JUL 25 2005

K050891

7. 510(k) SUMMARY

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Date Prepared April 5, 2005

Product and Trade Name *TOX A/B QUIK CHEK*™

Classification 21 CFR 866.2660

Predicate Devices

- *C. DIFFICILE TOX-B TEST* (K935296) - TECHLAB, Inc., Blacksburg, VA
- *C. difficile* toxin/antitoxin (K923463) - TECHLAB, Inc., Blacksburg, VA
- *C. DIFFICILE TOX A/B II™* (K003306 and K030404) - TECHLAB, Inc., Blacksburg, VA
- Premier™ Toxins A&B (K993914) - Meridian Bioscience, Inc., Cincinnati, OH
- ProSpecT® Clostridium difficile Toxin A/B (K033479) - Remel, Lenexa, KS
- Immuno*Card*® Toxins A&B (K041003) - Meridian Bioscience, Inc., Cincinnati, OH
- Xpect™ Clostridium difficile Toxin A/B (K041951) - Remel Inc., Lenexa, KS

Intended Use

The *TOX A/B QUIK CHEK™* test is a rapid immunoassay for detecting *Clostridium difficile* toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history.

Device Description

The *TOX A/B QUIK CHEK™* test uses antibodies specific for toxins A and B of *C. difficile*. The device contains a Reaction Window with two lines of immobilized antibodies. The test line ("T") contains antibodies against *C. difficile* toxins A and B. The other, representing a control line ("C"), contains anti-IgG antibodies. The *Conjugate* consists of antibodies to toxins A and B coupled to horseradish peroxidase. To perform the test, the fecal specimen is diluted with *Diluent*, and *Conjugate* is added to the diluted sample. The diluted sample-conjugate mixture is added to the Sample Well and the device is allowed to incubate at room temperature for 15 minutes. During the incubation, any toxin A and toxin B in the sample bind to anti-toxin antibody-peroxidase conjugate. The toxin-antibody complexes migrate through a filter pad to a membrane where they are captured by the immobilized anti-toxin antibodies in the line. The

Reaction Well is subsequently washed with *Wash Buffer*, followed by the addition of *Substrate*. After up to a 10 minute incubation, the "T" reaction is examined visually for the appearance of a blue line. A blue line indicates a positive test. A positive "C" reaction, indicated by a blue line, confirms that sample and all reagents were added in proper sequence and volume, that reagents were active at the time of performing the assay, and that proper sample migration occurred.

Comparative information of equivalent devices

Characteristics	510(k) Numbers	Intended Use	Format	Materials	Target Population
TOX A/B QUIK CHEK™ test	Subject to this 510(k)	Detection of *C. difficile* toxin in fecal specimens	Rapid membrane	Highly specific antibodies against *C. difficile* toxins A and B	Persons suspected of having *C. difficile* disease
Tissue culture assay (*TOX-B TEST*)	K935296	Detection of *C. difficile* toxin in fecal specimens	Tissue culture	Cell monolayer, specific neutralizing antiserum	Persons suspected of having *C. difficile* disease
C. DIFFICILE TOX A/B II™	K003306 and KK030404	Detection of *C. difficile* toxin in fecal specimens	Microtiter ELISA	Highly specific antibodies against *C. difficile* toxins A and B	Persons suspected of having *C. difficile* disease
Premier™ Toxins A&B	K993914	Detection of *C. difficile* toxin in fecal specimens	Microtiter ELISA	Highly specific antibodies against *C. difficile* toxins A and B	Persons suspected of having *C. difficile* disease
ProSpecT® Clostridium difficile Toxin A/B	K033479	Detection of *C. difficile* toxin in fecal specimens	Microtiter ELISA	Highly specific antibodies against *C. difficile* toxins A and B	Persons suspected of having *C. difficile* disease
Immuno*Card*® Toxins A&B	K041003	Detection of *C. difficile* toxin in fecal specimens	Rapid membrane	Highly specific antibodies against *C. difficile* toxins A and B	Persons suspected of having *C. difficile* disease
X/pect™ Clostridium difficile Toxin A/B	K041951	Detection of *C. difficile* toxin in fecal specimens	Rapid membrane	Highly specific antibodies against *C. difficile* toxins A and B	Persons suspected of having *C. difficile* disease

Summary of Performance Data

Clinical Accuracy

The tables below show a summary of the clinical performance of the *TOX A/B QUIK CHEK™* test. Results from 5 studies (2 in-house studies and 3 on-site studies) are included in the summary. Results from the *TOX A/B QUIK CHEK™* were compared to tissue culture assay. The *TOX A/B QUIK CHEK™* test exhibited a sensitivity and specificity of 90.2% and 99.7%, respectively. The predictive positive and negative values were 98.6% and 97.9%, respectively, and the correlation was 98.0%.

Summary of clinical performance comparing the *TOX A/B QUIK CHEK™* test versus tissue culture assay.

n=842	Tiss Cult pos	Tiss Cult neg
TOX A/B QUIK CHEK™ pos	138	2
TOX A/B QUIK CHEK™ neg	15	687

		95% CI
Sensitivity	90.2	84.1 - 94.2
Specificity	99.7	98.8 - 99.9
Predictive Positive Value	98.6	94.4 - 99.8
Predictive Negative Value	97.9	96.4 - 98.7
Correlation	98.0	97.8 - 98.2

Of the 2 tissue culture-negative/*TOX A/B QUIK CHEK™*-positive samples, 1 was negative in the *TOX A/B II™* test. Of the 15 specimens that were tissue culture-positive/*TOX A/B QUIK CHEK™*-negative, 12 were negative in a commercial A+B ELISA.

Analytical Sensitivity

The test was consistently positive at a concentration of 0.63 ng/mL for toxin A and 1.25 ng/mL for toxin B.

Cross-Reactivity

Strains of *C. difficile* that produce toxins A and B, or only toxin B, were demonstrated to react in the *TOX A/B QUIK CHEK™*. The specificity of the *TOX A/B QUIK CHEK™* test was evaluated by examining the reactivity of a wide range of common intestinal bacteria and intestinal pathogens in the assay. A summary of the results is shown below. The only non-*C. difficile* organism to react in the *TOX A/B QUIK CHEK™* test was *C. sordellii* VPI 9048, which produces toxin HT (hemorrhagic toxin) and toxin LT (lethal toxin) that are homologous to toxins A and B, respectively. All of the other organisms tested were negative in the *TOX A/B QUIK CHEK™*.

Bacterium	Strain	Reaction with *C. difficile* negative stool	Reaction with *C. difficile* positive stool
Aeromonas hydrophila	ATCC 7965	-	+
Bacillus cereus	ATCC 14579	-	+
Bacillus subtilis	ATCC 6051	-	+
Bacteroides fragilis	VPI 13785	-	+
Campylobacter coli	ATCC 49941	-	+
Campylobacter fetus	ATCC 25936	-	+
Campylobacter jejuni	ATCC 29428	-	+
Candida albicans	ATCC 10231	-	+
Clostridium bifermentans	VPI 2012	-	+
Clostridium butyricum	VPI 8260	-	+
Clostridium perfringens, types A	VPI 3624	-	+
Clostridium septicum	VPI 1524	-	+
Clostridium sordellii	VPI 9048	+	+
Clostridium sordellii	VPI 7319	-	+
Clostridium sporogenes	VPI 9743	-	+
Enterococcus faecalis	ATCC 19433	-	+
Escherichia coli EIEC	SD67	-	+
Escherichia coli	ATCC 25922	-	+
Escherichia coli O157 H7	B1409	-	+
Escherichia coli ETEC	E 2348169	-	+
Klebsiella pneumoniae	ATCC 9997	-	+
Peptostreptococcus anaerobius	ATCC 27337	-	+
Proteus vulgaris	ATCC 6380	-	+
Pseudomonas aeruginosa	ATCC 9027	-	+
Salmonella typhimurium	ATCC 14029	-	+
Shigella dysenteriae	ATCC 12022	-	+
Shigella flexneri	ATCC 12122	-	+
Shigella sonnei	ATCC 11060	-	+
Staphylococcus aureus	ATCC 6358	-	+

Bacterium	Strain	Reaction with *C. difficile* negative stool	Reaction with *C. difficile* positive stool
Staphylococcus aureus (Cowans)	ATCC 12598	-	+
Staphylococcus epidermidis	VPI 13140	-	+
Vibrio parahaemolyticus	ATCC 17802	-	+
Yersinia enterocolitica	ATCC 9610	-	+

Virus	ATCC#	Reaction with *C. difficile* negative stool	Reaction with *C. difficile* positive stool
Adenovirus type 1	VR-1	-	+
Adenovirus type 2	VR-846	-	+
Adenovirus type 3	VR-3	-	+
Adenovirus type 5	VR-5	-	+
Adenovirus type 40	VR-931	-	+
Adenovirus type 41	VR-930	-	+
Human coronavirus	VR-740	-	+
Coxsackievirus B2	VR-29	-	+
Coxsackievirus B3	VR-30	-	+
Coxsackievirus B4	VR-184	-	+
Coxsackievirus B5	VR-185	-	+
Echovirus 9	VR-1050	-	+
Echovirus 11	VR-1052	-	+
Echovirus 18	VR-48	-	+
Echovirus 22	VR-1063	-	+
Echovirus 33	VR-582	-	+
Enterovirus type 68	VR-1076	-	+
Enterovirus type 69	VR-1077	-	+
Enterovirus type 70	VR-836	-	+
Enterovirus type 71	VR-784	-	+

Interfering Substances

The following substances had no effect on test results, either with *C. difficile*-negative or *C. difficile*-positive specimens, when present in the stool in the concentrations indicated in the table.

Substance	Concentration	Reaction with *C. difficile* negative stool	Reaction with *C. difficile* positive stool
Hog gastric mucin	3.5% w/v	-	+
Human blood (O, Rh-)	40% v/v	-	+
Barium sulfate	5% w/v	-	+
Imodium®	5% w/v	-	+
Kaopectate®	5 mg/ml	-	+
Pepto-Bismol®	5% w/v	-	+
Steric/palmitic acid	40% w/v	-	+
Metronidazole	0.25% w/v	-	+
Vancomycin	0.25% w/v	-	+

Reproducibility

The reproducibility of the *TOX A/B QUIK CHEK™* test was determined using known positive (n=6) and negative (n=2) fecal specimens that were coded and sorted to prevent their identification during testing. Testing was performed on-site at 3 laboratories, which tested the samples on 3 days. The samples produced the expected results 100% of the time.

8. REFERENCES

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

JUL 25 2005

David M. Lyerly, Ph.D.
Vice President, Research and Development
TECHLAB®, Inc.
2001 Kraft Drive
Corporate Research Center
Blackburg, VA 24060-6358

Re: k050891
Trade/Device Name: TOX A/B QUICK CHECK™
Regulation Number: 21 CFR 866.2660
Regulation Name: Microorganism differentiation and identification device
Regulatory Class: Class I
Product Code: LLH
Dated: July 5, 2005
Received: July 8, 2005

Dear Dr. Lyerly:

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.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. 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 Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific information about the application of labeling requirements to your device, or questions on the promotion and advertising of your device, please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (240)276-0484. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). 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) 443-6597 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html

Sincerely yours,

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

REVISED

Indications for Use

510(k) Number (if known): K050891

Device Name: *TOX A/B QUIK CHEK™*

Indications For Use:

The *TOX A/B QUIK CHEK™* test is a rapid immunoassay for detecting *Clostridium difficile* toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history. FOR *IN VITRO* DIAGNOSTIC USE.

Prescription Use X AND/OR Over-The-Counter Use

(Part 21 CFR 801 Subpart D) (21 CFR 807 Subpart C)

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

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Freddie L. Poole

Division Sign-Off

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Office of In Vitro Diagnostic Device Evaluation and Safety

510(k) K050891

DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville, Maryland 20850

August 11, 2005

DAVID M LYERLY
Techlab, Inc.
2001 KRAFT DR.
BLACKSBURG, VA 24060-6358
US

Re: k050891
Received: April 8, 2005

Categorization Notification

Regulations codified at 42 CFR 493.17 et. seq., implementing the Clinical Laboratory Improvement Amendments of 1988, require the Secretary to provide for the categorization of specific clinical laboratory test systems by the level of complexity. Based upon these regulations, the following commercially marketed test system or assay for the analyte is categorized below:

Test System/Analyte (s) : (SEE ATTACHMENT)

This complexity categorization is effective as of the date of this notification. This categorization will be reported on FDA's home page http://www.fda.gov/cdrh/clia. This categorization information may be provided to the user of the commercially marketed test system or assay as specified for the analyte indicated. This categorization will also be announced in a Federal Register Notice, which will provide opportunity for comment on the decision. FDA reserves the right to reevaluate and recategorize this test based upon the comments received in response to the Federal Register Notice.

If you have any questions regarding this complexity categorization, please contact Freddie Poole at 240-276-0496.

Sincerely yours,

Steven Gutman

Steven I. Gutman, M.D., M.B.A.
Director
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

ATTACHMENT

Document Number : k050891

Test System: Techlab TOX A/B QUIK CHEK
Analyte : Clostridium Difficile
Complexity : MODERATE

Sure-Vue® C. difficile TOX A/B *Package Insert*

A rapid test for the detection of *C. difficile* toxins A and B in fecal specimens
Catalog No. 23900550 (25 Tests)
Patent Pending

INTENDED USE

The Sure-Vue® C. difficile TOX A/B test is a rapid immunoassay for detecting *Clostridium difficile* toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history.
For *in vitro* diagnostic use.

EXPLANATION

After treatment with antibiotics, many patients develop gastrointestinal problems ranging from mild diarrhea to severe pseudomembranous colitis. Many cases of the milder forms of gastrointestinal illness and most cases of pseudomembranous colitis are caused by toxigenic strains of *Clostridium difficile* (1). This organism is an opportunistic anaerobic bacterium that grows in the intestine once the normal flora has been altered by the antibiotic. Toxigenic strains of *C. difficile* carry the genes encoding the toxins while non-toxigenic strains do not carry the toxin genes. The disease results from the toxins that the toxigenic organism produces. The clinical symptoms associated with the disease are believed to be primarily due to toxin A, which is a tissue-damaging enterotoxin (2,3). *C. difficile* also produces a second toxin, designated toxin B. Toxin B, which has been referred to as the cytotoxin of the organism, is the toxin detected by the tissue culture assay currently used by many laboratories. Toxigenic *C. difficile* strains produce both toxins or only toxin B (4-7).

PRINCIPLE OF THE TEST

The Sure-Vue® C. difficile TOX A/B uses antibodies specific for toxins A and B of *C. difficile*. The device contains a *Reaction Window* with two vertical lines of immobilized antibodies (Fig. 1a). The test line ("T") contains antibodies against *C. difficile* toxins A and B. The control line ("C") contains anti-IgG antibodies. The *Conjugate* consists of antibodies to toxins A and B coupled to horseradish peroxidase. To perform the test, the sample is added to a tube containing a mixture of *Diluent* and *Conjugate*. The diluted sample-conjugate mixture is added to the *Sample Well* and the device is allowed to incubate at room temperature for 15 minutes. During the incubation, any toxin A and toxin B in the sample bind to anti-toxin antibody-peroxidase conjugate. The toxin-antibody complexes migrate through a filter pad to a membrane where they are captured by the immobilized anti-toxin antibodies in the line. The *Reaction Window* is subsequently washed with *Wash Buffer*, and the test is developed with the addition of *Substrate*. After a 10 minute incubation period, the "T" reaction is examined visually for the appearance of a vertical blue line on the "T" side of the *Reaction Window*. A blue line indicates a positive test. A positive "C" reaction, indicated by a vertical blue line on the "C" side of the *Reaction Window*, confirms that the test is working properly and the results are valid.

MATERIALS PROVIDED

Membrane Devices –25 pouches, each containing 1 device and a desiccant pack

Diluent **(14mL)** – Buffered protein solution containing 0.02% thimerosal with graduated dropper assembly

Wash Buffer **(10mL)** – A buffered solution containing 0.02% thimerosal with graduated dropper assembly

Substrate **(3.5mL)** – Solution containing tetramethylbenzidine

Conjugate **(2mL)** – Mouse monoclonal antibody specific for toxin A coupled to horseradish peroxidase and goat polyclonal antibody specific for toxin B coupled to horseradish peroxidase in a buffered protein solution containing 0.02% thimerosal

Positive Control **(1mL)** – Antigen in a buffered protein solution

Disposable plastic transfer pipettes – 50 (graduated at 25µL and 400µL)

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Small test tubes (e.g., plastic Eppendorf tubes)	*Applicator sticks*
Timer	*Vortex mixer*
Disposable gloves for handling fecal samples	*Pipettor and tips*

SHELF LIFE AND STORAGE

The expiration date of the kit is given on the label. Expiration dates for each component are listed on the individual labels. The kit should be stored between 2° and 8°C.

PRECAUTIONS

1. Reagents from different kits should not be mixed. Do not use a kit past the expiration date.
2. Bring all components to ROOM TEMPERATURE BEFORE USE!
3. Caps, tips and dropper assemblies are color-coded; do NOT mix or interchange!
4. Do not freeze the reagents. The kit should be stored between 2°C and 8°C.
5. The pouch containing the *Membrane Device* should be at room temperature before opening, and opened just before use. Keep the membrane devices dry before use.
6. Use fecal specimens within 72 hours of collection to obtain optimal results. Specimens that are frozen may lose activity due to freezing and thawing.
7. Specimens that have been preserved in 10% formalin, merthiolate formalin, sodium acetate formalin or polyvinyl alcohol cannot be used.
8. Specimens in transport media such as Cary Blair and C&S can be used as specified in the specimen preparation protocol.
9. Hold reagent bottles vertically to dispense reagents to ensure consistent drop size.
10. Specimens and membrane devices should be handled and disposed of as potential biohazards after use. Wear disposable gloves when doing the test.
11. Reagents contain thimerosal as a preservative and should be handled with normal laboratory caution.
12. Membrane devices cannot be reused.
13. The test has been optimized for sensitivity and specificity. Alterations of the specified procedure and/or test conditions may affect the sensitivity and specificity of the test. Do not deviate from the specified procedure.
14. Microbial contamination of reagents may decrease the accuracy of the assay. Avoid microbial contamination of reagents by using sterile disposable pipettes if removing aliquots from reagent bottles.
15. Be attentive to the total assay time when testing more than one fecal specimen. Add

Diluent first, then add the *Conjugate* to each tube of *Diluent*. Then add specimen to the tube of *Diluent/Conjugate*. Thoroughly mix all of the diluted specimens, and then transfer to the *Membrane Device*. The 15-minute incubation step begins after the last diluted sample-conjugate mixture has been transferred to the final *Membrane Device*.

COLLECTION AND HANDLING OF FECAL SPECIMENS

1. Standard collection and handling procedures used in-house for fecal specimens are appropriate. Specimens should be stored between 2° and 8°C; test specimens that are less than 24 hours old, whenever possible.
2. Store specimens frozen (≤-10°C) if the test cannot be performed within 72 hours of collection, but note that freezing and thawing of the specimen may result in loss of activity due to degradation of the toxins.
3. Make sure that specimens are thoroughly mixed PRIOR to performing the assay.
4. Storing fecal specimens in the *Diluent* is NOT recommended.
5. Do not allow the fecal specimens to remain in the *Diluent* and/or *Conjugate* for any extended period of time.

SPECIMEN PREPARATION

1. Bring all reagents and the required number of devices to room temperature before use.
2. Set up and label one small test tube for each specimen, and optional external controls as necessary.
3. Add 500µL *Diluent* to each tube for fecal specimens using the graduated black dropper assembly (or equivalent). For specimens in transport media such as Cary Blair or C&S, add 425µL of *Diluent* to the tube.
4. Add one drop of *Conjugate* (red capped bottle) to each tube.
5. Obtain one disposable plastic transfer pipette (supplied with the kit) for each sample – the pipettes have raised graduations at 25µL and 400µL.
6. Mix all specimens thoroughly regardless of consistency- it is essential that the specimens be evenly suspended before transferring.
   Liquid/Semi-solid specimens – pipette 25µL of specimen with a transfer pipette (graduated at 25µL and 400µL) and dispense into the *Diluent/Conjugate* mixture. Use the same transfer pipette to mix the diluted specimen.
   Formed/Solid specimens – Care must be taken to add the correct amount of formed feces to the sample mixture. Mix the specimen thoroughly using a wooden applicator stick and transfer a small portion (approximately 2mm diameter, the equivalent of 25µL) of the specimen into the *Diluent/Conjugate* mixture. Emulsify the specimen using the applicator stick.
   Fecal specimens in Cary Blair or C&S transport media - pipette 100µL of sample into the *Diluent/Conjugate* mixture.
7. **Optional External Control Samples:**
   External Positive Control - add one drop of *Positive Control* (gray-capped bottle) to the appropriate test tube.
   External Negative Control - add 25µL *Diluent* to the appropriate test tube.

NOTE: Transferring too little specimen, or failure to mix and completely suspend the specimen in the Diluent mixture, may result in a false-negative test result. The addition of too much fecal specimen may cause invalid results due to restricted sample flow.

TEST PROCEDURE

1. Obtain one *Membrane Device* per specimen, and one device per optional external

positive or negative control as necessary. The foil bags containing the devices should be brought to room temperature before opening. Label each device appropriately and orient it on a flat surface so the letter "C" on the device is on the left, the letter "T" is on the right, and the small *Sample Well* is located in the top right corner of the device (Fig. 1a).

2. Close each tube of diluted specimen and mix thoroughly. Proper mixing can be achieved by vortexing or inverting the tube. Immediately proceed to Step #3.
3. Using a transfer pipette (graduated at 25µL and 400µL), transfer 400µL of the diluted sample-conjugate mixture into the *Sample Well* (smaller hole in the top right corner of the device) of a *Membrane Device*, making certain to expel the liquid sample onto the wicking pad inside of the *Membrane Device*.
4. Incubate the device at room temperature for 15 minutes – the sample will wick through the device and a wet area will spread across the *Reaction Window* (larger hole in the middle of the device).

   NOTE FOR SAMPLES THAT FAIL TO MIGRATE:

   Occasionally, a diluted fecal specimen cannot be tested because it clogs the membrane and the Reaction Window does not wet properly. If the diluted fecal specimen fails to migrate properly within 5 minutes of adding the sample to the Sample Well (i.e. the membrane in the Reaction Window does not appear to be completely wet), then add 100µL (4 drops) of Diluent to the Sample Well and wait an additional 5 minutes (for a total of 20 minutes).
5. After the incubation, add 300µL of *Wash Buffer* to the *Reaction Window* using the graduated white dropper assembly (or equivalent). Allow the *Wash Buffer* to flow through the *Reaction Window* membrane and be absorbed completely.
6. Add 2 drops of *Substrate* (blue-capped bottle) to the *Reaction Window*. Read and record results visually after 10 minutes.

INTERPRETATION OF RESULTS

1. Interpretation of the test is most reliable when the device is read immediately at the end of the reaction period. Read the device at a normal working distance in a well-lit area. View with a line of vision directly over the device.
2. Observe device for the appearance of a blue line on the "C" side of the *Reaction Window* representing the internal positive control line. Observe device for the appearance of a blue line on the "T" side of the *Reaction Window* representing the test line. The lines may appear faint to dark in intensity.
3. **Positive Result:** A positive result may be interpreted at any time between the addition of *Substrate* and the 10-minute read time. Two blue lines are visible, the control line ("C") and the test line ("T"). The lines may appear faint to dark in intensity. The appearance of a blue line on the "T" side along with a blue control line is interpreted as a positive result. An obvious partial line is interpreted as a positive result. Do not interpret membrane discoloration or shadow as a positive result. A positive result indicates the presence of *C. difficile* toxin.
4. **Negative Result:** A test cannot be interpreted as negative or invalid until 10 minutes following the addition of *Substrate*. A single blue line is visible on the control ("C") side of the *Reaction Window* and no test line is visible on the "T" side of the *Reaction Window* (Fig. 1b). A negative result indicates *C. difficile* toxin is either absent in the specimen or is below the detection limit of the test.
5. **Invalid Result:** A single line is visible on the test ("T") side of the *Reaction Window*, or no lines are visible in the *Reaction Window* (Fig. 1c, 1d). The test result is invalid if a control line is not present at the completion of the reaction period.

FIGURE 1: Sure-Vue® C. difficile TOX A/B INTERPRETATION OF RESULTS

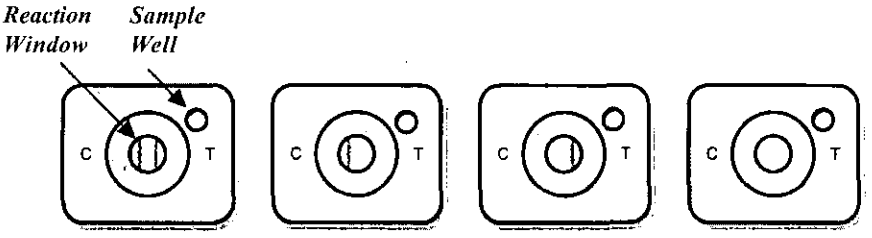

Fig. 1a. Positive *Fig 1b. Negative* *Fig. 1c. Invalid* *Fig. 1d. Invalid*

QUALITY CONTROL

Internal: A blue control line must be visible on the "C" side of the *Reaction Window* on every *Membrane Device* that is tested. The appearance of the blue control line confirms that the sample and reagents were added correctly, that the reagents were active at the time of performing the assay, and that the sample migrated properly through the *Membrane Device*. A clear background in the result area is considered an internal negative control. If the test has been performed correctly and reagents are working properly, the background will be clear to give a discernible result.

External: The reactivity of the Sure-Vue® C. difficile TOX A/B test should be verified on receipt using the *Positive Control* and negative control (*Diluent*). The *Positive Control* is supplied with the kit (gray-capped bottle). The *Positive Control* confirms the reactivity of the other reagents associated with the assay, and is not intended to ensure precision at the analytical assay cut-off. *Diluent* is used for the negative control. Additional tests can be performed with the controls to meet the requirements of local, state and/or federal regulations and/or accrediting organizations.

LIMITATIONS

1. The Sure-Vue® C. difficile TOX A/B test is used to detect *C. difficile* toxin(s) in fecal specimens. The test confirms the presence of toxin in feces and this information should be taken under consideration by the physician in light of the clinical history and physical examination of the patient. The Sure-Vue® C. difficile TOX A/B test will detect levels of toxin A at ≥0.63ng/mL and toxin B at ≥1.25ng/mL.
2. Fecal specimens are extremely complex. Optimal results with the Sure-Vue® C. difficile TOX A/B test are obtained with specimens that are less than 24 hours old. Most undiluted specimens can be stored between 2° and 8°C for 72 hours before significant degradation of the toxin is noted. If specimens are not assayed within this time period, they may be frozen and thawed. However, repeated freezing and thawing may result in loss in the immunoreactivity of toxins A and B.
3. Some specimens may give weak reactions. This may be due to a number of factors such as the presence of low levels of toxin, the presence of binding substances, or inactivating enzymes in the feces. *Under these conditions, a fresh specimen should be tested.* Additional tests that may be used in conjunction with the Sure-Vue® C. difficile TOX A/B test include culture with toxigenic testing or tissue culture cytotoxicity assay for the detection of *C. difficile* or its toxin(s).
4. Fecal specimens preserved in 10% formalin, merthiolate formalin, sodium acetate formalin, or polyvinyl alcohol cannot be used.

5. The Sure-Vue® C. difficile TOX A/B test is qualitative. The intensity of the color should not be interpreted quantitatively.
6. Some isolates of *C. sordellii* may react in the Sure-Vue® C. difficile TOX A/B test due to the production of immunologically related toxins (1).
7. Colonization rates of up to 50% have been reported in infants. A high rate has also been reported in cystic fibrosis patients (1,3).

EXPECTED VALUES

The reported incidence of *C. difficile*-associated disease in patients with antibiotic-associated diarrhea is 10 to 20%. In our studies, the incidence ranged from 10% to 22%. The prevalence of a positive Sure-Vue® C. difficile TOX A/B test will vary from location to location and hospitals may experience rates lower or higher than those observed at the sites used in this evaluation. *Clostridium difficile* disease is primarily a nosocomial disease of elderly patients, and hospitals that have higher numbers of elderly patients may experience higher rates. It is important to consider any test results in conjunction with clinical symptoms because some healthy adults and large numbers of healthy infants (up to 50%) will be positive for *C. difficile* toxin.

PERFORMANCE CHARACTERISTICS

The Sure-Vue® C. difficile TOX A/B test was compared with the tissue culture test at three U.S. hospitals and in-house at TECHLAB®, Inc. Specimens included in the evaluation were submitted to the clinical laboratory for routine testing. The tissue culture test was done according to the in-house procedure. The table below shows a summary of the clinical performance of the Sure-Vue® C. difficile TOX A/B test. The test exhibited a sensitivity and specificity of 90.2% and 99.7%, respectively. The predictive positive and negative values were 98.6% and 97.9%, respectively, and the correlation was 98.0%.

TABLE 1. Correlation of the Sure-Vue® C. difficile TOX A/B test with tissue culture.

N = 842	Tissue Culture positive	Tissue Culture negative
Sure-Vue® C. difficile TOX A/B test positive	138	2
Sure-Vue® C. difficile TOX A/B test negative	15	687

		95% CI
Sensitivity	90.2%	84.1 - 94.2
Specificity	99.7%	98.8 - 99.9
Predictive Positive Value	98.6%	94.4 - 99.8
Predictive Negative Value	97.9%	96.4 - 98.7
Correlation	98.0%	97.8 - 98.2

Of the 2 tissue culture-negative/Sure-Vue® C. difficile TOX A/B-positive samples, 1 was negative in a commercial toxin A+B ELISA. Of the 15 specimens that were tissue culture-positive/Sure-Vue® C. difficile TOX A/B-negative, 12 were negative in commercial toxin A+B ELISAs. There were 9 specimens that were unreadable. All of the specimens were negative by PCR analysis for the genes of toxin A (*tcdA*) and toxin B (*tcdB*).

A total of 51 fecal specimens diluted in Cary Blair and 32 fecal specimens diluted in C&S Transport Media were tested in the Sure-Vue® C. difficile TOX A/B test and the results were compared to those obtained by routine testing. The test exhibited an

agreement of 97.6% for the detection of *C. difficile* toxins in specimens prepared in Transport Media.

ANALYTICAL SENSITIVITY

The test was consistently positive at a concentration of 0.63ng/mL for toxin A and 1.25 ng/mL for toxin B.

REPRODUCIBILITY

The reproducibility of the Sure-Vue® C. difficile TOX A/B test was determined using known positive (n=6) and negative (n=2) fecal specimens that were coded and sorted to prevent their identification during testing. Testing was performed on-site at 3 laboratories, which tested the samples on 3 days. The samples produced the expected results 100% of the time.

CROSS REACTIVITY

Fecal specimens inoculated with the following microorganisms to a final concentration of approximately 10^8 or higher organisms per mL did not react in the Sure-Vue® C. difficile TOX A/B.

Bacteria: *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Bacteroides fragilis, Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, Candida albicans, Clostridium bifermentans, Clostridium butyricum, Clostridium perfringens, Clostridium septicum, Clostridium sordellii* (nontoxigenic), *Clostridium sporogenes, Enterococcus faecalis, Escherichia coli* EIEC, *Escherichia coli, Escherichia coli* 0157 H7, *Escherichia coli* ETEC, *Klebsiella pneumoniae, Peptostreptococcus anaerobius, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus aureus (Cowans), Staphylococcus epidermidis, Vibrio parahaemolyticus, Yersinia enterocolitica*

Viruses: Adenovirus types 1,2,3,5,40,41, Human coronavirus, Coxsackievirus B2,B3,B4,B5, Echovirus 9,11,18,22,33, Enterovirus type 68,69,70,71.

The only non-*C. difficile* organism to react with the Sure-Vue® C. difficile TOX A/B was *C. sordellii* VPI 9048. This strain produces toxins HT and LT, which are homologous to toxins A and B, respectively.

INTERFERING SUBSTANCES

The following substances had no effect on test results when present in feces in the concentrations indicated: mucin (3.5% w/v), human blood (40% v/v), barium sulfate (5% w/v), Imodium® (5% w/v), Kaopectate® (5mg/mL), Pepto-Bismol® (5% w/v), steric/ palmitic acid (40% w/v), Metronidazole (0.25% w/v), Vancomycin (0.25% w/v).

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TOX A/B QUIK CHEK ®

A rapid test for the detection of *C. difficile*
toxins A and B in fecal specimens
Patent Pending
Catalog No. T5033 (25 Tests)

ESPAÑOL p. 8

Test rápido para la detección de las toxinas A y B de
C. difficile en muestras fecales
Pendiente de Patente
Prod. No. T5033 (25 Pruebas)

DEUTSCH p. 15

Ein Schnelltest für den Nachweis von *C. difficile*-Toxin A und B in
Stuhlproben
Zum Patent angemeldet
Katalognummer. T5033 (25 Tests)

FRANCAISE p. 22

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Brevet en instance
Numéro de Catalogue T5033 (25 Analyses)

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International Symbol Key:

REF	Catalog Number		Temperature Limitation
IVD	*In Vitro* Diagnostic Medical Device		Use By/Expiration Date
LOT	Lot Information	CE	CE Symbol
Σ	Contains sufficient reagents for <n> tests	⚠	Caution, consult accompanying documents

TOX A/B QUIK CHEK®

INTENDED USE

The *TOX A/B QUIK CHEK®* test is a rapid immunoassay for detecting *Clostridium difficile* toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history.
FOR *IN VITRO* DIAGNOSTIC USE.

EXPLANATION

After treatment with antibiotics, many patients develop gastrointestinal problems ranging from mild diarrhea to severe pseudomembranous colitis. Many cases of the milder forms of gastrointestinal illness and most cases of pseudomembranous colitis are caused by toxigenic strains of *Clostridium difficile* (1). This organism is an opportunistic anaerobic bacterium that grows in the intestine once the normal flora has been altered by the antibiotic. Toxigenic strains of *C. difficile* carry the genes encoding the toxins while non-toxigenic strains do not carry the toxin genes. The disease results from the toxins that the toxigenic organism produces. The clinical symptoms associated with the disease are believed to be primarily due to toxin A, which is a tissue-damaging enterotoxin (2,3). *C. difficile* also produces a second toxin, designated toxin B. Toxin B, which has been referred to as the cytotoxin of the organism, is the toxin detected by the tissue culture assay currently used by many laboratories. Toxigenic *C. difficile* strains produce both toxins or only toxin B (4-7).

PRINCIPLE OF THE TEST

The *TOX A/B QUIK CHEK®* uses antibodies specific for toxins A and B of *C. difficile*. The device contains a *Reaction Window* with two vertical lines of immobilized antibodies (Fig. 1a). The test line ("T") contains antibodies against *C. difficile* toxins A and B. The control line ("C") contains anti-IgG antibodies. The *Conjugate* consists of antibodies to toxins A and B coupled to horseradish peroxidase. To perform the test, the sample is added to a tube containing a mixture of *Diluent* and *Conjugate*. The diluted sample-conjugate mixture is added to the *Sample Well* and the device is allowed to incubate at room temperature for 15 minutes. During the incubation, any toxin A and toxin B in the sample bind to anti-toxin antibody-peroxidase conjugate. The toxin-antibody complexes migrate through a filter pad to a membrane where they are captured by the immobilized anti-toxin antibodies in the line. The *Reaction Window* is subsequently washed with *Wash Buffer*, and the test is developed with the addition of *Substrate*. After a 10 minute incubation period, the "T" reaction is examined visually for the appearance of a vertical blue line on the "T" side of the *Reaction Window*. A blue line indicates a positive test. A positive "C" reaction, indicated by a vertical blue line on the "C" side of the *Reaction Window*, confirms that the test is working properly and the results are valid.

MATERIALS PROVIDED

MEM DEV — ***Membrane Devices*** –25 pouches, each containing 1 device and a desiccant pack

DIL SPE — ***Diluent* (14 mL)** – Buffered protein solution containing 0.02% thimerosal with graduated dropper assembly

WASH REAG — ***Wash Buffer* (10 mL)** – A buffered solution containing 0.02% thimerosal with graduated dropper assembly

SUBS REAG — ***Substrate* (3.5 mL)** – Solution containing tetramethylbenzidine

CONJ ENZ — ***Conjugate* (2 mL)** – Mouse monoclonal antibody specific for toxin A coupled to horseradish peroxidase and goat polyclonal antibody specific for toxin B coupled to horseradish peroxidase in a buffered protein solution containing 0.02% thimerosal

CONTROL + — ***Positive Control* (1 mL)** – Antigen in a buffered protein solution

Disposable plastic transfer pipettes – 50 (graduated at 25 µL and 400 µL)

11. Reagents contain thimerosal as a preservative and should be handled with normal laboratory caution.
12. Membrane devices cannot be reused.
13. The test has been optimized for sensitivity and specificity. Alterations of the specified procedure and/or test conditions may affect the sensitivity and specificity of the test. Do not deviate from the specified procedure.
14. Microbial contamination of reagents may decrease the accuracy of the assay. Avoid microbial contamination of reagents by using sterile disposable pipettes if removing aliquots from reagent bottles.
15. Be attentive to the total assay time when testing more than one fecal specimen. Add *Diluent* first, then add the *Conjugate* to each tube of *Diluent*. Then add specimen to the tube of *Diluent/Conjugate*. Thoroughly mix all of the diluted specimens, and then transfer to the *Membrane Device*. The 15-minute incubation step begins after the last diluted sample-conjugate mixture has been transferred to the final *Membrane Device*.

COLLECTION AND HANDLING OF FECAL SPECIMENS

1. Standard collection and handling procedures used in-house for fecal specimens are appropriate. Specimens should be stored between 2° and 8°C; test specimens that are less than 24 hours old, whenever possible.
2. Store specimens frozen (≤-10°C) if the test cannot be performed within 72 hours of collection, but note that freezing and thawing of the specimen may result in loss of activity due to degradation of the toxins.
3. Make sure that specimens are thoroughly mixed PRIOR to performing the assay.
4. Storing fecal specimens in the *Diluent* is NOT recommended.
5. Do not allow the fecal specimens to remain in the *Diluent* and/or *Conjugate* for any extended period of time.

SPECIMEN PREPARATION

1. Bring all reagents and the required number of devices to room temperature before use.
2. Set up and label one small test tube for each specimen, and optional external controls as necessary.
3. Add 500 µL *Diluent* to each tube for fecal specimens. For specimens in transport media such as Cary Blair or C&S, add 425 µL of *Diluent* to the tube.
4. Add one drop of Conjugate (red capped bottle) to each tube.
5. Obtain one disposable plastic transfer pipette (supplied with the kit) for each sample – the pipettes have raised graduations at 25 µL and 400 µL.
6. Mix all specimens thoroughly regardless of consistency- it is essential that the specimens be evenly suspended before transferring.
   Liquid/Semi-solid specimens – pipette 25 µL of specimen with a transfer pipette (graduated at 25 µL and 400 µL) and dispense into the *Diluent*. Use the same transfer pipette to mix the diluted specimen.
   Formed/Solid specimens – Care must be taken to add the correct amount of formed feces to the sample mixture. Mix the specimen thoroughly using a wooden applicator stick and transfer a small portion (approximately 2 mm diameter, the equivalent of 25 µL) of the specimen into the *Diluent*. Emulsify the specimen using the applicator stick.
   Fecal specimens in Cary Blair or C&S transport media - pipette 100 µL of sample into the *Diluent*.
7. **Optional External Control Samples:**
   External Positive Control - add one drop of *Positive Control* (gray-capped bottle) to the appropriate test tube.
   External Negative Control - add 25 µL *Diluent* to the appropriate test tube.

NOTE: Transferring too little specimen, or failure to mix and completely suspend the specimen in the Diluent *mixture, may result in a false-negative test result. The addition of too much fecal specimen may cause invalid results due to restricted sample flow.*

TEST PROCEDURE

1. Obtain one *Membrane Device* per specimen, and one device per optional external positive or negative control as necessary. The foil bags containing the devices should be brought to room temperature before opening. Label each device appropriately and orient it on a flat surface so the letter "C" on the device is on the left, the letter "T" is on the right, and the small *Sample Well* is located in the top right corner of the device (Fig. 1a).
2. Close each tube of diluted specimen and mix thoroughly. Proper mixing can be achieved by vortexing or inverting the tube. Immediately proceed to Step #3.
3. Using a transfer pipette (graduated at 25 µL and 400 µL), transfer 400 µL of the diluted sample-conjugate mixture into the ***Sample Well*** (smaller hole in the top right corner of the device) of a *Membrane Device*, making certain to expel the liquid sample onto the wicking pad inside of the *Membrane Device*.
4. Incubate the device at room temperature for 15 minutes – the sample will wick through the device and a wet area will spread across the *Reaction Window* (larger hole in the middle of the device). If the *Reaction Window* is not completely wet at the end of the 15-minute incubation, the test is considered invalid and the sample must be retested on a new device.
   NOTE FOR SAMPLES THAT FAIL TO MIGRATE:
   Occasionally, a diluted fecal specimen cannot be tested because it clogs the membrane and the Reaction Window *does not wet properly. If the diluted fecal specimen fails to migrate properly within 5 minutes of adding the sample to the* Sample Well *(i.e. the membrane in the* Reaction Window *does not appear to be completely wet), then add 100 µL of* Diluent *to the* Sample Well *and wait an additional 5 minutes (for a total of 20 minutes).*
5. After the incubation, add 300 µL of *Wash Buffer* to the ***Reaction Window***. Allow the *Wash Buffer* to flow through the *Reaction Window* membrane and be absorbed completely.
6. Add 2 drops of *Substrate* (blue-capped bottle) to the ***Reaction Window***. Read and record results visually after 10 minutes.

INTERPRETATION OF RESULTS

1. Interpretation of the test is most reliable when the device is read immediately at the end of the reaction period. Read the device at a normal working distance in a well-lit area. View with a line of vision directly over the device.
2. Observe device for the appearance of a blue line on the "C" side of the *Reaction Window* representing the internal positive control line. Observe device for the appearance of a blue line on the "T" side of the *Reaction Window* representing the test line. The lines may appear faint to dark in intensity.
3. **Positive Result:** A positive result may be interpreted at any time between the addition of *Substrate* and the 10-minute read time. Two blue lines are visible, the control line ("C") and the test line ("T"). The lines may appear faint to dark in intensity. The appearance of a blue line on the "T" side is interpreted as a positive result. An obvious partial line is interpreted as a positive result. Do not interpret membrane discoloration or shadow as a positive result. A positive result indicates the presence of *C. difficile* toxin.

2. Observe device for the appearance of a blue line on the "C" side of the *Reaction Window* representing the internal positive control line. Observe device for the appearance of a blue line on the "T" side of the *Reaction Window* representing the test line. The lines may appear faint to dark in intensity.
3. **Positive Result:** A positive result may be interpreted at any time between the addition of *Substrate* and the 10-minute read time. Two blue lines are visible, the control line ("C") and the test line ("T"). The lines may appear faint to dark in intensity. The appearance of a blue line on the "T" side along with a blue control line is interpreted as a positive result. An obvious partial line is interpreted as a positive result. Do not interpret membrane discoloration or shadow as a positive result. A positive result indicates the presence of *C. difficile* toxin.
4. **Negative Result:** A test cannot be interpreted as negative or invalid until 10 minutes following the addition of *Substrate*. A single blue line is visible on the control ("C") side of the *Reaction Window* and no test line is visible on the "T" side of the *Reaction Window* (Fig. 1b). A negative result indicates *C. difficile* toxin is either absent in the specimen or is below the detection limit of the test.
5. **Invalid Result:** A single line is visible on the test ("T") side of the *Reaction Window,* or no lines are visible in the *Reaction Window* (Fig. 1c, 1d). The test result is invalid if a control line is not present at the completion of the reaction period.

FIGURE 1: *TOX A/B QUIK CHEK*® INTERPRETATION OF RESULTS

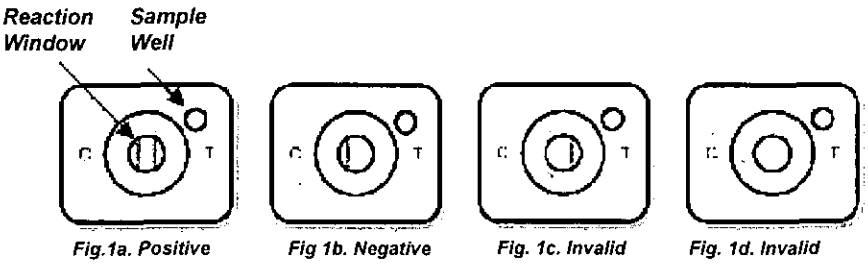

Fig.1a. Positive *Fig 1b. Negative* *Fig. 1c. Invalid* *Fig. 1d. Invalid*

QUALITY CONTROL

Internal: A blue control line must be visible on the "C" side of the *Reaction Window* on every *Membrane Device* that is tested. The appearance of the blue control line confirms that the sample and reagents were added correctly, that the reagents were active at the time of performing the assay, and that the sample migrated properly through the *Membrane Device*. A clear background in the result area is considered an internal negative control. If the test has been performed correctly and reagents are working properly, the background will be clear to give a discernible result.

External: The reactivity of the *TOX A/B QUIK CHEK*® test should be verified on receipt using the *Positive Control* and negative control (*Diluent*). The *Positive Control* is supplied with the kit (gray-capped bottle). The *Positive Control* confirms the reactivity of the other reagents associated with the assay, and is not intended to ensure precision at the analytical assay cut-off. *Diluent* is used for the negative control. Additional tests can be performed with the controls to meet the requirements of local, state and/or federal regulations and/or accrediting organizations.

LIMITATIONS

1. The *TOX A/B QUIK CHEK*® test is used to detect *C. difficile* toxin(s) in fecal specimens. The test confirms the presence of toxin in feces and this information should be taken under consideration by the physician in light of the clinical history and physical examination of the patient. The *TOX A/B QUIK CHEK*® test will detect levels of toxin A at ≥0.63 ng/mL and toxin B at ≥1.25 ng/mL.

2. Fecal specimens are extremely complex. Optimal results with the *TOX A/B QUIK CHEK®* test are obtained with specimens that are less than 24 hours old. Most undiluted specimens can be stored between 2° and 8°C for 72 hours before significant degradation of the toxin is noted. If specimens are not assayed within this time period, they may be frozen and thawed. However, repeated freezing and thawing may result in loss in the immunoreactivity of toxins A and B.
3. Some specimens may give weak reactions. This may be due to a number of factors such as the presence of low levels of toxin, the presence of binding substances, or inactivating enzymes in the feces. *Under these conditions, a fresh specimen should be tested.* Additional tests that may be used in conjunction with the *TOX A/B QUIK CHEK®* test include culture with toxigenic testing or tissue culture cytotoxicity assay for the detection of *C. difficile* or its toxin(s).
4. Fecal specimens preserved in 10% Formalin, merthiolate formalin, sodium acetate formalin, or polyvinyl alcohol cannot be used.
5. The *TOX A/B QUIK CHEK®* test is qualitative. The intensity of the color should not be interpreted quantitatively.
6. Some isolates of *C. sordellii* may react in the *TOX A/B QUIK CHEK®* test due to the production of immunologically related toxins (1).
7. Colonization rates of up to 50% have been reported in infants. A high rate has also been reported in cystic fibrosis patients (1,3).

EXPECTED VALUES

The reported incidence of *C. difficile*-associated disease in patients with antibiotic-associated diarrhea is 10 to 20%. In our studies, the incidence ranged from 10% to 22%. The prevalence of a positive *TOX A/B QUIK CHEK®* test will vary from location to location and hospitals may experience rates lower or higher than those observed at the sites used in this evaluation. *Clostridium difficile* disease is primarily a nosocomial disease of elderly patients, and hospitals that have higher numbers of elderly patients may experience higher rates. It is important to consider any test results in conjunction with clinical symptoms because some healthy adults and large numbers of healthy infants (up to 50%) will be positive for *C. difficile* toxin.

PERFORMANCE CHARACTERISTICS

The *TOX A/B QUIK CHEK®* test was compared with the tissue culture test at three U.S. hospitals and in-house at TECHLAB®, Inc. Specimens included in the evaluation were submitted to the clinical laboratory for routine testing. The tissue culture test was done according to the in-house procedure. The table below shows a summary of the clinical performance of the *TOX A/B QUIK CHEK®* test. The test exhibited a sensitivity and specificity of 90.2% and 99.7%, respectively. The predictive positive and negative values were 98.6% and 97.9%, respectively, and the correlation was 98.0%.

TABLE 1. Correlation of the *TOX A/B QUIK CHEK®* test with tissue culture.

N = 842	Tissue Culture positive	Tissue Culture negative
TOX A/B QUIK CHEK â positive	138	2
TOX A/B QUIK CHEK â negative	15	687

		95% CI
Sensitivity	90.2%	84.1 - 94.2
Specificity	99.7%	98.8 - 99.9
Predictive Positive Value	98.6%	94.4 - 99.8
Predictive Negative Value	97.9%	96.4 - 98.7
Correlation	98.0%	97.8 - 98.2

Of the 2 tissue culture-negative/*TOX A/B QUIK CHEK®*-positive samples, 1 was negative in a commercial toxin A+B ELISA. Of the 15 specimens that were tissue culture-positive/*TOX A/B QUIK CHEK®*-negative, 12 were negative in commercial toxin A+B ELISAs. There were 9 specimens that were unreadable. All of the specimens were negative by PCR analysis for the genes of toxin A (*tcdA*) and toxin B (*tcdB*).

A total of 51 fecal specimens diluted in Cary Blair and 32 fecal specimens diluted in C&S Transport Media were tested in the *TOX A/B QUIK CHEK®* test and the results were compared to those obtained by routine testing. The test exhibited an agreement of 97.6% for the detection of *C. difficile* toxins in specimens prepared in Transport Media.

ANALYTICAL SENSITIVITY

The test was consistently positive at a concentration of 0.63 ng/mL for toxin A and 1.25 ng/mL for toxin B.

REPRODUCIBILITY

The reproducibility of the *TOX A/B QUIK CHEK®* test was determined using known positive (n=6) and negative (n=2) fecal specimens that were coded and sorted to prevent their identification during testing. Testing was performed on-site at 3 laboratories, which tested the samples on 3 days. The samples produced the expected results 100% of the time.

CROSS REACTIVITY

Fecal specimens inoculated with the following microorganisms to a final concentration of approximately 10^5 or higher organisms per mL did not react in the *TOX A/B QUIK CHEK®*:
Bacteria: *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Bacteroides fragilis, Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, Candida albicans, Clostridium bifermentans, Clostridium butyricum, Clostridium perfringens, Clostridium septicum, Clostridium sordellii* (nontoxigenic), *Clostridium sporogenes, Enterococcus faecalis, Escherichia coli* EIEC, *Escherichia coli, Escherichia coli* 0157 H7, *Escherichia coli* ETEC, *Klebsiella pneumoniae, Peptostreptococcus anaerobius, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus aureus (Cowans), Staphylococcus epidermidis, Vibrio parahaemolyticus, Yersinia enterocolitica*
Viruses: Adenovirus types 1,2,3,5,40,41, Human coronavirus, Coxsackievirus B2,B3,B4,B5, Echovirus 9,11,18,22,33, Enterovirus type 68,69,70,71.

The only non-*C. difficile* organism to react with the *TOX A/B QUIK CHEK®* was *C. sordellii* VPI 9048. This strain produces toxins HT and LT, which are homologous to toxins A and B, respectively.

INTERFERING SUBSTANCES

The following substances had no effect on test results when present in feces in the concentrations indicated: mucin (3.5% w/v), human blood (40% v/v), barium sulfate (5% w/v), Imodium® (5% w/v), Kaopectate® (5 mg/mL), Pepto-Bismol® (5% w/v), steric/palmitic acid (40% w/v), Metronidazole (0.25% w/v), Vancomycin (0.25% w/v).

DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service
Food and Drug Administration
2098 Gaither Road
Rockville, Maryland 20850

February 8, 2008

Donna T Link
Quality Assurance Manager
TECHLAB, Inc.
2001 Kraft Drive
Blacksburg, VA 24060-6358 US

Re: k050891/A001
Received: January 15, 2008

Categorization Notification

Regulations codified at 42 CFR 493.17 et. seq., implementing the Clinical Laboratory Improvement Amendments of 1988, require the Secretary to provide for the categorization of specific clinical laboratory test systems by the level of complexity. Based upon these regulations, the following commercially marketed test system or assay for the analyte is categorized below:

Test System/Analyte (s) : (SEE ATTACHMENT)

This complexity categorization is effective as of the date of this notification and will be reported on FDA's home page http://www.fda.gov/cdrh/clia. This categorization information may be provided to the user of the commercially marketed test system or assay as specified for the analyte indicated. It will also be announced in a Federal Register Notice, which will provide opportunity for comment on the decision. FDA reserves the right to reevaluate and recategorize this test based upon the comments received in response to the Federal Register Notice.

If you change the test system name or your company's name or if a distributor's name replaces your name, you must request another categorization by sending in the revised labeling along with a letter to FDA referencing the document number above.

If you have any questions regarding this complexity categorization, please contact Freddie Poole at 240-276-0496.

Sincerely yours,

Steven Gutman

Steven I. Gutman, M.D., M.B.A.
Director
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

ATTACHMENT

Document Number : k050891/A001

Test System: Fisher Healthcare Sure-Vue C. difficile TOX A/B
Analyte : Clostridium Difficile
Complexity : MODERATE

CLIA Routing Slip

Document No : k050891/A001

Division: DMD
Branch: BACB

Applicant: TECHLAB, Inc.
Trade Name: Tox a/b quick chek

DMC Date Received: January 15, 2008
Division Date Received: January 18, 2008

Categorization Information

CLIA Reviewer: Tamara Felton [TMF]
Date Review Completed: February 8, 2008
Date Branch Concurred: February 8, 2008
Date Coordinator Concurred: FEB 8 2008
Effective Date:

Test Systems/Analytes/Grading

(See Attachment)

DMC 2/11

ATTACHMENT

Document Number : k050891/A001

Test System : Fisher Healthcare Sure-Vue C. difficile TOX A/B
Analyte : Clostridium Difficile
Complexity : MODERATE [10]
Knowledge [2]; Training and Experience [2]; Reagents Preparation [1];
Operational Steps [1]; Quality Control [1];
Troubleshooting and Maintenance [1]; Interpretation and Judgment [2]
Rationale : BA-026

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date: 1-16-08

From: DMC (HFZ-401)

Subject: Premarket Notification Number(s): K050891/A1

To: Division Director: MI/DMD

The attached information has been received by the 510(k) DMC on the above referenced 510(k) submission(s). Since a final decision has been rendered, this record is officially closed.

Please review the attached document and return it to the DMC, with one of the statements checked below.

_____Information does not change the status of the 510(k); no other action required by the DMC; please add to image file. (Prepare K-25) THIS DOES NOT APPLY TO TRANSFER OF OWNERSHIP. PLEASE BRING ANY TRANSFER OF OWNERSHIP TO POS.

_____Additional information requires a new 510(k); however, the information submitted is incomplete; (Noitify company to submit a new 510(k);[Prepare the K30 Letter on the LAN]

_____No response necessary (e.g., hard copy of fax for the truthful and accuracy statement, 510(k) statement, change of address, phone number, or fax number).

CLIA CATEGORIZATION refers to laboratory test system devices reviewed by the Division of Clinical Laboratory Devices (HFZ-440

__X__Information requires a **CLIA CATEGORIZATION**; the complexity may remain the same as the original 510(k) or may change as a result of the additional information (Prepare a CAT letter)

____Additional information requires a **CLIA CATEGORIZATION**; however, the information submitted is incomplete; (call or fax firm)

_____No response necessary

This information should be returned to the DMC within 10 working days from the date of this memorandum.

Reviewed by: TMF

Date: ~~02/08/08~~ 02/08/08
TMF

K050891/A1

TECHLAB®

January 14, 2008

CLIA Coordinator
Document Control Center (HFZ-401)
Food and Drug Administration
Center for Device and Radiological Health
Office of Device Evaluation
9200 Corporate Boulevard
Rockville, Maryland 20850

www.techlab.com

Subject: Sure-Vue® C. difficile TOX A/B Private Labeling of TECHLAB®, Inc. *TOX A/B QUIK CHEK®* (K050891) and request for CLIA categorization.

Dear CLIA Coordinator:

The purpose of this letter is to provide add-to-file notification that TECHLAB®, Inc. is to manufacture its *TOX A/B QUIK CHEK®* test under the brand name Sure-Vue® C. difficile TOX A/B (by Thermo Fisher Scientific) and to request CLIA characterization of the latter.

Enclosed is the proposed Package Insert (2 copies) for the Sure-Vue® C. difficile TOX A/B for CLIA Categorization purposes only. Please note that the Sure-Vue® C. difficile TOX A/B is identical in all respects (except product name).

Should you have any questions or require additional information regarding this matter, please contact me at:

Kimberly

TECHLAB®, Inc.
2001 Kraft Drive
Blacksburg, VA 24060
Tel: (540) 953-1664
Fax: (540) 953-1665

FDA CDRH DMC

JAN 15 2008

Received

Regards,

Donna T. Link
Quality Assurance Manager

FDA CDRH DMC

JAN 15 2008

Received

IC 12

Enclosure(s) 3

DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville, Maryland 20850

August 11, 2005

DAVID M LYERLY
Techlab, Inc.
2001 KRAFT DR.
BLACKSBURG, VA 24060-6358
US

Re: k050891
Received: April 8, 2005

Categorization Notification

Regulations codified at 42 CFR 493.17 et. seq., implementing the Clinical Laboratory Improvement Amendments of 1988, require the Secretary to provide for the categorization of specific clinical laboratory test systems by the level of complexity. Based upon these regulations, the following commercially marketed test system or assay for the analyte is categorized below:

Test System/Analyte (s) : (SEE ATTACHMENT)

This complexity categorization is effective as of the date of this notification. This categorization will be reported on FDA's home page http://www.fda.gov/cdrh/clia. This categorization information may be provided to the user of the commercially marketed test system or assay as specified for the analyte indicated. This categorization will also be announced in a Federal Register Notice, which will provide opportunity for comment on the decision. FDA reserves the right to reevaluate and recategorize this test based upon the comments received in response to the Federal Register Notice.

If you have any questions regarding this complexity categorization, please contact Freddie Poole at 240-276-0496.

Sincerely yours,

Steven Gutman

Steven I. Gutman, M.D., M.B.A.
Director
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

ATTACHMENT

Document Number : k050891

Test System: Techlab TOX A/B QUIK CHEK
Analyte : Clostridium Difficile
Complexity : MODERATE

CLIA Routing Slip

Document No : k050891

Division: DMD
Branch: BACB

Applicant: Techlab, Inc.
Trade Name: Tox a/b quick chek

DMC Date Received: April 8, 2005
Division Date Received: April 11, 2005

Categorization Information

CLIA Reviewer: Tara Goldman [TDG]
Date Review Completed: July 28, 2005

Date Branch Concurred: August 9, 2005
Date Coordinator Concurred:
Effective Date: AUG 11 2005

Test Systems/Analytes/Grading

(See Attachment)

DMC 8/11

ATTACHMENT

Document Number : k050891

Test System: Techlab TOX A/B QUIK CHEK
Analyte : Clostridium Difficile
Complexity : MODERATE [10]
Knowledge [2]; Training and Experience [2]; Reagents Preparation [1];
Operational Steps [1]; Quality Control [1];
Troubleshooting and Maintenance [1]; Interpretation and Judgment [2]
Rationale : BA-026

Tech kit

TOX A/B QUIK CHEK™

A rapid test for the detection of *C. difficile* toxins A and B in fecal specimens
Patent Pending

Catalog #T5033 (25 tests)

INTENDED USE

The *TOX A/B QUIK CHEK*™ test is a rapid immunoassay for detecting *Clostridium difficile* toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history.
FOR *IN VITRO* DIAGNOSTIC USE.

EXPLANATION

After treatment with antibiotics, many patients develop gastrointestinal problems ranging from mild diarrhea to severe pseudomembranous colitis. Many cases of the milder forms of gastrointestinal illness and most cases of pseudomembranous colitis are caused by toxigenic strains of *Clostridium difficile* (1). This organism is an opportunistic anaerobic bacterium that grows in the intestine once the normal flora has been altered by the antibiotic. Toxigenic strains of *C. difficile* carry the genes encoding the toxins while non-toxigenic strains do not carry the toxin genes. The disease results from the toxins that the toxigenic organism produces. The clinical symptoms associated with the disease are believed to be primarily due to toxin A, which is a tissue-damaging enterotoxin (2,3). *C. difficile* also produces a second toxin, designated toxin B. Toxin B, which has been referred to as the cytotoxin of the organism, is the toxin detected by the tissue culture assay currently used by many laboratories. Toxigenic *C. difficile* strains produce both toxins or only toxin B (4-7).

PRINCIPLE OF THE TEST

The *TOX A/B QUIK CHEK*™ uses antibodies specific for toxins A and B of *C. difficile*. The device contains a *Reaction Window* with two vertical lines of immobilized antibodies (Fig. 1a). The test line ("T") contains antibodies against *C. difficile* toxins A and B. The control line ("C") contains anti-IgG antibodies. The *Conjugate* consists of antibodies to toxins A and B coupled to horseradish peroxidase. To perform the test, the fecal specimen is diluted with *Diluent* and *Conjugate* is added to the diluted sample. The diluted sample-conjugate mixture is added to the *Sample Well* and the device is allowed to incubate at room temperature for 15 minutes. During the incubation, any toxin A and toxin B in the sample bind to anti-toxin antibody-peroxidase conjugate. The toxin-antibody complexes migrate through a filter pad to a membrane where they are captured by the immobilized anti-toxin antibodies in the line. The *Reaction Window* is subsequently washed with *Wash Buffer*, and the test is developed with the addition of *Substrate*. After a 10 minute incubation period, the "T" reaction is examined visually for the appearance of a vertical blue line on the "T" side of the *Reaction Window*. A blue line indicates a positive test. A positive "C" reaction, indicated by a vertical blue line on the "C" side of the *Reaction Window*, confirms that the test is working properly and the results are valid.

MATERIALS PROVIDED

Membrane Devices –25 pouches, each containing 1 device and a desiccant pack
***Diluent* (14 mL)** – Buffered protein solution containing 0.02% thimerosal
***Wash Buffer* (10 mL)** – A buffered solution containing 0.02% thimerosal
***Substrate* (3.5 mL)** – Solution containing tetramethylbenzidine
***Conjugate* (2 mL)** – Mouse monoclonal antibody specific for toxin A coupled to horseradish peroxidase and goat polyclonal antibody specific for toxin B coupled to horseradish peroxidase in a buffered protein solution containing 0.02% thimerosal
***Positive Control* (1 mL)** – Antigen in a buffered protein solution
Disposable plastic transfer pipettes – 50 (graduated at 25 µL and 400 µL)

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Small test tubes (e.g., plastic Eppendorf tubes)
Timer
Disposable gloves for handling fecal samples
Applicator sticks
Vortex mixer
Pipettor and tips

SHELF LIFE AND STORAGE

The expiration date of the kit is given on the label. Expiration dates for each component are listed on the individual labels. The kit should be stored between 2° and 8°C.

PRECAUTIONS

1. Reagents from different kits should not be mixed. Do not use a kit past the expiration date.
2. Bring all components to ROOM TEMPERATURE BEFORE USE!
3. Caps and tips are color-coded; do NOT mix!
4. Do not freeze the reagents. The kit should be stored between 2°C and 8°C.
5. The pouch containing the *Membrane Device* should be at room temperature before opening, and opened just before use. Keep the membrane devices dry before use.
6. Use fecal specimens within 72 hours of collection to obtain optimal results. Specimens that are frozen may lose activity due to freezing and thawing.
7. Specimens that have been preserved in 10% Formalin, merthiolate formalin, sodium acetate formalin or polyvinyl alcohol cannot be used.
8. Specimens in transport media such as Cary Blair and C&S can be used as specified in the specimen preparation protocol.
9. Hold reagent bottles vertically to dispense reagents to ensure consistent drop size.
10. Specimens and membrane devices should be handled and disposed of as potential biohazards after use. Wear disposable gloves when doing the test.

11. Reagents contain thimerosal as a preservative and should be handled with normal laboratory caution.
12. Membrane devices cannot be reused.
13. The test has been optimized for sensitivity and specificity. Alterations of the specified procedure and/or test conditions may affect the sensitivity and specificity of the test. Do not deviate from the specified procedure.
14. Microbial contamination of reagents may decrease the accuracy of the assay. Avoid microbial contamination of reagents by using sterile disposable pipettes if removing aliquots from reagent bottles.
15. Be attentive to the total assay time when testing more than one fecal specimen. Add *Diluent* first, then add the *Conjugate* to each tube of *Diluent*. Then add specimen to the tube of *Diluent/Conjugate*. Thoroughly mix all of the diluted specimens, and then transfer to the *Membrane Device*. The 15-minute incubation step begins after the last diluted sample-conjugate mixture has been transferred to the final *Membrane Device*.

COLLECTION AND HANDLING OF FECAL SPECIMENS

1. Standard collection and handling procedures used in-house for fecal specimens are appropriate. Specimens should be stored between 2° and 8°C; test specimens that are less than 24 hours old, whenever possible.
2. Store specimens frozen (≤-10°C) if the test cannot be performed within 72 hours of collection, but note that freezing and thawing of the specimen may result in loss of activity due to degradation of the toxins.
3. Make sure that specimens are thoroughly mixed PRIOR to performing the assay.
4. Storing fecal specimens in the *Diluent* is NOT recommended.
5. Do not allow the fecal specimens to remain in the *Diluent* and/or *Conjugate* for any extended period of time.

SPECIMEN PREPARATION

1. Bring all reagents and the required number of devices to room temperature before use.
2. Set up and label one small test tube for each specimen, and optional external controls as necessary.
3. Add 500 µL *Diluent* to each tube for fecal specimens. For specimens in transport media such as Cary Blair or C&S, add 425 µL of *Diluent* to the tube.
4. Add one drop of Conjugate (red capped bottle) to each tube.
5. Obtain one disposable plastic transfer pipette (supplied with the kit) for each sample – the pipettes have raised graduations at 25 µL and 400 µL.
6. Mix all specimens thoroughly regardless of consistency- it is essential that the specimens be evenly suspended before transferring.
   Liquid/Semi-solid specimens – pipette 25 µL of specimen with a transfer pipette (graduated at 25 µL and 400 µL) and dispense into the *Diluent*. Use the same transfer pipette to mix the diluted specimen.
   Formed/Solid specimens – Care must be taken to add the correct amount of formed feces to the sample mixture. Mix the specimen thoroughly using a wooden applicator stick and transfer a small portion (approximately 2 mm diameter, the equivalent of 25 µL) of the specimen into the *Diluent*. Emulsify the specimen using the applicator stick.
   Fecal specimens in Cary Blair or C&S transport media - pipette 100 µL of sample into the *Diluent*.
7. **Optional External Control Samples:**
   External Positive Control - add one drop of *Positive Control* (gray-capped bottle) to the appropriate test tube.
   External Negative Control - add 25 µL *Diluent* to the appropriate test tube.

NOTE: Transferring too little specimen, or failure to mix and completely suspend the specimen in the Diluent *mixture, may result in a false-negative test result. The addition of too much fecal specimen may cause invalid results due to restricted sample flow.*

TEST PROCEDURE

1. Obtain one *Membrane Device* per specimen, and one device per optional external positive or negative control as necessary. The foil bags containing the devices should be brought to room temperature before opening. Label each device appropriately and orient it on a flat surface so the letter "C" on the device is on the left, the letter "T" is on the right, and the small *Sample Well* is located in the top right corner of the device (Fig. 1a).
2. Close each tube of diluted specimen and mix thoroughly. Proper mixing can be achieved by vortexing or inverting the tube. Immediately proceed to Step #3.
3. Using a transfer pipette (graduated at 25 µL and 400 µL), transfer 400 µL of the diluted sample-conjugate mixture into the ***Sample Well*** (smaller hole in the top right corner of the device) of a *Membrane Device*, making certain to expel the liquid sample onto the wicking pad inside of the *Membrane Device*.
4. Incubate the device at room temperature for 15 minutes – the sample will wick through the device and a wet area will spread across the *Reaction Window* (larger hole in the middle of the device). If the *Reaction Window* is not completely wet at the end of the 15-minute incubation, the test is considered invalid and the sample must be retested on a new device.
   NOTE FOR SAMPLES THAT FAIL TO MIGRATE:
   Occasionally, a diluted fecal specimen cannot be tested because it clogs the membrane and the Reaction Window *does not wet properly. If the diluted fecal specimen fails to migrate properly within 5 minutes of adding the sample to the* Sample Well *(i.e. the membrane in the* Reaction Window *does not appear to be completely wet), then add 100 µL of* Diluent *to the* Sample Well *and wait an additional 5 minutes (for a total of 20 minutes).*
5. After the incubation, add 300 µL of *Wash Buffer* to the ***Reaction Window***. Allow the *Wash Buffer* to flow through the *Reaction Window* membrane and be absorbed completely.
6. Add 2 drops of *Substrate* (blue-capped bottle) to the ***Reaction Window***. Read and record results visually after 10 minutes.

INTERPRETATION OF RESULTS

1. Interpretation of the test is most reliable when the device is read immediately at the end of the reaction period. Read the device at a normal working distance in a well-lit area. View with a line of vision directly over the device.
2. Observe device for the appearance of a blue line on the "C" side of the *Reaction Window* representing the internal positive control line. Observe device for the appearance of a blue line on the "T" side of the *Reaction Window* representing the test line. The lines may appear faint to dark in intensity.
3. **Positive Result:** A positive result may be interpreted at any time between the addition of *Substrate* and the 10-minute read time. Two blue lines are visible, the control line ("C") and the test line ("T"). The lines may appear faint to dark in intensity. The appearance of a blue line on the "T" side is interpreted as a positive result. An obvious partial line is interpreted as a positive result. Do not interpret membrane discoloration or shadow as a positive result. A positive result indicates the presence of *C. difficile* toxin.

4. **Negative Result:** A test cannot be interpreted as negative or invalid until 10 minutes following the addition of *Substrate*. A single blue line is visible on the control ("C") side of the *Reaction Window* and no test line is visible on the "T" side of the *Reaction Window* (Fig. 1b). A negative result indicates *C. difficile* toxin is either absent in the specimen or is below the detection limit of the test.
5. **Invalid Result:** A single line is visible on the test ("T") side of the *Reaction Window,* or no lines are visible in the *Reaction Window* (Fig. 1c, 1d). The test result is invalid if a control line is not present at the completion of the reaction period.

FIGURE 1: *TOX A/B QUIK CHEK*™ INTERPRETATION OF RESULTS

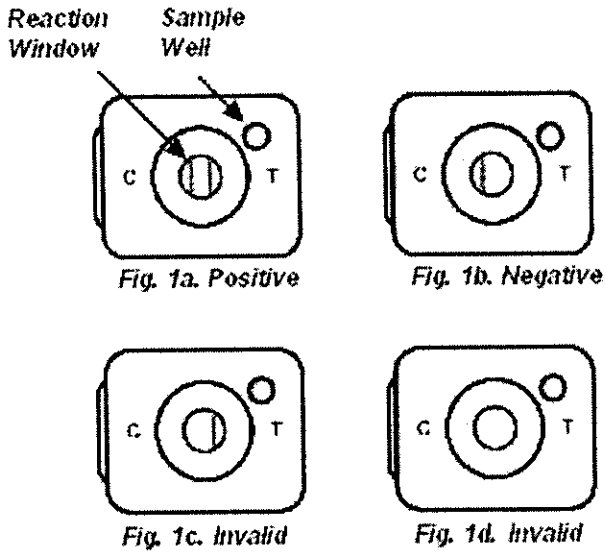

Fig. 1a. Positive *Fig. 1b. Negative*

Fig. 1c. Invalid *Fig. 1d. Invalid*

QUALITY CONTROL

Internal: A blue control line must be visible on the "C" side of the *Reaction Window* on every *Membrane Device* that is tested. The appearance of the blue control line confirms that the sample and reagents were added correctly, that the reagents were active at the time of performing the assay, and that the sample migrated properly through the *Membrane Device*.

External: The reactivity of the *TOX A/B QUIK CHEK*™ test should be verified on receipt using the *Positive Control* and negative control (*Diluent*). The *Positive Control* is supplied with the kit (gray-capped bottle). The *Positive Control* confirms the reactivity of the other reagents associated with the assay, and is not intended to ensure precision at the analytical assay cut-off.
Diluent is used for the negative control. Additional tests can be performed with the controls to meet the requirements of local, state and/or federal regulations and/or accrediting organizations.

LIMITATIONS

1. The *TOX A/B QUIK CHEK*™ test is used to detect *C. difficile* toxin(s) in fecal specimens. The test confirms the presence of toxin in feces and this information should be taken under consideration by the physician in light of the clinical history and physical examination of the patient. The *TOX A/B QUIK CHEK*™ test will detect levels of toxin A at ≥0.63 ng/mL and toxin B at ≥1.25 ng/mL.
2. Fecal specimens are extremely complex. Optimal results with the *TOX A/B QUIK CHEK*™ test are obtained with specimens that are less than 24 hours old. Most undiluted specimens can be stored between 2° and 8°C for 72 hours before significant degradation of the toxin is noted. If specimens are not assayed within this time period, they may be frozen and thawed. However, repeated freezing and thawing may result in loss in the immunoreactivity of toxins A and B.
3. Some specimens may give weak reactions. This may be due to a number of factors such as the presence of low levels of toxin, the presence of binding substances, or inactivating enzymes in the feces. *Under these conditions, a fresh specimen should be tested.* Additional tests that may be used in conjunction with the *TOX A/B QUIK CHEK*™ test include culture with toxigenic testing or tissue culture cytotoxicity assay for the detection of *C. difficile* or its toxin(s).
4. Fecal specimens preserved in 10% Formalin, merthiolate formalin, sodium acetate formalin, or polyvinyl alcohol cannot be used.
5. The *TOX A/B QUIK CHEK*™ test is qualitative. The intensity of the color should not be interpreted quantitatively.
6. Some isolates of *C. sordellii* may react in the *TOX A/B QUIK CHEK*™ test due to the production of immunologically related toxins (1).
7. Colonization rates of up to 50% have been reported in infants. A high rate has also been reported in cystic fibrosis patients (1,3).

EXPECTED VALUES

The reported incidence of *C. difficile*-associated disease in patients with antibiotic-associated diarrhea is 10 to 20%. In our studies, the incidence ranged from 10% to 22%. The prevalence of a positive *TOX A/B QUIK CHEK*™ test will vary from location to location and hospitals may experience rates lower or higher than those observed at the sites used in this evaluation. *Clostridium difficile* disease is primarily a nosocomial disease of elderly patients, and hospitals that have higher numbers of elderly patients may experience higher rates. It is important to consider any test results in conjunction with clinical symptoms because some healthy adults and large numbers of healthy infants (up to 50%) will be positive for *C. difficile* toxin.

PERFORMANCE CHARACTERISTICS

The *TOX A/B QUIK CHEK*™ test was compared with the tissue culture test at three U.S. hospitals and in-house at TECHLAB®, Inc. Specimens included in the evaluation were submitted to the clinical laboratory for routine testing. The tissue culture test was done according to the in-house procedure. The table below shows a summary of the clinical performance of the *TOX A/B QUIK CHEK*™ test.

The test exhibited a sensitivity and specificity of 90.2% and 99.7%, respectively. The predictive positive and negative values were 98.6% and 97.9%, respectively, and the correlation was 98.0%.

Table 1. Correlation of the *TOX A/B QUIK CHEK*™ test with tissue culture.

N = 842	Tiss cult pos	Tiss cult neg
***TOX A/B QUIK CHEK*™ pos**	138	2
***TOX A/B QUIK CHEK*™ neg**	15	687

		95% CI
Sensitivity	90.2	84.1 - 94.2
Specificity	99.7	98.8 - 99.9
Predictive Positive Value	98.6	94.4 - 99.8
Predictive Negative Value	97.9	96.4 - 98.7
Correlation	98.0	97.8 - 98.2

Of the 2 tissue culture-negative/*TOX A/B QUIK CHEK*™-positive samples, 1 was negative in a commercial toxin A+B ELISA. Of the 15 specimens that were tissue culture-positive/*TOX A/B QUIK CHEK*™-negative, 12 were negative in commercial toxin A+B ELISAs. There were 9 specimens that were unreadable. All of the specimens were negative by PCR analysis for the genes of toxin A (*tcdA*) and toxin B (*tcdB*).

A total of 51 fecal specimens diluted in Cary Blair and 32 fecal specimens diluted in C&S Transport Media were tested in the *TOX A/B QUIK CHEK*™ test and the results were compared to those obtained by routine testing. The test exhibited an agreement of 97.6% for the detection of *C. difficile* toxins in specimens prepared in Transport Media.

ANALYTICAL SENSITIVITY

The test was consistently positive at a concentration of 0.63 ng/mL for toxin A and 1.25 ng/mL for toxin B.

REPRODUCIBILITY

The reproducibility of the *TOX A/B QUIK CHEK*™ test was determined using known positive (n=6) and negative (n=2) fecal specimens that were coded and sorted to prevent their identification during testing. Testing was performed on-site at 3 laboratories, which tested the samples on 3 days. The samples produced the expected results 100% of the time.

CROSS REACTIVITY

Fecal specimens inoculated with the following microorganisms to a final concentration of approximately 10^8 or higher organisms per mL did not react in the *TOX A/B QUIK CHEK*™:

Bacteria: *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Bacteroides fragilis, Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, Candida albicans, Clostridium bifermentans, Clostridium butyricum, Clostridium perfringens, Clostridium septicum, Clostridium sordellii* (nontoxigenic), *Clostridium sporogenes, Enterococcus faecalis, Escherichia coli* EIEC, *Escherichia coli, Escherichia coli* 0157 H7, *Escherichia coli* ETEC, *Klebsiella pneumoniae, Peptostreptococcus anaerobius, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus aureus (Cowans), Staphylococcus epidermidis, Vibrio parahaemolyticus, Yersinia enterocolitica* **Viruses**: Adenovirus types 1,2,3,5,40,41, Human coronavirus, Coxsackievirus B2,B3,B4,B5, Echovirus 9,11,18,22,33, Enterovirus type 68,69,70,71.

The only non-*C. difficile* organism to react with the *TOX A/B QUIK CHEK*™ was *C. sordellii* VPI 9048. This strain produces toxins HT and LT, which are homologous to toxins A and B, respectively.

INTERFERING SUBSTANCES

The following substances had no effect on test results when present in feces in the concentrations indicated: mucin (3.5% w/v), human blood (40% v/v), barium sulfate (5% w/v), Imodium® (5% w/v), Kaopectate® (5 mg/mL), Pepto-Bismol® (5% w/v), steric/palmitic acid (40% w/v), Metronidazole (0.25% w/v), Vancomycin (0.25% w/v).

REFERENCES

1. Lyerly, D. M., H. C. Krivan, and T. D. Wilkins. 1988. *Clostridium difficile:* its disease and toxins. Clin. Microbiol. Rev. 1:1-18.
2. Lyerly, D. M., K. E. Saum, D. K. MacDonald, and T. D. Wilkins. 1985. Effects of *Clostridium difficile* toxins given intragastrically to animals. Infect. Immun. 47: 349-352.
3. Borriello, S. P., F. E. Barclay, A. R. Welch, J. M. Ketley, T. J. Mitchell, J. Stephen, and G. E. Griffin. 1985. Host and microbial determinants of the spectrum of *Clostridium difficile* mediated gastrointestinal disorders. Microecol. Ther. 15:231-236.
4. Lyerly, D. M., N. M. Sullivan, and T. D. Wilkins 1983 Enzyme-linked immunosorbent assay for *Clostridium difficile* toxin A. J. Clin. Microbiol. 17:72-78.
5. Laughon, B. E., R. P. Viscidi, S. L. Gdovin, R. H. Yolken, and J. G. Bartlett. 1984. Enzyme immunoassays for detection of *Clostridium difficile* toxins A and B in fecal specimens. J. Infect. Dis. 149: 781-788.
6. Lyerly, D. M., L. A. Barroso, and T. D. Wilkins. 1992. Characterization of a toxin A-/toxin B+ isolate of *Clostridium difficile*. Infect. Immun. 60: 4633-4639.
7. Dove, C. H., S.-Z. Wang, S. B. Price, C. J. Phelps, D. M. Lyerly, T. D. Wilkins, and J. L. Johnson. 1990. Molecular characterization of the *Clostridium difficile* toxin A gene. Infect. Immun. 58: 480-488.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

JUL 25 2005

David M. Lyerly, Ph.D.
Vice President, Research and Development
TECHLAB®, Inc.
2001 Kraft Drive
Corporate Research Center
Blackburg, VA 24060-6358

Re: k050891
Trade/Device Name: TOX A/B QUICK CHECK™
Regulation Number: 21 CFR 866.2660
Regulation Name: Microorganism differentiation and identification device
Regulatory Class: Class I
Product Code: LLH
Dated: July 5, 2005
Received: July 8, 2005

Dear Dr. Lyerly:

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.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. 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 Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific information about the application of labeling requirements to your device, or questions on the promotion and advertising of your device, please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (240)276-0484. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). 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) 443-6597 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html

Sincerely yours,

Sally A. Hojvat

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

REVISED

Indications for Use

510(k) Number (if known): K050891

Device Name: *TOX A/B QUIK CHEK™*

Indications For Use:

The *TOX A/B QUIK CHEK™* test is a rapid immunoassay for detecting *Clostridium difficile* toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history. FOR *IN VITRO* DIAGNOSTIC USE.

Prescription Use X AND/OR Over-The-Counter Use

(Part 21 CFR 801 Subpart D) (21 CFR 807 Subpart C)

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

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Freddie L. Poole

Division Sign-Off

Office of In Vitro Diagnostic Device Evaluation and Safety

510(k) K050891

DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

June 02, 2005

TECHLAB, INC.
2001 KRAFT DR.
BLACKSBURG, VA 24060
ATTN: DAVID M. LYERLY

510(k) Number: K050891
Product: TOX A/B QUICK CHEK

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 (HFZ-401) 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 www.fda.gov/cdrh/ode/a02-01.html.

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/cdrh/modact/leastburdensome.html

If after 30 days the requested information, or a request for an extension of time, is not received, we will discontinue review of your submission and proceed to delete your file from our review system. Please note our guidance document entitled, "Guidance for Industry and FDA Staff FDA and Industry Actions on Premarket Notification (510(k)) Submissions: Effect on FDA Review Clock and Performance Assessment". The purpose of this document is to assist agency staff and the device industry in understanding 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 and Modernization Act. You may review this document at http://www.fda.gov/cdrh/mdufma/guidance/1219.html. 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.

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 or policy questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301) 443-6597 or at their toll-free number (800) 638-2041, or contact me at (301) 594-1190.

Sincerely yours,

Marjorie Shulman
Supervisor Consumer Safety Officer
Premarket Notification Section
Office of Device Evaluation
Center for Devices and
Radiological Health

DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

April 11, 2005

TECHLAB, INC.
2001 KRAFT DR.
BLACKSBURG, VA 24060
ATTN: DAVID M. LYERLY

510(k) Number: K050891
Received: 08-APR-2005
Product: TOX A/B QUICK CHEK

The Food and Drug Administration (FDA), Center for Devices and Radiological Health (CDRH), has received the Premarket Notification you submitted in accordance with Section 510(k) of the Federal Food, Drug, and Cosmetic Act(Act) for the above referenced product. We have assigned your submission a unique 510(k) number that is cited above. Please refer prominently to this 510(k) number in any future correspondence that relates to this submission. We will notify you when the processing of your premarket notification 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.

On May 21, 2004, FDA issued a Guidance for Industry and FDA Staff entitled, "FDA and Industry Actions on Premarket Notification (510(k)) Submissions: Effect on FDA Review Clock and Performance Assessment". The purpose of this document is to assist agency staff and the device industry in understanding 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 and Modernization Act. Please review this document at http://www.fda.gov/cdrh/mdufma/guidance/1219.html.

Please remember that all correspondence concerning your submission MUST be sent to the Document Mail Center (DMC)(HFZ-401) 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 www.fda.gov/cdrh/ode/a02-01.html.

You should be familiar with the regulatory requirements for medical device available at Device Advice http://www.fda.gov/cdrh/devadvice/". If you have other procedural or policy questions, or want information on how to check on the status of your submission, please contact DSMICA at (301) 443-6597 or its toll-free number (800) 638-2041, or at their Internet address http://www.fda.gov/cdrh/dsmamain.html or me at (301)594-1190.

Sincerely yours,

Marjorie Shulman
Supervisory Consumer Safety Officer
Office of Device Evaluation
Center for Devices and Radiological Health

DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

April 08, 2005

TECHLAB, INC.
2001 KRAFT DR.
BLACKSBURG, VA 24060
ATTN: DAVID M. LYERLY

510(k) Number: K050891
Received: 08-APR-2005
Product: TOX A/B QUICK CHEK
User Fee ID Number: 6020484

The Food and Drug Administration (FDA) Center for Devices and Radiological Health (CDRH), has received the Premarket Notification you submitted in accordance with Section 510(k) of the Federal Food, Drug, and Cosmetic Act (Act) for the above referenced product. We have assigned your submission a unique 510(k) number that is cited above. Please refer prominently to this 510(k) number in any future correspondence that relates to this submission. YOU MAY NOT PLACE THIS DEVICE INTO COMMERCIAL DISTRIBUTION UNTIL YOU RECEIVE A LETTER FROM FDA ALLOWING YOU TO DO SO.

The Act, as amended by the Medical Device User Fee and Modernization Act of 2002 (MDUFMA) (Public Law 107-250), specifies that a submission shall be considered incomplete and shall not be accepted for filing until fees have been paid (Section 738(f)). Our records indicate that you have not submitted the user fee payment information and therefore your 510(k) cannot be filed and has been placed on hold. Please send a check to one of the addresses listed below:

By Regular Mail	By Private Courier (e.g., Fed Ex, UPS, etc.)
Food and Drug Administration P.O. Box 956733 St. Louis, MO 63195-6733.	U.S. Bank 956733 1005 Convention Plaza St. Louis, MO 63101 (314) 418-4983

The check should be made out to the Food and Drug Administration referencing the payment identification number, and a copy of the User Fee Cover sheet should be included with the check. A copy of the Medical Device User Fee Cover Sheet should be faxed to CDRH at (301) 594-2977 referencing the 510(k) number if you have not already sent it in with your 510(k) submission. After the FDA has been notified of the receipt of your user fee payment, your 510(k) will be filed and the review will begin. If payment has not been received within 30 days, your 510(k) will be deleted from the system. Additional information on user fees and how to submit your user fee payment may be found at http://www.fda.gov/oc/mdufma.

Please note that since your 510(k) has not been reviewed, additional information may be required during the review process and the file may be placed on hold once again. If you are unsure as to whether or not you need to file an application with FDA or what type of application to file, you should first telephone the Division of Small Manufacturers, International and Consumer Assistance (DSMICA), for guidance at (301)443-6597 or its toll-fee number (800)638-2041, or contact them at their Internet address http://www.fda.gov/cdrh/dsmamain.html, or you may submit a 513(g) request to the Document Mail Center at the address above. If you have any questions concerning the contents of this letter, you may contact me at (301) 594-1190.

Sincerely yours,

Marjorie Shulman
Consumer Safety Officer
Office of Device Evaluation
Center for Devices and
Radiological Health

K050891

510(k) Notification

TOX A/B QUIK CHEK™

ORIGINAL

TECHLAB®

Blacksburg, VA

M1

April 5, 2005

5K14

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Form Approved: OMB No. 0910-511 Expiration Date: August 31, 2005. See Instructions for OMB Statement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION
MEDICAL DEVICE USER FEE COVER SHEET

PAYMENT IDENTIFICATION NUMBER: (b) (4)
Write the Payment Identification number on your check.

A completed Cover Sheet must accompany each original application or supplement subject to fees. The following actions must be taken to properly submit your application and fee payment:

1. Electronically submits the completed Cover Sheet to the Food and Drug Administration (FDA) before payment is sent.
2. Include printed copy of this completed Cover Sheet with a check made payable to the Food and Drug Administration. Remember that the Payment Identification Number must be written on the check.
3. Mail Check and Cover Sheet to the US Bank Lock Box, FDA Account, P.O. Box 956733, St. Louis, MO 63195-6733. (*Note: In no case should payment be submitted with the application.*)
4. If you prefer to send a check by a courier, the courier may deliver the check and Cover Sheet to: US Bank, Attn: Government Lockbox 956733, 1005 Convention Plaza, St. Louis, MO 63101. (Note: This address is for courier delivery only. Contact the US Bank at 314-418-4821 if you have any questions concerning courier delivery.)
5. For Wire Transfer Payment Procedures, please refer to the MDUFMA Fee Payment Instructions at the following URL: http://www.fda.gov/cdrh/mdufma/faqs.html#3a. You are responsible for paying all fees associated with wire transfer.
6. Include a copy of the complete Cover Sheet in volume one of the application when submitting to the FDA at either the CBER or CDRH Document Mail Center.

-->

1. COMPANY NAME AND ADDRESS (include name, street address, city state, country, and post office code)

TECHLAB INC
2001 KRAFT DRIVE
BLACKSBURG VA 24060-6358
US

1.1 EMPLOYER IDENTIFICATION NUMBER (EIN)
541527427

2. CONTACT NAME
David Lyerly

2.1 E-MAIL ADDRESS
dlyerly@techlab.com

2.2 TELEPHONE NUMBER (include Area code)
540-953-1664

2.3 FACSIMILE (FAX) NUMBER (Include Area code)
540-953-1665

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/dc/mdufma

Select an application type:
[X] Premarket notification(510(k)); except for third party
[ ] Biologics License Application (BLA)
[ ] Premarket Approval Application (PMA)
[ ] Modular PMA
[ ] Product Development Protocol (PDP)
[ ] Premarket Report (PMR)

3.1 Select one of the types below
[X] Original Application
Supplement 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
[X] NO, I am not a small business
4.1 If Yes, please enter your Small Business Decision Number: null

5. 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, parents, and partner firms
[ ] This biologics application is submitted under secion 351 of the Public Health Service Act for a product licensed for further manufacturing use only
[ ] The sole purpose of the application is to support conditions of use for a pediatric population
[ ] The application is submitted by a state or federal government entity for a device that is not to be distributed commercially

6. 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 [X] NO

7. USER FEE PAYMENT AMOUNT SUBMITTED FOR THIS PREMARKET APPLICATION (FOR FISCAL YEAR 2005)
(b) (4)
06-Apr-2005

Form FDA 8601 (08/2003)

Close

4/06/05

TECHLAB®

April 5, 2005

Food and Drug Administration
Center for Devices & Radiological Health
510(k) Document Mail Center (HFZ-401)
9200 Corporate Boulevard
Rockville, MD 20850
ATTN: Microbiology Branch

RE: 510(k) Notification, *TOX A/B QUIK CHEK™*

To the Food and Drug Administration:

The following information constitutes a 510(k) premarket notification for the TECHLAB®, Inc. *TOX A/B QUIK CHEK*™, a rapid membrane immunoassay that detects *Clostridium difficile* toxins A and B in fecal specimens. The test is designed to aid in the diagnosis of *C. difficile* disease ranging from mild nosocomial diarrhea to pseudomembranous colitis. The test is a rapid membrane immunoassay that offers a rapid turn-around time. It also provides the clinical laboratory with a simple-to-use test format that is familiar to medical and laboratory personnel. The test will aid the physicians in the diagnosis of *C. difficile* disease, resulting in improved healthcare for the patients.

Please contact me by phone at (540) 953-1664, by fax at (540) 953-1665, or by e-mail at dlyerly@techlab.com for any questions or suggestions that you may have with this submission.

Sincerely,

David M. Lyerly, Ph.D.
Vice-President of Research and Development

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2001 Kraft Drive, Blacksburg, VA 24060-6358 • PH: (540) 953-1664 • FAX: (540) 953-1665 • e-mail: techlab@techlab.com
An ISO 9001:2000 and ISO 13485:96 Company with GLP Contract Research and GMP Manufacturing

PREMARKET NOTIFICATION
TRUTHFUL AND ACCURATE STATEMENT
(As required by 21 CFR 807.87(j))

I certify that, in my capacity as Vice President of Research and Development at

TECHLAB®, Inc., I believe to the best of my knowledge, that all

data and information submitted in the premarket notification are truthful and

accurate and that no material fact has been omitted.

David M. Lyerly

[Signature]

David M. Lyerly, Ph.D.

[Typed Name]

April 5, 2005

[Dated]

K050891

[Premarket Notification 510(k) Number]

60

(b)(4) Confidential and Proprietary Information

[redacted]

Contents

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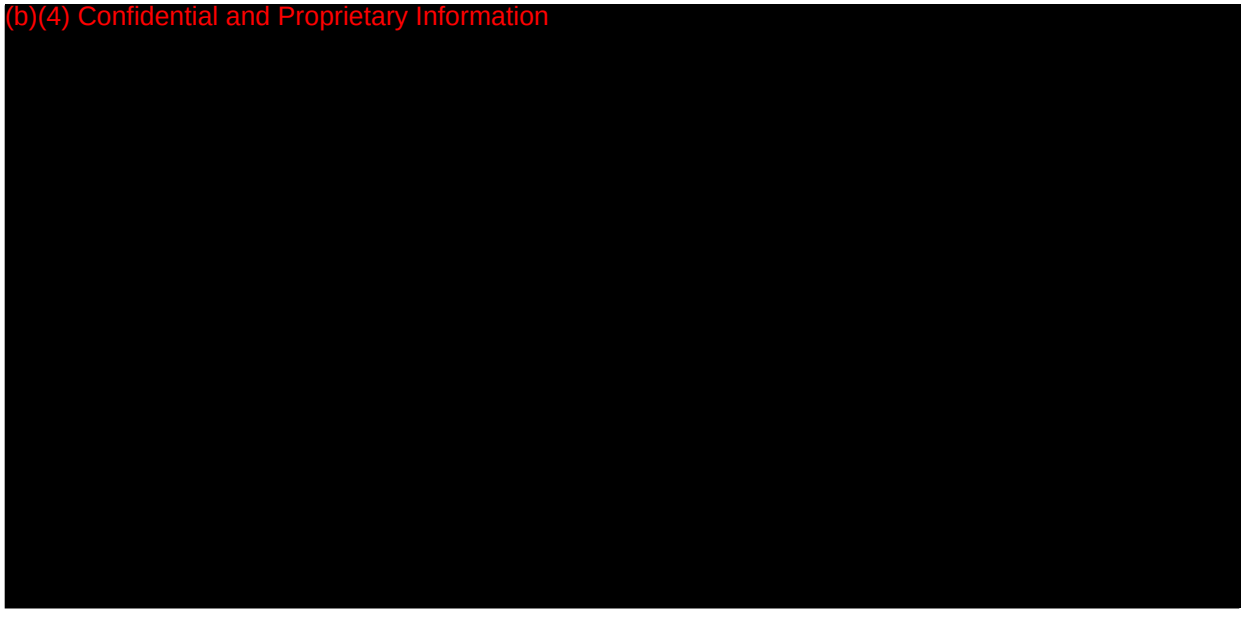
(b)(4) Confidential and Proprietary Information



1. GENERAL INFORMATION

1.1 Applicant

Date:	April 5, 2005
Name:	TECHLAB®, Inc.
Address:	2001 Kraft Drive Corporate Research Center Blacksburg, VA 24060-6364
Contact Person:	David M. Lyerly
Phone Number:	540-953-1664

Signature: David M. Lyerly

1.2 Product and Trade Name

TOX A/B QUIK CHEK™

1.3 Common Name or Classification Name

A rapid membrane immunoassay for detection of *Clostridium difficile* toxins A and B
Product Code: LLH

1.4 Established Registration Number

(b) (4)
Registration of Medical Device Establishment, #1122855

1.5 Manufacturing Facility Address

TECHLAB®, Inc.
2001 Kraft Drive
Corporate Research Center
Blacksburg, VA 24060-6364

1.6 Classification

Class I

1.7 Reason for Premarket Notification

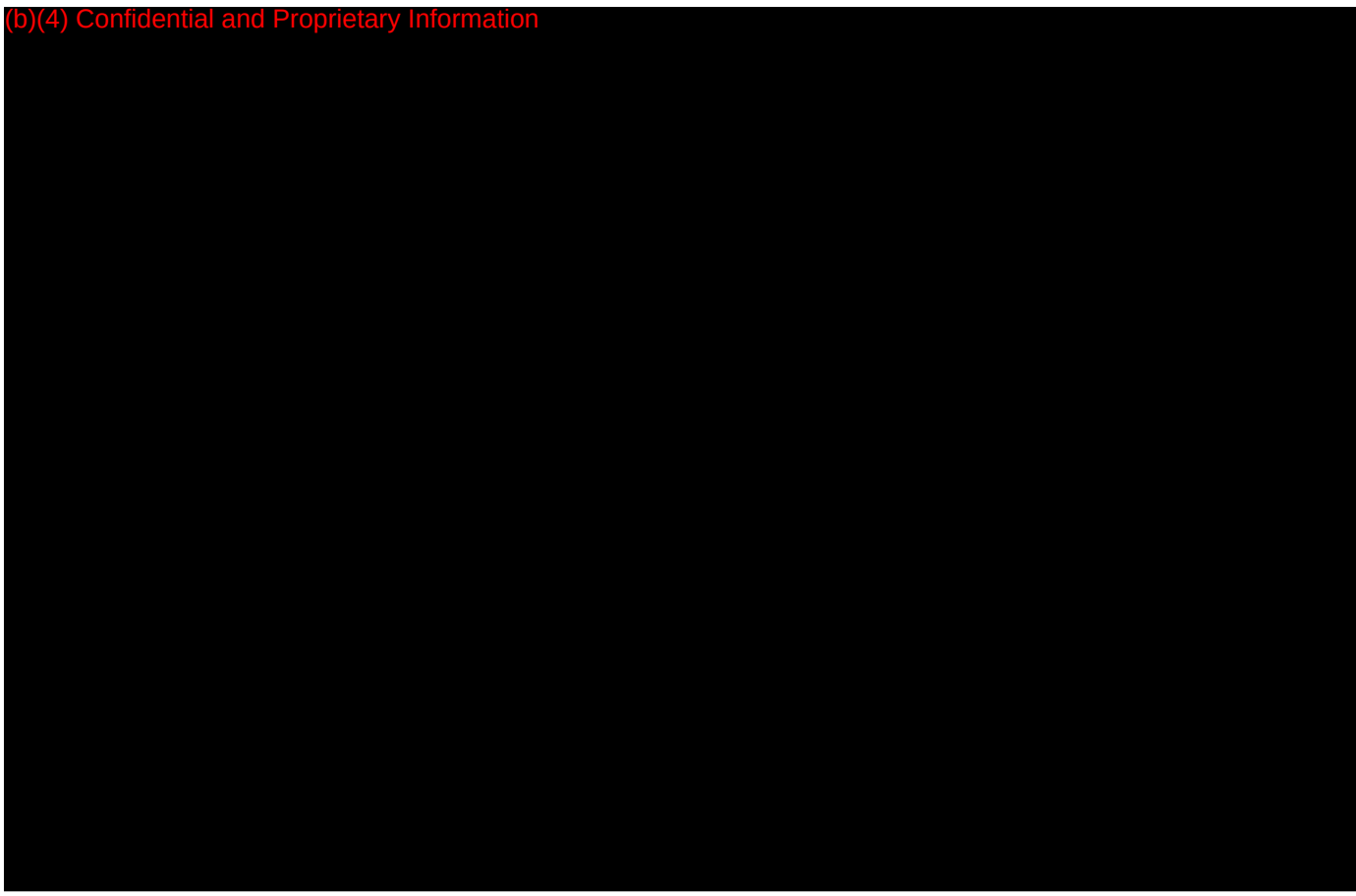
New rapid device for detection of *C. difficile* toxins A and B in fecal specimens

1.8 Predicate Device Description

Tissue culture assay is the current "Gold Standard" by FDA regulation for detecting *C. difficile* in fecal specimens. The assay is done using a commercial standardized tissue culture assay (e.g., the *C. difficile TOX-B TEST*, or *C. difficile* Toxin/Antitoxin kit) or in some cases, done using each laboratory's own protocol.

There are antibody-based devices commercially available for detection of *C. difficile* toxins A and B in fecal specimens. These antibody-based devices include ELISAs for the detection of toxins A and B in fecal specimens and rapid membrane-based formats for the detection of toxins A and B in fecal specimens. Both of the types of antibody-based devices use highly specific antibodies against toxins A and B. The turn-around time for the ELISA is about 1 hour and about 20 to 30 minutes for the rapid membrane-based tests.

(b)(4) Confidential and Proprietary Information



1.10 Predicate Device Companies

- *C. DIFFICILE TOX-B TEST* and *C. difficile* toxin/antitoxin are available from TECHLAB®, Inc. (Blacksburg, VA). Monolayer cells are available from Diagnostic Hybrids, Inc. (Athens, OH)
- TECHLAB®, Inc. (Blacksburg, VA)
- Meridian Bioscience, Inc. (Cincinnati, OH)
- Remel Inc. (Lenexa, KS)

1.11 Predicate Device 510(k) Numbers

- *C. DIFFICILE TOX-B TEST* - K935296
- *C. difficile* toxin/antitoxin - K923463
- *C. DIFFICILE TOX A/B II™* - K003306 and K030404
- Premier™ Toxins A&B - K993914
- ProSpecT® Clostridium difficile Toxin A/B - K033479
- Immuno*Card*® Toxins A&B - K041003
- X/pect™ Clostridium difficile Toxin A/B - K041951

1.12 Safety and Effectiveness Statement

The *TOX A/B QUIK CHEK*™ test is a rapid membrane immunoassay for the detection of toxins A and B of *C. difficile*. It is performed with a 25 minute turn-around time. The test detects toxins A and B produced by toxigenic strains of *C. difficile* in fecal specimens by using antibodies specific for *C. difficile* toxins A and B. The antibodies striped on the membrane are antigen-affinity purified antibodies against *C. difficile* toxins A and B. The detecting antibody consists of a monoclonal antibody specific to toxin A and polyclonal antibody to toxin B, both conjugated to horseradish peroxidase.

The *TOX A/B QUIK CHEK*™ test is highly sensitive for the detection of *C. difficile* toxins A and B in fecal specimens. The *TOX A/B QUIK CHEK*™ test offers the advantage of being a rapid test for clinical laboratories.

The *TOX A/B QUIK CHEK*™ test was compared to the tissue culture assay and discrepant results were analyzed by either the *C. DIFFICILE TOX A/B II*™ or the Premier™ Toxins A&B. The results of our clinical evaluations show that the *TOX A/B QUIK CHEK*™ test exhibited a sensitivity and specificity of 90.2% and 99.7%, respectively. The correlation with the tissue culture assay, which is considered the gold standard, was 98.0%.

These results demonstrate that the test is useful for the detection of *C. difficile* toxins A and B in fecal specimens.

2. STATEMENT OF INTENDED USE

510(k) Number (if known): ~~Not known~~ K050891

Device Name: *TOX A/B QUIK CHEK™*

Indications for Use

The *TOX A/B QUIK CHEK™* test is a rapid immunoassay for detecting *Clostridium difficile* toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history. FOR *IN VITRO* DIAGNOSTIC USE.

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

_____ _____ _____ _____ _____ _____

Concurrence of CDRH, Office of Device Evaluation (ODE)

Prescription Use_____ OR Over-The Counter Use_____
(Per 21 CFR 801.109)

(Optional format 1-2-96)

_____

Division Sign-Off

Office of In Vitro Diagnostic Device Evaluation and Safety

510(k)_____

3. DEVICE LABELING

3.1 Box Label

TOX A/B QUIK CHEK™

A Rapid Test for the Detection of *Clostridium difficile* Toxins A and B in Fecal Specimens.

IVD

FOR *IN VITRO* DIAGNOSTIC USE.

Store between 2° and 8°C / Do Not Freeze.

Test kit for 25 determinations.

Contents:	***Volumes:***
25 Membrane Devices	1 Test Each
1 Bottle Conjugate*	2 mL
1 Bottle Positive Control	1 mL
1 Bottle Diluent*	14 mL
1 Bottle Substrate	3.5 mL
1 Bottle Wash Buffer*	10 mL

*0.02% Thimerosal

TECHLAB®

TECHLAB®, Inc. Blacksburg, VA 24060
Tel.: (540) 953-1664 (800) 832-4522 USA
Fax: (540) 953-1665 www.techlab.com
Part No. 94-033-KT

Σ 25 | REF T5033 | LOT 0000000 | YYYY-MM-DD

2°C – 8°C

Do Not Freeze
Ne Pas Congeler
Nicht Einfrieren
Ne Debe Congelarse

CE EC REP Emergo Europe
PO Box 18510
2502 EM The Hague
The Netherlands

3.2 Component Labeling

[redacted]

For *In Vitro* Diagnostic Use. Store between 2° and 8°C.

IVD

Conjugate
Conjugué
Konjugat
Conjugado

REF 5033C
LOT 0000000
YYYY-MM-DD

TECHLAB®, Inc.
Blacksburg, VA 24060

2 mL

Part No. 94-033-C0

[redacted]

For *In Vitro* Diagnostic Use. Store between 2° and 8°C. TECHLAB®, Inc. Blacksburg, VA 24060

IVD

Diluent
Diluant
Verdünnungspuffer
Diluyente

14 mL

REF 5033D
LOT 0000000
YYYY-MM-DD

Part No. 94-033-D0

[redacted]

For *In Vitro* Diagnostic Use. Store between 2° and 8°C TECHLAB®, Inc. Blacksburg, VA 24060

IVD

Membrane Device
Dispositif De Membrane
Membrane Vorrichtung
Dispositivo De la Membrana

1 each

REF 5033MD
LOT 0000000
YYYY-MM-DD

Part No. 94-033-MD

[redacted]

For *In Vitro* Diagnostic Use. Store between 2° and 8°C.

IVD

Positive Control
Contrôle positif
Positive Kontrolle
Control positivo

REF 5029P
LOT 0000000
YYYY-MM-DD

TECHLAB®, Inc.
Blacksburg, VA 24060

1 mL

Part No. 94-000-P2

[redacted]

For *In Vitro* Diagnostic Use. Store between 2° and 8°C. **3.5 mL**

IVD

Substrate
Substrat / Substrat
Substrato

REF MS
LOT 0000000
YYYY-MM-DD

TECHLAB®, Inc.
Blacksburg, VA 24060

Part No. 94-000-MS

[redacted]

For *In Vitro* Diagnostic Use. Store between 2° and 8°C. TECHLAB®, Inc. Blacksburg, VA 24060

IVD

Wash Buffer
Tampon de lavage
Waschpuffer
Tampón de lavado

10 mL

REF MW
LOT 0000000
YYYY-MM-DD

Part No. 94-000-MW

[redacted]

A rapid test for the detection of *C. difficile* toxins A and B in fecal specimens

Patent Pending

Catalog #T5033 (25 tests)

INTENDED USE

The *TOX A/B QUIK CHEK*™ test is a rapid immunoassay for detecting *Clostridium difficile* toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history.

FOR *IN VITRO* DIAGNOSTIC USE.

EXPLANATION

After treatment with antibiotics, many patients develop gastrointestinal problems ranging from mild diarrhea to severe pseudomembranous colitis. Many cases of the milder forms of gastrointestinal illness and most cases of pseudomembranous colitis are caused by toxigenic strains of *Clostridium difficile* (1). This organism is an opportunistic anaerobic bacterium that grows in the intestine once the normal flora has been altered by the antibiotic. Toxigenic strains of *C. difficile* carry the genes encoding the toxins while non-toxigenic strains do not carry the toxin genes. The disease results from the toxins that the toxigenic organism produces. The clinical symptoms associated with the disease are believed to be primarily due to toxin A, which is a tissue-damaging enterotoxin (2,3). *C. difficile* also produces a second toxin, designated toxin B. Toxin B, which has been referred to as the cytotoxin of the organism, is the toxin detected by the tissue culture assay currently used by many laboratories. Toxigenic *C. difficile* strains produce both toxins or only toxin B (4-7).

PRINCIPLE OF THE TEST

The *TOX A/B QUIK CHEK*™ uses antibodies specific for toxins A and B of *C. difficile*. The device contains a *Reaction Window* with two vertical lines of immobilized antibodies (Fig. 1a). The test line ("T") contains antibodies against *C. difficile* toxins A and B. The control line ("C") contains anti-IgG antibodies. The *Conjugate* consists of antibodies to toxins A and B coupled to horseradish peroxidase. To perform the test, the fecal specimen is diluted with *Diluent* and *Conjugate* is added to the diluted sample. The diluted sample-conjugate mixture is added to the *Sample Well* and the device is allowed to incubate at room temperature for 15 minutes. During the incubation, any toxin A and toxin B in the sample bind to anti-toxin antibody-peroxidase conjugate. The toxin-antibody complexes migrate through a filter pad to a membrane where they are captured by the immobilized anti-toxin antibodies in the line. The *Reaction Window* is subsequently washed with *Wash Buffer*, and the test is developed with the addition of *Substrate*. After a 10 minute incubation period, the "T" reaction is examined visually for the appearance of a vertical blue line on the "T" side of the *Reaction Window*. A blue line indicates a positive test. A positive "C" reaction, indicated by a vertical blue line on the "C" side of the *Reaction Window*, confirms that the test is working properly and the results are valid.

MATERIALS PROVIDED

Membrane Devices –25 pouches, each containing 1 device and a desiccant pack
***Diluent* (14 mL)** – Buffered protein solution containing 0.02% thimerosal
***Wash Buffer* (10 mL)** – A buffered solution containing 0.02% thimerosal
***Substrate* (3.5 mL)** – Solution containing tetramethylbenzidine
***Conjugate* (2 mL)** – Mouse monoclonal antibody specific for toxin A coupled to horseradish peroxidase and goat polyclonal antibody specific for toxin B coupled to horseradish peroxidase in a buffered protein solution containing 0.02% thimerosal
***Positive Control* (1 mL)** – Antigen in a buffered protein solution
Disposable plastic transfer pipettes – 50 (graduated at 25 μL and 400 μL)

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Small test tubes (e.g., plastic Eppendorf tubes)
Timer
Disposable gloves for handling fecal samples
Applicator sticks
Vortex mixer
Pipettor and tips

SHELF LIFE AND STORAGE

The expiration date of the kit is given on the label. Expiration dates for each component are listed on the individual labels. The kit should be stored between 2° and 8°C.

PRECAUTIONS

1. Reagents from different kits should not be mixed. Do not use a kit past the expiration date.
2. Bring all components to ROOM TEMPERATURE BEFORE USE!
3. Caps and tips are color-coded; do NOT mix!
4. Do not freeze the reagents. The kit should be stored between 2°C and 8°C.
5. The pouch containing the *Membrane Device* should be at room temperature before opening, and opened just before use. Keep the membrane devices dry before use.
6. Use fecal specimens within 72 hours of collection to obtain optimal results. Specimens that are frozen may lose activity due to freezing and thawing.
7. Specimens that have been preserved in 10% Formalin, merthiolate formalin, sodium acetate formalin or polyvinyl alcohol cannot be used.
8. Specimens in transport media such as Cary Blair and C&S can be used as specified in the specimen preparation protocol.
9. Hold reagent bottles vertically to dispense reagents to ensure consistent drop size.
10. Specimens and membrane devices should be handled and disposed of as potential biohazards after use. Wear disposable gloves when doing the test.
11. Reagents contain thimerosal as a preservative and should be handled with normal laboratory caution.
12. Membrane devices cannot be reused.
13. The test has been optimized for sensitivity and specificity. Alterations of the specified procedure and/or test conditions may affect the sensitivity and specificity of the test. Do not deviate from the specified procedure.
14. Microbial contamination of reagents may decrease the accuracy of the assay. Avoid microbial contamination of reagents by using sterile disposable pipettes if removing aliquots from reagent bottles.
15. Be attentive to the total assay time when testing more than one fecal specimen. Prepare all fecal specimens in *Diluent* first, and then add the *Conjugate* to all of the diluted specimens. Thoroughly mix all of the diluted specimens, and then

transfer to the *Membrane Device*. The 15-minute incubation step begins after the last diluted sample-conjugate mixture has been transferred to the final *Membrane Device*.

COLLECTION AND HANDLING OF FECAL SPECIMENS

1. Standard collection and handling procedures used in-house for fecal specimens are appropriate. Specimens should be stored between 2° and 8°C; test specimens that are less than 24 hours old, whenever possible.
2. Store specimens frozen (≤-10°C) if the test cannot be performed within 72 hours of collection, but note that freezing and thawing of the specimen may result in loss of activity due to degradation of the toxins.
3. Make sure that specimens are thoroughly mixed PRIOR to performing the assay.
4. Storing fecal specimens in the *Diluent* is NOT recommended.
5. Dilute the fecal specimen in *Diluent*, add *Conjugate*, and immediately perform the test. Do not allow the fecal specimens to remain in the *Diluent* and/or *Conjugate* for any extended period of time.

SPECIMEN PREPARATION

1. Bring all reagents and the required number of devices to room temperature before use.
2. Set up and label one small test tube for each specimen, and optional external controls as necessary.
3. Add 500 µL *Diluent* to each tube for fecal specimens. For specimens in transport media such as Cary Blair or C&S, add 425 µL of *Diluent* to the tube.
4. Obtain one disposable plastic transfer pipette (supplied with the kit) for each sample – the pipettes have raised graduations at 25 µL and 400 µL.
5. Mix all specimens regardless of consistency- it is essential that the specimens be evenly suspended before transferring.
   Liquid/Semi-solid specimens – pipette 25 µL of specimen with a transfer pipette (graduated at 25 µL and 400 µL) and dispense into the *Diluent*. Use the same transfer pipette to mix the diluted specimen.
   Formed/Solid specimens – Care must be taken to add the correct amount of formed stool to the sample mixture. Mix the specimen thoroughly using a wooden applicator stick and transfer a small portion (approximately 2 mm diameter, the equivalent of 25 µL) of the specimen into the *Diluent*. Emulsify the specimen using the applicator stick.
   Fecal specimens in Cary Blair or C&S transport media - pipette 100 µL of sample into the *Diluent*.
6. **Optional External Control Samples:**
   External Positive Control - add one drop of *Positive Control* (gray-capped bottle) to the appropriate test tube.
   External Negative Control - add 25 µL *Diluent* to the appropriate test tube.

NOTE: Transferring too little specimen, or failure to mix and completely suspend the specimen in the Diluent *mixture, may result in a false-negative test result. The addition of too much fecal specimen may cause invalid results due to restricted sample flow.*

TEST PROCEDURE

1. Obtain one *Membrane Device* per specimen, and one device per optional external positive or negative control as necessary. The foil bags containing the devices should be brought to room temperature before opening. Label each

device appropriately and orient it on a flat surface so the letter "C" on the device is on the left, the letter "T" is on the right, and the small *Sample Well* is located in the top right corner of the device (Fig. 1a).

2. Add one drop of *Conjugate* (red-capped bottle) to all of the test tubes containing diluted specimen or control samples. Close each tube and mix thoroughly. Proper mixing can be achieved by vortexing or inverting the tube. Immediately proceed to Step #3.
3. Using a transfer pipette (graduated at 25 µL and 400 µL), transfer 400 µL of the diluted sample-conjugate mixture into the ***Sample Well*** (smaller hole in the top right corner of the device) of a *Membrane Device*, making certain to expel the liquid sample onto the wicking pad inside of the *Membrane Device.*
4. Incubate the device at room temperature for 15 minutes – the sample will wick through the device and a wet area will spread across the *Reaction Window* (larger hole in the middle of the device). If the *Reaction Window* is not completely wet at the end of the 15-minute incubation, the test is considered invalid and the sample must be retested on a new device.
   NOTE FOR SAMPLES THAT FAIL TO MIGRATE:
   Occasionally, a diluted fecal specimen cannot be tested because it clogs the membrane and the Reaction Window *does not wet properly. If the diluted fecal specimen fails to migrate properly within 5 minutes of adding the sample to the* Sample Well *(i.e. the membrane in the* Reaction Window *does not appear to be completely wet), then add 100 µL of* Diluent *to the* Sample Well *and wait an additional 5 minutes (for a total of 20 minutes).*
5. After the incubation, add 300 µL of *Wash Buffer* to the ***Reaction Window***. Allow the *Wash Buffer* to flow through the *Reaction Window* membrane and be absorbed completely.
6. Add 2 drops of *Substrate* (blue-capped bottle) to the ***Reaction Window***. Read and record results visually after 10 minutes.

INTERPRETATION OF RESULTS

1. Interpretation of the test is most reliable when the device is read immediately at the end of the reaction period. Read the device at a normal working distance in a well-lit area. View with a line of vision directly over the device.
2. Observe device for the appearance of a blue line on the "C" side of the *Reaction Window* representing the internal positive control line. Observe device for the appearance of a blue line on the "T" side of the *Reaction Window* representing the test line. The lines may appear faint to dark in intensity.
3. **Positive Result:** A positive result may be interpreted at any time between the addition of *Substrate* and the 10-minute read time. Two blue lines are visible, the control line ("C") and the test line ("T"). The lines may appear faint to dark in intensity. The appearance of a blue line on the "T" side is interpreted as a positive result. An obvious partial line is interpreted as a positive result. Do not interpret membrane discoloration or shadow as a positive result. A positive result indicates the presence of *C. difficile* toxin.
4. **Negative Result:** A test cannot be interpreted as negative or invalid until 10 minutes following the addition of *Substrate*. A single blue line is visible on the control ("C") side of the *Reaction Window* and no test line is visible on the "T" side of the *Reaction Window* (Fig. 1b). A negative result indicates *C. difficile* toxin is either absent in the specimen or is below the detection limit of the test.
5. **Invalid Result:** A single line is visible on the test ("T") side of the *Reaction Window*, or no lines are visible in the *Reaction Window* (Fig. 1c, 1d). The test result is invalid if a control line is not present at the completion of the reaction period.

FIGURE 1: *TOX A/B QUIK CHEK™* INTERPRETATION OF RESULTS

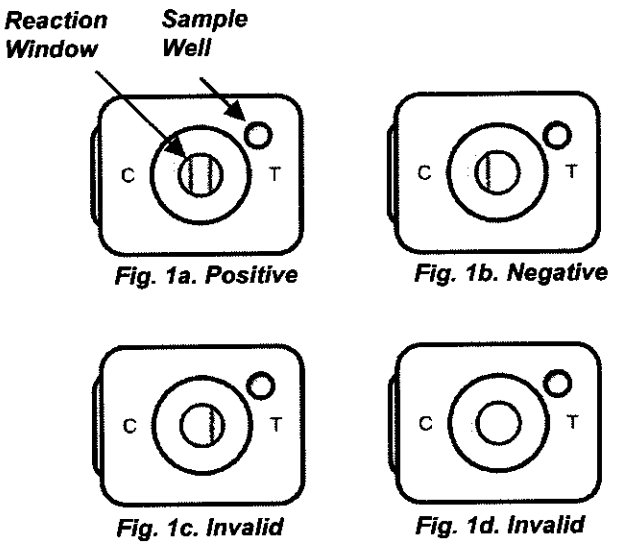


Fig. 1a. Positive *Fig. 1b. Negative*

Fig. 1c. Invalid *Fig. 1d. Invalid*

QUALITY CONTROL

Internal: A blue control line must be visible on the "C" side of the *Reaction Window* on every *Membrane Device* that is tested. The appearance of the blue control line confirms that the sample and reagents were added correctly, that the reagents were active at the time of performing the assay, and that the sample migrated properly through the *Membrane Device*.

External: The reactivity of the *TOX A/B QUIK CHEK™* test should be verified on receipt using the *Positive Control* and negative control (*Diluent*). The *Positive Control* is supplied with the kit (gray-capped bottle). The *Positive Control* confirms the reactivity of the other reagents associated with the assay, and is not intended to ensure precision at the analytical assay cut-off.
Diluent is used for the negative control. Additional tests can be performed with the controls to meet the requirements of local, state and/or federal regulations and/or accrediting organizations.

LIMITATIONS

1. The *TOX A/B QUIK CHEK™* test is used to detect *C. difficile* toxin(s) in fecal specimens. The test confirms the presence of toxin in feces and this information should be taken under consideration by the physician in light of the clinical history and physical examination of the patient. The *TOX A/B QUIK CHEK™* test will detect levels of toxin A at ≥0.63 ng/mL and toxin B at ≥1.25 ng/mL.
2. Fecal specimens are extremely complex. Optimal results with the *TOX A/B QUIK CHEK™* test are obtained with specimens that are less than 24 hours old. Most undiluted specimens can be stored between 2° and 8°C for 72 hours before significant degradation of the toxin is noted. If specimens are not assayed within this time period, they may be frozen and thawed. However, repeated freezing and thawing may result in loss of the immunoreactivity of toxins A and B.

3. Some specimens may give weak reactions. This may be due to a number of factors such as the presence of low levels of toxin, the presence of binding substances, or inactivating enzymes in the feces. *Under these conditions, a fresh specimen should be tested.* Additional tests that may be used in conjunction with the *TOX A/B QUIK CHEK*™ test include isolation of the organism on selective media, *C. DIFF CHEK*™ test, *C. DIFFICILE TOX A/B II*™ test, or tissue culture cytotoxicity assay (e.g., *C. DIFFICILE TOX-B TEST*) for the detection of *C. difficile* or its toxin(s).
4. Fecal specimens preserved in 10% Formalin, merthiolate formalin, sodium acetate formalin, or polyvinyl alcohol cannot be used.
5. The *TOX A/B QUIK CHEK*™ test is qualitative. The intensity of the color should not be interpreted quantitatively.
6. Some isolates of *C. sordellii* may react in the *TOX A/B QUIK CHEK*™ test due to the production of immunologically related toxins (1).
7. Colonization rates of up to 50% have been reported in infants. A high rate has also been reported in cystic fibrosis patients (1,3).

EXPECTED VALUES

The reported incidence of *C. difficile*-associated disease in patients with antibiotic-associated diarrhea is 10 to 20%. The prevalence of a positive *TOX A/B QUIK CHEK*™ test will vary from location to location and hospitals may experience rates lower or higher than those observed at the sites used in this evaluation. *Clostridium difficile* disease is primarily a nosocomial disease of elderly patients, and hospitals that have higher numbers of elderly patients may experience higher rates. It is important to consider any test results in conjunction with clinical symptoms because some healthy adults and large numbers of healthy infants (up to 50%) will be positive for *C. difficile* toxin.

PERFORMANCE CHARACTERISTICS

The *TOX A/B QUIK CHEK*™ test was compared with the tissue culture test at three U.S. hospitals and in-house at TECHLAB®, Inc. Specimens included in the evaluation were submitted to the clinical laboratory for routine testing. The tissue culture test was done according to the in-house procedure. The table below shows a summary of the clinical performance of the *TOX A/B QUIK CHEK*™ test. The test exhibited a sensitivity and specificity of 90.2% and 99.7%, respectively. The predictive positive and negative values were 98.6% and 97.9%, respectively, and the correlation was 98.0%.

Table 1. Correlation of the *TOX A/B QUIK CHEK*™ test with tissue culture.

n=842	Tissue Culture positive	Tissue Culture negative
***TOX A/B QUIK CHEK*™ positive**	138	2
***TOX A/B QUIK CHEK*™ negative**	15	687

		95% CI
Sensitivity	90.2	84.1 - 94.2
Specificity	99.7	98.8 - 99.9
Predictive Positive Value	98.6	94.4 - 99.8
Predictive Negative Value	97.9	96.4 - 98.7
Correlation	98.0	97.8 - 98.2

Of the 2 tissue culture-negative/*TOX A/B QUIK CHEK*™-positive samples, 1 was negative in a commercial toxin A+B ELISA. Of the 15 specimens that were tissue culture-positive/*TOX A/B QUIK CHEK*™-negative, 12 were negative in commercial toxin A+B ELISAs.

A total of 83 fecal specimens diluted in Cary Blair Transport Media were tested in the *TOX A/B QUIK CHEK*™ test and the results were compared to those obtained by routine testing. The test exhibited a sensitivity and specificity of 94.1% and 98.5%, respectively, for the detection of *C. difficile* toxins in Cary Blair specimens.

ANALYTICAL SENSITIVITY

The test was consistently positive at a concentration of 0.63 ng/mL for toxin A and 1.25 ng/mL for toxin B.

REPRODUCIBILITY

The reproducibility of the *TOX A/B QUIK CHEK*™ test was determined using known positive (n=6) and negative (n=2) fecal specimens that were coded and sorted to prevent their identification during testing. Testing was performed on-site at 3 laboratories, which tested the samples on 3 days. The samples produced the expected results 100% of the time.

CROSS REACTIVITY

Fecal specimens inoculated with the following microorganisms to a final concentration of approximately 10^8 or higher organisms per mL did not react in the *TOX A/B QUIK CHEK*™:

Bacteria: *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Bacteroides fragilis, Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, Candida albicans, Clostridium bifermentans, Clostridium butyricum, Clostridium perfringens, Clostridium septicum, Clostridium sordellii* (nontoxigenic), *Clostridium sporogenes, Enterococcus faecalis, Escherichia coli* EIEC, *Escherichia coli, Escherichia coli* 0157 H7, *Escherichia coli* ETEC, *Klebsiella pneumoniae, Peptostreptococcus anaerobius, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus aureus (Cowans), Staphylococcus epidermidis, Vibrio parahaemolyticus, Yersinia enterocolitica* **Viruses**: Adenovirus types 1,2,3,5,40,41, Human coronavirus, Coxsackievirus B2,B3,B4,B5, Echovirus 9,11,18,22,33, Enterovirus type 68,69,70,71.

The only non-*C. difficile* organism to react with the *TOX A/B QUIK CHEK*™ was *C. sordellii* VPI 9048. This strain produces toxins HT and LT, which are homologous to toxins A and B, respectively.

INTERFERING SUBSTANCES

The following substances had no effect on test results when present in feces in the concentrations indicated: mucin (3.5% w/v), human blood (40% v/v), barium sulfate (5% w/v), Imodium® (5% w/v), Kaopectate® (5 mg/mL), Pepto-Bismol® (5% w/v), steric/palmitic acid (40% w/v), Metronidazole (0.25% w/v), Vancomycin (0.25% w/v).

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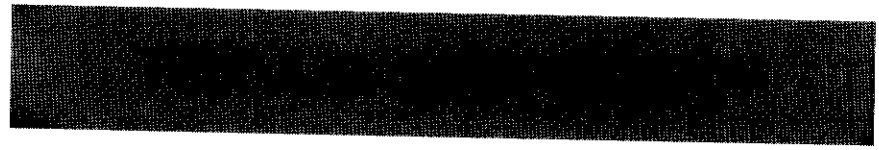
Developed and Manufactured by:

TECHLAB®

Blacksburg, VA 24060
U.S. only, 1-800-TECHLAB
TEL.: (540) 953-1664 FAX: (540) 953-1665

3.3 Package Insert



A rapid test for the detection of *C. difficile* toxins A and B in fecal specimens

Patent Pending

Catalog #T5033 (25 tests)

INTENDED USE

The *TOX A/B QUIK CHEK*™ test is a rapid immunoassay for detecting *Clostridium difficile* toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history.

FOR *IN VITRO* DIAGNOSTIC USE.

EXPLANATION

After treatment with antibiotics, many patients develop gastrointestinal problems ranging from mild diarrhea to severe pseudomembranous colitis. Many cases of the milder forms of gastrointestinal illness and most cases of pseudomembranous colitis are caused by toxigenic strains of *Clostridium difficile* (1). This organism is an opportunistic anaerobic bacterium that grows in the intestine once the normal flora has been altered by the antibiotic. Toxigenic strains of *C. difficile* carry the genes encoding the toxins while non-toxigenic strains do not carry the toxin genes. The disease results from the toxins that the toxigenic organism produces. The clinical symptoms associated with the disease are believed to be primarily due to toxin A, which is a tissue-damaging enterotoxin (2,3). *C. difficile* also produces a second toxin, designated toxin B. Toxin B, which has been referred to as the cytotoxin of the organism, is the toxin detected by the tissue culture assay currently used by many laboratories. Toxigenic *C. difficile* strains produce both toxins or only toxin B (4-7).

PRINCIPLE OF THE TEST

The *TOX A/B QUIK CHEK*™ uses antibodies specific for toxins A and B of *C. difficile*. The device contains a *Reaction Window* with two vertical lines of immobilized antibodies (Fig. 1a). The test line ("T") contains antibodies against *C. difficile* toxins A and B. The control line ("C") contains anti-IgG antibodies. The *Conjugate* consists of antibodies to toxins A and B coupled to horseradish peroxidase. To perform the test, the fecal specimen is diluted with *Diluent* and *Conjugate* is added to the diluted sample. The diluted sample-conjugate mixture is added to the *Sample Well* and the device is allowed to incubate at room temperature for 15 minutes. During the incubation, any toxin A and toxin B in the sample bind to anti-toxin antibody-peroxidase conjugate. The toxin-antibody complexes migrate through a filter pad to a membrane where they are captured by the immobilized anti-toxin antibodies in the line. The *Reaction Window* is subsequently washed with *Wash Buffer*, and the test is developed with the addition of *Substrate*. After a 10 minute incubation period, the "T" reaction is examined visually for the appearance of a vertical blue line on the "T" side of the *Reaction Window*. A blue line indicates a positive test. A positive "C" reaction, indicated by a vertical blue line on the "C" side of the *Reaction Window*, confirms that the test is working properly and the results are valid.

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4.3 Comparative Information of Equivalent Devices

Characteristics	510(k) Numbers	Intended Use	Format	Materials	Target Population
TOX A/B QUIK CHEK™ test	Subject to this 510(k)	Detection of *C. difficile* toxin in fecal specimens	Rapid membrane	Highly specific antibodies against *C. difficile* toxins A and B	Persons suspected of having *C. difficile* disease
Tissue culture assay (*TOX-B TEST*)	K935296	Detection of *C. difficile* toxin in fecal specimens	Tissue culture	Cell monolayer, specific neutralizing antiserum	Persons suspected of having *C. difficile* disease
C. DIFFICILE TOX A/B II™	K003306 and K030404	Detection of *C. difficile* toxin in fecal specimens	Microtiter ELISA	Highly specific antibodies against *C. difficile* toxins A and B	Persons suspected of having *C. difficile* disease
Premier™ Toxins A&B	K993914	Detection of *C. difficile* toxin in fecal specimens	Microtiter ELISA	Highly specific antibodies against *C. difficile* toxins A and B	Persons suspected of having *C. difficile* disease
ProSpecT® Clostridium difficile Toxin A/B	K033479	Detection of *C. difficile* toxin in fecal specimens	Microtiter ELISA	Highly specific antibodies against *C. difficile* toxins A and B	Persons suspected of having *C. difficile* disease
Immuno*Card*® Toxins A&B	K041003	Detection of *C. difficile* toxin in fecal specimens	Rapid membrane	Highly specific antibodies against *C. difficile* toxins A and B	Persons suspected of having *C. difficile* disease
X/pect™ Clostridium difficile Toxin A/B	K041951	Detection of *C. difficile* toxin in fecal specimens	Rapid membrane	Highly specific antibodies against *C. difficile* toxins A and B	Persons suspected of having *C. difficile* disease

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Intestinal bacteria that do not react in the *TOX A/B QUIK CHEK™* test.

Bacterium	Strain	Reaction in negative fecal specimen	Reaction in positive fecal specimen
Aeromonas hydrophila	ATCC 7965	-	+
Bacillus cereus	ATCC 14579	-	+
Bacillus subtilis	ATCC 6051	-	+
Bacteroides fragilis	VPI 13785	-	+
Campylobacter coli	ATCC 49941	-	+
Campylobacter fetus	ATCC 25936	-	+
Campylobacter jejuni	ATCC 29428	-	+
Candida albicans	ATCC 10231	-	+
Clostridium bifermentans	VPI 2012	-	+
Clostridium butyricum	VPI 8260	-	+
Clostridium perfringens, types A	VPI 3624	-	+
Clostridium septicum	VPI 1524	-	+
Clostridium sordellii	VPI 9048	+	+
Clostridium sordellii	VPI 7319	-	+
Clostridium sporogenes	VPI 9743	-	+
Enterococcus faecalis	ATCC 19433	-	+
Escherichia coli EIEC	SD67	-	+
Escherichia coli	ATCC 25922	-	+
Escherichia coli O157 H7	B1409	-	+
Escherichia coli ETEC	E 2348169	-	+
Klebsiella pneumoniae	ATCC 9997	-	+
Peptostreptococcus anaerobius	ATCC 27337	-	+
Proteus vulgaris	ATCC 6380	-	+
Pseudomonas aeruginosa	ATCC 9027	-	+
Salmonella typhimurium	ATCC 14029	-	+
Shigella dysenteriae	ATCC 12022	-	+
Shigella flexneri	ATCC 12122	-	+
Shigella sonnei	ATCC 11060	-	+
Staphylococcus aureus	ATCC 6358	-	+
Staphylococcus aureus (Cowans)	ATCC 12598	-	+
Staphylococcus epidermidis	VPI 13140	-	+
Vibrio parahaemolyticus	ATCC 17802	-	+
Yersinia enterocolitica	ATCC 9610	-	+

Intestinal viruses that do not react in the *TOX A/B QUIK CHEK™* test.

Virus	ATCC#	Reaction in negative stool	Reaction in positive stool
Adenovirus type 1	VR-1	-	+
Adenovirus type 2	VR-846	-	+
Adenovirus type 3	VR-3	-	+
Adenovirus type 5	VR-5	-	+
Adenovirus type 40	VR-931	-	+
Adenovirus type 41	VR-930	-	+
Human coronavirus	VR-740	-	+
Coxsackievirus B2	VR-29	-	+
Coxsackievirus B3	VR-30	-	+
Coxsackievirus B4	VR-184	-	+
Coxsackievirus B5	VR-185	-	+
Echovirus 9	VR-1050	-	+
Echovirus 11	VR-1052	-	+
Echovirus 18	VR-48	-	+
Echovirus 22	VR-1063	-	+
Echovirus 33	VR-582	-	+
Enterovirus type 68	VR-1076	-	+
Enterovirus type 69	VR-1077	-	+
Enterovirus type 70	VR-836	-	+
Enterovirus type 71	VR-784	-	+

Interfering substances

The following substances had no effect on test results, either with *C. difficile*-negative or *C. difficile*-positive specimens, when present in the stool in the concentrations indicated in the table: hog gastric mucin, human blood, barium sulfate, Imodium®, Kaopectate®, Pepto-Bismol®, steric/palmitic acid, metronidazole, and vancomycin.

Substances that do not interfere with the *TOX A/B QUIK CHEK™* test.

Substance	Concentration	Reaction in negative fecal specimen	Reaction in positive fecal specimen
Hog gastric mucin	3.5% w/v	-	+
Human blood (O, Rh-)	40% v/v	-	+
Barium sulfate	5% w/v	-	+
Imodium®	5% w/v	-	+
Kaopectate®	5 mg/ml	-	+
Pepto-Bismol®	5% w/v	-	+
Steric/palmitic acid	40% w/v	-	+
Metronidazole	0.25% w/v	-	+
Vancomycin	0.25% w/v	-	+

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A Toxin/Antitoxin Kit for the Detection of *C. difficile* Toxin B in Clinical Specimens

Catalog No. T5003/R5003

FRANCAISE p. 8

Un kit Toxine/Antitoxine pour la détection de la Toxine B de *C. difficile* dans les spécimens cliniques

Numéro de Catalogue T5003/R5003

Developed and Manufactured by

TECHLAB®

Blacksburg, VA 24060
U.S. only, 1-800-TECHLAB
TEL.: (540) 953-1664 FAX: (540) 953-1665

CE EC REP Emergo Europe
PO Box 18510
2502 EM The Hague
The Netherlands

International Symbol Key:

REF	Catalog Number		Temperature Range
IVD	*In Vitro* Diagnostics		Use By/Expiration Date
LOT	Lot Information	CE	CE Symbol

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or call 301-796-8118.

C. DIFFICILE TOX-B TEST

INTENDED USE

The *C. DIFFICILE TOX-B TEST* is intended for use in conjunction with the tissue culture cytotoxicity assay for the detection of *C. difficile* toxin B in patient specimens. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with patient history.

FOR *IN VITRO* DIAGNOSTIC USE.

EXPLANATION

After treatment with antibiotics, many patients develop gastrointestinal problems ranging from mild diarrhea to severe pseudomembranous colitis. Many cases of the milder forms of gastrointestinal illness and most cases of pseudomembranous colitis are caused by *Clostridium difficile* (1). This organism is an opportunistic anaerobic bacterium that grows in the intestine once the normal flora has been altered by the antibiotic. The disease results from the toxins that the organism produces. The clinical symptoms associated with the disease are believed to be primarily due to toxin A, which is a tissue-damaging enterotoxin (2,3).

Clostridium difficile also produces a second toxin, designated toxin B. Toxin B, which has been referred to as the cytotoxin of the organism, is the toxin detected by the tissue culture assay currently used by many laboratories. Most strains of *Clostridium difficile* either produce both toxins or neither toxin, although recently, toxin A negative/toxin B positive strains have been identified (4-7). Strains that produce high levels of toxin A also produce high levels of toxin B. Likewise, strains that produce low levels of toxin A produce low levels of toxin B, indicating that the toxin production may be similarly regulated. Tests that detect either toxin are being used as diagnostic aids in the identification of toxigenic strains. The genes for the toxins have been cloned and sequenced, and some properties of the toxins are now well-defined (8,9). Both toxins are large (molecular weight of toxin A, 308,000; molecular weight of toxin B, 279,000). Both toxins have a complex series of repeating units at the COOH-terminus of the molecule and these repeating units may serve as the binding portion that recognizes the receptor (10). There is some evidence suggesting that toxins A and B act synergistically and that the initial tissue damage caused by toxin A allows toxin B to exert its toxicity. Therefore, although toxin A is believed to cause most of the clinical signs, toxin B also may play an important role in the disease.

The disease caused by *Clostridium difficile* is complicated by a number of factors. Although the organism is responsible for almost all cases of pseudomembranous colitis, *C. difficile* is estimated to cause only about 25% of the cases of antibiotic-associated diarrhea. Thus, many cases of antibiotic-associated diarrhea are due to other causes. Toxigenic strains of *C. difficile* can range from weakly toxigenic to strains that produce extremely high levels of toxin. It is unclear if mild, self-limiting cases of diarrhea represent only milder forms of the disease caused by infection with weakly toxigenic strains or whether the presence of toxigenic *Clostridium difficile* occurs as a result of the diarrhea.

There are four methods currently used for the detection of *C. difficile* and its toxins in fecal specimens. These include (i) isolation of the organism, (ii) latex agglutination, (iii) tissue culture assay, and (iv) ELISA for detection of toxin A or both toxins A and B. Isolation of the organism and latex agglutination are useful for detecting the presence of the organism in fecal specimens. However, neither test demonstrates the presence of toxin. This has to be done using either the tissue culture test or an ELISA specific for the toxins. The tissue culture procedure has proved to be very useful because of the high activity of *C. difficile* toxin B in the assay. As little as one picogram of the toxin is sufficient to cause a positive reaction. Neutralization of the cytotoxic activity by *C. difficile* antitoxin confirms the presence of *C. difficile* toxin. The assay is to be used as an aid in the diagnosis of *C. difficile* disease and test results must be considered in conjunction with the patient history. This is due to the fact that some adults are asymptomatic carriers and can have detectable levels of toxin in their feces but have no clinical symptoms. In addition, up to 50% of healthy infants can have *C. difficile* toxin in their feces but show no sign of illness.

PRINCIPLE OF THE TEST

The *C. DIFFICILE TOX-B TEST* uses a tissue culture format to detect the presence of cytotoxic activity (noted by cell rounding) in fecal specimens and confirms the identification of *Clostridium difficile* toxin using specific antitoxin. Two reagents are provided in the *C. DIFFICILE TOX-B TEST*. One is the *Positive Toxin Control* reagent. The other is specific antitoxin to be used to confirm the presence of *C. difficile* toxin by neutralizing the cytotoxic activity. Also included is *Diluent* for preparing the fecal specimen. In the assay, an aliquot of a fecal specimen is emulsified in the *Diluent* and the diluted specimen is centrifuged and filtered. A sample of the filtrate is transferred to a tissue culture well containing phosphate-buffered saline (PBS) and to a well containing antitoxin. If *C. difficile* toxin is present, it will cause the cells in the well with PBS to become round, demonstrating the presence of the cytotoxic activity. The presence of *Clostridium difficile* toxin is confirmed if the cytotoxic activity is neutralized in the well containing antitoxin.

MATERIALS PROVIDED

Goat Antitoxin against *C. difficile*, 3.0 mL (with preservative)
Phosphate-buffered saline (PBS), 3.0 mL (with preservative)
Positive Toxin Control, 2.0 mL active *C. difficile* toxin in a buffered protein solution containing preservative.
Diluent, 100 mL (phosphate-buffered saline containing preservative and phenol red)
T5003 and R5003 contain sufficient reagents for two 48-Well Tissue Culture plates. T5003 also includes one 48-Well Tissue Culture plate containing mammalian cells.

MATERIALS REQUIRED BUT NOT PROVIDED

Microscope for observing cells	Incubator set at 37°C ± 2°C
Centrifuge and centrifuge tubes	Pipettes/pipettor
Test tubes for preparing dilutions	Syringe filters (0.45 to 0.8 µm)

SHELF LIFE AND STORAGE

The expiration date of the kit is given on the label. Expiration dates for each component are listed on the individual labels. Do not use reagents past the expiration date because the sensitivity decreases.

PRECAUTIONS

1. Use fecal specimens within 24 hours of collection to obtain optimal results. Specimens that are frozen (-20°C or lower) may lose activity due to freezing and thawing. AVOID REPEATED CYCLES OF FREEZING AND THAWING THE SPECIMEN. THIS MAY INACTIVATE THE TOXIN.
2. All specimens should be considered as potentially infectious and should be handled appropriately.
3. The *Positive Toxin Control* contains biologically active toxin and should be treated with caution. The antitoxin does not pose any known hazard.
4. Do not use the reagents beyond their expiration dates.
5. Store reagents according to the manufacturer's recommendations. This prolongs the shelf-life of the reagents.
6. Place reagents back in the refrigerator once the procedure has been completed.

COLLECTION AND HANDLING OF FECAL SPECIMENS

Standard collection and handling procedures used in-house for fecal specimens are appropriate. Specimens should be transported as soon as possible and stored between 2° and 8°C. Whenever possible, test fecal specimens which are less than 24 hours old. Store specimens at -20°C, or lower, if the test cannot be performed within 48 hours. **Freezing and thawing of the specimen, especially multiple times, may result in loss of activity due to degradation of the toxin.** Make sure that specimens are thoroughly mixed prior to performing the assay. This includes complete mixing of the specimen prior to preparing the fecal extract.

PROCESSING FECAL SPECIMENS

NOTE: Fecal specimens less than 24 hours old should be tested whenever possible. Specimens should be stored between 2° and 8°C until tested.

1. Set up one tube for each specimen to be tested. Add 1.8 mL *Diluent* to each tube. Label the tube directly on the side.
2. For formed fecal specimens, use a swab to transfer the fecal specimen to the tube. Coat the swab completely before transferring the specimen. Mix the swab in the diluent to remove as much sample as possible and squeeze the swab against the side of the tube to express any residual liquid. For liquid fecal specimens, use a plastic pipette to transfer 0.2 mL specimen to the tube (1/10 dilution). Make sure the liquid specimens are evenly suspended before transferring.
3. Vortex the tubes for 10 seconds. Transfer an aliquot of the specimen to a microcentrifuge tube (e.g., an Eppendorf tube) and centrifuge the specimen for 5 minutes at high speed (2,000 to 10,000 rpms) in a microcentrifuge.
4. Collect the supernatant fluid and filter it through a 0.45 µm membrane into a sterile tube. Store the filtrate between 2° and 8°C.

THE TISSUE CULTURE PROCEDURE

NOTE: The procedure described below may be used to detect *C. difficile* toxin in fecal specimens with the *C. DIFFICILE TOX-B TEST.* Alternatively, the kit may be used according to other modified in-house procedures. It is important, however, that the *Positive Toxin Control* and the antitoxin reagent be used according to the instructions.

1. Determine the number of wells that will be needed to perform the test. Two wells will be needed as control wells each time the test is performed. In addition, two wells will be needed for each specimen. Tissue culture cells that typically are used include human foreskin cells, MRC-5, WI-38, and Chinese Hamster Ovary cells.
2. Add 1 drop (50 µL) of PBS to one of the two wells in each set. Add 1 drop (50 µL) of *C. difficile* antitoxin to the other well in each set. Set up the wells as illustrated in table 1.

Table 1.

Well	PBS or Antitoxin	Test sample
Control Well 1	1 drop (50 µL) PBS	1 drop (50 µL) positive control
Control Well 2	1 drop (50 µL) antitoxin	1 drop (50 µL) positive control
Test Well 1	1 drop (50 µL) PBS	1 drop (50 µL) fecal filtrate
Test Well 2	1 drop (50 µL) antitoxin	1 drop (50 µL) fecal filtrate

The final dilution of fecal filtrate in the well is 1/50. Use new pipette tips between specimens. Please note that (i) one set of controls should be used each time the test is performed and (ii) the remaining fecal filtrate should be refrigerated between 2° and 8°C for up to 48 hours in case additional testing is required.

3. After the samples have been applied, incubate the wells at 37°C ± 2°C for 24 to 48 hours. Observe each well for cell rounding, indicating the presence of cytotoxic activity. The cytotoxic effect is considered positive if at least 50% of the cells in the well are rounded. Record the results for each well. The cytotoxic effect may be observed within 8 hours after applying the specimen if high amounts of toxin are present. However, specimens should not be considered negative until after 48 hours.

QUALITY CONTROL

A positive and negative control must be run with each series of test specimens. This includes the *Positive Toxin Control* added to a well containing buffer to demonstrate activity, and *Positive Toxin Control* added to a well containing antitoxin to demonstrate

neutralization of the cytotoxic activity. The positive control should show the typical rounding effect characteristic of *C. difficile* toxin. The test results are not valid unless these performance characteristics are met. If the performance characteristics are not met, please call Technical Services and do not report test results. Upon arrival, the kit should be inspected to ensure that components arrived in satisfactory condition. Test results along with control results should be recorded and reported according to in-house procedures and this information should be stored according to in-house procedures for future reference.

INTERPRETATION OF RESULTS

Well containing:		INTERPRETATION OF RESULTS
PBS	*C. difficile* Antitoxin	
-	-	Negative - No cytotoxic activity present
+	-	Cytotoxic activity present that is neutralized by *C. difficile* antitoxin, confirming the presence of *C. difficile* toxin in the specimen
+	+	Indeterminate results that may be caused by extremely high levels of *C. difficile* toxin. Fecal filtrate should be diluted an aditional 1/10 in *Diluent* and the test should be repeated to rule out this possibility. Upon repeat testing under these conditions, if the cells in the well containing PBS and highly diluted fecal filtrate are rounded and the cells in the well containing antitoxin and highly diluted fecal filtrate are not, *C. difficile* toxin is present. If the cells in both wells are round, the specimen contains cytotoxic activity but it is not due to *C. difficile toxin.*

Control Results: The cells in the well that contains PBS and *Positive Toxin Control* should be round. The cells in the well that contains *C. difficile* antitoxin and *Positive Toxin Control* should appear normal, demonstrating the neutralization of the *Positive Toxin Control* by the antitoxin.

Test Sample Results: When specimen filtrates show evidence of specific *C. difficile* toxin, report "Patient specimen positive for *Clostridium difficile* toxin." When specimen filtrates show no evidence of cytotoxic activity at 48 hours, report "No *Clostridium difficile* toxin detected." When specimen filtrates show evidence of cytotoxic activity that is not neutralized by *C. difficile* antitoxin, report "Nonspecific reaction in patient specimen, not characteristic of *Clostridium difficile* toxin." A repeat specimen may be helpful to rule out patient disease caused by *Clostridium difficile* toxin.

LIMITATIONS OF THE *C. DIFFICILE TOX-B TEST*

1. The *C. DIFFICILE TOX-B TEST* is used to detect *C. difficile* toxin in fecal specimens. Because of the complex nature of feces, specimens should be <24 hours old.
2. Detection of toxin by this test is dependent on the biological activity of the toxin. Specimens must be handled properly and stored as recommended to minimize inactivation of the toxin.
3. Some specimens may give reactions that are inconclusive (e.g., partial rounding of the cells, stretching instead of rounding). Under these conditions, the specimen should be retested or a fresh specimen should be tested. Additional tests that may be used in conjunction with the *C. DIFFICILE TOX-B TEST* include the ELISA for toxin A, ELISA for toxins A and B, isolation of the organism on selective media, and latex agglutination assay for the detection of the organism.

4. Certain isolates of *C. sordellii* produce the same type of rounding on tissue culture cells as toxigenic *C. difficile*. This is due to the similarities of the *C. sordellii* toxins and the *C. difficile* toxins. The strains of *C. sordellii* that produce these related toxins have not been detected in patients with antibiotic-associated diarrhea and colitis.

EXPECTED VALUES

The prevalence of a positive *C. DIFFICILE TOX-B TEST* in our clinical studies ranged from 5.8% to 19%. The prevalence will vary from location to location and hospitals may experience rates lower or higher than those observed at the sites used in the *C. DIFFICILE TOX-B TEST* evaluation. *Clostridium difficile* disease is primarily a nosocomial disease of elderly patients and hospitals that have higher numbers of elderly patients may experience higher rates. It is important to consider any test results in conjunction with clinical symptoms because some healthy adults and large numbers of healthy infants (up to 50%) will be positive for *C. difficile* toxin A or toxin B. In addition, *C. difficile* carriage rates of 22% to 32% have been reported in cystic fibrosis patients (11-13).

PERFORMANCE CHARACTERISTICS

The *C. DIFFICILE TOX-B TEST* was evaluated for its performance at four different locations in the United States. For the analysis, the *C. DIFFICILE TOX-B TEST* was compared with another commercial Cytotoxicity Test for *C. difficile* toxins, which also is a tissue culture-based assay. All tissue culture assays were done using human foreskin cells. A summary of the results from the clinical investigations is shown in table 2. The range of overall relative sensitivity, specificity, and correlation for the evaluations is presented in parentheses. In studies performed at reference centers in the northeastern and southeastern U.S. and a medical center on the East Coast involving a total of 427 specimens, the *C. DIFFICILE TOX-B TEST* exhibited an overall relative correlation of 100% with the other commercial test. In a study performed at a medical center in the Mid West involving 517 specimens, the *C. DIFFICILE TOX-B TEST* exhibited an overall relative sensitivity and specificity of 96.7% and 98.5%, respectively. The overall relative correlation was 98.3%. When discrepants were resolved by toxigenic culture, the overall relative sensitivity and specificity increased to 98.3% and 98.7%, with an overall relative correlation of 98.6%.

Table 2

		Commercial Cytotoxicity Assay/Toxigenic Culture	
		positive	negative
TOX-B TEST	positive	114	6
	negative	1	823

Overall relative sensitivity	99.1% (98.3% to 100%)
Overall relative specificity	99.3% (98.7% to 100%)
Overall relative correlation	99.3% (98.6% to 100%)
Number of specimens	944

CROSS-REACTIVITY

Toxic filtrates from *C. chauveoi*, *C. fallax*, *C. perfringens*, *C. septicum*, *and C. spiroforme* were not neutralized by the *C. difficile* antitoxin. In addition, *C. perfringens* alpha-toxin, *C. histolyticum* collagenase and clostripain, cholera toxin, diphtheria toxin, shiga-like toxin, and staphylococcal enterotoxin were not neutralized. The only organism known to cross-react in the *C. DIFFICILE TOX-B TEST* is *C. sordellii*. Certain toxigenic isolates of C. *sordellii* produce toxins that are very similar to the toxins of *C. difficile*. The hemorrhagic toxin (toxin HT) of *C. sordellii* is very similar to toxin A whereas the lethal toxin (toxin LT) is very similar to toxin B. Antibodies against the *C. difficile* toxins neutralize toxins HT and LT of *C. sordellii*. *Clostridium sordellii* has not been implicated in antibiotic-associated diarrhea and colitis.

REPRODUCIBILITY AND PRECISION

Identical sets of six fecal specimens were sent to three different clinical laboratories located in the Mid West, Southeast, and Northeast U.S. The *C. DIFFICILE TOX-B TEST* results from each laboratory were consistent with the results obtained in-house. In an in-house study, five positive fecal specimens were assayed at time 0, 24, 48, and 72 hours using the *C. DIFFICILE TOX-B TEST*. These specimens were shown to be consistently positive for *C. difficile* toxin at each of the time intervals, as determined by neutralization of the cytotoxic activity. The specimens were stored at 4°C during the testing. In additional in-house studies, three different lots of the positive control reagent were tested in duplicate and consistently gave cytotoxic titers of 10^5. Further evaluation demonstrated that different lots of the *C. difficile* antitoxin neutralized the *Positive Toxin Control*.

C. DIFFICILE TOX-B TEST - FRANCAIS

UTILISATION PRÉVUE

Le *C. DIFFICILE TOX-B TEST* est conçu pour être utilisé en conjonction avec une analyse de cytotoxicitée de culture de tissu pour la détection de la toxine B de *C. difficile* présente dans les spécimens des patients. Le test doit être utilisé pour aider dans le diagnostique de la maladie par *C. difficile*, et les résultats doivent être considérés en conjonction avec l'histoire médicale du patient.
POUR USAGE DIAGNOSTIQUE *IN VITRO*.

EXPLICATION

Suivant les traitements avec antibiotiques, plusieurs patients développent des problèmes gastro-intestinaux qui peuvent produire une diarrhée modérée ou même une colite pseudo-membraneuse sévère. Plusieur cas des formes plus tempérées des maladie gastro-intestinales et la majorité des cas de colite pseudo-membraneuse sont causés par *Clostridium difficile* (1). Cet organisme est une bactérie opportuniste anaérobique qui se multiplie dans l'intestin dès que la flore normale a été altérée par les antibiotiques. La maladie résulte des toxines que l'organisme produit. Les symptomes cliniques associés avec la maladie sont censés être provoqués principalement par la toxine A, qui endomage le tissu en tant qu'entérotoxine (2,3).

Clostridium difficile produit aussi une seconde toxine désignée toxine B. Toxine B, qui peut être représentée comme cytotoxine de l'organisme, est la toxine détectée par le test de culture de tissu qui est couramment en pratique dans plusieurs laboratoires. La majorité des variétés produisent soit les deux toxines, soit aucune des deux bien que, récemment, des variétés étant toxine A négative/toxine B positive ont été identifiés (4-7). De plus, les variétés de *Clostridium difficile* qui produisent des niveaux élevés de toxine A produisent aussi des niveaux élevés de toxine B. De la même manière, les variétés produisant des niveaux bas de toxine A produisent aussi des niveaux bas de toxine B, ce qui indique que la production de toxine peut être régulée d'une manière similaire. Les analyses qui détectent soit l'une des deux toxine, soit les des deux toxines sont en utilisation courante pour aider dans le diagnostique. Les gènes pour les toxines ont été copiés et leur séquences déterminées, et certaines de leur propriétées sont maintenant bien définies (8, 9). Les deux toxines sont larges (Mr de toxine A, 380.000; Mr de toxine B, 279.000). Toxine A a des séries complexes d'unitées qui se répètent au terminus-COOH de la molécule, et ces unitées sont probablement responsables pour la portion d'attachement qui identifie les récepteurs (10). Certaines données suggèrent la possibilité que toxine A et B agissent d'une manière synergétique et que, suivant le dommage initial causé par la toxine A, toxine B peut alors éxercer sa toxicitée. Par conséquent, bien que toxine A ait été proposé comme source de la majorité des signes cliniques, toxine B peut aussi jouer un rôle important dans la maladie.

La maladie causée par *Clostridium difficile* est compliquée par de nombreux facteurs. Bien que l'organisme soit responsable pour la plupart des cas de colite pseudo-membraneuse, il est estimé que seulement environ 25% des cas de diarrhées liés à l'usage des antibiotiques sont causés par *C. difficile*. Par conséquent, plusieurs cas de diarrhées liés à l'usage antibiotiques impliquent d'autres causes. Les variétés toxigéniques peuvent présenter un caractère faiblement toxigéniques alors que certaines variétés produisent des niveaux extrêmement élevés de toxine. Il n'est pas encore établi si la diahrrée représente une manifestation modérée naturellement limitée de la maladie causée par une infection par des variétés faiblement toxigéniques ou, alors, si la présence de *Clostridium difficile* toxigénique est le résultat de la diahrrée.

Il exist couramment quatre méthodes utilisées pour la détection de *C. difficile* et de ses toxines dans les spécimens fécaux. Elles inlcuent (i) l'isolation de l'organisme, (ii) l'Agglutionation au Latex, (iii) l'analyse par culture de tissu, et (iv) l'ELISA pour le détection de la toxine A ou toxine A et B. L'isolation de l'organisme et l'agglutination au latex sont des méthodes utiles pour détecter la présence de l'organisme dans les spécimens fécaux.

Cependant, aucune des deux méthodes ne peut démontrer la présence des toxines. Cela doit être fait en utilisant soit un test par culture de tissu ou alors un ELISA spécifique pour les toxines. La procédure par culture de tissu s'avère très utile à cause des niveaux élevés d'activités de la toxine B de *C. difficile* durant l'analyse. Des quantités très réduites d'environ un picogramme de toxine sont suffisantes pour causer une réaction positive. La neutralisation de l'activitée cytotoxique par l'antitoxine de *C.difficile* confirme la présence de la toxine de *C. difficile*. L'analyse doit être utilisé pour aider dans le diagnostique de la maladie par *C. difficile*, et les résultats doivent être considérés en conjonction avec l'histoire médicale du patient. Ceci est causé par le fait que certains adultes sont des porteurs asymptotiques et peuvent avoir des niveaux de toxines détectables dans leur matière fécale, même si ils ne présentent aucun symptomes cliniques. De plus, jusqu'à 50% des enfants en bas âge en bonne santés peuvent avoir des toxines de *C. difficile* dans leur matière fécale mais ne souffrent d'aucun signe de maladie.

PRINCIPE DE L'ANALYSE

Le *C. DIFFICILE TOX-B TEST* utilise une procédure de culture de tissu pour détecter la présence d'activitée cytotoxique (identifiée par l'arrondissage des céllules) dans les spécimens fécaux ce qui confirme l'identification de la toxine de *Clostridium difficile* en utilisant une antitoxine spécifique. Deux réactifs sont fournis dans le *C. DIFFICILE TOX-B TEST*. L'un est le réactif *Toxine Contrôle Positif*. L'autre est l'antitoxine spécifique utilisée pour confirmer la présence de la toxine de *C. difficile* en neutralisant son activitée cytotoxique. Aussi inclut est le *Diluant* pour préparer le spécimen fécal. Dans l'analyse, une partie aliquote d'un spécimen fécal est émulsifiée dans le *Diluant* puis le spécimen ainsi dilué est alors centrifugé et filtré. Un échantillon du filtrat est transféré dans un puit de culture de tissu contenant du tampon salin aux phosphates (TSP) et à un puit contenant de l'antitoxine. Si la toxine de *C. difficile* est présente, cela causera les céllules dans le puit avec le TSP de s'arrondir, démontrant la présence de l'activitée cytotoxique. La présence de la toxine de *C. difficile* est confirmée si l'activitée cytotoxique est neutralisée dans le puit contenant l'antitoxine.

MATÉRIAUX FOURNIS

Antitoxine de Chèvre contre *C. difficile*, 3,0 mL (avec préservatif)
Tampon salin aux phosphates (TSP), 3,0 mL (avec préservatif)
Toxine Contrôle Positif, 2,0 mL toxine de *C. difficile* active dans une solution de protéine tampon contenant du préservatif
Diluant, 100 mL (Tampon salin aux phosphates contenant du préservatif et du rouge de phénol)
T5003 et R5003 contiennent suffisamment de réactifs pour deux plaques de 48 puits chaque pour culture de tissu.
T5003 inclut aussi une plaque de 48 puits contenant des cultures de tissu avec céllules de mammifères.

MATÉRIAUX NÉCESSAIRES MAIS NON FOURNIS

Microscope pour observer les céllules
Centrifuge et tubes pour centrifuge
Tubes d'analyse pour préparer les dilutions
Filtres pour seringues (0,45 à 0,8 µm)
Incubateur à 37°C ± 2°C
Pipettes/pipetteur

DURÉE DE CONSERVATION ET STOCKAGE

La date d'échéance du kit est indiquée sur l'étiquette. La date d'échéance pour chaque composant est énumérée sur les étiquettes individuellement. Ne pas utiliser les réactifs aprés leurs dates d'échéances pour éviter une réduction de la sensitivité.

PRÉCAUTIONS

1. Utiliser les spécimens de matière fécale durant les premières 24 heures suivant la collection des spécimens afin d'obtenir des résultats optimaux. Les spécimens qui

ont été congelés (-20°C ou moins) peuvent perdre leurs activités à cause de la congélation et du dégel. ÉVITER LES CONGÉLATIONS ET DÉGELS RÉPÉTÉS DES SPÉCIMENS. CELA POURRAIT INACTIVER LA TOXINE.

2. Tous les spécimens doivent être considérés comme potentiellement infectieux et doivent donc être manipulés de façon appropriée.
3. Le réactif *Toxine Contrôle Positif* contient une toxine qui est biologiquement active et doit être traité avec prudence. L'antitoxine ne pose aucun danger connu.
4. Ne pas utiliser les réactifs aprés leurs dates d'échéances.
5. Stocker les réactifs selon les recommendations du fabricant. Cela prolonge la durée de conservation des réactifs.
6. Remettre les réactifs dans le réfrigérateur une fois que la procédure est complétée.

COLLECTION ET MANIPULATION DES SPÉCIMENS FÉCAUX

Les procédures standards internes de collection et de manipulation utilisées pour les spécimens fécaux sont appropriées. Les spécimens doivent être transportés aussitôt que possible et stockés entre 2° et 8°C. Quand cela est possible, éxaminer les échantillons qui ont moins de 24 heures. Stocker les spécimens à -20°C ou à des températures moindres si l'éxamen ne peut pas être éxécuté suivant les 48 heures aprés la collection de l'échantillon. La congélation et le dégel du spécimen, surtout si cela est répété plusieur fois, peut produire une perte d'activité causée par la dégradation de la toxine.

TRAITEMENT DES SPÉCIMENS FÉCAUX

NOTER: Les spécimens fécaux qui ont moins de 24 heures doivent être analysés dès que possible. Les spécimens doivent être stockés entre 2° et 8°C jusqu'à ce que leurs analyses soient performées.

1. Préparer un tube pour chaque spécimen à analyser. Ajouter 1,8 mL de *Diluant* à chaque tube. Marquer le tube directement sur le côté.
2. Pour les spécimens formés, utiliser une tige pour transférer le spécimen fécal au tube. Recouvrir la tige complètement avant de transférer le spécimen. Mélanger la tige dans du *Diluant* pour extraire autant d'échantillon que possible et appuyer la tige contre le côté du tube pour expulser le liquide résiduel. Pour les spécimens liquides, utiliser une pipette en plastique pour transférer 0,2 mL de spécimen au tube (dilution en rapport de 1/10). Assurez vous que les spécimens liquides soient suspendus d'une façon homogène avant de faire le transfert.
3. Vortéxer les tubes pendant 10 secondes. Transférer une partie aliquote du spécimen à un tube micro-centrifuge (par example un tube Eppendorf) et centrifuger le spécimen pendant 5 minutes à des vitesses élevées (2000 à 10000 rpms) avec une micro-centrifuge.
4. Collecter le fluide surnageant et filtrer à travers une membrane de 0,45 µm dans un tube stérile. Stocker le filtrat entre 2° et 8°C.

PROCÉDURE POUR LA CULTURE DE TISSU

NOTER: La procédure décrite ci-dessous peut être utilisée pour détecter la toxine de *C. difficile* dans les spécimens fécaux avec le *C. DIFFICILE TOX-B TEST*. Alternativement, le kit peut être utilisé selon d'autres procédures modifiées internement. Il est important, toutefois, que la *Toxine Contrôle Positif* et que le réactif antitoxine soient utilisés selon les instructions fournies.

1. Déterminer le nombre de puits qui seront nécessaires pour faire l'analyse. Deux puits seront nécessaires pour les puits de contrôles à chaque fois qu'une analyse est performée. De plus, deux puits seront nécessairess pour chaque spécimen. Les céllules pour culture de tissu qui sont typiquement utilisées incluent les céllules humaine du prépuce, MRC-5, WI-38, et les céllules d'ovaires de hamster chinois.
2. Ajouter 1 goutte (50 µL) de TSP à l'un des puits dans chaque série. Ajouter 1 goutte (50 µL) d'antitoxine de *C. difficile* à l'autre puit dans chaque série. Préparer les puits tel qu'il est illustré dans la Table 1.

Table 1.

Puit	TSP ou Antitoxine	Échantillon d'analyse
Puit de Contrôle 1	1 goutte (50 µL) de TSP	1 goutte (50 µL) de contrôle positif
Puit de Contrôle 2	1 goutte (50 µL) d'antitoxine	1 goutte (50 µL) de contrôle positif
Puit d'analyse 1	1 goutte (50 µL) de TSP	1 goutte (50 µL) de filtrat fécal
Puit d'analyse 2	1 goutte (50 µL) d'antitoxine	1 goutte (50 µL) de filtrat fécal

La dilution finale du filtrat fécal dans le puit est en rapport de 1/50. Utiliser des nouvelles pipettes pour chaque spécimen. Veuillez noter que (i) une série de contrôles doit être utilisée à chaque fois qu'un test est performé et (ii) que le reste du filtrat fécal doit être réfrigéré entre 2° et 8°C jusqu'à 48 heures dans l'éventualité ou des tests additionnels deviennent nécessaires.

3. Aprés que les échantillons aient été appliqués, incuber les puits à 37°C ± 2°C entre 24 et 48 heures. Observer chaque puit pour identifier l'arrondissage des céllules, ce qui indique la présence d'activitée cytotoxique. L'effet cytotoxique est considéré positif si au moins 50% des céllules deviennent arrondies. Enregistrer les résultats pour chaque puit. L'effet cytotoxique peut être observé durant les 8 heures aprés l'application du spécimen si des niveaux élevés de toxine sont présent. Cependant, les spécimens ne doivent pas être considérés négatifs jusqu'à aprés 48 heures d'observation.

CONTRÔLE DE QUALITÉ

Un contrôle positif et un contrôle négatif doit être fait avec chaque série de spécimens analysés. Cela inclut la *Toxine Contrôle Positif* ajoutée à un puit avec une solution tampon pour démontrer la présence de l'activitée, et la *Toxine Contrôle Positif* ajoutée à un puit contenant de l'antitoxine pour démontrer la neutralisation de l'activitée cytotoxique. Le contrôle positif doit provoquer l'effet d'arrondissage typique des céllules mais aussi caractéristique de la toxine de *C. difficile*. Les résultats des tests ne sont pas valides à moins que les caractéristiques de performance soient satisfaites. Si les caractéristiques de performance ne sont pas satisfaite, veuillez contacter les Services Techniques et ne pas rapporter les résultats. Dès son arrivée, le kit doit être inspecté pour s'assurer que ses articles sont en conditions satisfaisantes. Les résultats des tests de même que les résultats des contrôles doivent être enregistrés et rapportés selon les procédures internes et cette information doit être préservée selon les procédures internes pour future référence.

INTERPRETATION DES RESULTATS

Puit contenant:		INTERPRÉTATION DES RÉSULTATS
TSP	*C. difficile* Antitoxine	
-	-	Négatif - L'activitée cytotoxique n'est pas présente
+	-	L'activitée cytotoxique présente est neutralisée par l'antitoxine de *C. difficile*, confirmant la présence de la toxine de *C. difficile* dans le spécimen
+	+	Résultats indéterminés qui peuvent être causés par des niveaux de toxine *C. difficile* extrèmement élevés. Le filtrat fécal doit être dilué d'avantage avec du *Diluant* en rapport de 1/10 et le test doit être répété pour éliminer cette possibilité. Aprés avoir répété ce test sous ces conditions, si les céllules du puit contenant du TSP ainsi que du filtrat fécal hautement dilué sont arrondis et les céllules du puit contenant de l'antitoxine ainsi que du filtrat fécal hautement dilué ne le sont pas, alors, la toxine de *C. difficile* est présente. Si les céllules dans les deux puits sont arrondis, le spécimen possède donc une activitée cytotoxique qui n'est pas associée avec la présence de la toxine de *C. difficile*.

Résultats de Contrôle: Les céllules dans le puit contenant du TSP et de la *Toxine Contrôle Positif* devraient être arrondis. Les céllules dans le puit contenant l'antitoxine de *C. difficile* et de la *Toxine Contrôle Positif* devraient apparaitre normales, démontrant la neutralisation de la *Toxine Contrôle Positif* par l'antitoxine.

Résultats des Échantillons d'Analyse: Quand les filtrats du spécimen démontrent des signes spécifiques de la présence de toxine de *C. difficile*, rapporter: "Spécimen du patient positif pour la toxine de *Clostridium difficile*." Quand les filtrats du spécimen ne démontrent aucun signe d'activitée cytotoxique aprés 48 heures d'observation, rapporter: "Aucune toxine de *Clostridium difficile* détectée." Quand les filtrats du spécimen démontrent des signes d'activitée cytotoxique qui n'est pas neutralisée par l'antitoxine de *C. difficile*, rapporter: "Réaction Non-spécifique du spécimen du patient, la réaction caractéristique de la toxine de *Clostridium difficile* est absente." La répétition de l'analyse avec un spécimen additionnel peut être utile pour éliminer la possibilité d'une maladie causée par la toxine de *Clostridium difficile*.

LIMITATIONS DU *C. DIFFICILE TOX-B TEST*

1. Le *C. DIFFICILE TOX-B TEST* est utilisé pour détecter la toxine de *C. difficile* dans les spécimens fécaux. À cause de la nature complexe de la matière fécale, les spécimens de <24 heures doivent être utilisés.
2. La détection de la toxine par cette analyse dépend de l'activitée biologique de la toxine. Les spécimens doivent être manipulés de façon appropriée et stockés selon les recommendations fournies pour réduire au minimum l'inactivation de la toxine.
3. Certains spécimens peuvent produire des réactions qui ne sont pas définitivent (par example, des céllules partiellement arrondis, élongation au lieu d'arrondissage). Sous ces conditions, le spécimen doit être re-testé ou un spécimen frais doit être testé. Des tests additionnels peuvent être utilisés en conjonction avec le *C. DIFFICILE TOX-B TEST* tels que ELISA pour la toxine A, ELISA pour les toxines A et B, l'isolation de l'organisme sur des médias sélectifs, ou une analyse par agglutination au latex pour la détection de l'organisme.

4. Certains isolés de *C. sordellii* produisent le même type d'effet d'arrondissage des cellules en culture de tissu que ceux de *C. difficile* toxigénique. Cela est dû aux similarités des toxines de *C. sordellii* avec celles des toxines de *C. difficile*. Les variétés de *C. sordellii* qui produisent ces toxines similaires n'ont pas été détectées parmi les patients souffrant de diarrhées et colites associées avec traitement antibiotique.

LES VALEURS ANTICIPÉES

La prédominance d'un *C. DIFFICILE TOX-B TEST* positif dans nos études cliniques se situent entre 5,8% et 19%. La prédominance changera d'un milieu à un autre et les hôpitaux peuvent avoir des niveaux plus ou moins élevés que ceux observés parmi les sites utilisés pour l'évaluation du *C. DIFFICILE TOX-B TEST*. La maladie par *Clostridium difficile* est, principalement, une maladie nosocomial des patients agés et les hôpitaux qui ont un nombre élevés de patients agés peuvent aussi avoir des taux supérieurs à la norme. Il est important de considérer les résultats des analyses en conjonction avec les symptomes cliniques parceque certains adultes en bonne santés et un nombre important d'enfants en bas age (jusqu'à 50%) seront positifs pour les toxines A et B de *C. difficile*. De plus, des taux de contaminations par *C. difficile* ont été rapportés à hauteur de 22% à 32% parmi les patients atteints de fibreuse cystique (11-13).

CARACTÉRISTIQUES DE PERFORMANCE

La performance du *C. DIFFICILE TOX-B TEST* de *C. difficile* a été évalué à quatres endroits différents aux États Unis. Pour l'analyse, le *C. DIFFICILE TOX-B TEST* a été comparé à un autre Test de Cytotoxicité commercial pour les toxines de *C. difficile*, qui est aussi un test basé sur une analyse de culture. Toutes les analyses de culture ont été conduite avec des céllules provenant du prépuce. Un résumé des résultats des études cliniques est présenté dans la Table 2. Globalement, les intervalles de la sensitivité, de la spécificité, et de la corrélation pour l'évaluation sont présentés en parenthèse. Dans les études faites aux centres de références au Nord-Est et au Sud-Est des E.U. et à un centre médical sur la Côte Est (East Coast) utilisant 427 spécimens, le *C. DIFFICILE TOX-B TEST* a démontré une corrélation globale relative de 100% avec l'autre test commercial. Dans une étude faite dans un centre médical au Centre-Ouest (Mid West) du pays utilisant 517 spécimens, le *C. DIFFICILE TOX-B TEST* a démontré une sensitivité relative globale et une spécificité relative globale de 96,7% et de 98,5%, respectivement. La corrélation relative globale était de 98,3%. Les spécimens qui ont produit des résultats contradictoires ont été soumis à des analyses plus étendues utlilisant une analyse par culture toxigénique. La sensitivité relative globale et la spécificité ont, alors, toutes deux augmentées de 98,3% à 98,7%, et la corrélation relative globale était de 98,6%.

Table 2

		Analyse de Cytotoxicité Commerciale/Culture Toxigénique	
		positif	négatif
TOX-B TEST	positif	114	6
	négatif	1	823

Sensitivité relative globale	99,1% (98.3% à 100%)
Spécificité relative globale	99,3% (98.7% à 100%)
Corrélation relative globale	99,3% (98.6% à 100%)
Nombre de spécimens	944

LES RÉACTIONS CROISÉES

Les filtrats toxiques provenant de *C. chauveoi*, *C. fallax*, *C. perfringens*, *C. septicum*, *et de C. spiroforme* n'ont pas été neutralisés par l'antitoxine de *C. difficile*. De plus, l'alpha-toxine de *C. perfringens*, la collagénase et le clostripain de *C. histolyticum*, la toxine du choléra, la toxine de la diphthérie, la toxine shiga-like, et l'entérotoxine de staphylococcal n'ont pas été neutralisées. Le seule organisme reconnu pour produire une réaction

croisée avec le *C. DIFFICILE TOX-B TEST* est *C. sordellii*. Certains isolés toxigéniques de *C. sordellii* produisent des toxines qui sont similaires aux toxines de *C. difficile*. La toxine hémorragique (toxine HT) de *C. sordellii* est très similaire à la toxine A alors que la toxine létale (toxine LT) est très similaire à la toxine B. Les anticorps contre les toxines de *C. difficile* neutralisent les deux toxines HT et LT de *C. sordellii*. *Clostridium sordellii* n'est pas impliqué dans les diarrhées et colites associées avec traitement antibiotique.

REPRODUCTIBLITÉ ET PRÉCISION

Des séries identiques de 6 spécimens fécaux ont été envoyées à trois laboratoires cliniques différents se trouvant au Centre-Ouest (Mid West), au Sud-Est (Southeast), et au Nord-Est (Northeast) des E.U.. Les résultats du *C. DIFFICILE TOX-B TEST* provenant de chaque laboratoire sont en accord avec les résultats produits internement. Dans une étude interne, cinq spécimens fécaux positifs ont été analysé aprés 0, 24, 48, et 72 heures en utilisant le *C. DIFFICILE TOX-B TEST*. Ces spécimens ont été démontrés positifs systématiquement pour la toxine de *C. difficile* et pour les durées indiquées, ceci aprés détermination par neutralisation de l'activité cytotoxique. Les spécimens étaient stocké à 4°C durant le test. Dans des études internes additionnelles, trois lots différents du réactif contrôle positif ont été analysé en dupliqué et ont systématiquement produits des titres cytotoxiques de 10^5. Des évaluations supplémentaires ont démontrés que différents lots de l'antitoxine de *C. difficile* neutralisaient la *Toxine Contrôle Positif*.

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Product #	Description
T5000	*C. difficile Toxin/Antitoxin Kit*
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T5002	*LEUKO-TEST (latex agglutination)*
T5008	*IBD-CHEK®*
T5009	*IBD-SCAN®*
PT5012	*GIARDIA II*
T5014	*CRYPTOSPORIDIUM TEST*
T5015	*C. DIFFICILE TOX A/B II™*
T5017	*E. HISTOLYTICA II*
T5025	*C. DIFF CHEK™ - 60*
T5026	*C. DIFF CHEK™ - 30*
T9015	*STOOL-PREP™*

APPENDIX A. Protocol for *C. difficile* TOX-B TEST

(b)(4) Confidential and Proprietary Information

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APPENDIX C. *Clostridium difficile* cytotoxin assay

(b)(4) Confidential and Proprietary Information

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[redacted]

An ELISA for the detection of *Clostridium difficile* Toxins A and B.
Catalog No. T5015 (96 Tests)

Developed and Manufactured by

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International Symbol Key:

REF	Catalog Number		Temperature Range
IVD	*In Vitro* Diagnostics		Use By/Expiration Date
LOT	Lot Information	CE	CE Symbol

C. DIFFICILE TOX A/B II ™

INTENDED USE

The *C. DIFFICILE TOX A/B II* ™ test is an enzyme immunoassay for the detection of toxins A and B produced by toxigenic strains of *Clostridium difficile*. It can be used to detect toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history.
FOR *IN VITRO* DIAGNOSTIC USE.

EXPLANATION

After treatment with antibiotics, many patients develop gastrointestinal problems ranging from mild diarrhea to severe pseudomembranous colitis. Many cases of the milder forms of gastrointestinal illness and most cases of pseudomembranous colitis are caused by *Clostridium difficile (1)*. This organism is an opportunistic anaerobic bacterium that grows in the intestine once the normal flora has been altered by the antibiotic. The disease results from the toxins that the organism produces. The clinical symptoms associated with the disease are believed to be primarily due to toxin A, which is a tissue-damaging enterotoxin *(2,3)*. *Clostridium difficile* also produces a second toxin, designated toxin B. Toxin B, which has been referred to as the cytotoxin of the organism, is the toxin detected by the tissue culture assay currently used by many laboratories. Most strains either produce both toxins or neither toxin, although recently, toxin A negative/toxin B positive strains have been identified *(4,5)*. These toxin A negative/toxin B positive strains test positive in the *C. DIFFICILE TOX A/B II* ™ test *(6,7)*.

Clostridium difficile strains that produce high levels of toxin A also produce high levels of toxin B. Likewise, strains that produce low levels of toxin A produce low levels of toxin B, indicating that the toxin production may be regulated similarly. Tests that detect either or both toxins are being used as diagnostic aids. The genes for the toxins have been cloned and sequenced, and some properties of the toxins are now well-defined *(8,9)*. Both toxins are large *(M_r of toxin A, 308,000; M_r of toxin B, 279,000)*. Toxin A has a complex series of repeating units at the COOH-terminus of the molecule and these repeating units most likely serve as the binding portion that recognizes galactose-containing receptors *(10)*. There is some evidence suggesting that toxins A and B act synergistically and that the initial tissue damage caused by toxin A allows toxin B to exert its toxicity. Therefore, although toxin A is believed to cause most of the clinical signs, toxin B also may play an important role in the disease.

PRINCIPLE OF THE TEST

The *C. DIFFICILE TOX A/B II* ™ test uses antibodies to *Clostridium difficile* toxins A and B. The microassay wells supplied with the kit contain immobilized affinity-purified polyclonal goat antibody against toxins A and B. The detecting antibody consists of a mixture of toxin A monoclonal mouse antibody conjugated to horseradish peroxidase and toxin B polyclonal goat antibody conjugated to horseradish peroxidase. In the assay, an aliquot of a fecal specimen is emulsified in the *Diluent* and the diluted specimen is then transferred to the microassay well containing the detecting antibody. If toxins A and B are present in the specimen, they will bind to the detecting antibody and to the immobilized polyclonal antibody during the incubation phase. Any unbound material is removed during the washing steps. Following the addition of substrate, a color is detected due to the enzyme-antibody-antigen complexes that form in the presence of toxin.

MATERIALS PROVIDED

Diluent, 40 mL, buffered protein solution + 0.02% thimerosal. The *Diluent* is also to be used as the negative control solution (see TEST PROCEDURE).

Conjugate, 7 mL, mouse monoclonal antibody specific for toxin A coupled to horseradish peroxidase and goat polyclonal antibody specific for toxin B coupled to horseradish peroxidase in a buffered protein solution + 0.02% thimerosal

Substrate, 14 mL, solution containing tetramethylbenzidine and peroxide

Positive Control, 3.5 mL, inactivated toxins in a buffered protein solution containing 0.02% thimerosal

Wash Buffer Concentrate, 50 mL, 20X concentrate containing phosphate-buffered saline, detergent, and 0.2% thimerosal)

Stop Solution, 7 mL, 0.6N sulfuric acid. CAUTION: Avoid contact with skin; flush with water immediately if contact occurs.

Microassay Plate, 12 strips, each strip consisting of 8 wells, coated with affinity purified goat antibodies specific for toxins A and B (stored with desiccant)

Wash Solution Label, 1 label ***Applicator sticks,*** 50 sticks

ACCESSORIES

Disposable plastic pipettes	100 pipettes
Plastic adhesive sheets	2 sheets

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Wash bottle *Timer* *Vortex mixer*
Discard container *Distilled water* *Tubes for dilution of specimen*
Paper towels or absorbent sheets
Spectrophotometer capable of reading dual wavelength at 450/620 nm or single wavelength at 450 nm (a dual wavelength plate reader is recommended; absorbances should be measured at 450 nm and referenced at 620 nm)
Refrigerator set between 2° and 8°C *Incubator* set at 37°C ± 2°C

SHELF LIFE AND STORAGE

The expiration date of the kit is given on the label. Expiration dates for each component are listed on the individual labels. The kit containing the reagents with designated shelf life should be stored between 2° and 8°C and should be returned to the refrigerator as soon as possible after use.

PRECAUTIONS

1 Reagents from different kits should not be mixed. Do not use a kit past the expiration date.
2 Reagents should be at room temperature before use.
3 Caps and tips are color coded; do not mix!
4 When handling assay wells, avoid scratching the bottom of the wells because this may result in elevated absorbance readings.
5 Hold dropper bottles vertically to ensure proper drop size.
6 Handle specimens and used microassay wells as if capable of transmitting infectious agents. Wear gloves when doing the test.
7 Reagents contain 0.02% thimerosal as a preservative and should be handled with normal laboratory caution.
8 The *Stop Solution* contains 0.6N sulfuric acid. Flush with water immediately if contact occurs.
9 *Unused microwells must be placed back inside of the resealable pouch with the desiccant to protect them from moisture.*
10 Perform the washing procedure as directed to avoid high background reactions.
11 Use fecal specimens within 24 hours of collection to obtain optimal results. Specimens that are frozen *(-20°C or lower)* may lose activity due to freezing and thawing.
12 The *Substrate* is light sensitive and should be protected from direct sunlight or UV sources.
13 Optimal results are obtained by following the specified test procedure. The concentrations, incubation conditions, and processing specifications have been optimized for sensitivity and specificity. Alterations of the specified procedure and/or test conditions may affect the sensitivity and specificity of the test.

COLLECTION AND HANDLING OF FECAL SPECIMENS

NOTE: *Standard collection and handling procedures used in-house for fecal specimens are appropriate.* ***Specimens should be transported and diluted in the kit Diluent as soon***

as possible. Specimens should be stored between 2° and 8°C. Whenever possible, test samples which are less than 24 hours old. Store specimens at -20°C, or lower, if the test cannot be performed within 72 hours of collection (11). Freezing and thawing of the specimen, especially multiple times, may result in loss of activity due to degradation of the toxins. Fecal specimens that have been preserved in 10% Formalin, Merthiolate Formalin, Sodium Acetate Formalin, or Polyvinyl Alcohol cannot be used. Make sure that specimens are thoroughly mixed (vortexed) prior to performing the assay. This includes complete mixing of the specimen prior to transfer to Diluent as well as complete mixing of the diluted specimen prior to transfer to the microwell. The Diluent has been formulated to stabilize the toxins in fecal specimens and minimize degradation. Disposable pipettes which are included in the accessories are graduated at 50, 100, 200, and 300 µL. Specimens may be diluted in the optional STOOL-PREP™ device (Cat.#T9015, see instructions for use) and stored between 2° and 8°C.*

1 Set up one dilution tube for each specimen to be tested. Add 200 µL *Diluent* to each tube. *Label the tube directly on the side.*
2 For **formed fecal specimens**, use an applicator stick to transfer the fecal specimen to the tube. Transfer an amount equal to about 3 mm in diameter with the applicator stick to the *Diluent*. For **liquid fecal specimens**, use a plastic pipette to transfer 50 µL specimen to tube. Make sure the liquid specimens are evenly suspended before transferring.
3 Vortex the tubes for 10 seconds and store between 2° and 8°C until the ELISA is performed (within 72 hours of collection). Vortex again before transferring diluted specimen to microassay well. This ensures thorough mixing of the specimen.
4 Semi-automated washing equipment may be used with specimens that have been centrifuged (5000 x g for 10 minutes) to remove particulate matter.

PRELIMINARY PREPARATIONS

1 **IMPORTANT:** All reagents must be at room temperature prior to use in the assay.
2 Prepare 1X *Wash Solution*. The *Wash Buffer Concentrate* is supplied as a 20X concentrate *(a precipitate may be noticed)*. It should be diluted to a total volume of 1 liter by adding 50 mL of the concentrate to 950 mL of distilled water. Label the bottle. Store any unused 1X *Wash Solution* between 2° and 8°C.
3 **Assay Strip Preparation.** Each Strip contains 8 wells coated with affinity purified polyclonal antibodies specific for toxins A and B. Each specimen or control will employ one of these coated wells. Determine the number of wells to be used. Avoid contact with the base of the wells. Assay wells not used must be returned to the plastic bag and carefully resealed with desiccant.

TEST PROCEDURE

1 Add 1 drop (50 µL) of *Conjugate* (red cap) to each well. Be sure to hold each bottle vertically when adding the drops. Use 1 well for each fecal specimen, 1 well for the *Positive Control* and 1 well for the negative control *(i.e., Diluent)*. Identification marks may be written directly on side of well.
2 Transfer 100 µL (2 drops using a transfer pipette from the accessory kit) of diluted specimen to the assay well; or if using a *STOOL-PREP™** device, add diluted specimen according to the *STOOL-PREP™** device instructions. Add 1 drop (50 µL) of the *Positive Control* (black cap) to the positive control well and 50 µL (1 drop using a transfer pipette from the accessory kit) of the negative control *(i.e., Diluent)* to the negative control well.
3 Cut the adhesive plastic sheet to the size necessary to cover the wells. Cover the wells and incubate them at 37°C ± 2°C for 50 minutes.
4 Shake out the contents of the assay wells into a discard pan.
5 Wash each well using the 1X *Wash Solution* in a squirt bottle with a fine-tipped nozzle, directing the *Wash Solution* to the bottom of the well with force. Fill the wells, then shake the *Wash Solution* out of the well into a discard pan. Slap the inverted plate on a dry paper towel and repeat steps #4 and #5 **four times** using a dry paper towel each time. If any particulate matter is seen in the wells, continue washing until all the particulate matter is removed. **Note:** If using semi-automated or automated washing equipment, specimens must be centrifuged (5000 x g for 10 minutes) to remove any particulate matter. Add 350 µL of 1X *Wash Solution* to each well. Wash for a total of 5 times.

using optional *STOOL-PREP™** devices, wash the plate once by hand, slapping hard on dry paper towels and follow with 4 washes on an automated washer for a total of 5 washes. If any particulate matter is seen in the wells, continue washing until all the particulate matter is removed.

6 After washing, completely remove any residual liquid in the wells by striking the plate once again onto a dry paper towel until no liquid comes out. *Dispose of paper towels and specimen containers properly.*

7 Add 2 drops (100 µL) of *Substrate* (blue cap) to each well. Gently tap the wells to mix the substrate. Incubate the wells at room temperature for 10 minutes. Gently tap the wells at 5 minutes.

8 Add 1 drop (50 µL) of *Stop Solution* (yellow cap) to each well. Gently tap the wells and wait 2 minutes before reading. The addition of the *Stop Solution* converts the blue color to a yellow color which may be quantitated by measuring the optical density at 450 nm on a microplate ELISA reader. The instrument should be blanked against air. If a dual wavelength reader is used, blank against air at 620 and read at 450 nm. Wipe the underside of each well before measuring the optical density. If an ELISA reader is available, the test may be read visually in good light against a white background. Read within ten minutes after adding *Stop Solution.*

ALTERNATE TEST PROCEDURE/RAPID FORMAT

Perform the regular test procedure according to the instructions provided above replacing the 50 minute incubation at 37°C ± 2°C with 20 minutes at 37°C using the *Stat Fax 2200 Incubator/Shaker* or equivalent incubator/shaker. If the *Stat Fax 2200 Incubator/Shaker* is used, set the shaker to speed 7 and the temperature to 37°C. If other shakers are used, a speed of 1500 rpm is recommended. Shaking should not cause spillage. If spillage occurs, reduce the speed accordingly *(11).*

QUALITY CONTROL

1 A positive and negative control must be run with each series of test specimens.

2 Positive and negative controls must fall within their respective ranges or the test is not valid.
   a Positive Control must be a visible yellow color. If read on a spectrophotometer, the OD at 450 nm or using dual wavelength at 450/620 nm must be ≥0.500.
   b Negative Control must be visually clear. If read on a spectrophotometer, the OD at 450 nm must be <0.120. If read at 450/620 nm the absorbance must be <0.080.

3 Wells that are clear visually but give absorbance ≥0.120 should be wiped on the underside and remeasured.

4 Visual readings must be taken in good light against a white background.

5 A sample that yields a weak positive result (i.e., <0.200) and is adjacent to a strong positive should be repeated to assure carryover did not occur.

INTERPRETATION OF RESULTS

1 **Visual Reading**
   Negative = Colorless
   Positive = Any yellow color

2 **Spectrophotometric Single Wavelength at 450 nm**
   Negative = OD<0.120
   Positive = OD ≥0.120

3 **Spectrophotometric Dual Wavelength at 450/620 nm**
   Negative = OD<0.080
   Positive = OD ≥0.080

A positive test result indicates that *C. difficile* toxin A and/or toxin B are present in the fecal specimen.

LIMITATIONS OF THE *C. DIFFICILE TOX A/B II* ™ TEST

1 The *C. DIFFICILE TOX A/B II* ™ test is used to detect *C. difficile* toxin in fecal specimens. The test confirms the presence of toxin in feces and this information should be taken under consideration by the physician in light of the clinical history of the patient. Inability to

detect toxin A or B in fecal samples from patients suspected of having *C. difficile* disease may not preclude actual disease but may be caused by other factors (i.e., incorrect specimen collection, handling and/or storage, toxin levels lower than the kit detection limits). The *C. DIFFICILE TOX A/B II* ™ test will detect Toxin A at levels ≥0.8 ng/mL and Toxin B at levels ≥2.5 ng/mL.

2 Fecal specimens represent an extremely complex clinical specimen. Optimal results with the *C. DIFFICILE TOX A/B II* ™ test are obtained with specimens that are less than 24 hours old. Most specimens can be stored between 2° and 8°C for 72 hours before significant degradation of the toxin is noted. If specimens are not assayed within this time period, they may be frozen and thawed. However, freezing and thawing, especially multiple times, may cause specimens to lose their activity due to degradation of the toxin.

3 Some specimens may give weak reactions. This may be due to a number of factors such as the presence of a weakly toxigenic strain, low levels of toxin production *in vivo*, or the presence of binding substances or inactivating enzymes in the feces. *Under these conditions, the specimen should be retested or a fresh specimen should be tested.* Additional tests that may be used in conjunction with the *C. DIFFICILE TOX A/B II* ™ test include isolation of the organism on selective media, latex agglutination assay for the detection of *C. difficile* (the organism), or tissue culture cytotoxicity assay for the detection of *C. difficile* toxin. The isolation of the organism does not confirm that it is toxigenic. This must be confirmed by additional testing of the isolate using either the ELISA or tissue culture assay to demonstrate toxigenicity. Likewise, the latex agglutination assay, which detects a nontoxic protein of *C. difficile*, does not demonstrate the presence of toxigenic *C. difficile*.

4 Certain toxigenic isolates of *Clostridium sordellii* produce toxins that are similar in their biologic, physicochemical and immunologic properties to the toxins of *C. difficile*. These isolates, however, have not been detected in patients with antibiotic-associated diarrhea and colitis.

5 Fecal specimens that have been preserved in 10% Formalin, Merthiolate Formalin, Sodium Acetate Formalin, or Polyvinyl Alcohol cannot be used.

6 The performance characteristics of the *C. DIFFICILE TOX A/B II* ™ test have not been thoroughly established in the pediatric population.

EXPECTED VALUES

The prevalence of a positive *C. DIFFICILE TOX A/B TEST* plus positive tissue culture and/or toxigenic culture was 5.4% in one study and 8.6% in another study. The prevalence will vary from location to location and hospitals may experience rates lower or higher than those observed at the sites used in the *C. DIFFICILE TOX A/B TEST* evaluation. *Clostridium difficile* disease is primarily a nosocomial disease of elderly patients, and hospitals that have higher numbers of elderly patients may experience higher rates. It is important to consider any test results in conjunction with clinical symptoms because some healthy adults and large numbers of healthy infants (up to 50%) will be positive for *C. difficile* toxin (toxin-positive by either tissue culture or ELISA). In addition, *C. difficile* carriage rates of 22% to 32% have been reported in cystic fibrosis patients *(12-14)*.

PERFORMANCE CHARACTERISTICS

Clinical evaluation

The *C. DIFFICILE TOX A/B TEST* was compared with the tissue culture test at four U.S. hospitals and in-house at TECHLAB®, Inc. Specimens included in the evaluation were submitted to the clinical laboratory for routine testing. The tissue culture test was done according to the in-house procedure. Table 1 shows a comparison of the *C. DIFFICILE TOX A/B TEST* with tissue culture. Of the 1,152 specimens included in the evaluation, approximately 3.6% were from children ≤ 2 years of age. No *C. DIFFICILE TOX A/B TEST* (+)/tissue culture (-) specimens were identified. Overall, the sensitivity for the *C. DIFFICILE TOX A/B TEST* ranged from 83.3% to 96% (confidence interval of 87.4 to 95.0, p=0.05). The specificity was 100% in all studies. The predictive positive value was 100% in all studies and the predictive negative value ranged from 90% to 99.5% (confidence interval of 93.8 to 99.8, p=0.05). The correlation ranged from 94.9% to 99.5% (confidence interval of 96.6 to 99.4, p=0.05).

TABLE 1 Correlation of *C. DIFFICILE TOX A/B TEST* with Tissue Culture (n=1,152).

		Tissue Culture positive	Tissue Culture negative
C. DIFFICILE TOX A/B TEST	positive	165	0
	negative	14	973

Sensitivity	92.2%	Predictive Positive Value	100%
Specificity	100%	Predictive Negative Value	98.6%
Correlation	98.8%		

In-house comparison

In a study performed in-house the *C. DIFFICILE TOX A/B II* ™ test was compared to the *C. DIFFICILE TOX A/B TEST*. In the study, 218 clinical specimens representative of those routinely submitted to the clinical laboratory for *C. difficile* testing were evaluated. The results obtained with both tests were comparable.

Centrifugation

A total of 337 fecal specimens, including 30 positives and 307 negatives, were evaluated to determine the effect of centrifugation on performance. For the analysis, specimens were diluted and vortexed as described in the package insert. The specimens were centrifuged (5,000 x g) to remove insoluble material and the supernatant fluid was assayed in the *C. DIFFICILE TOX A/B TEST*. Results were compared to results obtained with the same panel of diluted and vortexed specimens that had not been centrifuged. The results demonstrated a correlation of 100% between centrifuged and noncentrifuged specimens.

CROSS-REACTIVITY

Various organisms were examined for cross-reactivity in the *C. DIFFICILE TOX A/B TEST*. For the analysis, broth cultures mixed with *Diluent* were evaluated. Broth cultures at log phase containing >10^8 bacteria per mL were used. A listing of the organisms that did not react under any of the conditions is shown in Table 2. The only organisms that reacted were toxigenic *C. difficile* and a toxigenic strain of *C. sordellii* (VPI strain 9048) which produces toxins that crossreact significantly with *C. difficile* toxins. A nontoxigenic strain of *C. sordellii* that does not produce toxin HT was negative in the test. *C. sordellii* has not been implicated in pseudomembranous colitis or antibiotic-associated diarrhea. The strains of toxigenic *C. difficile* that were analyzed included six toxigenic strains that ranged from weakly to highly toxigenic and two nontoxigenic strains. The *C. DIFFICILE TOX A/B TEST* detected all six of the toxigenic strains and did not react with the two nontoxigenic strains.

TABLE 2 Organisms that do not react in the *C. DIFFICILE TOX A/B TEST*

Aeromonas hydrophila	*Clostridium sporogenes*
Acinetobacter lwoffi	*Clostridium tetani*
Bacillus cereus	*Enterococcus faecalis*
Bacillus subtilis	*Escherichia coli*
Bacteroides fragilis (toxigenic/nontoxigenic)	*Escherichia coli* (enterohemmorrhagic)
Candida albicans	*Klebsiella pneumoniae*
Candida krusei	*Peptostreptococcus anaerobius*
Candida tropicalis	*Proteus vulgaris*
Clostridium bifermentans	*Pseudomonas aeruginosa*
Clostridium botulinum (Types A-G)	*Salmonella choleraesuis*
Clostridium chauvoei	*Salmonella enteritidis*
Clostridium difficile (nontoxigenic)	*Salmonella typhimurium*
Clostridium haemolyticum	*Shigella flexneri*
Clostridium histolyticum	*Shigella sonnei*
Clostridium novyi (Types A, B, C)	*Staphylococcus aureus* (Protein A-negative)
Clostridium perfringens (Types A-E)	*Staphylococcus aureus* (Protein A-positive @ <10^6)
Clostridium septicum	*Streptococcus pyogenes*
Clostridium sordellii (nontoxigenic)	*Vibrio parahaemolyticus*
Clostridium sporogenes	*Yersinia enterocolitica*

EFFECT OF FECAL SAMPLE CONSISTENCY

The reactions of fecal samples of varying consistencies in the *C. DIFFICILE TOX A/B TEST* and tissue culture assay are shown in Table 3. The rates of positive reactions were similar in all three types of fecal specimens. All of the specimens were submitted for *C. difficile* testing. The basis for submission is the clinical history of the patient and not the consistency of the specimen. In additional studies, highly purified toxin A and toxin B were used to spike liquid, semi-solid, and solid fecal specimens.

TABLE 3 Effect of sample consistency

Test	Liquid Samples	Semi-Solid Samples	Solid Samples
Number of specimens (n=435)	150	133	152
C. DIFFICILE TOX A/B TEST Positive	13 (8.7%)	11 (8.3%)	13 (8.6%)
Tissue Culture Positive	13 (8.7%)	14 (10.5%)	15 (9.9%)

The *C. DIFFICILE TOX A/B TEST* detected toxins A and B in liquid, semi-solid and solid fecal specimens at levels similar to those observed with toxin A and toxin B prepared in kit *Diluent*.

REPRODUCIBILITY AND PRECISION

Five fecal specimens (one negative specimen and four positive specimens) were sent to four independent laboratories for analysis using the *C. DIFFICILE TOX A/B TEST*. All specimens were kept frozen until the assay was performed. The results from each laboratory were compared with in-house results and found to be identical. The four positive specimens were confirmed positive and the negative specimen was confirmed negative at each site.

The intraassay coefficient of variation (CV) of the *C. DIFFICILE TOX A/B TEST* was determined by analyzing 32 positive and 32 negative control reactions along with 8 negative fecal specimens. Each fecal specimen was tested in 11 wells. The intraassay %CV was 7.190 with the positive control, 6.557 with the negative control, and 9.697 with the fecal specimens. The interassay CV was determined using four positive and one negative fecal specimens tested at time 0, 24, 48, and 72 hours. The %CV ranged from 9.9 to 29.6, with an average of 16.3.

C. DIFFICILE TOX A/B II ™ - ESPAÑOL

INDICACIÓN DE USO

La Prueba TOX A/B II de *C. difficile* es un inmunoensayo enzimático para a detección de las toxinas A y B producidas por las cepas toxigénicas de *Clostridium difficile*. Puede ser usado para detectar las toxinas A y B en especímenes fecales de personas sospechosas de estar enfermas por *C. difficile*. La prueba debe ser utilizada como ayuda en el diagnóstico de *C. difficile* y los resultados deben ser considerados en conjunto con la historia clínica del paciente. PARA USO DIAGNÓSTICO *IN VITRO*.

EXPLICACIÓN

Después del tratamiento con antibióticos, muchos pacientes desarrollan problemas gastrointestinales que van desde la diarrea moderada hasta la colitis pseudomembranosa severa. Muchos de los casos moderados de enfermedad gastrointestinal y la mayoría de los casos de colitis pseudomembranosa son causados por *Clostridium difficile* (1). Este microorganismo es una bacteria anaerobia oportunista que crece en el intestino una vez que la flora normal ha sido alterada por el antibiótico. La enfermedad es el resultado de las toxinas que el microorganismo produce. Se cree que los síntomas clínicos asociados con la enfermedad se deben principálmente a la toxina A, la cual es una enterotoxina destructora del tejido (2,3). *Clostridium difficile* también produce una segunda toxina, designada toxina B. La toxina B, que ha sido llamada citotoxina del microorganismo, es la toxina detectada por el ensayo de cultivo tisular usado en muchos laboratorios. La mayoría de las cepas producen ambas o ninguna toxina, aunque recientemente, cepas negativas a la toxina A y positivas a la toxina B han sido identificadas (4,5). Estas cepas toxina A negativas/ toxina B positivas son positivas en la Prueba TOX A/B II de *C. difficile* (6,7).

Las cepas de *C. difficile* que producen altos niveles de toxina A también producen altos niveles de toxina B. Asimismo, las cepas que producen niveles bajos de toxina A producen niveles bajos de toxina B, indicando que la producción de toxinas puede ser regulada en forma similar. Pruebas que detectan una o ambas toxinas están siendo empleadas como una ayuda en el diagnóstico. Los genes para las toxinas han sido clonados y secuenciados, y algunas de propiedades de las toxinas están actualmente bien definidas (8,9). Ambas toxinas son grandes (la porción Mr de la toxina A, 308,000; la porción Mr de la toxina B, 279,000). La toxina A tiene una compleja serie de unidades repetitivas en el extremo COOH- de la molécula y estas unidades repetitivas probablemente actuan como porciones de enlace que reconocen receptores con galactosa (10). Hay cierta evidencia que sugiere que las toxinas A y B actúan sinérgicamente y que el daño inicial del tejido causado por la toxina A permite a la toxina B ejercer su toxicidad. Por tanto, a pesar de que la toxina A es considerada la causante de la mayoria de los signos clínicos, la toxina B también juega un papel importante en la enfermedad.

PRINCIPIO DE LA PRUEBA

La Prueba TOX A/B II de *C. difficile* usa anticuerpos dirigidos contra las toxinas A y B de *Clostridium difficile*. Los pocillos de microtitulación provistos con el kit contienen inmovilizados, anticuerpos policlonales de cabra purificados por afinidad, dirigidos contra las toxinas A y B. El anticuerpo detector es una mezcla de anticuerpos monoclonales de ratón contra la toxina A conjugados con peroxidasa de rábano picante y anticuerpos policlonales de cabra dirigidos contra la tóxina B conjugados con peroxidasa de rábano picante. En el ensayo, una alícuota de espécimen fecal es emulsificado en el *Diluyente* y el espécimen diluido es entonces transferido a un pocillo de mitrotitulación que contiene el antígeno detector. Si las toxinas A y B están presentes en el espécimen, se unirán al anticuerpo detector y al anticuerpo policlonal inmovilizado durante la fase de incubación. Cualquier material no enlazado es removido durante los pasos de lavado. Siguiendo la adición del substrato, se puede detectar un color debido a los complejos de enzima-anticuerpo-antígeno que se forman en presencia de la toxina.

MATERIALES PROVISTOS

Diluyente, 40 mL, solución tampón proteica con 0.02% de timerosal. El Diluyente también

Conjugado, 7.0 mL, anticuerpos monoclonales de ratón específicos para toxina A acoplados con peroxidasa de rábano picante y anticuerpos policlonales de cabra específicos para toxina B acoplados con peroxidasa de rábano picante, en una solución tampón proteica con 0.02% de timerosal

Substrato, 14.0 mL (solución que contiene tetrametilbenzidina y peróxido)

Reactivo de Control Positivo, 3.5 mL (toxinas inactivadas en una solución tampón proteica con 0.02% de Timerosal).

Tampón de lavado concentrado 20X, 50 mL (concentrado 20X conteniendo solución tampón fosfato salina, detergente y 0.2% de timerosal).

Solución de Parada, 7.0 mL (ácido sulfúrico 0.6 N) PRECAUCIÓN: evite el contacto con la piel. En caso de contacto, lave inmediatamente con agua.

Placa para microanálisis, cada tira consiste de 8 pocillos recubiertos con anticuerpos de cabra purificados por afinidad específicos contra las toxinas A y B (almacenados con desecante).

Etiqueta para la Solución de Lavado	1 etiqueta
Palillos aplicadores	50 palillos

ACCESORIOS

100 Pipetas plásticas desechables
2 Hojas plásticas adhesivas

MATERIALES Y EQUIPOS REQUERIDOS PERO NO PROPORCIONADOS

Piceta para el agente de lavado	*Cronómetro*
Papel absorbente	*Contenedor para desechos*
Agua destilada	Tubos para dilución de muestra

Espectrofotómetro capaz de leer en longitud de onda dual a 450/620nm o 450/550nm, o longitud de onda única a 450nm (se recomienda un lector de longitud de onda dual; las absorbancias deben ser medidas a 450nm y referidas a 620nm o 550nm)

Mezclador vortex

Refrigerador ajustado entre 2°-8°C *Incubadora* ajustada a 37° C ± 2° C

DURACIÓN Y ALMACENAJE

La fecha de caducidad del kit está indicada en la etiqueta. Las fechas de expiración para cada componente están listadas en etiquetas individuales. El kit que contiene los reactivos con duración designada, debe ser almacenado entre 2° y 8° C y deberá ser regresado al refrigerador tan pronto como sea posible después de su uso.

PRECAUCIONES

1. No deben mezclarse los reactivos de kits diferentes. No use un kit pasada su fecha de caducidad.
2. Los reactivos deben estar a temperatura ambiente antes de usarse.
3. Las tapas y puntas están codificadas con colores; ¡No las mezcle!
4. Cuando maneje los pocillos de ensayo, evite raspar el fondo de los pocillos porque esto puede resultar en lecturas de absorbancia elevadas.
5. Mantenga los viales goteros en forma vertical para asegurar un tamaño de gota apropiado.
6. Maneje los especímenes y pocillos de microtitulación como si fueran capaces de trasmitir agentes infecciosos. Use guantes cuando realice la prueba.
7. Los Reactivos contienen 0.02% de timerosal como preservante y deben ser manejados con la precaución normal de laboratorio.
8. La *Solución de Parada* contiene ácido sulfúrico 0.6N. En caso de contacto, lave inmediatamente con agua.
9. *Los pocillos de ensayo sin utilizar deben ser puestos nuevamente dentro de la bolsa resellable con el desecante para protegerlas de la humedad.*
10. Realice el procedimiento de lavado tal y como se indica para evitar altas reacciones de fondo.
11. Use especímenes fecales dentro de las 24 horas de su recolección para obtener resultados óptimos. Los especímenes congelados (-20°C o menos) pueden perder

actividad debido al congelamiento y descongelación.

12. El Substrato es sensible a la luz y debe ser protegido de la radiación solar directa o de fuentes de radiación ultravioleta.
13. Resultados óptimos se obtienen al seguir los procedimientos de la prueba aqui especificados. Las concentraciones, condiciones de incubación, y especificaciones de procesamiento han sido optimizadas para sensibilidad y especificidad. Alteraciones del procedimiento especificado o de las condiciones de la prueba pueden afectar la sensibilidad y especificidad de ésta.

RECOLECCIÓN Y MANEJO DE LOS ESPECIMENES FECALES

NOTA: Los procedimientos estándares de recolección y manejo para especímenes fecales son apropiados. ***Los especímenes deberán ser transportados y diluídos en el diluyente del kit tan pronto como sea posible.*** *Los especímenes deben ser almacenados entre 2º y 8ºC. Si es posible, los especímenes de prueba deberán tener menos de 24 horas desde su recolección. Almacene los especímenes a - 20ºC, o menos, si la prueba no puede ser ejecutada dentro de las siguientes 72 horas de recolección (11). El congelamiento y descongelación de un espécimen, especialmente en múltiples ocasiones, puede resultar en la pérdida de actividad debido a la degradación de las toxinas. No pueden usarse especímenes fecales preservados con Formalina al 10%, Merthiolate Formalina, Formalina acetato de sodio o Alcohol polivinílico. Los especímenes fecales que se encuentran en medio de transporte como Cary Blair o C&S pueden ser usados; sin embargo, estas muestras llegan diluidas correctamente 1:5 y deben ser ensayados directamente del contenedor y no debe haber diluciones posteriores en diluyente (11). Asegúrese de que los especímenes estén completamente mezclados (vortex) antes de ejecutar el ensayo. Esto incluye un mezclado completo (vortex) del espécimen antes de transferirlo al diluyente al igual que se debe realizar un mezclado completo del espécimen diluido antes de transferir al pocillos de microtitulación. El diluyente ha sido formulado para estabilizar y minimizar la degradación de las toxinas en los especímenes fecales. Las pipetas desechables que se incluyen en los accesorios del kit están graduadas a 50, 100, 200 y 300 µL. Los especímenes pueden ser diluídos en el instrumento opcional STOOL-PREP ™ (# cat. T9015, ver instrucciones para uso) y almacenados entre 2º y 8ºC.*

1. Prepare un tubo de dilución por cada espécimen a examinar. Añada 200 µL de *Diluyente* a cada tubo. *Etiquete el tubo por un costado.*
2. Para **especímenes fecales formadas,** use un palillo aplicador para transferir el espécimen fecal al tubo. Transfiera una cantidad aproximadamente igual a 3mm en diámetro con el palillo aplicador al *Diluyente*. Para **especímenes fecales líquidas**, use una pipeta plástica para transferir 50 µL del espécimen al tubo. Asegúrese de que los especímenes líquidos estén uniformemente suspendidos antes de transferirlos.
3. Mezcle los tubos con vortex por 10 segundos y guarde entre 2º y 8ºC hasta que la prueba de ELISA se realice (dentro de las siguientes 72 horas de recolección). Agite nuevamente antes de transferir el espécimen diluido al pocillo de microtitulación. Esto asegura una correcta mezcla del espécimen.
4. Equipos de lavado semi-automáticos pueden ser usados con especímenes que han sido centrifugados (5000 x g por 10 minutos) para remover material particuladas.

PREPARACIONES PRELIMINARES

1. **IMPORTANTE**: Los contenidos del kit deben ser llevados a temperatura ambiente previo a su uso.
2. Prepare la *Solución de Lavado* 1X. El *Tampón de lavado concentrado 20X* es provisto como un concentrado al 20X (se puede observar un precipitado). Se debe diluir hasta aforar a un volumen de 1 litro, por adición de 50 mL del concentrado a 950 mL de agua destilada. Etiquete la botella. Almacene cualquier solución de lavado 1X no usada entre 2º y 8ºC.
3. **Prepare la tira de ensayo**. Cada tira contiene 8 pocillos recubiertos con anticuerpos policlonales purificados por afinidad específicos contra toxinas A y B. Cada espécimen o control requerirá uno de estos pocillos recubiertos. Determine el número de pocillos a utilizar. Evite el contacto con la base de los pocillos. Los pocilllos de ensayo no utilizados deben ser regresados a la bolsa plástica y cuidadosamente resellados con desecante.

PROCEDIMIENTO DE LA PRUEBA

1. Añada 1 gota (50 µL) del *Conjugado* (tapa roja) a cada pocillo. Asegúrese de sostener cada gotero en forma vertical cuando añada las gotas. Use un pocillo por cada espécimen fecal, un pocillo para el control positivo y uno para el negativo (p.e. *Diluyente*). Marcas de identificación pueden escribirse directamente en un costado del pocillo.
2. Transfiera 100 µL (2 gotas usando una pipeta de transferencia del kit accesorio) del espécimen diluido en el pozo de ensayo; o si está usando un instrumento *STOOL-PREP*™, agregue el espécimen diluido de acuerdo a las instrucciones del instrumento *STOOL-PREP*™. Añada 1 gota (50 µL) del control positivo (tapa negra) al pocillo de control positivo y 50 µL (1 gota usando pipeta de transferencia del del kit accesorio) de control negativo (p.e. *Diluyente*) al pozo del control negativo.
3. Corte la hoja adhesiva plástica al tamaño necesario para cubrir los pocillos. Cubra los pocillos e incúbelos a 37° C ± 2°C por 50 minutos.
4. Elimine los contenidos de los pocillos de ensayo mediante una inversión rápida dentro de un recipiente de desecho.
5. Lave cada pocillo usando la *Solución de Lavado* 1X en una piceta con punta fina, dirigiendo con fuerza la solución de lavado hacia el fondo del pocillo. Llene los pocillos, luego elimine la solución de lavado mediante inversión rápida dentro del recipiente de desecho. Golpee la placa invertida sobre un papel absorbente seco y repita los pasos #4 y #5 cuatro veces cada vez usando papel seco. Si se observa material particulado dentro de los pocillos, continúe los lavados hasta que haya sido eliminado. **Nota**: si está usando un equipo de lavado automático o semi-automático, los especímenes deben ser centrifugados (5000 x g por 10 minutos) para remover cualquier material particulado. Agregue 350 µL de la solución de lavado 1X a cada pocillo. Lave un total de 5 veces. Si utiliza los equipos optativos *STOOL-PREP* ™, lave la placa a mano una vez, golpenado fuertemente la placa invertida sobre toallas de papel secas, y luego continue con 4 lavados en una maquina de lavado automática para un total de 5 lavados. Si se observa cualquier material particulado en los pocillos, continúe lavando hasta que todo material particulado sea eliminado.
6. Después del lavado, elimine completamente cualquier líquido residual en los pozos golpeando la placa una vez mas sobre papel absorbente hasta que no salga líquido. *Deseche el papel y los contenedores con especimenes de forma apropiada.*
7. Añada 2 gotas (100 µL) del *Substrato* (tapa azul) a cada pozo. Golpee suavemente los pocillos para mezclar el substrato. Incube los pocillos a temperatura ambiente por 10 minutos. Golpeando suavemente a los 5 minutos.
8. Agregue 1 gota (50 µL) de la *Solución de Parada* (tapa amarilla) a cada pocillo. Golpee los pocillos suavemente y espere 2 minutos antes de leer. La adición de la *Solución de Parada* convierte el color azul en color amarillo el cual puede ser cuantificado midiendo la densidad óptica a 450 nm en un lector para microplacas de ELISA. En el instrumento se debe ajustar el blanco con aire. Si utiliza un lector de doble haz, ajuste el blanco con aire a 620 o 550 nm y lea a 450 nm. Limpie la parte de abajo de cada placa antes de medir la densidad óptica. Si un lector de ELISA no está disponible, el examen puede ser leído visualmente con buena luz contra un fondo blanco. Lea dentro de los diez minutos tras la adición de la *Solución de Parada*.

PROCEDIMIENTOS ALTERNATIVO DE PRUEBA/ FORMATO RÁPIDO

Ejecute el procedimiento regular de prueba de acuerdo a las instrucciones anteriores, reemplazando los 50 minutos de incubación a 37°C ± 2° C por 20 minutos a 32°C usando el *incubador/agitador Stat Fax 2200* o un incubador/agitador equivalente. Si el *incubador/ agitador Stat Fax 2200* es usado, fije el agitador a velocidad 7 y la temperatura a 37°C. Si se utilizan otros agitadores, se recomienda una velocidad de 1500 rpm. La agitación no debe causar derrames. Si el derrame ocurre, reduzca la velocidad adecuadamente (*11*).

CONTROL DE CALIDAD

1. Un control positivo y negativo deben ser corridos con cada serie de especimenes de prueba.
2. Los controles positivo y negativo deben caer en sus respectivos rangos o la prueba no será válida.

a) El control positivo debe dar un color visible amarillo. Si se lee en un espectrofotómetro, la DO a 450 nm o usando una longitud de onda dual 450/620 nm, o 450/550 nm debe ser ≥0.500.

b) El control negativo debe ser visualmente transparente. Si se lee en un espectrofotómetro, la DO a 450 nm debe ser <0.120. Si se lee a 450/620 nm, o 450/550 nm la absorbancia debe ser <0.080.

3. A los pocillos que son visualmente transparentes pero que dan absorbancia ≥0.120 se les debe limpiar el fondo y realizar nuevamente la lectura.
4. Las lecturas visuales deben ser tomadas con buena luz contra un fondo blanco.
5. Una muestra que arroja un resultado débil positivo (p.e. <0.200) y está junto a un pozo positivo intenso debe ser repetido para asegurarse de que no ocurrió un traspaso.

INTERPRETACIÓN DE RESULTADOS

1. **Interpretación Visual**
   Negativa: Incolora
   Positiva: Cualquier color amarillo
2. **Longitud de Onda Única Espectrofotométrica a 450 nm**
   Negativa= DO <0.120
   Positiva= DO ≥0.120
3. **Longitud de Onda Dual Espectrofotométrica a 450/620 nm o 450/550 nm**
   Negativa= DO <0.080
   Positiva= DO ≥0.080

Un resultado positivo indica que la toxina A y/o B de *C. difficile* está presente en el espécimen fecal.

LIMITACIONES DE LA PRUEBA TOX A/B II DE *C. DIFFICILE*.

1. La prueba TOX A/B II de *C. difficile* se usa para detectar la toxina de *C. difficile* en especímenes fecales. La prueba confirma la presencia de toxinas en heces y esta información debe ser tomada en consideración por un médico a la luz de historia clínica del paciente. La ausencia de detección de las toxina A o B en especimenes fecales de pacientes sospechosos de tener la enfermedad causada por *C. difficile*, no elimina la presencia de la enfermedad sino que puede deberse a otros factores. (p.e. incorrecta recolección de muestra, incorrecto manejo o almacenamiento, niveles de toxina inferiores a los límites de detección de la prueba). La prueba *C. DIFFICILE TOX A/B* II es capaz de detectar la Toxina A en niveles sobre ≥0.8 ng/mL y la toxina B en niveles sobre ≥2.5 ng/mL.
2. Los especímenes fecales representan un espécimen clínico extremadamente complejo. Los resultados óptimos con la prueba TOX A/B II de *C. difficile* son obtenidos con especímenes de menos de 24 horas. La mayoría de los especímenes pueden ser almacenados entre 2° y 8° C por 72 horas antes que se observe una significativa degradación de la toxina. Si los especímenes no son examinados dentro de este periodo, estos pueden ser congelados y descongelados. Sin embargo, congelar y descongelar, especialmente en múltiples ocasiones, puede causar que los especímenes pierdan su actividad debido al a degradación de las toxinas.
3. Algunos especímenes pueden dar reacciones débiles. Esto se puede deber a numerosos factores como la presencia de una cepa débilmente toxigénica, niveles bajos de producción de toxina *in vivo*, o la presencia de sustancias enlazantes o enzimas inactivantes en las heces. *Bajo estas condiciones, el espécimen debe ser reexaminado o una muestra fresca debe ser examinada.* Los exámenes adicionales que pueden ser usados en conjunto con la prueba TOX A/B II de *C. difficile* incluyen el aislamiento del microorganismo en medios selectivos, ensayo de aglutinación en látex para la detección de *C. difficile* (el microorganismo), o ensayo de citotoxicidad en cultivo de tejido para la detección de la toxina de *C. difficile*. El aislamiento del microorganismo no confirma que sea toxigénico. Esto debe ser confirmado por pruebas adicionales en la cepa aislada usando la prueba de ELISA o el ensayo de cultivo de tejido para demostrar toxigenicidad. Asimismo, el ensayo de aglutinación de látex, el cual detecta la proteína no tóxica de *C. difficile* no demuestra la presencia de *C. difficile* toxigénica.
4. Ciertas cepas de *Clostridium sordellii* producen toxinas que son similares en sus

propiedades biológicas, fisicoquímicas e inmunológicas a las toxinas de *C. difficile*. Sin embargo, estas cepas no han sido detectados en pacientes con diarrea asociada a antibióticos o colitis.

5. No se pueden usar especímenes fecales que han sido preservados en Formalina al 10%, Merthiolate Formalina, Formalina acetato de sodio o Alcohol polivinílico. Los especímenes de heces que están en medios de transporte como Cary Blair, o C&S pueden ser usados; pero, estas muestras llegan con la dilución correcta (1:5) y deben ser examinadas directamente del contenedor y no ser diluidas en el diluyente (11).
6. Las características de ejecución de la prueba TOX A/B II de *C. difficile* no han sido completamente establecidas en la población pediátrica.

VALORES ESPERADOS

La prevalencia de una prueba positiva TOX A/B TEST de *C. difficile* más cultivo de tejido positivo y/o cultivo toxigénico fue de 5.4% en un estudio y de 8.6% en otro estudio. La prevalencia variará de acuerdo a la locación y los hospitales pueden experimentar tasas más bajas o más altas que las observadas en los sitios de evaluación de la prueba TOX A/B II de *C. difficile*. La enfermedad por *Clostridium difficile* es primariamente nosocomial en pacientes ancianos, y los hospitales que tienen una gran cantidad de pacientes ancianos pueden observar una tasa alta. Es importante considerar cualquier resultado junto con los síntomas clínicos porque algunos adultos sanos y un gran número de niños sanos (cerca del 50%) serán positivos para la toxina de *C. difficile* (toxina-positivo tanto por cultivo de tejido como por ELISA). Además, tasas de portadores de *C. difficile* de entre 22% a 32% han sido reportadas en pacientes con fibrosis quística (*12-14*).

CARACTERÍSTICAS DE EJECUCIÓN

Evaluación clínica

La prueba TOX A/B TEST de *C. difficile* fue comparada con la prueba de cultivo de tejido en cuatro hospitales de E.U.A. e internamente en TECHLAB®. Los especímenes incluidos en la evaluación fueron entregados a los laboratorios clínicos para examen de rutina. Las pruebas de cultivo de tejido fueron realizadas de acuerdo al procedimiento interno. La tabla 1 muestra una comparación de la prueba TOX A/B TEST de *C. difficile* con el cultivo de tejido. De los 1,152 especímenes incluidos en la evaluación, aproximadamente 3.6% eran de niños ≤ 2 años de edad. No se identificaron especímenes con la prueba TOX A/B TEST de *C. difficile* (+)/ cultivo de tejido (-). En general, la sensibilidad de la prueba TOX A/B TEST de *C. difficile* varió entre un 83.3% a un 96% (intervalo de confianza de 87.4 a 95.0, p= 0.05). La especificidad fue de 100% en todos los estudios. El valor predictivo positivo fue de 100% en todos los estudios, el valor predictivo negativo varió de 90% a 99.5% (intervalo de confianza de 93.8 a 99.8, p=0.05). La correlación varió desde 94.9% a 99.5% (intervalo de confianza de 99.6 a 99.4, p= 0.05).

Tabla 1 Correlación de la prueba TOX A/B TEST de *C. difficile* con el cultivo de tejidos (n= 1,152).

		Cultivo de Tejidos	
		positivo	**negativo**
C. DIFFICILE TOX A/B TEST	**positivo**	**165**	**0**
	negativo	**14**	**973**

Sensibilidad 92.2% Valor predictivo positivo 100%
Especificidad 100% Valor predictivo negativo 98.6%
Correlación 98.8%

Comparación interna

En un estudio realizado internamente la prueba TOX A/B II de *C. difficile* fue comparada con la prueba TOX A/B TEST de *C. difficile*. En el estudio, 218 especímenes clínicos representativos de los entregados rutinariamente en el laboratorio clínico fueron evaluados para *C. difficile*. Los resultados obtenidos con ambos exámenes fueron comparables.

Centrifugación

Un total de 337 especímenes fecales, incluyendo 30 positivos y 307 negativos, fueron evaluados para determinar el efecto de la centrifugación en la ejecución. Para el análisis, los especímenes fueron diluidos y mezclados (vortex) como se describió en el inserto del paquete. Los especímenes fueron centrifugados (5,000 x g) para remover los materiales insolubles y el fluido sobrenadante fue examinado en la prueba TOX A/B TEST de *C. difficile*. Los resultados fueron comparados con los resultados obtenidos con el mismo panel de especímenes diluidos y mezclados que no habían sido centrifugados. Los resultados demostraron una correlación de 100% entre los especímenes centrifugados y no centrifugados.

REACCIONES CRUZADAS

Se examinaron varios organismos para determinar su capacidad de reacción cruzada en la prueba TOX A/B TEST de *C. difficile*. Para el análisis se evaluaron caldos de cultivo mezclados con *Diluyente*. Los caldos de cultivo fueron usados en una fase logarítmica que contenía $>10^8$ bacterias por mL. Una lista de los organismos que no reaccionaron bajo ninguna de las condiciones se muestra en la tabla 2. Los únicos microorganismos que reaccionaron fueron *C. difficile* toxigénico y una cepa toxigénica de *C. sordellii* (VPI cepa 9048) la cual produce toxinas que reaccionan significativamente en forma cruzada con *C. difficile*. Una cepa no toxigénica de *C. sordellii* que no produce toxina HT fue negativa en la prueba. *C. sordellii* no ha sido implicada en la colitis pseudomembranosa o diarrea asociada con antibióticos. Las cepas de *C. difficile* toxigénica que fueron analizadas incluían seis cepas toxigénicas que variaron de toxicidad débil a fuerte y dos cepas no toxigénicas. La prueba TOX A/B TEST de *C. difficile* detectó seis de las cepas toxigénicas y no reaccionó con las cepas no toxigénicas.

Tabla 2. Microorganismos que no reaccionaron en la prueba TOX A/B TEST de *C. difficile*

Aeromonas hydrophila	*Clostridium sporogenes*
Acinetobacter lwoffi	*Clostridium tetani*
Bacillus cereus	*Enterococcus faecalis*
Bacillus subtilis	*Escherichia coli*
Bacteroides fragilis (toxigenic/nontoxigenic)	*Escherichia coli* (enterohemmorrhagic)
Candida albicans	*Klebsiella pneumoniae*
Candida krusei	*Peptostreptococcus anaerobius*
Candida tropicalis	*Proteus vulgaris*
Clostridium bifermentans	*Pseudomonas aeruginosa*
Clostridium botulinum (Types A-G)	*Salmonella choleraesuis*
Clostridium chauvoei	*Salmonella enteritidis*
Clostridium difficile (nontoxigenic)	*Salmonella typhimurium*
Clostridium haemolyticum	*Shigella flexneri*
Clostridium histolyticum	*Shigella sonnei*
Clostridium novyi (Types A, B, C)	*Staphylococcus aureus* (Protein A-negative)
Clostridium perfringens (Types A-E)	*Staphylococcus aureus* (Protein A-positive @ $<10^8$)
Clostridium septicum	*Streptococcus pyogenes*
Clostridium sordellii (nontoxigenic)	*Vibrio parahaemolyticus*
Clostridium spiroforme	*Yersinia entercolitica*

EFECTOS DE LA CONSISTENCIA DE LOS ESPECIMENES FECALES

Las recciones de fecas de distintas consistencias en la prueba TOX A/B de *C. difficile* y cultivos de tejidos se muestran en la tabla 3. Las tasas de reacciones positivas fueron muy similares en los tres tipos de especímenes fecales. Todos los especímenes fueron enviados para examen de *C. difficile* en base a la historia clínica del paciente y no la consistencia del espécimen. En estudios adicionales, las toxinas A y B altamente purificadas fueron usadas para inocular especímenes líquidos, semisólidos y sólidos.

Tabla 3. Efecto de la consistencia de los especímenes.

Prueba	especímenes líquidas	especímenes semi-sólidas	especímenes sólidas
Número de especímenes (n=435)	150	133	152
Prueba TOX A/B de *C. difficile* positiva	13 (8.7%)	11 (8.3%)	13 (8.6%)
Cultivo de tejidos positivo	13 (8.7%)	14 (10.5%)	15 (9.9%)

La prueba TOX A/B TEST de *C. difficile* detectó las toxinas A y B en especímenes líquidas, semisólidas y sólidas a niveles similares de los observados con las toxinas A y B preparadas en el diluyente del kit.

REPRODUCIBILIDAD Y PRECISIÓN

Cinco especímenes fecales (un espécimen negativo y cuatro especímenes positivos) fueron enviados a cuatro laboratorios independientes para su análisis usando la prueba TOX A/B TEST de *C. difficile*. Todos los especímenes fueron mantenidos congelados hasta la ejecución del ensayo. Los resultados de cada laboratorio fueron comparados con los resultados internos, obteniéndose resultados idénticos. Los cuatro especímenes positivos fueron confirmados positivos y el espécimen negativo fue confirmado negativo en cada lugar.

El coeficiente de variación (CV) intra-ensayos de la prueba TOX A/B TEST de *C. difficile* fue determinado por análisis de 32 reacciones de control positivo y 32 de control negativos, junto con 8 especímenes fecales negativos. Cada espécimen fecal fue examinado en 11 pozos. El % CV intra-ensayos fue de 7.190 con el control positivo, 6.557 con el control negativo, y 9.697 con los especímenes fecales. El CV interensayos fue determinado usando cuatro especímenes fecales positivos y uno negativo, probados a tiempos de 0, 24, 48 y 72 horas. El % de CV varió desde 9.9 hasta 29.6, con un promedio de 16.3.

C. DIFFICILE TOX A/B II ™ - DEUTSCH

ANWENDUNGSZWECK

C. DIFFICILE TOX A/B II ™ ist eine Enzym-Immun-Analyse zum Nachweis der Toxine A und B, die von den toxigenen Arten des *Clostridium difficile* gebildet werden. Der TOX A/B TEST weist in Stuhlproben die *Clostridium difficile*-Toxine A und B bei Patienten mit Verdacht auf eine *C. difficile* assoziierte Durchfallerkrankung nach. Der Test sollte als Hilfsmittel zur Diagnose einer *C. difficile* Erkrankung benutzt werden, und die Resultate sollten in Zusammenhang mit der Krankheitsgeschichte des Patienten interpretiert werden. NUR FÜR *IN VITRO* DIAGNOSEZWECKE.

ERLÄUTERUNGEN

Nach einer Antibiotika-Behandlung entwickeln viele Patienten Magen-Darm-Beschwerden, die von mildem Durchfall bis zu schwerer pseudomembranöser Kolitis reichen. Viele der Durchfallerkrankungen und die meisten Fälle von pseudomembranöser Kolitis werden von *Clostridium difficile* verursacht (1). Dieser Organismus ist ein anärobes Bakterium, das sich in der Darmflora ansiedelt, sobald die normale Darmflora von den Antibiotika verändert wurde. Die Erkrankungen werden von den Toxinen verursacht, die dieser Organismus produziert. Die klinischen Symptome, die mit der Krankheit in Verbindung gebracht werden, werden nach allgemeiner Auffassung hauptsächlich durch das Toxin A, ein gewebeschädigendes Enterotoxin (2,3) verursacht. *Clostridium difficile* produziert ein weiteres Toxin, das als Toxin B bekannt ist. Toxin B, das auch als Cytotoxin des Organismus bezeichnet wird, ist dasjenige Toxin, das derzeit von den meisten laborüblichen Gewebeanalysen nachgewiesen wird. Die meisten Arten produzieren entweder beide Toxine oder keine der beiden Toxine, aber erst kürzlich wurden Toxin A negative/Toxin B positive Arten identifiziert (4,5). Diese Toxin A negativen/Toxin B positiven Arten testen positiv im *C. DIFFICILE TOX A/B II* ™ Test (6,7).

Clostridium difficile Arten, die grosse Mengen Toxin-A produzieren, bilden auch grosse Mengen von Toxin B. Genauso produzieren Arten, die nur wenig Toxin A bilden, auch nur wenig Toxin B, was darauf hinweist, dass die Toxin-Bildung für beide Varianten ähnlich reguliert wird. Für die Diagnose werden Tests benutzt, die entweder eines der Toxine oder beide nachweisen. Die Toxin-Gene wurden geklont und genetisch analysiert, und einige der Toxineigenschaften sind nun gut definiert (8,9). Beide Toxine sind relativ gross (M_r *des Toxin A, 308.000;* M_r *des Toxin B, 279.000*). Toxin A weisen eine komplexe Folge von sich wiederholenden Einheiten am COOH-Ende des Moleküls auf, und diese wiederholenden Einheiten dienen höchstwahrscheinlich als Bindeglied, das Galaktose-haltige Rezeptoren erkennen kann (10). Es gibt Anzeichen dafür, dass die beiden Toxine in Synergie agieren, und dass die von Toxin A verursachten Gewebeschäden dem Toxin B erlauben, ebenfalls aktiv zu werden. Obwohl Toxin A für die meisten klinischen Symptome verantwortlich gemacht wird, kann darum Toxin B ebenfalls eine bedeutende Rolle im Krankheitsverlauf spielen.

WIRKUNGSWEISE DES TESTS

Der *C. DIFFICILE TOX A/B II* ™ Test benutzt Antikörper gegen die *Clostridium difficile* Toxine A und B. Die Mikrotiterstreifen sind mit affinitätschromatographisch aufgereinigten polyklonalen Antikörpern (Ziege), die gegen die Toxine A und B gerichtet sind, beschichtet. Das Konjugat besteht aus einem monoklonalen Meerrettich-Peroxidase-markierten Antikörper (Maus) gegen Toxin A und einem polyklonalen Antikörper gegen Toxin B. In der Analyse wird eine kleine Menge einer Stuhlprobe im *Verdünnungspuffer* aufgelöst und dann in die Mikrotiter-Schalen übertragen, die Antikörper enthalten. Wenn die Toxine A oder B in der Stuhlprobe vorhanden sind, dann werden sie während der Inkubationsphase an die Antikörper und die polyklonalen immobilisierten Antikörper gebunden. Nicht gebundene Bestandteile werden dann weggewaschen. Daraufhin wird Substrat hinzugefügt, und gebundenes Enzymkonjugat in den Vertiefungen wandelt das farblose Substrat in ein blaues Endprodukt um.

PACKUNGSINHALT

Verdünnungspuffer, 40 mL, gepufferte Proteinlösung + 0,02% Thimerosal. Das *Verdünnungspuffer* kann auch als negatives Kontrollmittel benutzt werden (siehe TESTVERFAHREN).

Konjugat, 7 mL, monoklonale Antikörper (Maus) speziell für Toxin A gebunden an Meerrettich-Peroxydase und polylonale Antikörper (Ziege) speziell für Toxin B gebunden an Meerrettich-Peroxydase in einer gepufferten Proteinlösung + 0,02% Thimerosal

Substrat, 14 mL, Lösung enthält Tetramethylbenzidine and Peroxyde

Positive Kontrolle, 3,5 mL, inaktivierte Toxine in einer gepufferten Proteinlösung

Waschpufferkonzentrat, 50 mL, 20X Konzentrat, enthält Kochsalzlösung, Reinigungsmittel und 0,2% Thimerosal)

Stopplösung, 7 mL, 0,6N Schwefelsäure. ACHTUNG: Hautkontakt vermeiden sofort mit Wasser spülen, wenn Hautkontakt erfolgt.

Microanalyse-Platte, 12 streifen, jeder Streifen enthält 8 Schalenvertiefungen, die mit affinitätschromotographischen aufgereinigten Ziegen-Antikörpern für Toxine A und B beschichtet sind (mit Trockenmittel gelagert)

Reinigungsflüssigkeits-Etikette 1 Etikette

Holzstäbchen 50 Stäbchen

ZUBEHÖR

Einmal-Pipetten aus Plastik	100 Pipetten
Klebefolien	2 Folien

NICHT ENTHALTENE MATERIALIEN

Spritzflasche	*Timer*	*Vortex Schüttler*
Abfallbehälter	*Destilliertes Wasser*	*Geeichte Zylinder*

Papiertücher und saugfähiges Material

Reagenzgläser zur Verdünnung der Proben

Spektrophotometer mit dualer Lesekapazität für 450/620 nm oder singuläre Wellenlänge für 450 nm (ein duales Gerät sollte bei 450 nm abgelesen und mit 620 nm abgeglichen werden)

Kühlschrank, eingestellt auf 2° bis 8°C

Inkubator, eingestellt auf 37°C ± 2°C

HALTBARKEIT UND LAGERUNG

Das Verfalldatum ist auf dem Packungsetikett angegeben. Verfalldaten für die einzelnen Komponenten sind auf den Komponentenetiketten angegeben. Das Analyse-Set mit den verfallbaren Reagenzien sollte bei Temperaturen zwischen 2° und 8°C gelagert werden, und sollte so schnell wie möglich nach dem Gebrauch wieder in den Kühlschrank zurückgelegt werden.

VORSICHTSMASSNAHMEN

1. Reagenzien von verschiedenen Sets dürfen nicht gemischt werden. Benutzen Sie das Analyse-Set nicht nach dem Verfallsdatum.
2. Reagenzien sollten Raumtemperatur haben bevor sie verwendet werden.
3. Verschlüsse und Fläschchen sind farbkodiert; nicht vertauschen!
4. Vermeiden Sie, den Boden der Analyse-Schalen zu zerkratzen, sonst können erhöhte Absorbierungswerte resultieren.
5. Halten Sie die Tropfflaschen senkrecht damit die korrekte Tropfengröße sichergestellt wird.
6. Microtiter-Schalen und Stuhlproben sollten nach dem Gebrauch wie potentiell infektiöser Abfall behandelt und entsorgt werden. Tragen Sie immer Handschuhe während Sie den Test ausführen.
7. Reagenzien enthalten 0,02% Thimerosal als Konservierungsmittel und sollten mit der in Labors üblichen Vorsicht behandelt werden.
8. Die *Stopplösung* enthält 0,6 N Schwefelsäure. Spülen Sie sofort mit Wasser, wenn Hautkontakt erfolgen sollte.
9. Unbenutzte Miktotiter Analyse-Schalen müssen mit dem Trockenmittel in den wiederverschließbaren Plastikbeutel zurückgelegt werden, damit sie vor Feuchtigkeit geschützt sind.
10. Waschen Sie die Schalen wie vorgeschrieben um hohe Hintergrundreaktionen zu vermeiden.

11. Analysieren Sie die Stuhlproben innerhalb von 24 Stunden nach der Entnahme, um optimale Testergebnisse zu erzielen. Gefrorene Proben (-20°C oder niedriger) können aufgrund des Einfrierens und Auftauens Aktivitätsverluste aufweisen.
12. Das *Substrat* ist lichtempfindlich und sollte vor direkter Sonneneinstrahlung oder UV-Lichtquellen geschützt werden.
13. Optimale Ergebnisse werden erreicht, wenn die angegebenen Testverfahren eingehalten werden. Konzentrationen, Inkubationsbedingungen, und Verfahrensdetails wurden in Bezug auf Sensibilität und Genauigkeit optimiert. Abweichungen von den angebenen Verfahren und/oder Testbedingungen können die Sensibilität und die Genauigkeit des Tests beeinflussen.

ENTNAHME UND LAGERUNG DER STUHLPROBEN

Die üblichen Methoden für Abnahme und Behandlung der Stuhlproben sind akzeptabel. ***Stuhlproben sollten so schnell wie möglich in das Verdünnungspuffer gegeben werden.*** Stuhlproben sollten bei Temperaturen zwischen 2° und 8°C gelagert werden. Wenn möglich sollten die Stuhlproben weniger als 24 Stunden alt sein. Lagern Sie Stuhlproben bei Temperaturen – von 20 °C oder tiefer, wenn der Test nicht innerhalb von 72 Stunden nach der Probenentnahme durchgeführt werden kann. Mehrmaliges Einfrieren und Auftauen kann zu einem Aktivitätsverlust durch den Zerfall der Toxine führen. Stuhlproben, die in 10% Formalin, Merthiolat-Formalin, Natriumacetat-Formalin oder Polyvinylalkohol eingelegt wurden, können nicht benutzt werden. Die Stuhlproben müssen gerührt werden bevor sie ins *Verdünnungspuffer* gegeben werden und die verdünnten Proben werden nochmals durchgerührt bevor sie in die Analyse-Schalen gegeben werden. Das *Verdünnungspuffer* wurde so entwickelt, dass das Toxin in den Stuhlproben stabilisiert wird und der Abbau des Toxins minimiert wird. Einmal-Pipetten, die im Zubehör enthalten sind, sind auf 50, 100, 200 und 300 µL geeicht. Proben können wahlweise in *STOOL-PREP™* verdünnt werden (Katalognr. T9015, siehe Gebrauchsanweisung) und können zwischen 2-8 °C gelagert werden.

1. Benutzen Sie für jede zu testende Stuhlprobe ein eigenes Reagenzglas. Füllen Sie 200 µL *Verdünnungspuffer* in jedes Reagenzglas. *Kennzeichnen Sie das Reagenzglas direkt an der Seitenwand.*
2. **Für feste Stuhlproben (feste Stuhlproben von Personen ohne Durchfall sind normalerweise keine geeigneten Proben)** benutzen Sie ein Holzstäbchen um die Probe in das Reagenzglas zu übertragen. Übertragen Sie eine Probe mit etwa 3 mm Durchmesser in das *Verdünnungspuffer*. Für **flüssige Stuhlproben** benutzen Sie eine Plastikpipette um 50 µL der Probe ins Reagenzglas zu übertragen. Achten Sie darauf, dass die flüssigen Proben gleichmäßig in ihrer Konsistenz sind bevor sie übertragen werden.
3. Behandeln Sie die Reagenzgläser 10 Sekunden lang im Vortex-Schüttler und lagern Sie die Reagenzgläser anschließend bei Temperaturen zwischen 2° und 8°C bis ELISA abgeschlossen ist (innerhalb von 72 Stunden nach der Entnahme). Behandeln sie die Proben nochmals im Vortex-Schüttler bevor sie die verdünnten Proben in die Analyse-Schalen übertragen. Auf diese Weise werden die Proben gut durchgemischt.
4. Halbautomatische Waschgeräte können für Proben benutzt werden, die zentrifugiert wurden (5000 x g für 10 Minuten) um Restbestände zu entfernen.

TESTVORBEREITUNG

1. WICHTIG – Alle Reagenzien müssen Raumtemperatur haben bevor sie in der Analyse benutzt werden.
2. Bereiten Sie die 1X *Reinigungsflüssigkeit* vor. Das *Waschpufferkonzentrat* wird als 20X Konzentrat geliefert (Ausfall kann eventuell sichtbar sein). Es sollte auf ein Volumen von 1 Liter verdünnt werden, indem 50 mL des Konzentrats mit 950 mL destilliertem Wasser vermengt werden. Kennzeichnen Sie die Flasche. Lagern Sie eventuelle Restmengen der 1X *Reinigungsflüssigkeit* zwischen 2° und 8°C.
3. **Vorbereitung der Analysestreifen.** Jeder Streifen enthält 8 Schalen, die mit spezifischen affinitätschromatographisch aufgereinigten polyklonalen Antikörpern gegen Toxin A und B beschichtet sind. Jede Probe oder Kontrolle nimmt eine dieser beschichteten Schalen in Anspruch. Bestimmen Sie die Anzahl der benötigten Schalen,

Nicht benötigte Schalen werden zusammen mit dem Trockenmittel in den Plastikbeutel zurückgelegt und sorgfältig wieder verschlossen.

TESTVERFAHREN

1. Geben Sie einen Tropfen (50 µL) des *Konjugats* (roter Verschluss) in jede Schalenvertiefung. Achten Sie darauf jede Flasche senkrecht zu halten. Benutzen Sie je 1 Schalenvertiefung für jede Stuhlprobe, 1 für das *Positive Kontrollmittel* und 1 Schalenvertiefung für die Negativ-Kontrolle (z.B. *Verdünnungspuffer*). Markierungen zur Identifizierung können direkt auf die Seite der Schalenvertiefung geschrieben werden.
2. Übertragen Sie 100 µL (2 Tropfen mit der mitgelieferten Einwegpipette) der verdünnten Proben auf die Analyse-Schale; wenn Sie ein *STOOL-PREP™*-Gerät verwenden, folgen Sie den entsprechenden Geräteanweisungen. Geben Sie 1 Tropfen (50 µL) der *Positiven Kontroll Reagenzie* (schwarzer Verschluss) auf die Schalenvertiefung zur Positiv-Kontrolle und 50 µL (1 Tropfen mit der mitgelieferten Einwegpipette) der negativen Kontrollflüssigkeit (z.B. *Verdünnungspuffer)* auf die Schalenvertiefung zur Negativ-Kontrolle.
3. Schneiden Sie die Klebefolie so zu, dass das Schnittstück die Schalenvertiefungen vollständig abdeckt. Kleben Sie die Folie über die Schalenvertiefungen und inkubieren sie diese bei 37°C ± 2°C für 50 Minuten.
4. Schütteln Sie den Inhalt der Schalen in einen Abfallbehälter.
5. Waschen Sie jede Schalenvertiefung mit der 1X *Reinigungslösung* gut aus; benutzen Sie dazu eine Spritzflasche mit feiner Düse, und spritzen Sie die *Reinigungsflüssigkeit* kräftig auf den Boden der Schalenvertiefung. Füllen Sie die Schalenvertiefungen mit der *Reinigungsflüssigkeit* und entleeren Sie dann die Schalen in den Abfallbehälter. Klopfen Sie die umgedrehten Schalen auf ein trockenes Papiertuch und wiederholen Sie dann Schritte Nr.4 und Nr.5 **viermal** und benutzen Sie jedes Mal ein neues, trockenes Papiertuch. Wenn irgendwelche Restbestandteile noch in den Schalen zu sehen sind, waschen Sie diese solange aus bis alle Reste entfernt sind. **Hinweis:** Wenn halbautomatische oder automatische Reinigungsgeräte benutzt werden, müssen die Proben zentrifugiert werden (5000 x für 10 Minuten), um etwaige Restbestände zu entfernen. Geben Sie 350 µL der 1x *Reinigungsflüssigkeit* in jede Schalenvertiefung. Waschen Sie fünf Minuten lang. Wenn *STOOL-PREP™*- Geräte verwendet werden, waschen Sie die Schalen einmal von Hand, schlagen Sie die Schalen kräftig auf trockene Papiertücher und waschen Sie danach noch 4-mal mit dem automatischen Waschgerät, so dass insgesamt 5 Waschgänge erfolgen. Wenn Restbestände in den Schalen sichtbar sind, fahren Sie mit dem Waschen fort, bis sämtliche Reste entfernt sind.
6. Nach dem Waschen entfernen Sie alle Restflüssigkeit von den Schalen, indem Sie diese nochmals gegen ein trockenes Papiertuch klopfen bis keine Flüssigkeit mehr abgegeben wird. *Entsorgen Sie die Papiertücher und Stuhlproben in angemessener Weise.*
7. Geben Sie 2 Tropfen (100 µL) des *Substrats* (blauer Verschluss) in jede Schalenvertiefung. Inkubieren Sie die Schalen bei Raumtemperatur für 10 Minuten. Klopfen Sie nach 5 Minuten leicht an die Schalen.
8. Geben Sie 1 Tropfen (50 µL) der *Stopplösung* (gelber Verschluss) in jede Schalenvertiefung. Klopfen Sie leicht an die Analyse-Schalen und warten Sie 2 Minuten bis zum Ablesen. Hinzufügen der *Stopplösung* konvertiert die blaue Farbe in gelbe Farbe; die Farbveränderungen werden quantifiziert, indem die optische Dichte bei 450 nm auf einem ELISA Mikroplattenleser gemessen wird; Vergleichswerte sind 620 oder 550 nm auf einem dualen Wellenlänge-Lesegerät). Der Abgleich des Nullwertes erfolgt gegen Luft. Wischen Sie die Unterseite jeder Schalenvertiefung ab bevor die optische Dichte gemessen wird. Wenn ein ELISA-Lesegerät nicht verfügbar ist, kann das Testergebnis visuell in guten Lichtverhältnissen gegen einen weißen Hintergrund abgelesen werden. Wenn ein duales Lesegerät verwendet wird, dann führen Sie den Abgleich des Nullwertes bei 620 oder 550 nm gegen Luft durch und lesen Sie bei 450 nm das Testergebnis ab. Lesen Sie innerhalb von 10 Minuten nach Zugabe der *Stopplösung* ab.

ALTERNATIVES TESTVERFAHREN/SCHNELLVERFAHREN

Führen Sie das normale Testverfahren durch, aber anstelle der 50 Minuten Inkubation bei 37°C ± 2°C, inkubieren Sie bei 37°C für 20 Minuten im *Stat Fax 2200 Inkubator/Schüttler* oder

einem gleichartigen Inkubator/Schüttler. Wenn der *Stat Fax 2200 Inkubator/Schüttler* benutzt wird, stellen Sie die Geschwindigkeit auf Stufe 7 und die Temperatur auf 37°C ein. Wenn andere Geräte benutzt werden, wird eine Geschwindigkeit von 1500 rpm (U/min) empfohlen. Schütteln darf nicht zum Überlaufen führen. Sonst reduzieren Sie die Geschwindigkeit entsprechend (11).

QUALITÄTSKONTROLLE

1. Jede Testserie muss eine positive und eine negative Kontrolle einschließen.
2. Positive und negative Kontrollen müssen innerhalb der Toleranzgrenzen liegen, sonst ist der Test nicht gültig.
   a) Positive Kontrolle muss deutlich gelb sein.
      Wenn auf einem Spektrometer abgelesen wird, dann gelten folgende Toleranzwerte:
      OD_{450} oder $OD_{450/620 \text{ oder } 450/550} \geq 0{,}500$.
   b) Negative Kontrolle muss visuell klar sein.
      Wenn auf einem Spektrometer abgelesen wird, dann muss gelten:
      $OD_{450} < 0{,}120$, oder die $OD_{450/620 \text{ oder } 450/550} < 0{,}080$.
3. Schalen die visuell klar scheinen, aber eine Absorption von $\geq 0{,}120$ ausweisen, sollten auf der Unterseite abgewischt werden und die Absorbation erneut gemessen werden.
4. Visuelle Ablesung muss bei guten Lichtverhältnissen und gegen einen weißen Hintergrund durchgeführt werden.
5. Eine Probe, die nur ein schwaches positives Testergebnis zeigt (z.B. <0,200) und die neben einer stark positiven Probe liegt, sollte erneut getestet werden, damit sicher gestellt ist, dass keine versehentliche Übertragung erfolgte.

INTERPRETATION DER TESTERGEBNISSE

1. **Visuelles Ablesen**
   Negativ = Farblos
   Positiv = Jede gelbe Farbe
2. **Spektrophotometrische singuläre Wellenlänge bei 450 nm**
   Negativ = $OD_{450} < 0{,}120$
   Positiv = $OD_{450} \geq 0{,}120$
3. **Spektrophotometrische duale Wellenlänge bei 450/620 nm oder 450/550 nm**
   Negativ = $OD_{450/620 \text{ oder } 450/550} < 0{,}080$
   Positiv = $OD_{450/620 \text{ oder } 450/550} \geq 0{,}080$

Ein positives Testergebnis zeigt an, dass *C. difficile* Toxin A oder Toxin B in der Stuhlprobe vorhanden ist.

GRENZEN DES *C. DIFFICILE TOX A/B II*™ - VERFAHRENS

1. Das *C. DIFFICILE TOX A/B II* ™ Verfahren weist *C. difficile* Toxine in Stuhlproben nach. Der Test bestätigt das Vorhandensein des Organismus. Diese Information muss vom Arzt im Hinblick auf die Krankengeschichte des Patienten interpretiert werden. Wenn bei Verdacht auf *C. difficile* Erkrankung weder Toxin A noch Toxin B in den Stuhlproben des Patienten nachgewiesen werden kann, dann schließt das die Krankheit nicht aus, sondern kann auf andere Faktoren zurückzuführen sein (d.h. inkorrekte Probenentnahme, Behandlung und/oder Lagerung der Proben, oder geringe Toxin-Werte, die unter den Nachweisgrenzwerten des Analyse-Sets liegen). Der *C. DIFFICILE TOX A/B II* ™ - Test weist Toxin A bei Werten über 0,8 ng/mL und Toxin B bei Werten über 2,5 ng/mL nach.
2. Stuhlproben sind komplex. Optimale Ergebnisse werden im *C. DIFFICILE TOX A/B II* ™ – *Verfahren* mit Proben erzielt, die weniger als 24 Stunden alt sind. Die meisten Proben können bei 2°C - 8°C für 72 Stunden gelagert werden, bevor ein deutlicher Verfall der Toxine beobachtet werden kann. Wenn die Proben nicht innerhalb dieser Zeit analysiert werden können, dann können sie eingefroren und später wieder aufgetaut werden. Allerdings kann wiederholtes Einfrieren und Auftauen, die Aktivität der Proben reduzieren, weil das die Toxine allmählich zerfallen.
3. Einige Proben können schwach reagieren, so dass der Test unschlüssig ausfällt. Das kann auf mehrere Faktoren zurückgeführt werden, z. B. auf das Vorhandensein von

bindender Substanzen oder inaktivierenden Enzymen im Stuhl. *Unter diesen Bedingungen sollten die Proben erneut getestet werden oder eine neue Stuhlprobe sollte entnommen und getestet werden.* Zusätzliche Tests können zusammen mit dem *C. DIFFICILE TOX A/B II* ™ Test ausgeführt werden, einschließlich der Isolation des Organismus auf selektiven Medien, Latex-Agglutinations-Test zum Nachweis von *C. difficile* oder Kulturanlagen für Zytotoxin-Analysen zum Nachweis des *C. difficile* Toxins. Allein die Isolation des Organismus, läßt noch nicht den Schluß auf Toxine zu. Das muss durch zusätzliche Tests des Isolats mit ELISA oder Zellkultur-Analysen gezeigt werden. Auch der Latex-Agglutinatitons-Test, der ein nicht-toxisches Protein des *C. difficile* nachweist, läßt nicht auf das Vorhandensein des toxigenen *C. difficile* schließen.

4. Bestimmte toxigene Isolate des *Clostridium sordelli* produzieren Toxine, die *in* ihren biologischen, physiochemikalischen und immunologischen Eigenschaften den Toxinen des *C. difficile* ähnlich sind. Diese Isolate werden aber nicht in Stuhlproben von Patienten mit auf Antibiotika zurückzuführenden Durchfallerkrankungen und Kolitis gefunden.
5. Stuhlproben, die in 10% Formalin, Merthiolat-Formalin, Natriumacetat-Formalin oder Polyvinylalkohol gelagert wurden, können nicht verwendet werden.
6. Das Testverhalten des *C. DIFFICILE TOX A/B II* ™ Tests ist noch nicht ausreichend für pediatrische Patienten etabliert.

ERWARTUNGSWERTE

Positive Testergebnisse mit dem *C. DIFFICILE TOX A/B TEST* plus positive Zellkulturen und/oder toxigene Kulturen wurden in einer Studie mit 5,4% beobachtet, 8,6% in einer anderen Studie. Diese Ergebnisse werden stark variieren und je nach Ort, Krankenhaus können diese Quoten höher oder niedriger ausfallen. *Clostridium difficile* Erkrankungen sind vermehrt unter älteren Patienten zu beobachten, und Krankenhäuser, die einen grossen Anteil älterer Patienten haben, können höhere Erkrankungsraten haben. Testergebnisse müssen mit klinischen Symptomen gemeinsam interpretiert werden, denn einige gesunde Erwachsene und viele gesunde Kleinkinder (bis zu 50%) werden positiv für *C. difficile* Toxine testen (ELISA oder toxin-positive Zellkulturen). Außerdem wurde *C. difficile* in Patienten mit Zystofibrose mit Quoten von 22% bis 32% festgestellt (12-14).

LEISTUNGSMERKMALE

Klinische Bewertung

Der *C. DIFFICILE TOX A/B TEST* wurde in vier U.S. Krankenhäusern und bei TECHLAB® vor Ort mit Zellkultur-Tests verglichen. Die verwendeten Proben wurden den klinischen Labors zu Routineanalysen gegeben. Zellkultur-Tests wurden nach den laborüblichen Verfahren durchgeführt. Tabelle 1 zeigt einen Vergleich des *C. DIFFICILE TOX A/B TEST* mit Gewebekulturen. Im Vergleich wurden 1152 Proben analysiert, davon etwa 3,6% von Kindern unter 2 Jahren. Keine Probe war gleichzeitig im *C. DIFFICILE TOX A/B TEST* positiv und im Zellkultur-Test negativ. Insgesamt wurde die Sensibilität des *C. DIFFICILE TOX A/B TEST* mit 83,3% bis 96% angegeben (Konfidenzintervall 87,4-95,0 %, p = 0,05). Die Spezifität war 100% in allen Studien. Der positive prädiktive Wert betrug bei allen Studien 100 %. Der negative prädiktive Wert variierte zwischen 90 % und 99,5 % (Konfidenzintervall 93,8-99,8 %, p = 0,05). Die Übereinstimmung schwankte zwischen 94,9 % und 99,5 % (Konfidenzintervall 96,6-99,4 %, p = 0,05).

TABELLE 1 Korrelation zwischen *C. DIFFICILE TOX A/B TEST* im Vergleich zum Zellkultur-Test (n=1,152)

		Zellkultur-Test	
		positiv	**negativ**
C. DIFFICILE TOX A/B TEST	**positiv**	**165**	**0**
	negativ	**14**	**973**

Sensitivität:	92,2 %	Positiver prädiktiver Wert:	100 %
Spezifität:	100 %	Negativer prädiktiver Wert:	98,6 %
Korrelation:	98,8 %		

Interner Vergleich

In einer internen Studie wurden der *C. DIFFICILE TOX A/B II* ™ Test und der *C. DIFFICILE TOX A/B TEST* verglichen. 218 der zu routinemäßigen Laboruntersuchungen zum Nachweis von *C. difficile* eingereichten Stuhlproben wurden bewertet. Die Testergebnisse mit beiden Verfahren waren vergleichbar.

Zentrifugieren

337 Stuhlproben, davon 30 positive und 307 negative Proben, wurden bewertet, um den Einfluss des Zentrifugierens zu bestimmen. Im Versuch wurden die Proben wie vorgeschrieben verdünnt und gemixt. Dann wurden die Proben zentrifugiert (5.000 x g) um Restmaterialen und Restflüssigkeiten vom *C. DIFFICILE TOX A/B TEST* zu entfernen. Die Resultate wurden verglichen mit gleichartigen Proben, die nicht zentrifugiert wurden. Eine 100% Korrelation zwischen zentrifugierten und nicht-zentrifugierten Proben wurde festgestellt.

KREUZREAKTIVITÄT

Verschiedene Organismen wurden auf Kreuzreaktivität im *C. DIFFICILE TOX A/B TEST* untersucht. Für die Analyse wurden Kulturen vermischt mit *Verdünnungspuffer* untersucht. Kulturen mit mehr $>10^8$ Bakterien pro mL wurden benutzt. Eine Liste der Organismen, die nicht reagiert haben, ist in Tabelle 2 angegeben. Nur zwei Organismen zeigten Reaktionen, das toxigene *C. difficile* und eine toxigene Art des *C. sordellii* (VPI Art 9048), das Toxine bildet, die mit den *C. difficile* Toxinen deutlich kreuzreaktiv sind. Eine nicht-toxigene Art des *C. sordellii*, das kein Toxin HT bildet, war im Test negativ. *C. sordellii* wird nicht mit pseudomembranöser Kolitis oder mit durch Antibiotika verursachten Darmerkrankungen in Verbindung gebracht. Die toxigenen Arten des *C. difficile* wurden untersucht, einschließlich 6 schwach bis stark toxigener Arten und 2 nicht-toxigenen Arten. Der *C. DIFFICILE TOX A/B TEST* wies alle toxigenen Arten nach, und reagierte nicht mit den beiden nicht-toxigenen Arten.

TABELLE 2 zeigt alle Arten, die im *C. DIFFICILE TOX A/B TEST* nicht reagierten.

Aeromonas hydrophila
Acinetobacter lwoffi
Bacillus cereus
Bacillus subtilis
Bacteroides fragilis (toxigenic/nontoxigenic)
Candida albicans
Candida krusei
Candida tropicalis
Clostridium bifermentans
Clostridium botulinum (Types A-G)
Clostridium chauvoei
Clostridium difficile (nontoxigenic)
Clostridium haemolyticum
Clostridium histolyticum
Clostridium novyi (Types A, B, C)
Clostridium perfringens (Types A-E)
Clostridium septicum
Clostridium sordellii (nontoxigenic)
Clostridium spiroforme
Clostridium sporogenes
Clostridium tetani
Enterococcus faecalis
Escherichia coli
Escherichia coli (enterohemmorrhagic)
Klebsiella pneumoniae
Peptostreptococcus anaerobius
Proteus vulgaris
Pseudomonas aeruginosa
Salmonella choleraesuis
Salmonella enteritidis
Salmonella typhimurium
Shigella flexneri
Shigella sonnei
Staphylococcus aureus (Protein A-negative)
Staphylococcus aureus (Protein A-positive @ $<10^8$)
Streptococcus pyogenes
Vibrio parahaemolyticus
Yersinia entercolitica

AUSEIRKUNGEN DER STUHL-KONSISTENZ

Wie Stuhlproben mit unterschiedlicher Konsistenz im *C. DIFFICILE TOX A/B TEST* und in Gewebekultur-Analysen reagieren, ist in Tabelle 3 dargestellt. Alle drei Typen von Stuhlproben zeigen vergleichbare Ergebnisse. Alle Proben wurden auf *C. difficile* getestet. Die Krankengeschichte des Patienten und nicht die Konsistenz der Stuhlproben bestimmte ob die Proben getestet wurden oder nicht. In anderen Studien wurden hochwertige A- und B- Toxine in Stuhlproben von flüssiger, halb-fester und fester Konsistenz gegeben.

TABELLE 3 Auswirkung der Stuhl-Konsistenz

Test	Flüssiger Stuhl	Halb-fester Stuhl	Fester Stuhl
Anzahl Proben (n=435)	150	133	152
C. DIFFICILE TOX A/B TEST positiv	13 (8,7%)	11 (8,3%)	13 (8,6%)
Gewebekultur positiv	13 (8,7%)	14 (10,5%)	15 (9,9%)

Der *C. DIFFICILE TOX A/B TEST* wies Toxine A und B in flüssigen, halb-festen und festen Stuhlproben in vergleichbaren Mengen nach, wie sie in mit Toxinen A und B versetzte *Verdünnungspuffer* nachgewiesen wurden.

REPRODUZIERBARKEIT UND VERLÄSSLICHKEIT

Fünf Stuhlproben (eine negative und vier positive Proben) wurden zu vier unabhängigen Labors zum *C. DIFFICILE TOX A/B TEST* gesandt. Alle Proben wurden bis zum Analysebeginn eingefroren. Die Laborergebnisse wurden mit den internen Ergebnissen verglichen und sie waren identisch. Die vier positiven Proben wurden in allen Tests als positiv erkannt, und die negativen Proben testeten in allen Labors negativ.

Der Intra-Analyse-Koeffizient der Variation (CV) des *C. DIFFICILE TOX A/B TEST* wurde bestimmt, indem 32 positive und 32 negative Kontrollreaktionen zusammen mit 8 negativen Proben analysiert wurden. Jede Stuhlprobe wurde in 11 Schalen untersucht. Der Intra-Analyse %CV betrug 7,190 mit der Positiv-Kontrolle, 6,557 mit der Negativ-Kontrolle, und 9,697 mit den Stuhlproben. Der Intra-Analyse CV wurde bestimmt, indem vier positive und eine negative Stuhlprobe für jeweils 0, 24, 48 und 72 Stunden getestet wurden. Der CV-% Satz reichte von 9,9 bis 29,6, mit einem durchschnittlichen Wert von 16,3.

C. DIFFICILE TOX A/B II ™ - FRANCAIS

UTILISATION PRÉVUE

C. DIFFICILE TOX A/B II ™ test est une analyse immuno-enzymatique pour la détection des toxines A et B produit par des contraintes toxigènes de *Clostridium difficile*. L'analyse peut être utilisée pour la détection des toxines A et B de *C. difficile* présentes dans la matière fécale d'une personne suspectée d'avoir la maladie associée avec *C. difficile*. L'analyse doit être utilisée pour aider le diagnostique d'une infection par *C. difficile*, et les résultats doivent être considérés en conjonction avec l'histoire médicale du patient.
USAGE DIAGNOSTIQUE *IN VITRO*.

EXPLICATION

Suivant les traitements avec antibiotiques, plusieurs patients développent des problèmes gastro-intestinaux qui peuvent produire une diarrhée modérée ou même une colite pseudomembraneuse aiguë. Nombreux cas des formes les plus tempérées des maladies gastro-intestinales et la majorité des cas de pseudomembraneuse colite sont causés par *Clostridium difficile (1)*. Cet organisme est une bacterie opportuniste anaérobique qui se multiplie dans l'intestin dès que la flore normale a été altérée par les antibiotiques. La maladie résulte des toxines que l'organisme produit. Les symptomes cliniques associés avec la maladie sont censés être provoqués principalement par la toxine A, qui endomage le tissu en tant qu' entérotoxine *(2,3)*. *Clostridium difficile* produit aussi une seconde toxine designée toxine B. Toxine B, qui peut être représentée en tant que cytotoxine de l'organisme, est la toxine détectée par l'analyse de culture de tissu qui est actuellement en pratique dans plusieurs laboratoires. La majorité des variétés produisent soit les deux toxines, soit aucunes des deux bien que, récemment, des variétés étant toxine A négative/toxine B positive ont été identifiées *(4,5)*. Ces variétés de toxine A négative/toxine B positive sont positivement détectées par l'analyse de *C. DIFFICILE TOX A/B II* ™ test *(6,7)*.

Les variétés de *Clostridium difficile* qui produisent des niveaux élevés de toxine A produisent aussi des niveaux élevés de toxine B. De la même manière, les variétés produisant des niveaux bas de toxine A produisent aussi des niveaux bas de toxine B, ce qui indique que la production de toxine peut être régulée d'une manière similaire. Les analyses qui détectent soit l'une des deux toxine, soit les des deux toxines sont utilisées [en utilisation courante] pour aider dans le diagnostique. Les gènes pour les toxines ont été copiés et leur sequences déterminées, et certaines de leur propriétées sont maintenant bien définies *(8,9)*. Les deux toxines sont larges (Mr de toxine A, 380.000; Mr de toxine B, 279.000). Toxine A a des séries complexes d'unitées qui se répetent au terminus-COOH de la molécule, et ces unitées sont probablement responsables pour la partie d'attachement qui identifie les récepteurs contenant des unités de galactose *(10)*. Certaines données suggèrent la possibilité que toxine A et B agissent d'une manière synergétique et que, suivant le dommage initial causé par la toxine A, toxine B peut alors éxercer sa toxicité. Par conséquent, bien que toxine A ait été proposé comme produisant la majorité des signes cliniques, toxine B peut aussi jouer un rôle important dans la maladie.

PRINCIPE DE L'ANALYSE

C. DIFFICILE TOX A/B II ™ utilise des anticorps contre les toxines A et B de *Clostridium difficile*. Les puits de micro-titre fournis avec le kit contiennent des anticorps polyclonaux de chèvres immobilisés et purifiés par affinité, contre les toxines A et B. L'anticorps détectant consiste d'une mixture de l'anticorps monoclonal de toxine A attaché à l'enzyme péroxydase de raifort et de l'anticorps polyclonal de chèvre de toxine B attaché à l'enzyme péroxydase de raifort. Dans l'analyse, une portion de matière fécale est émulsifiée dans le *Diluant* et le spécimen, ainsi dilué, est transféré dans le puit de micro-titre qui contient l'anticorps détectant. Si les toxines A et B sont présentes dans le spécimen, elles s'attacheront à l'anticorps détectant et à l'anticorps polyclonal immobilisé durant la phase d'incubation. Le matériel non-attaché qui pourrait être présent est enlevé pendant des étapes de nettoyages. Suivant l'addition du substrat, une couleur est détectée, causée par le complex d'enzyme-anticorps-antigène qui se forme en la présence des toxines.

MATERIAUX FOURNIS

Diluant, 40 mL, solution tampon de protéine + 0,02 % thimerosal. Le *Diluant* est aussi utilisé comme solution pour le contrôle négatif (voir PROCÉDURE D'ANALYSE).

Conjugué, 7 mL, anticorps monoclonal de souris spécifique pour la toxine A attachée à l'enzyme péroxydase de raifort et anticorps polyclonal de chèvre spécific pour toxine B attachée à l'enzyme péroxydase de raifort dans une solution de protéine tampon + 0,02 % thimerosal.

Substrat, 14 mL, solution contenant tétramethylbenzidine et péroxyde

Contrôle Positif, 3,5 mL, toxines inactivées dans une solution de protéine tampon contenant 0,02% thimerosal

Tampon de lavage à concentration 20X, 50 mL, 20X concentré contenant du tampon salin aux phosphates, détergent, et 0,2 % thimerosal

Solution d'Arrêt, 7 mL, 0,6N d'acid sulfuric. ATTENTION: Éviter tout contact avec la peau; rinser immédiatement en cas de contact.

Microplaque de micropuits, 12 bandeau chacun contenant 8 puits couvert d'anticorps chèvre spécifique pour les toxines A et B (préserver avec matériel déshydratant) et purifié par affinité.

Étiquette pour Solution de Nettoyage, 1 étiquette

Tiges d'application, 50 tiges

ACCÉSSOIRES

Pipettes en plastic jetables	100 pipettes
Feuilles adhésives en plastique	2 feuilles

MATÉRIAUX ET ÉQUIPEMENT NÉCÉSSAIRES MAIS NON FOURNIS

Bouteille pour nettoyage *Temporisateur* *Mélangeur de vortex*
Récipient pour déchés *Eau distillée* *Cylindres gradués*
Serviettes en papier ou feuilles absorbantes *Tubes pour dilution des spécimens*
Spectrophotomètre pouvant mesurer à longueur d'ondes duel (450/620 nm) ou à une seule longueur d'ondes de 450 nm (un analyseur à longueur d'ondes multiples est recommendé; l'absorbance doit être mesuré à 450 nm et référencée à 620 nm)
Réfrigirateur réglé entre 2° et 8 °C *Incubateur* réglé à 37°C ± 2°C

DURÉE DE CONSERVATION ET STOCKAGE

La date d'échéance du kit est indiquée sur l'étiquette. La date d'échéance pour chaque composant est énumérée sur les étiquettes individuellement. Le kit contenant les réactifs avec les durées de conservation indiquées doit être stocké entre 2° et 8°C et doit être retourné au réfrigirateur aussitôt que possible aprés utilisation.

PRÉCAUTIONS

1 Les réactifs provenants de kit différents ne doivent pas être mélangés. N'utiliser pas le kit aprés sa date d'échéance.
2 Les réactifs doivent être à température ambiante avant utilisation.
3 Les chapeaux et les bouts de pipettes sont codé par couleur: ne les mélangez pas!
4 Quand vous manipulez les puits d'analyses, évitez d'égratiner le fond des puits pareceque des mesures élevées d'absorbance peuvent résulter.
5 La bouteille de compte-gouttes doit être maintenue verticallement pour assurer une goutte de taille appropriée.
6 Manipuler les spécimens et utiliser les puit de micro-titre en faisant attention de considérer qu'ils peuvent transmettre des maladies infectieuses. Utiliser des gants pendant les analyses.
7 Les réactifs contiennent 0,02 % de thimerosal comme préservatif et doivent être manipulés par des procédures [attentives] normales de laboratoire.
8 La *Solution d'Arrêt* contient 0,6N d'acid sulfurique. Rinser avec de l'eau immédiatement si un contact se produit.
9 *Les micropuits inutilisés doivent être placés à l'interieur de la poche rescellable avec le déshydratant pour les protéger de l'humidité.*
10 Éxécuter la procédure de nettoyage tel qu'il a été décris pour éviter des réactions non-spécifques élevées.

11 Utiliser les spécimens de matière fécale durant les premières 24 heures suivant la collection des spécimens afin d'obtenir des résultats optimaux. Les spécimens qui ont été congelés *(-20°C ou moins)* peuvent perdre leur activité à cause de la congélation et du dégel.
12 Le *Substrat* est sensible à la lumière et doit être protégé de la lumière provenant directement du soleil et des sources de lumière ultraviolet.
13 Des résultats optimaux seront obtenus si les procédures spécifiées sont suivies rigoureusement. Les concentrations, les conditions d'incubation, et les caractéristiques de traitement ont été optimisé spécifiquement vis à vis de la sensibilité et de la spécificité. L'altération de la procédure spécifiée ainsi que/ou alors des conditions d'analyses peut affecter le sensibilité et la spécificité de l'analyse.

COLLECTION ET MANIPULATION DES SPÉCIMENS FÉCAUX

NOTER: Les procédures standard internes de collection et de manipulation utilisées pour les spécimens fécaux sont appropriées. Les spécimens doivent être transportés et dilués dans le *Diluant* du kit aussitôt que possible. Les spécimens doivent être stockés entre 2° et 8°C. Quand cela est possible, éxaminer les échantillons qui ont moins de 24 heures. Stocker les spécimens à -20°C ou à des températures moindres si l'éxamen ne peut pas être éxécuté suivant les 72 heures aprés la collection de l'échantillon *(11)*. La congélation et le dégel du spécimen, surtout si cela est répété plusieur fois, peut produire une perte d'activité causée par la dégradation des toxines. Les spécimens fécaux qui ont été préservé dans 10% de Formaline, Merthiolate-Formaline, Formaline-Sodium Acétate, ou Alcool Polyvinyl ne peuvent pas être utilisés. Assurez vous que le spécimen ait été complètement mélangé (avec le mélangeur vortex) avant d'entreprendre une analyse. Cela inclue le mélange complet du spécimen avant le transfert au *Diluant* ainsi que le mélange complet du spécimen dilué avant le transfert au micropuit. Le *Diluant* a été formulé afin de stabiliser les toxines dans les spécimens fécaux et pour réduire au minimum leur dégradation. Les pipettes jetables qui sont inclues dans le kit d'accéssoires sont graduées à 50, 100, 200, et 300 µL. Les spécimens peuvent aussi être dilués dans l'appareil facultatif *STOOL-PREP*™* (numero de catalogue T9015, voir les instructions pour l'utilisation) et stockés entre 2° et 8°C.

1 Préparer un tube pour chaque échantillon à analyser. Ajouter 200 µL de *Diluant* à chaque tube. Metter l'étiquette directement sur le côté du tube.
2 Pour les spécimens solides, utiliser une tige d'application pour transférer l'échantillon de matière fécale au tube. Transférer, a l'aide d'une tige d'application, une quantitée d'environ 3 mm de diamètre au *Diluant*. Pour les spécimens liquides, utiliser une pipette en plastique pour transférer 50 µL d'échantillon au tube. Assurez vous que les spécimens liquides forment une suspension homogène avant de faire le transfert.
3 Mélanger le contenu des tubes à l'aide d'un mélangeur vortex pendant 10 secondes et maintener les à une température entre 2 et 8 °C jusqu'à ce que l'analyse par ELISA soit éxécuté (cependant, cela ne doit pas être fait aprés les 72 heures suivant la collection des échantillons). Mélanger le contenu dilué une fois de plus (à l'aide d'un mélangeur vortex) avant de transférer aux puits micro-titres. Cela garantit un bon mélange des spécimens.
4 Des instruments pour néttoyage semi-automatiques peuvent être utilisés pour centrifuger les échantillons (5000 ´ g pendant 10 minutes) et pour enlever toutes particules.

PRÉPARATIONS PRÉLIMINAIRES

1 IMPORTANT: Tous les réactifs doivent être à température ambiante avant de commencer l'analyse.
2 Preparer une *Solution de Nettoyage* 1X. Le *Tampon de lavage à concentration 20X* est fourni à 20X concentration (*un précipité peut être négligé*). Il doit être dilué dans un volume total de 1 litre en ajoutant 50 mL du concentré à 950 mL d'eau distillée. Mettre une étiquette décrivant le produit sur la bouteille. Stocker la *Solution de Nettoyage* inutilisée entre 2° et 8°C.
3 **Préparation des bandes d'analyses**. Chaque bande contient 8 puits couvert d'anticorps chèvre spécifique pour les toxines A et B et purifié par affinité. Chaque spécimen ou contrôle doit utiliser un de ces puits couverts. Déterminer le nombre de puits à utiliser. Éviter le contact avec le fond des puits. Les puits d'analyses non-utilisés

doivent être retournés dans le sac en plastic et soigneusement recollés avec le déshydratant.

PROCÉDURE D'ANALYSE

1 Ajouter 1 goutte (50 µL) de *Conjugué* (chapeau rouge) à chaque puit. Assurez vous de maintenir la bouteille verticalement quand vous ajoutez les gouttes. Utiliser 1 puit pour chaque spécimen fécal, 1 puit pour le *Contrôle Positif* et 1 puit pour le contrôle négatif (c.a.d. le *Diluant*). Des marques d'identifications peuvent être inscrites directement sur le coté des puits.

2 Transférer 100 µL (2 gouttes à l'aide d'une pipette de transfert du kit d'accéssoires) du spécimen dilué dans le puit d'analyse; ou si vous utilisez un appareil *STOOL-PREP™**, ajouter le spécimen dilué tel qu'il est indiqué dans les instructions pour le *STOOL-PREP™**. Ajouter 1 goutte (50 µL) du *Contrôle Positif* (chapeau noir) au puit désigné pour le contrôle positif et 50 µL (1 goutte à l'aide d'une pipette de transfert du kit d'accéssoires) du contrôle négatif (c.a.d. le *Diluant*) au puit désigné pour le contrôle négatif.

3 Couper la feuille en plastique adhésive afin d'obtenir les dimensions nécéssaires pour couvrir les puits. Couvrer les puits et incuber les à 37°C ± 2°C pour 50 minutes.

4 Secouer le contenu hors des puits d'analyse dans un récipient pour déché.

5 Laver chaque puit en utilisant la *Solution de Nettoyage* 1X dans une bouteille d'éjection à ouverture réduite, en dirigeant la *Solution de Nettoyage* au fond du puit vigoureusement. Remplisser les puits, puis secouer la *Solution de Nettoyage* en dehors du puit dans le recipi ent à déchés. Rabattre la plaque inversée sur une serviette en papier et répéter les étapes numero 4 et 5 **quatre fois** en utilisant une serviette en papier à chaque fois. Si des particules sont observables dans les puits, continuer le nettoyage jusqu'à ce que toutes les particules aient été enlevées. **Noter**: si vous utilisez des équipements de nettoyage automatique ou semi-automatique, les spécimens doivent être centrifugés (5000 ´ g pour 10 minutes) pour enlever les particules. Ajouter 350 µL de la *Solution de Nettoyage* 1X à chaque puit. Laver 5 fois au total. Si vous utilisez les appareils *STOOL-PREP™** facultatifs, laver la plaque une fois à la main, rabattre vigoureusement sur une serviette en papier puis ajouter 4 nettoyages avec un laveur automatique pour obtenir 5 nettoyages au total. Si des particules sont observables dans les puits, continuez à laver jusqu'à ce que toutes les particules aient été enlevées.

6 Aprés le nettoyage, enlever complètement le liquide residuel dans les puits en frappant la plaque une fois de plus sur une serviette sèche en papier jusqu'à ce que toutes traces du liquide aient disparu. *Disposer des serviettes en papier et du récipient pour le spécimen de manière adéquate.*

7 Ajouter 2 gouttes (100 µL) de *Substrat* (chapeau bleu) à chaque puit. Doucement tapoter les puits pour mélanger le substrat. Incuber les puits à température ambiante pour 10 minutes. Doucement tapoter les puits aprés 5 minutes.

8 Ajouter une goutte (50 µL) de *Solution d'Arrêt* (chapeau jaune) à chaque puit. Doucement tapoter les puits et attendre 2 minutes avant la mesure. L'addition de la *Solution d'Arrêt* transforme la couleur bleu en une couleur jaune qui peut être dosée en mesurant la densité optique à 450 nm sur un lecteur microplaque ELISA. L'instrument doit être calibré à son zéro à l'aide d'air. Si un lecteur de longueur d'onde duel est utilisé, demarquer le zero vis à vis de l'air à 620 nm et mesurer à 450 nm. Essuyer le dessous de chaque puit avant de mesurer la densité optique. Si un lecteur ELISA est disponible, l'analyse peut être evaluée visuellement sous de bonnes conditions d'exposition de lumière contre un fond blanc. La mesure doit être obtenue pas plus de dix minutes aprés l'addition de la *Solution d'Arrêt*.

PROCÉDURE D'ANALYSE ALTERNATIVE/PROCÉDURE RAPIDE

Éxecuter la procédure d'analyse regulière en accordance avec les instructions fournies ci-dessus en remplaçant les 50 minutes à 37°C ± 2°C par 20 minutes à 37°C en utilisant le *Stat Fax 2200 Incubator/Shaker* (incubateur/agitateur). Si le *Stat Fax 2200 Incubator/Shaker* est utilisé, ajuster l'agitateur à la vitesse 7 et la température à une température de 37°C. Si d'autres agitateurs sont utilisés, une vitesse de 1500 rpm est recommendée. L'agitation ne doit pas causer de débordements. Si il y a débordement, réduir la vitesse en accordance (???).

CONTRÔLE DE QUALITÉ

1 Un contrôle positif et négatif doit être fait avec chaque serie d'analyses de specimens.
2 Les contrôles positifs et négatifs doivent être compris dans leur marges respectives ou alors l'analyse n'est pas valide.
   a Le Contrôle Positif doit avoir une couleur evidente jaune. Si le control positif est lu à l'aide d'un spectrophotomètre, la DO à 450 nm ou la DO en utilisant des longueurs d'ondes duel à 450/620 nm doit être ≥0,500.
   b Le Contrôle Négatif doit être visuellement clair. Si le contrôle négatif est lu à l'aide d'un spectrophotomètre, la DO à 450 nm doit être <0,120. Si la mesure est obtenue à 450/620 nm l'absorbance doit être <0,080.
3 Les puits qui sont clairs visuellement mais qui produisent une absorbance de ≥0,120 doivent être éssuyés en dessous et remesurés.
4 Les évaluations visuelles doivent être obtenues sous de bonnes conditions d'exposition de lumière contre un fond blanc.
5 Un échantillon qui produit un resultat positif faible (c.a.d. <0,200) et qui est adjacent a un échantillon a signal positif fort, doit être répété pour s'assurer qu'un transfert ne s'est pas produit.

INTERPRÉTATIONS DES RÉSULTATS

1. **Évaluation Visuelle**
   Négatif = Sans couleur
   Positif = Toute couleur jaune
2. **Spectrophotométrique Longueur d'Onde Unique a 450 nm**
   Négatif = DO<0,120
   Positif = DO ≥0,120
3. **Spectrophotométrique Longueur d'Onde duel a 450/620 nm**
   Négatif = DO<0,080
   Positif = DO ≥0,080

Une analyse résultant en un positif indique que *C. difficile* toxine A et/ou toxine B sont présentes dans le spécimen fécal.

LIMITATIONS DE *C. DIFFICILE TOX A/B II* ™

1 L'analyse de *C. DIFFICILE TOX A/B II* ™ est utilisée pour détecter la toxine de *C. difficile* dans les spécimens fécaux. L'analyse confirme la présence de toxine dans la matière fecale et cette information doit être évaluée et considérée par un medecin en rapport avec l'histoire clinique du patient. L'inabilité de détecter les toxines A et B dans les échantillons fécaux des patients suspectés d'avoir la maladie associée avec *C. difficile*, ne peut pas exclure la présence réelle de la maladie, toutefois, elle peut être causée par d'autres facteurs (c.a.d., collection incorrecte de spécimen, manipulation et/ou stockage, des niveaux de toxines inferieurs aux limites de détection du kit). L'analyse de *C. DIFFICILE TOX A/B II* ™ détectera la Toxine A à des concentrations de ≥0,8 ng/mL et Toxine B at des concentrations de ≥2,5 ng/mL.
2 Les spécimens fécaux présentent une complexité extrême parmis les spécimens cliniques. Des résultats optimaux avec *C. DIFFICILE TOX A/B II* ™ seront obtenus avec des spécimens qui ont moins de 24 heures. La plupart des spécimens peuvent être stockés entre 2° et 8°C pour 72 heures avant que la dégradation significative des toxines soit observable. Si les spécimens ne sont pas analysés durant cette période, ils peuvent alors être congelés et dégelés. Toutefois, la congelation et le degel, surtout de façon répétée, peut produire une perte de l'activité des spécimens à cause de la dégradation des toxines.
3 Certains spécimens peuvent produire des réactions faibles. Plusieurs facteurs peuvent être à l'origine telle que la présence de variétés faiblement toxigéniques, des niveaux *in vivo* de production de toxines bas, ou la présence de substances qui s'associent ou d'enzymes inactivantes dans la matière fecale. *Sous ces conditions, les spécimens doivent être reanalysés ou un échantillon frais doit être analysé.* Des analyses additionelles qui peuvent être utilisées en conjonction avec l'analyse de *C. DIFFICILE TOX A/B II* ™ incluent l'isolation de l'organisme sur un média sélectif, l'analyse par l'agglutination du latex pour la détection de *C. difficile* (l'organisme), ou alors l'analyse de

la cytotoxicité de la culture de tissu pour la détection de la toxine de *C. difficile*. L'isolation de l'organisme ne confirme pas son caractère cytotoxigénique. Cela doit être confirmé par des analyses additionnelles de l'isolé en utilisant soit l'ELISA ou une analyse de tissu de culture pour démontrer un caractère cytotoxigénique. De la même manière, l'analyse par agglutination du latex, qui détecte une protéine de *C. difficile* non-toxique, ne démontre pas la présence d'un caractère cytotoxigénique de *C. difficile*.

4 Certains isolés cytotoxigéniques de *Clostridium sordellii* produisent des toxines similaires dans leur biologiques ainsi que dans leur propriétés physico-chimiques et immunologiques aux toxines de *C. difficile*. Ces isolés, toutefois, n'ont pas été détectés parmis les patients sous traitement antibiotique démontrant une diarrhée et colite.

5 Les spécimens fécaux qui ont été préservés dans 10% de Formaline, Merthiolate-Formaline, Formaline-Sodium Acétate, ou Alcool Polyvinyl ne peuvent pas être utilisés.

6 Les caractéristiques de performance de l'analyse de *C. DIFFICILE TOX A/B II* ™ n'ont pas été complètement établis au sein de la population pediatrique.

LES VALEURS ANTICIPÉES

La predominance d'une analyse *C. DIFFICILE TOX A/B TEST* positive ainsi que d'une culture de tissu cytotoxigénique positive était 5,4% dans une étude et 8,6% dans une autre étude. La predominance changera d'un endroit à un autre et les hôpitaux peuvent démontrer des taux plus ou moins importants que ceux qui ont été observé dans le site utilisé pour évaluer *C. DIFFICILE TOX A/B TEST*. La maladie associée avec *Clostridium difficile* est principalement une maladie nosocomial des patients agés, et les hôpitaux qui ont des nombres plus élevés de patients agés peuvent être confrontés avec des taux plus importants. Il est important de considérer les résultats des analyses en conjonction avec les symptomes cliniques, parceque certains adultes en bonne santé et un grand nombre d'enfants en bonne santé (jusqu'à 50%) seront positifs pour la toxine de *C. difficile* (positif pour la toxine soit par culture de tissu, soit par ELISA). De plus, des taux de contaminations par *C. difficile* ont été rapportés à hauteur de 22% à 32% parmis les patients avec fibrose cystique.

CARACTÉRISTIQUES DE PERFORMANCE

Évaluation clinique

L'analyse de *C. DIFFICILE TOX A/B TEST* a été comparée avec l'analyse de culture de tissu dans quatre hôpitaux des E.U. et de façon interne à TECHLAB®. Les spécimens inclus dans l'évaluation ont été soumis a des laboratoires cliniques pour des analyses de routines. L'analyse de culture de tissu a été faite selon les procédures internes. La Table 1 montre une comparaison entre l'analyse de *C. DIFFICILE TOX A/B TEST* et l'analyse de culture de tissu. Parmis les 1.152 spécimens inclus dans l'évaluation, approximativement 3,6% été parmis les enfants de ≤ 2 ans d'ages. Aucun spécimen analysé a produit des résultats d'analyse *C. DIFFICILE TOX A/B TEST* (+)/culture de tissu (-). De façon general, la sensibilité de l'analyse de *C. DIFFICILE TOX A/B TEST* se trouve entre 83,3% et 96% (intervalle de confiance entre 87,4 et 95,0, p=0.05). La spécificité était de 100% dans toutes les études. La valeur positive prédictive était de 100% dans toutes les études et la valeur négative prédictive se trouvait entre 90% et 99,5% (intervalle de confiance entre 93,8 et 99,8, p=0.05). La corrélation se trouvait entre 94,9% et 99,5% (intervalle de confiance entre 96,6 et 99,4, p=0.05).

TABLE 1 Corrélation de l'analyse de *C. DIFFICILE TOX A/B TEST* avec Culture de Tissu (n=1.152).

		Tissue Culture positive	Tissue Culture negative
C. DIFFICILE TOX A/B TEST	positive	165	0
	negative	14	973

Sensibilité 92.2% Valeur Positive Prédictive 100%
Spécificité 100% Valeur Négative Prédictive 98.6% Corrélation 98.8%

Comparaison interne

Dans une étude faite internement l'analyse de *C. DIFFICILE TOX A/B II* ™ a été

comparée à celle de *C. DIFFICILE TOX A/B TEST*. Dans l'étude, 218 spécimens cliniques représentatifs de ceux normalement soumis au laboratoire clinique pour l'analyse de *C. difficile* ont été évalués. Les résultats obtenus par les deux analyses sont comparables.

Centrifugation

Un total de 337 spécimens fécaux, en incluant 30 positifs et 307 négatifs, ont été évalués pour déterminer l'effet que la centrifugation pourrait avoir sur la performance. Pour cette analyse, des spécimens ont été dilués et mélangés avec un vortex tel qu'il est décrit dans les instructions. Les spécimens ont été centrifugés (5000 ´ g) pour enlever les matériaux insolubles et le fluide surnageant analysé avec le *C. DIFFICILE TOX A/B TEST*. Les résultats ont été comparés aux résultats obtenus avec la même serie de spécimens dilués et mélangés avec le vortex qui n'ont pas été centrifugés. Les résultats ont demontré une corrélation de 100% entre les spécimens centrifugés et ceux qui n'ont pas été centrifugés.

LES RÉACTIONS CROISÉES

Une variété d'organismes ont été examinés pour déterminer l'étendue des réactions croisées en utilisant l'analyse de *C. DIFFICILE TOX A/B TEST*. Pour ces analyses, des cultures de bouillon mélangées avec le *Diluant* ont été évaluées. Des cultures de bouillon durant leur phase logarithmique de croissance contenant $>10^8$ de bacteries par mL ont été utilisées. Une liste des organismes qui n'ont pas réagi sous aucunes des conditions est reproduite dans la Table 2. Les seuls organismes qui ont réagi étaient ceux de *C. difficile* toxigénique et ceux de *C. sordellii* (variété VPI 9048) qui ont produit des toxines réagissant de manière significative et non-spécifique avec les toxines de *C. difficile*. Une variété non-toxigénique de *C. sordellii* qui ne produit pas de toxines HT était négative dans l'analyse. *C. Sordellii* n'a pas été impliquée dans la colite pseudomembraneuse ou dans les diarrhée associées avec les antibiotiques. Les variétés *C. difficile* toxigéniques qui ont été analysées ont inclus six variétés toxigéniques à caractère entre faiblement toxigénique jusqu'à fortement toxigénique et deux representaient des variétés non-toxigéniques. L'analyse de *C. DIFFICILE TOX A/B TEST* a détecté toutes les six variétés toxigéniques et n'a pas reagi avec les deux variétés non-toxigéniques.

TABLE 2 Organismes qui n'ont pas reagi sous analyse de *C. DIFFICILE TOX A/B TEST*

Aeromonas hydrophila	*Clostridium sporogenes*
Acinetobacter lwoffi	*Clostridium tetani*
Bacillus cereus	*Enterococcus faecalis*
Bacillus subtilis	*Escherichia coli*
Bacteroides fragilis (toxigenic/nontoxigenic)	*Escherichia coli* (enterohemmorrhagic)
Candida albicans	*Klebsiella pneumoniae*
Candida krusei	*Peptostreptococcus anaerobius*
Candida tropicalis	*Proteus vulgaris*
Clostridium bifermentans	*Pseudomonas aeruginosa*
Clostridium botulinum (Types A-G)	*Salmonella choleraesuis*
Clostridium chauvoei	*Salmonella enteritidis*
Clostridium difficile (nontoxigenic)	*Salmonella typhimurium*
Clostridium haemolyticum	*Shigella flexneri*
Clostridium histolyticum	*Shigella sonnei*
Clostridium novyi (Types A, B, C)	*Staphylococcus aureus* (Protein A-negative)
Clostridium perfringens (Types A-E)	*Staphylococcus aureus* (Protein A-positive @ $<10^5$)
Clostridium septicum	*Streptococcus pyogenes*
Clostridium sordellii (nontoxigenic)	*Vibrio parahaemolyticus*
Clostridium spiroforme	*Yersinia entercolitica*

LES ÉFFETS DE LA CONSISTANCE DES SPÉCIMENS FÉCAUX

Les réactions des spécimens fécaux à consistance variables dans l'analyse de *C. DIFFICILE TOX A/B TEST* et dans l'analyse de la culture de tissu sont montrées dans la Table 3. Les taux de réactions positives étaient similaires parmis tous les trois types de spécimens fécaux. Tous les spécimens ont été soumis à l'analyse pour *C. difficile*. Les conditions de soumissions étaient l'histoire clinique du patient et non pas la consistance du spécimen. Dans des études additionnelles, les toxines A et B trés purifiés ont été utilisées pour contaminer le liquide, le semi-solide, et les spécimens fécaux solides.

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TABLE 3. Les effets de l'uniformité des spécimens fécaux

Analyse	Spécimens Liquides	Spécimens Semi-Solides	Spécimens Solides
Nombre de spécimens(n=435)	150	133	152
C. DIFFICILE TOX A/B TEST Positif	13 (8,7%)	11 (8,3%)	13 (8,6%)
Culture de Tissu Positif	13 (8,7%)	14 (10,5%)	15 (9,9%)

C. DIFFICILE TOX A/B TEST a détecté les toxines A et B dans le liquide, semi-solide, et dans les spécimens solides à des niveaux similaires à ceux qui ont été observés avec les toxines A et B préparées dans le *Diluant* du kit.

REPRODUCTIBLITÉ ET PRÉCISION

Cinq spécimens fécaux (un spécimen négatif et quatre positifs) ont été envoyés à quatre laboratoires indépendants pour analyse avec *C. DIFFICILE TOX A/B TEST.* Tous les spécimens ont été maintenus congelés jusqu'à ce que l'analyse ait été faite. Les résultats de chaque laboratoire ont alors été comparés avec les résultats internes et les résultats de chaque partie se sont avérés identiques. Les quatres spécimens positifs ont été confirmés positifs et le spécimen négatif a été confirmé négatif à chaque laboratoire.

Le coéfficient de variation (CV) d'intra-analyse de *C. DIFFICILE TOX A/B TEST* a été déterminé en analysant 32 positives et 32 négatives réactions de control de même que 8 négatifs spécimens fécaux. Chaque spécimen fecal a été analysé dans 11 puits. L'intra-analyse %CV était de 7,190 avec le contrôle positif, 6,557 avec le control négatif, et 9,697 avec les spécimens fécaux. L'inter-analyse CV a été établie en utilisant quatre spécimens fécaux positifs et 1 négatif analysés à des temps déterminés de 0, 24, 48, et 72 heures. Le %CV se trouve entre 9,9 et 29,6, avec une moyenne de 16,3.

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**STOOL-PREP™* is a trademark of Medical Packaging Corporation, Camarillo, CA

APPENDIX D. Protocol for *C. DIFFICILE TOX A/B II™ test*

Premier

Premier™ Toxins A&B

Enzyme Immunoassay for the Detection of *Clostridium difficile* Toxin A and Toxin B in Stool Specimens

Meridian Bioscience, Inc.

Premier™ Toxins A&B

Enzyme Immunoassay for the Detection of *Clostridium difficile* Toxin A and Toxin B in Stool Specimens

REF Catalog No. 616096 IVD For *in vitro* diagnostic use only.

INTENDED USE

Premier™ Toxins A&B is a qualitative enzyme immunoassay for the detection of *Clostridium difficile* toxin A and toxin B in stool from patients with antibiotic associated diarrhea. Premier Toxins A&B is intended for use as an aid in diagnosis of *C. difficile* associated disease.

EXPLANATION

Toxigenic *Clostridium difficile* is a major cause of antibiotic associated diarrhea and colitis[1] and is the causative agent for virtually all cases of pseudomembranous colitis.[2,3] Although about 2% of normal healthy adults are colonized with *C. difficile*,[4] many patients acquire this organism through nosocomial infection.[5] Exposure to most antibiotics is thought to allow proliferation of toxigenic *C. difficile* by disrupting the normal intestinal flora.[6] Two toxins, toxin A and toxin B, are associated with disease caused by *C. difficile*.[7] These toxins are immunochemically and biologically distinct. Antiserum prepared against purified toxin A or toxin B does not cross-react with the other toxin.[8] Toxin A has been described as an enterotoxin and causes an increase in intestinal permeability with subsequent enteric fluid accumulation and diarrhea.[9] Toxin B is a potent cytotoxin which causes rounding of cells in culture.[7,10] In hamsters, toxin B is lethal when administered intravenously by itself or intragastrically if combined with sublethal doses of toxin A.[11] The contribution of toxin B to the development of disease in the gut is not understood. It has, however, been hypothesized that the two proteins may act synergistically *in vivo*.[11,12]

The most frequently used test in the diagnosis of *C. difficile* colitis is determination of toxin B in patients' stool by cell culture with neutralization of the toxin by specific antiserum. Although this assay is extremely sensitive, and the presence of toxin B has a positive correlation to 90-100% of patients with severe disease,[1,7] it requires cell culture capability and up to 72 hours of incubation. In addition, the cytotoxin assay is not standardized and the procedures and cell lines used vary considerably.[9]

BIOLOGICAL PRINCIPLES

Premier Toxins A&B is an enzyme immunoassay for the direct detection of *Clostridium difficile* toxin A and toxin B in stool samples. Breakaway microwells are coated with toxin specific monoclonal and polyclonal antibodies. Diluted patient specimens and HRP-conjugated anti-toxin A and B polyclonal antibodies are added to microwells. If either toxin is present in the diluted patient samples, HRP-conjugated toxin polyclonal antibodies (specific for both toxins) complexes are formed which remain in the microwells after washing. After a final washing step, a substrate/chromagen (urea peroxide and tetramethylbenzidine) is added to the wells. Any bound conjugate converts the substrate/chromagen to a blue color. Addition of acid (Stop Solution) converts the blue to a yellow color.

MATERIALS PROVIDED

1. **96 Antibody coated Microwells** – Breakaway plastic wells coated with mouse monoclonal anti-toxin A antibody and polyclonal goat anti-toxin B antibody.
2. **Positive Control** (2.6ml) – Inactivated toxin A and B in buffered protein solution containing gentamicin and thimerosal (0.02%) as a preservative.
3. **Sample Diluent/Negative Control** (21.0ml) – Buffered protein solution containing gentamicin and thimerosal (0.02%) as preservative.
4. **20X Wash Buffer** (50ml) – Concentrated wash buffer containing thimerosal (0.2%) as a preservative.
5. **Enzyme Conjugate** (6.0ml) - Polyclonal goat anti-toxin A and anti-toxin B antibodies conjugated to horseradish peroxidase in buffered protein solution containing gentamicin and thimerosal (0.02%) as a preservative.
6. **Substrate** (12.5ml) - Buffered solution containing urea peroxide and tetramethyl benzidine.
7. **Stop Solution** (13.0ml) – 1M Phosphoric acid. CAUTION: Avoid contact with skin. Flush with water if contact occurs.
8. **96 Sample Transfer pipets**
9. **2 Microwell plate sealers**

MATERIALS NOT PROVIDED

1. Test tubes for dilution of sample.
2. Distilled or deionized water.
3. OPTIONAL: EIA microwell reader capable of reading absorbance at 450 nm or 450/630nm.
4. Squirt bottle.
5. Pipets and graduated cylinder for making 1X Wash Buffer.
6. Wooden applicator sticks.
7. Absorbent paper.
8. Incubator 37°± 2°C.
9. Timer.
10. Vortex Mixer.
11. Waste Container with disinfectant (i.e. 10% solution of household bleach) and / or autoclavable biohazard bags.
12. Disposable gloves.
13. OPTIONAL: Stat Fax™ - 2200 Incubator/Shaker for use in 20 minute incubation procedure.

Stat Fax™ is a trademark of Awareness Technology, Inc.

PRECAUTIONS

1. All reagents are for *in vitro* diagnostic use only.
2. Kit reagents should be warmed to room temperature (21°-27°C) and gently mixed before use.
3. Do not mouth pipet samples or reagents. Avoid contact with skin or mucous membranes.
4. Do not smoke, drink or eat in areas where specimens or kit reagents are handled.

5. Wear disposable gloves while handling specimens and thoroughly wash hands afterwards.
6. Use only the reagents provided with this kit. Do not interchange reagents from different kit lot numbers.
7. Do not use kit beyond the expiration date given on the kit label.
8. STOP SOLUTION contains 1M phosphoric acid. If contact with skin or mucous membranes occurs, flush with water immediately.
9. Patient specimens may contain infectious agents and should be handled at Biosafety Level 2 as recommended in the CDC/NIH manual "Biosafety in Microbiology and Biomedical Laboratories," 1988.
10. Avoid splashing or forming of aerosols when handling, diluting or transferring specimens.
11. Avoid microbial contamination of reagents or incorrect results may occur.
12. Transfer pipets provided must be used for specimen preparation and transfer. Use one per specimen. Cross contamination of samples or reagents may cause incorrect results.
13. **All stool samples must be mixed thoroughly, regardless of consistency, to insure a representative sample prior to pipeting.**
14. Reagent concentration, incubation times and temperatures have been optimized for sensitivity and specificity. Best results are obtained by adhering to these specifications.
15. Positive Control contains inactivated toxin A and toxin B. However, it should be handled as a potential biohazard.
16. Hold all vials vertically to insure proper drop size and delivery.
17. Do not allow the tips of any vial to touch the microwells.
18. Replace colored caps on correct vials.
19. All reagents are provided already diluted to the proper concentration (except the 20X Wash Buffer). Do not dilute further.
20. Any deviation below or above set incubation times may affect sensitivity and specificity and should be avoided.

SHELF LIFE AND STORAGE

1. Store all kit reagents at 2°-8°C. Return kit to the refrigerator promptly after use.
2. Keep microwells in pouch until the pouch reaches room temperature to avoid condensation. Return all unused microwells and the desiccant to ziplock pouch and seal tightly.
3. Diluted WASH BUFFER may be kept at room temperature and used for up to three months.

SPECIMEN HANDLING

Collect stool specimens into a clean, airtight container with no preservative. All stool specimens should be stored at 2°-8°C and tested as soon as possible. Ideally, stool specimens should be tested within 24 hours but specimens may be held at 2°-8°C for up to 72 hours prior to testing. If specimens cannot be tested within 72 hours, they should be frozen **immediately upon receipt** at -20°C or colder. A single freeze thaw cycle should not affect results.[13] Repeated freezing and thawing of samples should be avoided. Use only the SAMPLE DILUENT provided with this kit for diluting specimens. Specimen can be stored diluted in Sample Diluent in a sealed tube (as described in **SPECIMEN PREPARATION** below) for up to 8 hours at 2°-8°C.

REAGENT PREPARATION

1. Bring the entire kit, including microwell pouch, to room temperature (21°-27°C) before use. Return to 2°-8°C immediately after use.
2. Prepare decontamination vessel for discarding reagents and materials.
3. Do not allow microwells to dry out between steps.
4. Reproducibility in any EIA is largely dependent on the consistency and thoroughness with which the microwells are washed. Carefully follow the recommended wash procedure as outlined in the EIA test procedure. An automated washer may be used.
5. Prepare 1X Wash Buffer as needed. For example: 5.0ml of 20X Wash Buffer + 95.0ml of distilled or deionized wash is sufficient to wash one strip. Place in clean squirt bottle. The 1X Wash Buffer can be stored at 21°-27°C for up to three months.
6. One POSITIVE and one NEGATIVE CONTROL well must be included with each run of specimens. Use the POSITIVE CONTROL as provided. **DO NOT DILUTE.**
7. Use EIA plate sealers to cover assay during incubation steps. Cut to size, then remove paper backing before use.

SPECIMEN PREPARATION (1:5 Sample Dilution)

1. Add 200µl of Sample Diluent to a clean test tube with dropper assembly (or equivalent).
2. **Mix stool as thoroughly as possible prior to pipeting.**
   a. **For Liquid or semi-solid stool:** Using the disposable transfer pipets and measuring to the 1st calibration mark (50µl), transfer 50µl of stool into the dilution tube containing SAMPLE DILUENT. Rinse the transfer pipet by drawing the stool suspension in and out of the pipet several times. Remove pipet and vortex the suspension thoroughly (15 seconds), and replace pipet in tube.
   b. **Solid Stools:** Using a wooden applicator stick, transfer a small "BB" sized portion (3-4 mm diameter) of thoroughly mixed stool into Sample Diluent. Emulsify stool using the wooden applicator stick then vortex 15 seconds. Place a transfer pipet in the tube. Promptly proceed to assay.

Note: No centrifuging of specimen is needed.

PROCEDURE

This product is to be used by trained laboratory personnel only.

NOTE: With large numbers of specimens, repetitive or multichannel pipets may be used for dispensing the reagents.

1. After the pouch has reached 21°-27°C, break off the required number of microwells (one well for each specimen plus one positive and one negative control well per batch). Place the microwells in the microwell strip holder and record the location of all wells. Unused microwells must be resealed in the pouch immediately (see **SHELF LIFE AND STORAGE**).
2. Using the original transfer pipet, draw up diluted stool to the 100µl calibration point (second mark from tip of the pipet) and add to the appropriate well (place pipet tip halfway into well and let sample slowly run down side of well).
3. Add two free-falling drops of Positive or 100µl (second mark on transfer pipet) Negative Control (Sample Diluent) to the appropriate wells.
4. Add one free-falling drop of Enzyme Conjugate (50µl) to all wells. Mix wells by firmly shaking/swirling the plate for 30 seconds.
5. Cut plate sealer to size and press firmly onto top of microwells to seal. Incubate the plate for 50 minutes at 35°-39°C. **Alternatively, laboratories equipped with a heated plate shaker (Stat Fax™-2200) can incubate and rotate the plate for 20 minutes at 37°C at 1000 rpm (setting #5).**
6. **Carefully**, remove the plate sealer and wash wells (see **REAGENT PREPARATION**):
   a. Dump plate contents firmly into a biohazard receptacle.
   b. Bang the inverted plate on a clean stack of paper towels
   c. Fill all wells with 1X Wash Buffer directing stream of buffer to the sides of the wells to avoid foaming. Immediately proceed to Step 6.d.

d. Repeat wash cycle (dump, bang on fresh towels, fill) four to six times (for a total of 5-7 washes). After the last fill, dump and bang plates on fresh towels hard enough to remove as much excess wash buffer as possible but do not allow wells to completely dry at any time.

7. Clean the underside of all wells with a lint free tissue.
8. Add two free-falling drops of Substrate (100µl) to each well.
9. Firmly shake/swirl the plate for 10-15 seconds then incubate for 10 minutes at 21°-27°C.
10. Add two free-falling drops of Stop Solution (100µl) to all wells. Shake/swirl the plate firmly for 30 seconds to assure complete mixing. After addition of Stop Solution, wait 2 minutes before reading (Step 11). **NOTE**: initial color of positive reaction is blue, which changes to yellow upon addition of Stop Solution.
11. Observe reactions:
    a. Visual Determination – Read within 15 minutes after adding Stop Solution.
    b. Spectrophotometric Determination – Zero EIA reader on air. Wipe the underside of wells with a lint free tissue. Read absorbance at 450nm or 450/630nm within 30 minutes of adding Stop Solution.

QUALITY CONTROL

The Positive and Negative Controls must be used with each batch of specimens to provide quality assurance of the reagents and the procedure. It is suggested that the results of each quality control check be recorded in an appropriate log book to maintain high quality testing procedures and compliance with regulatory agencies.
The Negative Control should read <0.150 at 450nm and <0.100 at 450/630nm but greater than 0.00. If control is <0.00, re-blank the plate reader to air and reread the plate.
The Negative Control should be colorless to faint (barely visible) yellow when read visually.
The Positive Control should read less than 2.999 but greater than 0.600 at either 450nm or 450/630nm. The Positive Control should have a definite yellow color when read visually.
If the expected reactions are not observed, please contact Meridian Technical Services Department at 1-800-343-3858.
Any positive well without visible color should be repositioned, wiped on the underside of the well and reread.
At the time of each use, kit components should be visually examined for obvious signs of microbial contamination, freezing, or leakage.

INTERPRETATION OF RESULTS

1. **Visual Reading**
   Negative = colorless to faint (barely visible) yellow
   Positive = definite yellow color
2. **Spectrophotometric Single Wavelength (450nm)**
   Negative = OD450 < 0.150
   Positive = OD450 ≥ 0.150
3. **Spectrophotometric Dual Wavelength (450/630nm)**
   Negative = OD450/630 < 0.100
   Positive = OD450/630 ≥ 0.100

Extremely strong positive reaction may yield a purple precipitate within a few minutes of stopping the reaction.
A positive result indicates the presence of *C. difficile* toxin A and / or B. A negative result indicates the absence of toxins A and B, or that the level of toxin is below that which can be detected by the assay. The magnitude of the OD, above the cut-off, is not indicative of the severity or extent of the *C. difficile* infection.

LIMITATIONS OF THE PROCEDURE

Premier Toxins A&B detects the presence of *Clostridium difficile* toxin A and toxin B in stool. Failure to detect toxin A or toxin B in stool from patients with suspected *C. difficile* associated disease may not preclude actual disease but may be due to such factors as improper collection, handling and storage of the specimen or toxin concentrations in stool below the detection limit of this kit. The Premier Toxins A&B kit will detect toxin A at levels ≥ 1.4 ng/ml stool and toxin B at levels ≥ 2.4 ng/ml stool. Reactivity of positive samples as determined by Premier Toxins A&B may decrease with time due to degradation of the toxins. As with any diagnostic test, the results of Premier Toxins A&B should be interpreted with respect to patient history and other clinical and laboratory findings.
The performance of this assay has not been evaluated in a pediatric population.[14]
Certain strains of *C. sordellii* produce toxins which are immunologically cross reactive with *C. difficile* toxins A and B. *C. sordellii* has not been isolated, however, from stool obtained from patients with antibiotic associated diarrhea or pseudomembranous colitis while *C. difficile* was found to be present.[15,16]

EXPECTED VALUES

The frequency of antibiotic associated diarrhea caused by *C. difficile* is dependent on several factors including: patient population, type of institution and epidemiology. The reported incidence of *C. difficile* associated disease in patients suspected of having antibiotic associated diarrhea is 15-25%[1] although different facilities may find positivity rates outside this range.

PERFORMANCE CHARACTERISTICS

The performance of Premier Toxins A&B was evaluated in a clinical study performed at two sites in the United States. The kit was compared to the cellular cytotoxicity assay and discrepant results were resolved using toxigenic culture and a competitor's EIA for toxins A and B.

Premier Toxins A&B Results	Cytotoxin Result: Site 1		Cytotoxin Result: Site 2		Cytotoxin Result: All Sites	
	Pos	Neg	Pos	Neg	Pos	Neg
Pos	55	7	35	6	90	13
Neg	3	257	2	208	5	465
Performance Statistic	Value	95% CI	Value	95% CI	Value	95% CI
Sensitivity	94.8%	85.6-98.9%	94.6%	81.8-99.3%	94.7%	88.1-98.3%
Specificity	97.3%	94.6-98.9%	97.2%	94.0-99.0%	97.3%	95.4-98.5%
Positive Predictive Value	88.7%	78.1-95.3%	85.4%	70.8-94.4%	87.4%	81.0-93.8%
Negative Predictive Value	98.8%	96.7-99.8%	99.0%	96.6-99.9%	98.9%	97.5-99.7%
Correlation	96.9%	94.4-98.5%	96.8%	93.8-98.6%	96.9%	95.1-98.1%

Performance of the Premier Toxins A&B EIA was equivalent at each of the clinical trial sites. Overall sensitivity and specificity compared to the reference cytotoxin method were 94.7% and 97.3% respectively. Examination of the 13 false positive specimens revealed that one was positive by toxigenic culture (i.e. yielded a *C. difficile* isolate producing both toxins A and B). Four other specimens were positive by a competitor's toxin A+B EIA. Thus, 5/13 (38%) of the false positive specimens had additional independent evidence supporting the presence of toxigenic *C. difficile*. Toxigenic culture was negative on 3/5 of the Premier Toxins A&B negative, cytotoxin positive specimens. In addition, all five were negative by a competitor's toxin A+B EIA.

A competitor's A+B EIA (Wampole™ Laboratories) was also run on at each trial site. Results are compared to cytotoxin and Premier Toxins A&B below:

Premier Toxins A&B Results	Wampole A/B EIA	
	Pos	Neg
Pos	92	11
Neg	9	461
Relative Performance	Value	95% CI
Co-Positivity	91.1%	83.8-95.8%
Co-Negativity	97.7%	95.6-98.7%
Agreement	96.5%	94.5-97.7%

Wampole Toxins A/B Results	Cytotoxin Result	
	Pos	Neg
Pos	88	13
Neg	7	465
Performance Statistic	Value	95% CI
Sensitivity	92.6%	84.5-97.0%
Specificity	97.3%	95.4-98.5%
Positive Predictive Value	87.1%	80.6-93.7%
Negative Predictive Value	98.5%	97.0-99.4%
Correlation	96.5%	94.7-97.9%

Agreement between the two EIA's for toxins A&B was 96.5%. Two of the 11 specimens positive by Premier Toxins A&B and negative by the competitor's EIA were positive by cytotoxin and another specimen was positive by toxigenic culture. None of the 9 specimens positive by the competitor's EIA and negative by Premier Toxins A&B were positive by cytotoxin.

Wampole™ is a trademark of Wampole Laboratories

REPRODUCIBILITY

The reproducibility of the Premier Toxins A&B test was established using negative, low, medium and strong positive samples and controls tested in triplicate in three different batches at three sites. Inter- and intra-assay variances were calculated and are given below.

Source of Variance	Positive Control	Negative Control	High Positive	Medium Positive	Low Positive	Negative
Mean Absorbance	2.010	0.013	2.250	1.146	0.280	0.009
Within Run CV	4.1%	24.5%	7.3%	6.9%	15.9%	28.9%
Between Run CV	7.0%	16.2%	6.2%	13.9%	14.6%	31.7%

INTERFERING SUBSTANCES

Results of Premier Toxins A&B testing are not affected by blood, barium sulfate, metronidazole or vancomycin present in stool specimens.[13]

ASSAY SPECIFICITY

The specificity of **Premier Toxins A&B** was tested by utilizing the following bacterial or viral strains. Positive and negative stools were spiked with $\geq 1x10^8$ bacteria/ml and tested by **Premier Toxins A&B**. The only non-*C. difficile* microorganisms reactive with the Premier Toxins A&B were two strains of *Clostridium sordellii*, ATCC 9714 and VPI 9048. Both of these strains produce toxin A and B homologues HT and LT respectively. All other organisms were found to be negative when spiked into the negative stool. In addition, they did not interfere with the positive specimen.[13]

Microorganism or virus (# strains tested)

Adenovirus 40 & 41 (2)
Bacillus subtilis (1)
Campylobacter jejuni (1)
Clostridium difficile non-tox. (7)
Clostridium novyi (1)
Clostridium sporogenes (1)
Enterobacter cloacae (1)
Klebsiella pneumoniae (1)
Proteus vulgaris (1)
Salmonella typhimurium (1)
Shigella flexneri (1)
(Cowan I) (1)
Vibrio parahaemolyticus (1)
Aeromonas hydrophila (1)
Bacteroides fragilis (1)
Candida albicans (1)
Clostridium haemolyticum (1)
Clostridium perfringens (1)
Clostridium tetani (1)
Escherichia coli (3)
Peptostreptococcus anaerobius (1)
Pseudomonas aeruginosa (1)
Serratia liquefaciens (1)
Shigella sonnei (1)
Staphylococcus epidermidis (1)
Yersinia enterocolitica (1)
Bacillus cereus (1)
Campylobacter coli (1)
Clostridium butyricum (1)
Clostridium histolyticum (1)
Clostridium septicum (1)
Enterobacter aerogenes (1)
Helicobacter pylori (1)
Porphyromonas asaccharolytica (1)
Rotavirus (1)
Shigella dysenteriae (1)
Staphylococcus aureus (1)
Streptococcus faecalis (1)
Staphylococcus aureus
Vibrio cholerae (1)

The Premier Toxins A&B test was also evaluated using several reference strains of *C. difficile*. Strains were grown in BHI broth for 48 hours and tested for reactivity with the Premier Toxins A&B test. Results summarized below, indicated that the test correctly identified all toxigenic strains of *C. difficile*, even those producing only toxin B. No cross-reactivity was observed with non-toxigenic strains of *C. difficile*.

C. difficile Type	# of Premier Toxins A&B Pos / Total (% Correct)
A^+/B^+	25/25 (100%)
A^-/B^+	3/3 (100%)
A^-/B^-	0/14 (100%)

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APPENDIX E. Protocol for Meridian Premier™ Toxins A&B

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ImmunoCard® Toxins A & B

A Rapid Enzyme Immunoassay for the Detection of *Clostridium difficile* Toxins A and B in Stool Specimens

REF *Catalogue # 712050*

IVD *invitro diagnostic device*

INTENDED USE

ImmunoCard® Toxins A & B is a rapid, qualitative, horizontal-flow enzyme immunoassay (EIA) for detecting *Clostridium difficile* Toxins A and B in human stool. This assay is used as an aid in the diagnosis of *C. difficile*-associated disease.

SUMMARY AND EXPLANATION OF THE TEST

Toxigenic *Clostridium difficile* is the leading cause of nosocomial infectious diarrhea in developed countries. An estimated 300,000 cases of *C. difficile* associated disease (CDAD) are seen per year in U.S. hospitals alone. (1,2) *Clostridium difficile* is the etiologic agent in approximately 25% of all cases of antibiotic-associated diarrhea. Virtually any antibiotic can predispose a patient to CDAD. The clinical presentation for CDAD ranges from asymptomatic colonization to life-threatening pseudomembranous colitis and toxic megacolon. (2) Most pathogenic strains of *C. difficile* produce two biologically and immunologically distinct toxins: toxin A (enterotoxin) and toxin B (cytotoxin). Toxin A was once thought to be responsible for most of the pathology seen in human CDAD until reports of clinically relevant disease caused by strains of *C. difficile* that produce only toxin B began to appear in the late 1990's. (1) The most accurate assay overall for the detection of *C. difficile* is the cytotoxin assay, however the method requires tissue culture facilities, 48-72 hour incubation and is not standardized. (2) The use of a rapid test such as ImmunoCard Toxins A&B enables the physician to verify infection quickly, begin proper treatment and to initiate enteric isolation precautions in a hospital setting. (2)

PRINCIPLE OF THE TEST

ImmunoCard Toxins A & B consists of a membrane held in a plastic frame with two sample ports and two reaction ports. The membrane carries immobilized antibodies to toxins A and B. The Enzyme Conjugate Reagent consists of antibodies to toxins A and B coupled to horseradish peroxidase. To perform the test, patient stool sample is diluted with Specimen Diluent and Enzyme Conjugate and the mixture is incubated for 5 minutes. During the incubation, molecules of toxin, if present, are bound to the anti-toxin antibodies of the Conjugate. Following incubation, an aliquot of the mixture is added to each of the two sample ports and the test is incubated for an additional 5 minutes at 20-26 C. During the second incubation the toxin-conjugate complex is separated from particulate matter as the fluid portion of the sample flows through the membrane to the TEST and CONTROL reaction ports. The toxin-conjugate complexes are then captured at the TEST reaction port by immobilized antitoxin in the reaction membrane. (The second of the two reaction ports serves as an internal control.) Both reaction ports are subsequently washed with Wash Reagent to reduce interference by contaminating proteins before Substrate Reagent is added. The reaction ports are incubated for an additional 5 minutes during which time the enzyme conjugate modifies the Substrate Reagent. The result is the appearance of a blue color. Reactions are read visually. Development of a blue color in the TEST reaction port indicates a positive test. In the CONTROL port, the anti-toxin antibodies of the conjugate bind directly to the immobilized toxin. The appearance of blue in the CONTROL reaction port indicates that sample was added, that reagents were active at the time of use and that proper sample migration occurred.

REAGENTS:

ImmunoCard Toxins A & B Test Card: A membrane pad housed in a plastic frame and enclosed in a foil pouch with a desiccant. The pad carries immobilized monoclonal anti-toxin A and goat polyclonal anti-toxin B at the TEST reaction port and *C. difficile* toxin at the CONTROL reaction port. Store the test cards at 2-8 C when not in use.

Sample Diluent: A buffered protein solution containing thimerosal (0.02%) as a preservative. Store at 2-8 C when not in use.

Positive Control: Inactivated crude *C. difficile* toxin in a buffered solution containing thimerosal (0.02%) as a preservative. The reagent is supplied ready for use. Store at 2-8 C when not in use.

Enzyme Conjugate: A blend of goat polyclonal antibodies to toxins A and B conjugated to horseradish peroxidase and suspended in a buffered protein solution containing thimerosal (0.02%). Store at 2-8 C when not in use.

Wash Reagent: A buffered solution containing thimerosal (0.02%) as a preservative. Store at 2-8 C when not in use.

Substrate Reagent: A buffered solution containing tetramethyl-benzidine. Store at 2-8 C when not in use.

MATERIALS PROVIDED

1. ImmunoCard Toxins A & B Test Cards, in individual foil pouches with desiccants.
2. Sample Diluent, in a plastic dropper vial. Use as supplied.
3. Positive Control, in a plastic dropper vial. Use as supplied.
4. Wash Reagent, in a plastic dropper vial. Use as supplied.
5. Substrate Reagent, in a plastic dropper vial. Use as supplied.
6. Enzyme Conjugate, in plastic dropper vial. Use as supplied.
7. Plastic transfer pipettes with measuring marks for 25 µL and 150 µL.

MATERIALS NOT PROVIDED

1. Human stool specimens
2. Disposable latex gloves that should be used during the handling of the fecal samples as they are considered potentially biologically hazardous material
3. Vortex for suspending of the stool specimen in the Sample Diluent
4. Interval timer
5. Applicator sticks
6. Small test tubes, such as 10 x 75 mm or 12 x 75 mm

PRECAUTIONS

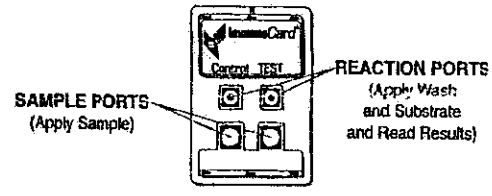
1. **All reagents are for *in vitro* diagnostic use only.**
2. **Do not store at temperatures above 2-8 C. Do not freeze.**
3. **Do not deviate from the established insert method or falsely positive or falsely negative results may occur.**
4. **Some patient specimens contain infectious agents; therefore all patient specimens should be handled and disposed of as if they are biologically hazardous.**
5. **DO NOT interchange the Test Card, Enzyme Conjugate and Positive Control reagents from different kit lot numbers. The other reagents (Sample Diluent, Wash Reagent, Substrate Reagent) can be interchanged between kits providing the reagents are within their expiration periods. Do not use any reagent beyond its labeled expiration date.**
6. **All stool samples must be mixed thoroughly before testing, regardless of consistency, to ensure a representative sample prior to testing.**
7. **Failure to bring specimens and reagents to room temperature (20-26 C) before testing may decrease assay sensitivity.**
8. **Inspect Test Cards before removing the foil pouch. Do not use Test Cards that have holes in the foil pouch or where the pouch has not been completely sealed. False-negative reactions may result due to deterioration of the improperly stored Test Cards.**
9. **Do not use the Sample Diluent or Positive Control if they are discolored or turbid. Discoloration or turbidity may be a sign of microbial contamination. It is normal for the Wash Reagent to appear slightly turbid.**
10. **The Substrate Reagent is light sensitive and should be stored in the dark. The appearance of the reagent should be clear and colorless. If the reagent exhibits any color or is turbid it should be discarded.**
11. **Hold reagent vials vertically when dispensing drops to ensure consistent drop size and delivery.**

SPECIMEN COLLECTION AND PREPARATION

The stool specimen should be transported in an airtight container and stored at 2-8 C until tested. The specimen should be tested as soon as possible, but may be held up to 4 days at 2-8 C prior to testing. If testing cannot be performed within this time frame, specimens should be frozen immediately on receipt and stored frozen (≤ -20 C) until tested. Specimens that are frozen and thawed up to three times are acceptable for use.

PROCEDURAL NOTES

1. The ImmunoCard® test card format is diagrammed below:

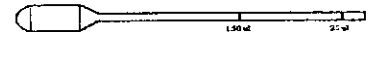


2. Batch processing of any number of samples or controls is possible provided that, for each card, the appropriate steps, sequence of reagent addition, incubation (wait) and result reading times are maintained. Each procedural step should be completed with each sample before the next step is started.
3. The **Control** (left) side of each card provides an internal control for each specimen. This tests the individual specimen for sufficient dilution and reagent performance.

TEST PROCEDURE

1. Bring all test cards, reagents and samples to room temperature (20-26 C) before testing. Remove the reagents from the kit box to warm. **Reagents may take up to 60 minutes to warm following refrigeration. RETURN TO 2-8 C IMMEDIATELY AFTER USE.**
2. Label one test tube for each patient specimen to be tested.
3. Add 200 µL of Specimen Diluent to a test tube using the dropper in the bottle.
4. Add three drops of Enzyme Conjugate (150 µL) to each tube.
5. Immediately add stool or control sample as follows:

5a. **Liquid/Semi-solid stools** – Mix the stool specimen thoroughly. Using a clean transfer pipette (included with the kit), draw the stool to the first mark from the end of the tip (25 µL). Dispense the stool into the Diluent/Conjugate mixture. Use the same pipette to mix the stool by gently aspirating, then dispensing the suspension several times. Vortex the final mixture for 10 seconds. Leave the pipette in the tube for use later in the test.*



5b. **Formed/Solid stools** – Mix the stool specimen thoroughly. Using a woode applicator stick, transfer a small portion (approximately 2 mm diameter) of th stool into the Diluent/Conjugate mixture. Emulsify the specimen using th applicator stick, and then thoroughly mix with a vortex for 10 seconds. Add pipette to the tube and leave for later use in testing.*

5c. **Optional control:** Using the container droppers, add 1 drop of Positiv Control or Negative Control (Specimen Diluent) (provided with the kit) to th Diluent/Conjugate mixture. Vortex 10 seconds. Add a pipette to the tube ar leave for later testing.

***NOTE: The transfer of too little specimen, or failure to mix and suspend th specimen in the Diluent/Conjugate mixture completely may result in a fals**

negative test result. Additional of too much stool specimen may cause invalid results due to restricted sample flow.

6. Let each diluted specimen stand for 5 ± 1 minutes at 20-26 C.
7. Use one ImmunoCard Test Card for each sample or control.
8. Remove the ImmunoCard Test Card from its foil pouch. Discard the pouch and desiccant. Label the device with the name of the patient or the control.
9. Vortex each specimen or control for 10 seconds before use.
10. Add 150 µL of each diluted specimen or control to each of the two sample ports on one test card. (150 µL represents the second mark on the transfer pipette.)
11. Incubate for 5 ± 1 minutes at 20-26 C. NOTE: At the end of incubation both reaction ports must appear completely wet. If either reaction port is not completely wet, discard the Test Card and repeat the procedure. Proceed with testing if sample remains in the sample ports yet both reaction ports are completely wetted.
12. Hold the Wash Reagent vial vertically and dispense exactly 3 drops to each of the reaction ports.
13. When the Wash Reagent has been completely absorbed, hold the vial vertically and add exactly three drops of Substrate Reagent.
14. Incubate the test card at 20-26 C for 5 minutes.
15. **Visually read the results of each card within 30 seconds of the end of incubation.**

INTERPRETATION OF RESULTS

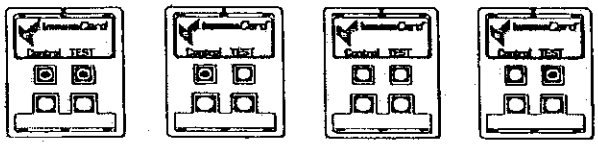


Figure 1 Positive Result | Figure 2 Negative Result | Figure 3 Invalid Result | Figure 4 Invalid Result

READ ONLY UPPER REACTION PORTS TO INTERPRET RESULTS

NOTE: Disregard color development in the sample ports. The color of the sample ports is not evaluated during testing.

Positive test result: (Figure 1) Blue color in the TEST (upper right) and CONTROL (upper left) reaction ports. The intensity of the blue color of the TEST port may vary from the bottom to the top of port. A positive test result indicates that toxin A and/or B is/are present in the sample. PLEASE NOTE: The TEST port may appear less blue than the CONTROL port.

Negative test result: (Figure 2) Blue color in the CONTROL reaction (upper left) port only. The TEST reaction (upper right) port should be colorless. (The wetting of the membrane may make the TEST port appear to be slightly grey.) A negative test result indicates that *C. difficile* toxins are absent or below the limit of detection of the assay. Occasionally the TEST PORT (upper right) may show evidence of a hint of blue color in the left or right side of the port, with the rest of the port colorless. This should be considered a negative test result.

Invalid test results:

1. No detectable blue color in the CONTROL reaction port (Figures 3 and 4). Failure of the CONTROL invalidates any test result. The invalid test may be due to the failure of a reagent or the Test Card to perform properly, failure to add sample, failure of the sample to migrate, or failure to dilute the specimen correctly leading to over inoculation of the Test Card. Samples with extremely high levels of toxins may also present with a positive TEST yet negative CONTROL port.
2. A blue ring on the plastic frame surrounding the TEST (upper right) port during the test procedure.

When invalid results are obtained, redilute the sample and repeat the test on a new card.

QUALITY CONTROL

Each Test Card contains an internal control. The appearance of a blue color in the CONTROL reaction port verifies the assay was active at the time of use, that sample was added and that there was adequate migration of the sample.

The reactivity of ImmunoCard Test Cards should be verified on receipt using external positive and negative controls samples. Positive Control reagent is supplied with the kit. The control is used to verify the reactivity of the other reagents associated with the assay, and is not intended to ensure precision at the analytical assay cut-off. Sample Diluent is used for the negative control. Additional tests can be performed with the controls to meet the requirements of local, state and/or federal regulations and/or accrediting organizations. The results expected with the Controls are described in the section on INTERPRETATION OF RESULTS. The Test Card should not be used if control tests do not produce the correct results. Proper results obtained with the Control Port, Positive Control and negative control (Sample Diluent) serve as indicators that the test was performed correctly, that the antibodies embedded in the membrane and the Enzyme Conjugate are active at the time of testing, and that the membrane supports proper sample flow. Failure of the internal and external control to produce the expected results suggests the test was not performed correctly (ie, incorrect volume of reagents added; incorrect incubation temperature or times used or that reagents were not brought to room temperature prior to testing). Repeat the control tests as the first step in determining the root cause of the failure.

LIMITATIONS OF THE PROCEDURE

1. The test is qualitative and no quantitative interpretation should be made with respect to the intensity of the color when reporting the result.
2. A positive test confirms the presence of toxins A and/or B only. A physician must use the test results in conjunction with other diagnostic procedures and the patient's clinical condition to establish a diagnosis of *C. difficile*-associated disease. The levels of toxin do not always correlate directly with the presence or severity of disease. (1)
3. Isolates of *C. sordellii* may react with this test due to the immunological identity of the *C. sordellii* toxins. (3)
4. The performance of specimens from pediatric patients has not been evaluated.
5. Two distinct groups have been identified that can harbor *C. difficile* asymptomatically at very high rates. Colonization rates of up to 50% and higher have been reported in infants (3) and rates of up to 32% in cystic fibrosis patients (4).

EXPECTED VALUES

The frequency of antibiotic-associated diarrhea caused by C. difficile is dependent on several factors, including patient population, type of institution and epidemiology. The reported incidence of C. difficile-associated disease in patients suspected of having antibiotic-associated dieases is 15-20%, although different facilities may find positive rates above or below this range. (2, 3, 5) A positive result in an uninfected patient is not expected.

SPECIFIC PERFORMANCE CHARACTERISTICS

Comparative studies

Three independent laboratories tested specimens with ImmunoCard Toxins A&B and the standard cell cytotoxicity assay with neutralization. The results of the parallel tests are given below.

Prospective Samples	ICTAB		
	Pos	Neg	Total
Cytotoxin Pos (Std)	67	5	72
Cytotoxin Neg (Std)	2	176	178
Total	69	181	250

		95% CI
Clinical ssensitivity	67/72 (93.1%)	87.1-98.9%
Clinical specificity	176/178 (98.9%)	97.6-100%
Predictive value positive test	67/69 (97.1%)	92.9-100%
Predictive value negative test	176/181 (97.2%)	94.5-99.5%

Retrospective Samples	ICTAB		
	Pos	Neg	Total
Cytooxin Pos (Std)	33	0	33
Cytotoxin Neg (Std)	5	303	308
Total	38	303	341

		95% CI
Clinical sensitivity	33/33 (100%)	N/A
Clinical specificity	303/303 (98.4%)	96.4-99.6%
Predictive value positive test	33/38 (86.8%)	76.2-97.8%
Predictive value negative test	303/303 (100%)	N/A

Analytical sensitivity

The lower limit of detection of this assay is approximately 3 ng of toxin A and 3 ng of Toxin B per mL of stool. This limit does not vary from solid to liquid/semi-solid stool.

Reproducibility

The reproducibility of ImmunoCard Toxins A & B was determined with known negative (n=2) and positive (n=6) samples that were coded and randomly sorted to prevent their identification during testing. Two of the positive samples were near the limit of detection for the assay. Three laboratories tested the samples on three consecutive days. The samples produced the expected results 100% of the time.

Analytical specificity

Stool specimens inoculated with the following microbial agents (to a final sample concentration of ≥ 1 X 10^6 organisms/mL) do not react with ImmunoCard Toxins A and B: Adenovirus 40, Adenovirus 41, *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis Bacteroides fragilis, Campylobacter coli, Campylobacter jejuni, Candida albicans Clostridium butyricum, nontoxigenic C. difficile (10 strains), Clostridium perfringens Clostridium septicum, Clostridium sordellii strain 9714, Clostridium sporogenes Enterobacter cloacae, Escherichia coli (four strains including two O157:H7 strains) Helicobacter pylori, Klebsiella pneumoniae, Peptostreptococcus anaerobius Porphyromonas asaccharolytica, Proteus vulgaris, Pseudomonas aeruginosa,* Rotavirus *Salmonella typhimurium, Serratia liquefaciens, Shigella dysenteriae, Shigella flexneri Shigella sonnei, Staphylococcus aureus, Staphylococcus aureus Cowan I Staphylococcus epidermidis, Streptococcus faecalis, Vibrio parahaemolyticus, Yersinia enterocolitica.* The only non-C. *difficile* microorganism reactive with ImmunoCard Toxin A & B was *C.sordellii* VPI 9048. This strain produces toxin A and B homologs HT and LT.

Interfering substances

The following substances had no effect on test results when present in stool in th concentrations indicated: fecal fat (4.8% w/v), whole blood (40% v/v), mucin (3.5% w/v Metronidazole (0.25% w/v), Vancomycin (0.25% w/v), barium sulfate (5% w/v), Imodiu AD® (5% w/v), Kaopectate® Caplets (5 mg/mL) Pepto Bismol® (5% v/v).

Additional information regarding the performance characteristics of this product can b obtained by contacting Meridian's Technical Support Department at (513) 271-3700.

ITALIANO

ImmunoCard® Toxins A &E

Test rapido Immunoenzimatico per la ricerca delle tossine A e B di Clostridiu difficile in campioni fecali

REF *Catalogo # 712050*

IVD *solo per uso diagnostico in vitr*

APPENDIX F. Protocol for the Immuno*Card*® Toxins A&B

 211

remel

x/pect™ Clostridium difficile Toxin A/B

INTENDED USE

REMEL's Xpect™ Clostridium difficile Toxin A/B test kit is a rapid *in vitro* immunochromatographic test for the direct, qualitative detection of *Clostridium difficile* Toxin A and/or B in human fecal specimens from patients suspected of having *Clostridium difficile*-associated disease (CDAD). The test is intended for use as an aid in diagnosis of CDAD. The test can also be used for confirmation of toxigenic *Clostridium difficile* from Brain Heart Infusion (BHI) broth culture.

SUMMARY AND EXPLANATION

Clostridium difficile was first described in 1935, but it was not until 1977 that links were made between the organism and disease. *Clostridium difficile* is a toxin-producing, spore-forming anaerobic gram-positive bacillus. The clinical presentation of *C. difficile* infection includes, in increasing order of severity, asymptomatic carriers, antibiotic-associated colitis, pseudomembranous colitis (PMC), and fulminant colitis.[1] A reduction of the normal microbial flora in the colon, usually caused by antibiotic therapy, allows overgrowth of *C. difficile*. Symptoms of antibiotic-associated colitis usually begin four to ten days after antibiotic treatment has begun.

Most pathogenic strains of *C. difficile* produce two toxins, Toxin A (enterotoxin) and Toxin B (cytotoxin), which are the main virulence factors for the organism. There appears to be a cascade of events, which result in the expression of the activity of these toxins. Toxin A is mildly cytopathic but induces large fluid shifts and mucosal inflammation. Toxin B is intensely cytopathic but its role in the disease process is not clearly understood. Variant strains which are Toxin A-negative, Toxin B-positive are known to exist, are fully pathogenic, and capable of producing the full spectrum of disease. The prevalence of these variant strains varies widely by institution and geographic location.[2,3,4,5,6,7]

Clostridium difficile-associated disease (CDAD) primarily occurs in hospitalized patients.[8,9] Individuals with CDAD shed spores in the stool, which can survive for as long as five months in the environment. Clinical and pathological features of CDAD are not easily distinguished from those of other gastrointestinal diseases, including ulcerative colitis, chronic inflammatory bowel disease, and Crohn's disease. Infection with toxigenic *C. difficile* is a potentially life-threatening disease process; however, when properly treated, patient mortality rates are low. Thus, rapid diagnosis allowing clinicians to initiate appropriate therapy and implement adequate measures to control nosocomial spread is important.

PRINCIPLE

The Xpect™ Clostridium difficile Toxin A/B test is a qualitative immunochromatographic assay that detects *C. difficile* Toxin A and Toxin B in stool specimens or cultures of toxigenic *C. difficile*. In performing the test, a specimen is first diluted with Specimen Diluent to help solubilize the toxins. A portion of the diluted sample is then mixed with a volume of Conjugate 1 containing antibodies to Toxin A and Toxin B coupled to colored microparticles, plus a volume of Conjugate 2 containing biotinylated antibodies to Toxin A and Toxin B. A volume of this mixture is transferred to a test device having immobilized streptavidin as a test line and goat anti-immunoglobulin antibody as a control line. Immunocomplexes of toxin and conjugated antibodies form a visible band as they flow across the test line. Excess colored particle conjugates form a visible band at the control line to document that the test is functioning properly.

STORAGE

Store test devices in sealed foil pouches at 2-30°C (room temperature or refrigerated). Store all kit reagent bottles and vials at 2-8°C. Do not freeze or overheat. Allow components to come to room temperature before use. Mix bottled reagents gently prior to use. Return the unused reagents to the refrigerator after use.

PRECAUTIONS

1. For *In Vitro* Diagnostic Use.
2. Standard precautions should be taken against the dangers of biological hazards by properly sterilizing specimens, containers, and test devices after their use. Consult appropriate references when necessary.[10]
3. Directions should be read and followed carefully.
4. Reagents are provided at the necessary working strength and are to be dispensed directly from the dropper bottles. Do not dilute reagents.
5. Do not interchange reagents between kits of different lots.
6. Do not use reagents beyond the printed expiration dates.
7. Microbial contamination of reagents may decrease the accuracy of the assay.

SPECIMEN COLLECTION, STORAGE AND TRANSPORTATION

Specimens should be collected in clean, airtight, leak-proof containers.

- Fresh, untreated stool specimens should be stored at 2-8°C and tested within 72 hours of collection. If fresh specimens cannot be tested within 72 hours, they should be frozen at -20°C or below in a non-defrosting freezer and tested within 2 months of collection. Avoid multiple freeze-thaw cycles.
- Fresh specimens diluted in the Sample Diluent provided in the kit can be stored refrigerated for up to 24 hours prior to testing.
- Stool specimens that have been concentrated or collected in Formalin, SAF, PVA, or Cary Blair are not suitable for use with this test.

REAGENTS AND MATERIALS SUPPLIED — 20 Tests

1. **Test Devices (20):** Each foil pouch contains one single-use test device with desiccant; membrane is striped with capture reagents
2. **Conjugate Reagent 1** (3.0 ml): Blue-black micro-particles coated with mouse anti-toxin A and rabbit anti-Toxin B
3. **Conjugate Reagent 2** (3.0 ml): Biotinylated goat anti-Toxin A and rabbit anti-Toxin B
4. **Specimen Diluent** (12.0 ml): Buffered solution with 0.03% ProClin™ 300
5. **Positive Control** (2.0 ml): Culture supernatant containing *C. difficile* Toxin A and B with preservative
6. **Negative Control** (2.0 ml): Buffered solution with preservative
7. **Disposable Transfer Pipettes** (40): Pipettes with marked graduations at 0.1 ml increments
8. **Dilution Tubes** (40): Tubes for specimen preparation with marked 0.5 ml volume
9. **Wooden Applicator Sticks** (20)
10. **Instructions for Use** (1)

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Stool specimen collection container(s)
2. Timer
3. BHI Broth (optional)

PROCEDURE

1. Allow kit components and stool specimens to come to room temperature before use.
2. Mix stool specimens **thoroughly** prior to testing (regardless of consistency).
3. Remove the test device from the foil pouch when ready to perform the test and place it on a flat surface.
4. Label the device (and dilution tubes) with patient or control identification.

Sample Preparation:

Stool Specimens

1. Add Specimen Diluent up to the line marked on the dilution tube (0.5 ml).
   a) For **solid** stool specimens, use a wooden applicator stick and sample from separate areas of the specimen. Transfer a 0.2 g (6-8 mm diameter) portion of stool to the dilution tube.
   b) For **semi-solid** or **liquid** stool specimens, use a transfer pipette to transfer 0.2 ml (second graduated mark from the tip) of specimen to the dilution tube.
2. Mix **thoroughly** and allow large particulates to settle.
3. Use a transfer pipette to dispense 0.1 ml (first molded graduated mark from the tip) of this preparation into another dilution tube.

BHI Broth Culture:

1. Use a transfer pipette to dispense 0.1 ml (first molded graduated mark from the tip) of a 72-hour BHI broth culture with suspected *Clostridium difficile* into a dilution tube.

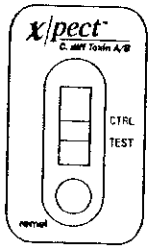
Test Procedure:

1. Add 0.1 ml (5 drops) of Conjugate Reagent 1 to the dilution tube containing the diluted specimen.
2. Add 0.1 ml (5 drops) of Conjugate Reagent 2 to the dilution tube containing the diluted specimen.
3. Mix the contents of the tube thoroughly.
4. Use a transfer pipette to dispense 0.2 ml (second graduated mark from the tip) of sample into the circular sample well of the test device.
5. Read and record the test results visually after 20 minutes according to the INTERPRETATION section. (Strong positive results may be apparent sooner than 20 minutes.). Results are invalid beyond 20 minutes.

INTERPRETATION OF THE TEST

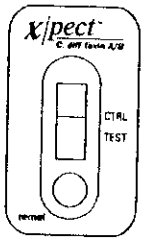
Positive Result (Toxin A and/or B present):

A positive test is indicated by two black-colored lines of any intensity; one in the TEST region and one in the control (CTRL) region. A positive test indicates the presence of *Clostridium difficile* Toxin A and/or Toxin B in the sample.



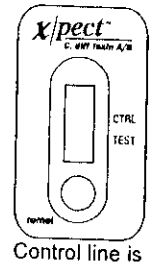
Negative Result (Toxin A and/or B not detected):

A negative test is indicated by only one black-colored line in the control (CTRL) region. A negative test indicates that *Clostridium difficile* Toxin A and/or Toxin B are absent or below the detection limit of the test.

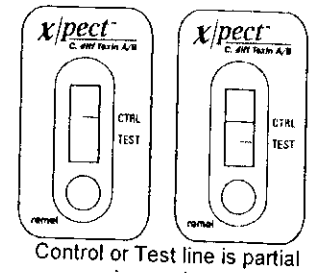


Invalid Result:

An invalid result occurs when the TEST line is partial or incomplete, or the control (CTRL) line is absent or incomplete at 20 minutes.



Control line is absent



Control or Test line is partial or incomplete

If invalid results occur due to restricted flow of the sample along the membrane, the specimen should be re-tested as follows:

Re-test Specimen Preparation

Further dilute the specimen as follows:

1. Combine, in a clean dilution tube:
   a) 0.1 ml (5 drops) of Specimen Diluent, and
   b) 0.1 ml (first molded graduated mark from the tip) of specimen from the initial specimen preparation in Specimen Diluent (without reagents added).
2. Mix the preparation by aspirating with the transfer pipette.
3. Use 0.1 ml of this diluted specimen preparation and repeat the Test Procedure.

QUALITY CONTROL

Internal: A procedural control is included in the test. The appearance of a control line in the CTRL region verifies that an intact conjugate has been added to the device, that the control line antibody is functionally active, and that adequate capillary flow has occurred. A clear background in the results area is considered an internal negative control. If the test has been performed correctly and reagents are working properly, the background will clear to give a discernible result.

External: The Positive and Negative Quality Controls provided with the kit should be run with each shipment and new kit lot number received. The Positive Control is used to verify reactivity of the reagents associated with the assay, and is not intended to ensure precision at the analytical assay cut-off. Each laboratory should follow their state and local requirements. To use, add 0.1 ml (5 drops) of the control to a dilution tube and process in accordance with Test Procedure outlined above (do not dilute controls with specimen diluent). If aberrant quality control results are noted, patient results should not be reported.

LIMITATIONS

1. A positive test does not define the presence of disease. The test detects the presence of Toxin A and/or Toxin B in fecal specimens. Results should be used in conjunction with other clinical findings to establish a diagnosis.
2. As with all *in vitro* diagnostic tests, a negative test result does not exclude the possibility of the presence of *C. difficile* Toxin A or Toxin B. This may occur when the toxin level in the sample is below the detection level of the test. The level of toxin has not been shown to correlate with either the presence or severity of disease. Test results should be interpreted by a physician in light of other laboratory results and clinical findings.
3. *Clostridium sordellii* is not a common inhabitant of the human intestine but does produce a toxin (HT) that has biological, physiochemical, and immunochemical properties similar to *Clostridium difficile* Toxin A. This similarity may cause cross-reactivity in any diagnostic test that detects *C. difficile* Toxin A and/or B.[11]

4. Proper specimen collection and processing are essential to achieve optimal performance of the test. See Specimen Collection, Storage and Transportation section.
5. The performance characteristics of this test have not been fully evaluated in pediatric populations.
6. A high dose Hook Effect was not observed during the BHI broth culture study in which *Clostridium difficile* was evaluated after growing in pure culture for 72-hours.

EXPECTED VALUES

Community-acquired CDAD cases are recognized, but the incidence is low (<1 case per 10,000 antibiotic prescriptions). This may be because diagnostic testing is not performed often enough in outpatient settings to detect CDAD. *C difficile* colitis occurs at a much higher frequency in patients who are hospitalized and is the fourth most common nosocomial disease reported to the Centers for Disease Control and Prevention. *C. difficile* is responsible for 20-30% of antibiotic-associated diarrhea and more than 90% of pseudomembranous colitis. The incidence rate of nosocomial CDAD may vary with hospital populations and is influenced by the presence of predisposing factors, such as increased patient age, type and duration of antimicrobial therapy, severity of underlying illness(es), and length of hospital stay. *C. difficile* is found in 3-5% of healthy adults and up to 50% of infants and young adults that asymptomatically carry both the bacteria and its toxins.[12] An overall prevalence rate of 16% was observed when the Xpect™ C. difficile Toxin A/B was evaluated in a prospective study conducted at four independent laboratories in the United States.

PERFORMANCE CHARACTERISTICS

Clinical Accuracy:

The performance of the Xpect™ Clostridium difficile Toxin A/B was evaluated at four geographically diverse regions of the United States. A total of eight hundred fifteen specimens were tested with the Xpect™ Clostridium difficile Toxin A/B test and compared to results obtained from the cytotoxin assay (CTA).

Overall		CTA Results	
		+	-
Xpect™ Results	+	132	25
	-	21	637
	TOTAL	153	662

Sensitivity: 86.3% (95% CI = 79.8-91.3%)
Specificity: 96.2% (95% CI = 94.5-97.5%)
Positive Predictive Value: 84.1% (95% CI = 77.4-89.4%)
Negative Predictive Value: 96.8% (95% CI = 95.2-98.0%)
% Correlation: 94.4% (95% CI = 92.5-95.8%)

Note : CI = Confidence Interval

Discordant results were further investigated by toxigenic culture and microwell enzyme immunoassay that detects both Toxin A and B. Four of 25 specimens that were cytotoxin negative and Xpect™ Clostridium difficile Toxin A/B positive on initial testing were positive by toxigenic culture and enzyme immunoassay. Ten of 21 (47.6%) specimens that were CTA positive and Xpect™ Clostridium difficile Toxin A/B negative on initial testing were negative by toxigenic culture and microwell enzyme immunoassay.

Performance Compared to Commercially Available Devices:

The Xpect™ C. difficile Toxin A/B test was also compared to two commercially available products. Each of the four clinical trial sites tested a chromatographic membrane assay that detects Toxin A only (Predicate Device), the Xpect™ C. difficile Toxin A/B test, and cytotoxin assay (CTA) for each sample. In addition, one clinical trial site tested a microwell enzyme immunoassay for the detection of both Toxin A and B for each sample. The results presented below are calculated using CTA as the reference.

Performance of Devices Compared to CTA

	n = 815		n = 267	
	Xpect™ C. difficile Toxin A/B	Predicate Device	Xpect™ C. difficile Toxin A/B	EIA
Sensitivity	86.3%	62.7%	91.0%	80.6%
Specificity	96.2%	98.8%	98.0%	97.5%

BHI Broth Culture Performance:

An in-house study was conducted using twenty-one known reference strains and thirty-six suspect *Clostridium difficile* isolates from stool specimens. BHI broth cultures were tested with the Xpect™ Clostridium difficile Toxin A/B test following 72-hours incubation. Under these conditions, the BHI broth culture of *Clostridium sordellii* ATCC® 9714 produced a positive reaction. There was 94.7% (54/57) agreement with expected values.

Analytical Sensitivity:

The analytical sensitivity was evaluated using purified *C. difficile* Toxin A and Toxin B. The Xpect™ C. difficile Toxin A/B test detects Toxin A at levels of $\geq$ 6.25 ng/ml (0.12 ng/test) and Toxin B at levels of $\geq$ 40.0 ng/ml (0.76 ng/test).

Cross-Reactivity:

Fifty-four microorganisms were evaluated with the Xpect™ C. difficile Toxin A/B test. No cross-reactivity was observed. Bacteria and yeast isolates were tested at 10^8 colony-forming units per ml concentration. Viral isolates were tested at concentrations of 10^4 to 10^5 $TCID_{50}$ (tissue culture infectious dose) per ml concentration. The following organisms were tested in the Xpect™ C. difficile Toxin A/B test.

Aeromonas hydrophila
Bacillus cereus
Bacillus subtilis
Bacteroides fragilis
Campylobacter coli
Campylobacter fetus subsp. *fetus*
Campylobacter jejuni subsp. *jejuni*
Campylobacter lari
Candida albicans
Clostridium botulinum (toxin 20 µg/ml)
Clostridium beijerinickii
Clostridium difficile (non-toxigenic)
Clostridium haemolyticum
Clostridium histolyticum
Clostridium innocuum
Clostridium novyi
Clostridium perfringens
Clostridium septicum
Clostridium sordellii
Clostridium sporogenes
Clostridium subterminale
Clostridiur.. tetani
Enterobacter aerogenes
Enterobacter cloacae
Enterococcus faecalis
Enterococcus faecium
Escherichia coli
Klebsiella pneumoniae
Peptostreptococcus anaerobius
Porphyromonas asaccharolytica
Proteus mirabilis
Proteus vulgaris
Pseudomonas aeruginosa
Salmonella Typhimurium
Serratia liquefaciens
Shigella boydii
Shigella dysenteriae
Shigella flexneri
Shigella sonnei
Staphylococcus aureus (Cowan)
Staphylococcus epidermidis
Vibrio cholerae
Vibrio parahaemolyticus
Yersinia enterocolitica
Giardia intestinalis
Entamoeba histolytica
Adenovirus type 2
Adenovirus type 40
Adenovirus type 41
Coxsackievirus B4
Cytomegalovirus
Echovirus (type 22)
Enterovirus (type 69)
Rotavirus

Interfering Substances:

The following substances were tested with the Xpect™ C. difficile Toxin A/B test and no interference was observed in the assay for any substance tested at the indicated levels: blood, mucous, fecal fat, Pepto-Bismol® (10%v/v), Imodium® AD (10%v/v), Kaopectate® (10%v/v), Castoria® (10%v/v), vancomycin (12.5 mg/ml), metronidazole (12.5 mg/ml), and barium sulfate (12.5 mg/ml).

Reproducibility:
Reproducibility testing was conducted at three sites, including one in-house site, on four separate days with six blinded samples. The samples consisted of known positive and negative stool specimens. The samples produced the expected result with the Xpect™ C. difficile Toxin A/B test 98.6% (71/72) of the time.

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PACKAGING
REF 24650, Xpect™ Clostridium difficile Toxin A/B.....20 Tests/Kit

SYMBOL LEGEND

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

IFU 24650, Revised November 17, 2004 PN 4889-A Printed in U.S.A.

12076 Santa Fe Drive, Lenexa, KS 66215, USA
General Information: (800) 255-6730 Technical Service: (800) 447-3641 Order Entry: (800) 447-3635
Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128
Website: www.remel.com Email: remel@remel.com

APPENDIX G. Protocol for the X/pect™ Clostridium difficile Toxin A/B

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Memorandum

From: Reviewer(s) - Name(s) MARIAN HEYLIGER

Subject: 510(k) Number K050891 / S1

To: The Record - It is my recommendation that the subject 510(k) Notification:

- ☐ Refused to accept.
- ☐ Requires additional information (other than refuse to accept).
- ☒ Is substantially equivalent to marketed devices.
- ☐ NOT substantially equivalent to marketed devices.
- ☐ Other (e.g., exempt by regulation, not a device, duplicate, etc.)

Is this device subject to Section 522 Postmarket Surveillance?	☐ YES	☒ NO
Is this device subject to the Tracking Regulation?	☐ YES	☒ NO
Was clinical data necessary to support the review of this 510(k)?	☐ YES	☒ NO
Is this a prescription device?	☒ YES	☐ NO
Was this 510(k) reviewed by a Third Party?	☐ YES	☒ NO
Special 510(k)?	☐ YES	☒ NO
Abbreviated 510(k)? Please fill out form on H Drive 510k/boilers	☐ YES	☒ NO

Truthful and Accurate Statement ☐ Requested ☑ Enclosed

☑ A 510(k) summary OR ☐ A 510(k) statement

☐ The required certification and summary for class III devices

☑ The indication for use form

Regulated under Sec 21 Part 866.2660 of the CFR Microorganism differentiation and identification device

Combination Product Category (Please see algorithm on H drive 510k/Boilers) N

Animal Tissue Source ☐ YES ☑ NO Material of Biological Origin ☐ YES ☑ NO

The submitter requests under 21 CFR 807.95 (doesn't apply for SEs):

☐ No Confidentiality ☐ Confidentiality for 90 days ☐ Continued Confidentiality exceeding 90 days

Predicate Product Code with class: LLH I

Additional Product Code(s) with panel (optional): 83

Reagents, Clostridium difficile toxin

Review: Freddie Jo Poole (Branch Chief) BRANCH (Branch Code) 7/19/05 (Date)

Final Review: for (Division Director) 7/20/05 (Date)

4

Revised:4/2/03

DEPARTMENT OF HEALTH & HUMAN SERVICES — Public Health Service
Food and Drug Administration
Memorandum

From: Reviewer(s) - Name(s) MARIAN HEYLIGER

Subject: 510(k) Number K050891

To: The Record - It is my recommendation that the subject 510(k) Notification:

On Telephone hold
See memo of 5/24/05
& email of 5/31/05

☐ Refused to accept.
☒ Requires additional information (other than refuse to accept).
☐ Is substantially equivalent to marketed devices.
☐ NOT substantially equivalent to marketed devices.
☐ Other (e.g., exempt by regulation, not a device, duplicate, etc.)

Is this device subject to Section 522 Postmarket Surveillance?	☐ YES	☐ NO
Is this device subject to the Tracking Regulation?	☐ YES	☐ NO
Was clinical data necessary to support the review of this 510(k)?	☐ YES	☐ NO
Is this a prescription device?	☐ YES	☐ NO
Was this 510(k) reviewed by a Third Party?	☐ YES	☐ NO
Special 510(k)?	☐ YES	☐ NO
Abbreviated 510(k)? Please fill out form on H Drive 510k/boilers	☐ YES	☐ NO

Truthful and Accurate Statement ☐ Requested ☐ Enclosed
☐ A 510(k) summary OR ☐ A 510(k) statement
☐ The required certification and summary for class III devices
☐ The indication for use form

Combination Product Category (Please see algorithm on H drive 510k/Boilers) _____

Animal Tissue Source ☐ YES ☐ NO Material of Biological Origin ☐ YES ☐ NO

The submitter requests under 21 CFR 807.95 (doesn't apply for SEs):
☐ No Confidentiality ☐ Confidentiality for 90 days ☐ Continued Confidentiality exceeding 90 days

Predicate Product Code with class: Additional Product Code(s) with panel (optional):

Review: Linda A. Yu for Paula BMCB 5/26/05
(Branch Chief) (Branch Code) (Date)

Final Review: Sally Hojvat DMD 5/31/05
(Division Director) (Date)

Revised:4/2/03

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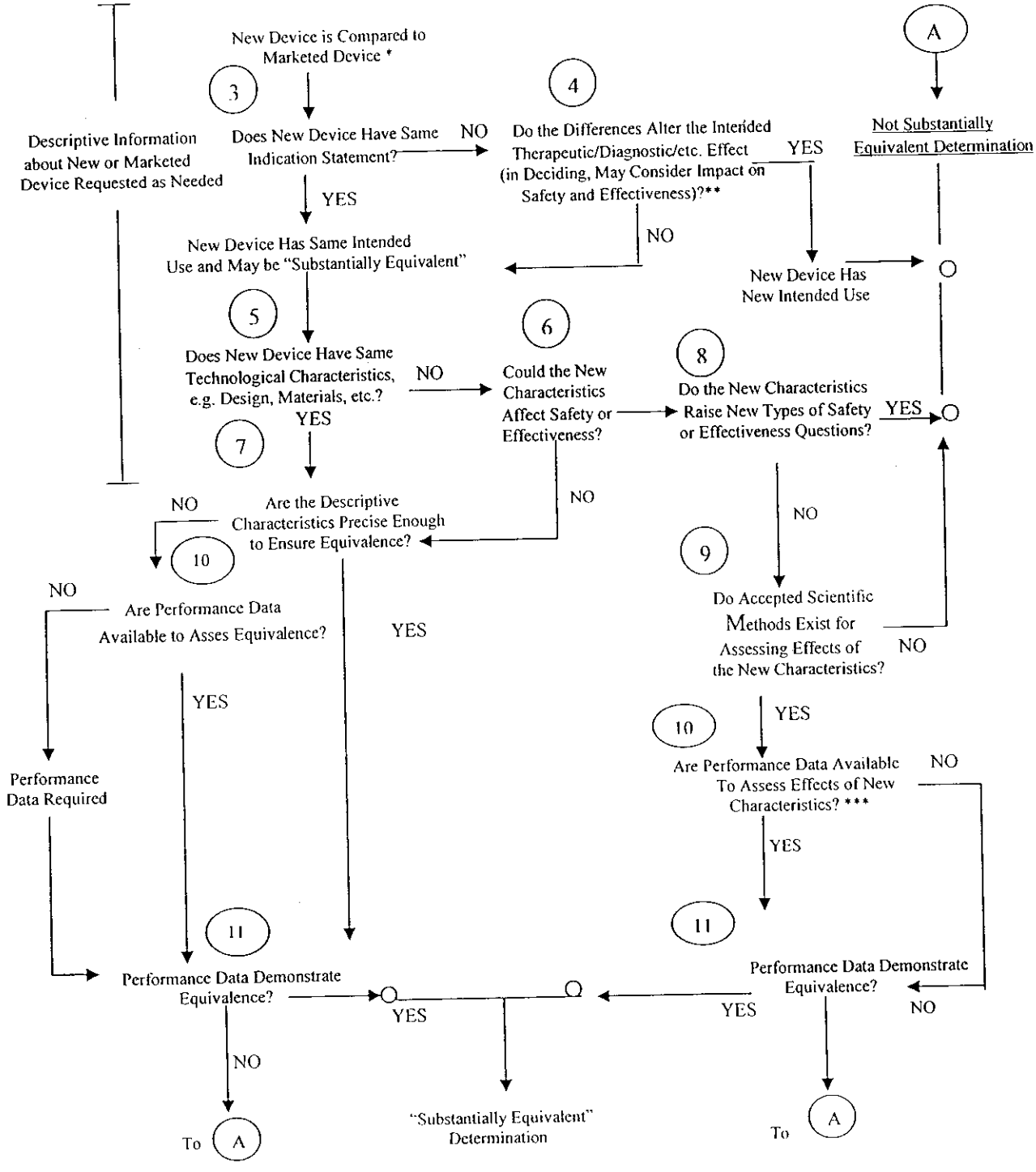
510(k) "SUBSTANTIAL EQUIVALENCE" DECISION-MAKING PROCESS



* 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.

Internal Administrative Form

	YES	NO
1. Did the firm request expedited review?		✓
2. Did we grant expedited review?		✓
3. Have you verified that the Document is labeled Class III for GMP purposes?		
4. If, not, has POS been notified?		
5. Is the product a device?	✓	
6. Is the device exempt from 510(k) by regulation or policy?		✓
7. Is the device subject to review by CDRH?	✓	
8. Are you aware that this device has been the subject of a previous NSE decision?		✓
9. If yes, does this new 510(k) address the NSE issue(s), (e.g., performance data)?		
10. Are you aware of the submitter being the subject of an integrity investigation?		✓
11. If, yes, consult the ODE Integrity Officer.		
12. Has the ODE Integrity Officer given permission to proceed with the review? (Blue Book Memo #I91-2 and Federal Register 90N0332, September 10, 1991.		

The deficiencies identified above 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/cdrh/modact/leastburdensome.html

REVISED:3/14/95

THE 510(K) DOCUMENTATION FORMS ARE AVAILABLE ON THE LAN UNDER 510(K) BOILERPLATES TITLED "DOCUMENTATION" AND MUST BE FILLED OUT WITH EVERY FINAL DECISION (SE, NSE, NOT A DEVICE, ETC.).

"SUBSTANTIAL EQUIVALENCE" (SE) DECISION MAKING DOCUMENTATION

K_____

Reviewer: _____

Division/Branch: _____

Device Name: _____

Product To Which Compared (510(K) Number If Known): _____

	YES	NO	
1. Is Product A Device			If **NO** = Stop
2. Is Device Subject To 510(k)?			If **NO** = Stop
3. Same Indication Statement?			If **YES** = Go To 5
4. Do Differences Alter The Effect Or Raise New Issues of Safety Or Effectiveness?			If **YES** = Stop **NE**
5. Same Technological Characteristics?			If **YES** = Go To 7
6. Could The New Characteristics Affect Safety Or Effectiveness?			If **YES** = Go To 8
7. Descriptive Characteristics Precise Enough?			If **NO** = Go To 10 If **YES** = Stop **SE**
8. New Types Of Safety Or Effectiveness Questions?			If **YES** = Stop **NE**
9. Accepted Scientific Methods Exist?			If **NO** = Stop **NE**
10. Performance Data Available?			If **NO** = Request Data
11. Data Demonstrate Equivalence?			Final Decision:

Note: In addition to completing the form on the LAN, "yes" responses to questions 4, 6, 8, and 11, and every "no" response requires an explanation.

1. Intended Use:

2. Device Description: Provide a statement of how the device is either similar to and/or different from other marketed devices, plus data (if necessary) to support the statement. Is the device life-supporting or life sustaining? Is the device implanted (short-term or long-term)? Does the device design use software? Is the device sterile? Is the device for single use? Is the device over-the-counter or prescription use? Does the device contain drug or biological product as a component? Is this device a kit? Provide a summary about the devices design, materials, physical properties and toxicology profile if important.

EXPLANATIONS TO "YES" AND "NO" ANSWERS TO QUESTIONS ON PAGE 1 AS NEEDED

1. Explain why not a device:

2. Explain why not subject to 510(k):

3. How does the new indication differ from the predicate device's indication:

4. Explain why there is or is not a new effect or safety or effectiveness issue:

5. Describe the new technological characteristics:

6. Explain how new characteristics could or could not affect safety or effectiveness:

7. Explain how descriptive characteristics are not precise enough:

8. Explain new types of safety or effectiveness questions raised or why the questions are not new:

9. Explain why existing scientific methods can not be used:

10. Explain what performance data is needed:

11. Explain how the performance data demonstrates that the device is or is not substantially equivalent:

ATTACH ADDITIONAL SUPPORTING INFORMATION

Heyliger, Marian

ɔm: Heyliger, Marian
ɹnt: Tuesday, May 31, 2005 12:45 PM
ſo: 'dlyerly@techlab.com'
Cc: Poole, Freddie M.; Heyliger, Marian
Subject: K050891 - Additional info request

(b)(4) Confidential and Proprietary Information

If you need further clarification, please feel free to contact me.

Thank you.

Marian Heyliger
Scientific Reviewer
mhh@cdrh.fda.gov
Phone: 240 276 0496
Fax: 240 276 0652

(b)(4) Confidential and Proprietary Information

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From: Marian Heyliger
Scientific Reviewer
OIVD/FDA

To: Dr. David Lyerly
Vice President of Research & Development
TechLab Inc.

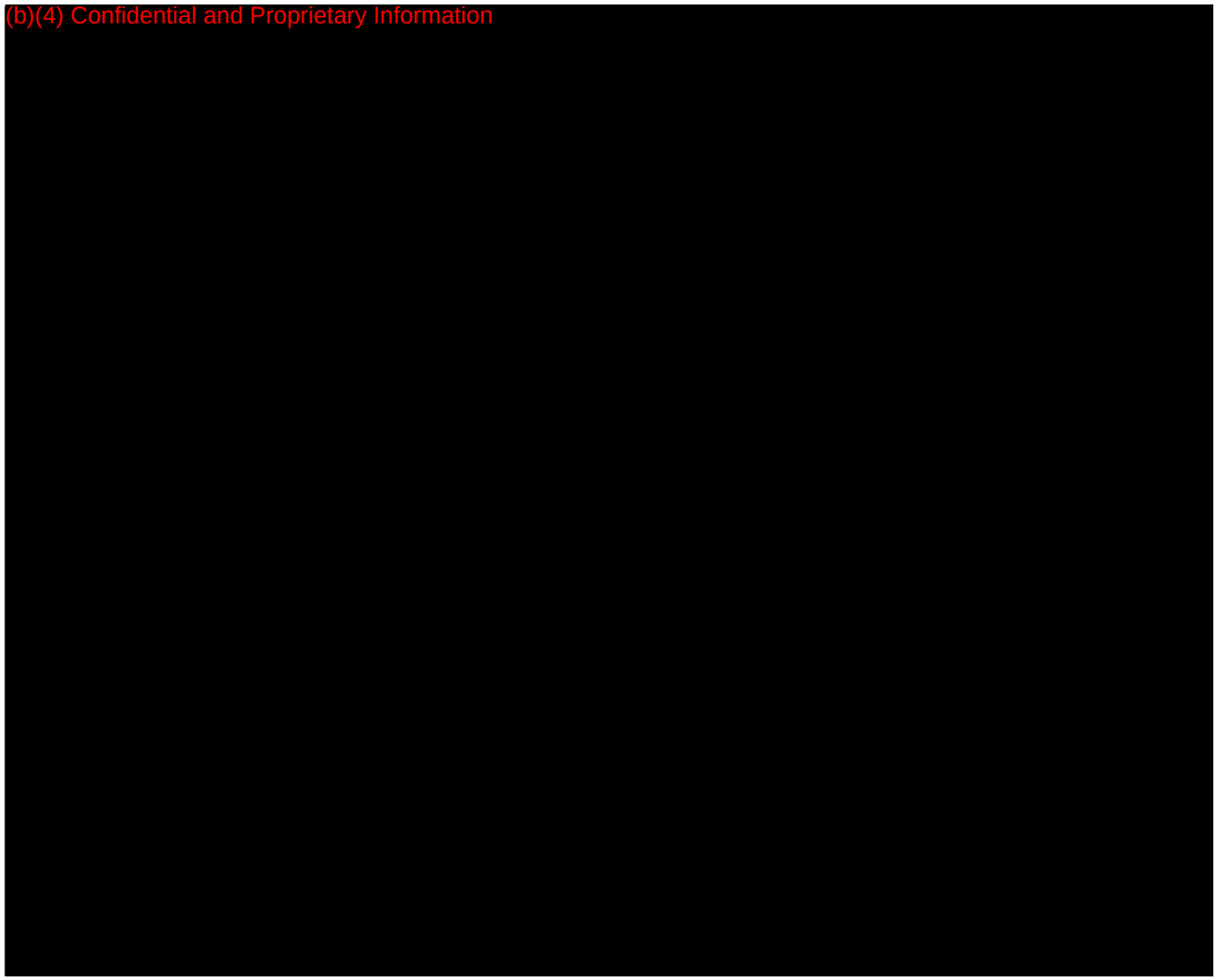
Date: May 24, 2005

Re: K 050891

Dear Dr. Lyerly,

We have completed the initial review of your submission. In order to continue the review, we require additional information as follows:

(b)(4) Confidential and Proprietary Information



(b)(4) Confidential and Proprietary Information

[redacted]

Indications for Use

510(k) Number (if known):

Device Name:

Indications For Use:

Prescription Use _____ AND/OR Over-The-Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 807 Subpart C)

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

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Date: May 20, 2005

From: Scientific Reviewer

Re: TechLab Tox A/B Quik Chek™

To the Record: K050891

Background

TechLab submitted this 510(k) on April 5, 2005. It was received in DMC on April 8, 2005 and assigned for review on April 12, 2005.

The Tox A/B Quik Chek is a rapid immunoassay for detecting Clostridium difficile toxins A & B in fecal specimens from persons suspected of having C. difficile disease. The test is to be used as an aid in the diagnosis of C. difficile disease and results should be considered in conjunction with patient history.

Review

(b)(4) Confidential and Proprietary Information and (b)(5)

[redacted]

50

(b)(4) Confidential and Proprietary Information and (b)(5)

[redacted]

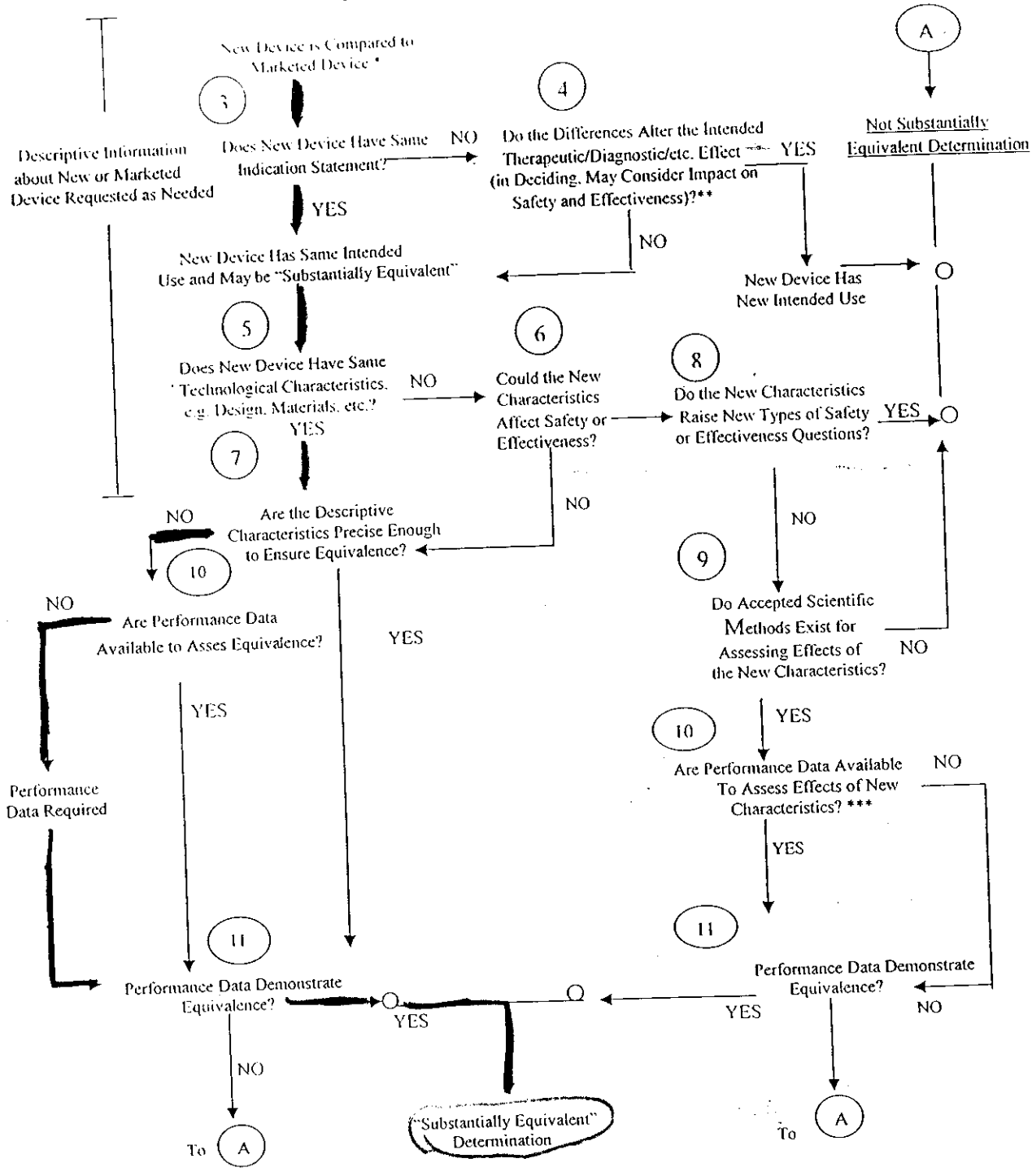
(b)(4) Confidential and Proprietary Information and (b)(5)

[redacted]

(b)(4) Confidential and Proprietary Information and (b)(5)

[redacted]

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.

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REVISED:3/14/95

"SUBSTANTIAL EQUIVALENCE" (SE) DECISION MAKING DOCUMENTATION

K 050891

Reviewer: M. Heyliger

Division/Branch: DMD

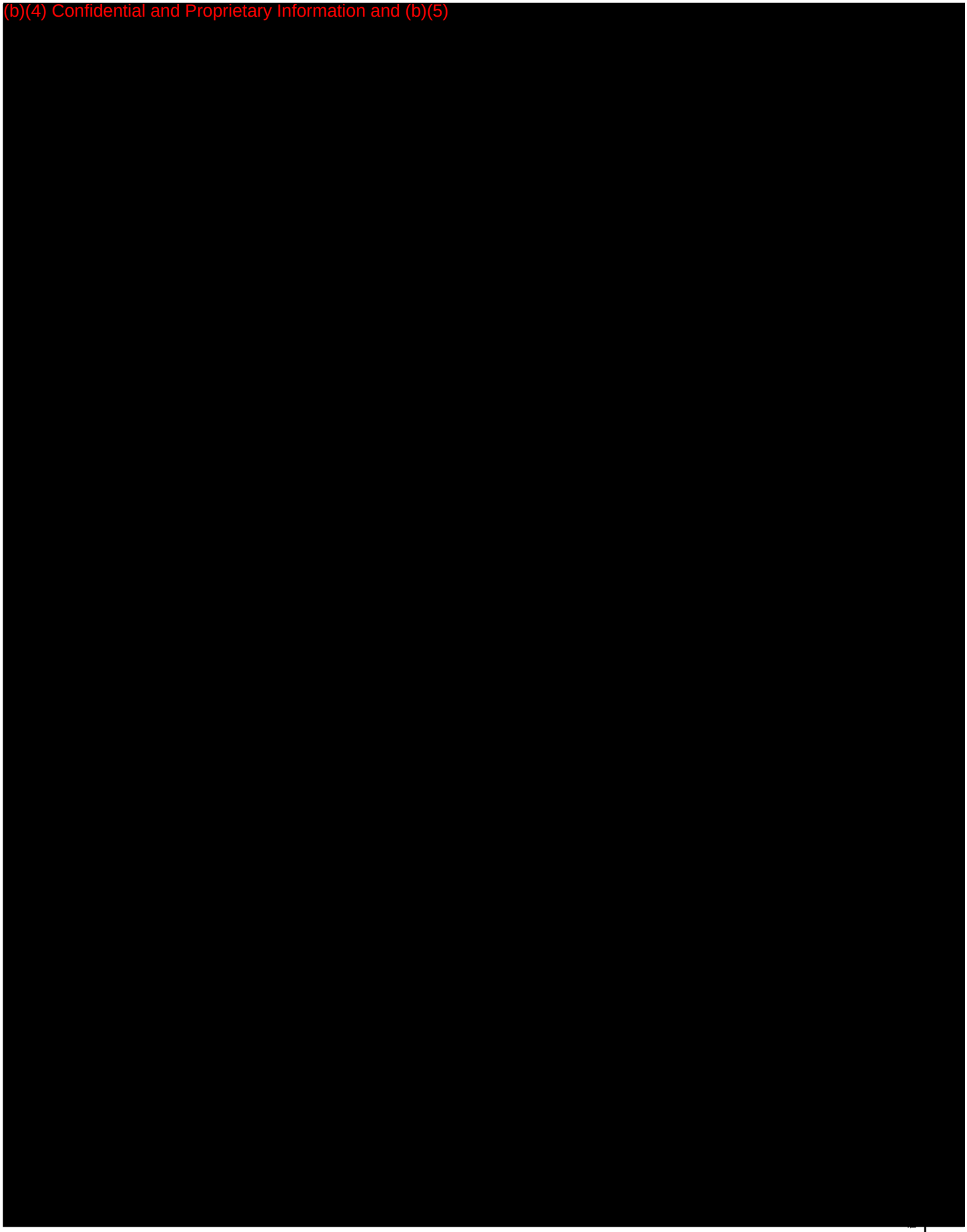
Device Name: TOX A/B QUIK CHEK

Product To Which Compared (510(K) Number If Known): Premier Toxins A&B
Meridian Bioscience Inc
K993914

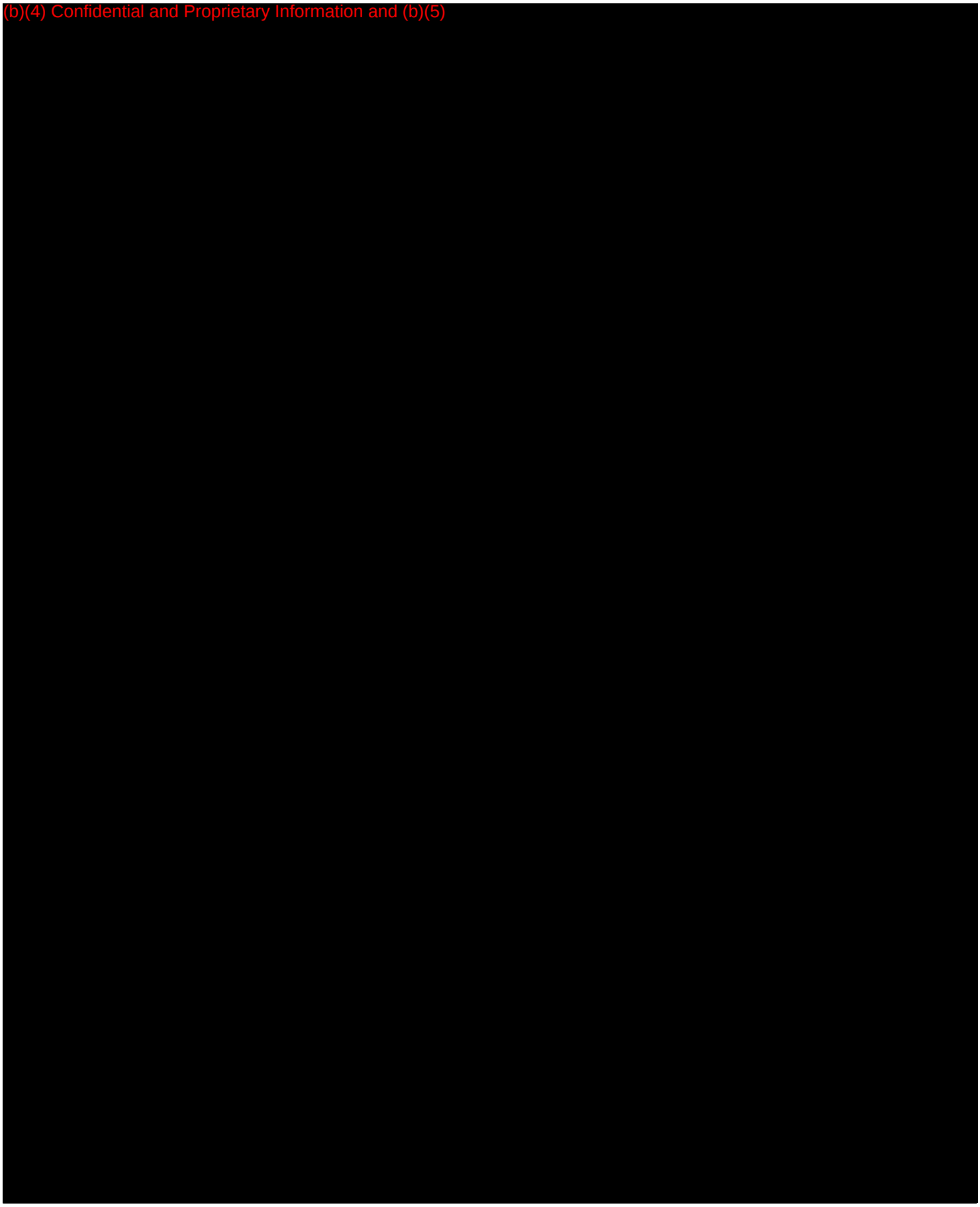
	YES	NO	
1. Is Product A Device	X		If **NO** = Stop
2. Is Device Subject To 510(k)?	X		If **NO** = Stop
3. Same Indication Statement?	X		If **YES** = Go To 5
4. Do Differences Alter The Effect Or Raise New Issues of Safety Or Effectiveness?			If **YES** = Stop **NE**
5. Same Technological Characteristics?	X		If **YES** = Go To 7
6. Could The New Characteristics Affect Safety Or Effectiveness?			If **YES** = Go To 8
7. Descriptive Characteristics Precise Enough?		X	If **NO** = Go To 10 If **YES** = Stop **SE**
8. New Types Of Safety Or Effectiveness Questions?			If **YES** = Stop **NE**
9. Accepted Scientific Methods Exist?			If **NO** = Stop **NE**
10. Performance Data Available?	X		If **NO** = Request Data
11. Data Demonstrate Equivalence?	X		Final Decision: SE

Note: In addition to completing the form on the LAN, "yes" responses to questions 4, 6, 8, and 11, and every "no" response requires an explanation.

(b)(4) Confidential and Proprietary Information and (b)(5)



(b)(4) Confidential and Proprietary Information and (b)(5)



510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number: K050891

B. Purpose for Submission:

Substantial equivalence determination

C. Measurand:

Clostridium difficile toxins A and B

D. Type of Test:

A qualitative rapid immunoassay

E. Applicant:

TechLab Inc.

F. Proprietary and Established Names:

TOX A/B Quik Chek

G. Regulatory Information:

1. Regulation section:

   21 CFR Part 866.2660 Microorganism Differentiation and Identification

2. Classification:

   I

3. Product code:

   LLH – Reagents, Clostridium difficile toxin

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use:

The *TOX A/B QUIK CHEK*™ test is a rapid immunoassay for detecting *Clostridium difficile* toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history.

2. Indication for use:

The *TOX A/B QUIK CHEK*™ test is a rapid immunoassay for detecting *Clostridium difficile* toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history.

3. Special conditions for use statement:

For prescription use only

4. Special instrument requirements:

Not applicable

I. Device Description:

The *TOX A/B QUIK CHEK*™ uses antibodies specific for toxins A and B of *C. difficile*. The device contains a *Reaction Window* with two vertical lines of immobilized antibodies. The test line ("T") contains antibodies against *C. difficile* toxins A and B. The control line ("C") contains anti-IgG antibodies. The *Conjugate* consists of antibodies to toxins A and B coupled to horseradish peroxidase. The kit contains:

Membrane Devices –25 pouches, each containing 1 device and a desiccant pack
Diluent **(14 mL)** – Buffered protein solution containing 0.02% thimerosal
Wash Buffer **(10 mL)** – A buffered solution containing 0.02% thimerosal
Substrate **(3.5 mL)** – Solution containing tetramethylbenzidine
Conjugate **(2 mL)** – Mouse monoclonal antibody specific for toxin A coupled to horseradish peroxidase and goat polyclonal antibody specific for toxin B coupled to horseradish peroxidase in a buffered protein solution containing 0.02% thimerosal
Positive Control **(1 mL)** – Antigen in a buffered protein solution

J. Substantial Equivalence Information:

1. Predicate device names:

   Premier toxins A & B, ImmunoCard Toxins A & B, C. difficile TOX A/B II, ProSpecT C. difficile toxin A/B, X/pect C. difficile toxin A/B

2. Predicate 510(k) numbers:

   K993914, K041003, K00306 and K030404, K033479 and K041951

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Detection of C.difficile toxins in fecal specimens	Same
Specimen type	Human stool	Same
Technology	Enzyme immunoassay	Same

Differences		
Item	Device	Predicate
Limit of detection	0.63 ng/mL for toxin A and 1.25 ng/ml for toxin B	$\geq$ 1.4 ng/ml of toxin A & $\geq$ 2.4 ng/ml of toxin B
Clinical sensitivity	90.2 % (84.1 - 94.2% C.I.)	94.7% (88.1 – 98.3% C.I.)
Clinical specificity	99.7 %(98.8 – 99.9% C.I.)	97.3% (95.4 – 98.5% C.I.)

K. Standard/Guidance Document Referenced (if applicable):

"Review Criteria for Devices assisting in the diagnosis of C. difficile associated disease" May 1990, ODE/CDRH Guidance Document

L. Test Principle:

The device contains a *Reaction Window* with two vertical lines of immobilized antibodies (Fig. 1a). The test line ("T") contains antibodies against *C. difficile* toxins A and B. The control line ("C") contains anti-IgG antibodies. The *Conjugate* consists of antibodies to toxins A and B coupled to horseradish peroxidase. To perform the test, the fecal specimen is diluted with *Diluent* and *Conjugate* is added to the diluted sample. The diluted sample-conjugate mixture is added to the *Sample Well* and the device is allowed

to incubate at room temperature for 15 minutes. During the incubation, any toxin A and toxin B in the sample bind to anti-toxin antibody-peroxidase conjugate. The toxin-antibody complexes migrate through a filter pad to a membrane where they are captured by the immobilized anti-toxin antibodies in the line. The *Reaction Window* is subsequently washed with *Wash Buffer*, and the test is developed with the addition of *Substrate*. After a 10 minute incubation period, the "T" reaction is examined visually for the appearance of a vertical blue line on the "T" side of the *Reaction Window*. A blue line indicates a positive test. A positive "C" reaction, indicated by a vertical blue line on the "C" side of the *Reaction Window*, confirms that the test is working properly and the results are valid.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The reproducibility of the *TOX A/B QUIK CHEK*™ test was determined using known positive (n=6) and negative (n=2) fecal specimens that were coded and masked. Testing was performed at 3 laboratories, which tested the samples on 3 days. The samples produced the expected results 100% of the time.

b. Linearity/assay reportable range:

N/A

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

N/A

d. Detection limit:

The analytical sensitivity was determined using serial two-fold dilutions of highly purified toxins A and B. Toxins were purified using standard in-house procedures. Six separate tests were conducted for each toxin and the test was consistently positive at a concentration of 0.63 ng/mL for toxin A and 1.25 ng/ml for toxin B.

e. Analytical specificity:

CROSS REACTIVITY

Fecal specimens inoculated with the following microorganisms to a final

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concentration of approximately 10^8 or higher organisms per mL did not react in the *TOX A/B QUIK CHEK*™:
Bacteria: *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Bacteroides fragilis, Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, Candida albicans, Clostridium bifermentans, Clostridium butyricum, Clostridium perfringens, Clostridium septicum, Clostridium sordellii* (nontoxigenic), *Clostridium sporogenes, Enterococcus faecalis, Escherichia coli* EIEC, *Escherichia coli, Escherichia coli* 0157 H7, *Escherichia coli* ETEC, *Klebsiella pneumoniae, Peptostreptococcus anaerobius, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus aureus (Cowans), Staphylococcus epidermidis, Vibrio parahaemolyticus, Yersinia enterocolitica* **Viruses**: Adenovirus types 1,2,3,5,40,41, Human coronavirus, Coxsackievirus B2,B3,B4,B5, Echovirus 9,11,18,22,33, Enterovirus type 68,69,70,71.

The only non-*C. difficile* organism to react with the *TOX A/B QUIK CHEK*™ was *C. sordellii* VPI 9048. This strain produces toxins HT and LT, which are homologous to toxins A and B, respectively.

INTERFERING SUBSTANCES

The following substances had no effect on test results when present in feces in the concentrations indicated: mucin (3.5% w/v), human blood (40% v/v), barium sulfate (5% w/v), Imodium® (5% w/v), Kaopectate® (5 mg/mL), Pepto-Bismol® (5% w/v), steric/palmitic acid (40% w/v), Metronidazole (0.25% w/v), Vancomycin (0.25% w/v).

f. Assay cut-off:

The assay was determined to detect Toxin A at 0.63ng/ml and Toxin B at 1.25 ng/ml

2. Comparison studies:

a. Method comparison with predicate device:

N/A

b. Matrix comparison:

N/A

3. Clinical studies:

a. Clinical Sensitivity:

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The *TOX A/B QUIK CHEK*™ test was compared with the tissue culture test at three U.S. hospitals and in-house at TECHLAB®, Inc. Specimens included in the evaluation were submitted to the clinical laboratory for routine testing. The tissue culture test was done according to the in-house procedure. The table below shows a summary of the clinical performance of the *TOX A/B QUIK CHEK*™ test. The test exhibited a sensitivity and specificity of 90.2% and 99.7%, respectively. The predictive positive and negative values were 98.6% and 97.9%, respectively, and the correlation was 98.0%.

Table 1. Correlation of the *TOX A/B QUIK CHEK*™ test with tissue culture.

N = 842	Tiss cult pos	Tiss cult neg
***TOX A/B QUIK CHEK*™ pos**	138	2
***TOX A/B QUIK CHEK*™ neg**	15	687

		95% CI
Sensitivity	90.2	84.1 - 94.2
Specificity	99.7	98.8 - 99.9
Predictive Positive Value	98.6	94.4 - 99.8
Predictive Negative Value	97.9	96.4 - 98.7
Correlation	98.0	97.8 - 98.2

Of the 2 tissue culture-negative/*TOX A/B QUIK CHEK*™-positive samples, 1 was negative in a commercial toxin A+B ELISA. Of the 15 specimens that were tissue culture-positive/*TOX A/B QUIK CHEK*™-negative, 12 were negative in commercial toxin A+B ELISAs. There were 9 specimens that were unreadable. Those specimens were negative by PCR analysis for the genes of toxin A (*tcdA*) and toxin B *(tcdB*).

A total of 51 fecal specimens diluted in Cary Blair and 32 fecal specimens diluted in C&S Transport Media were tested in the *TOX A/B QUIK CHEK*™ test and the results were compared to those obtained by routine testing. The test exhibited an agreement of 97.6% for the detection of *C. difficile* toxins in specimens prepared in Transport Media.

b. *Clinical specificity:*

See above Sec 3.a.

c. Other clinical supportive data (when a. and b. are not applicable):

N/A

4. Clinical cut-off:

Se limit of detection above Sec. M.1.d

5. Expected values/Reference range:

The reported incidence of *C. difficile*-associated disease in patients with antibiotic-associated diarrhea is 10 to 20%. In the studies conducted with this device, the incidence ranged from 10% to 22%. The prevalence of a positive *TOX A/B QUIK CHEK*^{TM} test will vary from location to location and hospitals may experience rates lower or higher than those observed at the sites used in this evaluation. *Clostridium difficile* disease is primarily a nosocomial disease of elderly patients, and hospitals that have higher numbers of elderly patients may experience higher rates. It is important to consider any test results in conjunction with clinical symptoms because some healthy adults and large numbers of healthy infants (up to 50%) will be positive for *C. difficile* toxin.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

1. The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

(b)(4) Confidential and Proprietary Information

[redacted]

Q. Administrative Information:

1. Applicant contact information:

   a. *Name of applicant:*

      TECHLAB Inc.

   b. *Mailing address:*

      2001 Kraft Drive

      Corporate Research Center

      Blacksburg, VA 24060-6364

   c. *Phone #:*

      540-953-1664

   d. *Fax #:*

      540-953-1665

   e. *E-mail address (optional):*

dlyerly@techlab.com

f. Contact:

Dr. David M. Lyerly, PhD.

Vice President of Research and Development

2. Review documentation:

(b)(4) Confidential and Proprietary Information and (b)(5)

[redacted]

R. Reviewer Name and Signature:

Marian Heyliger
CDRH/OIVD/DMD

DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

July 08, 2005

TECHLAB, INC.
2001 KRAFT DR.
BLACKSBURG, VA 24060
ATTN: DAVID M. LYERLY

510(k) Number: K050891
Product: TOX A/B QUICK CHEK

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 (HFZ-401) 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 www.fda.gov/cdrh/ode/a02-01.html.

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 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.

If you have procedural or policy questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301) 443-6597 or at their toll-free number (800) 638-2041, or contact me at (301) 594-1190.

Sincerely yours,

Marjorie Shulman
Supervisory Consumer Safety Officer
Premarket Notification Section
Office of Device Evaluation
Center for Devices and
Radiological Health

 K050891 / S1

TECHLAB®

ORIGINAL

TO: Marian Heyliger
Scientific Reviewer
OIVD/FDA

FROM: Dr. David Lyerly
Vice-President, Research & Development
TechLab, Inc.

DATE: July 5, 2005

RE: K 050891

Dear Ms. Heyliger,

Enclosed are revised pages 42 and 65. The revised package insert is included as a separate attachment. These revised pages and the package insert reflect the 9 specimens that were unreadable. The results in the tables and insert now show the changes as recommended.

Thank you for your assistance.

Sincerely,

David M. Lyerly, Ph.D.
Vice-President, Research & Development

S1

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REVISED Page 42

5.7.4 Summary of Results

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[redacted]

Summary of clinical performance comparing the *TOX A/B QUIK CHEK™* test versus tissue culture assay.

n=842	Tiss Cult pos	Tiss Cult neg
TOX A/B QUIK CHEK™ pos	138	2
TOX A/B QUIK CHEK™ neg	15	687

		95% CI
Sensitivity	90.2	84.1 - 94.2
Specificity	99.7	98.8 - 99.9
Predictive Positive Value	98.6	94.4 - 99.8
Predictive Negative Value	97.9	96.4 - 98.7
Correlation	98.0	97.8 - 98.2

(b)(4) Confidential and Proprietary Information

[redacted]

REVISED Page 65

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TOX A/B QUIK CHEK™

A rapid test for the detection of *C. difficile* toxins A and B in fecal specimens
Patent Pending

Catalog #T5033 (25 tests)

INTENDED USE

The *TOX A/B QUIK CHEK*™ test is a rapid immunoassay for detecting *Clostridium difficile* toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history.
FOR *IN VITRO* DIAGNOSTIC USE.

EXPLANATION

After treatment with antibiotics, many patients develop gastrointestinal problems ranging from mild diarrhea to severe pseudomembranous colitis. Many cases of the milder forms of gastrointestinal illness and most cases of pseudomembranous colitis are caused by toxigenic strains of *Clostridium difficile* (1). This organism is an opportunistic anaerobic bacterium that grows in the intestine once the normal flora has been altered by the antibiotic. Toxigenic strains of *C. difficile* carry the genes encoding the toxins while non-toxigenic strains do not carry the toxin genes. The disease results from the toxins that the toxigenic organism produces. The clinical symptoms associated with the disease are believed to be primarily due to toxin A, which is a tissue-damaging enterotoxin (2,3). *C. difficile* also produces a second toxin, designated toxin B. Toxin B, which has been referred to as the cytotoxin of the organism, is the toxin detected by the tissue culture assay currently used by many laboratories. Toxigenic *C. difficile* strains produce both toxins or only toxin B (4-7).

PRINCIPLE OF THE TEST

The *TOX A/B QUIK CHEK*™ uses antibodies specific for toxins A and B of *C. difficile*. The device contains a *Reaction Window* with two vertical lines of immobilized antibodies (Fig. 1a). The test line ("T") contains antibodies against *C. difficile* toxins A and B. The control line ("C") contains anti-IgG antibodies. The *Conjugate* consists of antibodies to toxins A and B coupled to horseradish peroxidase. To perform the test, the fecal specimen is diluted with *Diluent* and *Conjugate* is added to the diluted sample. The diluted sample-conjugate mixture is added to the *Sample Well* and the device is allowed to incubate at room temperature for 15 minutes. During the incubation, any toxin A and toxin B in the sample bind to anti-toxin antibody-peroxidase conjugate. The toxin-antibody complexes migrate through a filter pad to a membrane where they are captured by the immobilized anti-toxin antibodies in the line. The *Reaction Window* is subsequently washed with *Wash Buffer*, and the test is developed with the addition of *Substrate*. After a 10 minute incubation period, the "T" reaction is examined visually for the appearance of a vertical blue line on the "T" side of the *Reaction Window*. A blue line indicates a positive test. A positive "C" reaction, indicated by a vertical blue line on the "C" side of the *Reaction Window*, confirms that the test is working properly and the results are valid.

MATERIALS PROVIDED

Membrane Devices –25 pouches, each containing 1 device and a desiccant pack
***Diluent* (14 mL)** – Buffered protein solution containing 0.02% thimerosal
***Wash Buffer* (10 mL)** – A buffered solution containing 0.02% thimerosal
***Substrate* (3.5 mL)** – Solution containing tetramethylbenzidine
***Conjugate* (2 mL)** – Mouse monoclonal antibody specific for toxin A coupled to horseradish peroxidase and goat polyclonal antibody specific for toxin B coupled to horseradish peroxidase in a buffered protein solution containing 0.02% thimerosal
***Positive Control* (1 mL)** – Antigen in a buffered protein solution
Disposable plastic transfer pipettes – 50 (graduated at 25 µL and 400 µL)

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Small test tubes (e.g., plastic Eppendorf tubes)
Timer
Disposable gloves for handling fecal samples
Applicator sticks
Vortex mixer
Pipettor and tips

SHELF LIFE AND STORAGE

The expiration date of the kit is given on the label. Expiration dates for each component are listed on the individual labels. The kit should be stored between 2° and 8°C.

PRECAUTIONS

1. Reagents from different kits should not be mixed. Do not use a kit past the expiration date.
2. Bring all components to ROOM TEMPERATURE BEFORE USE!
3. Caps and tips are color-coded; do NOT mix!
4. Do not freeze the reagents. The kit should be stored between 2°C and 8°C.
5. The pouch containing the *Membrane Device* should be at room temperature before opening, and opened just before use. Keep the membrane devices dry before use.
6. Use fecal specimens within 72 hours of collection to obtain optimal results. Specimens that are frozen may lose activity due to freezing and thawing.
7. Specimens that have been preserved in 10% Formalin, merthiolate formalin, sodium acetate formalin or polyvinyl alcohol cannot be used.
8. Specimens in transport media such as Cary Blair and C&S can be used as specified in the specimen preparation protocol.
9. Hold reagent bottles vertically to dispense reagents to ensure consistent drop size.
10. Specimens and membrane devices should be handled and disposed of as potential biohazards after use. Wear disposable gloves when doing the test.

11. Reagents contain thimerosal as a preservative and should be handled with normal laboratory caution.
12. Membrane devices cannot be reused.
13. The test has been optimized for sensitivity and specificity. Alterations of the specified procedure and/or test conditions may affect the sensitivity and specificity of the test. Do not deviate from the specified procedure.
14. Microbial contamination of reagents may decrease the accuracy of the assay. Avoid microbial contamination of reagents by using sterile disposable pipettes if removing aliquots from reagent bottles.
15. Be attentive to the total assay time when testing more than one fecal specimen. Add *Diluent* first, then add the *Conjugate* to each tube of *Diluent*. Then add specimen to the tube of *Diluent/Conjugate*. Thoroughly mix all of the diluted specimens, and then transfer to the *Membrane Device*. The 15-minute incubation step begins after the last diluted sample-conjugate mixture has been transferred to the final *Membrane Device*.

COLLECTION AND HANDLING OF FECAL SPECIMENS

1. Standard collection and handling procedures used in-house for fecal specimens are appropriate. Specimens should be stored between 2° and 8°C; test specimens that are less than 24 hours old, whenever possible.
2. Store specimens frozen (≤-10°C) if the test cannot be performed within 72 hours of collection, but note that freezing and thawing of the specimen may result in loss of activity due to degradation of the toxins.
3. Make sure that specimens are thoroughly mixed PRIOR to performing the assay.
4. Storing fecal specimens in the *Diluent* is NOT recommended.
5. Do not allow the fecal specimens to remain in the *Diluent* and/or *Conjugate* for any extended period of time.

SPECIMEN PREPARATION

1. Bring all reagents and the required number of devices to room temperature before use.
2. Set up and label one small test tube for each specimen, and optional external controls as necessary.
3. Add 500 µL *Diluent* to each tube for fecal specimens. For specimens in transport media such as Cary Blair or C&S, add 425 µL of *Diluent* to the tube.
4. Add one drop of Conjugate (red capped bottle) to each tube.
5. Obtain one disposable plastic transfer pipette (supplied with the kit) for each sample – the pipettes have raised graduations at 25 µL and 400 µL.
6. Mix all specimens thoroughly regardless of consistency- it is essential that the specimens be evenly suspended before transferring.
   Liquid/Semi-solid specimens – pipette 25 µL of specimen with a transfer pipette (graduated at 25 µL and 400 µL) and dispense into the *Diluent*. Use the same transfer pipette to mix the diluted specimen.
   Formed/Solid specimens – Care must be taken to add the correct amount of formed feces to the sample mixture. Mix the specimen thoroughly using a wooden applicator stick and transfer a small portion (approximately 2 mm diameter, the equivalent of 25 µL) of the specimen into the *Diluent*. Emulsify the specimen using the applicator stick.
   Fecal specimens in Cary Blair or C&S transport media - pipette 100 µL of sample into the *Diluent*.
7. **Optional External Control Samples:**
   External Positive Control - add one drop of *Positive Control* (gray-capped bottle) to the appropriate test tube.
   External Negative Control - add 25 µL *Diluent* to the appropriate test tube.

NOTE: Transferring too little specimen, or failure to mix and completely suspend the specimen in the Diluent *mixture, may result in a false-negative test result. The addition of too much fecal specimen may cause invalid results due to restricted sample flow.*

TEST PROCEDURE

1. Obtain one *Membrane Device* per specimen, and one device per optional external positive or negative control as necessary. The foil bags containing the devices should be brought to room temperature before opening. Label each device appropriately and orient it on a flat surface so the letter "C" on the device is on the left, the letter "T" is on the right, and the small *Sample Well* is located in the top right corner of the device (Fig. 1a).
2. Close each tube of diluted specimen and mix thoroughly. Proper mixing can be achieved by vortexing or inverting the tube. Immediately proceed to Step #3.
3. Using a transfer pipette (graduated at 25 µL and 400 µL), transfer 400 µL of the diluted sample-conjugate mixture into the ***Sample Well*** (smaller hole in the top right corner of the device) of a *Membrane Device*, making certain to expel the liquid sample onto the wicking pad inside of the *Membrane Device*.
4. Incubate the device at room temperature for 15 minutes – the sample will wick through the device and a wet area will spread across the *Reaction Window* (larger hole in the middle of the device). If the *Reaction Window* is not completely wet at the end of the 15-minute incubation, the test is considered invalid and the sample must be retested on a new device.
   NOTE FOR SAMPLES THAT FAIL TO MIGRATE:
   Occasionally, a diluted fecal specimen cannot be tested because it clogs the membrane and the Reaction Window *does not wet properly. If the diluted fecal specimen fails to migrate properly within 5 minutes of adding the sample to the* Sample Well *(i.e. the membrane in the* Reaction Window *does not appear to be completely wet), then add 100 µL of* Diluent *to the* Sample Well *and wait an additional 5 minutes (for a total of 20 minutes).*
5. After the incubation, add 300 µL of *Wash Buffer* to the ***Reaction Window***. Allow the *Wash Buffer* to flow through the *Reaction Window* membrane and be absorbed completely.
6. Add 2 drops of *Substrate* (blue-capped bottle) to the ***Reaction Window***. Read and record results visually after 10 minutes.

INTERPRETATION OF RESULTS

1. Interpretation of the test is most reliable when the device is read immediately at the end of the reaction period. Read the device at a normal working distance in a well-lit area. View with a line of vision directly over the device.
2. Observe device for the appearance of a blue line on the "C" side of the *Reaction Window* representing the internal positive control line. Observe device for the appearance of a blue line on the "T" side of the *Reaction Window* representing the test line. The lines may appear faint to dark in intensity.
3. **Positive Result:** A positive result may be interpreted at any time between the addition of *Substrate* and the 10-minute read time. Two blue lines are visible, the control line ("C") and the test line ("T"). The lines may appear faint to dark in intensity. The appearance of a blue line on the "T" side is interpreted as a positive result. An obvious partial line is interpreted as a positive result. Do not interpret membrane discoloration or shadow as a positive result. A positive result indicates the presence of *C. difficile* toxin.

4. **Negative Result:** A test cannot be interpreted as negative or invalid until 10 minutes following the addition of *Substrate*. A single blue line is visible on the control ("C") side of the *Reaction Window* and no test line is visible on the "T" side of the *Reaction Window* (Fig. 1b). A negative result indicates *C. difficile* toxin is either absent in the specimen or is below the detection limit of the test.
5. **Invalid Result:** A single line is visible on the test ("T") side of the *Reaction Window*, or no lines are visible in the *Reaction Window* (Fig. 1c, 1d). The test result is invalid if a control line is not present at the completion of the reaction period.

FIGURE 1: *TOX A/B QUIK CHEK*™ INTERPRETATION OF RESULTS

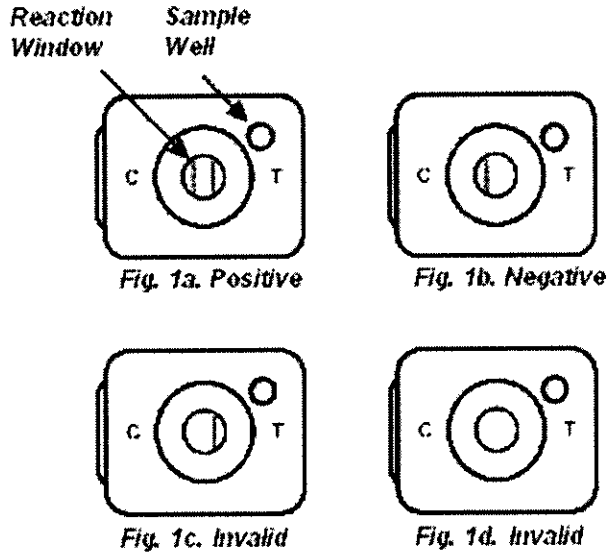

Fig. 1a. Positive *Fig. 1b. Negative*

Fig. 1c. Invalid *Fig. 1d. Invalid*

QUALITY CONTROL

Internal: A blue control line must be visible on the "C" side of the *Reaction Window* on every *Membrane Device* that is tested. The appearance of the blue control line confirms that the sample and reagents were added correctly, that the reagents were active at the time of performing the assay, and that the sample migrated properly through the *Membrane Device*.

External: The reactivity of the *TOX A/B QUIK CHEK*™ test should be verified on receipt using the *Positive Control* and negative control (*Diluent*). The *Positive Control* is supplied with the kit (gray-capped bottle). The *Positive Control* confirms the reactivity of the other reagents associated with the assay, and is not intended to ensure precision at the analytical assay cut-off.
Diluent is used for the negative control. Additional tests can be performed with the controls to meet the requirements of local, state and/or federal regulations and/or accrediting organizations.

LIMITATIONS

1. The *TOX A/B QUIK CHEK*™ test is used to detect *C. difficile* toxin(s) in fecal specimens. The test confirms the presence of toxin in feces and this information should be taken under consideration by the physician in light of the clinical history and physical examination of the patient. The *TOX A/B QUIK CHEK*™ test will detect levels of toxin A at ≥0.63 ng/mL and toxin B at ≥1.25 ng/mL.
2. Fecal specimens are extremely complex. Optimal results with the *TOX A/B QUIK CHEK*™ test are obtained with specimens that are less than 24 hours old. Most undiluted specimens can be stored between 2° and 8°C for 72 hours before significant degradation of the toxin is noted. If specimens are not assayed within this time period, they may be frozen and thawed. However, repeated freezing and thawing may result in loss in the immunoreactivity of toxins A and B.
3. Some specimens may give weak reactions. This may be due to a number of factors such as the presence of low levels of toxin, the presence of binding substances, or inactivating enzymes in the feces. *Under these conditions, a fresh specimen should be tested.* Additional tests that may be used in conjunction with the *TOX A/B QUIK CHEK*™ test include culture with toxigenic testing or tissue culture cytotoxicity assay for the detection of *C. difficile* or its toxin(s).
4. Fecal specimens preserved in 10% Formalin, merthiolate formalin, sodium acetate formalin, or polyvinyl alcohol cannot be used.
5. The *TOX A/B QUIK CHEK*™ test is qualitative. The intensity of the color should not be interpreted quantitatively.
6. Some isolates of *C. sordellii* may react in the *TOX A/B QUIK CHEK*™ test due to the production of immunologically related toxins (1).
7. Colonization rates of up to 50% have been reported in infants. A high rate has also been reported in cystic fibrosis patients (1,3).

EXPECTED VALUES

The reported incidence of *C. difficile*-associated disease in patients with antibiotic-associated diarrhea is 10 to 20%. In our studies, the incidence ranged from 10% to 22%. The prevalence of a positive *TOX A/B QUIK CHEK*™ test will vary from location to location and hospitals may experience rates lower or higher than those observed at the sites used in this evaluation. *Clostridium difficile* disease is primarily a nosocomial disease of elderly patients, and hospitals that have higher numbers of elderly patients may experience higher rates. It is important to consider any test results in conjunction with clinical symptoms because some healthy adults and large numbers of healthy infants (up to 50%) will be positive for *C. difficile* toxin.

PERFORMANCE CHARACTERISTICS

The *TOX A/B QUIK CHEK*™ test was compared with the tissue culture test at three U.S. hospitals and in-house at TECHLAB®, Inc. Specimens included in the evaluation were submitted to the clinical laboratory for routine testing. The tissue culture test was done according to the in-house procedure. The table below shows a summary of the clinical performance of the *TOX A/B QUIK CHEK*™ test.

The test exhibited a sensitivity and specificity of 90.2% and 99.7%, respectively. The predictive positive and negative values were 98.6% and 97.9%, respectively, and the correlation was 98.0%.

Table 1. Correlation of the *TOX A/B QUIK CHEK*™ test with tissue culture.

N = 842	Tiss cult pos	Tiss cult neg
***TOX A/B QUIK CHEK*™ pos**	138	2
***TOX A/B QUIK CHEK*™ neg**	15	687

		95% CI
Sensitivity	90.2	84.1 - 94.2
Specificity	99.7	98.8 - 99.9
Predictive Positive Value	98.6	94.4 - 99.8
Predictive Negative Value	97.9	96.4 - 98.7
Correlation	98.0	97.8 - 98.2

Of the 2 tissue culture-negative/*TOX A/B QUIK CHEK*™-positive samples, 1 was negative in a commercial toxin A+B ELISA. Of the 15 specimens that were tissue culture-positive/*TOX A/B QUIK CHEK*™-negative, 12 were negative in commercial toxin A+B ELISAs. There were 9 specimens that were unreadable. All of the specimens were negative by PCR analysis for the genes of toxin A (*tcdA*) and toxin B (*tcdB*).

A total of 51 fecal specimens diluted in Cary Blair and 32 fecal specimens diluted in C&S Transport Media were tested in the *TOX A/B QUIK CHEK*™ test and the results were compared to those obtained by routine testing. The test exhibited an agreement of 97.6% for the detection of *C. difficile* toxins in specimens prepared in Transport Media.

ANALYTICAL SENSITIVITY

The test was consistently positive at a concentration of 0.63 ng/mL for toxin A and 1.25 ng/mL for toxin B.

REPRODUCIBILITY

The reproducibility of the *TOX A/B QUIK CHEK*™ test was determined using known positive (n=6) and negative (n=2) fecal specimens that were coded and sorted to prevent their identification during testing. Testing was performed on-site at 3 laboratories, which tested the samples on 3 days. The samples produced the expected results 100% of the time.

CROSS REACTIVITY

Fecal specimens inoculated with the following microorganisms to a final concentration of approximately 10^8 or higher organisms per mL did not react in the *TOX A/B QUIK CHEK*™:

Bacteria: *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Bacteroides fragilis, Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, Candida albicans, Clostridium bifermentans, Clostridium butyricum, Clostridium perfringens, Clostridium septicum, Clostridium sordellii* (nontoxigenic), *Clostridium sporogenes, Enterococcus faecalis, Escherichia coli* EIEC, *Escherichia coli, Escherichia coli* 0157 H7, *Escherichia coli* ETEC, *Klebsiella pneumoniae, Peptostreptococcus anaerobius, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus aureus (Cowans), Staphylococcus epidermidis, Vibrio parahaemolyticus, Yersinia enterocolitica* **Viruses**: Adenovirus types 1,2,3,5,40,41, Human coronavirus, Coxsackievirus B2,B3,B4,B5, Echovirus 9,11,18,22,33, Enterovirus type 68,69,70,71.

The only non-*C. difficile* organism to react with the *TOX A/B QUIK CHEK*™ was *C. sordellii* VPI 9048. This strain produces toxins HT and LT, which are homologous to toxins A and B, respectively.

INTERFERING SUBSTANCES

The following substances had no effect on test results when present in feces in the concentrations indicated: mucin (3.5% w/v), human blood (40% v/v), barium sulfate (5% w/v), Imodium® (5% w/v), Kaopectate® (5 mg/mL), Pepto-Bismol® (5% w/v), steric/palmitic acid (40% w/v), Metronidazole (0.25% w/v), Vancomycin (0.25% w/v).

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TO : Marian Heyliger
Scientific Reviewer
OIVD/FDA

FROM: Dr. David Lyerly
Vice-President, Research & Development
TechLab, Inc.

DATE: May 31, 2005

RE: K 050891

Dear Ms. Heyliger,

Enclosed are responses to your questions concerning the above 510(k) submission. We believe that we have satisfactorily responded to your concerns and thank you for your assistance in this review.

Please note that revised pages from our 510(k) are found on the pages following our responses below. Your questions are in bold typeface.

(b)(4) Confidential and Proprietary Information and (b)(5)

[redacted]

(b)(4) Confidential and Proprietary Information and (b)(5)

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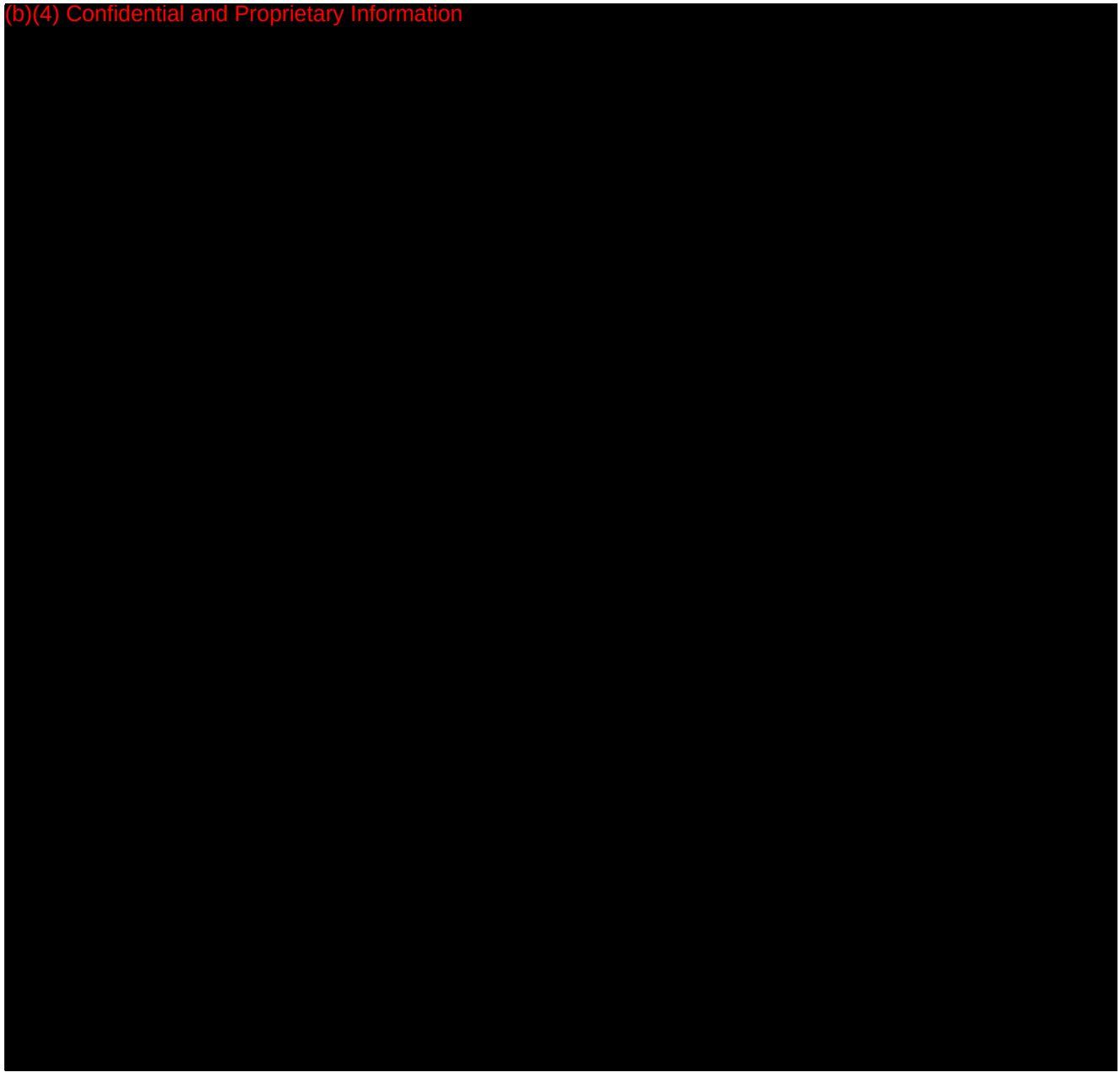
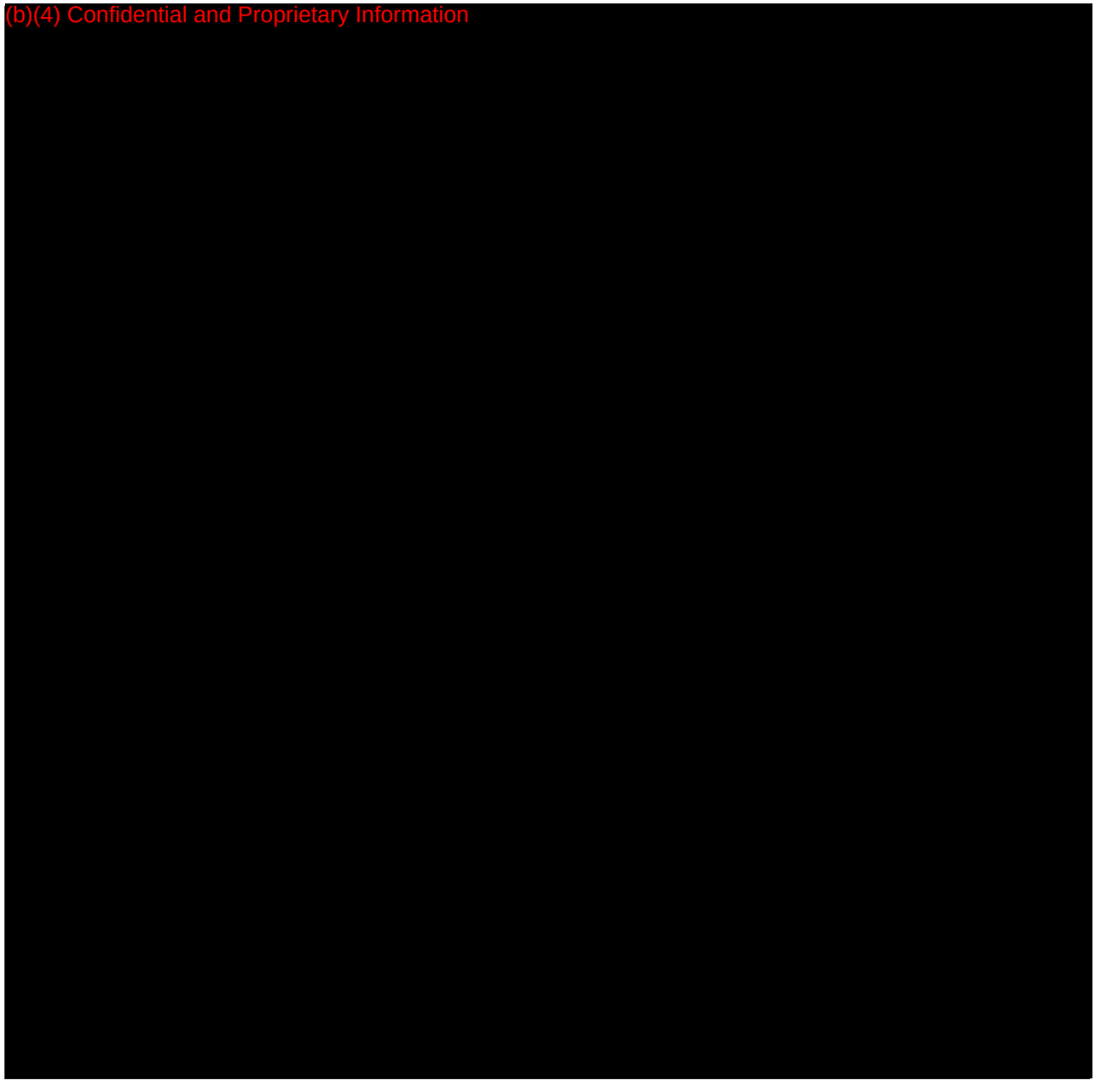
(b)(4) Confidential and Proprietary Information and (b)(5)





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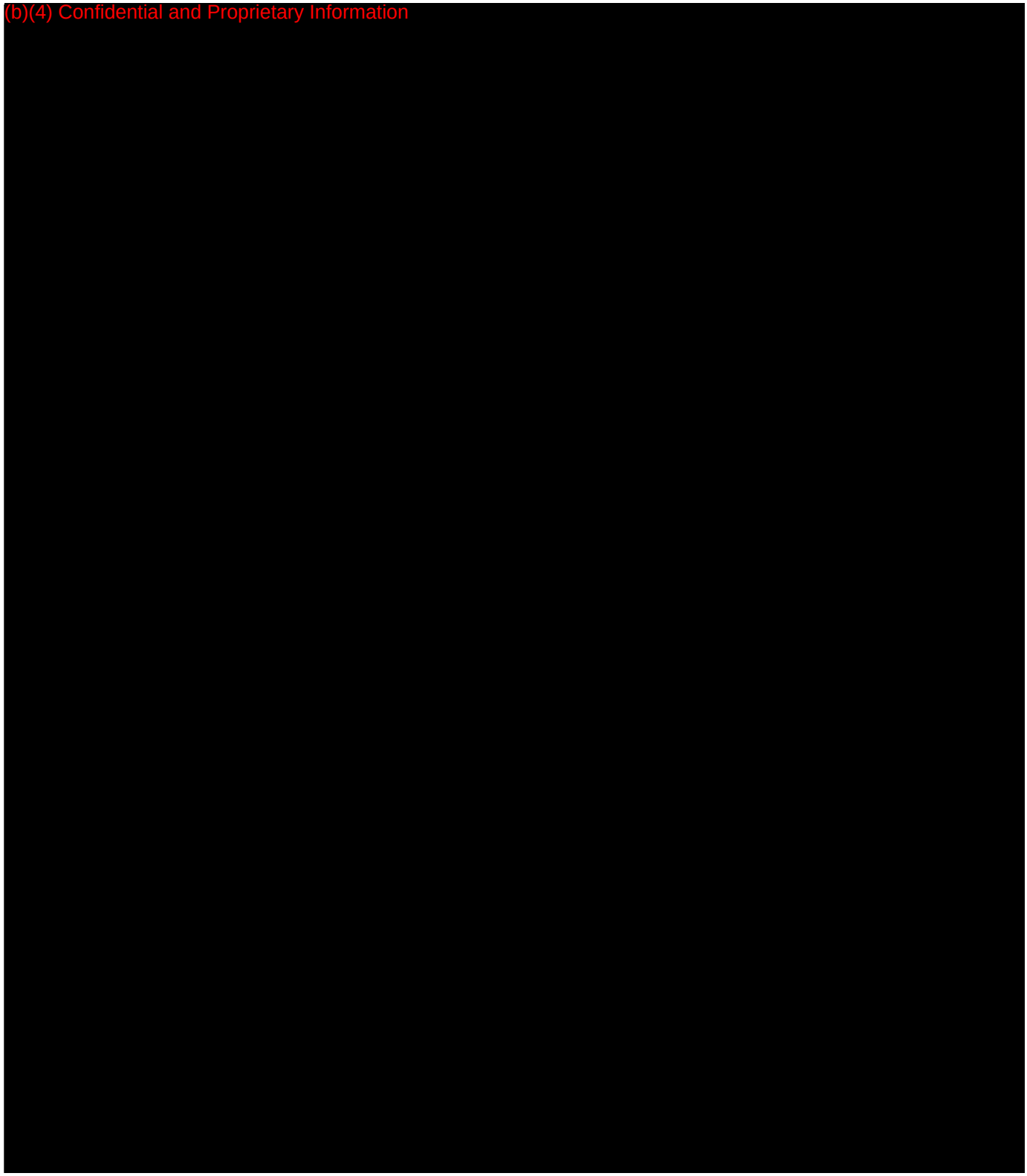

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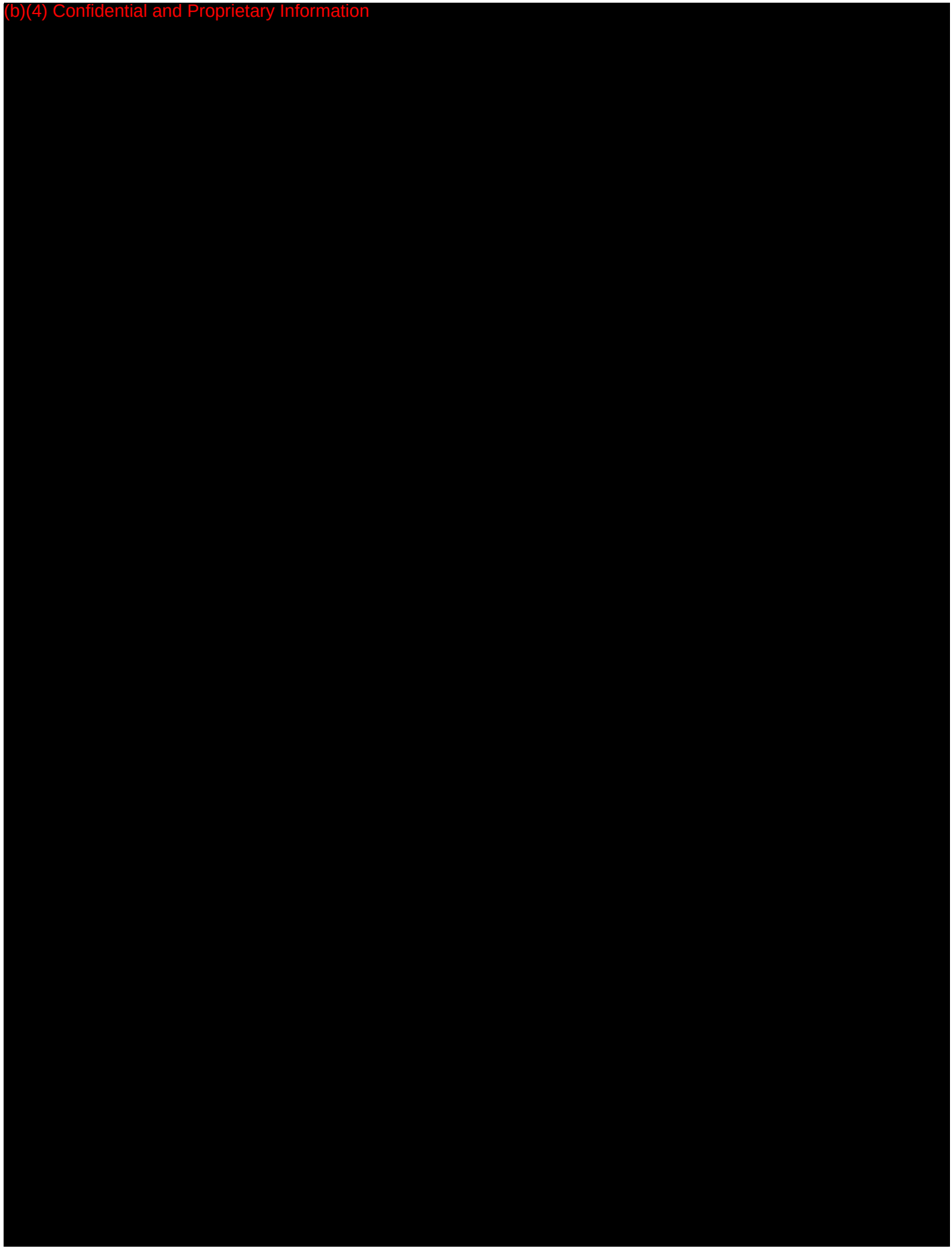

(b)(4) Confidential and Proprietary Information

[redacted]

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

CERTIFICATION: FINANCIAL INTERESTS AND ARRANGEMENTS OF CLINICAL INVESTIGATORS

Form Approved: OMB No. 0910-0396
Expiration Date: February 28, 2006.

TO BE COMPLETED BY APPLICANT

With respect to all covered clinical studies (or specific clinical studies listed below (if appropriate)) submitted in support of this application, I certify to one of the statements below as appropriate. I understand that this certification is made in compliance with 21 CFR part 54 and that for the purposes of this statement, a clinical investigator includes the spouse and each dependent child of the investigator as defined in 21 CFR 54.2(d).

Please mark the applicable checkbox.

☑ (1) As the sponsor of the submitted studies, I certify that I have not entered into any financial arrangement with the listed clinical investigators (enter names of clinical investigators below or attach list of names to this form) whereby the value of compensation to the investigator could be affected by the outcome of the study as defined in 21 CFR 54.2(a). I also certify that each listed clinical investigator required to disclose to the sponsor whether the investigator had a proprietary interest in this product or a significant equity in the sponsor as defined in 21 CFR 54.2(b) did not disclose any such interests. I further certify that no listed investigator was the recipient of significant payments of other sorts as defined in 21 CFR 54.2(f).

(b) (6) [redacted]

☐ (2) As the applicant who is submitting a study or studies sponsored by a firm or party other than the applicant, I certify that based on information obtained from the sponsor or from participating clinical investigators, the listed clinical investigators (attach list of names to this form) did not participate in any financial arrangement with the sponsor of a covered study whereby the value of compensation to the investigator for conducting the study could be affected by the outcome of the study (as defined in 21 CFR 54.2(a)); had no proprietary interest in this product or significant equity interest in the sponsor of the covered study (as defined in 21 CFR 54.2(b)); and was not the recipient of significant payments of other sorts (as defined in 21 CFR 54.2(f)).

☐ (3) As the applicant who is submitting a study or studies sponsored by a firm or party other than the applicant, I certify that I have acted with due diligence to obtain from the listed clinical investigators (attach list of names) or from the sponsor the information required under 54.4 and it was not possible to do so. The reason why this information could not be obtained is attached.

NAME	TITLE
DAVID LYERLY	VICE PRESIDENT
FIRM/ORGANIZATION	
TECHLAB, INC.	
SIGNATURE	DATE
David M Lyerly	MAY 31, 2005

Paperwork Reduction Act Statement

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. Public reporting burden for this collection of information is estimated to average 1 hour per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the necessary data, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information to the address to the right:

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Food and Drug Administration
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