as the test samples.

All cultures were incubated for 24 hours at 37°C ± 1°C with 4 - 6% CO₂. After incubation, the cells were stained, re-incubated for a minimum of 4 hours, and then examined microscopically for cytotoxic response.

Scoring:

All dishes were scored at each examination period using the following USP XXII Table:

Reactivity Grades for Agar Diffusion Test

<u>Grade</u>	<u>Reactivity</u>	<u>Conditions of all Cultures</u>
0	None	No detectable zone around or under specimen.
1	Slight	Some malformed or degenerated cells under specimen.
2	Mild	Zone limited to area under specimen.
3	Moderate	Zone extends 0.5 to 1.0 cm beyond specimen.
4	Severe	Zone extends greater than 1.0 cm beyond specimen but does not involve entire dish.

The average of the results of the two test dishes from each sample was used to determine the cytotoxic response.

Interpretation: The sample meets the requirements of the test if the cell culture treated with the sample does not score greater than a Mild Reactivity (Grade 2).



RESULTS

<u>Test Item</u>	<u>Reactivity</u>
2061-15 Black (FC770001) & 2061-15 Charcoal Grey (FC780546) with White Trans Tech Ink-PG2NT RDM Number: 13-2-0060-00534 Lot Number: IB1201QCN3 & IC0201QCM1	None
Postive Control	Severe
Negative Control	None

CONCLUSION

This sample meets the requirements of USP XXII for this cytotoxicity test.

**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number V4H023G were conducted by the Quality Assurance Unit on the following dates:

<u>NV Protocol</u>	<u>Phase of Study</u>	<u>Date</u>
15B-02	Sample placement on agar	08/11/94
15B-02	Sample removal and cell staining	08/12/94
15B-02	Scoring	08/12/94

QAU inspection findings are routinely reviewed by the management of Northview Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number V4H023G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.



Anne Riba
Quality Assurance Manager

8-22-94

Date



NORTHVIEW LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Alison Kondratiuk, Microbiologist
2. Cathy Burger, Technician
3. Kate Ford, Study Director

STATEMENT OF COMPLIANCE

This study, Northview Report Number V4H023G, has been conducted in accordance with applicable Good Laboratory Practice regulations.

Kate Ford
Study Director

8-22-94
Date

Opene Kuba
Quality Assurance Unit

8-22-94
Date

Martin Spalding
Management

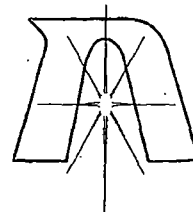
8-22-94
Date

:mc



Northview Laboratories, Inc.

1880 Holste Road ♦ Northbrook, IL 60062 ♦ 708/564-8181 ♦ Fax 708/564-8269



REPORT OF ANALYSIS

CLIENT 1733--1

REPORT DATE: 8/18/94

Ms. Mary K. Reinhard
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati OH 45242

NVL REPORT NUMBER: C4H077

YOUR P.O. NUMBER: A2442

SAMPLE IDENTIFICATION

2061-15 Black (FC770001) & 2061-15 Charcoal Grey (FC780546) with White Trans Tech Ink-PG2NT
175 pieces in a clear sealed Baxter Tower Sterileze® Gas Sterilization Pouch
Sterilization Data: Cobalt & ETO 6/30/94 & 7/6/94
RDM Number: 13-2-0060-00534
Lot Number: IB1201QCN3 & IC0201QCM1
Date Received: 8/3/94

ANALYSIS

USP PHYSICOCHEMICAL TEST FOR PLASTICS

RESULTS

<u>Test</u>	<u>USP Limits</u>	<u>Results</u>	<u>Status</u>
Non-Volatile Residue	≤ 15 mg*	< 15 mg	Conforms
Residue on Ignition	≤ 5 mg*	< 5 mg	Conforms
Heavy Metals	≤ 1 ppm in extract	< 1 ppm in extract	Conforms
Buffering Capacity	≤ 10.0 mL*	< 10.0 mL	Conforms

*Representing difference between sample and blank

PROCEDURE REFERENCE

USP XXII <661>, page 1572

NORTHVIEW LABORATORIES, INC.

Gayla Vokoun
Gayla Vokoun 8118194
Analytical Laboratory Services

:cm

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Atlantic, Midwest, and Pacific

F.D.A. REGISTRATION No. 14-18028

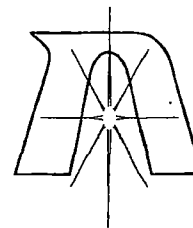
Reports are submitted to clients on a confidential basis. No reference to the work, the results, or to Northview Laboratories in any form of advertising, news release, or other public announcement may be made without our written authorization. Test results are applicable only to the samples being tested within the limits of the testing procedures identified and are not necessarily indicative of the characteristics of any other samples from the same or other lots. Northview Laboratories, Inc. shall not be liable under any circumstances for any amount in excess of the cost of the test performed.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS.GOV@fda.hhs.gov or 301-796-8188



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SPONSOR

Dr. Mary K. Reinhard
Team Leader of Biocompatibility/Pathology/GLP
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati OH 45242

NV REPORT NUMBER

V4H029G

STUDY COMPLETION DATE

August 22, 1994

TEST ARTICLE

2061-15 Black (FC770001) &
2061-15 Charcoal Grey (FC780546) with
White Trans Tech Ink-PG2NT
Lot Number: IB1201QCN3 & IC0201QCM1

TEST PERFORMED

CYTOTOXICITY - ELUTION TEST

STUDY DIRECTOR

Kate Ford
Laboratory Supervisor
Sterility Assurance Laboratory Services
Northview Laboratories, Inc.



STUDY DATES

Study Initiation Date: 08/08/94
Study Completion Date: 08/22/94
Date On Test: 08/09/94
Date Off Test: 08/12/94
Study Authorization: Signed Protocol
Date Sample Received: 8/3/94
Purchase Order: A2442

TEST ARTICLE IDENTIFICATION

2061-15 Black (FC770001) & 2061-15 Charcoal Grey (FC780546) with White Trans Tech Ink-PG2NT
15 pieces in a clear sealed Baxter Tower Sterileze Gas Sterilization Pouch
Sterilization Data: Cobalt & ETO 6/30/94 & 7/6/94

RDM Number: 13-2-0060-00534
Lot Number: IB1201QCN3 & IC0201QCM1

TEST PERFORMED

Cytotoxicity - Elution

OBJECTIVE

This study was designed to determine the *in-vitro* biological reactivity of mammalian cell cultures to specific extracts prepared from the test samples in physiological media.

PROTOCOL

This test was conducted according to Protocol Number V4H029G which incorporates by reference Northview SOP 15B-01, Rev. 3A.01 and is on file at Northview Laboratories, Inc. This protocol satisfies the requirements of:

USP XXII <87>, Supplement 5, page 2702

DEVIATIONS FROM PROTOCOL

There were no deviations.

DATA DISPOSITION

Raw data and final report from these studies are archived at Northview Laboratories, Inc., 1880 Holste Road, Northbrook, IL 60062, under Northview Report Number V4H029G. There were no remaining test samples. It is the responsibility of the client to maintain test samples in accordance with GLP regulations.



PROCEDURE

1. See NVL SOP 15B-01 *Cytotoxicity - Elution Test*

Amount Tested: 62.85 cm²

This sample was submitted as sterile.

2. Test Supplies:

Cell Line Identification: L-929 (ATCC CCL-1)
Cell Preparation Date: 8/8/94
MEM Lot Number: MEM 239
Positive Control Number: 41694PC
Negative Control Number: 41694NC
Incubator Code: VII
Caliper I.D.: 23

62.85 cm² of test material was immersed in 21.0 mL of serum supplemented culture medium and incubated for 24 hours at 37° ± 1°C with 4%-6% CO₂. Two positive controls and two negative controls were each extracted in 10 mL of medium under the same extraction conditions.

Tissue culture dishes containing a monolayer of L-929 mouse fibroblast cells were exposed to the test sample, positive control and negative control extracts. All cell cultures were incubated at 37°C ± 1°C with 4 - 6% CO₂. The cells were examined microscopically at 24 and 48 hours for cytotoxic response.

Scoring:

All dishes were scored at each examination period using the following USP XXII Table:

Reactivity Grades for Elution Test

<u>Grade</u>	<u>Reactivity</u>	<u>Conditions of all Cultures</u>
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present.
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; extensive cells lysis and empty areas between cells.
3	Moderate	Not more than 70% of the cells layers contain rounded cells and/or lysed.
4	Severe	Nearly complete destruction of the cell layers.

The average of the 48 hour results of the three test dishes from each sample was used to determine the cytotoxic response.

Interpretation: The sample meets the requirements of the test if the cell culture treated with the sample extract does not score greater than a Mild Reactivity (Grade 2).

RESULTS

<u>Test Item</u>	<u>Reactivity</u>
2061-15 Black (FC770001) & 2061-15 Charcoal Grey (FC780546) with White Trans Tech Ink-PG2NT RDM Number: 13-2-0060-00534 Lot Number: IB1201QCN3 & IC0201QCM1	None
Postive Control	Severe
Negative Control	None

CONCLUSION

This sample meets the requirements of USP XXII for this cytotoxicity test.

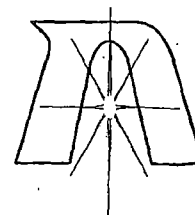


Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Part Name	Subject Device Material
Pad Print Ink	Acrylic Type PG/NT, Trans Tech America

Northview Laboratories, Inc.

1880 Holste Road ♦ Northbrook, IL 60062 ♦ 708/564-8181 ♦ Fax 708/564-8269



REPORT OF ANALYSIS

CLIENT 1733--1

REPORT DATE: 11/1/94

Ms. Mary K. Reinhard
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati OH 45242

NVL REPORT NUMBER: T4H011

YOUR P.O. NUMBER: A2442

SAMPLE IDENTIFICATION

2061-15 Black (FC770001) & 2061-15 Charcoal Grey (FC780546) with White Trans Tech Ink-PG2NT
4 pieces in a clear sealed Baxter Tower Sterileze Gas Sterilization Pouch
Sterilization Data: Cobalt & ETO 6/30/94 & 7/6/94
RDM Number: 13-2-0060-00534
Lot Number: IB1201QCN3 & IC0201QCM1
Date Received: 8/3/94

ANALYSIS

INFRARED SPECTROSCOPY - FTIR SPECTRA

RESULTS

An FTIR spectrum of the sample described above was recorded upon receipt and again about 60 days later, using a microscope accessory.

The spectra generally appear to be concordant and free from noticeable change; therefore, the sample may be considered to be stable over this time period as judged by this technique.

Copies of the spectra are attached to this report.

PROCEDURE REFERENCE

FTIR Spectroscopy

:tml

NORTHVIEW LABORATORIES, INC.

Paul Adlaf 11/2/94
Paul Adlaf, Ph. D., Director
Analytical Laboratory Services

A Northview Biosciences Company



Atlantic, Midwest, and Pacific

F.D.A. REGISTRATION No. 14-18028

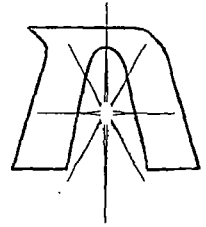
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Northview Laboratories, Inc.

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SPONSOR

Dr. Mary K. Reinhard
Team Leader of Biocompatibility/Pathology/GLP
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati OH 45242

NV REPORT NUMBER

C4H059G

STUDY COMPLETION DATE

August 18, 1994

TEST ARTICLE

2061-15 Black (FC770001) & 2061-15 Charcoal Grey (FC780546)
with White Trans Tech Ink-PG2NT
Lot Number: IB1201QCN3 & IC0201QCM1

TEST PERFORMED

HEMOLYSIS - EXTRACTION METHOD

STUDY DIRECTOR

Elaine Gruber
Laboratory Manager
Analytical Laboratory Services
Northview Laboratories, Inc.

A Northview Biosciences Company



Atlantic, Midwest, and Pacific

F.D.A. REGISTRATION No. 14-18028

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recycled paper

STUDY DATES

Study Initiation Date: 08/05/94
Study Completion Date: 08/18/94
Date On Test: 08/08/94
Date Off Test: 08/11/94
Study Authorization: Signed Protocol
Date Sample Received: 08/03/94
Purchase Order: A2442

TEST ARTICLE IDENTIFICATION

2061-15 Black (FC770001) & 2061-15 Charcoal Grey (FC780546) with White Trans Tech Ink-PG2NT
32 pieces in a clear sealed Baxter Tower Sterileze Gas Sterilization Pouch
Sterilization Data: Cobalt & ETO 6/30/94 & 7/6/94

RDM Number: 13-2-0060-00534
Lot Number: IB1201QCN3 & IC0201QCM1

TEST PERFORMED

Hemolysis - Extraction Method

OBJECTIVE

This study was designed to determine if the composition of the sample has the ability to cause hemolysis *in vitro*.

PROTOCOL

This test was conducted according to Protocol Number C4H059G which incorporates by reference Northview SOP 12F-11, Rev. 2B.00 and is on file at Northview Laboratories, Inc. This protocol satisfies the requirements of:

1. American National Standards/American Dental Association Document No. 41 for Recommended Standard Practices for Biological Evaluation of Dental Materials. Approved June 11, 1979.
2. Textbook of Microbiology, Bourdon, 3rd Edition, Revised. The Macmillan Company, New York (1952).
3. USP XXII, page 1498, Extraction Guidelines.

DEVIATIONS FROM PROTOCOL

There were no deviations.

DATA DISPOSITION

Raw data and final report from these studies are archived at Northview Laboratories, Inc., 1880 Holste Road, Northbrook, IL 60062, under Northview Report Number C4H059G. The remaining test samples were returned to the client. It is the responsibility of the client to maintain test samples in accordance with GLP regulations.



PROCEDURE

1. See NVL SOP 12F-11 *Hemolysis - Spectrophotometric Method*

2. Test Supplies:

Negative Control: 0.9% Sodium Chloride for Injection, USP
McGaw, Inc. Lot Number J4C466, Exp. Date: 9/96

Positive Control: Water for Irrigation, USP
McGaw, Inc. Lot Number J4C308, Exp. Date: 3/97

Rabbit Blood Source: Crane Laboratories
Lot Number 1190, Exp. Date: 8/23/94 (Rabbit Blood citrated)

Duplicate portions of 61.6 cm² of test material were transferred into separate test tubes. 20.5 mL of 0.9% Sodium Chloride for Injection, USP was added to each tube and 20 mL to two additional test tubes as negative controls. 20 mL of Water for Irrigation, USP was added to two tubes as positive controls. All tubes were extracted at 50°C for 72 hours. Following extraction, 10 mL of extract was taken from each tube and 0.2 mL of blood was added. The tubes were capped and gently inverted to mix. Following incubation of the test tubes at 37°C ± 2°C for 60 minutes, the contents of each tube was decanted into a centrifuge tube and centrifuged at 2000 RPM for 10 minutes. The supernatant was transferred to a cuvette using a pasteur pipet and the absorbance of the supernatant measured at 545 nm.

The percent hemolysis was calculated using the following formula, where A is absorbance.

$$\% \text{ Hemolysis} = \frac{A (\text{test sample}) - A (\text{negative control})}{A (\text{positive control}) - A (\text{negative control})} \times 100$$

RESULTSTest ItemResults

2061-15 Black (FC770001) &
2061-15 Charcoal Grey (FC780546)
with White Trans Tech Ink-PG2NT
RDM Number: 13-2-0060-00534
Lot Number: IB1201QCN3 & IC0201QCM1

< 1% Hemolysis

CONCLUSION

A test sample with 5% or less hemolysis is considered non-hemolytic.

**QUALITY ASSURANCE UNIT
GLP INSPECTION & AUDIT SUMMARY**

Comparisons of the raw data and laboratory operations with the protocol for Northview Report Number C4H059G were conducted by the Quality Assurance Unit on the following dates:

<u>NV Protocol</u>	<u>Phase of Study</u>	<u>Date</u>
12F-11	Sample Preparation	08/08/94
12F-11	Extract Transfer/Addition of Blood	08/11/94
12F-11	Absorbance Readings	08/11/94

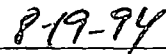
QAU inspection findings are routinely reviewed by the management of Northview Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

FINAL REPORT AUDIT

Northview Report Number C4H059G represents an accurate description of the conduct and final results of this study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practices Regulations.



Anne Riba
Quality Assurance Manager



Date

NORTHVIEW LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Marita Ocampo, Analyst
2. Rose-Marie Tobin, Chemistry Supervisor
3. Elaine Gruber, Study Director

STATEMENT OF COMPLIANCE

This study, Northview Report Number C4H059G, has been conducted in accordance with applicable Good Laboratory Practice regulations.

Elaine Gruber
Study Director

8-19-94
Date

Chene Luba
Quality Assurance Unit

8-19-94
Date

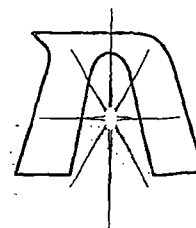
Martin Spalding
Management

8-19-94
Date

:mc

Northview Pacific Laboratories, Inc.

2800 Seventh Street ♦ Berkeley, CA 94710 ♦ 510/548-8440 ♦ Fax 510/548-5425



SPONSOR

Mary K. Reinhard, D.V.M.
Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

NV REPORT NUMBER

U4H006G

STUDY COMPLETION DATE

September 19, 1994

TEST ARTICLE IDENTIFICATION

2061-15 Black (FC770001)
2061-15 Charcoal Grey (FC780546) with
White Trans Tech Ink-PG2NT

Code Identification: RDM #13-2-0060-00534
Lot Number: IB1201QCN3 & IC0201QCM1
Sterilization Data: Cobalt & ETO 6/30/94 & 7/6/94

TEST PERFORMED

Ames

Northview Pacific Laboratories, Inc.



NV Report Number U4H006G

Page ii of ii

STUDY DATES

Testing Facility: Nelson Laboratories, Inc.
P.O. Box 17557
4535 South 2300 East
Salt Lake City, UT 84117

Study Completion Date: September 19, 1994
Date Sample Received: July 29, 1994
Purchase Order: A2442

Study Authorization: Signed Protocol

Test Procedure: See Attached

CONCLUSION

The results are summarized in Tables 1 - 8. The tester strains were sensitive to selected marker mutagens both with and without S-9 activation using a rat liver homogenate. The tester strains were also tested for proper strain performance. These tests included UV light sensitivity to verify uvrB mutation, crystal violet sensitivity to verify increased cell and ampicillin resistance to verify presence or absence of the R-factor plasmid.

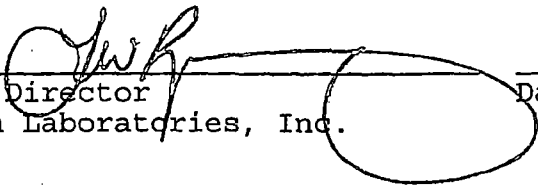
None of the 5 tester strains produced two-fold increases in the number of revertants. The spot tests showed no zone of increased reversion or of inhibition.

In summary, the extract tested against the five strains did not meet the criteria for a potential mutagen.



I CERTIFY THAT THIS STUDY WAS PERFORMED IN ACCORDANCE
WITH THE U.S. FDA GOOD LABORATORY PRACTICES.
(GLP REGULATIONS)

LABORATORY NO. 66171


Study Director
Nelson Laboratories, Inc.

12 Sep 94
Date

SOP/QAU/018B-9/0493



QAU AUDIT STATEMENT

SALMONELLA TYPHIMURIUM REVERSE MUTATION ASSAY
AMES TEST

Study Director:

Final Report Dated:

Teri A. Roming, M.S. RM(AAM)

07 Sep 94

1. The test was conducted in accordance with USFDA Good Laboratory Practices Regulations [21 CFR Part 58]. All laboratory results which pertain to this study are recorded in Data File Number 66171.
2. In accordance with the Good Laboratory Practice regulations, this study was inspected by the Quality Assurance Unit on: 23 Aug 94. The findings of the inspection(s) were reported to management and to the study director on: 23 Aug 94.
3. The Quality Assurance Unit has reviewed this report and has determined that the methods and standard operating procedures are accurately described, and that the reported results accurately reflect the raw data.

M. Kaye Markel
M. Kaye Markel
Quality Assurance Unit

13 Sep. 94
Date

SOP/QAU/018B-10/0493



SALMONELLA TYPHIMURIUM REVERSE MUTATION ASSAY
AMES TEST

LABORATORY NUMBER: 66171
PROTOCOL NUMBER: 942161-1 (As Per SOP/CTX/014)
SAMPLE SOURCE: Northview Pacific Laboratories,
Inc.
SAMPLE IDENTIFICATION: 2061-15 Black (FC770001) &
2061-15 Charcoal Grey
(FC780546) with White
Trans Tech Ink- PG2NT
Lot Numbers: IB1201QCN3 &
IC0201QCM1
Code Identification:
RDM #13-2-0060-00534
Sterilization Data: Cobalt and
ETO 6/30/94 & 7/6/94
Reference #U4H006G
P.O. #NVP 122
START DATE: 19 Aug 94
COMPLETION DATE: 02 Sep 94
REPORT DATE: 07 Sep 94
INTRODUCTION:

The Ames test employs several strains of *Salmonella typhimurium* which have been selected based on their sensitivity to mutation because of: 1) an increased cell wall permeability, due to mutation which causes partial loss of the lipopolysaccharide barrier that coats the bacterial surface and results in increased permeability to large molecules (rfa mutation); 2) a mutation in the bacterial cell system to excise and repair defects in the DNA, resulting in the inability to repair damaged or mutated sections (uvrB mutation); and 3) R-factor plasmids (some strains) and a multicopy plasmid (some strains) which contain error-prone DNA repair systems.

The *Salmonella* reverse mutation (Ames) tests are performed by mixing the test substance or an extract of the test substance and the test organism together in a soft agar solution, which contains only small amounts of histidine. The histidine permits the inoculated test organism to undergo a limited number of divisions, but is insufficient to permit normal growth. The tester strains require histidine for growth, due to a mutation in the gene which controls production of histidine. If, however, the strain undergoes a reverse mutation, (spontaneous or induced by the test substance or a positive control material), the organism no longer requires histidine to grow and can produce a visible colony or revertant. Only mutations to the test organism in the region

Northview Pacific Laboratories, Inc.
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of the histidine gene will cause the test organism to undergo a reverse mutation to an organism which then no longer requires histidine. The tester strains are selected to detect various types of mutagens. The tester strains employed were TA97A, TA98, TA100, TA102 and TA1535.

The test extract was evaluated against the five strains listed. The tests were performed both with and without S-9 activation. The S-9 activation system is designed to simulate mammalian liver enzyme systems and is used to detect substances which undergo metabolic activation from non-mutagenic forms.

The test extract was evaluated in plate incorporation assays and in spot tests with and without S-9 activation.

PROCEDURE:

Strain Verification: Test strains were checked for presence of the desired properties prior to all tests. These tests included verification of the following:

- Presence of uvrB mutation
- Presence or absence of R-factor plasmid
- Presence of rfa mutation
- Requirement for histidine

The uvrB mutation was verified by demonstrating UV sensitivity (lack of repair system). The R-factor was checked by determining sensitivity or resistance to ampicillin (0.08% in 0.02 NaOH). The presence of the rfa mutation was verified by demonstrating sensitivity to crystal violet (0.1% in water) on nutrient agar plates. The histidine requirement was assured by plating onto minimal glucose agar plates spread with 0.1 mL of 0.5 mM biotin and both with and without 0.1 mL of 0.5 mM histidine.

Sample Preparation: 4 g of the sample was extracted in 20 mL of 0.9% saline at 70°C for 24 hours.

Metabolic Activation System: The S-9 activation system was used to screen for the presences of mutagens from byproducts of the test sample. Rat liver S-9, Lot #38276, liver homogenate was obtained from Organon Teknika, Durham, NC. The homogenate was kept frozen at <-70°C and has been well characterized in our laboratory.

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The material is prepared from 8-10 week old Sprague Dawley male rats. The technique for preparation of rat liver microsomal enzymes (S-9) was identical to that described by Ames. The animals were injected intraperitoneally with Aroclor 1254 in corn oil at a dose of 500 mg/kg five days prior to sacrifice. The S-9 was prepared as a KCl homogenate and stored at -70°C until use. All procedures were carried out at $0-4^{\circ}\text{C}$. Immediately before use, the S-9 preparation was mixed with 0.5 mL of S-9 base containing 20 μL S-9 preparation to each 2.0 mL aliquot agar overlay. When working with soft agar, temperatures never exceeded 47°C , and plates were poured within 2 minutes of the addition of S-9.

Plate Incorporation Tests: Minimal agar plates containing histidine-deficient Vogel Bonner Medium E (VBME) fortified with 2% glucose were prepared. Aliquots of top agar were melted and maintained at $45^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Each 100 mL aliquot of top agar was fortified with approximately 10 mL of 0.5 mM biotin and 0.5 mM histidine immediately before use. Five to ten mL of a thirteen-hour nutrient broth culture of the appropriate bacterial tester strain was added to the top agar, and 2.0 mL aliquots of this mixture were transferred to sterile, disposable capped tubes. One-tenth mL of the sample extract was added to 2 mL aliquots of top agar. The sample extract was tested with and without the S-9 activation system.

Spot Tests: The test material was also tested in spot tests on plates with and without the S-9 activation system. Two mL aliquots of the top agar mixture containing histidine-biotin solution and the broth culture of the appropriate tester strain were added to agar plates. The plates were allowed to harden then 10 μL spots were added to the plates. All strains were tested with the extract using 10 μL spots. The plates were placed into the incubator within an hour of plating.

Positive Control Materials: The following positive control materials were used: Sodium Azide, 4-nitro-0-phenylene-diamine (NPD), and 2 aminofluorene (2AF). The controls were tested with and without the S-9 activation system. The controls were tested with all of the strains using incorporation and spot tests.

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Test Evaluation:

Test criteria: 1) Tested extract, spot test and plate incorporation. 2) Tested against tester strains TA97A, TA98, TA100, TA102 and TA1535. 3) Tested in the presence and the absence of liver microsomal enzymes preparation.

Criteria for a mutagen: 1) A two-fold increase over the spontaneous reversion rate. 2) Demonstration of a dose-response curve when dilutions are tested.

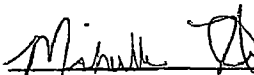
Criteria for a non-mutagen: 1) A less than two-fold increase over spontaneous reversion rate. 2) No dose response curve when dilutions are tested.

CONCLUSION:

The results are summarized in Tables 1-8. The tester strains were sensitive to selected marker mutagens both with and without S-9 activation using a rat liver homogenate. The tester strains were also tested for proper strain performance. These tests included UV light sensitivity to verify uvrB mutation, crystal violet sensitivity to verify increased cell wall permeability and corresponding loss of cell coat material, and ampicillin resistance to verify presence or absence of the R-factor plasmid.

None of the 5 tester strains produced two-fold increases in the number of revertants. The spot tests showed no zone of increased reversion or of inhibition.

In summary, the extract tested against the five strains did not meet the criteria for a potential mutagen.


Michelle A. Hudson, B.S.
Study Director


Teri A. Roming, M.S. RM(AAM)
Biocompatibility Section Leader

jww

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TABLE 1. TA97A Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	131	141	160	125	139
S-9 Control	133	153	170	125	145

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	103	108	116	135	116
Sample with S-9	155	196	137	177	166

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TABLE 2. TA98 Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	28	34	37	43	36
S-9 Control	40	51	56	48	49

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	35	31	34	35	34
Sample with S-9	28	51	43	47	42

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TABLE 3. TA100 Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	77	62	81	105	81
S-9 Control	98	112	107	91	102

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	74	98	75	94	85
Sample with S-9	96	94	81	94	91

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TABLE 4. TA102 Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	179	188	185	195	187
S-9 Control	182	195	184	197	190

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	205	197	227	201	208
Sample with S-9	237	234	214	192	219

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TABLE 5. TA1535 Results

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Control	23	25	17	22	22
S-9 Control	23	18	24	17	21

IDENTIFICATION	PLATE NUMBER				AVERAGE
	1	2	3	4	
Sample	24	23	20	17	21
Sample with S-9	18	20	16	22	19

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TABLE 6. Spot Test Results

SAMPLE EXTRACT	ACTIVATION	97A	98	100	102	1535
	None	-	-	-	-	-
	S-9	-	-	-	-	-

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TABLE 7. Strain Verification Results

PARAMETER	STRAINS				
	TA97A	TA98	TA100	TA102	TA1535
uvrB	+	+	+	-	+
R-factor	+	+	+	+	-
rfa	+	+	+	+	+
Histidine Requirement	+	+	+	+	+

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TABLE 8. Positive Controls

CONTROL ID	ACTIVATION	97A	98	100	102	1535
Sodium Azide (Expected Result)	No S-9	-	-	+	-	+
	No S-9	(-)	(-)	(+)	(-)	(+)
NPD (Expected Result)	No S-9	+	+	+	-	-
	No S-9	(+)	(+)	(+)	(-)	(-)
2AF (Expected Result)	S-9	+	+	+	-	-
	S-9	(+)	(+)	(+)	(-)	(-)

Positive controls were analyzed by incorporation assays and spot tests.

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Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Part Name	Subject Device Material
Entire Device	Cytotoxicity



FINAL STUDY REPORT

STUDY TITLE: ISO MEM Endpoint Dilution Assay
Using L-929 Mouse Fibroblast Cells

PROTOCOL NUMBER: 140350-15

TEST ARTICLE IDENTIFICATION: Trade Name: Garfunkel 35cm Curved Distal
End Jaw & Shaft, Built according to Assembly:
PRC055882, Sterilization: 90-110 kGy,
Reference Number: EE2734
Lot # PRC055882

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Ethicon Endo-Surgery
4545 Creek Road
Cincinnati, OH 45242

STUDY NUMBER: 172325

CLIENT MNEMONIC: ETH02

RESULT SUMMARY: The test article is considered **non-cytotoxic** at the
neat concentration under the conditions of this test.

**QUALITY ASSURANCE UNIT SUMMARY**

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR part 58) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Each inspection was performed to assure the quality and integrity of the study.

<u>Phase Inspected</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Addition of Extract	07/05/12	07/06/12	07/20/12
Final Report	07/20/12	07/20/12	07/20/12

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: _____

Sandra DeaneDate: 07/20/12**GOOD LABORATORY PRACTICE STATEMENT**

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: _____

Jaime HaueterDate: 7-20-12Professional personnel involved:

Lisa Olson, BS
Teri Tanquist, BS
Jean Kringstad, BS
Jaime Haueter, BS
Jean Mesarich, AA

Vice President, Testing and Service Development
Vice President of Process Improvement and Operations
Director, *In Vitro* Testing Services
Study Director
Client Relations Manager

**PURPOSE**

The purpose of this study was to evaluate a serially diluted extract of a test article for cytotoxicity to mammalian cells in culture by a method compliant with the requirements specified in ISO 10993-5:2009.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

TEST ARTICLE RECEIVED: 07/05/12
INITIATION DATE: 07/05/12
COMPLETION DATE: 07/20/12

TEST ARTICLE IDENTIFICATION

Test Article Name:	Trade Name: Garfunkel 35cm Curved Distal End Jaw & Shaft, Built according to Assembly: PRC055882, Sterilization: 90-110 kGy, Reference Number: EE2734
Lot #:	PRC055882
Sterilization Method:	Gamma 90-110 kGy
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature
Intended Use/Application:	Medical Device
Physical Description:	According to the Sponsor, the test article consisted of metal jaw attached to black shaft with white printing ink.

CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.



EXPERIMENTAL DESIGN

Experimental Summary

The test article was incubated in the appropriate volume of E-MEM + 5% FBS in a sterile vessel for 24 ± 2 hours at 37 ± 1 °C. At the end of the extraction period, the extract was decanted from the test article. A series of eight dilutions of the extract were prepared in E-MEM + 5% FBS in a two-fold dilution. After the dilutions were prepared, the maintenance culture media was removed from test culture wells and replaced with 1.0 ml of test media dilutions in triplicate wells. The positive control media, test article extract, and control extracts were added at the same time to the culture plate in triplicate wells. The plates were incubated for 72 hours at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air. The cultures were evaluated for cytotoxic effects by microscopic examination at 24, 48 and 72 ± 4 hours of incubation and the results scored and recorded.

Justification For Selection Of The Test System

The MEM Endpoint Dilution Assay is one of several *in vitro* mammalian cell tests which have been used to screen materials for their potential cytotoxicity. The L-929 cell line has a history of use in assays of this type.

AMENDMENTS/DEVIATIONS: None.

IDENTIFICATION OF THE TEST SYSTEM

Cultures of L-929 cells (mouse fibroblast) were obtained from American Type Culture Collection (ATCC # CCL-1). Mycoplasma-free cell lines which were purchased from the vendor and kept frozen in the lab until used. To maintain the sensitivity, they are only sub-cultured for up to 15 passages and then discarded.

Cultures were grown and used as monolayers in disposable tissue culture labware at 37 ± 1 °C in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air at WuXi AppTec, Inc. The media used for growth of cells is Eagle's minimal essential medium (E-MEM) supplemented with 5% (v/v) fetal bovine serum (FBS). The medium is also supplemented with the following: 2 mM L-glutamine, 10 mM HEPES, 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 units/mL penicillin, and 1% 2.50 ug/mL amphotericin B (Fungizone). WuXi AppTec Inc. has a long-standing history using antibiotics in culture medium. The use of antibiotics in culture media has not shown adverse effects when used for this assay, exhibited by the varying degrees of toxicity seen in the assay controls.

TEST MATERIAL PREPARATION

The test article was cut according to the Sponsor provided instructions (see Appendix). The remaining test article was not cut further, was placed into an extraction vessel, and prepared at a ratio of 4 g to 20 mL of extraction vehicle.

TABLE 1: TEST ARTICLE RECORD

VEHICLE	TEST ARTICLE AMOUNT (g)	VEHICLE AMOUNT (mL)	QUANTITY OF TEST ARTICLE USED
E-MEM + 5% FBS	3.1	15.5	1

EXTRACTION MEDIA

Eagle's minimal essential medium (E-MEM), supplemented with 5% (v/v) fetal bovine serum (FBS), was used to extract the test article. The medium was also supplemented with the following: 2 mM L-glutamine, 10 mM HEPES, 0.01 mg/mL vancomycin, 0.01 mg/mL gentamicin, 1% 1000 units/mL penicillin, and 1% 2.50 ug/mL amphotericin B (Fungizone). The pH was confirmed to be 7.26.



CONTROL ARTICLE PREPARATION

A negative, intermediate, positive, and cell control were run in parallel with the test article. A negative control (high density polyethylene (HDPE)) known to be non-toxic under the test conditions was prepared at a ratio of 120 cm² to 20 mL of extraction vehicle. An intermediate control (black rubber) known to be mildly toxic under the test conditions was prepared and extracted at a ratio of 4 g to 20 mL of extraction vehicle. A positive control (100 µM CdCl₂) known to be toxic under the test conditions was prepared by diluting cadmium chloride stock powder with 100 mL of sterile water to create a 10 mM solution. The solution was then prepared at a ratio of 0.1 mL to 9.9 mL of extraction vehicle. A cell control (E-MEM + 5% FBS) was incubated in parallel with the test sample and controls:

TABLE 2: CONTROL / CELL LINE RECORD

CONTROL IDENTIFICATION	CLASS	LOT #	SUPPLIED BY:	EXPIRATION
100 µM CdCl ₂	Positive Control	TDSI1074	WXAT	09/20/12
Black Rubber Stopper	Intermediate Control	WXAT010710	Fisher	02/28/13
High Density Polyethylene (HDPE)	Negative Control	C-091	Hatano	02/2016
E-MEM + 5% FBS	Medium	TDSI1177	WXAT	07/10/12
L-929	Cell Line	L070212	ATCC	NA

NA = Not applicable

TEST ARTICLE EXTRACTION

The extraction mixture and controls were incubated for 24 ± 2 hours at 37 ± 1 °C. At the start of the extraction, the solutions appeared clear and free of particulates. The extracts were agitated during the course of the extraction period. At the end of the extraction period, the vessels were well shaken. The test article was observed after extraction to be intact with no macroscopically observable degradation and the extract was clear. The extract was not filtered and was used immediately. See Tables 1-3.

TABLE 3: EXTRACTION RECORD

VEHICLE	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END
E-MEM + 5% FBS	37.0 °C	07/05/12 2:45 pm	37.0 °C	07/06/12 12:46 pm

EXPERIMENTAL PROCEDURE

L-929 cells were plated at 2.5 x 10⁴ cells/mL at least 24 hours before use. Prior to inoculation, the cell cultures were examined to ensure they had formed a nearly confluent monolayer and that they appeared uniform and viable.

The test article and controls were extracted with the appropriate amount of E-MEM + 5% FBS for 24 ± 2 hours at 37 ± 1 °C. At the end of the extraction period, the vessels were well shaken and the extraction media was used immediately. A series of eight dilutions of the extract were prepared in E-MEM + 5% FBS in a two-fold dilution. One mL aliquots of the test article and control extract and the series of dilutions were plated in triplicate onto the cell line.

The cell cultures were incubated in a humidified atmosphere for 72 hours. The monolayers were evaluated for cytotoxic effects at 24, 48, and 72 hours. At each incubation period, the cell cultures were observed for signs of cytopathic effect (CPE) including lysis, crenation, plaques, and excessive rounding of cells.

TEST EVALUATION

Criteria for evaluating cytotoxicity will include morphologic changes in cells, such as granulation, crenation, or rounding, and loss of viable cells from the monolayer by lysis or detachment. The



validity of the test requires that negative control cultures maintain a healthy normal appearance throughout the duration of the test. Degrees of toxicity were scored as described in Table 4.

TABLE 4: SCORING

GRADE	REACTIVITY	CONDITIONS OF ALL CULTURES
0	None	Discrete intracytoplasmic granules; no cell lysis, no reduction of cell growth
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cells layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers

According to the current ISO guidelines, test articles scoring '0', '1', or '2' are considered NON-CYTOTOXIC. Test articles scoring '3' or '4' are considered CYTOTOXIC.

VALIDITY CRITERIA

Validity of the assay and the test article result was based upon the criteria listed below and scientific judgment.

The negative and cell control should display no cytotoxic reaction. The intermediate control should display a mild to moderate cytotoxic reaction, a score of '2' or '3'. The positive control should display a moderate to severe cytotoxic reaction, a score of '3' or '4'.

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS: Not applicable.

STATISTICAL METHODS: None used.

RECORD RETENTION

A certified copy of this report and data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

COMPLIANCE

This study was performed in compliance with the following International Standards:

ISO 10993-5:2009 Biological Evaluation of Medical Devices, Part 5: Tests for *In Vitro* Cytotoxicity.

ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

TEST ARTICLE DISPOSITION

Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

RESULTS

The negative and cell controls exhibited healthy growth throughout the experiment. The monolayers exposed to CdCl₂ exhibited a toxic response while the black rubber intermediate control induced a mild level of toxicity. Therefore, the test system was responding normally and met the criteria for a valid assay. See Table 5.



When scoring lysis, each sample was rated relative to the amount of lysis displayed by the negative control. The negative and cell control were considered '0', the cells displayed at least 70% viability. The intermediate control displayed a mild cytotoxic reaction resulting in a score of '2'. The positive control displayed a severe cytotoxic reaction, resulting in a score of '4'. Therefore, the test system was responding normally and met the criteria for a valid assay. See Table 5.

In this assay, the test article did not induce cytotoxicity. No abnormal events such as pH change or debris were noted.

TABLE 5: RESULTS

TEST ARTICLE AND CONTROLS	SAMPLE EXTRACT		CYTOTOXICITY SCORES		
	SIZE	(mL)	24 HOURS	48 HOURS	72 HOURS
Test Article	3.1 g	15.5	0/0/0	0/0/0	0/0/0
Test Article – 1:2	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:4	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:8	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:16	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:32	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:64	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:128	NA	NA	0/0/0	0/0/0	0/0/0
Test Article – 1:256	NA	NA	0/0/0	0/0/0	0/0/0
Positive Control	0.1 mL	9.9	4/4/4	4/4/4	4/4/4
Intermediate Control	1.9 g	9.5	2/2/2	2/2/2	2/2/2
Negative Control	30 cm ²	5.0	0/0/0	0/0/0	0/0/0
Cell Control	NA	10.0	0/0/0	0/0/0	0/0/0

NA = Not Applicable

ANALYSIS AND CONCLUSION

The test article at the neat concentration, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256 ratios scored '0' at the 24, 48 and 72 ± 4 hours observation periods. Therefore the test article is considered **non-cytotoxic** at the neat concentration.

REFERENCES

U.S. Pharmacopeia, Section 87, current revision.

WuXi AppTec Reference Library Contents, Form ALS-4650-1

WuXi AppTec SOP: MED-8750, Minimum Essential Medium (MEM) Elution Assay



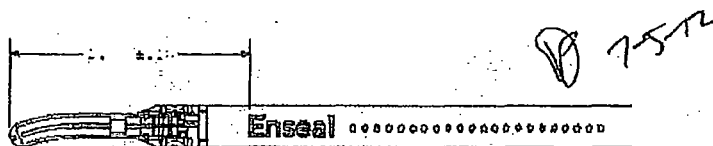
APPENDIX

Attachment 1

172325

82H 7-5-12

Here is the artwork, as defined on the drawing (Inches):



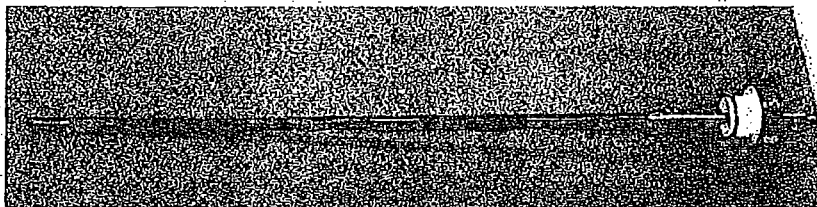
Actual image of sample, showing where 4cm would stop (note that this will not include all of the pad printing if cut at 4cm)



The sample that is received by lab will be same as above except will have white printing ink with the logo from the top picture.

The lab is to cut the shaft at 4 cm from the metal tip end so that some of the pad print is included on the shaft.

Overall image of the sample that I will provide for processing and testing:



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Attachment 5: 510(k) Summary

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

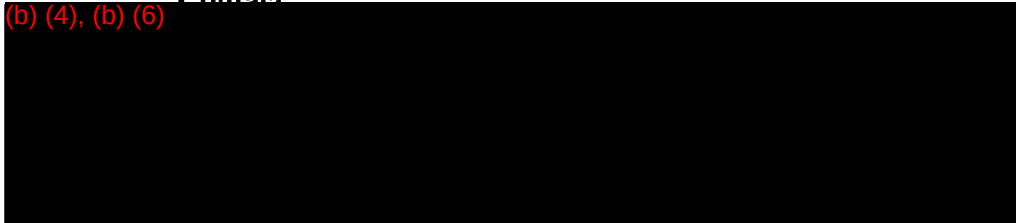
510(k) Summary

Company

Ethicon Endo-Surgery, LLC
475 Calle C
Guaynabo, PR 00969

Contact

(b) (4), (b) (6)



Date Prepared: September 10, 2012

Device Name

Trade Name: ENSEAL® G2 Articulating Tissue Sealers
Common Name: Electrosurgical Cutting and Coagulation Instruments

Classification Names

- Electrosurgical, Cutting & Coagulation & Accessories
(21 CFR 878.4400, Product code GEI)
- Electrocautery, Gynecologic and Accessories
(21 CFR 884.4120, Product code HGI)

Predicate Device

ENSEAL® G2 Tissue Sealers, K112033

Device Description:

The Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers are sterile, single-use surgical instruments designed to seal and cut vessels, and to cut, grasp and dissect soft tissue during open and laparoscopic surgery.

The energy activation and knife lock are activated by a single button. The instrument shaft can be rotated using the rotation knob to facilitate visualization and enable easy access to targeted tissue. The power cord is permanently attached to the device and connects the instrument to the generator. The ENSEAL G2 Articulating Tissue Sealers include an articulation capability that allows the user to articulate the end-effector during surgical procedures.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Indication for Use:

The ENSEAL G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with the Generator GEN11 (GEN11). They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7mm and tissue and/or vascular bundles as large as will fit in the jaws of the instruments.

The ENSEAL G2 Articulating Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures.

Technological Characteristics:

The Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers components are similar to the Predicate. The ENSEAL G2 Articulating Tissue Sealers have a 5 mm diameter shaft and are available in two shaft lengths: 35 and 45cm; and two jaw types: 3mm curved jaw and 5mm straight jaw. The jaws are normally in the opened position and can be partially or fully closed by squeezing the closing handle. The jaws have atraumatic teeth for grasping and holding targeted tissue when clamped. The handles of the Subject ENSEAL G2 Articulating Tissue Sealers have an ergonomic interface for the user. Like the Predicate, the Subject devices include an integrated energy activation button. The devices use a combination of the application of bipolar electrosurgical energy in conjunction with the I-BLADE knife, to compress, coagulate, and transect tissue.

In addition to the similar technological characteristics as the Predicate, the ENSEAL G2 Articulating Tissue Sealers instrument shaft device articulates approximately 60° to the left and the right of center. The instrument shaft can be articulated using the articulation wheel to gain additional access to tissue and facilitate the user's ability to target tissue and vascular bundles up to 60°. The top of the articulation wheel has a white arrow to indicate when the device shaft is straight and not articulated. When the articulation wheel is rotated clockwise, the shaft articulates to the right; when the articulation wheel is rotated counterclockwise, the shaft articulates to the left. While articulated, the device jaws seal and cut vessels, and cut, grasp and dissect soft tissue as intended. The Subject ENSEAL G2 Articulating Tissue Sealers performance is equivalent to the Predicate ENSEAL G2 Tissue Sealers.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Performance Data:

Device performance testing was assessed against the predicate to demonstrate equivalent performance.

Ex-vivo and in-vivo tests were performed to verify that the performance of the ENSEAL G2 Articulating Tissue Sealers perform as intended and meets the definition of substantial equivalence to the Predicate device, ENSEAL G2 Tissue Sealers.

The Ex-vivo tests (bench) include Electromagnetic Compatibility, Electrical Safety, Biocompatibility, Force to Activate Energy, and Sealed Vessel Burst Pressure. Electrical Safety and Electromagnetic Compatibility Testing is in accordance with the standard for Electrical Basic Safety, Electromagnetic Compatibility, and Safety of High-frequency surgical equipment, and performed and subsequently certified for all applicable sections of IEC 60601-1-2:2007, IEC 60601-1:2005, and IEC 60601-2-2:2009. Biocompatibility testing for all materials is in accordance with the standards AAMI/ANSI/ISO 10993-1:2009 and on FDA General Program Memorandum #G95-1: Use of International Standard ISO 10993, "Biological Evaluation of Medical Devices - Part 1: Evaluation and Testing." In-vivo testing (acute and 30-day survival) were conducted to evaluate vessel seal integrity and various tissue effects. Ex-vivo and In-vivo test results demonstrate that the Subject Devices meet requirements and exhibit equivalent performance to the Predicate Devices.



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

Ethicon Endo-Surgery, LLC

January 25, 2013

(b) (4), (b) (6)

Re: K122797

Trade/Device Name: ENSEAL® G2 Articulating Tissue Sealers

Regulation Number: 21 CFR 878.4400

Regulation Name: Electrosurgical cutting and coagulation device and accessories

Regulatory Class: Class II

Product Code: GEI, HGI

Dated: January 16, 2013

Received: January 22, 2013

(b) (4), (b) (6)

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments; or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA).

You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you; however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical

Page 2 – Mrs. Emily Kruetzkamp

device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please go to <http://www.fda.gov/AboutFDA/CentersOffices/CDRH/CDRHOffices/ucm115809.htm> for the Center for Devices and Radiological Health's (CDRH's) Office of Compliance. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to <http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm> for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address <http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm>.

Sincerely yours,

Peter D. Rumm-S

Mark N. Melkerson
Acting Director
Division of Surgical Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

Page 3 – Mrs. Emily Kruetzkamp

Concurrence & Template History Page
[THIS PAGE IS INCLUDED IN IMAGE COPY ONLY]

Full Submission Number:

For Office of Compliance Contact Information:

http://insideportlets.fda.gov:9010/portal/page?_pageid=197,415881&_dad=portal&_schema=PORTAL&org=318

For Office of Surveillance and Biometrics Contact Information:

http://insideportlets.fda.gov:9010/portal/page?_pageid=197,415881&_dad=portal&_schema=PORTAL&org=423

Digital Signature Concurrence Table	
Reviewer Sign-Off	
Branch Chief Sign-Off	
Division Sign-Off	Peter D. Rumm -S 2013.01.25 11:44:03 -05'00'

Template Name: K1(A) – SE after 1996

Template History:

Date of Update	By	Description of Update
7/27/09	Brandi Stuart	Added Updates to Boiler Table
8/7/09	Brandi Stuart	Updated HFZ Table
1/11/10	Diane Garcia	Liability/Warranty sentence added at bottom of 1 st page
10/4/11	M. McCabe Janicki	Removed IFU sheet and placed in Forms
9/25/12	Edwena Jones	Added digital signature format
12/12/12	M. McCabe Janicki	Added an extra line between letter signature block and the word "Enclosure". Also, added a missing digit in 4-digit extension on letterhead zip code: "002" should be "0002".

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Indications for Use Form

Indications for Use

510(k) Number (if known): _____

Device Name: ENSEAL® G2 Articulating Tissue Sealers

Indications for Use:

The ENSEAL® G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with the Generator GEN11 (GEN11). They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

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Prescription Use X
(Part 21 CFR 801 Subpart D)

AND/OR

Over-The-Counter Use _____
(21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE
OF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Page 1 of 1

Dwight Yen

2013.01.31 08:06:31 -05'00'

(Division Sign-Off)

Division of Surgical Devices

510(k) Number K122797

FAX HEADER 1:

FAX HEADER 2:

TRANSMITTED/STORED : JAN. 31. 2013 2:25PM
FILE MODE OPTION

ADDRESS

RESULT

PAGE

2860 MEMORY TX

EES Fax Server

OK

3/3

REASON FOR ERROR
E-1) HANG UP OR LINE FAIL
E-3) NO ANSWERE-2) BUSY
E-4) NO FACSIMILE CONNECTION

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center - WO66-G609
Silver Spring, MD 20993-0002

Ethicon Endo-Surgery, LLC

(b) (4), (b) (6)

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Food and Drug Administration
Office of Device Evaluation &
Office of In Vitro Diagnostics

COVER SHEET MEMORANDUM

***** NOTE: This form is OPTIONAL for holds, and REQUIRED for final decisions *****

From: Reviewer Name Nosovskyy, Mykhaylo
Subject: 510(k) Number K122797
To: The Record

Please list CTS decision code SE

- ☐ Refused to accept (Note: this is considered the first review cycle, See Screening Checklist
http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPremarketNotification510kProgram/0_5631/Screening%20Checklist%207%202%2007.doc)
- ☐ Hold (Additional Information or Telephone Hold).
- ☒ Final Decision (SE) SE with Limitations, NSE (select code below), Withdrawn, etc.).

Not Substantially Equivalent (NSE) Codes

- | | |
|-----------------------------|---|
| ☐ NO | NSE for lack of predicate |
| ☐ NI | NSE for new intended use |
| ☐ NQ | NSE for new technology that raises new questions of safety and effectiveness |
| ☐ NU | NSE for new intended use AND new technology raising new questions of safety and effectiveness |
| ☐ NP | NSE for lack of performance data |
| ☐ NS | NSE no response |
| ☐ NL | NSE for lack of performance data AND no response |
| ☐ NM | NSE pre-amendment device call for PMAs (515i) |
| ☐ NC | NSE post-amendment device requires PMAs |
| ☐ NH | NSE for new molecular entity requires PMA |
| ☐ TR | NSE for transitional device |

Please complete the following for a final clearance decision (i.e., SE, SE with Limitations, etc.):		YES	NO
Indications for Use Page	<i>Attach IFU</i>	X	
510(k) Summary /510(k) Statement	<i>Attach Summary</i>	X	
Truthful and Accurate Statement.	<i>Must be present for a Final Decision</i>	X	
Is the device Class III?			X
If yes, does firm include Class III Summary?	<i>Must be present for a Final Decision</i>		
Does firm reference standards? (If yes, please attach form from http://www.fda.gov/opacom/morechoices/fdaforms/FDA-3654.pdf)		X	
Is this a combination product? (Please specify category _____, see http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPremarketNotification510kProgram/0_413b/COMBINATION%20PRODUCT%20ALGORITHM%20(REVISED%203-12-03).DOC)			X
Is this a reprocessed single use device? (Guidance for Industry and FDA Staff – MDUFMA - Validation Data in 510(k)s for Reprocessed Single-Use Medical Devices, http://www.fda.gov/cdrh/ode/guidance/1216.html)			X
Is this device intended for pediatric use only?			X
Is this a prescription device? (If both prescription & OTC, check both boxes.)			X
Did the application include a completed FORM FDA 3674, Certification with Requirements of ClinicalTrials.gov Data Bank?			X
Is clinical data necessary to support the review of this 510(k)?			X

For United States-based clinical studies **only**: Did the application include a completed FORM FDA 3674, *Certification with Requirements of ClinicalTrials.gov Data Bank?* (If study was conducted in the United States, and FORM FDA 3674 was not included or incomplete, then applicant must be contacted to obtain completed form.)

Does this device include an Animal Tissue Source?	X
All Pediatric Patients age<=21	X
Neonate/Newborn (Birth to 28 days)	X
Infant (29 days -< 2 years old)	X
Child (2 years -< 12 years old)	X
Adolescent (12 years -< 18 years old)	X
Transitional Adolescent A (18 - <21 years old) Special considerations are being given to this group, different from adults age ≥ 21 (different device design or testing, different protocol procedures, etc.)	X
Transitional Adolescent B (18 -<= 21; No special considerations compared to adults => 21 years old)	X
Nanotechnology	X
Is this device subject to the Tracking Regulation? (Medical Device Tracking Guidance, http://www.fda.gov/cdrh/comp/guidance/169.html)	X

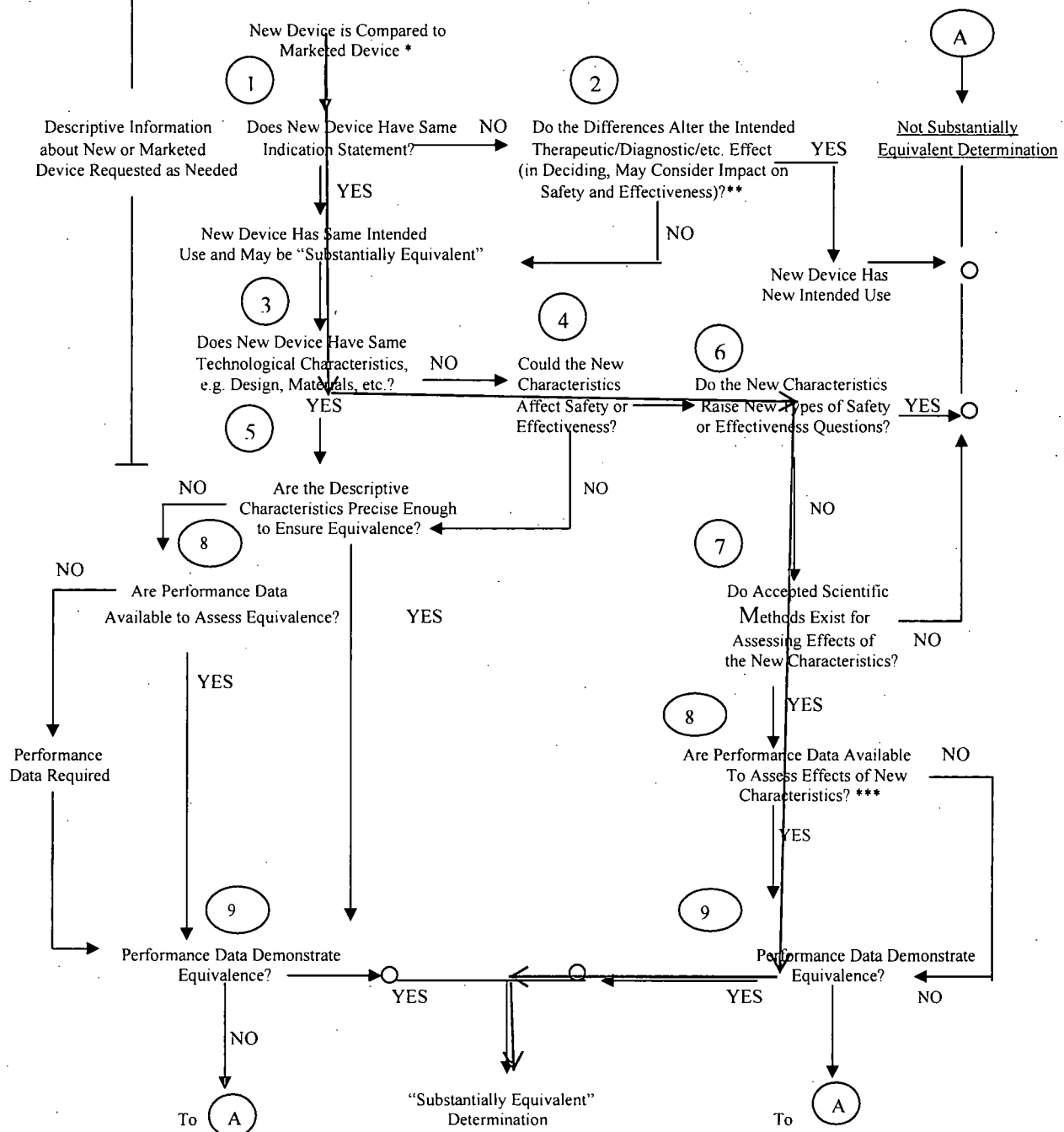
Regulation Number	Class*	Product Code
21 CFR 878.4400, 21 CFR 884.4120	II	GEI, HGI

(*If unclassified, see 510(k) Staff)

Additional Product Codes: _____

Digital Signature Concurrence Table	
Reviewer Sign-Off	Mykhaylo B. Nosovskyy 2013.01.24 17:24:21 -05'00'
Branch Chief Sign-Off	Dwight Yen 2013.01.25 06:51:04 -05'00'
Division Sign-Off	Peter D. Rumm -S 2013.01.25 11:40:11 -05'00'

510(k) "SUBSTANTIAL EQUIVALENCE" DECISION-MAKING PROCESS



K122797

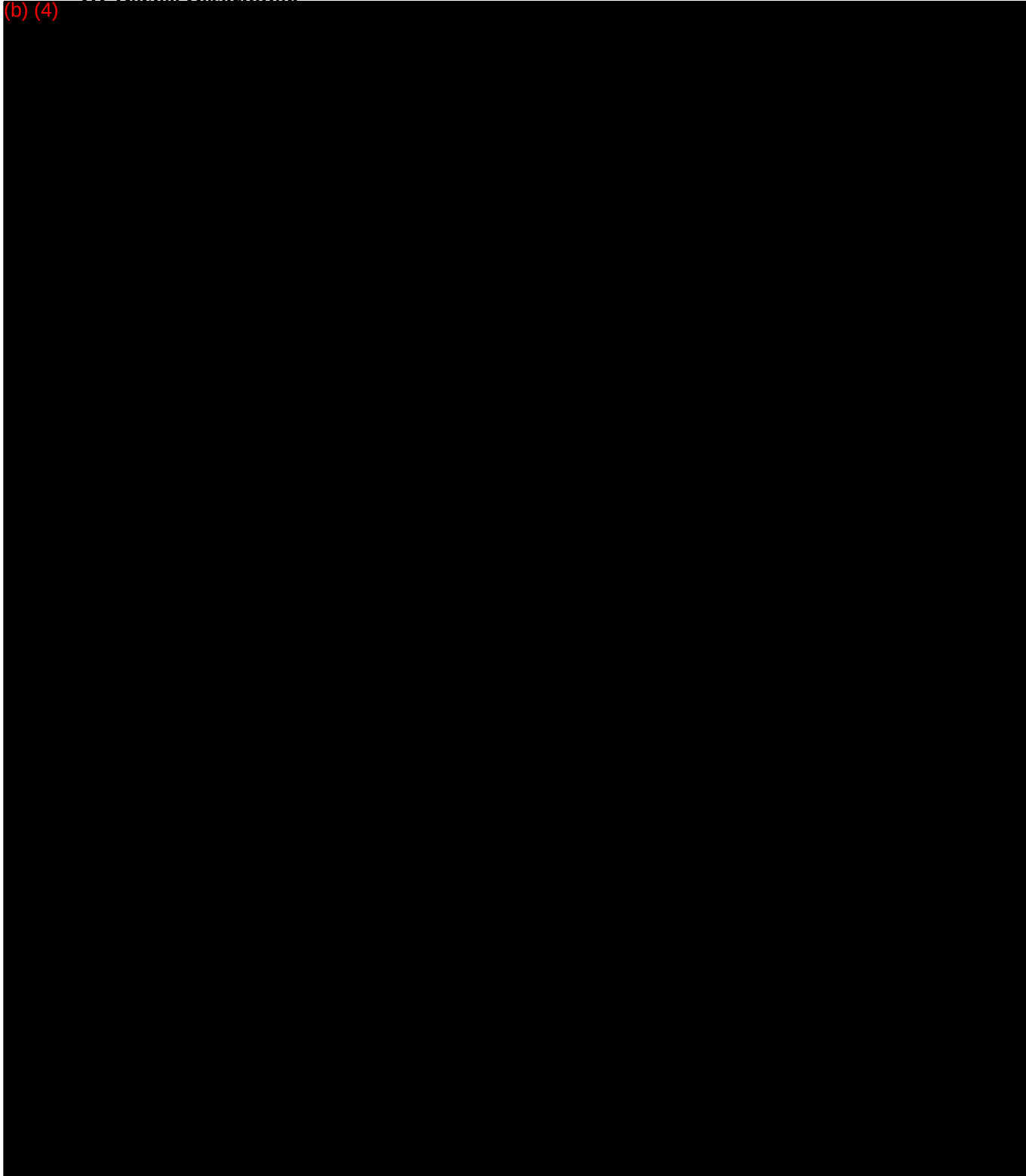
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2013.01.24 16:31:50 -05'00'

Dwight Yen
2013.01.25 06:49:54 -05'00'

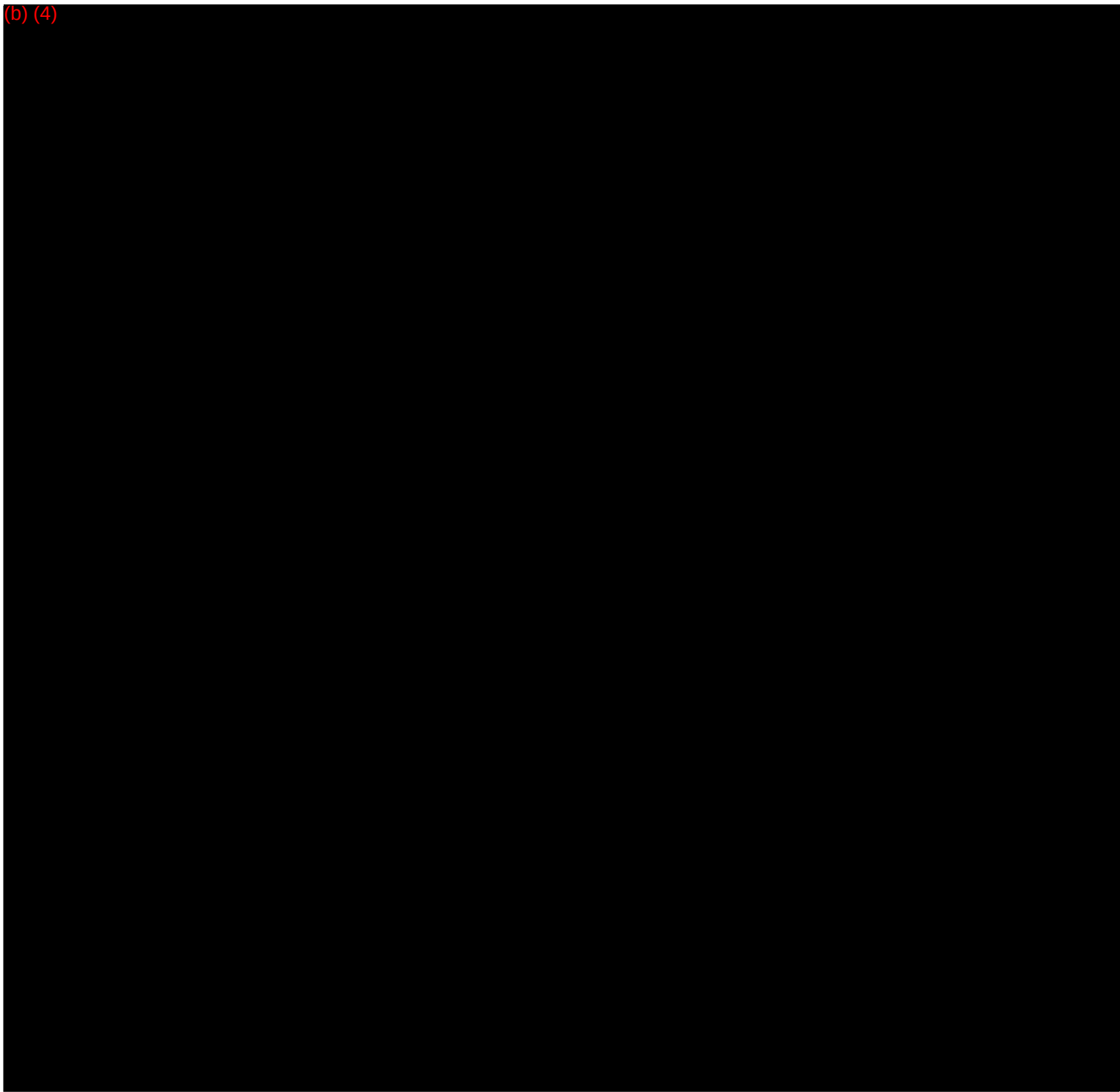
- * 510(k) Submissions compare new devices to marketed devices. FDA requests additional information if the relationship between marketed and "predicate" (pre-Amendments or reclassified post-Amendments) devices is unclear.
- ** This decision is normally based on descriptive information alone, but limited testing information is sometimes required.
- *** Data maybe in the 510(k), other 510(k)s, the Center's classification files, or the literature.

III. Device Description

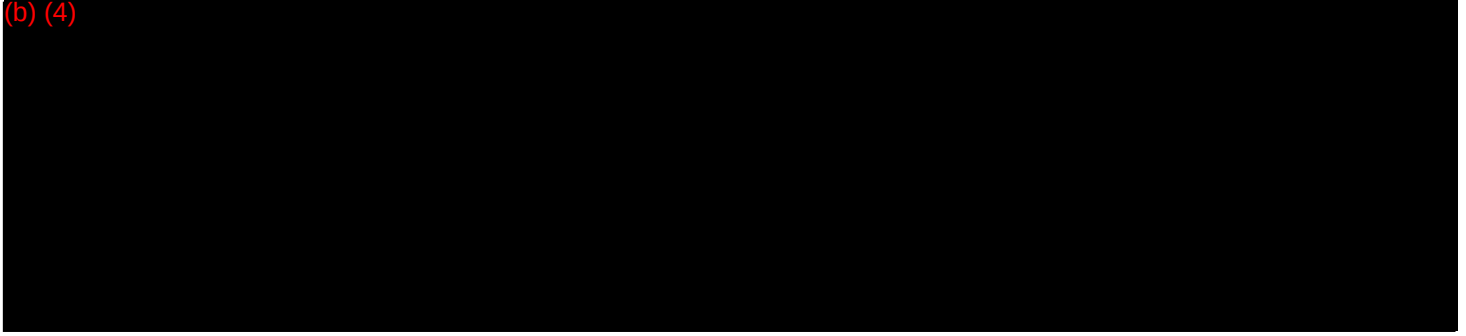
(b) (4)



(b) (4)

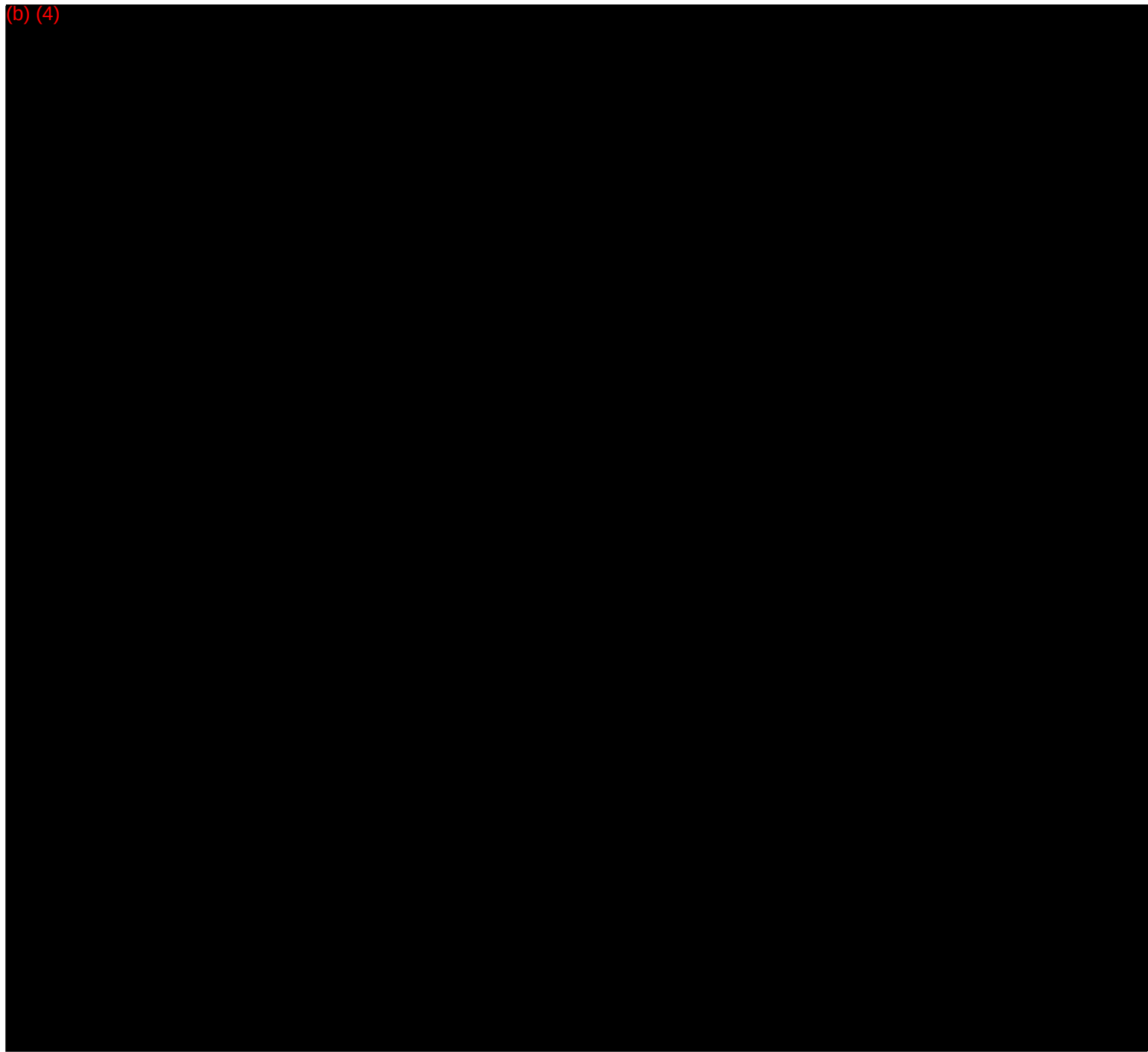


(b) (4)



XI. Performance Testing – Bench

(b) (4)





Food and Drug Administration
Center for Devices and Radiological Health
Document Control Center WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

January 22, 2013

ETHICON ENDO-SURGERY, LLC
C/O ETHICON ENDO-SURGERY, INC.
4545 CREEK ROAD
CINCINNATI, OHIO 45242

510k Number: K122797

Product: ENSEAL G2 ARTICULATING TISSUE

(b) (4), (b) (6)

The additional information you have submitted has been received.

We will notify you when the processing of this submission has been completed or if any additional information is required. Please remember that all correspondence concerning your submission **MUST** be sent to the Document Mail Center at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review. Please refer to this guidance for information on current fax and e-mail practices at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089402.htm>. On August 12, 2005 CDRH issued the Guidance for Industry and FDA Staff: Format for Traditional and Abbreviated 510(k)s. This guidance can be found at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm084365.htm>. Please refer to this guidance for assistance on how to format an original submission for a Traditional or Abbreviated 510(k).

The Safe Medical Devices Act of 1990, signed on November 28, states that you may not place this device into commercial distribution until you receive a letter from FDA allowing you to do so. As in the past, we intend to complete our review as quickly as possible. Generally we do so in 90 days. However, the complexity of a submission or a requirement for additional information may occasionally cause the review to extend beyond 90 days. Thus, if you have not received a written decision or been contacted within 90 days of our receipt date you may want to check with FDA to determine the status of your submission.

Please ensure that whether you submit a 510(k) Summary as per 21 CFR 807.92, or a 510(k) Statement as per 21 CFR 807.93, it meets the content and format regulatory requirements.

If you have procedural questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301)796-7100 or at their toll-free number (800)638-2041, or contact the 510k staff at (301)796-5640.

Sincerely,

510(k) Staff

Mcdonald, Lisa *

From: Mcdonald, Lisa *
Sent: Tuesday, January 22, 2013 7:33 PM
:
Cc: DCCLetters
Subject: K122797 AI Letter
Attachments: CrystalViewer[2].rtf

Tracking:

Recipient

ekruetzk@its.jnj.com
DCCLetters

Delivery

Delivered: 1/22/2013 7:33 PM

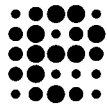
Mcdonald, Lisa *

From: Microsoft Outlook
To: DCCLetters
It: Tuesday, January 22, 2013 7:33 PM
Subject: Delivered: K122797 AI Letter

Your message has been delivered to the following recipients:

DCCLetters (DCCLetters@fda.hhs.gov)

Subject: K122797 AI Letter



**Ethicon
Endo-Surgery**

PART OF THE JOHNSON & JOHNSON FAMILY OF COMPANIES

K-90

K122797/SD

Ethicon Endo-Surgery, Inc.
4545 Creek Road
Cincinnati, OH 45242

January 18, 2013

Mykhaylo B. Nosovskyy, Ph.D
Mechanical Engineer
General Surgery Devices Branch
US Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

Tel. # 301-796-3745
Fax # 301-847-8119
mykhaylo.nosovskyy@fda.hhs.gov

FDA CDRH DMC

JAN 22 2013

Received

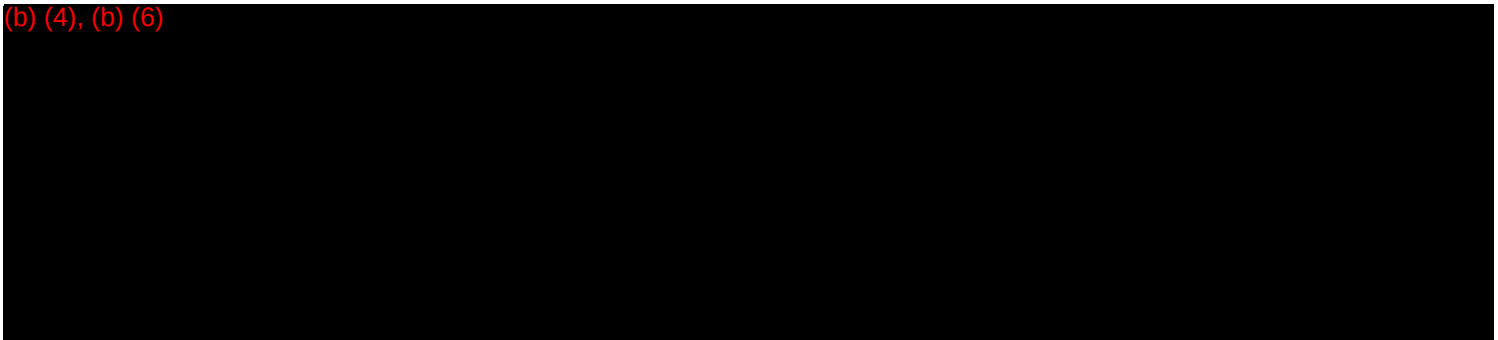
Re: K122797, ENSEAL G2 Articulating Tissue Sealers

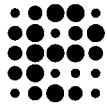
Dear Dr. Nosovskyy:

Thank you for your review of the 510(k) submission K122797. I have addressed your questions and made the requested changes in response to your request dated December 19, 2012. Please see the attached pages with the questions and responses. The Section 4 Indications For Use and Section 5 510k Summary have been included for your reference, there are no changes to these documents.

An electronic copy (eCopy) of the response is included with this submission. The eCopy is an exact duplicate of the paper submission. The eCopy is accompanied by an extra copy of the cover letter.

(b) (4), (b) (6)





**Ethicon
Endo-Surgery**

PART OF THE JOHNSON & JOHNSON FAMILY OF COMPANIES

Ethicon Endo-Surgery, Inc.
4545 Creek Road
Cincinnati, OH 45242

January 18, 2013

Mykhaylo B. Nosovskyy, Ph.D
Mechanical Engineer
General Surgery Devices Branch
US Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

Tel. # 301-796-3745
Fax # 301-847-8119
mykhaylo.nosovskyy@fda.hhs.gov

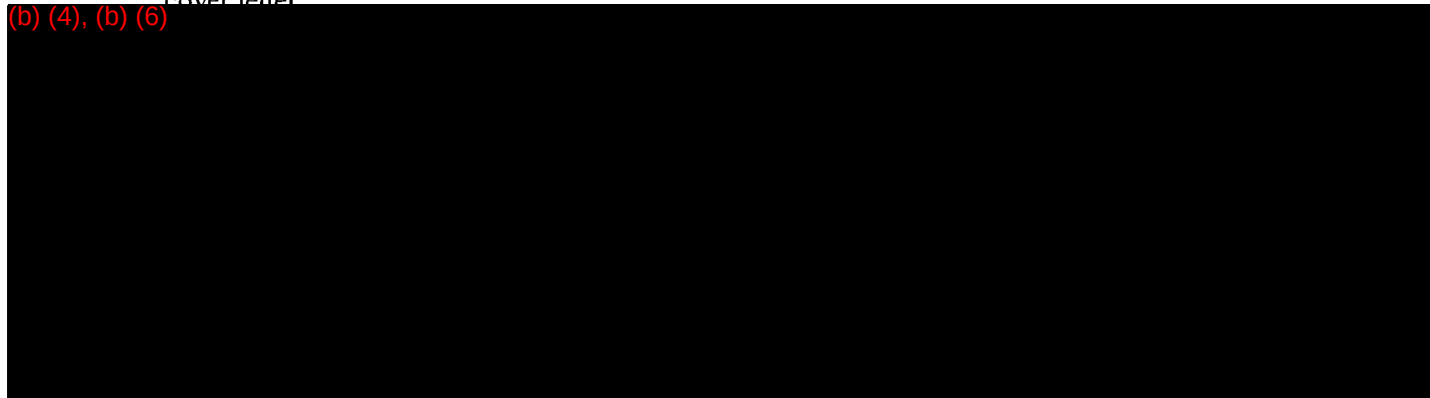
Re: K122797, ENSEAL G2 Articulating Tissue Sealers

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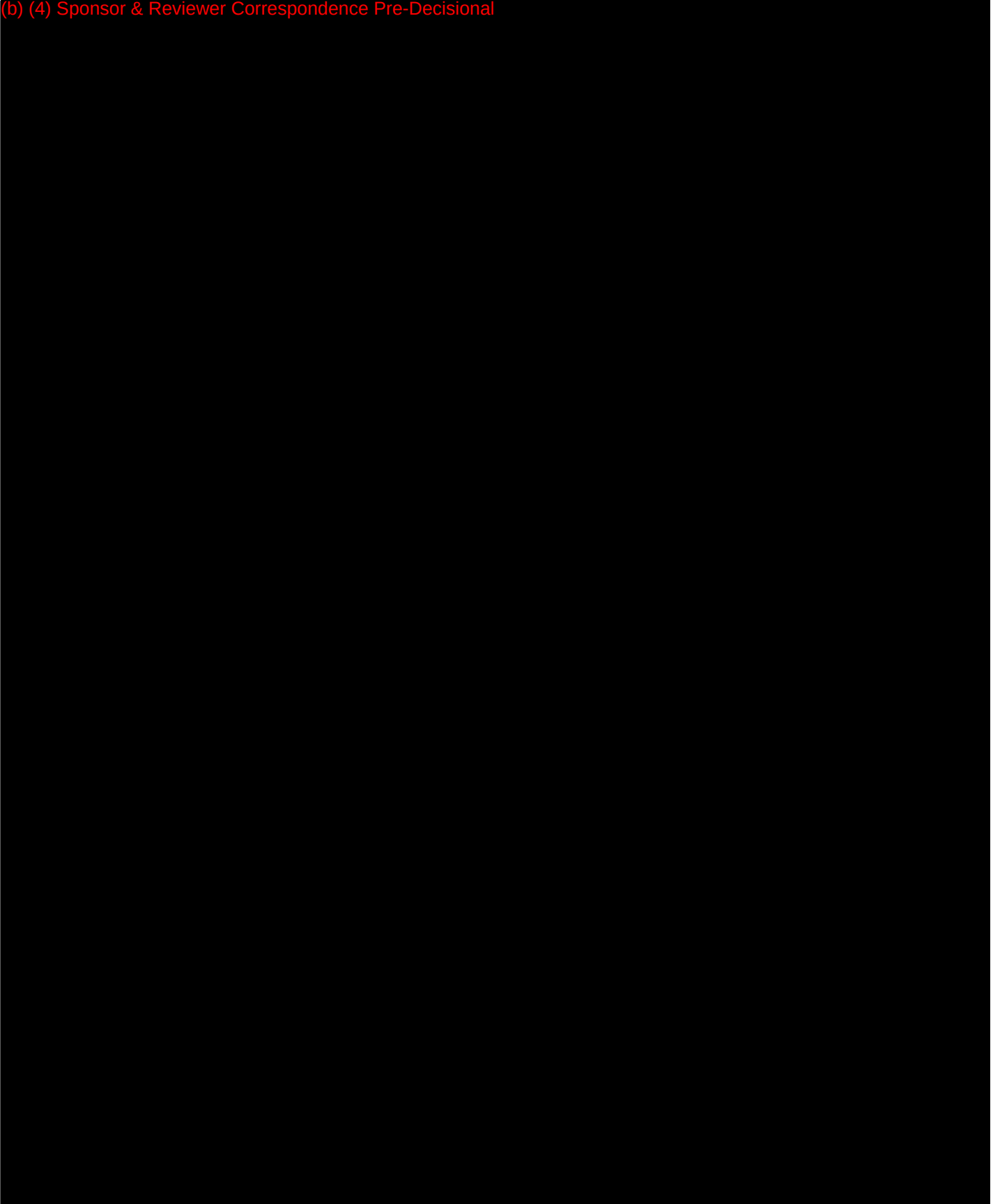
An electronic copy (eCopy) of the response is included with this submission. The eCopy is an exact duplicate of the paper submission. The eCopy is accompanied by an extra copy of the cover letter.

(b) (4), (b) (6)



1. Biocompatibility

(b) (4) Sponsor & Reviewer Correspondence Pre-Decisional



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Section 4: Indications for Use Statement

The Indication for Use statements for the proposed devices are provided on the following page.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Indications for Use Form

Indications for Use

510(k) Number (if known): _____

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Indications for Use:

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Prescription Use X
(Part 21 CFR 801 Subpart D)

AND/OR

Over-The-Counter Use _____
(21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE
OF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Page 1 of 1

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Section 5: 510(k) Summary

The 510(k) Summary for the Subject devices is on the following pages.

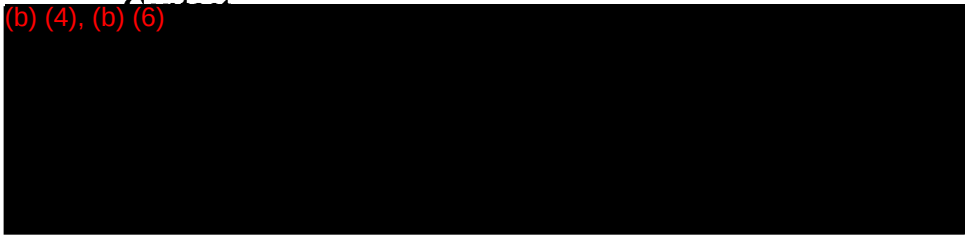
Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

510(k) Summary

Company

Ethicon Endo-Surgery, LLC
475 Calle C
Guaynabo, PR 00969

(b) (4), (b) (6)



Date Prepared: September 10, 2012

Device Name

Trade Name: ENSEAL® G2 Articulating Tissue Sealers
Common Name: Electrosurgical Cutting and Coagulation Instruments

Classification Names

- Electrosurgical, Cutting & Coagulation & Accessories
(21 CFR 878.4400, Product code GEI)
- Electrocautery, Gynecologic and Accessories
(21 CFR 884.4120, Product code HGI)

Predicate Device

ENSEAL® G2 Tissue Sealers, K112033

Device Description:

The Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers are sterile, single-use surgical instruments designed to seal and cut vessels, and to cut, grasp and dissect soft tissue during open and laparoscopic surgery.

The energy activation and knife lock are activated by a single button. The instrument shaft can be rotated using the rotation knob to facilitate visualization and enable easy access to targeted tissue. The power cord is permanently attached to the device and connects the instrument to the generator. The ENSEAL G2 Articulating Tissue Sealers include an articulation capability that allows the user to articulate the end-effector during surgical procedures.

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Indication for Use:

The ENSEAL® G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with the Generator GEN11 (GEN11). They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7mm and tissue and/or vascular bundles as large as will fit in the jaws of the instruments.

The ENSEAL® G2 Articulating Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures.

Technological Characteristics:

The Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers components are similar to the Predicate. The ENSEAL G2 Articulating Tissue Sealers have a 5 mm diameter shaft and are available in two shaft lengths: 35 and 45cm; and two jaw types: 3mm curved jaw and 5mm straight jaw. The jaws are normally in the opened position and can be partially or fully closed by squeezing the closing handle. The jaws have atraumatic teeth for grasping and holding targeted tissue when clamped. The handles of the Subject ENSEAL G2 Articulating Tissue Sealers have an ergonomic interface for the user. Like the Predicate, the Subject devices include an integrated energy activation button. The devices use a combination of the application of bipolar electrosurgical energy in conjunction with the I-BLADE knife, to compress, coagulate, and transect tissue.

In addition to the similar technological characteristics as the Predicate, the ENSEAL G2 Articulating Tissue Sealers instrument shaft device articulates approximately 60° to the left and the right of center. The instrument shaft can be articulated using the articulation wheel to gain additional access to tissue and facilitate the user's ability to target tissue and vascular bundles up to 60°. The top of the articulation wheel has a white arrow to indicate when the device shaft is straight and not articulated. When the articulation wheel is rotated clockwise, the shaft articulates to the right; when the articulation wheel is rotated counterclockwise, the shaft articulates to the left. While articulated, the device jaws seal and cut vessels, and cut, grasp and dissect soft tissue as intended. The Subject ENSEAL G2 Articulating Tissue Sealers performance is equivalent to the Predicate ENSEAL G2 Tissue Sealers.

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Performance Data:

Ex-vivo and in-vivo tests were performed to verify that the performance of the ENSEAL G2 Articulating Tissue Sealers meets the definition of substantial equivalence to the Predicate device, ENSEAL G2 Tissue Sealers. Device performance was assessed against design requirements, including a 30-day chronic survival study, to demonstrate that the Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers perform as intended.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers**Biocompatibility Evaluation of New Material****Biocompatibility Evaluation of Sodium Stearate, Vegetable**

Sodium Stearate, Vegetable is used as a lubricant on the flex joint. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. Biocompatibility of this material was evaluated on titanium 6Al-4V wire, and in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.2

Table 15.2 Biocompatibility Evaluation of Sodium Stearate, Vegetable

Test Type	Test Name	Test Report Number	Lab Name	Result
Cytotoxicity	Cytotoxicity (MEM-Elution)	Y2E019G	Northview Pacific	Pass
Analytical Test	Analytical Tests (IR)	F3730	Evans Analytical	Pass
Pyrogenicity	Pyrogenicity (LAL)	CTX011145.F	WuXi AppTec Laboratory Services	Pass
Pyrogenicity	Pyrogenicity (Pyrogenicity)	Project: 57911 Protocol: 9000770N	WuXi AppTec Laboratory Services	Pass

Sodium Stearate, Vegetable is considered biologically equivalent to Calcium Stearate. Biocompatibility of calcium stearate was evaluated on titanium 6Al-4V wire, and in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.3.

Table 15.3 Biocompatibility Evaluation of Calcium Stearate, Vegetable

Test Type	Test Name	Test Report Number	Lab Name	Result
Implantation	Implantation (119 day with Histopathology)	TU014-120, 13-2-0355-00074-TEST	NAMSA	Pass
Implantation	Implantation (60 day with Histopathology)	TU014-860, 13-2-0355-00074-TEST	NAMSA	Pass
Implantation	Implantation (30 day-muscle)	TU014-830, 13-2-0355-00074-TEST	NAMSA	Pass
Implantation	Implantation (7 day muscle)	TU014-807/s TU035-800, 13-2-0355-00074 TEST	NAMSA	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	V4J033G	Northview Laboratories Inc.	Pass
Hemocompatibility	Hemocompatibility (Hemolysis)	C4J138G	Northview Laboratories Inc.	Pass

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Test Type	Test Name	Test Report Number	Lab Name	Result
Cytotoxicity	Cytotoxicity (Agarose overlay)	V4J032G	Northview Laboratories Inc.	Pass
Pyrogenicity	Systemic Toxicity (Material-Mediated Pyrogenicity - rabbit)	X4J126G	Northview Pacific	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute Systemic Toxicity)	X4J128G	Northview Pacific	Pass
Genotoxicity	Genotoxicity (Ames Mutagenicity)	U4J010G	Northview Pacific	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	X4J127G	Northview Pacific	Pass
Irritation/ Intra cutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/ Irritation)	X4J128G	Northview Pacific	Pass

The Sodium Stearate, Vegetable and Calcium Stearate, Vegetable materials passed all biocompatibility tests recommended under ISO 10993-1 for Externally Communicating Devices in contact with Tissue, Bone, and Dentin for less than 24 hours ("Limited") as shown in Table 15.2; as well as the test suggested by the FDA (Acute Systemic Toxicity) for this patient contact category, and those considered supplementary tests.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers**Biocompatibility Evaluation of Polycarbonate, Calibre 2081-15**

The polycarbonate is used for the flex joint. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. Biocompatibility of this material was evaluated in isolation, in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.4

Table 15.4 Biocompatibility Evaluation of Polycarbonate, Calibre 2081-15

Test Type	Test Name	Test Report Number	Lab Name	Result
Cytotoxicity	Cytotoxicity (MEM-Elution)	V4E026G	Northview Laboratories Inc.	Pass
Cytotoxicity	Cytotoxicity (Agarose overlay)	V4E025G	Northview Laboratories Inc.	Pass
Hemocompatibility	Hemocompatibility (Hemolysis)	C4E026G	Northview Laboratories Inc.	Pass
Physico-Chemical	Physico-Chemical (USP Phys Chem)	C4E027G	Northview Laboratories Inc.	Pass
Irritation/ Intracutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/ Irritation)	X4D273G	Northview Pacific	Pass
Implantation	Implantation (Implantation (with Histo.)	X4D273G	Northview Pacific	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute Systemic Toxicity)	X4D273G	Northview Pacific	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	X4D275G	Northview Pacific	Pass
Pyrogenicity	Systemic Toxicity (Material-Mediated Pyrogenicity - rabbit)	X4D274G	Northview Pacific	Pass
Genotoxicity	Genotoxicity (Ames Mutagenicity)	U4D005G	Northview Pacific	Pass
Analytical Test	Analytical Tests (IR)	T4E008	Northview Pacific	Pass

The polycarbonate, Calibre 2081-15 material passed all biocompatibility tests required under ISO 10993-1 for Externally Communicating Devices in contact with Tissue, Bone or Dentin for less than 24 hours ("Limited"); as well as for the test suggested by the FDA (Acute Systemic Toxicity) for this patient contact category, and those considered supplementary tests.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers**Biocompatibility Evaluation of PolyEtherEtherKetone (PEEK), Victrex 381G / PEEK**

The PolyEtherEtherKetone (PEEK), Victrex 381G / PEEK is on the RF Sleeve Insulator. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. Biocompatibility of this material was evaluated partially in isolation (Implantation Test) and partially in combination with another device, and in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.5.

Table 15.5 Biocompatibility Evaluation of PolyEtherEtherKetone (PEEK), Victrex 381G / PEEK

Test Type	Test Name	Test Report Number	Lab Name	Result
Physico-Chemical	Physico-Chemical (USP Phys Chem)	ISR: 30681, Reference: 92093-4, 91798-15, File: 1572	In House Study	Pass
Hemocompatibility	Hemocompatibility (Partial Thromboplastin Time, PTT)	97G-0375	Toxikon	Pass
Hemocompatibility	Hemocompatibility (<i>In vitro</i> Hemocompatibility)	97G-0386	Toxikon	Pass
Implantation	Implantation (7 day muscle)	958340	Liberco Testing	Pass
Hemocompatibility	Hemocompatibility (Hemolysis (Direct))	Project: 3243, Protocol: HMD100, File: 1572	ViroMED	Pass
Genotoxicity	Genotoxicity (Ames Mutagenicity)	Project: 3275, Protocol: AM100, File 1572	ViroMED	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	Project:3165, Protocol: GM100 File: 1572	ViroMED	Pass
Pyrogenicity	Pyrogenicity (Pyrogenicity)	Project:3240 Protocol: RP100 File: 1572	ViroMED	Pass
Irritation/ Intracutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/Irritation)	Project: 3253 Protocol: IC100 File: 1572	ViroMED	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute)	Project: 3167 Protocol: ST100 File: 1572	ViroMED	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	Project: 3242, Protocol: MEM929, File: 1572	ViroMED	Pass

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers**Biocompatibility Evaluation of Titanium Nitride**

Titanium Nitride is the plating used on the I-Blade. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. Biocompatibility of this material was evaluated in combination with 17-4 stainless steel, which is the material used on the I-Blade. Biocompatibility of this material was evaluated in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.6.

Table 15.6 Biocompatibility Evaluation of Titanium Nitride (I-Blade Plating)

Test Type	Test Name	Test Report Number	Lab Name	Result
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	Lab: 12T_29166_04	NAMSA	Pass
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	Lab: 12T_29166_01	NAMSA	Pass
Analytical Test	Analytical Tests (Gas Chromatography Mass Spectrometry(GC/MS))	Lab: 12T_29166_05	NAMSA	Pass
Cytotoxicity	Cytotoxicity (Extract Colony Assay (JMHLW))	Study: 067720 Protocol: C11662-1	WuXi AppTec Laboratory Services	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	Study: 167721, Protocol: 140350-15	WuXi AppTec Laboratory Services	Pass
Hemocompatibility	Hemocompatibility (Hemolysis (Direct))	Study:167725 Protocol: 150300-12	WuXi AppTec Laboratory Services	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute)	Study: 167724 Protocol: 901770-21	WuXi AppTec Laboratory Services	Pass
Irritation/ Intracutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/Irritation)	Study:167723 Protocol: 9107015-2	WuXi AppTec Laboratory Services	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	Study:167722 Protocol: 900850-25	WuXi AppTec Laboratory Services	Pass

Titanium Nitride passed all biocompatibility tests required under ISO 10993-1 for Externally Communicating Devices in contact with Tissue, Bone, and Dentin for less than 24 hours ("Limited"); as well as the test suggested by the FDA (Acute Systemic Toxicity) for this patient contact category. In addition, Titanium Nitride was considered non-hemolytic in a supplementary Hemocompatibility test.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers**Biocompatibility Evaluation of Nickel-Tin Plating**

The Nickel-Tin Plating is used on the on the PTC Clip. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. This material was previously cleared in the Predicate Device Submission K112033. Biocompatibility of this material was evaluated in accordance with ISO 10993-1.

The biocompatibility for the Nickel-Tin Plating was evaluated in combination with additional materials. The test article subassembly, titled “silver metal jaw attached to black shaft with white printing,” included the 4cm distal end of the Predicate Device, which has the same materials as the Subject Device, except for the Titanium Nitride, PEEK, and Sodium Stearate, as listed in Table 15.1. The test methods and results are summarized in Tables 15.7 through 15.10.

Table 15.7 Biocompatibility Evaluations of Distal End Sub-Assembly, Including Nickel-Tin Plating

Test Type	Test Name	Test Report Number	Lab Name	Result
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	Lab: 11T_44631_02	NAMSA	Pass
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	Lab: 11T_44631_01	NAMSA	Pass
Analytical Test	Analytical Tests (ICP-Nickel Analysis)	Lab: 11T_44631_05, Lab: 11T_44631_06	NAMSA	Pass
Analytical Test	Analytical Tests (Gas Chromatography Mass Spectrometry(GCMS))	Lab: 11T_44631_08	NAMSA	Pass
Analytical Test	Analytical Tests (Gas Chromatography Mass Spectrometry(GCMS))	Lab: 11T_44631_07	NAMSA	Pass
Analytical Test	Analytical Tests (IR)	Lab: 11T_44631_03, 11T_44631_04	NAMSA	Pass
Cytotoxicity	Cytotoxicity (Extract Colony Assay (JMHLW))	Study:157895 Protocol: C11662-1	WuXi AppTec Laboratory Services	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	Study:157891 Protocol:140350-15	WuXi AppTec Laboratory Services	Pass
Hemocompatibility	Hemocompatibility (Hemolysis (Direct))	Study: 157896 Protocol: 150300-12	WuXi AppTec Laboratory Services	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute)	Study: 157894, Protocol: 901770-21	WuXi AppTec Laboratory Services	Pass

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Test Type	Test Name	Test Report Number	Lab Name	Result
Irritation/ Intracutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/Irritation)	Study: 157893 Protocol: 9107015-2	WuXi AppTec Laboratory Services	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	Study: 157892 Protocol: 900850-24	WuXi AppTec Laboratory Services	Pass

The ICP Report 11T_44631_05, ICP analysis of the water extract for the Nickel-Tin Plating, results were significantly below toxicologically acceptable exposure level, as shown in Table 15.8.

Table 15.8 ICP Spectroscopy Results for Water Extract of Nickel-Tin Plating

Element	Amount Detected (ppm)	Amount/device ^a	Acceptable Dose ^c	Comment
Molybdenum	2.5	23.5 µg (0.405 µg/kg/day) ^b	4 µg/kg/day (RfD)	No patient impact, > 9 times less than the RfD
Nickel	<0.05 BDL	< 0.47 µg	30 µg/day (PDE)	No patient impact, >3500 times less than PDE
Calcium	1.4	13.2 µg	250,000 µg/day (RDA)	No patient impact, > 1.8 x 10 ⁴ times less than the RDA
Magnesium	0.36	3.4 µg	350 µg/day (RDA)	No patient impact, > 1.0 x 10 ² times less than the RDA
Potassium	0.22	2.1 µg (0.036 µg/kg/day) ^b	30,000 µg/kg/day (RDI)	No patient impact, > 8 x 10 ⁵ times less than RDI

BDL: Below detection limit, PDE: Permitted Daily Exposure, RDA: Recommended Daily Allowance, RfD: Reference Dose, RDI: Recommended Daily Intake

^a Example calculation: (1.4 µg/g x 9.4 grams total test article) = 13.2 µg

^b Based on a 58 kg adult

^c The source for the acceptable dose limits is A. Wallace Hayes, Editor, Principles and Methods of Toxicology, Third Edition, New York: Raven Press, Pages 422, 425, and 429.

Calcium, Magnesium and Potassium naturally occur in humans at levels much in excess than the amount that leached from the device during testing. In addition, these elements

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are ingested in foods and nutritional supplements. Clinical chemistry reference ranges for serum levels are:

- Magnesium 1.7-2.2 mg/dL
- Calcium 8.5-10.2mg/dl
- Potassium 3.5-5.5 mEq/L

The patient exposure to the Molybdenum and Nickel from the device sub-assembly testing (which includes the PTC Clip composed of the 304 stainless steel and Nickel-Tin Plating) was compared to the acceptable dose as stated by Principles and Methods of Toxicology, Third Edition, New York and is further supported by the permissible daily exposure (PDE) as defined in the EMEA CHMP Guideline on the Specification Limits for Residues of Metal Catalysts or Metal Reagents. The results from the ICP analysis of the extract demonstrate that the patient exposure to elements found in this sub-assembly testing is less than the Acceptable Dose. The remaining elements, including Nickel, are Below Detection Limit (BDL). The elements from this testing, which includes the Nickel-Tin Plating, were either not detectable or detected in residual amounts that are significantly less than these values. Therefore, the Nickel-Tin Plating of the PTC Clip of the ENSEAL G2 Articulating Tissue Sealers can be considered biocompatible and poses no increased risk to the patient.

The ICP Report 11T_44631_08, GC/MS analysis of isopropyl alcohol extract for the Nickel-Tin Plating, results were significantly below toxicologically acceptable exposure level, as shown in Table 15.9.

Table 15.9 GC/MS Results for Isopropyl Extract of Nickel-Tin Plating

Isopropyl Extract	Amount Detected (ppm)	Amount/device ^a
1-phenyl-4-(3, 5-dimethylphenyl) buta-1, 3-diene	3.14 µg/mL	50.24 µg/device
benzene, 1, 1- (1, 5- hexadiene – 1, 6-diyl) bis-	3.00 µg/mL	48µg/device

^a Example calculation: (3.14 µg/mL x 48mL) /3 devices evaluated = 50.24 µg/device

Two organic compounds were observed in the isopropyl alcohol extract: 1-phenyl-4-(3, 5-dimethylphenyl) buta-1, 3-diene (3.14 µg/mL or 50.24 µg/device); and (CAS# 4439-45-6): benzene, 1, 1- (1, 5- hexadiene – 1, 6-diyl) bis- (3.00 µg/mL or 48µg/device). The levels of the two chemicals in the isopropyl extract are extremely low (approximately 3 ppm). The comprehensive in vitro and in vivo studies of the device, as discussed below, account for overall biocompatibility of bioavailable substances and showed no toxicity. The biocompatibility of these 2 organic compounds was tested for cytotoxicity, Sensitization, and Irritation as part of the in vitro and in vivo studies, which were provided in support of biocompatibility for the Nickel-Tin Plating on the sub-assembly. The evaluations include in vitro toxicity reports: Cytotoxicity (Report 157891), Cytotoxicity (Report 157895), and Hemolysis (Report 157896), and the in vivo toxicity reports: Systemic Toxicity (Report 157894), Irritation (Report 157893), and Sensitization

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(Report 157892). Under the conditions of each study, the 4cm distal end was considered non-cytotoxic.

In addition, based on structure-activity evaluation (using Derek Nexus 3.0 Lhasa Limited software), no structural alerts for these chemicals were noted for multiple toxicological endpoints. These endpoints include irritation, sensitization, mutagenicity, and carcinogenicity. Therefore, the presence of these chemicals does not represent any additional risk to the patient. Additionally, GC/MS analysis of the water extract revealed no semi-volatile compound above 2ppm, the limit of detection in the purified water extract.

The supplier of the Nickel-Tin Plating changed since the biocompatibility evaluation of the predicate device. Limited testing was performed on samples from each supplier to ensure biological equivalency. Biocompatibility of this material was evaluated in accordance with ISO 10993-1 studies. The test methods and results are summarized in Table 15.10.

Table 15.10 ICP Biocompatibility Evaluation of Nickel-Tin Plating, Bridge Testing

Test Type	Test Name	Test Report Number	Lab Name	Result
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	Lab: 12T_37839_01, Lab: 12T_37838_02	NAMSA	Pass
Analytical Test	Analytical Tests (IR)	Lab: 12T_37838_01 Lab: 12T_37839_02	NAMSA	Pass
Cytotoxicity	Cytotoxicity (Extract Colony Assay (JMHLW))	Study: 171502 Study: 171504	WuXi AppTec Laboratory Services	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	Study: 171505 Study: 171503	WuXi AppTec Laboratory Services	Pass

The Nickel-Tin Plating passed all biocompatibility tests recommended under ISO 10993-1 for Externally Communicating Devices in contact with Tissue, Bone or Dentin for less than 24 hours ("limited"); as well as the test suggested by the FDA (Acute Systemic Toxicity) for this patient contact category, and those considered supplementary tests. The Nickel-Tin Plating of the new supplier is biological equivalent to the plating in the predicate device.

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**Biocompatibility Testing of Patient –Contacting Components of the Device:
Evaluation for Potential Material Interactions**

The distal end of the subject production device was evaluated to account for potential interaction of patient-contacting materials (ISO 10993-1). Similar to the predicate device, no-adverse effects were expected from the combination of materials used in the Subject Device. However, a supplementary cytotoxicity test was conducted to confirm this assumption. This test was considered toxicologically adequate for this objective. The test title and result are summarized in Table 15.11.

Table 15.11 Biocompatibility Testing of Patient-Contacting Components of the Device: Evaluation for Potential Material Interactions

Test Type	Test Name	Test Report Number	Lab Name	Result
Cytotoxicity	Cytotoxicity (MEM-Endpoint Dilution)	Study: 172325 Protocol: 140350-15	WuXi AppTec Laboratory Services	Pass

As expected, no cytotoxicity was observed in this test. Based on the comprehensive absence of toxicity, no biologically relevant interactions of patient-contact materials were observed. Similar to the predicate device, the assumption of no adverse material interactions was confirmed. Based on the absence of cytotoxicity, no biologically relevant interactions of patient-contacting materials were observed for the device.

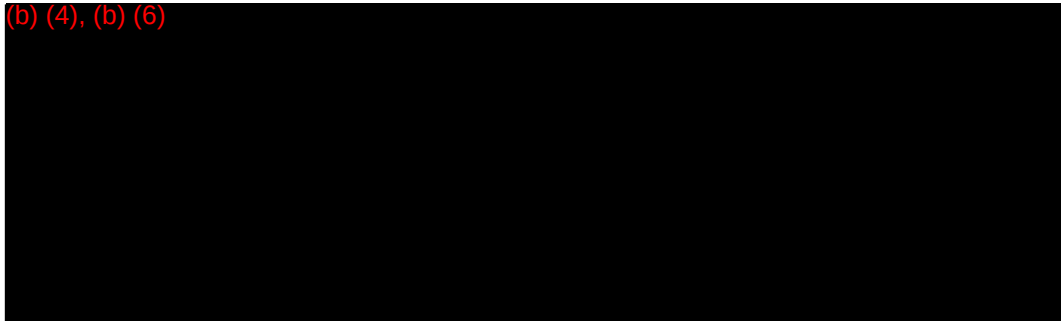


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January 17, 2013

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The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) acknowledges receipt of your submission. This submission has been assigned the following unique document control number. Failure to reference this assigned number in future correspondence may result in processing delays.

510(k) Number: K122797/S002

Device: Enseal G2 Articulating Tissue Sealers, Straight Jaw, Enseal G2 Articulating Tissue Sealers, Curved Jaw

Dated: 16-JAN-2013

Received: 17-JAN-2013

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Sincerely yours,
Samie Allen
Policy Analyst
Office of Device Evaluation
Center for Devices and Radiological Health

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K122797/S002

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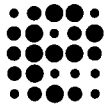
Mcdonald, Lisa *

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**Ethicon
Endo-Surgery**

PART OF THE JOHNSON & JOHNSON FAMILY OF COMPANIES

Ethicon Endo-Surgery, Inc.
4545 Creek Road
Cincinnati, OH 45242

January 16, 2013

Mykhaylo B. Nosovskyy, Ph.D
Mechanical Engineer
General Surgery Devices Branch
US Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

FDA CDRH DMC

JAN 17 2013

Received

Tel. # 301-796-3745
Fax # 301-847-8119
mykhaylo.nosovskyy@fda.hhs.gov

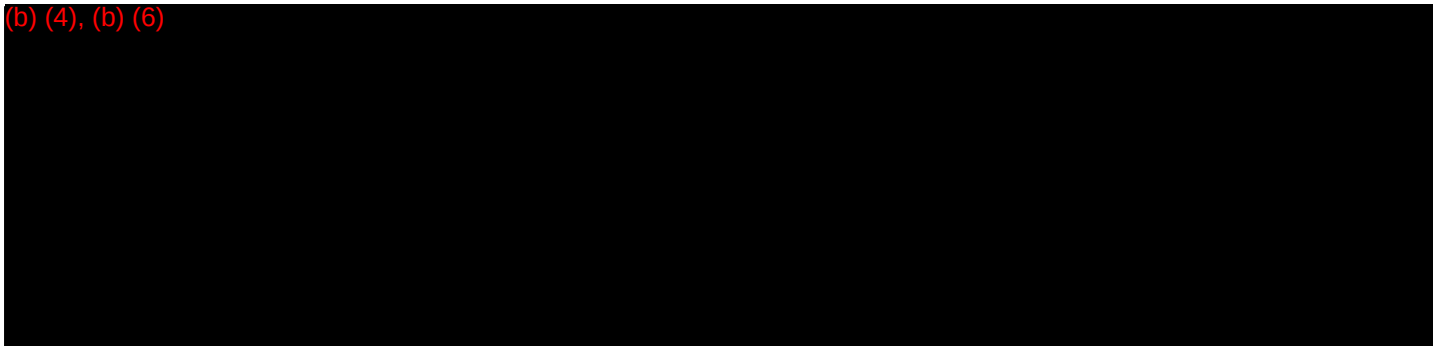
Re: K122797, ENSEAL G2 Articulating Tissue Sealers

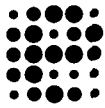
Dear Dr. Nosovskyy:

Thank you for your review of the 510(k) submission K122797. I have addressed your questions and made the requested changes in response to your request dated December 19, 2012. Please see the attached pages with the questions and responses. The Section 4 Indications For Use and Section 5 510k Summary have been included for your reference, there are no changes to these documents.

An electronic copy (eCopy) of the response is included with this submission. The eCopy is an exact duplicate of the paper submission. The eCopy is accompanied by an extra copy of the cover letter.

(b) (4), (b) (6)





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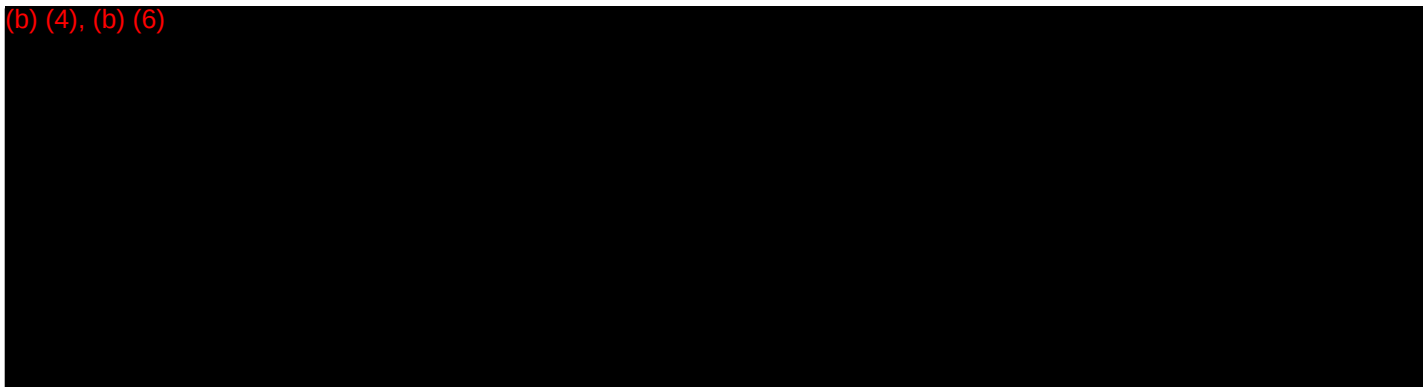
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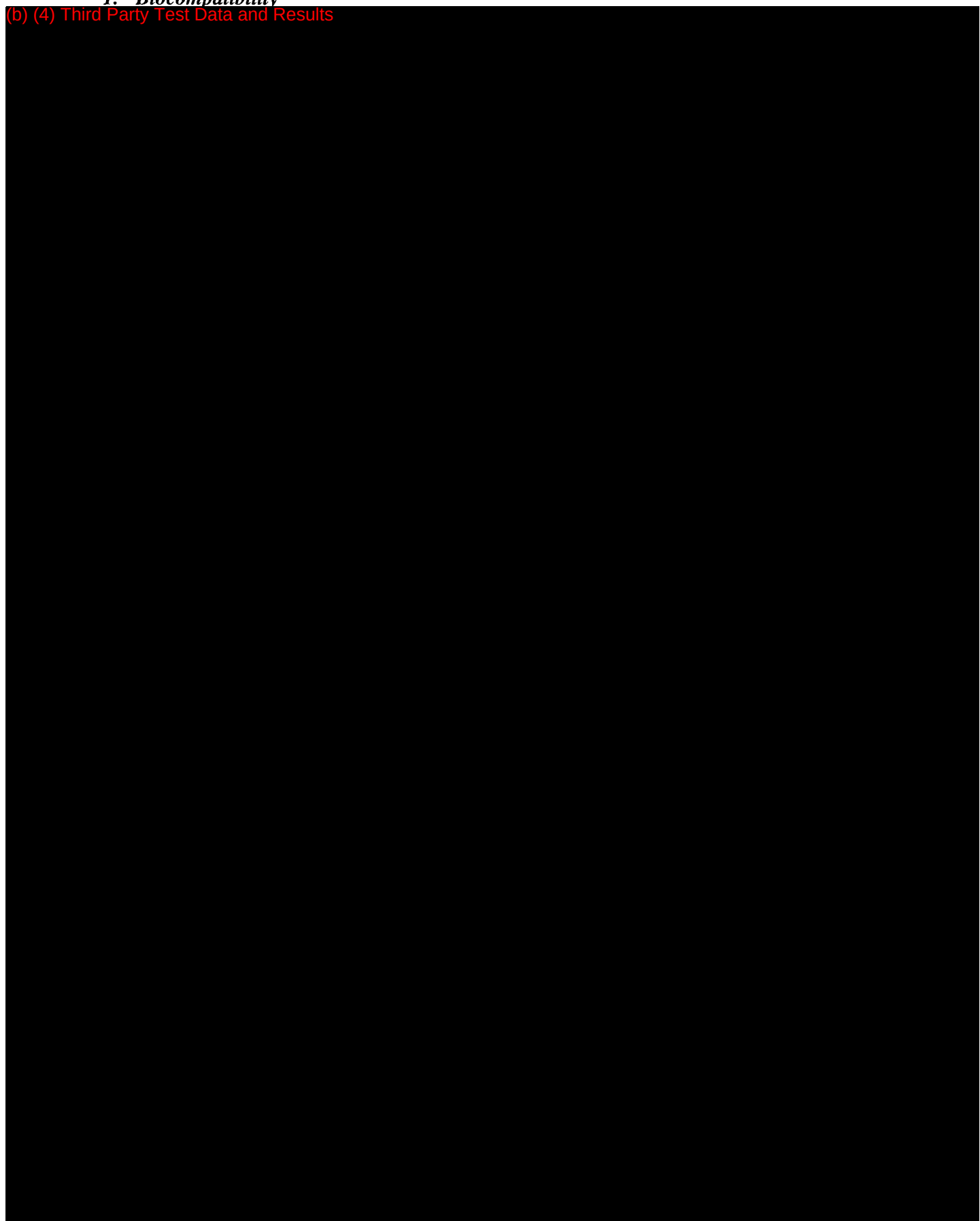
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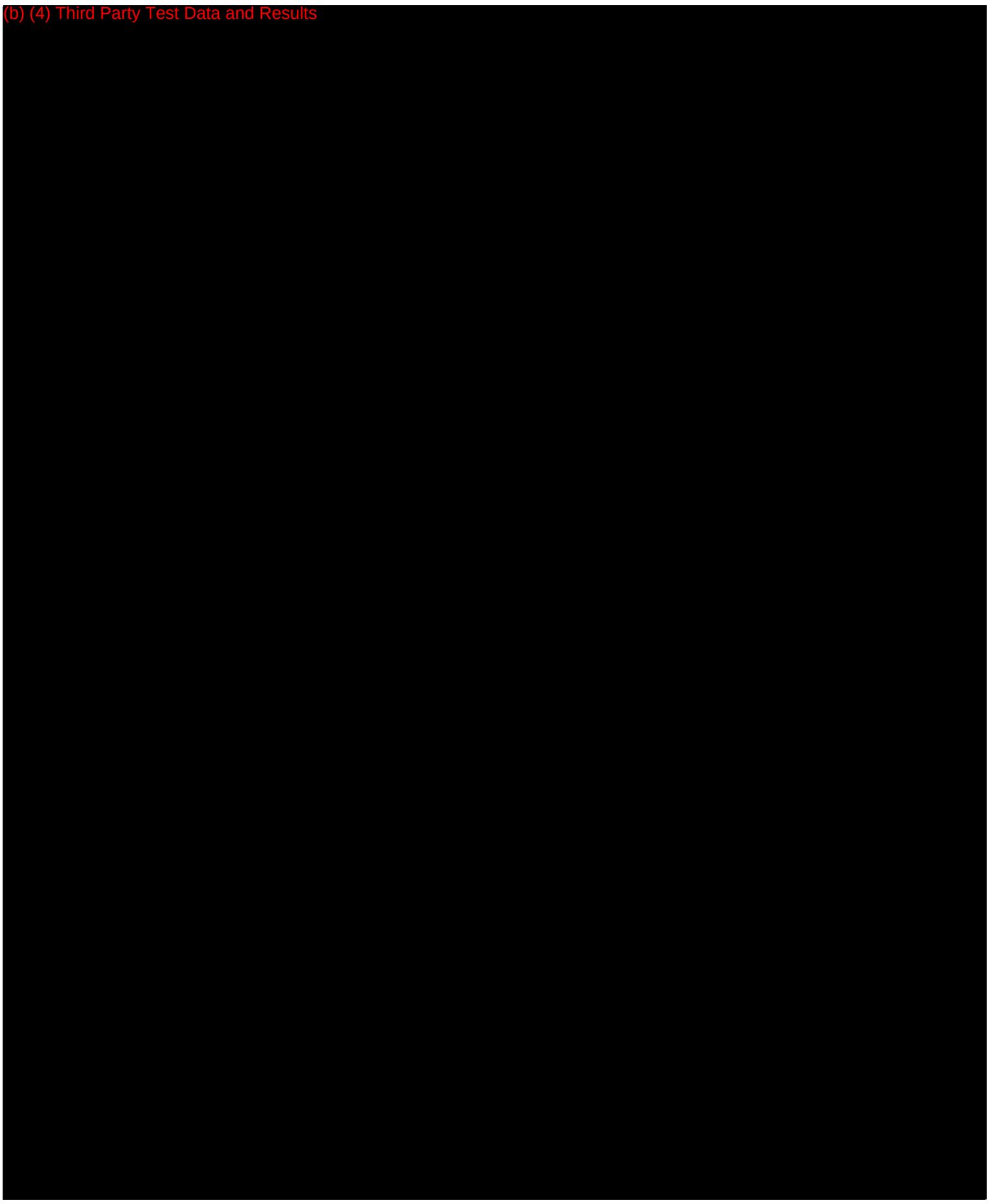


1. Biocompatibility

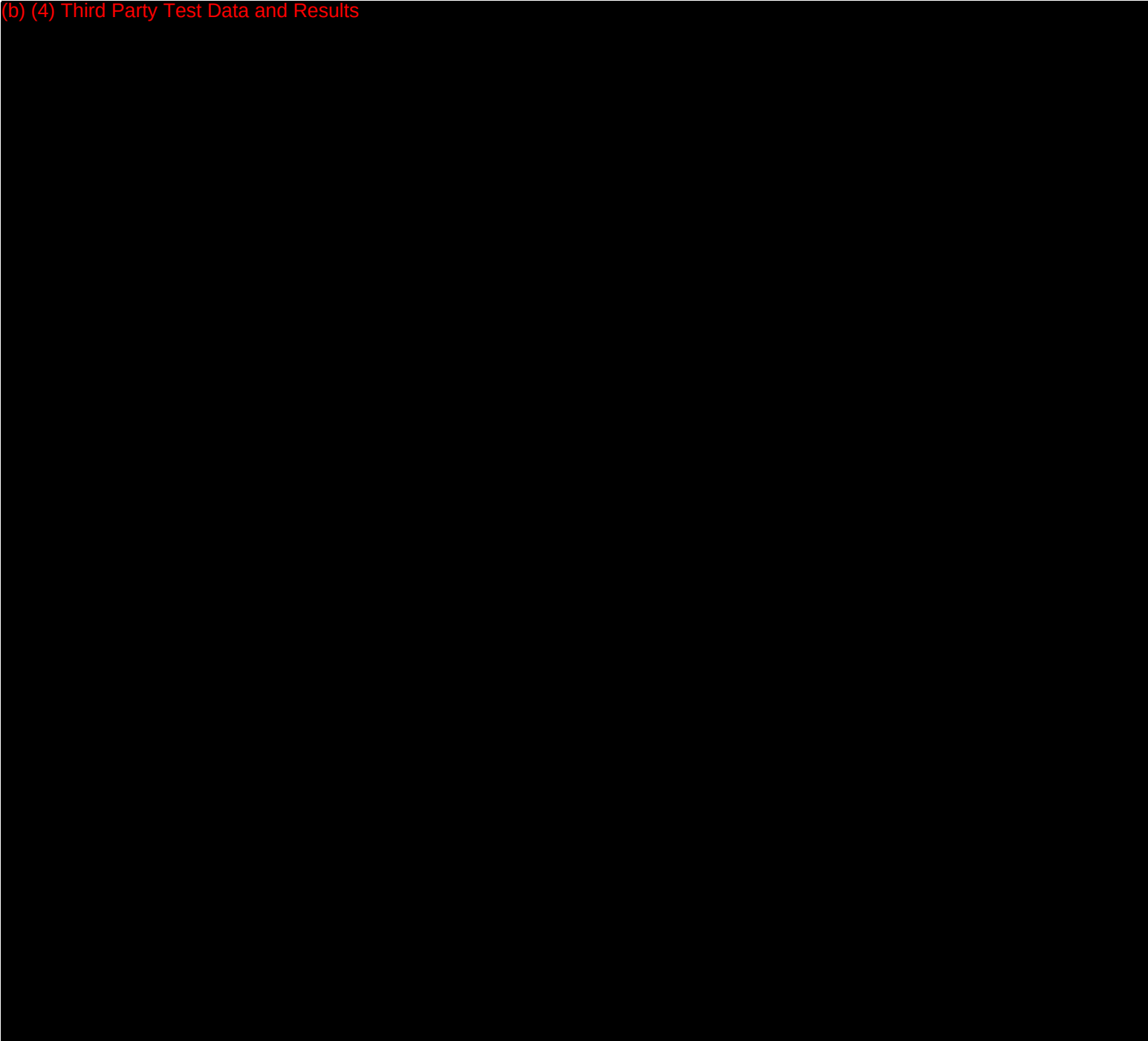
(b) (4) Third Party Test Data and Results



(b) (4) Third Party Test Data and Results



(b) (4) Third Party Test Data and Results



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Section 4: Indications for Use Statement

The Indication for Use statements for the proposed devices are provided on the following page.

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Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Indications for Use Form

Indications for Use

510(k) Number (if known): _____

Device Name: ENSEAL® G2 Articulating Tissue Sealers

Indications for Use:

The ENSEAL® G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with the Generator GEN11 (GEN11). They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7mm and tissue and/or vascular bundles as large as will fit in the jaws of the instruments.

The ENSEAL® G2 Articulating Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures.

Prescription Use X
(Part 21 CFR 801 Subpart D)

AND/OR

Over-The-Counter Use _____
(21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE
OF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Page 1 of 1

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Section 5: 510(k) Summary

The 510(k) Summary for the Subject devices is on the following pages.

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Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

510(k) Summary

Company

Ethicon Endo-Surgery, LLC
475 Calle C
Guaynabo, PR 00969

(b) (4), (b) (6)



Date Prepared: September 10, 2012

Device Name

Trade Name: ENSEAL® G2 Articulating Tissue Sealers
Common Name: Electrosurgical Cutting and Coagulation Instruments

Classification Names

- Electrosurgical, Cutting & Coagulation & Accessories
(21 CFR 878.4400, Product code GEI)
- Electrocautery, Gynecologic and Accessories
(21 CFR 884.4120, Product code HGI)

Predicate Device

ENSEAL® G2 Tissue Sealers, K112033

Device Description:

The Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers are sterile, single-use surgical instruments designed to seal and cut vessels, and to cut, grasp and dissect soft tissue during open and laparoscopic surgery.

The energy activation and knife lock are activated by a single button. The instrument shaft can be rotated using the rotation knob to facilitate visualization and enable easy access to targeted tissue. The power cord is permanently attached to the device and connects the instrument to the generator. The ENSEAL G2 Articulating Tissue Sealers include an articulation capability that allows the user to articulate the end-effector during surgical procedures.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Indication for Use:

The ENSEAL® G2 Articulating Tissue Sealers are indicated for bipolar coagulation and mechanical transection of tissue during laparoscopic and open procedures.

The devices are bipolar electrosurgical instruments for use with the Generator GEN11 (GEN11). They are intended for use during open or laparoscopic, general and gynecological surgery to cut and seal vessels, and to cut, grasp and dissect tissue during surgery.

Indications for use include open and laparoscopic, general and gynecological surgical procedures (including urologic, thoracic, plastic and reconstructive, bowel resections, hysterectomies, cholecystectomies, gall bladder procedures, Nissen fundoplication, adhesiolysis, oophorectomies, etc.), or any procedure where vessel ligation (cutting and sealing), tissue grasping and dissection is performed. The devices can be used on vessels up to (and including) 7mm and tissue and/or vascular bundles as large as will fit in the jaws of the instruments.

The ENSEAL® G2 Articulating Tissue Sealers have not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures. Do not use these devices for these procedures.

Technological Characteristics:

The Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers components are similar to the Predicate. The ENSEAL G2 Articulating Tissue Sealers have a 5 mm diameter shaft and are available in two shaft lengths: 35 and 45cm; and two jaw types: 3mm curved jaw and 5mm straight jaw. The jaws are normally in the opened position and can be partially or fully closed by squeezing the closing handle. The jaws have atraumatic teeth for grasping and holding targeted tissue when clamped. The handles of the Subject ENSEAL G2 Articulating Tissue Sealers have an ergonomic interface for the user. Like the Predicate, the Subject devices include an integrated energy activation button. The devices use a combination of the application of bipolar electrosurgical energy in conjunction with the I-BLADE knife, to compress, coagulate, and transect tissue.

In addition to the similar technological characteristics as the Predicate, the ENSEAL G2 Articulating Tissue Sealers instrument shaft device articulates approximately 60° to the left and the right of center. The instrument shaft can be articulated using the articulation wheel to gain additional access to tissue and facilitate the user's ability to target tissue and vascular bundles up to 60°. The top of the articulation wheel has a white arrow to indicate when the device shaft is straight and not articulated. When the articulation wheel is rotated clockwise, the shaft articulates to the right; when the articulation wheel is rotated counterclockwise, the shaft articulates to the left. While articulated, the device jaws seal and cut vessels, and cut, grasp and dissect soft tissue as intended. The Subject ENSEAL G2 Articulating Tissue Sealers performance is equivalent to the Predicate ENSEAL G2 Tissue Sealers.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

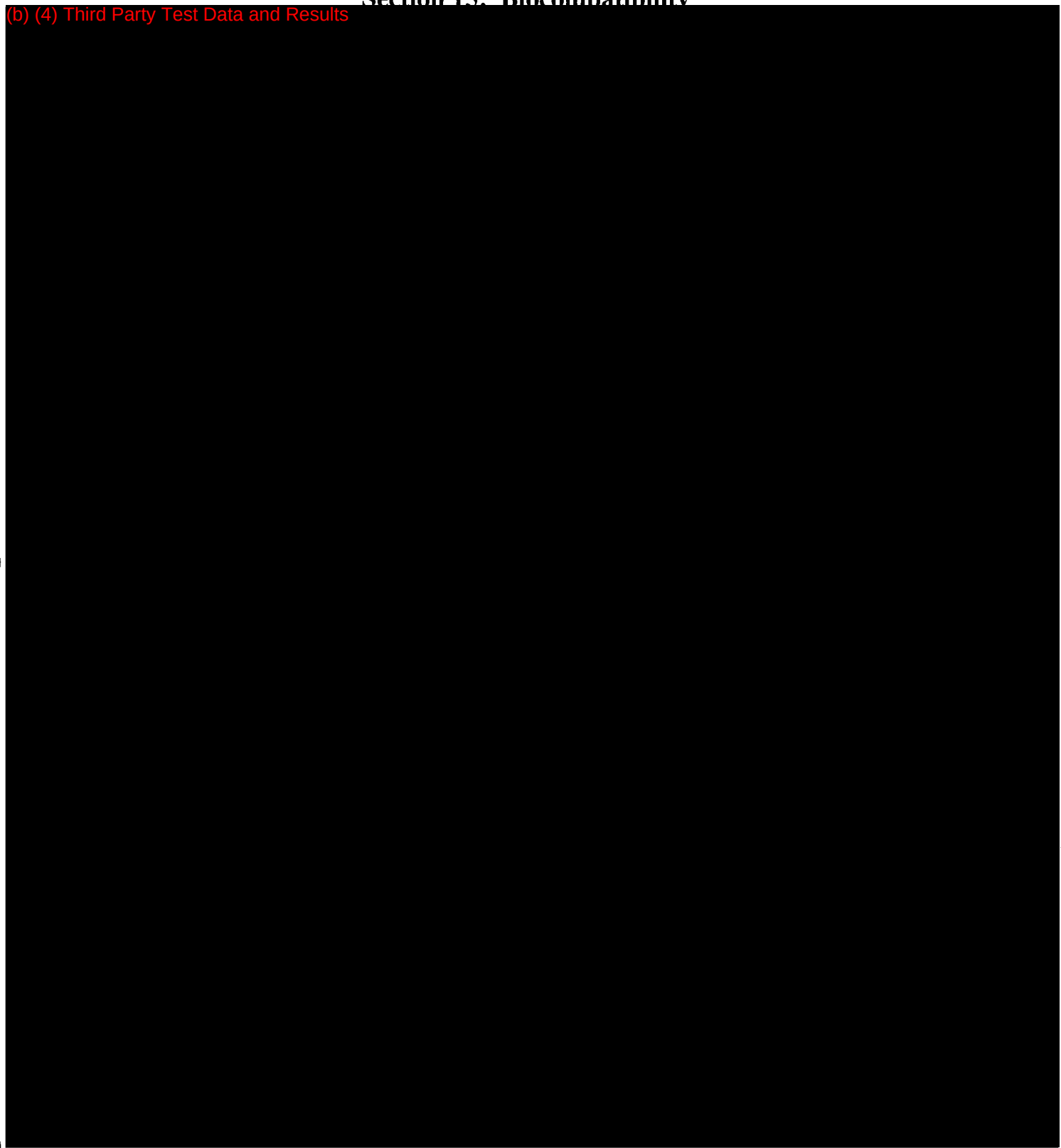
Performance Data:

Ex-vivo and in-vivo tests were performed to verify that the performance of the ENSEAL G2 Articulating Tissue Sealers meets the definition of substantial equivalence to the Predicate device, ENSEAL G2 Tissue Sealers. Device performance was assessed against design requirements, including a 30-day chronic survival study, to demonstrate that the Ethicon Endo-Surgery ENSEAL G2 Articulating Tissue Sealers perform as intended.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Section 15: Biocompatibility

(b) (4) Third Party Test Data and Results



Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Biocompatibility Evaluation of New Material

Biocompatibility Evaluation of Sodium Stearate, Vegetable

Sodium Stearate, Vegetable is used as a lubricant on the flex joint. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. Biocompatibility of this material was evaluated on titanium 6Al-4V wire, and in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.2

Table 15.2 Biocompatibility Evaluation of Sodium Stearate, Vegetable

Test Type	Test Name	Test Report Number	Lab Name	Result
Cytotoxicity	Cytotoxicity (MEM-Elution)	Y2E019G	Northview Pacific	Pass
Analytical Test	Analytical Tests (IR)	F3730	Evans Analytical	Pass
Pyrogenicity	Pyrogenicity (LAL)	CTX011145.F	WuXi AppTec Laboratory Services	Pass
Pyrogenicity	Pyrogenicity (Pyrogenicity)	Project: 57911 Protocol: 9000770N	WuXi AppTec Laboratory Services	Pass

Sodium Stearate, Vegetable is considered biologically equivalent to Calcium Stearate. Biocompatibility of calcium stearate was evaluated on titanium 6Al-4V wire, and in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.3.

Table 15.3 Biocompatibility Evaluation of Calcium Stearate, Vegetable

Test Type	Test Name	Test Report Number	Lab Name	Result
Implantation	Implantation (119 day with Histopathology)	TU014-120, 13-2-0355-00074-TEST	NAMSA	Pass
Implantation	Implantation (60 day with Histopathology)	TU014-860, 13-2-0355-00074-TEST	NAMSA	Pass
Implantation	Implantation (30 day-muscle)	TU014-830, 13-2-0355-00074-TEST	NAMSA	Pass
Implantation	Implantation (7 day muscle)	TU014-807/s TU035-800, 13-2-0355-00074 TEST	NAMSA	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	V4J033G	Northview Laboratories Inc.	Pass
Hemocompatibility	Hemocompatibility (Hemolysis)	C4J138G	Northview Laboratories Inc.	Pass

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Test Type	Test Name	Test Report Number	Lab Name	Result
Cytotoxicity	Cytotoxicity (Agarose overlay)	V4J032G	Northview Laboratories Inc.	Pass
Pyrogenicity	Systemic Toxicity (Material-Mediated Pyrogenicity - rabbit)	X4J126G	Northview Pacific	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute Systemic Toxicity)	X4J128G	Northview Pacific	Pass
Genotoxicity	Genotoxicity (Ames Mutagenicity)	U4J010G	Northview Pacific	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	X4J127G	Northview Pacific	Pass
Irritation/ Intra cutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/ Irritation)	X4J128G	Northview Pacific	Pass

The Sodium Stearate, Vegetable and Calcium Stearate, Vegetable materials passed all biocompatibility tests recommended under ISO 10993-1 for Externally Communicating Devices in contact with Tissue, Bone, and Dentin for less than 24 hours ("Limited") as shown in Table 15.2; as well as the test suggested by the FDA (Acute Systemic Toxicity) for this patient contact category, and those considered supplementary tests.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers**Biocompatibility Evaluation of Polycarbonate, Calibre 2081-15**

The polycarbonate is used for the flex joint. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. Biocompatibility of this material was evaluated in isolation, in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.4

Table 15.4 Biocompatibility Evaluation of Polycarbonate, Calibre 2081-15

Test Type	Test Name	Test Report Number	Lab Name	Result
Cytotoxicity	Cytotoxicity (MEM-Elution)	V4E026G	Northview Laboratories Inc.	Pass
Cytotoxicity	Cytotoxicity (Agarose overlay)	V4E025G	Northview Laboratories Inc.	Pass
Hemocompatibility	Hemocompatibility (Hemolysis)	C4E026G	Northview Laboratories Inc.	Pass
Physico-Chemical	Physico-Chemical (USP Phys Chem)	C4E027G	Northview Laboratories Inc.	Pass
Irritation/ Intracutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/ Irritation)	X4D273G	Northview Pacific	Pass
Implantation	Implantation (Implantation (with Histo.)	X4D273G	Northview Pacific	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute Systemic Toxicity)	X4D273G	Northview Pacific	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	X4D275G	Northview Pacific	Pass
Pyrogenicity	Systemic Toxicity (Material-Mediated Pyrogenicity - rabbit)	X4D274G	Northview Pacific	Pass
Genotoxicity	Genotoxicity (Ames Mutagenicity)	U4D005G	Northview Pacific	Pass
Analytical Test	Analytical Tests (IR)	T4E008	Northview Pacific	Pass

The polycarbonate, Calibre 2081-15 material passed all biocompatibility tests required under ISO 10993-1 for Externally Communicating Devices in contact with Tissue, Bone or Dentin for less than 24 hours ("Limited"); as well as for the test suggested by the FDA (Acute Systemic Toxicity) for this patient contact category, and those considered supplementary tests.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Biocompatibility Evaluation of PolyEtherEtherKetone (PEEK), Victrex 381G / PEEK

The PolyEtherEtherKetone (PEEK), Victrex 381G / PEEK is on the RF Sleeve Insulator. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. Biocompatibility of this material was evaluated partially in isolation (Implantation Test) and partially in combination with another device, and in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.5.

Table 15.5 Biocompatibility Evaluation of PolyEtherEtherKetone (PEEK), Victrex 381G / PEEK

Test Type	Test Name	Test Report Number	Lab Name	Result
Physico-Chemical	Physico-Chemical (USP Phys Chem)	ISR: 30681, Reference: 92093-4, 91798-15, File: 1572	In House Study	Pass
Hemocompatibility	Hemocompatibility (Partial Thromboplastin Time, PTT)	97G-0375	Toxikon	Pass
Hemocompatibility	Hemocompatibility (<i>In vitro</i> Hemocompatibility)	97G-0386	Toxikon	Pass
Implantation	Implantation (7 day muscle)	958340	Liberco Testing	Pass
Hemocompatibility	Hemocompatibility (Hemolysis (Direct))	Project: 3243, Protocol: HMD100, File: 1572	ViroMED	Pass
Genotoxicity	Genotoxicity (Ames Mutagenicity)	Project: 3275, Protocol: AM100, File 1572	ViroMED	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	Project: 3165, Protocol: GM100 File: 1572	ViroMED	Pass
Pyrogenicity	Pyrogenicity (Pyrogenicity)	Project: 3240 Protocol: RP100 File: 1572	ViroMED	Pass
Irritation/ Intracutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/Irritation)	Project: 3253 Protocol: IC100 File: 1572	ViroMED	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute)	Project: 3167 Protocol: ST100 File: 1572	ViroMED	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	Project: 3242, Protocol: MEM929, File: 1572	ViroMED	Pass

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers**Biocompatibility Evaluation of Titanium Nitride**

Titanium Nitride is the plating used on the I-Blade. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. Biocompatibility of this material was evaluated in combination with 17-4 stainless steel, which is the material used on the I-Blade. Biocompatibility of this material was evaluated in accordance with ISO 10993-1. The test methods and results are summarized in Table 15.6.

Table 15.6 Biocompatibility Evaluation of Titanium Nitride (I-Blade Plating)

Test Type	Test Name	Test Report Number	Lab Name	Result
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	Lab: 12T_29166_04	NAMSA	Pass
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	Lab: 12T_29166_01	NAMSA	Pass
Analytical Test	Analytical Tests (Gas Chromatography Mass Spectrometry(GC/MS))	Lab: 12T_29166_05	NAMSA	Pass
Cytotoxicity	Cytotoxicity (Extract Colony Assay (JMHLW))	Study: 067720 Protocol: C11662-1	WuXi AppTec Laboratory Services	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	Study: 167721, Protocol: 140350-15	WuXi AppTec Laboratory Services	Pass
Hemocompatibility	Hemocompatibility (Hemolysis (Direct))	Study: 167725 Protocol: 150300-12	WuXi AppTec Laboratory Services	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute)	Study: 167724 Protocol: 901770-21	WuXi AppTec Laboratory Services	Pass
Irritation/ Intracutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/Irritation)	Study: 167723 Protocol: 9107015-2	WuXi AppTec Laboratory Services	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	Study: 167722 Protocol: 900850-25	WuXi AppTec Laboratory Services	Pass

Titanium Nitride passed all biocompatibility tests required under ISO 10993-1 for Externally Communicating Devices in contact with Tissue, Bone, and Dentin for less than 24 hours ("Limited"); as well as the test suggested by the FDA (Acute Systemic Toxicity) for this patient contact category. In addition, Titanium Nitride was considered non-hemolytic in a supplementary Hemocompatibility test.

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers**Biocompatibility Evaluation of Nickel-Tin Plating**

The Nickel-Tin Plating is used on the on the PTC Clip. This component comes in contact with Tissue, Bone or Dentin for less than 24 hours. This material was previously cleared in the Predicate Device Submission K112033. Biocompatibility of this material was evaluated in accordance with ISO 10993-1.

The biocompatibility for the Nickel-Tin Plating was evaluated in combination with additional materials. The test article subassembly, titled "silver metal jaw attached to black shaft with white printing," included the 4cm distal end of the Predicate Device, which has the same materials as the Subject Device, except for the Titanium Nitride, PEEK, and Sodium Stearate, as listed in Table 15.1. The test methods and results are summarized in Tables 15.7 through 15.10.

Table 15.7 Biocompatibility Evaluations of Distal End Sub-Assembly, Including Nickel-Tin Plating

Test Type	Test Name	Test Report Number	Lab Name	Result
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	Lab: 11T_44631_02	NAMSA	Pass
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	Lab: 11T_44631_01	NAMSA	Pass
Analytical Test	Analytical Tests (ICP-Nickel Analysis)	Lab: 11T_44631_05, Lab: 11T_44631_06	NAMSA	Pass
Analytical Test	Analytical Tests (Gas Chromatography Mass Spectrometry(GCMS))	Lab: 11T_44631_08	NAMSA	Pass
Analytical Test	Analytical Tests (Gas Chromatography Mass Spectrometry(GCMS))	Lab: 11T_44631_07	NAMSA	Pass
Analytical Test	Analytical Tests (IR)	Lab: 11T_44631_03, 11T_44631_04	NAMSA	Pass
Cytotoxicity	Cytotoxicity (Extract Colony Assay (JMHLW))	Study:157895 Protocol: C11662-1	WuXi AppTec Laboratory Services	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	Study:157891 Protocol:140350-15	WuXi AppTec Laboratory Services	Pass
Hemocompatibility	Hemocompatibility (Hemolysis (Direct))	Study: 157896 Protocol: 150300-12	WuXi AppTec Laboratory Services	Pass
Acute Systemic Toxicity	Systemic Toxicity (Acute)	Study: 157894, Protocol: 901770-21	WuXi AppTec Laboratory Services	Pass

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

Test Type	Test Name	Test Report Number	Lab Name	Result
Irritation/ Intracutaneous Reactivity	Irritation/Intracutaneous Reactivity (Intracutaneous/Irritation)	Study: 157893 Protocol: 9107015-2	WuXi AppTec Laboratory Services	Pass
Sensitization	Sensitization (Guinea Pig, Maximization)	Study: 157892 Protocol: 900850-24	WuXi AppTec Laboratory Services	Pass

The ICP Report 11T_44631_05, ICP analysis of the water extract for the Nickel-Tin Plating, results were significantly below toxicologically acceptable exposure level, as shown in Table 15.8.

Table 15.8 ICP Spectroscopy Results for Water Extract of Nickel-Tin Plating

Element	Amount Detected (ppm)	Amount/device ^a	Acceptable Dose ^c	Comment
Molybdenum	2.5	23.5 µg (0.405 µg/kg/day) ^b	4 µg/kg/ day (RfD)	No patient impact, > 9 times less than the RfD
Nickel	<0.05 BDL	< 0.47 µg	30 µg/day (PDE)	No patient impact, >3500 times less than PDE
Calcium	1.4	13.2 µg	250,000 µg/day (RDA)	No patient impact, > 1.8 x 10 ⁴ times less than the RDA
Magnesium	0.36	3.4 µg	350 µg/day (RDA)	No patient impact, > 1.0 x 10 ² times less than the RDA
Potassium	0.22	2.1 µg (0.036 µg/kg/day) ^b	30,000 µg/kg/day (RDI)	No patient impact, > 8 x 10 ⁵ times less than RDI

BDL: Below detection limit, PDE: Permitted Daily Exposure, RDA: Recommended Daily Allowance, RfD: Reference Dose, RDI: Recommended Daily Intake

^a Example calculation: (1.4 µg/g x 9.4 grams total test article) = 13.2 µg

^b Based on a 58 kg adult

^c The source for the acceptable dose limits is A. Wallace Hayes, Editor, Principles and Methods of Toxicology, Third Edition, New York: Raven Press, Pages 422, 425, and 429.

Calcium, Magnesium and Potassium naturally occur in humans at levels much in excess than the amount that leached from the device during testing. In addition, these elements

Ethicon Endo-Surgery, LLC, 510(k) Premarket Notification (Traditional) for ENSEAL® G2 Articulating Tissue Sealers

are ingested in foods and nutritional supplements. Clinical chemistry reference ranges for serum levels are:

- Magnesium 1.7-2.2 mg/dL
- Calcium 8.5-10.2mg/dl
- Potassium 3.5-5.5 mEq/L

The patient exposure to the Molybdenum and Nickel from the device sub-assembly testing (which includes the PTC Clip composed of the 304 stainless steel and Nickel-Tin Plating) was compared to the acceptable dose as stated by Principles and Methods of Toxicology, Third Edition, New York and is further supported by the permissible daily exposure (PDE) as defined in the EMEA CHMP Guideline on the Specification Limits for Residues of Metal Catalysts or Metal Reagents. The results from the ICP analysis of the extract demonstrate that the patient exposure to elements found in this sub-assembly testing is less than the Acceptable Dose. The remaining elements, including Nickel, are Below Detection Limit (BDL). The elements from this testing, which includes the Nickel-Tin Plating, were either not detectable or detected in residual amounts that are significantly less than these values. Therefore, the Nickel-Tin Plating of the PTC Clip of the ENSEAL G2 Articulating Tissue Sealers can be considered biocompatible and poses no increased risk to the patient.

The ICP Report 11T_44631_08, GC/MS analysis of isopropyl alcohol extract for the Nickel-Tin Plating, results were significantly below toxicologically acceptable exposure level, as shown in Table 15.9.

Table 15.9 GC/MS Results for Isopropyl Extract of Nickel-Tin Plating

Isopropyl Extract	Amount Detected (ppm)	Amount/device ^a
1-phenyl-4-(3, 5-dimethylphenyl) buta-1, 3-diene	3.14 µg/mL	50.24 µg/device
benzene, 1, 1- (1, 5- hexadiene – 1, 6-diyl) bis-	3.00 µg/mL	48µg/device

^a Example calculation: (3.14 µg/mL x 48mL) /3 devices evaluated = 50.24 µg/device

Two organic compounds were observed in the isopropyl alcohol extract: 1-phenyl-4-(3, 5-dimethylphenyl) buta-1, 3-diene (3.14 µg/mL or 50.24 µg/device); and (CAS# 4439-45-6): benzene, 1, 1- (1, 5- hexadiene – 1, 6-diyl) bis- (3.00 µg/mL or 48µg/device). The levels of the two chemicals in the isopropyl extract are extremely low (approximately 3 ppm). The comprehensive in vitro and in vivo studies of the device, as discussed below, account for overall biocompatibility of bioavailable substances and showed no toxicity. The biocompatibility of these 2 organic compounds was tested for cytotoxicity, Sensitization, and Irritation as part of the in vitro and in vivo studies, which were provided in support of biocompatibility for the Nickel-Tin Plating on the sub-assembly. The evaluations include in vitro toxicity reports: Cytotoxicity (Report 157891), Cytotoxicity (Report 157895), and Hemolysis (Report 157896), and the in vivo toxicity reports: Systemic Toxicity (Report 157894), Irritation (Report 157893), and Sensitization

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(Report 157892). Under the conditions of each study, the 4cm distal end was considered non-cytotoxic.

In addition, based on structure-activity evaluation (using Derek Nexus 3.0 Lhasa Limited software), no structural alerts for these chemicals were noted for multiple toxicological endpoints. These endpoints include irritation, sensitization, mutagenicity, and carcinogenicity. Therefore, the presence of these chemicals does not represent any additional risk to the patient. Additionally, GC/MS analysis of the water extract revealed no semi-volatile compound above 2ppm, the limit of detection in the purified water extract.

The supplier of the Nickel-Tin Plating changed since the biocompatibility evaluation of the predicate device. Limited testing was performed on samples from each supplier to ensure biological equivalency. Biocompatibility of this material was evaluated in accordance with ISO 10993-1 studies. The test methods and results are summarized in Table 15.10.

Table 15.10 ICP Biocompatibility Evaluation of Nickel-Tin Plating, Bridge Testing

Test Type	Test Name	Test Report Number	Lab Name	Result
Analytical Test	Analytical Tests (Non-volatile residuals/FTIR)	Lab: 12T_37839_01, Lab: 12T_37838_02	NAMSA	Pass
Analytical Test	Analytical Tests (IR)	Lab: 12T_37838_01 Lab: 12T_37839_02	NAMSA	Pass
Cytotoxicity	Cytotoxicity (Extract Colony Assay (JMHLW))	Study: 171502 Study: 171504	WuXi AppTec Laboratory Services	Pass
Cytotoxicity	Cytotoxicity (MEM-Elution)	Study: 171505 Study: 171503	WuXi AppTec Laboratory Services	Pass

The Nickel-Tin Plating passed all biocompatibility tests recommended under ISO 10993-1 for Externally Communicating Devices in contact with Tissue, Bone or Dentin for less than 24 hours ("limited"); as well as the test suggested by the FDA (Acute Systemic Toxicity) for this patient contact category, and those considered supplementary tests. The Nickel-Tin Plating of the new supplier is biological equivalent to the plating in the predicate device.

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**Biocompatibility Testing of Patient –Contacting Components of the Device:
Evaluation for Potential Material Interactions**

The distal end of the subject production device was evaluated to account for potential interaction of patient-contacting materials (ISO 10993-1). Similar to the predicate device, no-adverse effects were expected from the combination of materials used in the Subject Device. However, a supplementary cytotoxicity test was conducted to confirm this assumption. This test was considered toxicologically adequate for this objective. The test title and result are summarized in Table 15.11.

Table 15.11 Biocompatibility Testing of Patient-Contacting Components of the Device: Evaluation for Potential Material Interactions

Test Type	Test Name	Test Report Number	Lab Name	Result
Cytotoxicity	Cytotoxicity (MEM-Endpoint Dilution)	Study: 172325 Protocol: 140350-15	WuXi AppTec Laboratory Services	Pass

As expected, no cytotoxicity was observed in this test. Based on the comprehensive absence of toxicity, no biologically relevant interactions of patient-contact materials were observed. Similar to the predicate device, the assumption of no adverse material interactions was confirmed. Based on the absence of cytotoxicity, no biologically relevant interactions of patient-contacting materials were observed for the device.