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RESEARCH**

*APPLICATION NUMBER:*

**21-586**

**MICROBIOLOGY REVIEW**

**Office of Nonprescription Products  
Clinical Microbiology Review**

**NDA Number:** 21-586

**Sponsor:** 3M Medical Division  
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St. Paul, MN 55144-1000  
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**Product Name(s):**

**Code Name:** Not available

**Non-proprietary/USAN Name:** Iodine Povacrylex

**Generic Name:** Two active ingredients: iodophor acrylate copolymer (0.7% available iodine) and 74% w/w isopropyl alcohol

**Proprietary Name:** DuraPrep™ Surgical Solution

**Pharmacological Category:** Healthcare Antiseptic/ Patient preoperative skin preparation

**Dosage Form:** Paint-on solution

**Route of Administration:** Topical

**Submission/Review Dates:**

**Submission numbers:** S-010 AZ; S-011 BI

**Dates of Submission:** March 28, 2006; June 22, 2006

**CDER Stamp Date:** March 29, 2006

**Date Received by Reviewer:** April 10, 2006; June 22, 2006

**Date Written Review Completed:** July 20, 2006

**Material Reviewed:** 3M study No. I2MS 05-010214, "Study to assess the antimicrobial effectiveness of 3M™ DuraPrep™ surgical solution against resident human skin flora on the groin region," 3M study No. I2MS 05-010346, "Pilot study to assess the antimicrobial effectiveness of Hibiclens cleanser and ChloroPrep Skin Prep against resident human skin flora on the groin region," 3M Study No. I2MS 05-010125, "Evaluation of Durability and Antimicrobial Persistence of DuraPrep Surgical Solution and ChloroPrep One-Step Skin Preparation Following Exposure to Saline Using a Bacterial Challenge Method," and the package insert.

**Related Documents:** IND 49,411/PN-057 and PN-058.

**Reviewer:** Colleen Kane Rogers, Ph.D.

### **Background:**

3M filed an NDA (N21-586) for DuraPrep surgical solution in October 2003. After reviewing the application, FDA issued an approvable letter to 3M on August 27, 2004. The primary deficiency in the application was that the data failed to demonstrate that DuraPrep solution achieves the expected mean 3-log<sub>10</sub> reduction of bacterial counts on the groin at 10 minutes after application. Two pivotal studies were submitted in support of DuraPrep solution for patient preoperative preparation (LIMS 8304 and LIMS 8918). Both studies used Hibiclens, an NDA-approved chlorhexidine-containing preoperative preparation, as an active comparator. While DuraPrep and Hibiclens both met the 2-log<sub>10</sub> reduction on the abdomen, neither product met the 3-log<sub>10</sub> reduction at the groin.

At the January 10, 2005, End-of-Review conference for DuraPrep, 3M was asked to conduct a clinical study which demonstrates a mean 3-log<sub>10</sub> reduction in skin flora on the groin at 10 minutes post-application. At that meeting FDA stated that a two-arm study would be acceptable. A draft protocol for the requested study was submitted on March 21, 2005. 3M modified the protocol based on teleconferences with FDA on May 25, 2005, and July 6, 2005.

On March 28, 2006, 3M submitted an amendment to the NDA that provides the results for the requested study (I2MS 10214). Hibiclens was chosen as the active comparator for this study based on results of a pilot study (I2MS 10346), which is also included in the submission.

### **Conclusions:**

- Both DuraPrep™ solution (iodine povacrylex in 74% isopropanol) and Hibiclens® cleanser (chlorhexidine gluconate 4%) met the 1994 Tentative Final Monograph (TFM)<sup>1</sup> criteria of a 3-log<sub>10</sub> reduction of bacterial counts in the groin at 10 minutes post-application and remained below baseline at 6 hours.
- The bacterial counts for both DuraPrep™ solution and Hibiclens® remained below baseline for up to 24 hours post-application.
- When the log reductions achieved with DuraPrep™ solution were compared with those of Hibiclens®, there was no significant difference in bacterial reductions at 10 minutes post-application between the two products. However, Hibiclens® was significantly more effective than DuraPrep™ solution at 6 and 24 hours post-application.
- The results of pilot study I2MS 10346 suggest that both Hibiclens and ChlorPrep would perform equally well as active controls at this testing laboratory.

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<sup>1</sup> Tentative Final Monograph for Healthcare Antiseptic Drug Products; 59 FR 31402, June 17, 1994.

**Recommendations to be conveyed to the sponsor:**

Based on the efficacy data provided in this amendment, DuraPrep™ solution meets the patient preoperative skin preparation efficacy criteria. This application may be approved contingent upon compliance with the indicated changes to the Microbiology section of the Package Insert.

The following are recommendations on the Microbiology section of the Package Insert:

- Define the Multi-Drug Resistant *E. faecalis* in Tables 1 and 2 more fully by listing the specific antibiotic resistances of this strain in the footnote.
- Include a statement regarding the unknown clinical significance of the clinical simulation studies at the beginning of the Clinical Studies section.
- You may provide log reduction data for DuraPrep™ solution in the text as long as you also include a description of the TFM efficacy criteria.
- If log reduction data is provided in the text, it should reflect the data that is provided in the figures. In other words, do not provide data averaged from separate study arms in the text when this is not shown in the figure.
- Do not use log reduction data from the 2 minute time point in the text since this information is not part of the TFM efficacy criteria and is not available for all treatment groups.

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**Microbiology Executive Summary:**

Clinical simulation testing for patient preoperative skin preparations involves meeting specific bacterial log reduction criteria at both a dry skin site (e.g., abdomen) and a moist skin site (e.g., groin). In the two pivotal trials originally submitted in support of DuraPrep™ solution (iodine povacrylex in 74% isopropanol) for patient preoperative preparation, the product met the bacterial log reduction criteria on the abdomen, but failed at the groin site. Before DuraPrep™ solution can be approved, the sponsor was asked to demonstrate a mean 3-log<sub>10</sub> reduction in skin flora on the groin at 10 minutes post-application. The basis for approval is a patient preoperative skin preparation study performed by ██████████ (study # 05-010214). The study consisted of two arms: DuraPrep™ solution and Hibiclens® cleanser (chlorhexidine gluconate 4%). Only the groin site was tested.

Both DuraPrep™ solution and Hibiclens® cleanser met the Tentative Final Monograph criteria of a 3-log<sub>10</sub> reduction of bacterial counts in the groin at 10 minutes post-application and remained below baseline at 6 hours. The bacterial counts for both products also remained below baseline for up to 24 hours post-application. Based on the efficacy data provided in this amendment, DuraPrep™ solution meets the TFM patient preoperative skin preparation efficacy criteria and this application may be approved.

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## I. Introduction

Prior to surgery or other invasive procedures, the skin is treated with topical antimicrobial products to reduce the risk of nosocomial infections by reducing the number of microorganisms on the skin. Preoperative skin preparation of a surgical patient is an important step in the prevention of wound infection. The goal of the preoperative skin preparation is to create an operative field that is as close to sterile as possible and to do so efficiently with minimal damage to the skin at the site of the proposed incision. Though skin sterility is impossible to achieve, the skin preparation is intended to effect the highest possible reduction of skin flora, to suppress the growth of residual skin flora, and to suppress the growth of transient organisms that enter into the operative field. In the ideal case, the preoperative skin preparation would continue to maintain antimicrobial activity at and around the incision site for the duration of the surgical procedure and until sealing of the incision.

## II. Clinical Simulation Study

The sponsor submitted a patient preoperative skin preparation study titled "Study to assess the antimicrobial effectiveness of 3M™ DuraPrep™ surgical solution against resident human skin flora on the groin region" (Final Report # 05-010214 dated January 17, 2006). This study was conducted by \_\_\_\_\_ for the sponsor.

### Study Objectives

The primary objectives of the study were to demonstrate that DuraPrep solution meets the 1994 Tentative Final Monograph (TFM) criteria of a 3- $\log_{10}$  reduction in the groin at 10 minutes following application, with counts remaining significantly below baseline at 6 hours post-application. The secondary objectives were to a) demonstrate the 24-hour efficacy of DuraPrep solution (i.e., that counts remain significantly below baseline) and b) compare the log reduction achieved by DuraPrep solution to that of Hibiclens.

***Reviewer's comment:*** *Although the TFM efficacy criteria for patient preoperative skin preparations include a 2- $\log_{10}$  bacterial reduction on the abdomen, a 3- $\log_{10}$  bacterial reduction on the groin, and counts not to exceed baseline at 6 hours post-application, the sponsor was told that testing of abdominal sites in this study was not necessary. The secondary objectives are not required by the TFM and are acceptable.*

### Scope

The study evaluated the antimicrobial efficacy of DuraPrep solution (iodine povacrylex in 74% isopropanol) and Hibiclens (chlorhexidine gluconate (CHG) 4%) each using one product application procedure. Hibiclens was used as the active comparator.

***Reviewer's comment:*** *Hibiclens was chosen as the active comparator based on the results of a pilot study, which is reviewed below (see section V). Previously, we recommended that the sponsor use ChlorPrep as the active control rather*

*than Hibiclens since Hibiclens had been having problems meeting the 3-log reduction on the groin. The pilot study was undertaken to compare the efficacy of Hibiclens and ChloroPrep, and suggests that both products would perform equally well at this testing laboratory.*

### **Subjects**

A total of 107 subjects were assigned screening numbers for the study (designated as S01 through S99 followed by 601 through 607); 102 subjects were screened for microbial counts, 81 subjects were randomized and 80 received study treatment. Of the 81 randomized subjects, 80 (98.8%) were evaluable for safety and 66 (81.5%) were evaluable for efficacy. A total of 62 (76.5%) subjects completed the study and 19 (23.5%) subjects did not complete the study. Fourteen (17.3%) subjects did not complete the study because treatment day baseline criteria were not met. Of the remaining 5 subjects who did not complete the study, 4 subjects (Subjects 001G, 002G, 022G, and 024G) had site contamination at one or more of the sampling sites and 1 subject (Subject 030G) was excluded prior to treatment due to acne on the treatment site.

**Reviewer's comment:** *A sufficient number of subjects completed the study to fulfill the original proposal of 62 evaluable groin regions. The sponsor provided a description of why subjects were excluded from the final evaluation; although they did not explain how the sites were contaminated (did the subject or investigator contaminate the sites?). For the subjects that were excluded due to site contamination, three subjects are missing the 6- and 24-hour samples for Hibiclens, and one subject is missing the 6-hour sample for Hibiclens as well as the 24-hour samples for both treatments. These subjects could not complete the study because it is not known whether they fulfilled the 6-hour requirement of not exceeding baseline counts. If the data for the 10-minute samples from these four subjects were included, both DuraPrep and Hibiclens would have met the 3-log reduction in 3 of 4 of these subjects.*

### **Inclusion Criteria**

Subjects were eligible for participation in the study if they met all of the following criteria:

- were healthy volunteers of either gender, any race, and who were at least 18 years of age;
- satisfied all Inclusion/Exclusion criteria and voluntarily signed the ICF;
- had baseline bacterial counts on screening and treatment days of at least 5.0 log<sub>10</sub> CFU/cm<sup>2</sup> per groin site;
- had skin within 6 inches of the test areas that was free from cuts, acne, abrasions, and skin irritation;
- were willing to follow instructions for the study; and
- were willing to return within 6 hours of treatment and again the next day for the 24-hour sampling.

### Exclusion Criteria

Subjects were excluded from participation in the study if they met any of the following criteria:

- had any form of dermatitis, acne, open wounds, or other skin disorders on the groin test areas;
- had a history of skin allergies;
- had a known sensitivity to any acrylate-, iodine-, chlorhexidine gluconate-, or alcohol-containing products, or to medical tape or natural rubber latex;
- had used antimicrobial soaps, lotions, dandruff shampoos, deodorants, or topical or systemic antibiotic medications within 14 days of the scheduled screening or treatment day;
- had exposure to any other topical medications, creams, or ointments on the test areas within 14 days of the scheduled screening or treatment day;
- had a history of skin cancer within 6 inches of the test areas;
- had contact with biocide-treated swimming pools or hot tubs, tanning beds, hot waxes, or depilatories in the groin area within 14 days of the scheduled screening or treatment day;
- had bathed or showered the test areas within 48 hours prior to the scheduled screening or treatment day;
- had contact with solvents, acids, bases, or other household chemicals in the test areas within 14 days of the screening or treatment day; or
- were pregnant, possibly pregnant, attempting pregnancy, or nursing.

**Reviewer's comment:** *In previous discussions with FDA, the sponsor was asked to allow subjects over 65 years of age to participate in the study. In the current study there is no upper age limit for study participants and this is acceptable. Other inclusion and exclusion criteria are standard for this type of study.*

*There is a discrepancy in the description of the baseline inclusion criteria. The protocol (p. 15) states that subjects must have screening day baseline counts of at least  $5 \log_{10}$  CFU/cm<sup>2</sup> per groin site. The clinical study report (p. 20) states that subjects must have baseline counts on screening and treatment days of at least  $5 \log_{10}$  CFU/cm<sup>2</sup> per groin site. This change was not listed in the protocol amendments. Based on the fact that 14 subjects did not complete the study because treatment day baseline criteria were not met, it appears that the inclusion criteria listed in the protocol are incorrect.*

### Randomization

On the treatment day, a subject number (starting with #001) was sequentially assigned to each subject who satisfied the inclusion/exclusion criteria, including screening bacterial counts. Test areas within each groin region were assigned to treatment according to the subject number per a computer-generated randomization schedule to receive DuraPrep solution on one side of the groin region and Hibiclens cleanser on the contralateral side. Randomization was balanced between left and right sides. Baseline and post-preparation sampling times were randomized to sites within each groin test area.

**Reviewer's comment:** *The randomization scheme for placement of treatments was not followed for one subject (002G). The sponsor claims valid samples were obtained and the effect of this deviation was minimal. I agree that this deviation has little effect on the outcome of the study.*

### **Blinding**

The investigational materials were not blinded from the investigator due to differences in application technique, color, and other physical characteristics. The study staff performing the bacterial enumeration was not involved in the application of the investigational material or in the collection of samples. In order to keep the enumeration of the bacteria blinded, the bacterial plates were not identified by the investigational material used. Therefore, the clinical microbiology technicians who evaluated the bacterial cultures were kept blinded to study treatment.

**Reviewer's comment:** *The investigator or subinvestigator who collected the samples also performed bacterial enumerations for three subjects (013G, 015G, and 026G). Two of these subjects (015G and 026G) did not complete the study because they did not meet the baseline count criteria. The third subject did complete the study. In this case, the investigator was not blinded to study treatment. Subject 013G did not meet the 3-log reduction criteria for either DuraPrep or Hibiclens at 10 minutes; therefore, it seems unlikely that there was investigator bias for these unblinded samples.*

### **Pretreatment Phase**

Prior to the scheduled screening day, subjects underwent a minimum 14-day pretreatment phase to allow for the removal of any antimicrobial agents from their skin. During this phase, subjects were to refrain from the use of products containing antimicrobial agents in accordance with the Subject Instructions. Subjects were given product kits containing non-antimicrobial soaps, deodorants, and shampoos for use during the pretreatment phase through the treatment phase or until notified by the investigator.

In addition, subjects avoided the use of systemic or topical antibiotics or medication and contact with chemically-treated swimming pools or hot tubs, hot waxes and depilatories on the test areas for 14 days before the scheduled screening day. If it became necessary for a subject to take systemic antibiotics or to apply topical medications to the test areas, the subject was to contact the investigator as soon as possible so that another volunteer could be recruited.

A visual skin assessment of the test areas was performed during the pretreatment phase. If subjects required hair removal to facilitate sample collection, the subject was asked to return to the test facility at least 48 hours before the screening day. Subjects were not allowed to shower or bathe the test areas for 48 hours prior to their scheduled screening day. On screening day, a visual assessment of the test areas was performed and the screening baseline samples were collected. Baseline samples were taken from the centers of each of the 2 contralateral test areas using modified sampling solution.

**Reviewer's comment:** *This pretreatment phase is standard for this type of study and is acceptable.*

### **Treatment Day**

The test site within the groin region (groin test area) was defined as the inner aspect of the upper thigh within and parallel to the inguinal crease below the groin. Using a 2" x 5" sterile template, the corners of each groin test area were marked directly on the skin using a non-toxic skin marker. Four sampling sites were numbered within each groin test area. The positioning and numbering of the groin sampling sites were standard for all subjects. Sampling sites on the contralateral side of the groin were numbered in a mirror-image orientation. The 4 sampling sites within each groin test area represented a baseline (pre-preparation) site and 3 post-preparation sample sites (10 minutes, 6 hours, and 24 hours).

After groin test areas were marked and sample sites were numbered, baseline samples were collected from the appropriate site per the randomization schedule in each test area using the appropriate sampling solution for the treatment to be applied and the cup scrub technique.

Following collection of the baseline sample, randomly assigned contralateral groin test areas were prepped with DuraPrep solution or Hibiclens cleanser. Treatments were randomized between left and right test areas, and baseline and post-preparation sampling times were randomized among the sampling sites within each test area. The duration of each preparation procedure was recorded on the case report form.

### **Sample Collection**

Quantitative cultures were obtained from skin sites at 10 minutes, 6 hours, and 24 hours using a modification of the cup scrub method of Williamson and Kligman. The treatment day baseline and post-treatment samples taken from Hibiclens test regions were collected using standard sampling solution (SSS). SSS contains

~~\_\_\_\_\_~~  
~~\_\_\_\_\_~~  
All screening day baseline samples and the treatment day baseline and post-treatment samples taken from DuraPrep solution test regions were collected using modified sampling solution (MSS). ~~\_\_\_\_\_~~

**Reviewer's comment:** *This sample collection scheme is acceptable. In some previous protocols the sponsor proposed collecting Hibiclens samples in Hibiclens sampling solution (HSS). HSS differs from SSS in that it*

*Since the DuraPrep samples are collected in SSS with \_\_\_\_\_, collecting Hibiclens samples in SSS rather than HSS is preferable.*

### Sample Processing and Plating of Bacteria

Raw colony counts from each of the dilutions were recorded on the appropriate case report forms for each subject. The average CFU/mL and CFU/cm<sup>2</sup> of skin were determined for baseline samples (screening and treatment). The average CFU/mL was determined and recorded for post-preparation samples from each test area.

**Reviewer's comment:** *The sample processing and plating methods are acceptable.*

### Results

Only subjects who met the minimum baseline inclusion criteria on the screening and treatment days of the study on both sides of the body were considered evaluable for efficacy. In the event of missing efficacy data at some but not all time points, paired data from the available time points were included in the analysis. If data from a treatment pair were not available, the data from a single treatment were not included in the analysis, since the design of the study was paired. Sixty-six subjects were evaluable for efficacy. Log reductions were calculated by subtracting the post-treatment log counts from the average of the screening day and treatment day baseline log-transformed bacterial counts.

For DuraPrep solution, a mean 3.32-log reduction of bacterial counts was achieved at 10 minutes, thereby satisfying the 3-log reduction criteria of the TFM. Log counts remained significantly below baseline at 6 hours (mean log reduction = 2.67), satisfying the TFM criteria for counts to remain below baseline. At the 10 minute and 6 hour time points, the changes from baseline were statistically significant ( $p < 0.0001$ ). The 24-hour efficacy of DuraPrep solution was also confirmed at the groin site since there was a mean log reduction of 2.28 at 24 hours, a statistically significant reduction from baseline ( $p < 0.0001$ ).

A secondary objective of the study was to compare the log reduction of bacterial counts achieved with the application of DuraPrep solution with those achieved with the application of Hibiclens cleanser. At 10 minutes, DuraPrep solution reduced the bacteria by 3.32 logs compared with 3.41 logs for Hibiclens cleanser. Both of these preparations met the 3-log reduction criteria of the TFM. The difference in the log reduction between DuraPrep solution and Hibiclens cleanser was not statistically significant ( $p=0.4716$ ). Hibiclens cleanser was significantly more effective than DuraPrep solution at 6 and 24 hours ( $p=0.0499$  and  $p=0.0004$ , respectively). Overall, DuraPrep solution and Hibiclens cleanser can be considered similar, with Hibiclens cleanser showing greater efficacy at 6 and 24 hours.

**Table 1: Summary of the log<sub>10</sub> transformed bacterial counts from DuraPrep**

Sample	Sample size	Mean	SD	Log <sub>10</sub> reduction	95% CI
Baseline *	66	5.42	0.317	NA	NA
10 minutes	66	2.10	0.913	3.32	3.09 to 3.55
6 hours	66	2.74	0.729	2.67	2.48 to 2.87
24 hours	65	3.14	0.660	2.28	2.12 to 2.44

**Table 2: Summary of the log<sub>10</sub> transformed bacterial counts from Hibiclens**

Sample	Sample size	Mean	SD	Log <sub>10</sub> reduction	95% CI
Baseline *	66	5.37	0.262	NA	NA
10 minutes	66	1.96	0.987	3.41	NA
6 hours	62	2.57	0.714	2.81	NA
24 hours	59	2.79	0.621	2.59	NA

\* Average of screening and treatment day baseline counts

**Reviewer's comment:** *Both DuraPrep solution and Hibiclens cleanser met the TFM criteria of a 3-log reduction of bacterial counts at 10 minutes post-application and remained below baseline at 6 hours. Both products also met one of the secondary objectives – counts for both treatments remained below baseline for up to 24 hours post-application. For the other secondary objective, the log reductions achieved with DuraPrep solution were compared with those of Hibiclens. Based on the sponsor's calculations, there was no significant difference in bacterial reductions at 10 minutes post-application between the two products. However, Hibiclens was significantly more effective than DuraPrep solution at 6 and 24 hours post-application.*

*DuraPrep solution would also meet the 3-log reduction criteria using a 95% confidence interval approach (95%CI was 3.09 to 3.55 at 10 minutes). Confidence intervals were not provided for Hibiclens, but since the variation appears similar to that seen with DuraPrep solution, we would expect Hibiclens to meet the 3-log reduction criteria using a 95% CI approach as well.*

*Finally, if we analyze the data using a percent responders approach, nearly two-thirds of all subjects met the 3-log reduction criteria. For subjects treated with*

*DuraPrep solution, 60.6% (40/66) of the subjects met the TFM criteria compared to 67.7% (42/62) of the subjects treated with Hibiclens.*

### **Protocol Deviations**

No subjects had protocol deviations with respect to sampling time. Six subjects had other protocol deviations but no subjects discontinued from the study due to protocol deviations. For subject 002G, the randomization scheme for placement of treatments was not followed. Since valid samples were obtained, the effect of this deviation on the study was determined to be minimal. For subjects 013G, 015G, and 026G, the investigator or subinvestigator who collected the samples also performed bacterial enumeration. Therefore, for these subjects, the person enumerating the bacteria was not blinded to study treatment. No action was taken as this deviation was believed to have no negative impact on the study. For subjects 059G and 137G, the incorrect amount of sampling solution was used for sample sites 4 and 3, respectively. To correct this, the formula used to calculate the CFU/cm<sup>2</sup> was adjusted accordingly; therefore, the effect of this deviation on the study was determined to be minimal.

**Reviewer's comment:** *For the subjects where the investigator was not blinded to study treatment, two of the subjects did not complete the study and the third subject did not meet the 3-log reduction criteria. As mentioned above, it seems unlikely that there was investigator bias for these unblinded samples. The other protocol deviations (change in randomization scheme, incorrect amount of sampling solution) likely had no effect on the study outcome.*

### **III. Neutralizer Validation**

The neutralizer validation procedure and results are presented in Module 5 (section 5.3.9). The original neutralization protocol is provided in section 13.8 of the clinical study protocol. Six subjects were treated at two 5" x 5" sites for the neutralizer validation samples. One site was treated with DuraPrep solution and the other with Hibiclens cleanser. The treated sites were sampled using the scrub cup technique and MSS (DuraPrep solution) or SSS (Hibiclens cleanser).

One numbers control (i.e., viability control) and 2 toxicity control tubes were also prepared. Several changes were made to the controls as listed in protocol amendments dated June 8, 2005, and August 2, 2005. The numbers control was changed from

Both protocol amendments describe changes to the MSS toxicity control procedure. The MSS toxicity control was ultimately changed as follows:

The results of the neutralizer validation studies are presented in the tables below.

**Table 3: Neutralizer Toxicity Control Results Expressed as Log CFU/mL**

Subject No.	Time	Numbers Control <sup>1</sup>	Toxicity Control SSS <sup>1,2</sup>	Difference From Numbers Control	Toxicity Control MSS <sup>1,3</sup>	Difference From Numbers Control
NE 1	<1 minute	2.58	2.50	0.08	2.52	0.06
	30 minutes	2.59	2.49	0.10	2.52	0.07
NE 2	<1 minute	2.58	2.50	0.08	2.52	0.06
	30 minutes	2.59	2.49	0.10	2.52	0.07
NE 3	<1 minute	2.58	2.50	0.08	2.52	0.06
	30 minutes	2.59	2.49	0.10	2.52	0.07
NE 4	<1 minute	2.58	2.50	0.08	2.52	0.06
	30 minutes	2.59	2.49	0.10	2.52	0.07
NE 5	<1 minute	2.58	2.50	0.08	2.52	0.06
	30 minutes	2.59	2.49	0.10	2.52	0.07
NE 6	<1 minute	2.58	2.50	0.08	2.52	0.06
	30 minutes	2.59	2.49	0.10	2.52	0.07

<sup>1</sup>All results were an average of Samples 1 and 2

<sup>2</sup>Standard Sampling Solution; <sup>3</sup>Standard Sampling Solution containing

**Table 4: Neutralizer Effectiveness DuraPrep Solution Results Expressed as Log CFU/mL**

Subject No.	Time	Numbers Control <sup>1</sup>	DuraPrep Solution with MSS <sup>1</sup>	Difference From Numbers Control
NE 1	<1 minute	2.58	2.46	0.12
	30 minutes	2.59	2.46	0.13
NE 2	<1 minute	2.58	2.43	0.15
	30 minutes	2.59	2.43	0.16
NE 3	<1 minute	2.58	2.42	0.16
	30 minutes	2.59	2.42	0.17
NE 4	<1 minute	2.58	2.44	0.14
	30 minutes	2.59	2.43	0.16
NE 5	<1 minute	2.58	2.44	0.14
	30 minutes	2.59	2.44	0.15
NE 6	<1 minute	2.58	2.43	0.15
	30 minutes	2.59	2.43	0.16

<sup>1</sup>All results were an average of Samples 1 and 2 from 2 treatment sites

**Table 5: Neutralizer Effectiveness Hibiclens Antiseptic Skin Cleanser Results Expressed as Log CFU/mL**

Subject No.	Time	Numbers Control <sup>1</sup>	Hibiclens cleanser with SSS <sup>1</sup>	Difference From Numbers Control
NE 1	<1 minute	2.58	2.43	0.15
	30 minutes	2.59	2.43	0.16
NE 2	<1 minute	2.58	2.42	0.16
	30 minutes	2.59	2.42	0.17
NE 3	<1 minute	2.58	2.43	0.15
	30 minutes	2.59	2.41	0.18
NE 4	<1 minute	2.58	2.43	0.15
	30 minutes	2.59	2.44	0.15
NE 5	<1 minute	2.58	2.44	0.14
	30 minutes	2.59	2.45	0.14
NE 6	<1 minute	2.58	2.43	0.15
	30 minutes	2.59	2.42	0.17

All results were an average of Samples 1 and 2 from 2 treatment sites

**Reviewer's comment:** *Standard neutralizer validation assays, such as ASTM E1054, include four separate tests: test organism viability (numbers control), neutralizer effectiveness, neutralizer toxicity, and a product control. The sponsor assessed three of these, but did not run a product control. To be complete, the sponsor should also have tested the ability of DuraPrep and Hibiclens to inhibit the growth of the test organism under the conditions used in this assay.*

*The summary data presented in Table 3 above is misleading since only one tube each of the numbers control and toxicity controls were evaluated, rather than six samples as the table implies. This explains the identical results for each subject.*

*The sponsor provided the plate count data for the neutralizer validation study at the request of this reviewer (supplement 011). There were a few computational errors in the submitted data. However, through conversion to log<sub>10</sub> and rounding, these errors did not affect the mean log<sub>10</sub> CFU/mL or the log<sub>10</sub> differences from the numbers control. The difference between the test samples and the numbers control did not exceed 0.17 log<sub>10</sub> for DuraPrep and 0.18 log<sub>10</sub> for Hibiclens. The sponsor considered any difference less than 0.3 logs as effective; therefore, both DuraPrep and Hibiclens were effectively neutralized.*

*It is unclear what volume of pooled sample was used for the six test subjects. Module 5 (section 5.3.9) states that 4.5 mL of pooled sample was inoculated and the clinical study protocol (section 13.8.7.2.5) states that 5 mL of pooled sample was inoculated. The control tubes all contained 5 mL. The protocol changes made to the controls, especially the MSS toxicity control, are mainly as previously requested by FDA and are acceptable.*

*It is unfortunate that only a gram-negative organism was tested in this assay since the clinical simulation uses resident flora, which is more likely to include gram-positive organisms. However, the sponsor previously stated that the \_\_\_\_\_ used in the sampling solution for DuraPrep is toxic to Staphylococcus, so Staphylococcus could not be used in the pivotal trial neutralizer validation study.*

*Previously FDA recommended that both types of samples be plated and incubated in the same manner; however, we agreed that the sponsor could plate the samples as described in the protocol.*

#### **IV. Additional Clinical Studies**

In addition to the pivotal study (reviewed above), the sponsor submitted two additional clinical studies and two pilot studies:

- 3M Study No. I2MS 10125, "Evaluation of Durability and Antimicrobial Persistence of DuraPrep Surgical Solution and ChlorPrep One-Step Skin Preparation Following Exposure to Saline Using a Bacterial Challenge Method."
- 3M Study No. I2MS 10417, "Comparative Study on the Efficacy and Cost Between DuraPrep Skin Preparation and Conventional Povidone-Iodine Skin Preparation in Coronary Artery Bypass Surgery: A Prospective Randomized Trial."
- 3M Study No. I2MS 9981, "Pilot Study to Evaluate the Durability and Antimicrobial Persistence of 3M DuraPrep Surgical Solution and ChlorPrep One-Step Skin Preparation Following Exposure to Saline Using a Bacterial Challenge Method."
- 3M Study No. I2MS 10346, "Pilot Study to Assess the Antimicrobial Effectiveness of Hibiclens Cleanser and ChlorPrep Skin Prep Against Resident Human Skin Flora on the Groin Region."

Only one of the two additional clinical studies that were provided in this submission will be reviewed here. The other clinical study will not be reviewed because it is not relevant to the microbiology of the pivotal trial. One of the two pilot studies is reviewed below in section V.

The 3M study No. I2MS 10125, "Evaluation of Durability and Antimicrobial Persistence of DuraPrep Surgical Solution and ChlorPrep One-Step Skin Preparation Following Exposure to Saline Using a Bacterial Challenge Method" was included in this review because it is relevant to information included in the professional product labeling

(package insert). The data from this study was not included in the draft package insert, but the study is similar to a study that the sponsor wishes to include in the package insert.

The purpose of this study was to compare the persistent antimicrobial activity of iodophor in DuraPrep solution versus CHG in ChloroPrep preparation against transient organisms (modeled by a marker organism) after a saline rinse and a saline soak. The rationale is that saline and other anionic solutions inactivate CHG in solution. Because ChloroPrep preparation is water-soluble, two potential causes of reduced effectiveness after challenge with saline are that the product may be washed away or the CHG may be inactivated during surgery. In contrast, DuraPrep solution is designed to resist wash-off after skin application, forming an insoluble film on the skin. Previous studies have shown that the iodophor in DuraPrep solution remains active after the prep is dried and rinsed with various solutions (LIMS 8197 and 9302).

### **Study Objectives**

The primary objective of this study was to evaluate the resistance to wash-off and/or inactivation of chlorhexidine gluconate in ChloroPrep One-Step skin preparation compared to DuraPrep solution by demonstrating the persistence of antimicrobial activity of CHG and iodine following contact with saline using a bacterial challenge method. The secondary objective was to determine the presence or absence of CHG or iodine on the saline-soaked gauze collected after exposure.

***Reviewer's comment:*** *The study title and purpose mention evaluating the antimicrobial persistence of DuraPrep solution and ChloroPrep after saline treatment; however, only the 10 minute time point is assessed in the study. A 10 minute time point is not appropriate for evaluating the persistence of these products. Antimicrobial persistence is usually evaluated at least 6 hours post-application.*

### **Overall Study Design**

Healthy subjects were entered into a 7-day pretreatment phase during which standardized, non-antimicrobial soaps, shampoos, and deodorants were used. Standard inclusion and exclusion criteria were used, with one exception. To be included in the study, subjects had to have volar forearms that were a minimum of 8 inches long and had minimal hair.

For each subject, one forearm was prepped with ChloroPrep preparation, the other forearm with DuraPrep solution. When the preparations had dried for 10 minutes, randomized individual sites within each test area were treated with either saline rinse or saline soak. There were four sites on each volar forearm: one recovery control site to collect the baseline counts and three sites within the prepped area (prep control, saline rinse and saline soak). The recovery control was always closest to the wrist and received a saline rinse prior to inoculation. The prepped control, saline rinse, and saline soak were randomized among the remaining three sites. A fifth site (above the prepped area) was used for the saline soak control on unprepped skin for chemical testing only.

For the saline rinse sites, the forearm:

COI

### Subjects

61 volunteers were enrolled in the pretreatment phase, such that a total of 36 subjects were evaluable for efficacy at completion of the study.

**Reviewer's comment:** *The sponsor provided the values used in the sample size calculation, which were based on a pilot study. Based on these values, the sample size seems appropriate.*

### Statistical Methods

Log reductions were calculated as the log counts from the recovery control site minus the log counts from each of the prepped sites. For each of the prep control, saline rinse and saline soak sites, the paired difference in log reduction between the test products were calculated for each subject. Resistance to wash-off was calculated by subtracting the log count of the prepped control from the log count for saline rinse and saline soak. For both the saline rinse and saline soak, the paired difference in wash-off between the test products was calculated for each subject. Since the design of the study was paired, if the data from a treatment pair were not available, the data from a single treatment were not included in the analysis.

The significance of the difference in log reduction or wash-off between treatments was assessed using a paired t-test. Success was defined as a significantly greater log reduction or significantly less wash-off for DuraPrep-treated sites compared to

ChlorPrep-treated sites. Significance was assessed at alpha = 0.05 (2-sided). In addition, the 95% confidence limits for the paired difference between treatments were calculated. A nonparametric analysis (Wilcoxon signed rank test) was also conducted to verify the results.

**Reviewer’s comment:** *The sponsor designed the study as a paired comparison and did not use data from single treatments. However, recently ONP statisticians have recommended that studies of this type should follow the intent-to-treat principle of analysis to include data from all treated subjects.*

**Results**

DuraPrep solution had significantly stronger resistance to wash-off by saline rinse or soak treatments compared with ChlorPrep skin prep. The log reduction of seeded organisms was higher for ChlorPrep than for DuraPrep for each condition (prep control, saline rinse, and saline soak). The p-values from the paired t-tests, as well as the Wilcoxon signed rank tests, were all less than 0.0001.

**Table 6: Study summary data**

	n	Mean log “Baseline”	Mean log recovered	Log Reduction	Wash-off (postprep - saline trmt)
<b>DuraPrep</b>		6.10 ± 0.219			
Saline soak	36		4.64 ± 1.172	1.46 ± 1.202	0.41 ± 1.127
Prep control	36		4.23 ± 1.515	1.88 ± 1.486	
Saline rinse	36		4.56 ± 1.384	1.55 ± 1.385	0.33 ± 1.423
<b>ChlorPrep</b>		6.14 ± 0.191			
Saline soak	36		3.42 ± 0.974	2.72 ± 0.969	2.65 ± 1.295
Prep control	36		0.77 ± 1.191	5.37 ± 1.210	
Saline rinse	36		3.45 ± 1.206	2.69 ± 1.198	2.68 ± 1.714

**Reviewer’s comment:** *The sponsor concludes that DuraPrep solution is more resistant to wash-off by saline rinse or soak than ChlorPrep. This is not an unexpected result since ChlorPrep is an alcohol-based product and is water-soluble. ChlorPrep would not be expected to remain on the skin after rinsing and is not the most appropriate comparator for this study. Furthermore, the results of this study may be misleading since we don’t know what “durability” on the skin means in a clinical setting. In addition, this study does not assess residual antimicrobial activity as a result of “durability” on the skin. We do not know whether there is any clinical meaning to having the prep remain on the skin for a certain amount of time post-prep. Finally, this study design is not standard for patient preoperative skin preparations and was never discussed at any of the public Nonprescription Drugs Advisory Committee meetings on clinical study design of healthcare antiseptics.*

## V. Pilot Studies

Only one of the two pilot studies that were provided in this submission will be reviewed here. The 3M study No. I2MS 05-010346, "Pilot study to assess the antimicrobial effectiveness of Hibiclens Cleanser and ChloroPrep Skin Prep against resident human skin flora on the groin region," was included in this review because it is relevant to the conduct of the pivotal trial. The results of this pilot study were used to choose the active comparator for the pivotal trial.

***Reviewer's comment: Previously, we suggested that the sponsor use an active comparator they feel confident will pass the effectiveness criteria. We pointed out that Hibiclens was having problems meeting the 3-log reduction on the groin and suggested that ChloroPrep may be a more appropriate active control. The sponsor felt that Hibiclens is considered the "gold standard" and also pointed out that ChloroPrep was never tested against an appropriate active control and therefore, would not be an acceptable control. This pilot study was undertaken to compare the efficacy of Hibiclens and ChloroPrep and determine which product to use as the active comparator in the pivotal trial.***

### Study Objectives

The primary objective of this pilot study was to demonstrate that Hibiclens cleanser and ChloroPrep skin prep meet the 1994 TFM criteria for log reduction in the groin with each of two sampling solutions (3 log<sub>10</sub>/cm<sup>2</sup> reduction at 10 minutes post-prep).

***Reviewer's comment: The TFM performance criteria for preoperative skin preps on the groin include two parts: a 3-log reduction at 10 minutes post-application and bacterial counts not exceeding baseline at 6 hours post-application. Here the sponsor only evaluated one of the two performance criteria for preoperative skin preps.***

### Overall Study Design

This was a randomized, partially blinded, paired-comparisons study in which each subject received Hibiclens cleanser and ChloroPrep skin prep. A sufficient number of subjects who met the entrance criteria were enrolled into the treatment phase of the study such that a minimum of 14 groin regions (left and right) were evaluable for efficacy. Screening day baseline microbial samples and the treatment day baseline and post-treatment samples were collected using both Hibiclens sampling solution (HSS) and standard sampling solution (SSS). Antimicrobial effectiveness was evaluated by measuring the log reduction of resident skin flora on groin sites at 10 minutes following application of the test material.

### Subjects

Because this was a pilot study, there was no statistical justification of the sample size. Twenty-one subjects were screened for microbial counts. Nineteen of these passed the screening baselines. Of those, 14 subjects were randomized and treated and all 14 subjects completed the study.

**Reviewer's comment:** *There is no formal guidance for pilot studies of this type, including the appropriate number of subjects to use. However, recently ONP has recommended that a pilot study that provides 20-25 degrees of freedom for error is usually adequate to give reasonably reliable sample size estimates. Since FDA had already agreed that the sponsor could use a sample size of 62 subjects in the pivotal trial, a pilot study designed to help determine the appropriate sample size was not necessary.*

#### **Inclusion and Exclusion Criteria**

The inclusion and exclusion criteria were the same as for the pivotal trial, except that only subjects between 18 and 65 years of age were included. Subjects had to have screening and treatment day baseline counts of at least 5 log CFU/cm<sup>2</sup> per groin site with both sampling solutions to be included in the study.

**Reviewer's comment:** *The inclusion and exclusion criteria are similar to those in the pivotal study and are acceptable.*

#### **Pretreatment Phase and Screening Day**

Healthy subjects were entered into a 14-day pretreatment phase during which standardized, non-antimicrobial soaps, shampoos, and deodorants were used. Prior to collection of the screening baseline samples, a visual skin assessment of each test area was performed. The screening baseline samples (2 per groin site) were collected using the cup scrub technique and two sampling solutions (HSS and SSS). Subjects were not allowed to shower or bathe the test areas for 48 hours prior to the treatment day.

#### **Treatment Phase**

The treatment phase was scheduled no sooner than 72 hours and no later than 7 days from the time when screening baseline samples were collected. On treatment day, each subject was prepared for 2 test areas on the groin with 4 sampling sites each. Two baseline samples were collected from randomized sites, one per sampling solution (HSS or SSS). Following the baseline sample collection, randomly assigned contralateral test areas were prepped with either Hibiclens cleanser or ChloroPrep skin prep as described below. The test materials were applied per the randomization schedule and study personnel used sterile gloves to apply the solutions. Two microbial samples were collected per test area at 10 minutes ( $\pm$  30 seconds) postprep, one with HSS and one with SSS. Two technicians collected microbial samples concurrently using the cup scrub technique.

#### **Treatment A = ChloroPrep® One-Step Skin Preparation**

1. Pinch the wings on the applicator to break the ampule and release the antiseptic.
2. Wet the sponge by repeatedly pressing and releasing the sponge against the treatment area until liquid is visible on the skin.
3. Use repeated back-and-forth strokes of the sponge for approximately 2 minutes.
4. Completely wet the treatment area with antiseptic.
5. Allow the area to air dry for approximately 1 minute. Do not blot or wipe away.
6. Contact time begins after step 5.

**Treatment B = Hibiclens® Antiseptic Skin Cleanser**

1. Measure 5 mL of Hibiclens cleanser onto a sterile gauze pad.
2. Apply the product to the 2" x 5" treatment area for 2 minutes, then dry with a sterile gauze.
3. Repeat steps 1-2.
4. Contact time begins after the site is dried a second time.

**Sample Collection**

**Reviewer's comment: *The sample collection process is the same as for the Hibiclens control in the pivotal trial and is acceptable.***

**Sample Processing and Plating of Bacteria**

**Reviewer's comment: *The sample processing and plating procedure is the same as in the pivotal trial and is acceptable.***

**Statistical Methods**

Log reductions were calculated by subtracting the post-treatment log recovery from the average of the screening and treatment day baseline log recovery. The primary objective, to demonstrate that the products met the TFM criteria for log reduction, was assessed by calculating the mean log reduction for each prep and sampling solution combination. The 95% confidence limits around the log reductions were also calculated. In addition, the percent of subjects who met the 3 log reduction criteria was calculated for each treatment and sampling solution. Differences between sampling solutions for each prep and differences between preps for each sampling solution were assessed using the Wilcoxon signed rank test.

**Efficacy Results**

Both ChloroPrep and Hibiclens met the 3-log reduction criteria at 10 minutes on the groin with both sampling solutions. There were no significant differences in log reduction between the two preps or the two sampling solutions. The log reduction for ChloroPrep was 3.37 with SSS and 3.32 with HSS compared to 3.40 and 3.11 respectively, for Hibiclens. When ChloroPrep was sampled with SSS or HSS, 71.4% of subjects had a 3 log reduction or higher. When Hibiclens was sampled with HSS, 57.1% of the subjects had a 3-log reduction or higher compared to 71.4% when sampled with SSS.

**Table 7: Pilot study summary data (10 minutes post-prep)**

Sample	Std. Sampling Soln.		Hibiclens Sampling Soln.	
	ChloroPrep	Hibiclens	ChloroPrep	Hibiclens
Sample size	14	14	14	14
Baseline *	5.75 ± 0.33	5.81 ± 0.32	5.72 ± 0.40	5.76 ± 0.41
Mean log count	2.38 ± 0.64	2.41 ± 0.70	2.41 ± 0.72	2.65 ± 0.50
Mean log reduc.	3.37 ± 0.66	3.40 ± 0.77	3.32 ± 0.72	3.11 ± 0.54
95% CI	2.99-3.75	2.95-3.84	2.90-3.73	2.80-3.42
% responders	10/14 (71%)	10/14 (71%)	10/14 (71%)	8/14 (57%)

\* Average of screening and treatment day baseline counts

**Reviewer’s comment:** *ChloroPrep and Hibiclens performed similarly in this pilot study. Both products achieved a 3-log<sub>10</sub> reduction at 10 minutes post-application; however, the sponsor did not test the sites at 6 hours post-application. The TFM performance criteria for preoperative skin preps on the groin include two parts: a 3-log reduction at 10 minutes post-application and bacterial counts not exceeding baseline at 6 hours post-application. Although it is very unlikely either product would have exceeded baseline counts by 6 hours post-prep, this time point was not assessed. Therefore, these products cannot be considered to have fully met the TFM criteria in this study.*

*There does not appear to be any statistical difference between the log reductions achieved by ChloroPrep or Hibiclens using the same sampling buffer (SSS or HSS). Also, there does not appear to be any statistical difference between sampling one product with either SSS or HSS.*

*When we look at the percent responders, greater than two-thirds of subjects treated with ChloroPrep met or exceeded a 3-log reduction (71%). This was true regardless of the sampling solution. The same percentage of subjects met or exceeded the 3-log reduction when treated with Hibiclens and sampled with SSS. However, sampling Hibiclens-treated subjects with HSS was not as effective (only 57% met the 3-log criteria) as the other treatments.*

*If we look at the data using a confidence interval approach, both ChloroPrep and Hibiclens would just miss the 3-log cut-off of the lower bound. The lower bound*

*of the confidence interval was slightly closer to 3 for subjects sampled with SSS regardless of the treatment.*

*The sponsor has demonstrated that Hibiclens can be an appropriate active comparator for their pivotal trial. Although the differences were not significant, sampling using SSS appears to lead to slightly greater bacterial recovery.*

#### **Protocol deviations**

There was one protocol deviation. For subject 010, only 4 mL of SSS was used for both right and left treatment day baselines. Therefore, the calculations for the treatment day baselines for SSS was  $(\text{CFU/mL} \times 4 \text{ mL}) / 3.80 \text{ cm}^2$ .

**Reviewer's comment:** *This protocol deviation was adequately addressed by changing the calculations and does not adversely affect the outcome of the study.*

#### **Neutralizer Validation**

This neutralization study determined the ability of SSS and HSS to completely neutralize the active ingredients in Hibiclens cleanser and ChloroPrep skin prep when applied to the abdomen of the test subject without exhibiting toxicity to the marker organism. Only one subject participated in this neutralization verification. The test organism used in the evaluation was *Serratia marcescens* (ATCC 14756).

There was no significant difference between the numbers control, the neutralizer effectiveness samples for the products with each sampling solution, and the toxicity controls. These results indicated that the neutralizers in both solutions were effective and non-toxic.

**Table 8: Neutralization results for pilot study 05-010346**

Sample	Time (min)	Log CFU/mL	Difference from Numbers Control
Numbers Control	< 1	2.64	--
	30	2.65	--
SSS Toxicity Control	< 1	2.64	0
	30	2.64	0.01

HSS Toxicity Control	< 1	2.63	0.01
	30	2.63	0.02
ChlorPrep Skin Prep with SSS	< 1	2.61	0.03
	30	2.60	0.05
ChlorPrep Skin Prep with HSS	< 1	2.54	0.10
	30	2.54	0.11
Hibiclens Cleanser with SSS	< 1	2.54	0.10
	30	2.55	0.10
Hibiclens Cleanser with HSS	< 1	2.61	0.03
	30	2.61	0.04

All results above are averages of Sample 1 and Sample 2 from 2 treatment sites per product.

**Reviewer's comment:** *Only summary data was provided for this neutralizer validation study. The difference between the numbers control and the toxicity controls was very low (0.02 log CFU/mL or less). Therefore, both SSS and HSS are non-toxic to S. marcescens. The differences between the numbers control and the antiseptics were also low (0.03 to 0.11 log CFU/mL), demonstrating that both antiseptics were effectively neutralized. It is unfortunate that only a gram-negative organism was tested in this assay since the clinical simulation uses resident flora, which is more likely to include gram-positive organisms. However, the sponsor previously stated that the — used in the sampling solution for DuraPrep is toxic to Staphylococcus, so Staphylococcus could not be used in the pivotal trial neutralizer validation study.*

## VI. Package Insert (Target Product Information (TPI))

The sponsor provided a draft Package Insert (Target Product Information (TPI)), including new data and responses to FDA comments (dated July 20, 2004, and August 17, 2004) on the previous TPI. The in vitro Microbiology studies and Clinical studies will be reviewed here. Please refer to the Labeling Review and Medical Officer Review for comments on other sections of the Package Insert. This reviewer proposes the following changes to the TPI. Additions are underlined and deletions are in ~~striketrough~~ type.

### CLINICAL PHARMACOLOGY *In vitro* Microbiology Studies

7   Page(s) Withheld

       § 552(b)(4) Trade Secret / Confidential

  X   § 552(b)(4) Draft Labeling

       § 552(b)(5) Deliberative Process

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Colleen Kane Rogers, Ph.D.  
Interdisciplinary Scientist

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Concurrence  
Debbie Lumpkins  
Team Leader

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/s/

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Colleen Rogers  
7/20/2006 10:51:37 AM  
MICROBIOLOGIST

Debbie Lumpkins  
7/20/2006 11:41:53 AM  
INTERDISCIPLINARY

Appears This Way  
On Original

Division of Anti-Infective Drug Products  
Clinical Microbiological Review # 1

**NDA:** 21-586

**Date Completed:** July 14, 2004

**Applicant (NDA):**

3M Medical Division

3M Center

Bldg. 275-5W-06

St. Paul, MN 55144-1000

651-733-1110

**Chem/Ther. Type:** Antimicrobial

**Submission Reviewed:** NDA 21-586

**Providing for:** Preparation of the skin prior to surgery; helps reduce bacteria that potentially can cause skin infection.

**Product Names:**

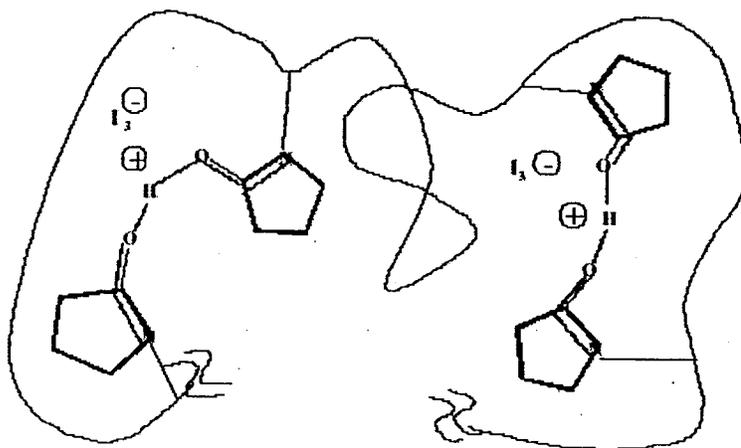
Proprietary: DuraPrep™ Surgical Solution

Non-proprietary/USAN: Iodophor (0.7% available Iodine) and Isopropyl Alcohol (74% w/w)

**Chemical name:** Isopropanol, Iodine Acrylate Copolymer Solution

**Structural formula:** Isopropanol:  $\text{CH}_3\text{CHOHCH}_3$

**DuraPrep Copolymer-Iodine Complex:**



**Molecular formula:**  $C_3H_8O; (I_3)_n (C_6H_9O_2)_n (C_4H_8O_2)_n (C_{10}H_{20}O_2)_n$

**Dosage form:** Iodophor (0.7% available iodine) and isopropanol (74% w/w) solution

**Routes of administration:** Topical

**Pharmacological Category:** Topical Antiseptic

**Dispensed:** Rx \_\_\_\_\_ OTC  X

**Initial Submission Dates**

Received by CDER: October 27, 2003

Received by Reviewer: November 6, 2003

Review Completed: July 14, 2004

**Related Documents:** IND 49,411

**Remarks:**

This review of NDA 21-586 describes the findings and recommendations of the Clinical Microbiology Reviewer. These recommendations are for evaluation by the Division Director for the determination of a decision whether to approve this drug application.

**Executive Summary:**

The subject of this application is DuraPrep, a patient preoperative skin preparation containing an iodophor (0.7% available iodine) and isopropanol (74% w/w). Each of these ingredients contributes different attributes to the function of the final product. The isopropanol is a wide spectrum antimicrobial providing the final product with a rapid antimicrobial effect as it evaporates from the skin. The iodine is a wide spectrum antimicrobial that acts to augment suppression of the resident skin flora and is believed to function as a protective barrier against the transient flora that may be acquired during surgical procedures. Since this product contains two active ingredients, the drug product must meet the drug combination policy. This policy requires the Applicant demonstrate the contribution of each active ingredient in adequate and well-controlled clinical studies. Thus, this NDA was reviewed with this regulatory perspective.

***In Vitro* Studies**

***Spectrum of Activity.*** The *in vitro* antimicrobial spectrum and MBC (minimal bacterial concentration) of DuraPrep solution was determined against 50 different microbial isolates (25 laboratory strains and 25 fresh clinical isolates) of 21 different organisms in the pivotal study, LIMS 7720. These organisms included both Gram-positive bacteria, Gram-negative bacteria, and yeast. For all isolates tested, the MBCs are well below the use concentration of iodine in DuraPrep solution and Betadine solution. DuraPrep w/o  $I_2$  is bactericidal against only a few isolates of five of the organisms tested and only at higher concentrations according to data generated at \_\_\_\_\_

The MBC study design for LIMS 7720 was based on the Tentative Final Monograph (TFM) for Topical Antimicrobial Drug Products for Over-the-Counter Human Use (Federal Register 59[116]:31444-31445; 17 Jun 94), using a modification of methodology established by the National Committee for Clinical Laboratory Standards (entitled "Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically," Document M7-A5, 5th edition, 20:2, 2000). This modification is explained on p28 of this review.

**Contribution of Iodine.** Due to technical constraints on testing, the Applicant found it necessary to make some modifications to the methods in the TFM. Primarily, the formulation of DuraPrep solution puts inherent limits on the ability to isolate the separate contribution of alcohol. The copolymer in DuraPrep solution is soluble in a mixture of IPA (isopropanol) and water as long as \_\_\_\_\_ . Therefore, it is not possible to create a formulation of DuraPrep solution that contains a concentration of alcohol that is \_\_\_\_\_ . To study the contribution of IPA to the antimicrobial activity of DuraPrep solution, the activity of DuraPrep solution was compared with that of the dried film (after the IPA had evaporated off). LIMS 7311 was a time-kill study using the dried film method and LIMS 8919 was a time-kill study conducted without evaporating off the alcohol. The contribution of alcohol to the antimicrobial activity of DuraPrep solution could be indirectly ascertained by comparing the results of these two studies.

**Time-Kill Kinetics.** The microbial kill rate of DuraPrep solution as measured by time-kill kinetics was determined against 15 different organisms in LIMS 8919. Time-kill kinetics was not conducted against all of the organisms listed in the TFM but several species responsible for surgical site infections were included. It was determined that the method used for this study was inappropriate for testing Betadine solution; therefore, the testing of Betadine solution was discontinued. After one minute of exposure to DuraPrep, a range of log<sub>10</sub> reduction of 4.0 or greater (4.0 to 7.1) in microbial counts was shown with 14 of the 15 test organisms. The microbial kill rate of the iodine from dried DuraPrep film was determined against 27 different organisms in LIMS 7311.

LIMS 7311 demonstrated the contribution of iodine in DuraPrep solution. LIMS 8919 demonstrated the effect of both iodine and alcohol in the formulation. The complete formulation in LIMS 8919 always had a higher log reduction of bacterial counts compared with the dried film in LIMS 7311, indicating the contribution of IPA to the microbial activity of the complete formulation.

The three *in vitro* pivotal studies showed that DuraPrep solution is an effective bactericidal agent. Furthermore, the dried films of DuraPrep solution and Betadine solution exhibited similar kill rates against the majority of the organisms tested.

**Pilot Studies.** In addition to these three pivotal studies, five pilot studies (LIMS 7215, SRFE 1623, SRFE 1624, SRFE 1625, and SRFE 1263) were conducted to examine the MBC and time-kill characteristics of DuraPrep solution, and to optimize the methods for completion of the pivotal studies.

**Microbial Resistance.** The development of resistance to DuraPrep solution was not examined through any specific *in vitro* studies but was instead evaluated by a review of the literature. Minimal information regarding resistance to iodine or IPA was found. No development of resistance has been determined for iodine in povidone-iodine.

**In Vivo Studies**

**Efficacy Against Resident Skin Flora.** Two pivotal studies, LIMS 8304 and LIMS 8918, demonstrated effectiveness of DuraPrep solution against resident skin flora on the abdomen *but not* the groin. Both studies confirmed that DuraPrep solution reduced resident skin flora and maintained counts below baseline for 24 hours. In LIMS 8304, only DuraPrep solution met the TFM reduction criteria of a 2-log reduction on the abdomen; neither DuraPrep nor Hibiclens met the 3-log reduction on the groin. In LIMS 8918, while both products met the 2-log reduction on the abdomen, neither product met the 3-log reduction on the groin, although both products performed equivalently. Results are shown in Table A (Table 5.13.1 of the NDA submission).

**Table A. LIMS 8304 and LIMS 8918 Data Summary: Mean Log Reduction of Bacterial Counts (CFU/cm<sup>2</sup>) (SD)**

	LIMS 8304		LIMS 8918	
	Hibiclens Cleanser	DuraPrep Solution	Hibiclens Cleanser	DuraPrep Solution
<b>Abdomen Data</b>	(N=31)	(N=31)	(N=34)	(N=34)
Baseline Log Counts	3.83 (0.491)	3.84 (0.678)	3.51 (0.329)	3.52 (0.433)
Log Reductions:				
2 Minutes	2.52 (1.595)	2.45 (1.377)	2.16 (1.229)	2.42 (1.294)
10 Minutes	1.83 (1.647)	2.48 (1.444)	2.15 (1.302)	2.47 (1.146)
6 Hours	2.02 (1.522)	2.34 (1.520)	1.75 (1.149)	2.31 (1.266)
24 Hours	2.01 (1.456)	1.70 (1.669)	1.78 (0.883)	1.57 (1.154)
<b>Groin Data</b>	(N=39)	(N=39)	(N=47)	(N=47)
Baseline Log Counts	6.39 (0.478)	6.40 (0.486)	5.89 (0.480)	5.82 (0.511)
Log Reductions:				
10 Minutes	2.93 (1.168)	2.95 (1.265)	1.94 (0.964)	2.37 (1.085)
6 Hours	3.36 (1.087)	2.70 (1.318)	2.31 (0.947)	2.29 (0.971)
24 Hours	2.92 (1.222)	2.51 (1.411)	2.69 (0.882)	2.13 (0.796)

SD = Standard deviation.

Note: Log Reduction = average of Screening and Treatment Day baseline log-transformed bacterial counts minus post-treatment log-transformed bacterial counts.

Note: Only subjects with data available from a treatment pair for a given sampling time point were included in this summary table.

Source: Tables 9 and 10, LIMS 8304 and Tables 7 and 8, LIMS 8918 Final CSRs.

In a teleconference dated June 12, 2003, the Applicant noted that the criteria outlined in the TFM for the groin site were not attained in LIMS 8918 but that the results of the product were equivalent to those of Hibiclens. The Division concluded that it was not necessary to repeat the study; the Division clarified that this does not mean that the application will be approved but only that repeating the study would not be beneficial. However the Division recommended that the Applicant submit the data in their NDA along with a *rationale for the acceptance of this data* by the Division. This Reviewer

assumes that the rationale for the acceptance of this data by the Division is that the log reductions demonstrated by DuraPrep were greater than the log reductions demonstrated by the positive control, Hibiclens.

In either study, both the positive control (Hibiclens) and the test product (DuraPrep) failed to meet the TFM criteria for log reduction in the groin site. However, both the positive control and the test product did meet the 2-log reduction criterion for the abdominal site in LIMS 8918; only DuraPrep met the 2-log reduction criterion for the abdominal site in LIMS 8304. However, reexamination of this data (taken from the study reports) in a different format reveals a possible explanation for the log reduction data from the individual subjects as well as the mean log reduction. In addition, the new format identifies whether the log reductions for the *individual subjects* met the TFM criterion and the *percentage* of individual subjects who did meet the TFM criterion.

**Table B. Mean log reductions and percentage of individuals meeting the TFM log reduction threshold**

		mean log reduction	% meeting threshold
<b>LIMS 8304</b>			
abdomen			
	DuraPrep	2.52	67.90
	Hibiclens	2.09	52.50
inguinal			
	DuraPrep	2.78	35.71
	Hibiclens	2.93	51.28
<b>LIMS 8919</b>			
abdomen			
	DuraPrep	2.4	68.96
	Hibiclens	2.11	58.97
inguinal			
	DuraPrep	2.23	20.89
	Hibiclens	1.94	12.00

From Table B, it is clear that the *majority of individuals* as well as *the mean of those individuals* from both studies met the 2-log reduction at the abdominal site for both the test product and the positive control. In both studies, both DuraPrep and Hibiclens easily reached the 2-log reduction criterion for the abdominal site. In fact, DuraPrep outperformed Hibiclens in both the ~~\_\_\_\_\_~~ and ~~\_\_\_\_\_~~ studies with log reductions of 2.52 and 2.4 log reductions, respectively. *Clearly, reaching the TFM 2-log reduction criterion for the abdominal site is readily achievable.*

It is also clear from Table B that in both studies, neither the majority of individuals nor the mean of those individuals met the 3-log reduction at the inguinal site. DuraPrep only outperformed the positive control, Hibiclens, in one study, yet, in both studies neither DuraPrep nor Hibiclens meet the TFM 3-log reduction criterion for the inguinal site. *Clearly, reaching the TFM 3-log reduction criterion for the inguinal site is not readily achievable but indeed, difficult to achieve for individual test subjects.*

This phenomenon, in which the TFM criteria for the abdominal but not the inguinal site is met by either test product or positive control (Hibiclens), is not unique to this product, DuraPrep. Therefore, there must be a reason common to most or all topical antiseptics which explains why topical antiseptics fail in the wet skin site (inguinal site) but not in the dry skin site (abdominal site).

The reasons may be difficult to determine due to the plethora of variables that could affect the success of a topical antiseptic in the dry skin sites versus the wet skin sites. These variables may include: different normal skin flora, different numbers of bacteria, and different immunological responses. For example, there are different varieties and numbers of bacteria that comprise the normal skin flora in dry and wet sites. Wet sites generally possess higher bacterial counts and the inguinal site would be expected to contain a higher percentage of Gram-negative bacteria due to the proximity to the perianal region. Most antiseptics are generally more effective against either Gram-negative or Gram-positive bacteria.

It is important to recognize that the *in vivo* studies for topical antiseptics rely on clinical simulations that measure the reduction in numbers of normal resident skin flora, not pathogens. In addition, there has been no direct correlation made between bacterial log reduction by the use of a topical antiseptic and the risk of infection via the skin during surgery. Studies that might be more useful in demonstrating the efficacy of topical antiseptics might include *in vivo* studies in animal models and studies that utilize bacterial challenge to the skin with organisms known to cause surgical infections. To their credit, the Applicant has supplied data from bacterial challenge methods in both animal models and human clinical simulations.

At the End of Phase 2 meeting held November 6, 2000, the Agency agreed with the Applicant that the bacterial challenge test method is acceptable to demonstrate the contribution of iodine if no difference is seen in standard TFM testing. However, the Agency stated that two studies would be required, at separate laboratories if the study was used to demonstrate the contribution of iodine.

The Applicant has supplied data from such studies in which surgical site pathogens such as tetracycline resistant *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecalis* were used in bacterial challenge methodology in a pig skin model. LIMS 8626, LIMS 8676, and LIMS 8690 pilot studies tested DuraPrep solution and DuraPrep w/o I<sub>2</sub> with such methodology. Using this methodology in three pilot studies, the Applicant shows that DuraPrep demonstrates log reductions against three different surgical site pathogens, *E. coli*, *S. aureus*, and *E. faecalis*. While the list of surgical site pathogens tested in the animal model is by no means comprehensive, it is a positive and innovative step in the direction of determining a correlation between microbial log reductions and risk of surgical site infection.

***Efficacy Against a Bacterial Challenge and Contribution of Iodine.*** The contribution of iodine to the antimicrobial efficacy of the formulation was demonstrated by the greater

reduction of a bacterial challenge using DuraPrep solution compared with DuraPrep w/o I<sub>2</sub> in studies LIMS 8197 and LIMS 9302. Both studies demonstrated significant mean log reductions for DuraPrep solution relative to DuraPrep w/o I<sub>2</sub> although the magnitude of the reductions differed somewhat. Results are summarized in Table C (Table 5.13.2 of the NDA submission).

**Table C. LIMS 8197 and LIMS 9302 Data Summary: Mean Log Reduction of a Bacterial Challenge (CFU/cm<sup>2</sup>) (SD)**

Inoculation Time/ Contact Time	LIMS 8197		LIMS 9302	
	DuraPrep w/o I <sub>2</sub> (N=30)	DuraPrep Solution (N=30)	DuraPrep w/o I <sub>2</sub> (N=24)	DuraPrep Solution (N=24)
<b>When Preparation is Dry</b>				
5 Minutes <sup>1</sup>	-0.05 (0.507)	1.45 (1.550)	-0.02 (0.136)	0.51 (1.346)
30 Minutes <sup>2</sup>	-0.67 (0.895)	2.82 (1.924)	-0.39 (0.701)	3.47 (1.905)
<b>2 Hours Post-Preparation</b>				
5 Minutes	0.22 (1.083)	1.26 (1.621)	-0.02 (0.139)	0.75 (1.485)
30 Minutes	-0.52 (0.804)	3.04 (1.782)	-0.03 (1.261)	3.39 (1.702)
<b>6 Hours Post-Preparation</b>				
5 Minutes	0.03 (0.194)	1.82 (1.781)	0.02 (0.111)	0.71 (1.146)
30 Minutes	-0.18 (0.841)	2.96 (1.761)	0.05 (0.612)	3.77 (1.699)

<sup>1</sup> Subject 011 was missing the assessment at 5-minute residence time when preparation was dry due to technician error.

<sup>2</sup> Subject 205 was missing the assessment at 30-minute residence time when preparation was dry, due to technician error.

SD = standard deviation.

Source: Table 7, LIMS 8197 and Table 7, LIMS 9302 Final CSRs.

**Conclusion:**

*Despite the inability of both DuraPrep and Hibiclens to meet the 3-log reduction criterion in the TFM, DuraPrep had larger bacterial log reductions than the positive control (Hibiclens) at either the abdominal or inguinal sites in the clinical simulations. Coupled with the success of DuraPrep against three surgical site pathogens in a bacterial challenge method in a pig skin model and human clinical simulations, this Reviewer deems that this NDA application is approvable contingent upon compliance with the indicated changes to the Microbiology Section of the Package Insert.*

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## INTRODUCTION

DuraPrep solution is a preoperative skin preparation that contains two active ingredients, an iodophor (0.7% available iodine) and IPA (isopropanol, 74% w/w). Iodine was first

used as an antiseptic in 1839. It has a wide spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria and is fungicidal, protozoacidal, cysticidal, virucidal, and also demonstrates some sporicidal activity (1,2). Iodine tinctures (1.0 to 2.0% iodine in 70% alcohol) were frequently used in the past and were considered to be effective and relatively fast drying (2). When used in the presence of organic matter (serum and tissue debris), some iodine is bound by covalent bonds. Even in the presence of this binding, a solution as dilute as 0.1% demonstrates adequate bactericidal actions.

Iodine solutions are not stable, are irritating to tissues, and have the potential to cause allergic reactions. These adverse effects have led to the development of iodophors, which are carriers of iodine that are usually complexes of iodine and organic compounds. Povidone-iodine is a complex of iodine and the nonsurfactant polymer polyvinylpyrrolidone (PVP). Povidone-iodine possesses similar bactericidal, virucidal, and protozoacidal effects as tincture of iodine or the stronger iodine in Lugol's solution (3). The efficacy and safety of iodophores has been established for many years in the United States (4, 5).

Isopropyl alcohol, the other active ingredient of DuraPrep solution, is a short-chain alcohol ( $C_3H_7OH$ ). Alcohols, in general, have been used for centuries as antiseptics (6). Larson and Morton reviewed the effectiveness of alcohol as an antimicrobial agent (7). According to Ehrenkranz, alcoholic-based antiseptics provides a "quick kill" of infectious microbes thereby limiting the risk of development of antimicrobial resistance (8).

## **PRECLINICAL EFFICACY--*IN VITRO***

### **Mechanism of Action**

McDonnell and Russell recently reviewed the mechanisms of action of iodine and IPA (9). Iodine is rapidly bactericidal, fungicidal, tuberculocidal, virucidal, and sporicidal. Even at low concentrations the antimicrobial effects of iodine are rapid. Although iodine-containing products have been used as antiseptics for 150 years, the exact mechanism of action remains unknown. Iodine rapidly penetrates microorganisms and attacks key groups of proteins (especially the sulfur-containing amino acids cysteine and methionine), nucleotides, and fatty acids, resulting in destruction of the microorganism. Less is known about the antiviral action of iodine. Non-lipid viruses and parvoviruses are less sensitive to the effects of iodine than are viruses with a lipid envelope. Therefore, it is believed that iodine reacts with the surface proteins on enveloped viruses and may destabilize membrane fatty acids by reacting with unsaturated carbon bonds.

The mechanism of action of alcohols is also not completely understood. The most commonly used alcohols for disinfection are ethanol, isopropyl alcohol (IPA), and *n*-propanol. All are rapid, broad-spectrum, antimicrobial agents with activity against vegetative bacteria, viruses, and fungi, but not spores. IPA is considered slightly more bactericidal than the other alcohols, although this depends on the concentration and the test organism. Isopropyl alcohol is more lipophilic than ethanol and therefore is less active against hydrophilic viruses such as polio. The optimal concentration at which

alcohols are antimicrobial is between 60 and 90%. Since alcohols are more effective in the presence of water, it is believed that they cause membrane damage and rapid denaturation of proteins leading to interference with metabolism and eventual cell lysis.

#### Microbial Resistance

The development of resistance to DuraPrep solution was not examined through any specific *in vitro* studies. The Applicant asserts that a search of published literature reveals minimal information regarding resistance to iodine or isopropanol. It is unclear whether true resistance to iodine exists (10,11). According to Ehrenkranz (8), alcohol-based antiseptics provide a quick kill rate thereby limiting the risk of development of microbial resistance.

#### Antimicrobial Spectrum of Activity

Table 1 lists all *in vitro* studies performed that demonstrate the antimicrobial activity of DuraPrep. These include various MBC (minimal bactericidal concentration) determinations and time-kill studies.

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Table 1. Table of Studies Assessing the Antimicrobial Activity of DuraPrep Solution <i>In Vitro</i>		
Protocol Number/Site	Study Title	Primary Objectives
LIMS 7720	Minimum Bactericidal Concentration of 3M™ DuraPrep™ Surgical Solution	To determine the minimum concentration of DuraPrep solution that results in complete kill of test organisms (the MBC) after a 30-minute contact time.

LIMS 8919	3M DuraPrep Surgical Solution and Betadine Solution Using a Time-Kill Procedure	To determine the <i>in vitro</i> microbial kill rate by DuraPrep solution and Betadine solution after 15 second, 30 second, and 1 minute contact times.
LIMS 7311	Time-Kill Assay "Dried Film" Filter Method	To determine the microbial kill rate by iodine released from dried films of DuraPrep solution and Betadine solution after 1-, 5-, and 15-minute contact times. DuraPrep w/o I <sub>2</sub> was included as a non-iodophor control product.
LIMS 7215	Determination of Minimum Bactericidal Concentration	To determine the minimum concentration of DuraPrep solution, DuraPrep w/o I <sub>2</sub> , and Betadine solution resulting in complete kill of test organisms after a 30-minute contact time.
SRFE 1623 3M Health Care	Determination of Antimicrobial Activity of DuraPrep Surgical Solution and Betadine Using a Membrane Filter Assay	To document and compare the efficacy of DuraPrep solution, Betadine solution, Hibiclens cleanser, and povidone-iodine topical gel against clinically important, antibiotic-resistant, Gram-positive bacteria.
SRFE 1624 3M Health Care	Determination of the Antimicrobial Activity of Iodine Released from DuraPrep and Povidone-Iodine Dried Films	To measure and compare the bactericidal activity of free iodine that is released from dried films of povidone-iodine and DuraPrep solution.
SRFE 1625 3M Health Care	Determination of Minimum Bactericidal Concentration of Iodine in DuraPrep and Povidone-Iodine Tincture	To determine and compare the minimum bactericidal concentration of iodine in DuraPrep solution and a tincture of povidone-iodine, both containing 0.7% available iodine, compared with DuraPrep w/o I <sub>2</sub> .
SRFE 1263 3M Health Care	<i>In Vitro</i> Bactericidal Efficacy of DuraPrep Surgical Solution Compared to Betadine Solution	To demonstrate <i>in vitro</i> bactericidal efficacy of DuraPrep solution compared with Betadine solution against the 4 pathogens most frequently isolated from surgical infections: <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>E. faecalis</i> .
LIMS 8628 3M Health Care	Pilot Study to Evaluate the Bacterial Challenge Method (with <i>Staphylococcus aureus</i> ) Using <i>in vivo</i> Pig Skin as a Substrate	The purpose of this pilot study was to evaluate the bacterial challenge methodology in an <i>in vivo</i> pig skin model using DuraPrep Surgical Solution and DuraPrep w/o I <sub>2</sub> . A tetracycline-resistant strain of <i>Staphylococcus aureus</i> (ATCC # 14154) was the test organism used in this study. The effect of inoculum contact time and volume were also considered.
LIMS 8676 3M Health Care	Pilot Study to Evaluate the Bacterial Challenge Method (with <i>Escherichia coli</i> ) Using <i>in vivo</i> Pig Skin as a Substrate	The purpose of this pilot study was to evaluate the bacterial challenge methodology in an <i>in vivo</i> pig skin model using DuraPrep Surgical Solution and DuraPrep w/o I <sub>2</sub> . The test organism used in this study was <i>Escherichia coli</i> (ATCC # 15221). The effect of inoculum contact time and volume were also considered.
LIMS 8690 3M Health Care	Pilot Study to Evaluate the Bacterial Challenge Method (with <i>Enterococcus faecalis</i> ) Using <i>in vivo</i> Pig Skin as a Substrate	The purpose of this pilot study was to evaluate the bacterial challenge methodology in an <i>in vivo</i> pig skin model using DuraPrep Surgical Solution and DuraPrep w/o I <sub>2</sub> . The test organism used in this study was <i>Enterococcus faecalis</i> (ATCC # 10741). The effect of inoculum contact time and volume were also considered.

**Pivotal Studies:**

**Minimum Bactericidal Concentration**

The LIMS 7720 study determined the minimum concentration of DuraPrep solution that results in complete kill of test organisms (the MBC) after a 30-minute contact time. The study includes Betadine solution as an iodine-containing control product. The non-iodine vehicle control product is DuraPrep w/o I<sub>2</sub>. The study fulfills the Tentative Final

Monograph (TFM) requirement to assess the *in vitro* spectrum of DuraPrep solution. Twenty-five laboratory organisms (when available) and 25 or more clinical isolates of each organism listed in the TFM were tested against DuraPrep solution with five laboratory and five clinical isolates of each organism also tested against DuraPrep w/o I<sub>2</sub> and Betadine solution. A total of 468 laboratory strains and 583 clinical isolates were tested against DuraPrep solution for a total of 1,051 organisms. A total of 105 laboratory organisms and 106 clinical isolates were tested against both DuraPrep w/o I<sub>2</sub> and Betadine solution for a total of 211 organisms. Neutralization of the DuraPrep was verified for one isolate of each of the 21 organisms prior to the start of the study.

The MBCs of iodine-containing antiseptics were determined. The test method was modified to allow for the \_\_\_\_\_

\_\_\_\_\_ The final available iodine concentration ranged from \_\_\_\_\_  $\mu\text{g/mL}$  of iodine.

\_\_\_\_\_. These plates were incubated for the appropriate time and temperature required by the isolate.

\_\_\_\_\_ The MBC values were determined from the replicate plates and recorded as a range. Records of incubation time, temperature and recovery medium were maintained for each organism and the test organism concentration was verified for each isolate.

To monitor the reproducibility of the test method, five internal control organisms were run (*Staphylococcus aureus* ATCC 29213, and *Escherichia coli* ATCC 25922 for organisms tested with cation adjusted Mueller Hinton Broth (CAMHB), *Bacteroides fragilis* ATCC 25285 for Fluid Thioglycollate Medium (FTM), *Haemophilus influenzae* ATCC 49247 for Haemophilus Test Medium (HTM), and *Streptococcus pneumoniae* ATCC 49619 for CAMHB + Lysed Horse Blood (CAMHB + LHB)). The expected MBC fell within range for all organisms on all but a single day. On the single day on which the

control organism was out of range, the MBC was one-dilution stop off. A one-dilution test variability is standard for microdilution methods.

Table 2 provides the MBC range ( $\mu\text{g/mL}$ ) for all laboratory strains and clinical isolates of each organism tested against DuraPrep solution, DuraPrep w/o  $\text{I}_2$  and Betadine solution. The MBC values were obtained for all products for all isolates tested. The determined MBCs for each species were variable, although most isolates had MBCs within 1-dilution of one another. MBCs for duplicate samples were either identical or within 1 dilution of one another. Most organisms tested against DuraPrep w/o  $\text{I}_2$  had an MBC of  $\geq 16 \mu\text{g/mL}$ . A few of the isolates had one or more of the replicate MBC values lower than  $16 \mu\text{g/mL}$  as indicated in the range listed.

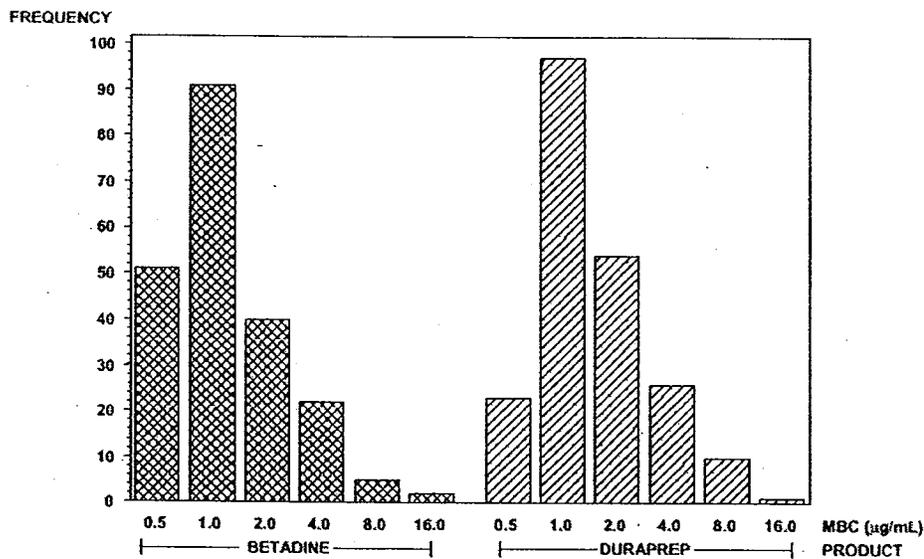
Microorganism	Ranges for DuraPrep Solution and Betadine Solution					
	Laboratory Strains ( $\mu\text{g/mL}$ )			Clinical Isolates ( $\mu\text{g/mL}$ )		
	Betadine Solution	DuraPrep Solution	DuraPrep w/o $\text{I}_2$ Solution	Betadine Solution	DuraPrep Solution	DuraPrep w/o $\text{I}_2$ Solution
<i>Acinetobacter</i> sp.	0.5-4	0.25-2	>16	0.5-1	0.25-4	>16
<i>B. fragilis</i> + <i>B. sp.</i>	0.5-1	0.25-2	8->16	0.5-8	0.25-8	16->16
<i>Haemophilus influenzae</i>	0.25-0.5	0.125-2	4->16	0.25-0.5	0.125-1	4->16
<i>Enterobacter</i> sp.	0.5-1	0.5-2	16->16	0.5-2	0.5-2	>16
<i>Escherichia coli</i>	0.5-1	0.5-1	16->16	0.5-1	0.5-2	>16
<i>Klebsiella</i> sp.	0.5-1	0.25-1	16->16	0.5-1	0.5-2	>16
<i>Pseudomonas aeruginosa</i>	1-4	0.5-8	16->16	1-4	1-4	>16
<i>Proteus mirabilis</i>	0.5-1	0.5-2	8->16	0.5-1	0.5-4	>16
<i>Serratia marcescens</i>	0.25-4	0.5-2	16->16	0.25-2	0.25-4	>16
<i>Staphylococcus aureus</i>	1-2	0.5-2	16->16	0.5-1	0.5-4	16->16
<i>Staphylococcus. epidermidis</i>	0.5-1	0.125-2	16->16	0.5-1	0.25-1	>16
<i>S. hominis</i> + CNS	0.5-1	0.5-2	>16	0.5-1	0.25-4	>16
<i>Staphylococcus haemolyticus</i> + CNS	0.5-1	0.5-1	8->16	0.5	0.5-2	>16
<i>Staphylococcus saprophyticus</i> + CNS	0.5-4	1-2	>16	0.5-2	0.5-2	>16
<i>Micrococcus</i> sp. ( <i>M. luteus</i> )	0.25-4	0.5-4	>16	0.5-2	0.5-4	>16
<i>Streptococcus pyogenes</i>	0.5-4	0.25-16	>16	0.5-8	0.5-8	>16
<i>Enterococcus faecalis</i>	1-2	0.5-4	>16	1-2	1-4	>16
<i>Enterococcus faecium</i>	1-2	1-4	>16	1-4	1-4	>16
<i>Streptococcus pneumoniae</i>	0.5-1	0.125-8	16->16	0.5-2	0.25-4	4->16
<i>Candida</i> sp.	1-16	1-16	>16	4-16	2-16	>16
<i>Candida albicans</i>	2-8	2-8	>16	2-8	2-8	>16

Note: MBC values include the full range of replicates for each organism.  
MBC = minimum bactericidal concentration; CNS = coagulase negative staphylococci; sp = species.  
Source: Tables 3 and 4, Clinical Study Report LIMS 7720.

The MBC frequency histogram for strains tested against both DuraPrep and Betadine are shown in Figure 1. The frequency distributions are similar, with a slight shift to the right for DuraPrep solution. The MBCs for both test products were well below the product use concentrations. Although 211 total isolates were tested against both

DuraPrep solution and Betadine solution, the number tested for each organism was only 10 to 11, which did not allow for conclusions regarding comparative MBC distribution for a given organism.

**Figure 1. Frequency Histogram - DuraPrep Solution vs Betadine Solution - Total Number of Isolates Tested (N = 211) - All Organisms.**



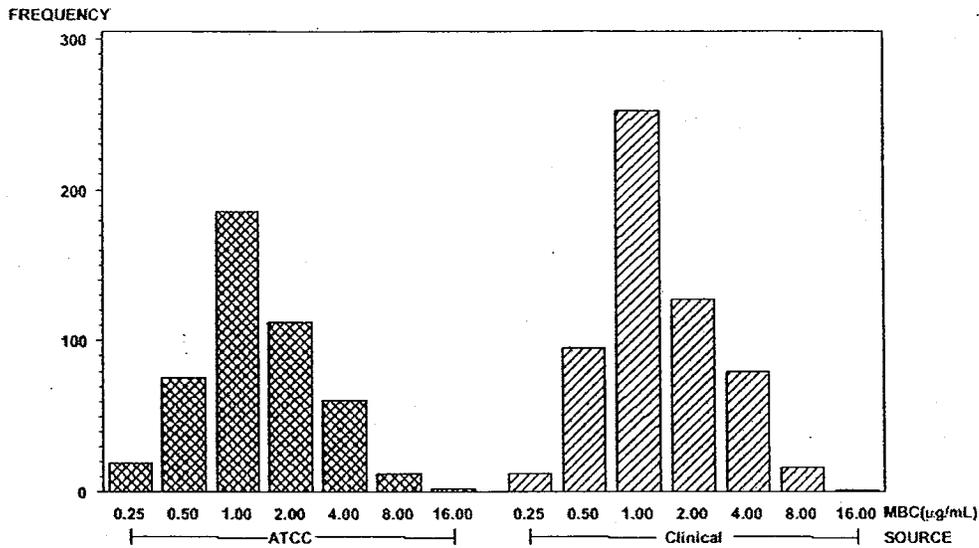
MBC = minimum bactericidal concentration  
Source: Figure 1, Clinical Study Report LIMS 7720.

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The MBC frequency histogram for all organisms (Figure 2) suggests comparable MBC distribution for the clinical isolates and the American Type Culture Collection (ATCC) laboratory microorganisms.

**Figure 2. Frequency Histogram for DuraPrep Solution - ATCC (N = 468) vs**

**Clinical (N = 583) - All Organisms.**

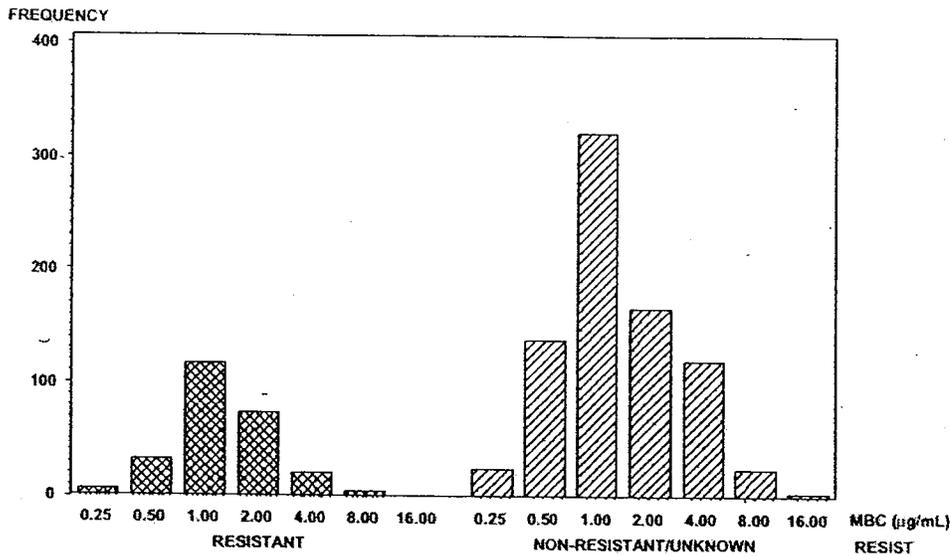


MBC = minimum bactericidal concentration  
Source: Figure 2, Clinical Study Report LIMS 7720.

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Figure 3 indicates the MBC ranges and distributions are similar for isolates with known antibiotic resistance (single- or multi-drug) and isolates with no or unknown resistance.

**Figure 3. Frequency Histogram for DuraPrep Solution - Antibiotic-Resistant (N = 254) vs Non-Antibiotic Resistant/Unknown (N = 797) - All Organisms.**



MBC = minimum bactericidal concentration  
Source: Figure 3, Clinical Study Report LIMS 7720.

In conclusion, MBCs were obtained for all the isolates tested. All MBCs were well below the use concentration of iodine in each product (6,020 µg/mL for DuraPrep solution and 10,200 µg/mL for Betadine solution). DuraPrep w/o I<sub>2</sub> exhibited a slight bactericidal effect at higher concentrations with isolates of *H. influenzae*, *B. fragilis*, *P. mirabilis*, *S. haemolyticus*, and *S. pneumoniae*, and no bactericidal effect with the remaining organisms at the dilutions tested. The data generated with control organisms indicated acceptable test method variation.

**Reviewer's Comments:** Although the TFM requests the use of a MIC study to demonstrate the spectrum of activity, a MBC study was conducted because the iodophor is bound to the microtiter plate thus preventing the performance of a MIC study. At the End of Phase 2 meeting (November 6, 2000), the Agency agreed that a MBC study is more rigorous than the MIC study and thus acceptable.

#### Neutralization Validation for LIMS 7720

Neutralization of the antimicrobial properties of the products is verified for one isolate of each of the 21 organisms prior to the start of the study. This is accomplished by \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
Growth in all wells of the receiver plate following incubation is interpreted as evidence of

adequate neutralization. Successful neutralization is observed for each product. Results are included in the final report for each organism. The following ATCC laboratory organisms are used for neutralization verification:

**Table 3. ATCC Strains used in Neutralization Validation.**

ATCC Number	Organism
19606	<i>Acinetobacter baumannii</i>
25285	<i>Bacteroides fragilis</i>
33391	<i>Haemophilus influenzae</i>
13048	<i>Enterobacter aerogenes</i>
11229	<i>Escherichia coli</i>
11296	<i>Klebsiella pneumoniae</i>
15442	<i>Pseudomonas aeruginosa</i>
4630	<i>Proteus mirabilis</i>
14756	<i>Serratia marcescens</i>
6538	<i>Staphylococcus aureus</i>
12228	<i>Staphylococcus epidermidis</i>
27844	<i>Staphylococcus hominis</i>
29970	<i>Staphylococcus haemolyticus</i>
15305	<i>Staphylococcus saprophyticus</i>
7468	<i>Micrococcus luteus</i>
12344	<i>Streptococcus pyogenes</i>
29212	<i>Enterococcus faecalis</i>
19434	<i>Enterococcus faecium</i>
33400	<i>Streptococcus pneumoniae</i>
18804	<i>Candida albicans</i>
2001	<i>Candida glabrata</i>

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**Reviewer's comments:** The Applicant has not supplied data for validation of the neutralization of the MBC method.

#### **Time-Kill Kinetic Studies**

The LIMS 8919 study determined the *in vitro* rate of microbial kill by DuraPrep solution after 15-second, 30-second, and 1-minute contact times. Ten organisms designated in the TFM and five additional organisms (four antibiotic-resistant organisms and one yeast) were tested (see Table 4).

Surviving bacteria were enumerated and the log<sub>10</sub> reduction from the numbers control at each time point was calculated.

The numbers control was performed in the same manner with \_\_\_\_\_ replacing the \_\_\_\_\_. The inoculum suspension was enumerated at the beginning and end of each test period. Neutralization of the DuraPrep was verified prior to study start.

Time-kill curves were obtained for each of the 15 organisms tested with DuraPrep solution. The log reductions in bacterial counts after treatment with DuraPrep solution at each time point are presented in Table 4. A few of the organisms were retested on different days; therefore, there were replicate data for those organisms. Initial testing of several organisms with Betadine solution showed inconsistent counts between dilutions and probable inhibition of the organisms when exposed in this study procedure. Neutralization of the DuraPrep was effective; however, short exposure of some organisms to Betadine solution seemed to result in sublethal injury, which manifested itself in erratic test results. It was determined that this method was inappropriate so testing of Betadine solution was discontinued.

<sup>1</sup> In older study reports, the ratio was referred to as 1:3 for 1 part proprietary ingredient to 3 parts sampling solution. In more recent study reports it was referred to as a ratio of 1:4 for the same 1 part plus 3 parts.

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**Table 4. Log Reductions for DuraPrep Solution During Time-Kill Studies.**

Microorganism	ATCC #	15 Seconds	Log Reductions at:	
			30 Seconds	1 Minute
<i>E. faecalis</i>	29212	7.08	7.08	6.49
<i>E. faecalis</i> (MDR)	51299	5.86	3.34	4.90
<i>E. faecium</i> (VRE)	51559	7.08	7.15	6.92
<i>E. coli</i>	11229	5.30	4.45	6.64
<i>E. coli</i>	25922	6.58	6.68	6.65
<i>M. luteus</i>	7468	1.96	2.62	4.28

<i>P. aeruginosa</i>	15442	6.92	7.04	7.11
<i>P. aeruginosa</i>	27853	4.15	6.26	5.90
<i>S. marcescens</i>	14756	5.30	6.78	6.49
<i>S. aureus</i> *	29213	5.88	7.08	5.16
		7.00	4.24	5.48
<i>S. aureus</i>	6538	7.00	6.82	6.07
<i>S. aureus</i> (MRSA)*	33592	6.39	5.19	3.63
		7.04	4.71	7.08
<i>S. epidermidis</i>	12228	6.62	6.30	6.68
<i>S. epidermidis</i> (MRSE)*	51625	5.20	6.52	5.66
		1.52	7.11	6.51
<i>C. albicans</i>	10231	2.78	5.21	6.21

\* There are replicate data for these organisms since they were retested on different days.

MDR = multi-drug resistant; VRE = vancomycin-resistant enterococci; MRSA = methicillin-resistant *Staphylococcus aureus*; MRSE = methicillin-resistant *Staphylococcus epidermidis*.

Source: Table 2, Clinical Study Report LIMS 8919.

After 15 seconds of exposure to DuraPrep solution, a log<sub>10</sub> reduction of 4.0 or greater (4.0 to 7.1) in microbial counts is shown with 12 of the 15 test organisms. After 30 seconds of exposure, a log<sub>10</sub> reduction of 4.0 or greater (4.0 to 7.1) in microbial counts is shown with 13 of the 15 test organisms. After one minute of exposure, a log<sub>10</sub> reduction of 4.0 or greater (4.0 to 7.1) in bacterial counts is shown with 14 of the 15 test organisms. One replicate test of methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) shows a 3.63-log reduction after one minute of exposure.

In conclusion, time-kill curves were successfully generated for all 15 organisms tested. The neutralizer is effective in neutralizing the test product and is not toxic to the test organisms.

**Reviewer's comments:** Microbial counts for three of the test organisms demonstrate less than 4.0 log<sub>10</sub> reductions at 15 sec. These organisms are *M. luteus*, MRSE, and *C. albicans*. Of these, only *M. luteus* does not show a 4.0 log<sub>10</sub> reduction at the next time point, 30 sec. However, *M. luteus* does eventually show a 4.0 log<sub>10</sub> reduction at the one min time point. While *E. faecalis* (MDR) shows less than a 4.0 log<sub>10</sub> reduction at 30 sec, the organism does show a 4.0 log<sub>10</sub> reduction at one min. However, while MRSA shows a >4.0 log<sub>10</sub> reduction at 15 sec and 30 sec, at one minute, the organism shows less than a 4.0 log<sub>10</sub> reduction. This observation is troubling as it may indicate that the IPA component is responsible for the initial reductions in microbial counts but after evaporation, the microbial counts increase suggesting the iodine component of the product is not efficacious against MRSA. Normally, enumerations are done at 3, 6, 9, 12, 20 and 30 min. Data for these time points would provide a better idea of kill activity of a period of time that would more closely approximate surgery. Enumerations of microbial cells at these time points would be instructive regarding the efficacy of the iodine component of the product against MRSA.

**Neutralization Validation For LIMS 8919.**

Prior to initiating this study, a neutralization verification test is conducted to ensure the effectiveness of the neutralizer in inactivating the iodine and to affirm that the neutralizer is not toxic to the test organisms. Modified Neutralizing Solution (MNS) is 1:4 ratio<sup>2</sup> (one part proprietary ingredient to three parts neutralizing solution). The neutralizing solution is ~~\_\_\_\_\_~~. The test organisms used are *E. coli* (ATCC 11229) and *S. aureus* (ATCC 6538).

For toxicity testing, the same method as above is used, ~~\_\_\_\_\_~~  
~~\_\_\_\_\_~~ For the numbers control, the same method as above is used, ~~\_\_\_\_\_~~  
Following incubation, CFUs/mL are calculated and converted to log<sub>10</sub> CFU/mL.

<sup>2</sup> In older study reports, the ratio is referred to as 1:3 for one part proprietary ingredient to three parts sampling solution. In more recent study reports it is referred to as a ratio of 1:4 for the same one part proprietary ingredient plus three parts sampling solution.

The neutralization is considered effective if the post-preparation sample recovered was not more than 0.2 log<sub>10</sub> less than the Numbers Control sample. The SSS is considered non-toxic if the Toxicity Control sample is not more than 0.2 log<sub>10</sub> less than the Numbers Control sample.

Neutralizer toxicity and effectiveness data are presented in Table 5.

Table 5. Summary of Neutralizer Toxicity and Effectiveness for LIMS 8919

	<i>E. coli</i> ATCC 11229		<i>S. aureus</i> ATCC 6538	
	Log <sub>10</sub> CFU/mL Recovered	Log Difference from Numbers Control	Log <sub>10</sub> CFU/mL Recovered	Log Difference from Numbers Control

Numbers Control	(T=0)	1.88	NA	1.87	NA
	(T=30)	1.81	NA	1.91	NA
Toxicity	(T=0)	1.81	0.07	1.94	-0.07
	(T=30)	1.79	0.02	1.81	0.10
Effectiveness	(T=0)	1.94	-0.06	2.04	-0.17
	(T=30)	1.86	-0.05	1.99	-0.08

Source : Table 1, LIMS 8919 Final CSR.

In conclusion, the neutralizer used in this study, MNS, is considered effective since the  $\log_{10}$  CFU/mL of the test sample is not more than 0.2 logs less than the numbers control. The neutralizer is considered non-toxic since the toxicity control is not more than 0.2 logs less than the numbers control.

#### Time-Kill Assay "Dried Film" Filter Method

The objective of the LIMS 7311 study is to determine the rate of microbial kill by iodine released from dried films of DuraPrep solution and Betadine solution after 1-, 5-, and 15-minute contact times. DuraPrep w/o  $I_2$  is included as a non-iodophor control product. Twenty-seven organisms are tested which include: 13 Gram-positive bacterial isolates, 13 Gram-negative bacterial isolates, and one yeast isolate.

All test conditions, including the untreated controls, are run in duplicate. The data from each treatment are originally expressed as average  $\log_{10}$  reduction from the control filters (no antiseptic film); however, for the Study Sponsor Report the average  $\log_{10}$  microbial recovery is calculated and graphed. Neutralization was verified for DuraPrep solution, using two organisms on the day those organisms are tested.

The data for this study can be found in Table I, pp 6-9 of the LIMS 7311 study report. Overall, DuraPrep solution and Betadine solution films exhibit similar kill rates against the majority of the organisms tested. Betadine solution demonstrates a slightly faster rate of kill against *K. pneumoniae* and *S. marcescens*, as shown by a greater kill at one minute, and against *C. albicans* and *E. aerogenes* at one and five minutes, respectively. DuraPrep solution demonstrates a slightly faster kill against MDR *E. faecalis*, and *S. pyogenes*, as shown by greater kill at five and 15 minutes, respectively. The significance, if any, of these differences is unknown. DuraPrep w/o  $I_2$  exhibits little or no antimicrobial

activity, regardless of the test organism or contact time, demonstrating the activity of the iodine in the DuraPrep solution final product formulation.

#### Neutralization Validation for LIMS 7311

The neutralizing buffer used in this study is \_\_\_\_\_  
\_\_\_\_\_ The test organisms used in this evaluation are *S. aureus* (ATCC 33592) and *E. coli* (ATCC 11229).

Neutralization of DuraPrep solution was verified at \_\_\_\_\_

When this study was conducted in 1996, it was the general procedure of \_\_\_\_\_  
— to verify neutralization for only the target product, in this case, DuraPrep solution. The only criterion was that the bacterial counts recovered be comparable. Table 6 presents the raw data for DuraPrep solution neutralization.

Table 6. Summary of Neutralizer Effectiveness for LIMS 7311: DuraPrep Solution

	<i>E. coli</i> ATCC 11229				<i>S. aureus</i> ATCC 33592			
	CFUs per Filter		Average CFUs	Log <sub>10</sub> CFU Recovery	CFUs per Filter		Average CFUs	Log <sub>10</sub> CFU Recovery
0 min	58	63	60.5	1.78	23	25	24	1.38
30 min	63	64	63.5	1.80	37	33	35	1.54
Confirmation	61	69	65	1.81	29	34	31.5	1.50

The number of organisms recovered from both post-inoculation time points is calculated and compared to confirm that the iodine contained in DuraPrep solution is effectively neutralized. The results from this test confirm that the antiseptic is effectively neutralized.

**Reviewer's comments:** Note that although the Applicant has supplied neutralization effectiveness data, the neutralization toxicity data is not present.

#### Pilot Studies:

**The Contribution of Alcohol to the Antimicrobial Activity of DuraPrep Solution**  
LIMS 7311 and LIMS 8919 examine the contribution of IPA to the antimicrobial activity of DuraPrep solution. LIMS 7311 is a time-kill study using the dried film method and LIMS 8919 is a time-kill study conducted without evaporating off the alcohol. The

contribution of alcohol to the antimicrobial activity of DuraPrep solution can be indirectly ascertained by comparing the results of these two studies.

The objective of the LIMS 7311 study is to determine the microbial kill rate by iodine released from dried films of DuraPrep solution. This method evaluates the activity of the iodophor component of DuraPrep solution in the absence of IPA. The product is applied to a sterile membrane filter and dried (evaporating off the alcohol) prior to inoculation with the test organism. The organism contact times evaluated are at 1, 5, and 15 minutes. At each time point, the filter is transferred into neutralizer and surviving bacteria are enumerated using standard methods. Twenty-seven organisms were tested.

LIMS 8919 used a wet-filter method. The objective of this study was to determine the *in vitro* microbial kill rate of DuraPrep solution. Test organisms were applied to a membrane filter prior to application of the product. The organism contact times were 15 seconds, 30 seconds, and 1 minute (much shorter due to the anticipated activity of the IPA). Iodine was neutralized and surviving bacteria were enumerated using standard methods. Fifteen organisms were tested but data for only eight are shown.

The common time point between the two studies is one minute. This is the shortest time point for LIMS 7311 and the longest for LIMS 8919. Table 7 compares the log<sub>10</sub> reductions for DuraPrep solution after a one-minute contact time for eight organisms tested in both studies.

The effect of alcohol in the time-kill study can be calculated by subtracting the results of LIMS 7311 from LIMS 8919. These results are in the last column of Table 7. The contribution of alcohol ranges from 0.61 logs through 6.15 logs for the eight organisms tested.

**Table 7. Log<sub>10</sub> Reduction by DuraPrep Solution in Two Time-Kill Studies at One Minute (LIMS 7311 and LIMS 8919)**

Organism	ATCC #	LIMS 7311 Iodine contribution	LIMS 8919 Alcohol + Iodine contribution	Alcohol contribution (column 4 – column 3)
<i>E. coli</i>	11229	6.03	6.64	0.61
<i>E. coli</i>	25922	4.12	6.65	2.53
<i>S. marcescens</i>	14756	2.47	6.49	4.02
<i>E. faecalis</i> (MDR)	51299	2.16	4.90	2.74
<i>S. aureus</i>	6538	1.65	6.07	4.42
<i>S. aureus</i> (MRSA)	33592	0.93	3.63*	2.70
			7.08	6.15
<i>S. epidermidis</i>	12228	5.12	6.68	1.56
<i>C. albicans</i>	10231	0.91	6.21	5.30

\* Replicate testing on 2 different days

Notes: MDR = Multiple drug resistant; MRSA = Methicillin-resistant *Staphylococcus aureus*

Source: Table 1, LIMS 7311 and Table 2, LIMS 8919 Final CSRs.

**Reviewer's comments:** Note that not all the organisms tested in the previous time-kill studies were employed in this dried-film study. Also, there is no testing of alcohol alone, however, this is inferred by subtracting the results of the iodine

contribution (column 3) from the results of the iodine plus alcohol contribution (column 4).

The data from LIMS 7311 demonstrate the contribution of iodine in DuraPrep solution. The data from LIMS 8919 demonstrate the effect of both iodine and alcohol in the formulation. The complete formulation in LIMS 8919 always has a higher log reduction of bacterial counts compared to the dried film in LIMS 7311, indicating the contribution of IPA to the antimicrobial activity of the complete formulation.

#### **Determination of Minimum Bactericidal Concentration**

The objective of the LIMS 7215 pilot study is to determine the minimum concentration of DuraPrep solution, DuraPrep w/o I<sub>2</sub>, and Betadine solution resulting in complete kill of test organisms after a 30-minute contact time. In this pilot study, ten isolates of six bacterial strains (*Burkholderia cepacia*, *E. faecalis*, *E. coli*, *S. epidermidis*, methicillin-sensitive *S. aureus*, and MRSA) were tested. Fresh clinical isolates were used when available.

A one-dilution margin of error is standard for microdilution methods used to determine the minimum inhibitory concentration of antibiotics. *S. aureus* ATCC 6538 was run as a control strain with each set of isolates evaluated to assess the reproducibility of the MBC method used in this study. Results of MBC determinations on the control strain demonstrated that the test method used was performed correctly, with MBCs routinely being within one dilution of one another, both within duplicate samples and between repeated tests.

The MBCs for each species were somewhat variable, although most isolates had MBCs that were within one dilution of each other. When available, clinical isolates were run preferentially to determine the degree of variability within these strains. There was no discernible difference in MBCs between clinical and ATCC isolates. The greatest degree of variability was seen against isolates of *B. cepacia*. The MBCs for Betadine solution

against this organism ranged from 2 µg/mL to 16 µg/mL and for DuraPrep solution ranged from 8 µg/mL to >16 µg/mL. For most other species, the MBCs differed by only one or two dilutions.

The MBCs of DuraPrep solution were usually one to two dilutions higher than the MBCs of Betadine solution against the same organism. All MBCs observed were well below the use concentration of each product. DuraPrep w/o I<sub>2</sub> exhibited no bactericidal activity.

#### **Determination of the Antimicrobial Activity of DuraPrep Surgical Solution and Betadine Solution Using a Membrane Filter Assay**

The objective of the SRFE 1623 pilot study is to compare the efficacy of DuraPrep solution, Betadine solution, and Hibiclens cleanser against clinically important, antibiotic-resistant, Gram-positive bacteria. Pharmaseal® Povidone-Iodine Topical Gel was compared with DuraPrep solution against only one organism, *E. faecium*.

DuraPrep solution, Betadine solution, and Hibiclens cleanser all reduce *S. epidermidis* to undetectable levels within one minute. *S. aureus* is reduced to undetectable levels within 3-5 minutes with Betadine solution and one minute with DuraPrep solution. The only *S. aureus* strain tested with Hibiclens cleanser is reduced to undetectable levels within two minutes. Enterococcal isolates survive up to ten minutes with Betadine solution but were reduced to undetectable levels for all strains within one minute with DuraPrep solution. Hibiclens cleanser appears to be more effective than Betadine solution but not as effective as DuraPrep solution against enterococcal strains. Although there was a three-log reduction in bacterial counts of *E. faecium* at two minutes following treatment with the povidone-iodine gel, there was no reduction to undetectable levels by ten minutes. Control data comparing DuraPrep solution with DuraPrep w/o I<sub>2</sub> indicated that the rapid kill rate observed with DuraPrep solution was due in large part to the antimicrobial effect of the IPA.

In conclusion, the rate of kill by DuraPrep solution (with the exception of the methicillin-resistant strain of *S. epidermidis*) is faster than the rates of kill of the aqueous iodophors and aqueous chlorhexidine.

#### **Determination of Antimicrobial Activity of Iodine Released from DuraPrep and Povidone-Iodine Dried Films**

The objective of the SRFE 1624 pilot study is to measure and compare the bactericidal activity of iodine that is released from dried films of povidone-iodine and DuraPrep solution. A secondary objective is to assess the microbiological activity, if any, of

DuraPrep w/o I<sub>2</sub>. The bacterial strains evaluated are *E. faecalis*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, and *E. coli*.

Maximum bacterial reduction is achieved within a one-minute contact time against all isolates, with the exception of *E. faecalis*, for both dried DuraPrep solution and dried povidone-iodine. The dried DuraPrep solution achieves a two- to three-log reduction of *E. faecalis* within one minute of contact time and a complete reduction within five minutes, compared with povidone-iodine film that achieves complete kill within one minute. This difference is presumably due to the water-insolubility of the DuraPrep polymer and the relatively high "resistance" of enterococci to killing by iodine compared to other bacteria. There is no appreciable reduction of any of the test organisms on filters treated with DuraPrep w/o I<sub>2</sub> at any of the time points.

In conclusion, the Applicant demonstrates the ability of dried films of DuraPrep solution and povidone-iodine to release iodine in concentrations sufficient to kill a heavy bacterial inoculum within five minutes. Comparison of the activity of DuraPrep solution with the vehicle control (DuraPrep w/o I<sub>2</sub>) demonstrates that the activity seen with DuraPrep solution is due solely to the release of iodine and not to the acrylate subunits of the polymer.

#### **Determination of Minimum Bactericidal Concentration of Iodine in DuraPrep and Povidone-Iodine Tincture**

The objective of the SRFE 1625 pilot study is to determine and compare the MBC of iodine in DuraPrep solution and a tincture of povidone-iodine, both containing 0.7% available iodine, and DuraPrep w/o I<sub>2</sub>. A secondary objective is to characterize the activity, if any, of DuraPrep w/o I<sub>2</sub>. Minimum bactericidal concentrations were determined against 31 different bacterial strains.

The method used to determine MBCs for the test substances was a modification of the National Committee on Clinical Laboratory Standards (NCCLS) microdilution method. All test substances were diluted using a \_\_\_\_\_ dilution procedure to prevent precipitation of the DuraPrep polymer / \_\_\_\_\_

Antiseptic concentrations ranged

from  $\mu\text{g/mL}$  iodine and were run in duplicate. The DuraPrep w/o  $\text{I}_2$  served as a control for the IPA as well as for the polymer.

The threshold for measuring growth was set at a change in OD of 0.005 compared with the media control. The MBC was defined as the lowest concentration of iodine that resulted in complete kill of the organism at each time point.

The MBC of iodine for all bacteria ranges from 1 to 16  $\mu\text{g/mL}$  depending on the bacterial strain and the contact time. Complete kill occurs in 30 minutes for all organisms at an iodine concentration of  $\leq 4 \mu\text{g/mL}$ , with *E. faecalis* and *S. aureus* requiring slightly higher concentrations of iodine to achieve complete kill than the other test strains. The MBCs of DuraPrep solution and povidone-iodine solution against all strains tested were equivalent within one-dilution of the test method. There was no apparent antimicrobial activity of the DuraPrep w/o  $\text{I}_2$  (control) against any of the test strains, as indicated by growth in all test wells at all time points, indicating that neither the IPA nor the DuraPrep polymer contributed to the antimicrobial activity.

#### ***In Vitro* Bactericidal Efficacy of DuraPrep Solution Compared to Betadine Solution**

The objective of the SRFE 1263 pilot study is to demonstrate the *in vitro* bactericidal efficacy of DuraPrep solution compared with Betadine solution against the four pathogens most frequently isolated from surgical infections: *E. coli*, *S. aureus*, *P. aeruginosa*, and *E. faecalis*.

Active bacterial cultures were dispersed evenly over a sterile cellulose acetate membrane surface and allowed to incubate overnight. The membranes were exposed to 200  $\mu\text{L}$  of either DuraPrep solution or Betadine solution for one and two minutes. After each exposure time, the membranes were placed into 100 mL of sterile 0.1% sodium thiosulfate and macerated for five minutes. Duplicate membranes were run for each product at each time point. Surviving bacteria were enumerated using standard procedures.

Both test materials have a 100% kill rate at both time points against *E. coli*, *S. aureus*, and *P. aeruginosa* ( $> \text{six-log}_{10}$  reduction). DuraPrep solution is more effective against *E. faecalis* ( $> \text{six-log}_{10}$  reduction at both one and two minutes) than Betadine solution (one- $\text{log}_{10}$  reduction at one minute and three- $\text{log}_{10}$  reduction at two minutes).

In conclusion, both test materials are similar in activity against *E. coli*, *S. aureus*, and *P. aeruginosa*. From this study, the Applicant concludes DuraPrep solution is more effective than Betadine solution against *E. faecalis*.

## PRECLINICAL EFFICACY--*IN VIVO*

### Pharmacokinetics

Neither the active components nor the final DuraPrep solution are intended for systemic use, therefore no clinical pharmacokinetic (PK) studies were conducted. To assess the potential for iodine absorption, DuraPrep solution was compared with Betadine solution (Betadine Surgical Scrub and Betadine solution [a scrub and paint]) and an iodine-enriched meal in 12 subjects in Study SRFE 1621. The study report for SRFE 1621 is provided in Module 5.3.3 of the NDA submission.

Only one study conducted during the nonclinical testing program contained any PK assessments. In Study SRFE 1620, blood and urine levels of iodine were determined following dermal application in the hairless rat. The results from this study demonstrate that there is no difference in the absorption or elimination of iodine following treatment with DuraPrep solution or Betadine solution, or when DuraPrep solution is applied to either normal or abraded skin.

### Animal Prophylactic and Therapeutic Studies--Evaluation of the Bacterial Challenge Method Using *in vivo* Pig Skin as a Substrate

The objective of the LIMS 8628, LIMS 8676, and LIMS 8690 pilot studies are to evaluate the bacterial challenge methodology in an *in vivo* pig skin model using DuraPrep solution and DuraPrep w/o I<sub>2</sub>. The test organisms used in these studies are a tetracycline-resistant strain of *S. aureus* (ATCC 14154), *E. coli* (ATCC 15221), and *E. faecalis* (ATCC 10741) for pilot studies LIMS 8628, LIMS 8676, and LIMS 8690, respectively. Neutralization was verified prior to study start.

Prior to the application of the test materials, the lateral surface of the anesthetized pig is cleaned with 70% IPA from front to back legs and from spine to just above the teat area. One pig is used for multiple sampling on multiple days. Three replicates are required for per study. DuraPrep solution or DuraPrep w/o I<sub>2</sub> are randomly applied to one of the two test areas on the cleaned skin and allowed to air dry (three to five minutes). Within each test area, four individual test sites measuring approximately one inch in diameter are marked and inoculated with either 25 or 50 µL (approximately 10<sup>6</sup> to 10<sup>7</sup> CFU) of the specified bacteria. The other test area (control) is also inoculated with either 25 or 50 µL of the specified bacteria. After inoculation, the bacteria are allowed to remain *in situ* for five minutes or 30 minutes. Surviving bacteria are collected using a cup scrub method and sampling solution.

Quantification uses standard methods. For *S. aureus*, Vogel Johnson agar containing potassium tellurite and tetracycline is used. For *E. coli*, MacConkey agar is used. For *E. faecalis*, m-Enterococcus agar is used.

A toxicity control is run by inoculating an aliquot of the sampling solution with the challenge organism to confirm that the sampling solution does not adversely affect the growth of the organism. The variables investigated are the inoculum contact time (five

and 30 minutes) and the inoculum volume (25 or 50  $\mu\text{L}$ ).  $\text{Log}_{10}$  reductions for each variable studied are calculated by subtracting the  $\text{log}_{10}$  bacterial recovery of the DuraPrep solution or DuraPrep w/o  $\text{I}_2$  site from that of the 30 minute untreated-control site.

DuraPrep solution-treated sites demonstrate bacterial reduction of *S. aureus* at both time periods. At five minutes, a 3.6-mean  $\text{log}_{10}$  reduction is observed for both inoculum levels (25  $\mu\text{L}$  and 50  $\mu\text{L}$ ) and at 30 minutes, 3.9-(25  $\mu\text{L}$ ) and 4.3-(50  $\mu\text{L}$ ) mean  $\text{log}_{10}$  reductions are observed. DuraPrep w/o  $\text{I}_2$  shows no activity against the bacterial challenge at either time point. There is a statistically significant difference in log reduction between DuraPrep and DuraPrep w/o  $\text{I}_2$  at both five minutes and 30 minutes ( $p < 0.0001$ ).

Bacterial reduction of *E. coli* is observed on DuraPrep solution-treated sites under all of the test conditions. At five minutes, the 25- $\mu\text{L}$  inoculum level shows a mean  $\text{log}_{10}$  reduction of 3.0 and the 50- $\mu\text{L}$  level shows a mean  $\text{log}_{10}$  reduction of 1.9. At 30 minutes, the mean  $\text{log}_{10}$  reduction is 3.1 for 25  $\mu\text{L}$  and 4.1 for 50  $\mu\text{L}$ . DuraPrep w/o  $\text{I}_2$  shows no activity against the bacterial challenge under any test condition. There is a statistically significant difference in log reduction between DuraPrep solution and DuraPrep w/o  $\text{I}_2$  at both five minutes and 30 minutes ( $p < 0.0001$ ).

Significant bacterial reduction of *E. faecalis* is observed on DuraPrep solution-treated sites at both time periods. At five minutes, the 25- $\mu\text{L}$  inoculum level shows a mean  $\text{log}_{10}$  reduction of 1.6 and the 50- $\mu\text{L}$  inoculum level shows a mean  $\text{log}_{10}$  reduction of 2.2. At 30 minutes, the mean  $\text{log}_{10}$  reduction is 3.3 (25  $\mu\text{L}$ ) and 3.8 (50  $\mu\text{L}$ ). DuraPrep w/o  $\text{I}_2$  shows no activity against the bacterial challenge at either time period. There is a statistically significant difference in log reduction between DuraPrep and DuraPrep w/o  $\text{I}_2$  at both five minutes ( $p < 0.0006$ ) and 30 minutes ( $p < 0.0001$ ).

In conclusion, the results of this study demonstrate the contribution of iodine in DuraPrep solution when tested against *S. aureus*, *E. coli*, and *E. faecalis*. In addition, the results indicate the *in vivo* pig skin model is a potentially valid model for bacterial challenge studies.

#### **Enzyme Hydrolysis Rates**

A search of the literature indicates that no studies investigating the effect of enzyme hydrolysis on iodine and IPA have been conducted on human skin.

## **CLINICAL EFFICACY**

### ***In Vivo* Studies Conducted During the Clinical Trial**

The antimicrobial activity of DuraPrep solution has been evaluated *in vivo*, as described in Table 8.

**Table 8. Table of Studies Assessing the Antimicrobial Activity of DuraPrep Solution *In Vivo***

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Protocol # / Site	Study Title	Primary Objectives
LIMS 8304	Pivotal Study to Assess the Antimicrobial Effectiveness of 3M DuraPrep Surgical Solution against Resident Human Skin Flora on Abdomen and Groin Regions (Study 1)	To demonstrate that DuraPrep solution meets the 1994 TFM criteria for log reduction of resident bacterial flora and to demonstrate the contribution of iodine to the formulation by showing a significantly greater log reduction at 24 hours on sites treated with DuraPrep solution compared with those treated with DuraPrep w/o I <sub>2</sub> .
LIMS 8918	Pivotal Study to Assess the Antimicrobial Effectiveness of 3M DuraPrep Surgical Solution Against Resident Human Skin Flora on Abdomen and Groin Regions (Study 2)	To demonstrate that DuraPrep solution meets the 1994 TFM criteria for log reduction of resident bacterial flora.
LIMS 8197	Evaluation of the Persistent Antimicrobial Activity of 3M DuraPrep Surgical Solution and DuraPrep w/o I <sub>2</sub> Control Using a Bacterial Challenge Method (Study 1)	To assess the contribution of iodine to the antimicrobial activity of DuraPrep solution.
LIMS 9302	Evaluation of the Persistent Antimicrobial Activity of 3MduraPrep Surgical Solution and DuraPrep w/o I <sub>2</sub> Control Using a Bacterial Challenge Method (Study 2)	To assess the contribution of iodine to the antimicrobial activity of DuraPrep solution.
LIMS 8198	Evaluation of the Durability and Persistent Antimicrobial Activity of 3M DuraPrep Surgical Solution and Betadine Scrub and Solution following Exposure to Blood and Saline Using a Bacterial Challenge Method	To compare the durability and persistence of antimicrobial activity of DuraPrep film and Betadine combination following a wash with autologous blood and saline.

**Pivotal Studies to Assess the Antimicrobial Effectiveness of 3M Duraprep™ Surgical Solution Against Resident Human Skin Flora on Abdomen and Groin Regions**

LIMS 8304 is a randomized, partially blinded, paired-comparison study in which each subject receives DuraPrep solution and either Hibiclens cleanser or DuraPrep w/o iodine (I<sub>2</sub>). LIMS 8918 is a randomized, partially blinded, paired-comparison study in which each subject received DuraPrep solution and either Hibiclens cleanser or Betadine® Surgical Scrub and Betadine® Solution (hereafter called Betadine combination). Antimicrobial effectiveness is evaluated by measuring the log reduction of resident skin flora (on abdomen sites and on groin sites) following investigational material application. Neutralization of the test materials by MSS (modified sampling solution) and SSS (standard sampling solution) is verified prior to study start.

Healthy subjects are entered into a 14-day Pretreatment Phase during which standardized, non-antimicrobial soaps, shampoos, and deodorants are used. Following the Pretreatment Phase, each subject is required to visit the test facility on an arranged day for collection of screening baseline samples from the abdomen and groin regions. A visual skin assessment of each test area is performed and the screening baseline samples are collected using the cup scrub technique. Screening baseline samples are taken from each of the two contralateral test areas within each body region using a modified sampling solution (MSS). Subjects whose screening baseline samples meet the minimum values for inclusion in the study are notified and invited to participate in the Treatment Phase of the study. Following the baseline sample collection, randomly assigned contralateral test

areas are prepped with DuraPrep solution and either Hibiclens cleanser or DuraPrep w/o I<sub>2</sub> (LIMS 8304) or Betadine combination (LIMS 8918).

Microbial samples were collected at two minutes ( $\pm 30$  seconds), ten minutes ( $\pm 1$  min), six hours ( $\pm 15$  min), and 24 hours ( $\pm 30$  min) post-preparation (abdomen) and at ten minutes ( $\pm 1$  min), six hours ( $\pm 15$  min), and 24 hours ( $\pm 30$  min) post-preparation (groin). Post-preparation timing begins upon completion of the investigational material application. Microbial samples are collected using the cup scrub technique as described in the TFM. DuraPrep solution-treated, DuraPrep w/o I<sub>2</sub>-treated, and Betadine combination-treated sites are sampled with MSS. Hibiclens cleanser-treated sites are sampled with standard sampling solution (SSS). Bacterial counts are performed by individuals who are blinded to the identities of the test product associated with each sample.

#### Study 1— (LIMS 8304)

The log reduction data for the resident skin flora for all abdomen and groin subjects treated with DuraPrep solution are summarized in Table 9. The following experiments present the log reduction data for subjects treated with DuraPrep, DuraPrep w/o I<sub>2</sub>, and Hibiclens. Log reductions are calculated by subtracting the post-treatment log-transformed bacterial counts from the average of the Screening Day and baseline Treatment Day log-transformed bacterial counts. The primary objective is to satisfy the criteria in the TFM, which requires a two-log reduction of bacterial counts on the abdomen and a three-log reduction on the groin at ten minutes, and in both cases the bacterial counts must not return to baseline within six hours.

For the group where DuraPrep solution is applied to the abdominal site, a mean 2.65-log reduction of bacterial counts is achieved at ten minutes, and at six hours the mean log reduction is 2.49, thereby satisfying and exceeding the two-log reduction criteria of the TFM. The actual reduction of bacterial counts is achieved more rapidly than required by the TFM since there is a mean log reduction of 2.57 at two minutes. At all time points, the changes from baseline are statistically significant ( $p < 0.0001$ ). A secondary objective is to demonstrate the 24-hour efficacy of DuraPrep solution. At 24 hours, the log reduction of bacterial counts is 1.95, a statistically significant reduction from baseline ( $p < 0.0001$ ).

For the group where DuraPrep solution is applied to the groin site, a mean log reduction of 2.76 is achieved at ten minutes, and at six hours, the mean log reduction is 2.86. These reductions if rounded to a three-log reduction, thereby satisfying the TFM criteria. At all time points, the changes from baseline are statistically significant ( $p < 0.0001$ ). The 24-hour efficacy of DuraPrep solution is also confirmed at the groin site since there is a mean log reduction of 2.36 at 24 hours, a statistically significant reduction from baseline ( $p < 0.0001$ ).

**Table 9. Summary of Log Reduction of Bacterial Counts (CFU/cm<sup>2</sup>) For DuraPrep Solution-Treated Sites (Efficacy-Evaluable Population) – Abdomen and Groin Subjects**

	Abdomen	Groin
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	DuraPrep Solution (N = 61)	p-value <sup>1</sup>	DuraPrep Solution (N = 70)	p-value <sup>1</sup>
<b>Baseline Value<sup>2</sup></b>				
Mean (SD)	3.83 (0.613)	N/A	6.40 (0.476)	N/A
<b>Log Reduction<sup>3</sup> at:</b>				
2 Minutes				
Mean (SD)	2.57 (1.357)	<0.0001	ND	
95% CI	(2.22, 2.92)			
10 Minutes				
Mean (SD)	2.65 (1.371)	<0.0001	2.76 (1.110)	<0.0001
95% CI	(2.30, 3.00)		(2.50, 3.03)	
6 Hours				
Mean (SD)	2.49 (1.512)	<0.0001	2.86 (1.359)	<0.0001
95% CI	(2.10, 2.88)		(2.52, 3.19)	
24 Hours				
Mean (SD)	1.95 (1.740)	< 0.0001	2.36 (1.385)	< 0.0001
95% CI	(1.50, 2.39)		(2.02, 2.69)	

SD = standard deviation; Min = minimum; Max = maximum; CI = confidence interval; ND = not done; N/A = not applicable.

<sup>1</sup> Based on paired t-test (1-tailed) on the log reduction (difference between baseline and the post-preparation log counts).

<sup>2</sup> Baseline = average of Screening and Treatment Day baseline log-transformed bacterial counts.

<sup>3</sup> Log Reduction = average of Screening and baseline Treatment Day log transformed bacterial counts minus post-treatment log-transformed bacterial counts.

Source: Table 6, LIMS 8304 CSR.

The contribution of iodine to the bactericidal activity of DuraPrep solution is assessed by comparing the log reduction of bacterial counts achieved with DuraPrep solution with the log reduction of bacterial counts achieved with DuraPrep w/o I<sub>2</sub>. The primary comparison is at 24 hours, which is in contrast to the other experiments shown. At 24 hours, the log reduction of resident bacterial flora for DuraPrep solution is not statistically significantly different from the log reduction for DuraPrep w/o I<sub>2</sub> on either the abdomen (p = 0.8817) or the groin (p = 0.9742). The results of these comparisons for the abdomen are shown in Table 10; comparisons for the groin are shown in Table 11.

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**Table 10. Summary of Log Reduction of Bacterial Counts (CFU/cm<sup>2</sup>) For DuraPrep Solution-Treated Sites versus DuraPrep w/o I<sub>2</sub>-Treated Sites (Efficacy-Evaluable Population) - Abdomen Subjects**

	Treatment Group		
	DuraPrep w/o I <sub>2</sub>	DuraPrep Solution	

Sampling Time	(N = 30)	(N = 30)	Difference	p-value <sup>1</sup>
<b>Baseline Value<sup>2</sup></b>				
Mean (SD)	3.72 (0.558)	3.82 (0.549)	0.10 (0.403)	0.1929
95% CI			(-0.05, 0.25)	
<b>Log Reduction<sup>3</sup> at:</b>				
<b>2 Minutes</b>				
Mean (SD)	2.44 (1.315)	2.70 (1.347)	0.27 (1.360)	0.2939
95% CI			(-0.24, 0.77)	
<b>10 Minutes</b>				
Mean (SD)	2.53 (1.233)	2.83 (1.291)	0.30 (1.345)	0.2352
95% CI			(-0.20, 0.80)	
<b>6 Hours</b>				
Mean (SD)	2.19 (1.604)	2.64 (1.513)	0.45 (1.314)	0.0688
95% CI			(-0.04, 0.94)	
<b>24 Hours</b>				
Mean (SD)	2.16 (1.592)	2.20 (1.804)	0.04 (1.581)	0.8817
95% CI			(-0.55, 0.63)	

SD = standard deviation; CI = confidence interval.

<sup>1</sup> Based on paired t-test (2-tailed) on difference between DuraPrep solution and DuraPrep w/o I<sub>2</sub> post-preparation log counts.

<sup>2</sup> Baseline = average of Screening and Treatment Day baseline log-transformed bacterial counts.

<sup>3</sup> Log Reduction = average of Screening and baseline Treatment Day log-transformed bacterial counts minus post-treatment log transformed bacterial counts.

Note: Only subjects with data available from a treatment pair for a given sampling time point are included in this summary table.

Source: Table 7, LIMS 8304 CSR.

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**Table 11. Summary of Log Reduction of Bacterial Counts (CFU/cm<sup>2</sup>) For DuraPrep Solution-Treated Sites versus DuraPrep w/o I<sub>2</sub>-Treated Sites (Efficacy-Evaluable Population) Groin Subjects**

	Treatment Group		
	DuraPrep w/o I <sub>2</sub>	DuraPrep Solution	Paired

Sampling Time	(N = 31)	(N = 31)	Difference	p-value <sup>1</sup>
<b>Baseline Value<sup>2</sup></b>				
Mean (SD) 95% CI	6.38 (0.550)	6.41 (0.472)	0.03 (0.292) (-0.08, 0.14)	0.5508
<b>Log Reduction<sup>3</sup> at:</b>				
<b>10 Minutes</b>				
Mean (SD) 95% CI	2.58 (0.935)	2.53 (0.839)	-0.06 (1.109) (-0.46, 0.35)	0.7837
<b>6 Hours</b>				
Mean (SD) 95% CI	2.72 (1.396)	2.97 (1.381)	0.25 (1.525) (-0.32, 0.82)	0.3772
<b>24 Hours</b>				
Mean (SD) 95% CI	2.26 (1.068)	2.27 (1.478)	0.01 (1.176) (-0.43, 0.45)	0.9742

SD = standard deviation; CI = confidence interval.

<sup>1</sup> Based on paired t-test (2-tailed) on difference between DuraPrep solution and DuraPrep w/o I post-preparation log counts.

<sup>2</sup> Baseline = average of Screening and Treatment Day baseline log-transformed bacterial counts.

<sup>3</sup> Log Reduction = average of Screening and baseline Treatment Day log-transformed bacterial counts minus post-treatment log transformed bacterial counts.

Note: Only subjects with data available from a treatment pair for a given sampling time point are included in this summary table.

Source: Table 8, LIMS 8304 CSR.

Generally, for both the abdomen and the groin, the difference in the log reduction between DuraPrep solution and Hibiclens cleanser groups is not statistically significant ( $p \geq 0.0616$ ), as shown in Table 12 and Table 13. The one exception occurs at the six-hour time point for the groin. At this time point, Hibiclens cleanser is significantly more effective than DuraPrep solution ( $p = 0.0115$ ). However, since this difference is not detected at either the 10-minute or the 24-hour time point, the clinical relevance of this difference is unclear.

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**Table 12. Summary of Log Reduction of Bacterial Counts (CFU/cm<sup>2</sup>) For DuraPrep Solution-Treated Sites versus Hibiclens Cleanser-Treated Sites (Efficacy- Evaluable Population) - Abdomen Subjects**

	Treatment Group	
	Hibiclens	DuraPrep

Sampling Time	Cleanser (N = 31)	Solution (N = 31)	Paired Difference	p-value <sup>1</sup>
<b>Baseline Value<sup>2</sup></b>				
Mean (SD)	3.83 (0.491)	3.84 (0.678)	0.00 (0.488)	0.9665
95% CI			(-0.18, 0.18)	
<b>Log Reduction<sup>3</sup> at:</b>				
<b>2 Minutes</b>				
Mean (SD)	2.52 (1.595)	2.45 (1.377)	-0.07 (1.499)	0.7916
95% CI			(-0.62, 0.48)	
<b>10 Minutes</b>				
Mean (SD)	1.83 (1.647)	2.48 (1.444)	0.65 (1.872)	0.0616
95% CI			(-0.03, 1.34)	
<b>6 Hours</b>				
Mean (SD)	2.02 (1.522)	2.34 (1.520)	0.32 (1.657)	0.2960
95% CI			(-0.29, 0.92)	
<b>24 Hours</b>				
Mean (SD)	2.01 (1.456)	1.70 (1.669)	-0.31 (1.281)	0.1887
95% CI			(-0.78, 0.16)	

SD = standard deviation; CI = confidence interval.

<sup>1</sup> Based on paired t-test (2-tailed) on difference between DuraPrep solution and Hibiclens cleanser post-preparation log counts.

<sup>2</sup> Baseline = average of Screening and Treatment Day baseline log-transformed bacterial counts.

<sup>3</sup> Log Reduction = average of Screening and baseline Treatment Day log-transformed bacterial counts minus post-treatment log-transformed bacterial counts.

Note: Only subjects with data available from a treatment pair for a given sampling time point are included in this summary table.

Source: Table 9, LIMS 8304 CSR.

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**Table 13. Summary of Log Reduction of Bacterial Counts (CFU/cm<sup>2</sup>) For DuraPrep Solution- Treated Sites versus Hibiclens Cleanser-Treated Sites (Efficacy-Evaluable Population) - Groin Subjects**

	Treatment Group		
	Hibiclens Cleanser	DuraPrep Solution	Paired

Sampling Time	(N = 39)	(N = 39)	Difference	p-value <sup>1</sup>
<b>Baseline Value<sup>2</sup></b>				
Mean (SD)	6.39 (0.478)	6.40 (0.486)	0.01 (0.332)	0.8893
95% CI			(-0.10, 0.11)	
<b>Log Reduction<sup>3</sup> at:</b>				
<b>10 Minutes</b>				
Mean (SD)	2.93 (1.168)	2.95 (1.265)	0.03 (1.137)	0.8843
95% CI			(-0.34, 0.40)	
<b>6 Hours</b>				
Mean (SD)	3.36 (1.087)	2.70 (1.318)	-0.66 (1.477)	0.0115
95% CI			(-1.16, -0.16)	
<b>24 Hours</b>				
Mean (SD)	2.92 (1.222)	2.51 (1.411)	-0.42 (1.490)	0.1251
95% CI			(-0.95, 0.12)	

SD = standard deviation; CI = confidence interval.

- <sup>1</sup> Based on paired t-test (2-tailed) on difference between DuraPrep solution and Hibiclens cleanser post-preparation log counts.
- <sup>2</sup> Baseline = average of Screening and Treatment Day baseline log-transformed bacterial counts.
- <sup>3</sup> Log Reduction = average of Screening and baseline Treatment Day log-transformed bacterial counts minus post-treatment log-transformed bacterial counts.

Note: Only subjects with data available from a treatment pair for a given sampling time point are included in this summary table.

Source: Table 10, LIMS 8304.

The results of this study demonstrate that DuraPrep solution satisfies the criteria defined in the TFM for demonstrating antimicrobial activity on the abdomen site. On the abdomen, there is a greater than two-log reduction of bacterial counts by ten minutes that does not return to baseline by six hours. On the groin site, the reduction of bacterial counts at ten minutes (2.76-log reduction), when rounded, satisfies the criteria defined in the TFM (three-log reduction) according to the Applicant; at six hours post-preparation, the log reduction of bacterial counts is 2.86, indicating bacterial counts remain below baseline.

As expected from earlier pilot study results, the contribution of iodine to the bactericidal activity of DuraPrep solution is not demonstrated using the methods outlined in this study. There are no statistically significant differences between DuraPrep solution and DuraPrep w/o I<sub>2</sub> in the log reduction of resident bacterial flora at any time point on either the abdomen or the groin.

**Reviewer's comments:** While the test product (2.48) met the TFM requirements for the 2-log reduction at ten minutes at the abdominal site, the comparator (1.83) did not. Neither test product (2.95) nor comparator (2.93) met the TFM criteria for the 3-log reduction at the groin site. These numbers *cannot* be rounded up to 3-log in order to reach the TFM criteria.

Neutralization Validation for Lims 8304 / Study 1)

*Neutralizer and Test Organisms.* The SSS (standard sampling solution) and MSS (modified sampling solution) contain

*S. marcescens* (ATCC 14756). The test organism used in the evaluation test is

*Preparation*

*Sample Site Preparation.*

*Sample Inoculation and Plating.*

*Numbers Control.*

Colonies are counted and recorded.  
The average CFU count for each sample is calculated and converted to log<sub>10</sub> CFU/mL.

*Toxicity Control – MSS.*

The average CFU count for each sample is calculated and converted to  $\log_{10}$  CFU/mL.

*Toxicity Control – SSS.*

Colonies are counted and recorded. The average CFU count for each sample are calculated and converted to  $\log_{10}$  CFU/mL.

*Neutralization Criteria.* The neutralization is considered effective if the post-preparation sample recovered is not more than 0.2  $\log_{10}$  less than the Numbers Control sample. The sampling solutions are considered non-toxic if the Toxicity Control sample is not more than 0.2  $\log_{10}$  less than the Numbers Control sample.

*Results.* Table 14 presents the  $\log_{10}$  values recovered from all neutralization samples plated, including the numbers and toxicity controls, and evaluates whether these samples are within 0.2  $\log_{10}$  of the numbers control.

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Table 14. Table of Neutralization Results for LIMS 8304

		Log <sub>10</sub> Difference from Numbers	Within 0.2 Log <sub>10</sub> of
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Test	Log <sub>10</sub> Value	Control	Numbers Control
Numbers Control (<1 Minute)	2.76	N/A	N/A
Numbers Control (30 Minutes)	2.73	0.03	Yes
Numbers Control (Final)	2.74	0.02	Yes
Toxicity Control, MSS (<1 Minute)	2.75	0.01	Yes
Toxicity Control, MSS (30 Minutes)	2.73	0.03	Yes
Toxicity Control, SSS (<1 Minute)	2.73	0.03	Yes
Toxicity Control, SSS (30 Minutes)	2.71	0.05	Yes
Neutralization Effectiveness Subject N9 DuraPrep Solution (<1 Minute)	2.72	0.04	Yes
Neutralization Effectiveness Subject N9 DuraPrep Solution (30 Minutes)	2.78	-0.02	Yes
Neutralization Effectiveness Subject N9 Hibiclens cleanser (<1 Minute)	2.73	0.03	Yes
Neutralization Effectiveness Subject N9 Hibiclens cleanser (30 Minutes)	2.82	-0.06	Yes
Neutralization Effectiveness Subject N10 DuraPrep Solution (<1 Minute)	2.69	0.07	Yes
Neutralization Effectiveness Subject N10 DuraPrep Solution (30 Minutes)	2.68	0.08	Yes
Neutralization Effectiveness Subject N10 Hibiclens cleanser (<1 Minute)	2.69	0.07	Yes
Neutralization Effectiveness Subject N10 Hibiclens cleanser (30 Minutes)	2.74	0.02	Yes
Neutralization Effectiveness Subject N11 DuraPrep Solution (<1 Minute)	2.75	0.01	Yes
Neutralization Effectiveness Subject N11 DuraPrep Solution (30 Minutes)	2.73	0.03	Yes
Neutralization Effectiveness Subject N11 Hibiclens cleanser (<1 Minute)	2.68	0.08	Yes
Neutralization Effectiveness Subject N11 Hibiclens cleanser (30 Minutes)	2.67	0.09	Yes
Neutralization Effectiveness Subject N12 DuraPrep Solution (<1 Minute)	2.71	0.05	Yes

Table 14. Table of Neutralization Results for LIMS 8304 (continued)

	Log <sub>10</sub> Difference
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Test	Log <sub>10</sub> Value	from Numbers Control	Within 0.2 Log <sub>10</sub> of Numbers Control
Neutralization Effectiveness Subject N12 DuraPrep Solution (30 Minutes)	2.70	0.06	Yes
Neutralization Effectiveness Subject N12 Hibiclens cleanser (<1 Minute)	2.73	0.03	Yes
Neutralization Effectiveness Subject N12 Hibiclens cleanser (30 Minutes)	2.71	0.05	Yes
Neutralization Effectiveness Subject N13 DuraPrep Solution (<1 Minute)	2.73	0.03	Yes
Neutralization Effectiveness Subject N13 DuraPrep Solution (30 Minutes)	2.73	0.03	Yes
Neutralization Effectiveness Subject N13 Hibiclens cleanser (<1 Minute)	2.71	0.05	Yes
Neutralization Effectiveness Subject N13 Hibiclens cleanser (30 Minutes)	2.68	0.08	Yes
Neutralization Effectiveness Subject N14 DuraPrep Solution (<1 Minute)	2.66	0.10	Yes
Neutralization Effectiveness Subject N14 DuraPrep Solution (30 Minutes)	2.62	0.14	Yes
Neutralization Effectiveness Subject N14 Hibiclens cleanser (<1 Minute)	2.70	0.06	Yes
Neutralization Effectiveness Subject N14 Hibiclens cleanser (30 Minutes)	2.71	0.05	Yes

Source: LIMS 8304 Final CSR.

In conclusion, the MSS completely neutralizes any available iodine that is recovered from the DuraPrep solution. The SSS completely neutralizes any chlorhexidine gluconate that is recovered from the Hibiclens cleanser. The MSS and the SSS are non-toxic to the test organism.

**Reviewer's comments:** In a teleconference dated April 4, 2002, the Applicant confirmed that the neutralization validation would be conducted with a known concentration of DuraPrep. In addition, the use of *Serratia marcescens* with MSS in the neutralization validation was accepted by the Division.

**Study 2— (LIMS 8918)**

The log reductions of the resident skin flora for all abdomen and groin subjects treated with DuraPrep solution are summarized in Table 15. Log reductions are calculated by

subtracting the post-treatment log-transformed bacterial counts from the average of the Screening Day and Treatment Day baseline log-transformed bacterial counts. The primary objective of the study is for DuraPrep to meet the criteria in the TFM, which requires a two-log reduction of bacterial counts on the abdomen and a three-log reduction on the groin at ten minutes, and in both cases the bacterial counts must not return to the baseline level within six hours.

For the group where DuraPrep solution is applied to the abdominal site, a 2.35-log reduction of bacterial counts was achieved at ten minutes, and at six hours the mean log reduction is 2.31, thereby satisfying and exceeding the two-log reduction TFM criterion. The actual reduction of bacterial counts is achieved more rapidly than required by the TFM since there is a mean log reduction of 2.38 at two minutes. At all time points, the changes from baseline are statistically significant ( $p < 0.0001$ ). A secondary objective is to demonstrate the persistence of DuraPrep solution over 24 hours. At 24 hours, the mean log reduction of bacterial counts is 1.27, a statistically significant reduction from baseline ( $p < 0.0001$ ).

For the group where DuraPrep solution is applied to the groin site, a mean log reduction of 2.23 is achieved at ten minutes, and at six hours the mean log reduction is 2.27. This reduction is less than the three-log reduction criterion of the TFM. At all time points, the changes from baseline are statistically significant ( $p < 0.0001$ ). The 24-hour efficacy of DuraPrep solution is also confirmed at the groin site since there was a mean log reduction of 2.19 at 24 hours, a statistically significant reduction from baseline ( $p < 0.0001$ ).

**Table 15. Summary of Log Reduction of Bacterial Counts (CFU/cm<sup>2</sup>) For DuraPrep Solution-Treated Sites (Efficacy-Evaluable Population) - Abdomen and Groin Subjects**

	Abdomen		Groin	
	DuraPrep Solution (N = 45)	p-value <sup>1</sup>	DuraPrep Solution (N = 60)	p-value <sup>1</sup>
<b>Baseline Value<sup>2</sup></b>				
Mean (SD)	3.53 (0.415)	N/A	5.83 (0.487)	N/A
<b>Log Reduction<sup>3</sup> at:</b>				
<b>2 Minutes</b>				
Mean (SD)	2.38 (1.268)	<0.0001	ND	
95% CI	(2.00, 2.76)			
<b>10 Minutes</b>				
Mean (SD)	2.35 (1.251)	<0.0001	2.23 (1.059)	<0.0001
95% CI	(1.98, 2.73)		(1.96, 2.50)	
<b>6 Hours</b>				
Mean (SD)	2.31 (1.196)	<0.0001	2.27 (0.972)	<0.0001
95% CI	(1.95, 2.66)		(2.02, 2.53)	
<b>24 Hours</b>				
Mean (SD)	1.27 (1.233)	<0.0001	2.19 (0.879)	<0.0001
95% CI	(0.90, 1.64)		(1.95, 2.43)	

SD = standard deviation; CI = confidence interval; ND = not done; N/A = not applicable.

<sup>1</sup> Based on paired t-test (1-tailed) on the log reduction (difference between baseline and the post-preparation log counts at a given sampling time point).

- <sup>2</sup> Baseline = average of Screening and Treatment Day baseline log-transformed bacterial counts.  
<sup>3</sup> Log Reduction = average of Screening and baseline Treatment Day log-transformed bacterial counts minus post-treatment log-transformed bacterial counts.

Source: Table 6, LIMS 8918 CSR.

DuraPrep solution is significantly more effective than Hibiclens cleanser on the abdomen at 6 hours (p=0.0221) and on the groin at ten minutes (p=0.0030), as shown in Table 16 and Table 17. On the groin at 24 hours, Hibiclens cleanser is significantly more effective than DuraPrep solution (p=0.0061). At the rest of the time points on both the abdomen and the groin, the differences in the log reduction between the DuraPrep solution group and the Hibiclens cleanser group are not statistically significant (p≥0.2132).

In the small number of subjects studied in the DuraPrep solution versus Betadine combination group, both preparations meet the TFM requirement of a two-log reduction on the abdomen. Neither preparation meet the TFM requirement of a three-log reduction on the groin.

**Table 16. Summary of Log Reduction of Bacterial Counts (CFU/cm<sup>2</sup>) For DuraPrep Solution-Treated Sites versus Hibiclens Cleanser-Treated Sites (Efficacy-Evaluable Population) - Abdomen Subjects**

Sampling Time	Treatment Group			p-value <sup>1</sup>
	Hibiclens Cleanser (N = 34)	DuraPrep Solution (N = 34)	Paired Difference	
<b>Baseline Value<sup>2</sup></b>				
Mean (SD)	3.51 (0.329)	3.52 (0.433)	0.01 (0.358)	0.8193
95% CI			(-0.11, 0.14)	
<b>Log Reduction<sup>3</sup> at:</b>				
<b>2 Minutes</b>				
Mean (SD)	2.16 (1.229)	2.42 (1.294)	0.26 (1.415)	0.3064
95% CI			(-0.25, 0.76)	
<b>10 Minutes</b>				
Mean (SD)	2.15 (1.302)	2.47 (1.146)	0.32 (1.581)	0.2433
95% CI			(-0.23, 0.87)	
<b>6 Hours</b>				
Mean (SD)	1.75 (1.149)	2.31 (1.266)	0.56 (1.329)	0.0221
95% CI			(0.09, 1.03)	
<b>24 Hours</b>				
Mean (SD)	1.78 (0.883)	1.57 (1.154)	-0.21 (0.940)	0.2132
95% CI			(-0.55, 0.13)	

SD = standard deviation; CI = confidence interval.

<sup>1</sup> Based on paired t-test (2-tailed) on difference between DuraPrep solution and Hibiclens cleanser post-preparation log counts.

<sup>2</sup> Baseline = average of Screening and Treatment Day baseline log-transformed bacterial counts.

<sup>3</sup> Log Reduction = average of Screening and Treatment Day baseline log-transformed bacterial counts minus post-treatment log-transformed bacterial counts.

Note: Only subjects with data available from a treatment pair for a given sampling time point are included in this summary table.

Source: Table 7, LIMS 8918 CSR.

**Table 17. Summary of Log Reduction of Bacterial Counts (CFU/cm<sup>2</sup>) For DuraPrep Solution-Treated Sites versus Hibiclens Cleanser-Treated Sites (Efficacy-Evaluable Population) - Groin Subjects**

Sampling Time	Treatment Group			
	Hibiclens Cleanser (N = 47)	DuraPrep Solution (N = 47)	Paired Difference	p-value <sup>1</sup>
<b>Baseline Value<sup>2</sup></b>				
Mean (SD)	5.89 (0.480)	5.82 (0.511)	-0.07 (0.387)	0.2481
95% CI			(-0.18, 0.05)	
<b>Log Reduction<sup>3</sup> at:</b>				
<b>10 Minutes</b>				
Mean (SD)	-1.94 (0.964)	2.37 (1.085)	0.43 (0.940)	0.0030
95% CI			(0.15, 0.71)	
<b>6 Hours</b>				
Mean (SD)	2.31 (0.947)	2.29 (0.971)	-0.02 (0.743)	0.8566
95% CI			(-0.25, 0.21)	
<b>24 Hours</b>				
Mean (SD)	2.69 (0.882)	2.13 (0.796)	-0.56 (1.077)	0.0061
95% CI			(-0.95, -0.17)	

SD = standard deviation; CI = confidence interval.

<sup>1</sup> Based on paired t-test (2-tailed) on difference between DuraPrep solution and Hibiclens cleanser post-preparation log counts.

<sup>2</sup> Baseline = average of Screening and Treatment Day baseline log-transformed bacterial counts.

<sup>3</sup> Log Reduction = average of Screening and Treatment Day baseline log-transformed bacterial counts minus post-treatment log-transformed bacterial counts.

Note: Only subjects with data available from a treatment pair for a given sampling time point are included in this summary table.

Source: Table 8, LIMS 8918 CSR.

The studies demonstrate the antimicrobial effectiveness of DuraPrep solution meets the criteria defined in the TFM for sites on the abdomen since a greater than two-log reduction of resident bacterial counts is reached ten minutes post-preparation and counts do not return to baseline values at six or 24 hours post-preparation. For the groin site, a mean log reduction of 2.23 is achieved at ten minutes. This is less than the three-log reduction TFM criterion. Neither of the control products, Hibiclens cleanser nor Betadine combination, meet the three-log criterion for the groin site. The Applicant states that at all time points, the changes from baseline for DuraPrep solution are statistically significant ( $p < 0.0001$ ), indicating that bacterial counts remain below baseline.

Generally, for both the abdomen and the groin at most time points, the difference in the log reduction between DuraPrep solution and Hibiclens cleanser is not statistically significant. At the six-hour time point for the abdomen site and the ten-minute time point for the groin site, DuraPrep solution is significantly more effective than Hibiclens cleanser ( $p = 0.0221$  and  $p = 0.0030$ , respectively). At the 24-hour time point for the groin site, Hibiclens cleanser is significantly more effective than DuraPrep solution ( $p = 0.0061$ ).

Overall, DuraPrep solution and Hibiclens cleanser can be considered similar, with DuraPrep solution showing greater efficacy up to six hours and Hibiclens cleanser showing greater efficacy at 24 hours.

*Reviewer's comments:* While both the test product and the comparator met the TFM requirements for the 2-log reduction at ten minutes at the abdominal site, neither test product (2.37) nor comparator (1.94) met the TFM criteria for the 3-log reduction at the groin site.

Both the positive control and the test product failed to meet the TFM criteria for log reduction in the inguinal site. However, both the positive control and the test product did meet the 2-log reduction criterion for the abdominal site. The Applicant has supplied the mean log reductions for the inguinal and abdominal sites in Tables 16 and 17. Reexamination of this data in a different format reveals a possible explanation for the log reduction data from the individual subjects as well as the mean log reduction. In addition, the new format identifies whether the log reductions for the individual subjects met the TFM criterion and the percentage of individual subjects who did meet the TFM criterion.

Tables 18-26 present the ten minute log reduction data from both the \_\_\_\_\_ and \_\_\_\_\_ clinical simulation studies for both the abdominal and inguinal sites for each individual subject. Table 18 demonstrates the 10 min. log reduction data for Hibiclens at the inguinal site in study LIMS 8304 \_\_\_\_\_. Table 19 demonstrates the 10 min log reduction data for Hibiclens at the abdominal site in study LIMS 8304 \_\_\_\_\_. Table 20 demonstrates the 10 min. log reduction data for DuraPrep at the inguinal site in study LIMS 8304 \_\_\_\_\_. Table 21 demonstrates the 10 min. log reduction data for DuraPrep at the abdominal site in study LIMS 8304 \_\_\_\_\_. Table 22 demonstrates the 10 min. log reduction data for Hibiclens at the inguinal site in study LIMS 8918 \_\_\_\_\_. Table 23 demonstrates the 10 min. log reduction data for Hibiclens at the abdominal site in study LIMS 8918 \_\_\_\_\_. Table 24 demonstrates the 10 min. log reduction data for Hibiclens at the inguinal site in study LIMS 8918 \_\_\_\_\_. Table 25 demonstrates the 10 min. log reduction data for DuraPrep at the inguinal site in study LIMS 8918 \_\_\_\_\_. Table 26 demonstrates the 10 min log reduction data for DuraPrep at the abdominal site in study LIMS 8918 \_\_\_\_\_. The mean log reductions and percentages of individuals above threshold were determined and assembled into a summary table found as Table 27.

<b>Table 18. LIMS 8304: 10 min. log reduction using Hibiclens at the Inguinal Site</b>
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Subject Number	Scheduled Sampling Time	Treatment	Side	Site Number	Log Reduction^ (CFU/cm2)	Above Threshold?*
002G	10 Minutes	Y	LEFT	2	-	
003G	10 Minutes	Y	RIGHT	4	-	
007G	10 Minutes	Y	LEFT	1	3.21	1
008G	10 Minutes	Y	RIGHT	2	1.66	
009G	10 Minutes	Y	RIGHT	2	2.81	
011G	10 Minutes	Y	LEFT	1	1.9	
013G	10 Minutes	Y	RIGHT	4	2.66	
015G	10 Minutes	Y	RIGHT	4	0.75	
017G	10 Minutes	Y	RIGHT	4	1.89	
018G	10 Minutes	Y	LEFT	2	3.96	1
023G	10 Minutes	Y	LEFT	4	2.01	
024G	10 Minutes	Y	RIGHT	1	3.36	1
026G	10 Minutes	Y	LEFT	1	2.85	
027G	10 Minutes	Y	RIGHT	1	3.38	1
029G	10 Minutes	Y	RIGHT	4	1.6	
030G	10 Minutes	Y	LEFT	2	1.79	
032G	10 Minutes	Y	LEFT	2	3.19	1
033G	10 Minutes	Y	LEFT	2	2.96	
035G	10 Minutes	Y	RIGHT	4	1.63	
042G	10 Minutes	Y	LEFT	4	3.5	1
043G	10 Minutes	Y	RIGHT	2	2.75	
048G	10 Minutes	Y	LEFT	4	3.08	1
049G	10 Minutes	Y	RIGHT	1	3.06	1
051G	10 Minutes	Y	LEFT	1	3.22	1
052G	10 Minutes	Y	LEFT	4	1.94	
053G	10 Minutes	Y	LEFT	1	2.77	
054G	10 Minutes	Y	RIGHT	1	2.15	
057G	10 Minutes	Y	LEFT	4	3.45	1
060G	10 Minutes	Y	RIGHT	2	1.53	
102G	10 Minutes	Y	LEFT	2	3.02	1
103G	10 Minutes	Y	RIGHT	2	1.4	
106G	10 Minutes	Y	RIGHT	1	3.47	1
111G	10 Minutes	Y	LEFT	1	3.9	1
113G	10 Minutes	Y	RIGHT	4	3.13	1
126G	10 Minutes	Y	LEFT	1	3.09	1
129G	10 Minutes	Y	RIGHT	4	5.75	1
152G	10 Minutes	Y	LEFT	4	3.88	1
206G	10 Minutes	Y	RIGHT	1	6.35	1
211G	10 Minutes	Y	LEFT	1	1.91	
213G	10 Minutes	Y	RIGHT	4	5.15	1
306G	10 Minutes	Y	RIGHT	1	4.14	1
For above threshold,		1=yes				
Mean=					2.93	
% above threshold=					51.28	

<b>Table 19. LIMS 8304: 10 min. log Reduction using Hibiclens at the Abdomen</b>						
<b>Subject Number</b>	<b>Scheduled Sampling Time</b>	<b>Treatment</b>	<b>Side</b>	<b>Site Number</b>	<b>Log Reduction^ (CFU/cm2)</b>	<b>Above Threshold?*</b>
003A	10 Minutes	Y	LEFT	3	2.41	1
004A	10 Minutes	Y	LEFT	4	1.2	
007A	10 Minutes	Y	RIGHT	2	0.75	
009A	10 Minutes	Y	RIGHT	2	2.87	1
010A	10 Minutes	Y	RIGHT	3	3.09	1
011A	10 Minutes	Y	RIGHT	4	0.32	
014A	10 Minutes	Y	LEFT	2	3.57	1
015A	10 Minutes	Y	RIGHT	1	-0.01	
016A	10 Minutes	Y	LEFT	4	4.06	1
017A	10 Minutes	Y	LEFT	3	-0.35	
018A	10 Minutes	Y	RIGHT	1	2.53	1
022A	10 Minutes	Y	RIGHT	3	0.89	
023A	10 Minutes	Y	LEFT	1	3.39	1
025A	10 Minutes	Y	LEFT	1	0.91	
026A	10 Minutes	Y	RIGHT	1	2.59	1
029A	10 Minutes	Y	LEFT	4	-0.44	
030A	10 Minutes	Y	LEFT	2	3.36	1
032A	10 Minutes	Y	LEFT	1	3.35	1
034A	10 Minutes	Y	RIGHT	2	1.76	
035A	10 Minutes	Y	RIGHT	4	3.83	1
036A	10 Minutes	Y	RIGHT	2	0.76	
037A	10 Minutes	Y	LEFT	1	3.55	1
043A	10 Minutes	Y	LEFT	4	0.38	
048A	10 Minutes	Y	RIGHT	3	3.88	1
050A	10 Minutes	Y	LEFT	2	1.99	
051A	10 Minutes	Y	LEFT	2	1.47	
053A	10 Minutes	Y	RIGHT	4	-1.49	
056A	10 Minutes	Y	RIGHT	1	0.01	
057A	10 Minutes	Y	RIGHT	4	-0.69	
058A	10 Minutes	Y	LEFT	3	3.61	1
109A	10 Minutes	Y	RIGHT	2	3.18	1
118A	10 Minutes	Y	RIGHT	1	3.05	1
135A	10 Minutes	Y	RIGHT	4	3.11	1
143A	10 Minutes	Y	LEFT	4	4.2	1
150A	10 Minutes	Y	LEFT	2	1.52	
158A	10 Minutes	Y	LEFT	3	1.36	
209A	10 Minutes	Y	RIGHT	2	4.23	1
309A	10 Minutes	Y	RIGHT	2	4.13	1
915A	10 Minutes	Y	RIGHT	1	0.8	
922A	10 Minutes	Y	RIGHT	3	4.29	1
*For above threshold, 1= yes						
					Mean=	2.09
						% above threshold= 52.50

**Table 20. LIMS 8304: 10 min. log reduction using DuraPrep at the inguinal site.**

Subject Number	Scheduled Sampling Time	Treatment	Side	Site Number	Log Reduction^ (CFU/cm2)	Above Threshold?
006G	10 Minutes	W	LEFT	1	2.21	
007G	10 Minutes	W	RIGHT	1	3.97	1
008G	10 Minutes	W	LEFT	2	1.3	
009G	10 Minutes	W	LEFT	2	1.65	
010G	10 Minutes	W	RIGHT	2	2.58	
011G	10 Minutes	W	RIGHT	1	3.74	1
012G	10 Minutes	W	RIGHT	1	1.43	
013G	10 Minutes	W	LEFT	4	5.62	1
014G	10 Minutes	W	LEFT	2	3.03	1
015G	10 Minutes	W	LEFT	4	3.34	1
016G	10 Minutes	W	LEFT	2	1.79	
017G	10 Minutes	W	LEFT	4	2.5	
018G	10 Minutes	W	RIGHT	2	4.1	1
019G	10 Minutes	W	LEFT	1	1.62	
020G	10 Minutes	W	RIGHT	1	2.79	
021G	10 Minutes	W	RIGHT	1	1.73	
022G	10 Minutes	W	LEFT	2	0.75	
023G	10 Minutes	W	RIGHT	4	2.21	
024G	10 Minutes	W	LEFT	1	4.76	1
025G	10 Minutes	W	LEFT	2	2.1	
026G	10 Minutes	W	RIGHT	1	4.4	1
027G	10 Minutes	W	LEFT	1	3.03	1
028G	10 Minutes	W	RIGHT	2	2.29	
029G	10 Minutes	W	LEFT	4	1.59	
030G	10 Minutes	W	RIGHT	2	2.61	
031G	10 Minutes	W	LEFT	2	3.45	1
032G	10 Minutes	W	RIGHT	2	2.81	
033G	10 Minutes	W	RIGHT	2	2.21	
034G	10 Minutes	W	LEFT	1	4.48	1
035G	10 Minutes	W	LEFT	4	1.64	
036G	10 Minutes	W	RIGHT	1	2.6	
037G	10 Minutes	W	RIGHT	1	1.03	
038G	10 Minutes	W	LEFT	2	1.38	
039G	10 Minutes	W	LEFT	4	2.79	
040G	10 Minutes	W	RIGHT	4	2.56	
041G	10 Minutes	W	LEFT	1	4.25	1
042G	10 Minutes	W	RIGHT	4	3.6	1
043G	10 Minutes	W	LEFT	2	1.36	
044G	10 Minutes	W	LEFT	1	3.05	1
045G	10 Minutes	W	RIGHT	2	2.93	
046G	10 Minutes	W	RIGHT	4	2.97	
047G	10 Minutes	W	RIGHT	4	2.91	
048G	10 Minutes	W	RIGHT	4	2.52	
049G	10 Minutes	W	LEFT	1	1.81	
050G	10 Minutes	W	RIGHT	1	2.56	

051G	10 Minutes	W	RIGHT	1	3.13	1
052G	10 Minutes	W	RIGHT	4	0.6	
053G	10 Minutes	W	RIGHT	1	2.92	
054G	10 Minutes	W	LEFT	1	2.1	
055G	10 Minutes	W	RIGHT	4	3.13	1
056G	10 Minutes	W	LEFT	1	2.77	
057G	10 Minutes	W	RIGHT	4	1.72	
058G	10 Minutes	W	LEFT	4	3.34	1
059G	10 Minutes	W	LEFT	4	2.8	
060G	10 Minutes	W	LEFT	2	1.1	
101G	10 Minutes	W	RIGHT	2	1.63	
102G	10 Minutes	W	RIGHT	2	2.95	
103G	10 Minutes	W	LEFT	2	2.14	
104G	10 Minutes	W	RIGHT	4	2.62	
105G	10 Minutes	W	LEFT	4	2.83	
106G	10 Minutes	W	LEFT	1	3.69	1
111G	10 Minutes	W	RIGHT	1	3.73	1
113G	10 Minutes	W	LEFT	4	3.41	1
126G	10 Minutes	W	RIGHT	1	5.01	1
129G	10 Minutes	W	LEFT	4	4.16	1
152G	10 Minutes	W	RIGHT	4	2.34	
206G	10 Minutes	W	LEFT	1	4.58	1
211G	10 Minutes	W	RIGHT	1	1.38	
213G	10 Minutes	W	LEFT	4	4.93	1
306G	10 Minutes	W	LEFT	1	4.63	1
Mean=						25
2.77						
% above threshold=						35.71

**Table 21. LIMS 8304: 10 min. log reduction using DuraPrep at the abdominal site.**

Subject Number	Scheduled Sampling Time	Treatment	Side	Site Number	Log Reduction <sup>^</sup> (CFU/cm2)	Above Threshold?
003A	10 Minutes	Y	LEFT	3	2.41	1
004A	10 Minutes	Y	LEFT	4	1.2	
007A	10 Minutes	Y	RIGHT	2	0.75	
009A	10 Minutes	Y	RIGHT	2	2.87	1
010A	10 Minutes	Y	RIGHT	3	3.09	1
011A	10 Minutes	Y	RIGHT	4	0.32	
014A	10 Minutes	Y	LEFT	2	3.57	1
015A	10 Minutes	Y	RIGHT	1	-0.01	
016A	10 Minutes	Y	LEFT	4	4.06	1
017A	10 Minutes	Y	LEFT	3	-0.35	
018A	10 Minutes	Y	RIGHT	1	2.53	1
022A	10 Minutes	Y	RIGHT	3	0.89	
023A	10 Minutes	Y	LEFT	1	3.39	1

025A	10 Minutes	Y	LEFT	1	0.91	
026A	10 Minutes	Y	RIGHT	1	2.59	1
029A	10 Minutes	Y	LEFT	4	-0.44	
030A	10 Minutes	Y	LEFT	2	3.36	1
032A	10 Minutes	Y	LEFT	1	3.35	1
034A	10 Minutes	Y	RIGHT	2	1.76	
035A	10 Minutes	Y	RIGHT	4	3.83	1
036A	10 Minutes	Y	RIGHT	2	0.76	
037A	10 Minutes	Y	LEFT	1	3.55	1
043A	10 Minutes	Y	LEFT	4	0.38	
048A	10 Minutes	Y	RIGHT	3	3.88	1
050A	10 Minutes	Y	LEFT	2	1.99	
051A	10 Minutes	Y	LEFT	2	1.47	
053A	10 Minutes	Y	RIGHT	4	-1.49	
056A	10 Minutes	Y	RIGHT	1	0.01	
057A	10 Minutes	Y	RIGHT	4	-0.69	
058A	10 Minutes	Y	LEFT	3	3.61	1
109A	10 Minutes	Y	RIGHT	2	3.18	1
118A	10 Minutes	Y	RIGHT	1	3.05	1
135A	10 Minutes	Y	RIGHT	4	3.11	1
143A	10 Minutes	Y	LEFT	4	4.2	1
150A	10 Minutes	Y	LEFT	2	1.52	
158A	10 Minutes	Y	LEFT	3	1.36	
209A	10 Minutes	Y	RIGHT	2	4.23	1
309A	10 Minutes	Y	RIGHT	2	4.13	1
915A	10 Minutes	Y	RIGHT	1	0.8	
922A	10 Minutes	Y	RIGHT	3	4.29	1
Mean=					2.09	
% above threshold=						52.50

**Table 22. LIMS 8304: 10 min. log reduction using Hibiclens at the inguinal site.**

Subject Number	Scheduled Sampling Time	Treatment	Side	Site Number	Log Reduction <sup>^</sup> (CFU/cm <sup>2</sup> )	Above Threshold?
007G	10 Minutes	Y	LEFT	1	3.21	1
008G	10 Minutes	Y	RIGHT	2	1.66	
009G	10 Minutes	Y	RIGHT	2	2.81	
011G	10 Minutes	Y	LEFT	1	1.9	
013G	10 Minutes	Y	RIGHT	4	2.66	
015G	10 Minutes	Y	RIGHT	4	0.75	
017G	10 Minutes	Y	RIGHT	4	1.89	
018G	10 Minutes	Y	LEFT	2	3.96	1
023G	10 Minutes	Y	LEFT	4	2.01	
024G	10 Minutes	Y	RIGHT	1	3.36	1
026G	10 Minutes	Y	LEFT	1	2.85	
027G	10 Minutes	Y	RIGHT	1	3.38	1

029G	10 Minutes	Y	RIGHT	4	1.6	
030G	10 Minutes	Y	LEFT	2	1.79	
032G	10 Minutes	Y	LEFT	2	3.19	1
033G	10 Minutes	Y	LEFT	2	2.96	
035G	10 Minutes	Y	RIGHT	4	1.63	
042G	10 Minutes	Y	LEFT	4	3.5	1
043G	10 Minutes	Y	RIGHT	2	2.75	
048G	10 Minutes	Y	LEFT	4	3.08	1
049G	10 Minutes	Y	RIGHT	1	3.06	1
051G	10 Minutes	Y	LEFT	1	3.22	1
052G	10 Minutes	Y	LEFT	4	1.94	
053G	10 Minutes	Y	LEFT	1	2.77	
054G	10 Minutes	Y	RIGHT	1	2.15	
057G	10 Minutes	Y	LEFT	4	3.45	1
060G	10 Minutes	Y	RIGHT	2	1.53	
102G	10 Minutes	Y	LEFT	2	3.02	1
103G	10 Minutes	Y	RIGHT	2	1.4	
106G	10 Minutes	Y	RIGHT	1	3.47	1
111G	10 Minutes	Y	LEFT	1	3.9	1
113G	10 Minutes	Y	RIGHT	4	3.13	1
126G	10 Minutes	Y	LEFT	1	3.09	1
129G	10 Minutes	Y	RIGHT	4	5.75	1
152G	10 Minutes	Y	LEFT	4	3.88	1
206G	10 Minutes	Y	RIGHT	1	6.35	1
211G	10 Minutes	Y	LEFT	1	1.91	
213G	10 Minutes	Y	RIGHT	4	5.15	1
306G	10 Minutes	Y	RIGHT	1	4.14	1
Mean=					2.93	
% above threshold=						51.28

**Table 23. LIMS 8918: 10 min. log reduction using Hibiclens at the abdominal site.**

Subject Number	Scheduled Sampling Time	Treatment	Side	Site Number	Log Reduction <sup>^</sup> (CFU/cm <sup>2</sup> )	Meets Threshold?
002A	10 Minutes	Y	LEFT	1	3.88	1
003A	10 Minutes	Y	RIGHT	3	2.27	1
004A	10 Minutes	Y	LEFT	4	1.73	
005A	10 Minutes	Y	LEFT	3	2.51	1
006A	10 Minutes	Y	RIGHT	2	3.27	1
007A	10 Minutes	Y	LEFT	4	0.54	
008A	10 Minutes	Y	RIGHT	4	1.51	
009A	10 Minutes	Y	RIGHT	2	3.49	1
011A	10 Minutes	Y	LEFT	4	0.7	
012A	10 Minutes	Y	RIGHT	1	2.38	1
014A	10 Minutes	Y	RIGHT	2	1.83	
015A	10 Minutes	Y	RIGHT	1	4.46	1
016A	10 Minutes	Y	LEFT	1	1.52	

017A	10 Minutes	Y	RIGHT	2	3.02	1
018A	10 Minutes	Y	LEFT	3	0.59	
020A	10 Minutes	Y	RIGHT	1	2.66	1
021A	10 Minutes	Y	RIGHT	3	3.69	1
022A	10 Minutes	Y	RIGHT	4	3.27	1
023A	10 Minutes	Y	LEFT	2	2.55	1
024A	10 Minutes	Y	LEFT	3	2.5	1
025A	10 Minutes	Y	RIGHT	2	3.12	1
027A	10 Minutes	Y	RIGHT	4	1.31	
028A	10 Minutes	Y	LEFT	1	1.12	
029A	10 Minutes	Y	RIGHT	4	1.07	
031A	10 Minutes	Y	LEFT	2	2.96	1
032A	10 Minutes	Y	LEFT	4	2	1
033A	10 Minutes	Y	LEFT	1	-0.18	
035A	10 Minutes	Y	RIGHT	1	2.35	1
037A	10 Minutes	Y	LEFT	2	2.94	1
040A	10 Minutes	Y	RIGHT	3	2.72	1
104A	10 Minutes	Y	LEFT	4	0.83	
120A	10 Minutes	Y	RIGHT	1	3.71	1
122A	10 Minutes	Y	RIGHT	4	0.33	
124A	10 Minutes	Y	LEFT	3	2.48	1
127A	10 Minutes	Y	RIGHT	4	1	
135A	10 Minutes	Y	RIGHT	1	3.55	1
137A	10 Minutes	Y	LEFT	1	-0.21	
222A	10 Minutes	Y	RIGHT	4	2.28	1
422A	10 Minutes	Y	RIGHT	4	0.67	
Mean=					2.11	
% above threshold=						58.97

**Table 24. LIMS 8918: 10 min. log reduction using Hibiclens at the inguinal site.**

Subject Number	Scheduled Sampling Time	Treatment	Side	Site Number	Log Reduction^ (CFU/cm2)	Meets Threshold?
001G	10 Minutes	Y	RIGHT	1	0.92	
002G	10 Minutes	Y	LEFT	1	3.07	1
003G	10 Minutes	Y	RIGHT	4	0.74	
004G	10 Minutes	Y	RIGHT	2	1.44	
005G	10 Minutes	Y	RIGHT	1	1.88	
006G	10 Minutes	Y	LEFT	4	2.54	
007G	10 Minutes	Y	LEFT	1	2.13	
008G	10 Minutes	Y	RIGHT	1	0.54	
009G	10 Minutes	Y	LEFT	2	2.77	
010G	10 Minutes	Y	RIGHT	2	1.67	
011G	10 Minutes	Y	LEFT	1	1.46	
012G	10 Minutes	Y	LEFT	1	4.45	1
014G	10 Minutes	Y	RIGHT	4	1.19	

015G	10 Minutes	Y	LEFT	4	2.18	
016G	10 Minutes	Y	LEFT	2	0.45	
019G	10 Minutes	Y	RIGHT	4	2.13	
020G	10 Minutes	Y	LEFT	2	1.91	
021G	10 Minutes	Y	RIGHT	4	2.49	
022G	10 Minutes	Y	RIGHT	2	1.02	
028G	10 Minutes	Y	RIGHT	4	2.27	
029G	10 Minutes	Y	RIGHT	2	2.04	
031G	10 Minutes	Y	LEFT	4	2.51	
032G	10 Minutes	Y	RIGHT	2	2.49	
033G	10 Minutes	Y	LEFT	2	1.29	
035G	10 Minutes	Y	LEFT	4	3.9	1
036G	10 Minutes	Y	RIGHT	1	3.9	1
037G	10 Minutes	Y	LEFT	2	2.14	
038G	10 Minutes	Y	LEFT	4	1.6	
039G	10 Minutes	Y	RIGHT	1	1.64	
040G	10 Minutes	Y	LEFT	1	1.91	
106G	10 Minutes	Y	LEFT	4	1.8	
107G	10 Minutes	Y	LEFT	1	2.76	
109G	10 Minutes	Y	LEFT	2	1.87	
111G	10 Minutes	Y	LEFT	1	2.95	
116G	10 Minutes	Y	LEFT	2	1.84	
120G	10 Minutes	Y	LEFT	2	3.18	1
133G	10 Minutes	Y	LEFT	2	1.56	
136G	10 Minutes	Y	RIGHT	4	1.71	
138G	10 Minutes	Y	LEFT	4	1.23	
139G	10 Minutes	Y	RIGHT	1	0.82	
140G	10 Minutes	Y	LEFT	1	3.95	1
211G	10 Minutes	Y	LEFT	1	1.71	
216G	10 Minutes	Y	LEFT	2	2.39	
233G	10 Minutes	Y	LEFT	2	1.6	
239G	10 Minutes	Y	RIGHT	1	1.7	
311G	10 Minutes	Y	LEFT	1	1.5	
339G	10 Minutes	Y	RIGHT	1	1.66	
411G	10 Minutes	Y	LEFT	1	0.52	
511G	10 Minutes	Y	LEFT	1	0.36	
611G	10 Minutes	Y	LEFT	1	1	
Mean=					1.94	
% meets threshold=						12.00

**Table 25. LIMS 8918: 10 min. log reduction using DuraPrep at the inguinal site.**

Subject Number	Scheduled Sampling Time	Treatment	Side	Site Number	Log Reduction^ (CFU/cm2)	Meets Threshold?
001G	10 Minutes	W	LEFT	1	1.51	
002G	10 Minutes	W	RIGHT	1	2.22	
003G	10 Minutes	W	LEFT	4	2.11	
004G	10 Minutes	W	LEFT	2	1.28	
005G	10 Minutes	W	LEFT	1	4.21	1
006G	10 Minutes	W	RIGHT	4	2.79	
007G	10 Minutes	W	LEFT	1	3.23	1
008G	10 Minutes	W	RIGHT	2	1.5	
009G	10 Minutes	W	LEFT	2	2.47	
010G	10 Minutes	W	RIGHT	1	3.07	1
011G	10 Minutes	W	RIGHT	1	2.73	
012G	10 Minutes	W	LEFT	2	1.42	
013G	10 Minutes	W	LEFT	4	1.96	
014G	10 Minutes	W	RIGHT	4	3.64	1
015G	10 Minutes	W	RIGHT	2	0.86	
016G	10 Minutes	W	RIGHT	4	1.14	
017G	10 Minutes	W	RIGHT	2	2.68	
018G	10 Minutes	W	LEFT	4	5.4	1
019G	10 Minutes	W	RIGHT	2	3.5	1
020G	10 Minutes	W	LEFT	2	1.14	
022G	10 Minutes	W	LEFT	2	0.79	
023G	10 Minutes	W	LEFT	1	1.66	
024G	10 Minutes	W	LEFT	4	0.46	
025G	10 Minutes	W	RIGHT	4	3.79	1
026G	10 Minutes	W	RIGHT	2	1.53	
027G	10 Minutes	W	LEFT	4	2.82	
028G	10 Minutes	W	LEFT	2	2.36	
029G	10 Minutes	W	LEFT	1	1.17	
030G	10 Minutes	W	RIGHT	4	3.02	1
031G	10 Minutes	W	LEFT	2	1.67	
032G	10 Minutes	W	RIGHT	2	1.68	
033G	10 Minutes	W	RIGHT	1	2.96	
034G	10 Minutes	W	RIGHT	4	2.06	
035G	10 Minutes	W	LEFT	1	4.07	1
036G	10 Minutes	W	RIGHT	2	2.17	
037G	10 Minutes	W	RIGHT	4	3.25	1
038G	10 Minutes	W	LEFT	1	3.23	1
039G	10 Minutes	W	RIGHT	1	2.66	
040G	10 Minutes	W	RIGHT	4	2.67	
106G	10 Minutes	W	RIGHT	1	3.2	1
107G	10 Minutes	W	RIGHT	2	1.81	
109G	10 Minutes	W	RIGHT	1	4.52	1
111G	10 Minutes	W	RIGHT	2	2.11	
116G	10 Minutes	W	RIGHT	2	2.89	
120G	10 Minutes	W	LEFT	2	1.72	
123G	10 Minutes	W	RIGHT	4	2.93	

126G	10 Minutes	W	LEFT	4	2.58	
131G	10 Minutes	W	RIGHT	2	1.65	
133G	10 Minutes	W	RIGHT	1	1.21	
134G	10 Minutes	W	LEFT	1	2	
136G	10 Minutes	W	RIGHT	4	1.58	
138G	10 Minutes	W	LEFT	1	0.46	
139G	10 Minutes	W	RIGHT	1	5.13	1
140G	10 Minutes	W	RIGHT	1	2.64	
211G	10 Minutes	W	RIGHT	2	1.86	
216G	10 Minutes	W	LEFT	2	0.96	
223G	10 Minutes	W	RIGHT	4	2.37	
226G	10 Minutes	W	RIGHT	2	1.55	
233G	10 Minutes	W	RIGHT	1	1.19	
234G	10 Minutes	W	LEFT	1	1.2	
239G	10 Minutes	W	RIGHT	1	1.33	
311G	10 Minutes	W	LEFT	2	1.92	
323G	10 Minutes	W	LEFT	1	2.36	
339G	10 Minutes	W	RIGHT	1	1.72	
411G	10 Minutes	W	LEFT	2	0.91	
423G	10 Minutes	W	RIGHT	1	1.15	
511G	10 Minutes	W	RIGHT	1	1.51	
Mean=					2.23	
% meet threshold=						20.89

Table 26. LIMS 8918: 10 min. log reduction using DuraPrep at the abdominal site.

Subject Number	Scheduled Sampling Time	Treatment	Side	Site Number	Log Reduction <sup>^</sup> (CFU/cm2)	Meets Threshold?
001A	10 Minutes	W	LEFT	1	2.97	1
002A	10 Minutes	W	RIGHT	1	3.8	1
003A	10 Minutes	W	LEFT	3	3.41	1
004A	10 Minutes	W	RIGHT	4	3.04	1
005A	10 Minutes	W	RIGHT	3	1.31	
006A	10 Minutes	W	LEFT	2	-0.05	
007A	10 Minutes	W	RIGHT	4	3.13	1
008A	10 Minutes	W	LEFT	4	2.56	1
009A	10 Minutes	W	LEFT	2	0.73	
010A	10 Minutes	W	LEFT	3	2.83	1
011A	2 Minutes	W	RIGHT	3	0.4	
012A	10 Minutes	W	LEFT	1	1.95	
013A	10 Minutes	W	LEFT	3	1.85	
014A	10 Minutes	W	LEFT	2	3.9	1
015A	10 Minutes	W	LEFT	1	4.17	1
016A	10 Minutes	W	RIGHT	1	3.65	1
017A	10 Minutes	W	LEFT	2	2.81	1
018A	10 Minutes	W	RIGHT	3	0.87	

019A	10 Minutes	W	RIGHT	2	2.94	1
020A	10 Minutes	W	LEFT	1	0.18	
021A	10 Minutes	W	LEFT	3	2.36	1
022A	10 Minutes	W	LEFT	4	3.04	1
023A	10 Minutes	W	RIGHT	2	2.52	1
024A	10 Minutes	W	RIGHT	3	2.76	1
025A	10 Minutes	W	LEFT	2	3.24	1
026A	10 Minutes	W	RIGHT	3	3.95	1
027A	10 Minutes	W	LEFT	4	2.43	1
028A	10 Minutes	W	RIGHT	1	3.08	1
029A	10 Minutes	W	LEFT	4	2.88	1
030A	10 Minutes	W	LEFT	2	2.79	1
031A	10 Minutes	W	RIGHT	2	3.47	1
032A	10 Minutes	W	RIGHT	4	3.09	1
033A	10 Minutes	W	RIGHT	1	1.02	
034A	10 Minutes	W	RIGHT	4	3.32	1
035A	10 Minutes	W	LEFT	1	0.97	
036A	10 Minutes	W	LEFT	4	0.42	
037A	10 Minutes	W	RIGHT	2	3.3	1
038A	10 Minutes	W	RIGHT	3	3.73	1
039A	10 Minutes	W	RIGHT	1	3.8	1
040A	10 Minutes	W	LEFT	3	3.02	1
104A	10 Minutes	W	RIGHT	4	3.07	1
110A	10 Minutes	W	LEFT	3	-0.14	
120A	10 Minutes	W	LEFT	1	0.93	
122A	10 Minutes	W	LEFT	4	-0.29	
124A	10 Minutes	W	RIGHT	3	2.85	1
126A	10 Minutes	W	RIGHT	2	-0.04	
127A	10 Minutes	W	LEFT	4	1.92	
134A	10 Minutes	W	RIGHT	4	2.1	1
135A	10 Minutes	W	LEFT	1	1.97	
137A	10 Minutes	W	RIGHT	2	3.7	1
138A	10 Minutes	W	RIGHT	3	3.74	1
210A	10 Minutes	W	LEFT	3	3.07	1
222A	10 Minutes	W	LEFT	4	2.17	1
234A	10 Minutes	W	RIGHT	4	3.09	1
322A	10 Minutes	W	LEFT	4	3.49	1
334A	10 Minutes	W	RIGHT	4	2.95	1
422A	10 Minutes	W	LEFT	4	2.41	1
434A	10 Minutes	W	RIGHT	4	0.44	
mean					2.40	40
% above threshold						68.96

Table 27. Mean log reductions and percentage of individuals

meeting the TFM log reduction threshold.

		mean log reduction	% meeting threshold
<b>LIMS 8304</b>			
abdomen			
	DuraPrep	2.52	67.90
	Hibiclens	2.09	52.50
inguinal			
	DuraPrep	2.78	35.71
	Hibiclens	2.93	51.28
<b>LIMS 8919</b>			
abdomen			
	DuraPrep	2.4	68.96
	Hibiclens	2.11	58.97
inguinal			
	DuraPrep	2.23	20.89
	Hibiclens	1.94	12.00

From Table 27, it is clear that the *majority of individuals* as well as *the mean of those individuals* from both studies met the 2-log reduction at the abdominal site for both the test product and the positive control. In the \_\_\_\_\_ study, 68% and 53% of individuals met the TFM criterion of a 2-log reduction for DuraPrep and Hibiclens, respectively. In the \_\_\_\_\_ study, 69% and 59% of individuals met the TFM criterion of a 2-log reduction for DuraPrep and Hibiclens, respectively. In both studies, both DuraPrep and Hibiclens easily reached the 2-log reduction criterion for the abdominal site. In fact, DuraPrep outperformed Hibiclens in both the \_\_\_\_\_ and \_\_\_\_\_ studies with log reductions of 2.52 and 2.4 log reductions, respectively. *Clearly, reaching the TFM 2-log reduction criterion for the abdominal site is readily achievable.*

It is also clear from Table 27 that the in both studies, neither the *majority of individuals* nor the *mean of those individuals* met the 3-log reduction at the inguinal site. In the \_\_\_\_\_ study, only 36% and 51% of individuals met the TFM criterion of a 3-log reduction for DuraPrep and Hibiclens, respectively. In the \_\_\_\_\_ study, only 21% and 12% of individuals met the TFM criterion of a 3-log reduction for DuraPrep and Hibiclens, respectively. DuraPrep only outperformed the positive control, Hibiclens, in one study yet, in both studies neither DuraPrep nor Hibiclens meet the TFM 3-log reduction criterion in the inguinal site. *Clearly, reaching the TFM 3-log reduction criterion for the inguinal site is not readily achievable but indeed, difficult to achieve for individual subjects.*

**Neutralization Validation for Lims 8918 ( \_\_\_\_\_, Study 2)**

**Methodology.** Two abdominal sites of protocol specified size from each of six subjects are treated with one of the test materials. Each subject has one site treated with DuraPrep solution and the other site is treated with either Hibiclens cleanser or Betadine combination. The treated sites are sampled using either MSS (DuraPrep solution and Betadine combination) or SSS (Hibiclens cleanser) and sampled by the scrub cup technique.

*Results.* The results of the LIMS 8918 neutralization assay are shown in Table 28.

<b>Table 28. Recovery of <i>S. marcescens</i> in LIMS 8918 Neutralization Assay</b>				
<b>Article</b>	<b>Time</b>	<b>Average CFU/mL</b>	<b>Log<sub>10</sub> CFU/mL</b>	<b>Log<sub>10</sub> Difference</b>
Numbers	Immediate	3.1 x 10 <sup>2</sup>	2.49	NA
Control	30 Minutes	3.1 x 10 <sup>2</sup>	2.49	NA
Toxicity Control (TC-MSS)	Immediate	3.5 x 10 <sup>2</sup>	2.54	0.05
	30 Minutes	3.4 x 10 <sup>2</sup>	2.53	0.04
Toxicity Control (TC-SSS)	Immediate	3.3 x 10 <sup>2</sup>	2.52	0.03
	30 Minutes	3.4 x 10 <sup>2</sup>	2.53	0.04
Subject N-01 Left Abdomen	Immediate	2.9 x 10 <sup>2</sup>	2.46	0.03
	30 Minutes	3.2 x 10 <sup>2</sup>	2.50	0.01
Subject N-01 Right Abdomen	Immediate	3.3 x 10 <sup>2</sup>	2.52	0.03
	30 Minutes	3.3 x 10 <sup>2</sup>	2.52	0.03
Subject N-02 Left Abdomen	Immediate	4.2 x 10 <sup>2</sup>	2.62	0.13
	30 Minutes	3.8 x 10 <sup>2</sup>	2.58	0.09
Subject N-02 Right Abdomen	Immediate	3.4 x 10 <sup>2</sup>	2.53	0.04
	30 Minutes	3.4 x 10 <sup>2</sup>	2.53	0.04
Subject N-03 Left Abdomen	Immediate	3.2 x 10 <sup>2</sup>	2.50	0.01
	30 Minutes	3.2 x 10 <sup>2</sup>	2.50	0.01
Subject N-03 Right Abdomen	Immediate	3.0 x 10 <sup>2</sup>	2.48	0.01
	30 Minutes	3.1 x 10 <sup>2</sup>	2.49	0.00

**Table 28. Recovery of *S. marcescens* in LIMS 8918 Neutralization Assay**

(continued)				
Article	Time	Average CFU/mL	Log <sub>10</sub> CFU/mL	Log <sub>10</sub> Difference
Subject N-04 Left Abdomen	Immediate	3.6 x 10 <sup>2</sup>	2.56	0.07
Subject N-04 Left Abdomen	30 Minutes	3.0 x 10 <sup>2</sup>	2.48	0.01
Subject N-04 Right Abdomen	Immediate	3.3 x 10 <sup>2</sup>	2.52	0.03
Subject N-04 Right Abdomen	30 Minutes	3.1 x 10 <sup>2</sup>	2.49	0.00
Subject N-05 Left Abdomen	Immediate	3.4 x 10 <sup>2</sup>	2.53	0.04
Subject N-05 Left Abdomen	30 Minutes	3.3 x 10 <sup>2</sup>	2.52	0.03
Subject N-05 Right Abdomen	Immediate	3.2 x 10 <sup>2</sup>	2.50	0.01
Subject N-05 Right Abdomen	30 Minutes	2.9 x 10 <sup>2</sup>	2.46	0.03
Subject N-06 Left Abdomen	Immediate	3.6 x 10 <sup>2</sup>	2.56	0.07
Subject N-06 Left Abdomen	30 Minutes	3.4 x 10 <sup>2</sup>	2.53	0.04
Subject N-06 Right Abdomen	Immediate	3.6 x 10 <sup>2</sup>	2.56	0.07
Subject N-06 Right Abdomen	30 Minutes	3.4 x 10 <sup>2</sup>	2.53	0.04

The neutralizer system is considered effective if recovery of the organism in the antiseptic sample is not more than 0.2 logs different than the corresponding number control. The neutralizer system is not considered toxic if recovery of the organism in the toxicity controls is not more than 0.2 logs different than the corresponding number control. In this study, the neutralizer system is effective and not toxic.

#### ***Pilot Studies***

#### **Evaluation of the Persistent Antimicrobial Activity of 3M Duraprep™ Surgical Solution and DuraPrep Without Iodine Control Using a Bacterial Challenge Method**

Both LIMS 8197 and LIMS 9302 are randomized, partially blinded, paired-comparison studies designed to evaluate the contribution of iodine to the antimicrobial activity of DuraPrep solution. Antimicrobial effectiveness is evaluated by measuring the log reduction of a bacterial challenge with four different challenge organisms (*Staphylococcus aureus*, *Serratia marcescens*, *Enterococcus faecalis*, *Escherichia coli*). Antibiotic susceptibilities are obtained for each organism prior to study initiation. Log reduction of organisms recovered from the prepped test sites (calculated from corresponding untreated control sites) are determined at three post-preparation time points and two organism residence times. Log reductions of the bacterial challenge achieved with DuraPrep film are compared with those achieved with DuraPrep w/o I<sub>2</sub>

film. Betadine combination is tested for information only. Neutralization of the test materials by SSS is verified prior to study start.

Protocols for the pilot studies are similar but different from the protocols for the pivotal studies. Healthy subjects are entered into a minimum 7-day Pretreatment Phase during which standardized, non-antimicrobial soaps, shampoos, and deodorants are used. Following the Pretreatment Phase, subjects meeting all inclusion and no exclusion criteria are assigned treatment numbers and randomized to treatment and bacterial strain on Treatment Day. On the Treatment Day, each subject is prepared for four test areas on the back, one for each treatment (DuraPrep solution, DuraPrep w/o I<sub>2</sub>, Betadine combination, and an untreated recovery control). Each test area contains six individual test sites (three inoculation times and two bacterial residence times). When the preparations are dry, individual sites within each test area are inoculated with 50 µL (approximately 10<sup>8</sup> colony forming units [CFU]/mL) of the challenge organism. After inoculation, the test organism remains *in situ* for 5 or 30 minutes prior to sample collection. The organisms are recovered using a modified cup scrub technique and SSS. The inoculation of individual sites within each test area and recovery of organisms is repeated in the same manner at approximately two hours and six hours post-preparation. After sample collection, the inoculated sites are disinfected with 70% IPA. Bacterial counts are performed by individuals who are blinded to the identities of the test product associated with each sample.

Four to eight days following treatment, subjects return for a dermatological evaluation of the test sites. At this visit, a qualified individual visually examines the test area of the skin to ensure that there is no infection present.

#### Study 1—LIMS 8197

The contribution of iodine to the antimicrobial activity of DuraPrep solution is assessed by comparing the log reduction of a bacterial challenge (with different organisms) on sites prepped with either DuraPrep solution or DuraPrep w/o I<sub>2</sub>. At the primary analysis time point (six hours post-preparation, 30-minute residence time), the log reduction of the bacterial challenge is significantly greater on DuraPrep film (mean log reduction = 2.96) than on DuraPrep w/o I<sub>2</sub> film (mean log reduction = -0.18; p<0.0001, based on a paired t-test). These results demonstrate that iodine contributes significantly to the antimicrobial activity of DuraPrep solution. A summary of log reduction of bacterial challenge for the efficacy evaluable population is found in Table 29.

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**Table 29. Summary of Log Reduction of Bacterial Challenge(CFU/cm<sup>2</sup>) – DuraPrep**

**Solution Versus DuraPrep w/o I<sub>2</sub> (Efficacy-Evaluable Population)**

Inoculation Time/ Contact Time	DuraPrep w/o I <sub>2</sub> (N = 31)	DuraPrep Solution (N = 31)	Paired Difference in Log Reduction <sup>1</sup>	P-value <sup>2</sup>	P-value <sup>3</sup>
<b>When Preparation is Dry</b>					
5 Minutes <sup>4</sup>					
Mean (SD)	-0.05 (0.507)	1.45 (1.550)	1.49 (1.486)	<0.0001	<0.0001
95% CI			(0.94, 2.05)		
30 Minutes <sup>5</sup>					
Mean (SD)	-0.67 (0.895)	2.82 (1.924)	3.49 (2.165)	<0.0001	<0.0001
95% CI			(2.68, 4.30)		
<b>2-Hours Post-Preparation</b>					
5 Minutes					
Mean (SD)	0.22 (1.083)	1.26 (1.621)	1.05 (1.416)	0.0003	<0.0001
95% CI			(0.53, 1.56)		
30 Minutes					
Mean (SD)	-0.52 (0.804)	3.04 (1.782)	3.56 (2.000)	<0.0001	<0.0001
95% CI			(2.83, 4.30)		
<b>6-Hours Post-Preparation</b>					
5 Minutes					
Mean (SD)	0.03 (0.194)	1.82 (1.781)	1.79 (1.782)	<0.0001	<0.0001
95% CI			(1.14, 2.45)		
30 Minutes					
Mean (SD)	-0.18 (0.841)	2.96 (1.761)	3.14 (2.061)	<0.0001	<0.0001
95% CI			(2.38, 3.89)		

SD = standard deviation; CI = confidence interval.

<sup>1</sup> Calculated by subtracting the log reduction of DuraPrep w/o I<sub>2</sub> from the log reduction of DuraPrep solution.

<sup>2</sup> Based on a paired t-test.

<sup>3</sup> Based on a Wilcoxon Signed Rank Test.

<sup>4</sup> Subject 011 was missing the assessment at 5-minute residence time when preparation was dry due to technician error.

<sup>5</sup> Subject 205 was missing the assessment at 30-minute residence time when preparation was dry due to technician error.

Source: Table 7, LIMS 8197 CSR.

At the primary analysis time point, the mean log reduction of the bacterial challenge is greater for DuraPrep film than for DuraPrep w/o I<sub>2</sub> film (p ≤ 0.0009) for each of the bacterial organisms tested except *E. faecalis* for which there is no statistically significant difference. These findings were confirmed with the non-parametric signed rank test. At the primary analysis time point, the paired difference is the greatest with *S. aureus* (4.22) and the least with *E. faecalis* (0.95). Although the difference in log reduction for *E. faecalis* is not statistically significant at the primary time point, it is statistically significant (favoring DuraPrep film over DuraPrep w/o I<sub>2</sub> film) at all other time points (p ≤ 0.03).

Data for the Betadine combination-treated sites are summarized for informational purposes only. At all time points, there is a substantial mean log reduction of the bacterial counts on Betadine combination-treated sites compared with the untreated control sites (mean log reductions ranged from 4.96 to 5.83).

In conclusion, iodine contributes to the antimicrobial activity of DuraPrep solution, as assessed by the mean log reduction of a bacterial challenge against four different bacteria. The contribution of iodine is demonstrated for all four bacterial organisms tested, although the magnitude of the contribution differed depending on the organism. The antimicrobial activity of Betadine combination is confirmed using the methods in this study.

**Neutralization Validation for Lims 8197 (Study 1)**

*Neutralization Method.* This neutralization study determines the ability of the SSS to neutralize completely any available iodine that is recovered from the DuraPrep solution and from the Betadine combination in the process of sampling post-product application. Two human subjects are designated for neutralization validation only. Four challenge microorganisms are used: *E. faecalis* (ATCC 10741), *E. coli* (ATCC 25922), *S. marcescens* (ATCC 14756), and *S. aureus* (ATCC 27217). One subject is used to test *S. aureus* and *E. faecalis* and the other subject is used to test *E. coli* and *S. marcescens*. Two post-inoculation times are tested, immediate (< 1 minute) and 30 minutes, in order to detect any potential neutralizer effect between the time of sample collection and the time of plating.

*Results.* The neutralization is considered effective if the post-preparation sample recovered is not more than 0.3 log<sub>10</sub> less than the Numbers Control sample. The Standard Sampling Solution is considered non-toxic if the Toxicity Control sample is not more than 0.2 log<sub>10</sub> less than the Numbers Control sample.

The log<sub>10</sub> values recovered for the four test organisms from all neutralization samples plated, including the numbers and toxicity controls, and whether these samples are within 0.2 log<sub>10</sub> of the numbers control are presented in Table 30, Table 31, Table 32, and Table 33.

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**Table 30. Neutralization Results for *Staphylococcus aureus* (ATCC 27217)**

Test	Log <sub>10</sub> Value	Log <sub>10</sub> Difference from Numbers Control	Within 0.2 Log <sub>10</sub> of Numbers Control?
Numbers Control (<1 Minute)	1.48	NA	Yes
Numbers Control (30 Minutes)	1.63	-0.15	Yes
Numbers Control (Final)	1.58	-0.10	Yes
Toxicity Control, SSS (<1 Minute)	1.61	-0.13	Yes
Toxicity Control, SSS (30 Minutes)	1.63	-0.15	Yes
Neutralization Effectiveness-Subject N1 Betadine—5 Minutes-Replication 1- (<1 Minute)	1.69	-0.21	No
Neutralization Effectiveness-Subject N1 Betadine—5 Minutes-Replication 1- (30 Minutes)	1.66	-0.18	Yes
Neutralization Effectiveness-Subject N1 Betadine—5 Minutes-Replication 2- (<1 Minute)	1.62	-0.14	Yes
Neutralization Effectiveness-Subject N1 Betadine—5 Minutes-Replication 2- (30 Minutes)	1.61	-0.13	Yes
Neutralization Effectiveness-Subject N1 Betadine—30 Minutes-Replication 1- (<1 Minute)	1.56	-0.08	Yes
Neutralization Effectiveness-Subject N1 Betadine—30 Minutes-Replication 1- (30 Minutes)	1.61	-0.13	Yes
Neutralization Effectiveness-Subject N1 Betadine—30 Minutes-Replication 2- (<1 Minute)	1.63	-0.15	Yes
Neutralization Effectiveness-Subject N1 Betadine—30 Minutes-Replication 2- (30 Minutes)	1.66	-0.18	Yes
Neutralization Effectiveness-Subject N1 DuraPrep—5 Minutes-Replication 1- (<1 Minute)	1.63	-0.15	Yes
Neutralization Effectiveness-Subject N1 DuraPrep —5 Minutes-Replication 1- (30 Minutes)	1.60	-0.12	Yes
Neutralization Effectiveness-Subject N1 DuraPrep —5 Minutes-Replication 2- (<1 Minute)	1.57	-0.09	Yes
Neutralization Effectiveness-Subject N1 DuraPrep —5 Minutes-Replication 2- (30 Minutes)	1.67	-0.19	Yes
Neutralization Effectiveness-Subject N1 DuraPrep —30 Minutes-Replication 1- (<1 Minute)	1.63	-0.15	Yes
Neutralization Effectiveness-Subject N1 DuraPrep —30 Minutes-Replication 1- (30 Minutes)	1.62	-0.14	Yes
Neutralization Effectiveness-Subject N1 DuraPrep —30 Minutes-Replication 2- (<1 Minute)	1.63	-0.15	Yes
Neutralization Effectiveness-Subject N1 DuraPrep —30 Minutes-Replication 2- (30 Minutes)	1.62	-0.14	Yes

Source: LIMS 8197 Final CSR

**Table 31. Neutralization Results for *Serratia marcescens* (ATCC 14756)**

Test	Log <sub>10</sub> Value	Log <sub>10</sub> Difference from Numbers Control	Within 0.2 Log <sub>10</sub> of Numbers Control?
Numbers Control (<1 Minute)	2.30	NA	NA
Numbers Control (30 Minutes)	2.26	0.04	Yes
Numbers Control (Final)	2.34	-0.04	Yes
Toxicity Control, SSS (<1 Minute)	2.32	-0.02	Yes
Toxicity Control, SSS (30 Minutes)	2.29	0.01	Yes
Neutralization Effectiveness-Subject N2 Betadine—5 Minutes-Replication 1- (<1 Minute)	2.36	-0.06	Yes
Neutralization Effectiveness-Subject N2 Betadine—5 Minutes-Replication 1- (30 Minutes)	2.30	0.00	Yes
Neutralization Effectiveness-Subject N2 Betadine—5 Minutes-Replication 2- (<1 Minute)	2.29	0.01	Yes
Neutralization Effectiveness-Subject N2 Betadine—5 Minutes-Replication 2- (30 Minutes)	2.33	-0.03	Yes
Neutralization Effectiveness-Subject N1 Betadine—30 Minutes-Replication 1- (<1 Minute)	2.29	0.01	Yes
Neutralization Effectiveness-Subject N2 Betadine—30 Minutes-Replication 1- (30 Minutes)	2.27	0.03	Yes
Neutralization Effectiveness-Subject N2 Betadine—30 Minutes-Replication 2- (<1 Minute)	2.31	-0.01	Yes
Neutralization Effectiveness-Subject N2 Betadine—30 Minutes-Replication 2- (30 Minutes)	2.32	-0.02	Yes
Neutralization Effectiveness-Subject N2 DuraPrep—5 Minutes-Replication 1- (<1 Minute)	2.31	-0.01	Yes
Neutralization Effectiveness-Subject N2 DuraPrep —5 Minutes-Replication 1- (30 Minutes)	2.31	-0.01	Yes
Neutralization Effectiveness-Subject N2 DuraPrep —5 Minutes-Replication 2- (<1 Minute)	2.28	0.02	Yes
Neutralization Effectiveness-Subject N2 DuraPrep —5 Minutes-Replication 2- (30 Minutes)	2.29	0.01	Yes
Neutralization Effectiveness-Subject N2 DuraPrep —30 Minutes-Replication 1- (<1 Minute)	2.28	0.02	Yes
Neutralization Effectiveness-Subject N2 DuraPrep —30 Minutes-Replication 1- (30 Minutes)	2.25	0.05	Yes
Neutralization Effectiveness-Subject N2 DuraPrep —30 Minutes-Replication 2- (<1 Minute)	2.29	0.01	Yes
Neutralization Effectiveness-Subject N2 DuraPrep —30 Minutes-Replication 2- (30 Minutes)	2.25	0.05	Yes

Source: LIMS 8197 Final CSR

**Table 32. Neutralization Results for *Enterococcus faecalis* (ATCC 10741)**

Test	Log <sub>10</sub> Value	Log <sub>10</sub> Difference from Numbers Control	Within 0.2 Log <sub>10</sub> of Numbers Control?
Numbers Control (<1 Minute)	2.05	NA	NA
Numbers Control (30 Minutes)	2.05	0.00	Yes
Numbers Control (Final)	2.11	-0.06	Yes
Toxicity Control, SSS (<1 Minute)	2.03	0.02	Yes
Toxicity Control, SSS (30 Minutes)	2.06	-0.01	Yes
Neutralization Effectiveness-Subject N1 Betadine—5 Minutes-Replication 1- (<1 Minute)	2.07	-0.02	Yes
Neutralization Effectiveness-Subject N1 Betadine—5 Minutes-Replication 1- (30 Minutes)	2.05	0.00	Yes
Neutralization Effectiveness-Subject N1 Betadine—5 Minutes-Replication 2- (<1 Minute)	1.92	0.13	Yes
Neutralization Effectiveness-Subject N1 Betadine—5 Minutes-Replication 2- (30 Minutes)	1.99	0.06	Yes
Neutralization Effectiveness-Subject N1 Betadine—30 Minutes-Replication 1- (<1 Minute)	2.03	0.02	Yes
Neutralization Effectiveness-Subject N1 Betadine—30 Minutes-Replication 1- (30 Minutes)	2.00	0.05	Yes
Neutralization Effectiveness-Subject N1 Betadine—30 Minutes-Replication 2- (<1 Minute)	2.00	0.05	Yes
Neutralization Effectiveness-Subject N1 Betadine—30 Minutes-Replication 2- (30 Minutes)	2.16	-0.11	Yes
Neutralization Effectiveness-Subject N1 DuraPrep—5 Minutes-Replication 1- (<1 Minute)	1.99	0.06	Yes
Neutralization Effectiveness-Subject N1 DuraPrep —5 Minutes-Replication 1- (30 Minutes)	2.04	0.01	Yes
Neutralization Effectiveness-Subject N1 DuraPrep —5 Minutes-Replication 2- (<1 Minute)	1.98	0.07	Yes
Neutralization Effectiveness-Subject N1 DuraPrep —5 Minutes-Replication 2- (30 Minutes)	2.00	0.05	Yes
Neutralization Effectiveness-Subject N1 DuraPrep —30 Minutes-Replication 1- (<1 Minute)	1.98	0.07	Yes
Neutralization Effectiveness-Subject N1 DuraPrep —30 Minutes-Replication 1- (30 Minutes)	NP	NA	NA
Neutralization Effectiveness-Subject N1 DuraPrep —30 Minutes-Replication 2- (<1 Minute)	2.04	0.01	Yes
Neutralization Effectiveness-Subject N1 DuraPrep —30 Minutes-Replication 2- (30 Minutes)	2.16	-0.11	Yes

NP= Not plated; NA= Not applicable  
Source: LIMS 8197 Final CSR

**Table 33. Neutralization Results for *Escherichia coli* (ATCC 25922)**

Test	Log <sub>10</sub> Value	Log <sub>10</sub> Difference from Numbers Control	Within 0.2 Log <sub>10</sub> of Numbers Control?
Numbers Control (<1 Minute)	1.94	NA	NA
Numbers Control (30 Minutes)	1.95	-0.01	Yes
Numbers Control (Final)	1.91	0.03	Yes
Toxicity Control, SSS (<1 Minute)	2.05	-0.11	Yes
Toxicity Control, SSS (30 Minutes)	2.08	-0.14	Yes
Neutralization Effectiveness-Subject N2 Betadine—5 Minutes-Replication 1- (<1 Minute)	2.03	-0.09	Yes
Neutralization Effectiveness-Subject N2 Betadine—5 Minutes-Replication 1- (30 Minutes)	2.06	-0.12	Yes
Neutralization Effectiveness-Subject N2 Betadine—5 Minutes-Replication 2- (<1 Minute)	2.02	-0.08	Yes
Neutralization Effectiveness-Subject N2 Betadine—5 Minutes-Replication 2- (30 Minutes)	2.05	-0.11	Yes
Neutralization Effectiveness-Subject N1 Betadine—30 Minutes-Replication 1- (<1 Minute)	2.06	-0.12	Yes
Neutralization Effectiveness-Subject N2 Betadine—30 Minutes-Replication 1- (30 Minutes)	2.09	-0.15	Yes
Neutralization Effectiveness-Subject N2 Betadine—30 Minutes-Replication 2- (<1 Minute)	2.03	-0.09	Yes
Neutralization Effectiveness-Subject N2 Betadine—30 Minutes-Replication 2- (30 Minutes)	2.05	-0.11	Yes
Neutralization Effectiveness-Subject N2 DuraPrep—5 Minutes-Replication 1- (<1 Minute)	2.03	-0.09	Yes
Neutralization Effectiveness-Subject N2 DuraPrep —5 Minutes-Replication 1- (30 Minutes)	2.09	-0.15	Yes
Neutralization Effectiveness-Subject N2 DuraPrep —5 Minutes-Replication 2- (<1 Minute)	2.07	-0.13	Yes
Neutralization Effectiveness-Subject N2 DuraPrep —5 Minutes-Replication 2- (30 Minutes)	2.05	-0.11	Yes
Neutralization Effectiveness-Subject N2 DuraPrep —30 Minutes-Replication 1- (<1 Minute)	2.06	-0.12	Yes
Neutralization Effectiveness-Subject N2 DuraPrep —30 Minutes-Replication 1- (30 Minutes)	2.07	-0.13	Yes
Neutralization Effectiveness-Subject N2 DuraPrep —30 Minutes-Replication 2- (<1 Minute)	2.05	-0.11	Yes
Neutralization Effectiveness-Subject N2 DuraPrep —30 Minutes-Replication 2- (30 Minutes)	2.05	-0.11	Yes

Source: LIMS 8197 Final CSR

In conclusion, all neutralization results are within 0.2 log<sub>10</sub> of the numbers control; the neutralization is effective and non-toxic.

**Study 2—LIMS 9302**

The contribution of iodine to the antimicrobial activity of DuraPrep solution is assessed by comparing the log reduction of a bacterial challenge on sites prepped with either DuraPrep solution or DuraPrep w/o I<sub>2</sub>. At the primary analysis time point (six hours post-preparation, 30-minute residence time) the log reduction of the bacterial challenge is significantly greater on DuraPrep film (mean log reduction = 3.77) than on DuraPrep w/o I<sub>2</sub> film (mean log reduction = 0.05, p<0.0001, based on a paired t-test). These results demonstrate that iodine contributes to the antimicrobial activity of DuraPrep solution. A summary of log reduction of bacterial challenge for the efficacy evaluable population is presented in Table 34. These organisms included *S. aureus*, *E. coli*, *E. faecalis*, and *S. marcescens*.

**Table 34. Summary of Log Reduction of Bacterial Challenge (CFU/cm<sup>2</sup>) – DuraPrep Solution Versus DuraPrep w/o I<sub>2</sub> (Efficacy-Evaluable Population)**

Inoculation Time/ Contact Time	DuraPrep w/o I <sub>2</sub> (N=24)	DuraPrep Solution (N=24)	Paired Difference in Log Reduction <sup>1</sup>	P-value <sup>2</sup>	P-value <sup>3</sup>
<b>When Preparation is Dry</b>					
5 Minutes					
Mean (SD)	-0.02 (0.136)	0.51 (1.346)	0.53 (1.326)	0.0626	<0.0001
95% CI			(-0.03, 1.09)		
30 Minutes					
Mean (SD)	-0.39 (0.701)	3.47 (1.905)	3.86 (2.243)	<0.0001	<0.0001
95% CI			(2.91, 4.80)		
<b>2-Hours Post-Preparation</b>					
5 Minutes					
Mean (SD)	-0.02 (0.139)	0.75 (1.485)	0.77 (1.482)	0.0185	<0.0001
95% CI			(0.14, 1.39)		
30 Minutes					
Mean (SD)	-0.03 (1.261)	3.39 (1.702)	3.42 (2.354)	<0.0001	<0.0001
95% CI			(2.43, 4.42)		
<b>6-Hours Post-Preparation</b>					
5 Minutes					
Mean (SD)	0.02 (0.111)	0.71 (1.146)	0.69 (1.159)	0.0079	<0.0001
95% CI			(0.20, 1.18)		
30 Minutes					
Mean (SD)	0.05 (0.612)	3.77 (1.699)	3.72 (1.601)	<0.0001	<0.0001
95% CI			(3.04, 4.39)		

Note: Pooled data for all 4 bacterial strains (*S. aureus*, *S. marcescens*, *E. faecalis*, and *E. coli*).  
SD = standard deviation; CI = confidence interval.

<sup>1</sup> Calculated by subtracting the log reduction of DuraPrep w/o I<sub>2</sub> from the log reduction of DuraPrep solution.

<sup>2</sup> Based on a paired t-test.

<sup>3</sup> Based on a Wilcoxon Signed Rank Test.

Source: Table 7, LIMS 9302 CSR.

At the primary analysis time point, the mean log reduction of the individual bacterial challenges is significantly greater on DuraPrep film than on DuraPrep w/o I<sub>2</sub> film (p≤ 0.0034, based on a paired t-test) for each of the four bacterial organisms. This is confirmed with the non-parametric signed rank test. The paired difference is the greatest with *E. coli* (4.86) and the least with *E. faecalis* (2.32).

Data for the Betadine combination-treated sites are summarized for informational purposes only. At all time points, there is a substantial mean log reduction of the bacterial counts on Betadine combination-treated sites compared with the untreated control sites (mean log reductions ranged from 5.69 to 6.56).

In conclusion, iodine contributes significantly to the antimicrobial activity of DuraPrep solution, as assessed by the mean log reduction of a bacterial challenge. The contribution of iodine is demonstrated for all four bacterial organisms tested, although the magnitude of the contribution differs depending on the organism. The antimicrobial activity of Betadine combination is confirmed using the methods in this study.

**Neutralization Validation for Lims 9302 (\_\_\_\_\_, Study 2)**

**Methodology.**

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*Results.* The neutralization plate counts, average CFU/mL, and log<sub>10</sub> CFU/mL are shown in Table 35 for the four test organisms. The average log<sub>10</sub> CFU/mL and log<sub>10</sub> difference are shown in 36.

**Table 35. Plate Count, Average CFU/mL, and Log<sub>10</sub> CFU/mL**

SUBJECT NUMBER: N2		ORGANISM: <i>S. marcescens</i> ATCC 14756												
Area - Site	Treatment HTR Code	Sampling Time	Plating Time	Replicate 1			Replicate 2			Average CFU/mL	Log <sub>10</sub> CFU/mL			
NA	NA	Number Control A	Immediate	47	32	31	21	34	36	1.0 x 10 <sup>2</sup>	2.00			
				27	39	28	32	20	24	8.5 x 10 <sup>1</sup>	1.93			
NA	NA	Number Control B	Immediate	24	38	21	35	35	24	8.8 x 10 <sup>1</sup>	1.94			
				29	30	32	35	27	25	8.9 x 10 <sup>1</sup>	1.95			
NA	NA	Toxicity Control A	Immediate	27	28	24	44	39	32	9.7 x 10 <sup>1</sup>	1.99			
				34	22	26	29	20	26	7.8 x 10 <sup>1</sup>	1.89			
NA	NA	Toxicity Control B	Immediate	30	28	32	32	29	45	9.8 x 10 <sup>1</sup>	1.99			
				30	32	36	26	37	28	9.4 x 10 <sup>1</sup>	1.97			
X-3	A	5A	Immediate	27	31	24	36	37	28	9.2 x 10 <sup>1</sup>	1.96			
				25	24	36	25	31	33	8.7 x 10 <sup>1</sup>	1.94			
X-6	A	5B	Immediate	28	29	29	36	33	34	9.4 x 10 <sup>1</sup>	1.97			
				34	26	29	25	30	26	8.5 x 10 <sup>1</sup>	1.93			
X-1	A	30A	Immediate	24	30	27	30	38	23	8.6 x 10 <sup>1</sup>	1.93			
				32	34	38	33	35	36	1.0 x 10 <sup>2</sup>	2.00			
X-4	A	30B	Immediate	35	27	20	33	37	31	9.2 x 10 <sup>1</sup>	1.96			
				43	24	32	33	32	32	9.8 x 10 <sup>1</sup>	1.99			
Z-3	D	5A	Immediate	30	31	27	21	27	36	8.6 x 10 <sup>1</sup>	1.93			
				30	33	24	38	35	37	9.8 x 10 <sup>1</sup>	1.99			
Z-6	D	5B	Immediate	27	33	43	38	34	25	1.0 x 10 <sup>2</sup>	2.00			
				22	24	35	21	32	30	8.2 x 10 <sup>1</sup>	1.91			
Z-1	D	30A	Immediate	27	39	29	34	35	27	9.6 x 10 <sup>1</sup>	1.98			
				24	37	25	33	39	34	9.6 x 10 <sup>1</sup>	1.98			
Z-4	D	30B	Immediate	27	30	33	32	30	29	9.0 x 10 <sup>1</sup>	1.95			
				44	38	30	27	23	25	9.4 x 10 <sup>1</sup>	1.97			
INOCULUM COUNTS				10 <sup>-1</sup>			10 <sup>-2</sup>			10 <sup>-3</sup>			Average CFU/mL	
Beginning Inoculum				A			T	T	47	41	6	4	4.4 x 10 <sup>3</sup>	
Beginning Inoculum				B			T	T	40	41	2	6	4.0 x 10 <sup>3</sup>	
End Inoculum							T	T	31	43	2	2	3.7 x 10 <sup>3</sup>	

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Table 35. Plate Count, Average CFU/mL, and Log<sub>10</sub> CFU/mL (continued)

SUBJECT NUMBER: N2			ORGANISM: <i>S. aureus</i> ATCC 27217								
Area - Site	Treatment HTR Code	Sampling Time	Plating Time	Plate Counts						Average CFU/mL	Log <sub>10</sub> CFU/mL
				Replicate 1			Replicate 2				
NA	NA	Number Control A	Immediate	27	32	23	19	23	28	7.6 x 10 <sup>1</sup>	1.88
			30 Minutes	30	18	23	29	20	22	7.1 x 10 <sup>1</sup>	1.85
NA	NA	Number Control B	Immediate	26	25	26	23	26	32	7.9 x 10 <sup>1</sup>	1.90
			30 Minutes	19	26	26	34	19	26	7.5 x 10 <sup>1</sup>	1.88
NA	NA	Toxicity Control A	Immediate	34	21	29	35	15	25	8.0 x 10 <sup>1</sup>	1.90
			30 Minutes	31	19	24	24	21	19	6.9 x 10 <sup>1</sup>	1.84
NA	NA	Toxicity Control B	Immediate	18	24	26	21	17	28	6.7 x 10 <sup>1</sup>	1.83
			30 Minutes	26	18	19	19	15	22	6.0 x 10 <sup>1</sup>	1.78
W-3	A	5A	Immediate	16	22	19	13	19	25	5.7 x 10 <sup>1</sup>	1.76
			30 Minutes	30	27	25	23	21	12	6.9 x 10 <sup>1</sup>	1.84
W-6	A	5B	Immediate	21	25	19	26	25	23	7.0 x 10 <sup>1</sup>	1.84
			30 Minutes	18	25	17	21	21	28	6.5 x 10 <sup>1</sup>	1.81
W-1	A	30A	Immediate	27	20	19	23	23	20	6.6 x 10 <sup>1</sup>	1.82
			30 Minutes	15	26	31	22	24	16	6.7 x 10 <sup>1</sup>	1.83
W-4	A	30B	Immediate	24	20	27	24	17	19	6.6 x 10 <sup>1</sup>	1.82
			30 Minutes	21	21	19	30	18	21	6.5 x 10 <sup>1</sup>	1.81
Y-3	D	5A	Immediate	23	22	26	24	17	21	6.6 x 10 <sup>1</sup>	1.82
			30 Minutes	29	22	18	22	30	27	7.4 x 10 <sup>1</sup>	1.87
Y-6	D	5B	Immediate	22	30	15	17	29	17	6.5 x 10 <sup>1</sup>	1.81
			30 Minutes	21	20	16	30	18	26	6.6 x 10 <sup>1</sup>	1.82
Y-1	D	30A	Immediate	23	27	28	23	26	37	8.2 x 10 <sup>1</sup>	1.91
			30 Minutes	13	37	23	26	24	25	7.4 x 10 <sup>1</sup>	1.87
Y-4	D	30B	Immediate	20	14	20	16	24	23	5.8 x 10 <sup>1</sup>	1.76
			30 Minutes	20	28	22	28	16	15	6.4 x 10 <sup>1</sup>	1.81
INOCULUM COUNTS				10 <sup>-1</sup>		10 <sup>-2</sup>		10 <sup>-3</sup>		Average CFU/mL	
Beginning Inoculum				A		T		T		47 50 2 6 4.8 x 10 <sup>3</sup>	
Beginning Inoculum				B		T		T		36 34 4 1 3.5 x 10 <sup>3</sup>	
End Inoculum				T		T		T		30 58 2 3 4.4 x 10 <sup>3</sup>	

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Table 35. Plate Count, Average CFU/mL, and Log<sub>10</sub> CFU/mL (continued)

SUBJECT NUMBER: N3			ORGANISM: <i>E. coli</i> ATCC 25922								
Area Site	Treatment HTR Code	Sampling Time	Plating Time	Plate Counts						Average CFU/mL	Log <sub>10</sub> CFU/mL
				Replicate 1			Replicate 2				
NA	NA	Number	Immediate	41	47	49	44	26	41	1.2 x 10 <sup>2</sup>	2.08
		Control A	30 Minutes	42	53	33	46	53	40	1.3 x 10 <sup>2</sup>	2.11
NA	NA	Number	Immediate	34	50	38	30	36	44	1.2 x 10 <sup>2</sup>	2.08
		Control B	30 Minutes	38	37	45	52	40	35	1.2 x 10 <sup>2</sup>	2.08
NA	NA	Toxicity	Immediate	41	38	40	55	42	60	1.4 x 10 <sup>2</sup>	2.15
		Control A	30 Minutes	50	60	49	42	56	53	1.6 x 10 <sup>2</sup>	2.20
NA	NA	Toxicity	Immediate	49	41	33	40	48	67	1.4 x 10 <sup>2</sup>	2.15
		Control B	30 Minutes	34	43	22	61	44	51	1.3 x 10 <sup>2</sup>	2.11
Z-3	A	5A	Immediate	50	40	51	48	35	38	1.3 x 10 <sup>2</sup>	2.11
			30 Minutes	46	59	40	47	66	67	1.6 x 10 <sup>2</sup>	2.20
Z-6	A	5B	Immediate	38	66	56	48	48	45	1.5 x 10 <sup>2</sup>	2.18
			30 Minutes	61	66	38	58	54	37	1.6 x 10 <sup>2</sup>	2.20
Z-1	A	30A	Immediate	49	49	69	52	50	67	1.7 x 10 <sup>2</sup>	2.23
			30 Minutes	72	45	67	64	77	78	2.0 x 10 <sup>2</sup>	2.30
Z-4	A	30B	Immediate	46	56	52	45	33	40	1.4 x 10 <sup>2</sup>	2.15
			30 Minutes	68	61	67	59	71	69	2.0 x 10 <sup>2</sup>	2.30
Y-3	D	5A	Immediate	59	45	45	40	38	52	1.4 x 10 <sup>2</sup>	2.15
			30 Minutes	69	31	57	67	60	62	1.7 x 10 <sup>2</sup>	2.23
Y-6	D	5B	Immediate	37	40	36	48	63	44	1.3 x 10 <sup>2</sup>	2.11
			30 Minutes	43	71	41	67	73	62	1.8 x 10 <sup>2</sup>	2.26
Y-1	D	30A	Immediate	33	42	47	42	44	50	1.3 x 10 <sup>2</sup>	2.11
			30 Minutes	61	48	48	58	42	57	1.6 x 10 <sup>2</sup>	2.20
Y-4	D	30B	Immediate	44	54	41	40	35	52	1.3 x 10 <sup>2</sup>	2.11
			30 Minutes	50	57	59	61	57	54	1.7 x 10 <sup>2</sup>	2.23
INOCULUM COUNTS				10 <sup>-1</sup>		10 <sup>-2</sup>		10 <sup>-3</sup>		Average CFU/mL	
Beginning Inoculum			A	T	T	53	47	6	10	5.0 x 10 <sup>3</sup>	
End Inoculum			B	T	T	78	41	7	8	6.0 x 10 <sup>3</sup>	

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Table 35. Plate Count, Average CFU/mL, and Log<sub>10</sub> CFU/mL  
(continued)

SUBJECT NUMBER: N4				ORGANISM: <i>E. faecalis</i> ATCC 10741								
Area - Site	Treatment HTR Code	Sampling Time	Plating Time	Plate Counts						Average CFU/mL	Log <sub>10</sub> CFU/mL	
				Replicate 1			Replicate 2					
NA	NA	Number	Immediate	24	27	29	28	33	26	8.4 x 10 <sup>1</sup>	1.92	
		Control A	30 Minutes	18	17	24	36	32	41	8.4 x 10 <sup>1</sup>	1.92	
NA	NA	Number	Immediate	24	32	31	37	30	28	9.1 x 10 <sup>1</sup>	1.96	
		Control B	30 Minutes	17	36	17	32	25	29	7.8 x 10 <sup>1</sup>	1.89	
NA	NA	Toxicity	Immediate	29	26	31	23	22	29	8.0 x 10 <sup>1</sup>	1.90	
		Control A	30 Minutes	24	32	17	29	21	36	8.0 x 10 <sup>1</sup>	1.90	
NA	NA	Toxicity	Immediate	31	22	22	28	33	41	8.8 x 10 <sup>1</sup>	1.94	
		Control B	30 Minutes	28	34	28	26	33	37	9.3 x 10 <sup>1</sup>	1.97	
Z-3	A	5A	Immediate	27	23	27	23	24	40	8.2 x 10 <sup>1</sup>	1.91	
			30 Minutes	24	31	23	32	27	32	8.4 x 10 <sup>1</sup>	1.92	
Z-6	A	5B	Immediate	25	29	19	33	23	23	7.6 x 10 <sup>1</sup>	1.88	
			30 Minutes	33	34	32	28	40	26	9.6 x 10 <sup>1</sup>	1.98	
Z-1	A	30A	Immediate	31	27	28	34	31	26	8.8 x 10 <sup>1</sup>	1.94	
			30 Minutes	28	29	27	27	29	24	8.2 x 10 <sup>1</sup>	1.91	
Z-4	A	30B	Immediate	38	32	20	29	27	25	8.6 x 10 <sup>1</sup>	1.93	
			30 Minutes	36	26	26	30	32	25	8.8 x 10 <sup>1</sup>	1.94	
Y-3	D	5A	Immediate	19	29	20	29	28	29	7.7 x 10 <sup>1</sup>	1.89	
			30 Minutes	36	39	23	35	32	30	9.8 x 10 <sup>1</sup>	1.99	
Y-6	D	5B	Immediate	34	24	32	29	35	25	9.0 x 10 <sup>1</sup>	1.95	
			30 Minutes	31	28	31	38	18	29	8.8 x 10 <sup>1</sup>	1.94	
Y-1	D	30A	Immediate	17	23	18	28	33	30	7.4 x 10 <sup>1</sup>	1.87	
			30 Minutes	32	25	34	32	29	40	9.6 x 10 <sup>1</sup>	1.98	
Y-4	D	30B	Immediate	29	37	23	32	41	30	9.6 x 10 <sup>1</sup>	1.98	
			30 Minutes	32	33	30	19	35	27	8.8 x 10 <sup>1</sup>	1.94	
INOCULUM COUNTS				10 <sup>-1</sup>			10 <sup>-2</sup>			10 <sup>-3</sup>		Average CFU/mL
Beginning Inoculum				T	T	22	46	7	9	3.4 x 10 <sup>3</sup>		
End Inoculum				T	T	38	27	3	4	3.2 x 10 <sup>3</sup>		

NA = Not Applicable; A = DuraPrep solution; D = Betadine combination; T = TNTC.

Source: 9302 Final CSR

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**Table 36. Neutralization Average Log<sub>10</sub> CFU/mL and Log<sub>10</sub> Difference**

SUBJECT NUMBER: N02		ORGANISM: <i>S. marcescens</i> ATCC 14756		
PRODUCT	SAMPLING TIME OR CONTROL	PLATING TIME	AVERAGE Log <sub>10</sub> CFU/mL	LOG <sub>10</sub> DIFFERENCE
NA	Number Control	Immediate	1.97	NA
		30 Minutes	1.94	NA
NA	Toxicity Control	Immediate	1.99	-0.02
		30 Minutes	1.93	0.01
Betadine Combination	5 Minutes	Immediate	1.96	0.01
		30 Minutes	1.95	-0.01
Betadine Combination	30 Minutes	Immediate	1.96	0.01
		30 Minutes	1.98	-0.04
DuraPrep Solution	5 Minutes	Immediate	1.96	0.01
		30 Minutes	1.94	0.00
DuraPrep Solution	30 Minutes	Immediate	1.94	0.03
		30 Minutes	2.00	-0.06

SUBJECT NUMBER: N02		ORGANISM: <i>S.aureus</i> ATCC 27217		
NA	Number Control	Immediate	1.89	NA
		30 Minutes	1.86	NA
NA	Toxicity Control	Immediate	1.86	0.03
		30 Minutes	1.81	0.05
Betadine Combination	5 Minutes	Immediate	1.82	0.07
		30 Minutes	1.84	0.02
Betadine Combination	30 Minutes	Immediate	1.84	0.05
		30 Minutes	1.84	0.02
DuraPrep Solution	5 Minutes	Immediate	1.80	0.09
		30 Minutes	1.82	0.04
DuraPrep Solution	30 Minutes	Immediate	1.82	0.07
		30 Minutes	1.82	0.04

SUBJECT NUMBER: N03		ORGANISM: <i>E. coli</i> ATCC 25922		
PRODUCT	SAMPLING TIME OR CONTROL	PLATING TIME	AVERAGE Log <sub>10</sub> CFU/mL	Log <sub>10</sub> DIFFERENCE
NA	Number Control	Immediate	2.08	NA
		30 Minutes	2.10	NA
NA	Toxicity Control	Immediate	2.15	-0.07
		30 Minutes	2.16	-0.06
DuraPrep Solution	5 Minutes	Immediate	2.14	-0.06
		30 Minutes	2.20	-0.10
DuraPrep Solution	30 Minutes	Immediate	2.19	-0.11
		30 Minutes	2.30	-0.20
Betadine Combination	5 Minutes	Immediate	2.13	-0.05
		30 Minutes	2.24	-0.14
Betadine Combination	30 Minutes	Immediate	2.11	-0.03
		30 Minutes	2.22	-0.12

**Table 36. Neutralization Average Log<sub>10</sub> CFU/mL and Log<sub>10</sub> Difference (continued)**

SUBJECT NUMBER: N04		ORGANISM: <i>E. faecalis</i> ATCC 10741		
NA	Number Control	Immediate	1.94	NA
		30 Minutes	1.90	NA
NA	Toxicity Control	Immediate	1.92	0.02
		30 Minutes	1.94	- 0.04
Betadine Combination	5 Minutes	Immediate	1.92	0.02
		30 Minutes	1.96	- 0.06
Betadine Combination	30 Minutes	Immediate	1.92	0.02
		30 Minutes	1.96	- 0.06
DuraPrep Solution	5 Minutes	Immediate	1.90	0.04
		30 Minutes	1.95	- 0.05
DuraPrep Solution	30 Minutes	Immediate	1.94	0.00
		30 Minutes	1.92	- 0.02

NA = Not Applicable  
Source: 9302 Final CSR

The neutralizer system is considered effective if recovery of the organism in the antiseptic sample is not more than 0.2 logs different than the corresponding number control. The neutralizer system is considered non-toxic if recovery of the organism in the toxicity controls is not more than 0.2 logs different than the corresponding number control. In this study, the neutralizer system is both effective and non-toxic.

**Evaluation of the Durability and Persistent Antimicrobial Activity of 3M™ Duraprep™ Surgical Solution and Betadine Scrub and Solution Following Exposure to Blood and Saline Using a Bacterial Challenge Method**

LIMS 8198 is a randomized, partially blinded, paired-comparison study to evaluate the durability and persistence of the antimicrobial activity of DuraPrep film (DuraPrep solution once it is dry) and Betadine combination following a wash with autologous blood and saline. Antimicrobial effectiveness is evaluated by measuring the log reduction of a bacterial challenge with tetracycline-resistant *S. aureus* after a wash-off procedure simulating surgery. The log reduction of organisms recovered from the prepped test sites (compared with corresponding untreated control sites) is determined at two post-preparation time points and two organism residence times. Log reductions of the bacterial challenge achieved with DuraPrep film are compared with those achieved with Betadine combination. Neutralization of the test materials by SSS is verified prior to study start. Antibiotic susceptibilities are obtained for the challenge organism prior to study initiation.

Healthy subjects are entered into a 7-day Pretreatment Phase during which standardized, non-antimicrobial soaps, shampoos, and deodorants are used. Following the Pretreatment Phase, subjects meeting all inclusion and no exclusion criteria are assigned treatment numbers and randomized to treatment on the Treatment Day. On the Treatment Day, each subject is prepared for three test areas on the back, one for each treatment (DuraPrep solution, Betadine combination, and an untreated control). Each test area contains four individual test sites (two inoculation times and two bacterial residence times). Ten

minutes after treatment (when the preparations were expected to be dry), test areas are washed with autologous blood and saline to simulate exposure to fluids during surgery.

Individual sites within each test area are inoculated with the challenge organism approximately 15 minutes post-preparation (including completion of the blood and saline wash). After inoculation, the test organism remains *in situ* for 5 or 30 minutes prior to sample collection. The organisms are recovered using a modified cup scrub technique and SSS. The inoculation of individual sites within each test area and recovery of organisms are repeated in the same manner at approximately six hours post-preparation. Enumeration of bacterial counts is performed by individuals who are blinded to the identities of the test product associated with each sample.

At the primary analysis time point (six hours post-preparation, 30-minute residence time), the log reduction of the bacterial challenge is statistically significantly greater on DuraPrep film (mean log reduction = 4.191) than on Betadine combination (mean log reduction = 2.667) ( $p = 0.0098$ , based on paired t-test). There is also a greater log reduction of the bacterial challenge on DuraPrep film than on Betadine combination at six hours post-preparation, five-minute residence time, and at 15 minutes post-preparation, 30-minute residence time, but the differences at these time points are not statistically significant. A summary of log reduction of bacterial counts is presented in Table 37.

**Table 37. Summary of Log Reduction<sup>1</sup> of Bacterial Counts Efficacy (Evaluable Population)**

	Betadine Combination (N = 14)	DuraPrep Solution (N = 14)	Paired Difference In Log Reduction <sup>2</sup>	Paired t-test p-value	Wilcoxon Signed Rank Test p-value
<b>15-Minutes -</b>					
5-minute residence time					
Mean (SD)	2.84 (1.858)	1.73 (1.376)	-1.11 (2.581)	0.1325	0.1228
95% CI			(-2.60, 0.38)		
30-minute residence time					
Mean (SD)	3.33 (1.727)	3.75 (1.380)	0.42 (1.983)	0.4566	0.5693
95% CI			(-0.78, 1.62)		
<b>6-Hours Post-Preparation</b>					
5-minute residence time					
Mean (SD)	2.37 (1.610)	2.59 (1.865)	0.22 (2.617)	0.7589	0.6698
95% CI			(-1.29, 1.73)		
30-minute residence time					
Mean (SD)	2.67 (1.772)	4.19 (0.941)	1.52 (1.888)	0.0098	0.0139
95% CI			(0.43, 2.61)		

CI = confidence interval; SD = standard deviation.

<sup>1</sup> Calculated by subtracting the recovery log count from the treated sample from that of the appropriate untreated recovery control.

<sup>2</sup> Calculated by subtracting the log reduction of Betadine from the log reduction of DuraPrep.

Source: Table 6, LIMS 8198 Final CSR.

Prior to the blood and saline wash, the color of the Betadine combination and the DuraPrep solution preparations are clearly visible on all 16 subjects. Both immediately

following the blood and saline wash and at 6 hours following the application of the preparations, the color on 100% of the DuraPrep-treated sites still remains clearly visible. The color of the Betadine combination preparations is less evident; none of the sites has clearly visible color at either time point, approximately 75% of the Betadine-combination-treated sites have no visible color and approximately 25% have only slightly visible color at both time points following the wash. The difference in the visual assessment of the color of the DuraPrep film and the Betadine combination preparations is statistically significant both immediately following the blood and saline wash ( $p < 0.0001$ ) and at six hours following the preparation ( $p = 0.0001$ ). A summary of the color of the preparation in the treated area, as assessed by the study coordinator, is presented in Table 38.

**Table 5.7.13 Summary of Color of Preparation in Treated Area (All Randomized Subjects)**

Color of Preparation	Betadine Combination (N = 16) n (%)	DuraPrep Solution (N = 16) n (%)	p-value <sup>1</sup>
<b>When Dry</b>			
Not Visible	0	0	—
Slightly Visible	0	0	
Clearly Visible	16 (100.0)	16 (100.0)	
<b>After Saline and Blood Wash</b>			
Not Visible	12 (75.0)	0	< 0.0001
Slightly Visible	4 (25.0)	0	
Clearly Visible	0	16 (100.0)	
<b>6 Hours Post-Preparation</b>			
Not Visible	11 (78.6)	0	0.0001
Slightly Visible	3 (21.4)	0	
Clearly Visible	0	14 (100.0)	
NA	2	2	

<sup>1</sup> Based on non-parametric Wilcoxon signed rank test.

NA = Not applicable (these subjects did not complete the 6-hour time points).

— = Unevaluable.

Source: Table 7, LIMS 8198 Final CSR.

In conclusion, the antimicrobial effectiveness of DuraPrep film persists for at least 6 hours following a wash with autologous blood and saline, as assessed by the log reduction of bacterial counts following a bacterial challenge. DuraPrep film is insoluble in water and resists wash-away, as demonstrated by the retention of both antimicrobial activity and color intensity following the wash. In addition, DuraPrep film is significantly superior to Betadine combination in antimicrobial effectiveness against transient organisms at six hours following the wash, as assessed by log reduction of a 30-minute bacterial challenge. DuraPrep film is visually more evident than Betadine combination following a wash with autologous blood and saline, as assessed by appearance of the preparation just following the wash and after six hours.

#### **Neutralization Validation for LIMS 8198**

This neutralization study determines the ability of SSS to neutralize completely any available iodine that is recovered from DuraPrep solution and Betadine combination in

the process of sampling post-product application. Only one human subject is designated for neutralization validation. A tetracycline-resistant strain of *Staphylococcus aureus* (ATCC 27217) is used as the challenge microorganism. Two organism contact times are tested, immediate (< 1 minute) and thirty minutes, in order to detect any potential neutralizer effect between the time of sample collection and the time of plating.

*Preparation*

*Sample Site Preparation*

Following completion of all sampling, the residual investigational materials are removed from the subject's back using 70% IPA.

*Sample Inoculum and Plating*

*Numbers Control*

*Toxicity Control.*

*Neutralization Criteria.* The neutralization is considered effective if the post-preparation sample recovered is not more than 0.2 log<sub>10</sub> less than the Numbers Control sample. The SSS is considered non-toxic if the Toxicity Control sample is not more than 0.2 log<sub>10</sub> less than the Numbers Control sample.

*Results.* Table 39 presents the log<sub>10</sub> values recovered from all neutralization samples plated, including the numbers and toxicity controls, and the table evaluates whether these samples were within 0.3 log<sub>10</sub> of the numbers control. In conclusion, the SSS completely neutralized any available iodine that is recovered from the DuraPrep solution and from the Betadine solution combination. In addition, the study demonstrates the SSS is non-toxic to the test organism.

**Table 39. Table of Neutralization Results for LIMS 8198**

Test	Log <sub>10</sub> Value	Log <sub>10</sub> Difference from Numbers Control	Within 0.2 Log <sub>10</sub> of Numbers Control
Numbers Control (<1 Minute)	2.07	N/A	N/A
Numbers Control (30 Minutes)	2.09	-0.02	Yes
Numbers Control (Final)	2.09	-0.02	Yes
Toxicity Control, SSS (<1 Minute)	2.11	-0.04	Yes
Toxicity Control, SSS (30 Minutes)	2.01	0.06	Yes
Neutralization Effectiveness DuraPrep Solution 5 Minutes , inoculation (<1 Minute)	2.09	-0.02	Yes
Neutralization Effectiveness DuraPrep Solution 5 Minutes , inoculation (30 Minutes s)	2.07	0.00	Yes
Neutralization Effectiveness DuraPrep Solution 30 Minutes , inoculation (<1 Minute)	2.07	0.00	Yes
Neutralization Effectiveness DuraPrep Solution 30 Minutes , inoculation (30 Minutes)	2.06	0.01	Yes
Neutralization Effectiveness Betadine Combination 5 Minutes , inoculation (<1 Minutes)	2.05	0.02	Yes

Neutralization Effectiveness Betadine Combination 5 Minutes , inoculation (30 Minutes)	2.04	0.03	Yes
Neutralization Effectiveness Betadine Combination 30 Minutes , inoculation (<1 Minute)	2.10	-0.03	Yes
Neutralization Effectiveness Betadine Combination 30 Minutes , inoculation (30 Minutes)	2.08	-0.01	Yes

Source: LIMS 8198 Final CSR.

### Clinical Laboratory Test Methods

Standard microbiological techniques are used for bacterial sampling and enumeration. The methods used in studies are based on methods described in the Tentative Final Monograph (TFM) for Topical Antimicrobial Drug Products for Over-the-Counter Human Use (Federal Register 59[116]:31444-31445; 17 Jun 94) and ASTM E 1054-02 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents".

### Pilot Efficacy Studies - Methods Development

#### Effects of \_\_\_\_\_ on the Recovery of Normal Skin Flora

LIMS 7179 is a controlled, comparative pilot study in which each subject's skin is sampled with both MSS and SSS. A proprietary ingredient ( \_\_\_\_\_  
 \_\_\_\_\_ -to be redacted) is a candidate for use in dissolving the water-insoluble DuraPrep film to allow microbial sampling of the skin. The objective of the study is to determine the antimicrobial effect, if any, of MSS (1:3) (one part proprietary ingredient to three parts SSS) on the recovery of resident human skin flora. Aerobic microbial recovery from unprepped skin sites using the SSS is compared with that of MSS (1:3).

LIMS 7721 is a controlled, comparative pilot study utilizing a paired design where each subject received both MSS (1:1) and SSS sampling solutions. The objective of the study is to compare the recovery of resident aerobic skin flora using the MSS (1:1) versus SSS. No DuraPrep solution is used in these methods development studies.

Ten subjects are enrolled in each study. Prior to Treatment Day, all subjects undergo a 14-day pretest phase to provide stabilization of resident skin flora. During this time, subjects are to refrain from using antimicrobial products and swimming or bathing in chlorinated pools or hot tubs. On Treatment Day, eight sites are marked onto the skin of each subject's unprepped back. For LIMS 7179, four microbial samples are collected using SSS and the remaining four sites were sampled using MSS (1:3). For LIMS 7721, four microbial samples are collected using the traditional recovery solution (SSS) and the remaining four sites are sampled using MSS (1:1). Microbial samples are collected using a cup scrub sampling technique. Aerobic microbial recovery from both treatment groups are determined and statistically analyzed.

For LIMS 7179, the log<sub>10</sub> microbial recovery using the SSS is 3.58 compared with a log<sub>10</sub> recovery of 3.45 using MSS (1:3). Both a paired t-test and confidence limits indicate there is no statistically significant difference in recovery between the two solutions. Therefore, the MSS, at a ratio of 1:3 with SSS, has no significant effect on the recovery of resident human skin flora when compared with SSS alone.

For LIMS 7721, the mean log<sub>10</sub> microbial recovery using the SSS is 3.72 compared with a mean log<sub>10</sub> recovery of 2.95 using the MSS (1:1). The difference in mean log<sub>10</sub> recovery between the two solutions is 0.77 ± 0.44 logs. The 90% confidence interval around the difference is 0.538 to 1.0 log. This difference is statistically significant (p<0.0004). Thus, the MSS at a ratio of 1:1 with SSS has a significant negative effect on the recovery of resident aerobic skin flora. Therefore, MSS (1:1) does not appear to be an ideal microbial recovery solution for DuraPrep solution efficacy studies.

**A Pilot Study to Assess Recovery of Organisms from Skin Prepped with DuraPrep Surgical Solution**

LIMS 7448 is a paired-comparison, pilot study in which each subject receives all study treatments. The primary objective of this study is to test the ability of MSS (1:1) to dissolve the DuraPrep copolymer and allow recovery of organisms (*Bacillus subtilis* spores) from beneath the DuraPrep film. Bacterial spores are distributed over the surface of five alcohol-prepped skin sites on each of the volar forearms of subjects. DuraPrep solution or a control (Betadine solution or 74% [w/w] IPA) is applied to the surface of three individually seeded skin sites. Sterile saline is applied to the remaining two sites to simulate the preparation application; these sites are referred to as “untreated” and serve as recovery controls. The treated sites and one of the untreated sites are sampled using MSS (1:1). The other untreated site is sampled using SSS. After the test sites has dried, spores are recovered using a cup scrub technique and quantified using standard methods. Five subjects were enrolled in and completed the study.

Recovery of spores from under the DuraPrep film is very similar to recovery of spores from IPA-treated sites and from the untreated control sites sampled with MSS (1:1). The recovery is somewhat higher from Betadine-treated sites and from the untreated sites sampled with SSS. The means and standard deviations of the various treatments are shown in Table 40 and Table 41, along with the paired comparison of the difference between DuraPrep solution and the other treatments.

**Table 40. Log Counts from Sites Sampled 10 Minutes Post-Preparation Sample**

Betadine MSS (N=5)		DuraPrep MSS (N=5)		IPA MSS (N=5)		Untreated MSS (N=5)		Untreated SSS (N=5)	
Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
5.85	0.15	5.58	0.28	5.62	0.22	5.58	0.29	5.92	0.23

IPA = isopropyl alcohol; MSS = modified sampling solution; SD = standard deviation; SSS = standard sampling solution.

Source: Table 1, LIMS 7448.

**Table 41. Paired Comparisons Between Treatments from Sites Sampled 10 Minutes Post-Preparation**

Paired Comparison DuraPrep MSS versus	Difference Between Log Means	SD	P-value
Isopropyl Alcohol MSS	-0.041	0.201	0.6707
Betadine MSS	-0.272	0.239	0.0634
Untreated MSS	-0.001	0.246	0.9937
Untreated SSS	-0.341	0.253	0.0394

MSS = modified sampling solution; SD = standard deviation; SSS = standard sampling solution.  
Source: Table 2 and Appendix 16.1.6, LIMS 7448 CSR.

There is a significant effect of treatment on spore recovery ( $p < 0.002$ ). A multiple comparisons t-test is used to assess pairwise comparisons. The IPA-, untreated MSS-, and DuraPrep solution-treated sites have similar recovery and all have significantly lower recovery than from Betadine solution-treated and untreated SSS sites.

In conclusion, recovery of spores from beneath the DuraPrep film is similar to recovery of spores from IPA-treated sites and from the untreated sites sampled with the MSS, indicating that the DuraPrep film is adequately dissolved to allow for recovery of spores. However, recovery of spores is somewhat higher from Betadine solution-treated sites sampled with MSS and from the untreated sites sampled with SSS.

#### **Comparison of Recovery of Seeded Organisms Using Various Microbial Sampling Solutions: Modified Sampling Solution (1:3) and (1:1) versus Standard Sampling Solution**

LIMS 7824 is a controlled, comparative pilot study where each subject receives all study treatments. The objective of the study is to test the ability of MSS (1:3) and (1:1) to dissolve the DuraPrep solution copolymer film to allow for the recovery of test organisms (*B. subtilis* spores) from beneath the film.

Ten healthy subjects are recruited, enrolled into the study, and randomized to a treatment scheme. Bacterial spores are evenly distributed over nine sampling sites on each subject's back following an alcohol skin preparation. DuraPrep solution, Betadine solution, 74% IPA, or sterile phosphate-buffered saline (PBS) are applied to the surface of the seeded sites. After the test sites dry completely, samples are collected using MSS (1:3), MSS (1:1), or SSS using a cup scrub technique and quantified using standard techniques. An analysis of variance is conducted to assess the effects of sampling solution, preparation, and interaction.

There are main effects of preparation and sampling solution, but there is also an interaction of preparation and sampling solution ( $p < 0.0003$ ). Analysis is stratified by type of sampling solution to assess the recovery from each preparation. Recovery of spores differed among preparations when using MSS (1:1) ( $p < 0.0001$ ). Recovery is highest from Betadine solution-treated sites followed by untreated sites, then DuraPrep solution-treated sites. Recovery also differs when using MSS (1:3) ( $p < 0.0001$ ), with the highest recovery from Betadine solution-treated sites, followed by untreated sites, IPA-treated sites, and DuraPrep solution-treated sites. There is no difference in the recovery between Betadine-

solution treated and untreated sites sampled with SSS. The means and standard deviations of the various treatments are shown in Table 42.

**Table 42. Effects of Preparation and Sampling Solution**

Treatment	Bacterial Recovery (Mean Log <sub>10</sub> CFU)		
	MSS (1:3) Mean (±SD)	MSS (1:1) Mean (±SD)	SSS Mean (±SD)
DuraPrep Solution	5.86 (±0.06)	5.82 (±0.06)	ND
74% IPA	5.92 (±0.09)	ND	ND
PBS (control)	5.98 (±0.05)	6.02 (±0.05)	6.12 (±0.03)
Betadine Solution	6.11 (±0.04)	6.12 (±0.03)	6.11 (±0.07)

CFU = colony forming unit; IPA = isopropyl alcohol; ND = not determined; MSS = modified sampling solution; SD = standard deviation; SSS = standard sampling solution PBS = phosphate-buffered saline.  
Source: Table 1, CSR LIMS 7824.

In conclusion, recovery of bacterial spores from Betadine solution-treated sites sampled with MSS at either ratio is equivalent to control sites sampled with SSS, but higher than recovery from DuraPrep solution-treated, IPA-treated, and control sites sampled with MSS. Bacterial spore recovery from DuraPrep solution-treated sites sampled with MSS (1:3) is similar to that of DuraPrep solution-treated sites sampled with MSS (1:1). Recovery of spores from DuraPrep solution-treated sites sampled with MSS (1:3) is slightly lower than from saline-treated and from IPA-treated sites. Although the differences are quite small, 0.12 and 0.06 log<sub>10</sub>, respectively, they are statistically significant due to the low variability of the data. Similar results are observed from DuraPrep solution-treated sites sampled with MSS (1:1) compared with the saline-treated site (difference of 0.2 log<sub>10</sub>).

**Pilot Study to Assess Microbial Methodology Used in the Evaluation of the Antimicrobial Effectiveness of a DuraPrep™ Surgical Solution versus Vehicle Control (DuraPrep w/o I<sub>2</sub>) in Normal Human Skin Flora on Abdomen and Groin Skin Sites**

LIMS 7727 is a prospective, randomized, paired-comparison, pilot study to measure the reduction of normal aerobic bacterial flora on the abdominal and groin test areas following the application of DuraPrep solution or DuraPrep w/o I<sub>2</sub> for up to six hours post-preparation. A secondary objective is to assess the microbial sampling procedures (MSS 1:1) that are to be used in future pivotal studies planned to evaluate the antimicrobial effectiveness of DuraPrep solution. The measure of efficacy is the log reduction of skin bacterial counts from baseline after treatment with DuraPrep solution or DuraPrep w/o I<sub>2</sub> at various time points.

The study begins with a 14-day pretest washout period for bacterial stabilization. Following the washout period, microbial screening samples are collected from right and left abdominal and groin test sites to determine eligibility for the Treatment Day of the study. Randomly assigned contralateral abdominal and groin sites are prepped with DuraPrep solution or DuraPrep w/o I<sub>2</sub>. Screening and Treatment Day microbial samples are collected using a cup scrub technique and MSS (1:1). Subjects who meet screening

criteria (bacterial counts of  $3 \log_{10}/\text{cm}^2$  per abdominal site and  $5 \log_{10}/\text{cm}^2$  per groin site) qualify for the study. On Treatment Day, baseline samples are collected and test article applied to each treatment area. Microbial samples are collected at 2 minutes, 10 minutes, 30 minutes, and 6 hours post-preparation from the abdominal site and at 2 minutes and 6 hours post-preparation from the groin site.

Results for the log reduction of bacterial counts at the abdomen site are shown in Table 43 for the five subjects from the \_\_\_\_\_ site and in Table 44 for one subject from the \_\_\_\_\_ site. A paired t-test is used to test the differences between treatments. None of the differences (from the \_\_\_\_\_ site subjects) are statistically significant.

**Table 43. Abdomen Sites: Log Reduction - \_\_\_\_\_**

	Number of Subjects	Sample			
		DuraPrep Solution		DuraPrep w/o I <sub>2</sub>	
Time		Mean	SD	Mean	SD
2 minutes	5	3.2	1.6	3.0	1.3
10 minutes	5	3.4	0.7	3.3	0.9
30 minutes	5	3.9	0.6	3.3	1.0
6 hours	5	2.9	0.7	2.9	0.8

SD = Standard deviation.

Source: Table 1, CSR LIMS 7727.

**Table 44. Abdomen Sites: Log Reduction - \_\_\_\_\_**

	Number of Subjects	Sample			
		DuraPrep Solution		DuraPrep w/o I <sub>2</sub>	
Time		Mean	SD	Mean	SD
2 minutes	1	0.4	-	3.1	-
10 minutes	1	2.0	-	3.1	-
30 minutes	1	3.4	-	3.1	-
6 hours	1	1.5	-	2.5	-

SD = Standard deviation.

Source: Table 2, CSR LIMS 7727.

Results for the log reduction of bacterial counts at the groin site are shown in Table 45 for the five subjects from the \_\_\_\_\_ site and Table 46 for the three subjects from the \_\_\_\_\_ site. None of the differences are statistically significant.

**Table 45. Groin Sites: Log Reduction - \_\_\_\_\_**

	Number of Subjects	Sample			
		DuraPrep Solution		DuraPrep w/o I <sub>2</sub>	
Time		Mean	SD	Mean	SD
2 minutes	5	2.5	1.5	2.5	1.2
6 hours	5	2.7	1.2	3.0	1.4

SD = Standard deviation.

Source: Table 3, CSR LIMS 7727.

**Table 46. Groin Sites: Log Reduction –**

Time	Number of Subjects	Sample			
		DuraPrep Solution		DuraPrep w/o I <sub>2</sub>	
		Mean	SD	Mean	SD
2 minutes	3	1.9	0.2	2.1	0.3
6 hours	3	2.7	1.3	2.2	0.7

SD = Standard deviation.

Source: Table 4, CSR LIMS 7727.

Although this pilot study is not intended to demonstrate efficacy, both treatments studied showed similar bacterial reductions. This suggests that log reduction of resident bacterial counts may not be the ideal method for assessing the contribution of iodine to the antimicrobial activity of DuraPrep solution.

**Pilot Study to Assess Microbial Methodology Used in the Evaluation of the Antimicrobial Effectiveness of a Preoperative Skin Preparation in Normal Human Skin Flora**

LIMS 7449 is a prospective, randomized, paired-comparison, evaluator-blinded pilot study to assess MSS (1:1) and the test methods to be used in future pivotal studies to evaluate the antimicrobial effectiveness of DuraPrep solution.

Randomly assigned contralateral abdominal sites are prepped with DuraPrep solution and either DuraPrep w/o I<sub>2</sub> or Betadine combination. Microbial samples are collected at 2, 10, and 30 minutes, and at 6 hours post-preparation from the abdomen. From the groin area, samples are taken at 2, 10, and 30 minutes post-preparation. The microbial samples are collected using a cup scrub technique and MSS (1:1) to dissolve the DuraPrep film. Bacteria in samples are enumerated using standard techniques. The measure of efficacy in this study is the log reduction of skin bacterial counts from baseline after treatment with DuraPrep solution, DuraPrep w/o I<sub>2</sub>, or Betadine combination at various time points.

Ten subjects completed both the abdomen and groin portions of the study. The average log reductions from baseline for DuraPrep solution versus Betadine combination on the abdomen are shown in Table 47. By six hours, the application of DuraPrep solution results in a 2.5-log reduction of counts compared to a 1.7-log reduction for Betadine combination, although the variability between subjects is high for both preparations.

**Table 47. Log Reduction on the Abdomen - DuraPrep Solution vs Betadine Combination**

Time	Sample			
	Betadine Combination (N=5)		DuraPrep Solution (N=5)	
	Mean	SD	Mean	SD
2 minutes	2.3	0.9	2.2	1.3
10 minutes	2.4	1.0	2.1	1.3
30 minutes	1.8	1.2	1.6	1.4
6 hours	1.7	1.5	2.5	1.0

SD = Standard deviation.

Source: Table 1, CSR LIMS 7449.

The average log reductions from baseline for DuraPrep solution versus DuraPrep w/o I<sub>2</sub> on the abdomen are shown in Table 48. Both preparations demonstrate a similar log reduction of bacteria at all time points.

**Table 48. Log Reduction on the Abdomen - DuraPrep Solution versus DuraPrep w/o I<sub>2</sub>**

Time	Sample			
	DuraPrep Solution (N=5)		DuraPrep w/o I <sub>2</sub> (N=5)	
	Mean	SD	Mean	SD
2 minutes	1.1	0.7	1.0	0.7
10 minutes	1.0	0.8	1.6	1.2
30 minutes	1.6	1.4	1.7	1.5
6 hours	1.0	0.7	0.7	0.9

SD = Standard deviation.

Source: Table 2, CSR LIMS 7449.

The average log reductions from baseline for DuraPrep solution versus Betadine combination on the groin at 2 minutes and 30 minutes are shown in Table 49. Both preparations result in a similar log reduction of bacteria at all time points.

**Table 49. Log Reduction on the Groin - DuraPrep Solution versus Betadine (2 Minutes)**

Time	Sample			
	Betadine Combination (N=5)		DuraPrep Solution (N=5)	
	Mean	SD	Mean	SD
2 minutes	2.7	1.1	3.3	1.9
30 minutes	2.9	0.7	2.6	1.1

SD = Standard deviation.

Source: Table 3, CSR LIMS 7449.

The average log reductions from baseline for DuraPrep solution versus Betadine combination on the groin at 10 minutes and 30 minutes are shown in Table 50. Both preparations result in a similar log reduction of bacteria at all time points though the variability of the data between individuals is high for both preparations.

**Table 50. Log Reduction on the Groin - DuraPrep Solution versus Betadine Combination (10 Minutes)**

Time	Sample			
	Betadine Combination (N=5)		DuraPrep Solution (N=5)	
	Mean	SD	Mean	SD
10 minutes	3.1	1.1	3.7	1.5
30 minutes	4.0	0.8	3.0	1.2

SD = Standard deviation.

Source: Table 4, CSR LIMS 7449.

Although the study is not intended to demonstrate efficacy, general trends are seen. DuraPrep solution, DuraPrep w/o I<sub>2</sub>, and Betadine combination produce similar log reductions on the abdomen at all time points. DuraPrep solution and Betadine combination produce similar log reductions on the groin sites at all time points. The variability in the individual skin counts is high, making the comparison of antimicrobial efficacy at the various time points difficult without a larger sample size.

**Assessment of a Modified Sampling Solution for Evaluating Antimicrobial Effectiveness of DuraPrep Surgical Solution Against Resident Human Skin Flora on Abdomen and Groin Sites**

LIMS 8058 is an open-label, multicenter, paired-comparison, randomized pilot study to confirm the use of MSS (1:3) for collection of microbial samples from skin sites prepped with DuraPrep solution or Betadine combination. Following a 14-day pretest washout period, microbial screening samples are collected from right and left abdominal and groin test sites to determine subject eligibility for the Treatment Day of the study. Qualified subjects are randomly assigned treatment with DuraPrep solution or Betadine combination to contralateral test areas on their abdomen and/or groin. On Treatment Day, baseline samples are collected and test articles are applied to each test area. Microbial samples are collected using MSS on the abdominal area at 2, 10, and 30 minutes, and 6 hours post-preparation. Samples are collected on the groin test areas at 10 minutes and 6 hours post-preparation. All microbial samples are collected using a cup scrub technique. Bacteria are enumerated using standard techniques.

Five subjects are evaluable for efficacy on the abdomen and five on the groin. The log reductions of bacterial counts after DuraPrep solution or Betadine combination treatment of abdomen sites are shown in Table 51. The log reductions of bacterial counts after DuraPrep solution or Betadine combination treatment of groin sites are shown in Table 52. All results are from the \_\_\_\_\_ site. For both the abdomen sites and the groin sites, the log reductions achieved after treatment with DuraPrep solution or Betadine combination met the criteria defined in the TFM.

**Table 51. DuraPrep Solution and Betadine Combination Log Reduction-Abdomen Sites**

Log Reduction	Sample			
	DuraPrep Solution (N=5)		Betadine Combination (N=5)	
	Mean	SD	Mean	SD
2 Minutes	2.7	1.7	3.4	1.0
10 Minutes	3.1	1.8	2.6	2.5
30 Minutes	3.2	1.7	3.1	1.7
6 Hours	3.3	1.0	3.1	1.2

SD = Standard deviation.

Source: Table 3, CSR LIMS 8058.

**Table 52. DuraPrep Solution and Betadine Combination Log Reduction-Groin Sites**

Log Reduction	Sample			
	DuraPrep Solution (N=5)		Betadine Combination (N=5)	
	Mean	SD	Mean	SD
10 Minutes	3.8	2.4	4.9	1.1
6 Hours	3.6	1.8	3.9	1.2

SD = Standard deviation.

Source: Table 5, CSR LIMS 8058.

DuraPrep solution and Betadine combination show persistent antimicrobial activity against normal skin flora up to six hours post-preparation. Both DuraPrep solution and

Betadine combination treatment result in log reductions of bacterial counts that satisfy the TFM criteria.

*Reviewer's comments:* Although the Applicant states that this study was a multicentered study, only data from the \_\_\_\_\_ site is presented.

**Log Reduction of Normal Flora using Hibiclens - Recovery with Modified Sampling Solution versus Standard Sampling Solution using TFM Method**

LIMS 8786 is a randomized, paired-comparison pilot study where each subject receives DuraPrep solution and Hibiclens cleanser. The primary objective of the study is to verify that Hibiclens cleanser produces a 3-log reduction of bacterial counts within 10 minutes on the groin when sampled with MSS. The secondary objective is to evaluate the neutralization validation protocol; Betadine solution is included in the neutralization validation for informational purposes only.

Each subject participates in a 14-day washout period where no antimicrobial products or exposures are used. Following the washout period, subjects are screened to meet minimum baseline bacterial counts ( $4.0 \log_{10}/\text{cm}^2$  per groin site). On Treatment Day, randomly assigned contralateral groin sites are prepped with DuraPrep solution and Hibiclens cleanser. The test sites are sampled at 10 minutes post-preparation using a cup scrub technique and MSS is used to sample DuraPrep solution and Hibiclens cleanser-treated sites; a second Hibiclens cleanser-treated site was sampled with SSS.

Nine subjects completed the study. Table 53 shows the average log baseline counts (average of screening and Treatment Day baseline counts) recovered from the groin site with MSS. Also shown are the log counts recovered using MSS at 10 minutes post-preparation. The overall mean log reduction of bacterial counts was similar for both treatments, 3.28 logs for DuraPrep solution and 3.32 logs for Hibiclens cleanser.

**Table 53. DuraPrep Solution and Hibiclens Cleanser Log Counts and Log Reduction with MSS**

Log Counts	Sample			
	DuraPrep Solution (N=7)		Hibiclens Cleanser (N=7)	
	Mean	SD	Mean	SD
Baseline*	4.47	0.67	4.40	0.56
10 Minutes	1.19	1.86	1.08	1.46
Log Reduction	3.28	1.35	3.32	1.29

\*Baseline is the average of the screening and Treatment Day baseline bacterial counts.  
SD = Standard deviation.

Source: Table 1, CSR LIMS 8786.

**Table 54. Hibiclens Log Counts and Log Reduction MSS Versus SSS**

(N = 8)	MSS		SSS		SSS - MSS	
	Mean	SD	Mean	SD	Mean	SD
Treatment Day Baseline*	3.78	1.05	4.07	0.94	0.28	0.46
10 minutes	0.94	1.41	2.10	1.63	1.16	2.36
Log Reduction	2.84	0.98	1.97	1.31	-0.87	1.98

\*Screening baseline data were obtained with MSS only.

SD = Standard deviation.

MSS = modified sampling solution; SSS = standard sampling solution; SD = Standard deviation.  
Source: Table 2, CSR LIMS 8786.

Evaluation of the log reduction of bacterial counts for Hibiclens cleanser-treated sites shows that at 10 minutes post-preparation the log reduction is 2.84 when MSS is used and 1.97 when SSS is used (Table 54). The bacterial count recovered at 10 minutes is 0.94 logs with MSS compared with 2.10 logs with SSS. The difference in the use of MSS versus SSS is a mean log reduction of 0.87 logs in favor of MSS.

Since this is a pilot study, no efficacy conclusions are drawn. There appears to be an interaction of MSS with Hibiclens cleanser as evidenced by the lower recovery of organisms from sites sampled with MSS compared with those sampled with SSS. The neutralization validation method works well and was incorporated into the pivotal studies.

### **Log Reduction of Normal Flora using Hibiclens and Betadine - Recovery with Modified Sampling Solution and Standard Sampling Solution using TFM**

#### **Method: a Pilot Study**

LIMS 8986 is a randomized, paired-comparison pilot study where subjects received two of the following treatments: DuraPrep solution, Hibiclens cleanser, or Betadine combination. The primary objective is to compare the log reductions (on the groin) produced by Betadine combination using MSS versus SSS. Secondary objectives are 1) to verify the results of a pilot study in which differences in bacterial recovery are seen depending on whether MSS or SSS is used after treatment with Hibiclens cleanser and 2) to assess the log reductions of bacteria achieved by each of the different preparations compared with the TFM criteria. DuraPrep solution is included as a historical reference.

Following a 14-day pretest washout period, microbial screening samples are collected from groin test sites to determine subject eligibility for the Treatment Day of the study. Qualified subjects are randomly assigned treatment with DuraPrep solution, Hibiclens cleanser, or Betadine combination to contralateral test areas on the groin. On Treatment Day, baseline samples are collected with SSS and MSS and test articles are applied to each test area. Microbial samples are collected at ten minutes post-preparation. Microbial samples are collected using a cup scrub method and MSS for DuraPrep solution, Hibiclens cleanser, or Betadine combination, or SSS for Hibiclens cleanser and Betadine combination. Bacteria are enumerated using standard techniques.

Twelve subjects completed the study. Descriptive statistics including 95% confidence intervals around the difference in log reductions (MSS vs. SSS and between preparations) are calculated. The log counts and log reductions of bacterial counts for Hibiclens cleanser and Betadine combination and the differences between the data collected with MSS and SSS are shown in Table 55.

**Table 55. Log Counts and Log Reductions for Preparations Sampled With MSS Versus SSS**

	Sample			
	Hibiclens Cleanser (N=8)		Betadine Combination (N=8)	
	Mean	SD	Mean	SD
Baseline MSS	6.0	0.28	5.9	0.33
Baseline SSS	6.0	0.46	6.0	0.51
Post-Preparation MSS	0.2	0.00	2.4	1.55
Post-Preparation SSS	2.4	1.40	3.0	0.82
Log Reduction MSS	5.8	0.28	3.5	1.72
Log Reduction SSS	3.6	1.62	2.9	1.06
Baseline Difference (SSS-MSS)	0.0	0.25	0.1	0.27
Post-Prep Difference (SSS-MSS)	2.2	1.40	0.6	1.94
Log Reduction Difference (SSS-MSS)	-2.2	1.45	-0.5	1.81

MSS = Modified sampling solution; SD = Standard deviation; SSS = Standard sampling solution.  
Source: Table 2, CSR LIMS 8986.

The baseline bacterial recovery with MSS and SSS is similar indicating that MSS has little, if any, toxic effect on normal skin flora and is equivalent to SSS. When sampled using SSS, both Hibiclens cleanser and Betadine combination meet the 3-log reduction required by the TFM. The interaction of MSS with Hibiclens cleanser seen in an earlier study (see Section 5.8.1.8 of the NDA submission) is confirmed since there is an average 2.2-log greater reduction of bacteria when sites are sampled at ten minutes post-preparation using MSS compared with sites sampled using SSS. The log reduction of bacteria seen after Betadine-combination treatment is the same whether MSS or SSS was used as the sampling solution.

At ten minutes post-preparation, all treatments (DuraPrep solution, Betadine combination, and Hibiclens cleanser) sampled with MSS meet the 3-log reduction criterion defined in the TFM. Log bacterial counts and reductions at all test sites sampled with MSS are shown in Table 56.

**Table 56. Log Counts and Reductions for Sites Sampled with MSS Sample**

	DuraPrep Solution (N=8)		Hibiclens Cleanser (N=8)		Betadine Combination (N=8)	
	Mean	SD	Mean	SD	Mean	SD
Screening Baseline	5.7	0.26	6.0	0.37	6.0	0.41
Treatment Day Baseline	5.9	0.49	6.0	0.49	5.9	0.45
Baseline Average	5.8	0.27	6.0	0.28	5.9	0.33
10 Minutes	2.4	1.63	0.2	0.00	2.4	1.55
Log Reduction	3.4	1.71	5.8	0.28	3.5	1.72

SD = Standard deviation.  
Source: Table 6, LIMS 8986 CSR.

In conclusion, the log reduction of resident flora at ten minutes after sites are treated with Betadine combination is not statistically significantly different when MSS or SSS is used as the sampling solution. DuraPrep solution-treated sites sampled with MSS meet the TFM criteria (at least a 3-log reduction on the groin). Betadine combination-treated sites and Hibiclens cleanser-treated sites sampled with MSS and SSS also meet the TFM

criterion. When Hibiclens cleanser-treated sites are sampled with MSS, there is an average 2.2-log greater reduction than when sites are sampled with SSS. This confirms the results from an earlier study (LIMS 8786) where an interaction between Hibiclens cleanser and MSS was seen.

**Pilot Study to Evaluate the Antimicrobial Persistence of DuraPrep™ Surgical Solution, DuraPrep Vehicle Control (DuraPrep w/o I<sub>2</sub>), and Betadine® Solution and to Evaluate Resistance to Wash-off by Blood and Saline**

LIMS 7820 is a 2-phase, paired-comparison, pilot study to evaluate the test methodology used to assess the antimicrobial persistence of DuraPrep solution using a tetracycline-resistant strain of *S. aureus* as a challenge organism at different time points following application of the preparation (when dry and at 6 and 12 hours post-preparation). In addition, during the second phase of the study, the methodology used to assess the antimicrobial persistence of DuraPrep solution following a wash with blood and saline is evaluated. The primary measure of efficacy is the log reduction from baseline of viable organisms from the test sites treated with preparations alone or treated with preparations and washed with blood and saline.

Test areas on each subject's back are prepped with DuraPrep solution, DuraPrep w/o I<sub>2</sub>, or Betadine combination. After the preparations dry, half of the subjects' backs remain untreated (Phase I) and the other half (Phase II) are washed with blood and saline to simulate exposure to fluids during surgery. Individual test sites within each prepped area are inoculated with a challenge organism after the preparations (Phase I) and blood and saline wash (Phase II) are dry, and at 6 and 12 hours post-preparation. The challenge organism remains *in situ* for either two or five minutes. The organisms are recovered using a cup scrub technique.

Six subjects completed the study, three in each phase. The mean and standard deviation of the log bacterial reduction are given for each treatment and inoculum exposure time in Table 57. The log reductions of bacterial counts following treatment with DuraPrep solution are consistently greater than those achieved with DuraPrep w/o I<sub>2</sub> both with and without a blood and saline wash. Following the wash with blood and saline, the antimicrobial activity of Betadine combination is decreased, activity of DuraPrep solution is increased, and the activity of DuraPrep w/o I<sub>2</sub> remains unchanged.

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**Table 57. Log Reduction - All Treatment Conditions**

Wash	Time	Contact Time	Preparation					
			Betadine Combination		DuraPrep Solution		DuraPrep w/o I <sub>2</sub>	
			Mean	SD	Mean	SD	Mean	SD
No (N=3)	0 hours	2 minutes	6.36	0.40	0.09	0.13	-0.08	0.04
		5 minutes	6.65	0.12	0.94	0.74	-0.05	0.21
	6 hours	2 minutes	6.82	0.06	0.91	0.20	-0.09	0.14
		5 minutes	6.76	0.11	1.44	0.77	-0.06	0.25
Yes (N=3)	0 hours	2 minutes	0.91	0.96	0.50	0.18	0.01	0.16
		5 minutes	0.09	0.08	1.61	1.17	0.00	0.00
	6 hours	2 minutes	1.40	2.28	1.31	0.37	0.00	0.09
		5 minutes	0.53	0.29	3.99	1.08	0.18	0.15
	12 hours	5 minutes	1.24	1.74	2.93	2.16	-0.07	0.14

SD = Standard deviation.

Source: Table 2, LIMS 7820 CSR.

In conclusion, although this pilot study is not intended to demonstrate efficacy, since the log reductions achieved with DuraPrep solution are consistently greater than those achieved with DuraPrep w/o I<sub>2</sub>, the contribution of iodine is suggested. DuraPrep solution activity is resistant to wash-off with blood and saline.

**Reviewer comments:** A definitive conclusion cannot be made as the N=3 is too low for such a conclusion.

**Pilot Study to Evaluate the Persistent Antimicrobial Activity of 3M™ DuraPrep™ Surgical Solution, DuraPrep without Iodine, and Betadine® Solution using a Bacterial Challenge Method**

LIMS 8089 is a paired comparison pilot study designed to evaluate the bacterial challenge test methodology used to assess the antimicrobial persistence of DuraPrep solution. Three subjects were enrolled and completed the study. Test areas located on the upper-to-mid back are treated randomly with either DuraPrep solution, DuraPrep w/o I<sub>2</sub>, Betadine combination, or left untreated as a control. Test sites are inoculated with 50 µL of a tetracycline-resistant *S. aureus* (containing 10<sup>5</sup> to 10<sup>6</sup> CFU) at 6 hours post-preparation. After inoculation, the bacteria are allowed to remain *in situ* for 5, 15, or 30 minutes and then microbial samples are collected using the cup scrub technique and quantified.

DuraPrep solution and Betadine combination are able to reduce the bacterial challenge by more than five logs at six hours post-preparation at all organism residence times (5, 15, or 30 minutes), as shown in Table 58. DuraPrep solution and Betadine combination show persistence against transient organisms for six hours post-preparation. DuraPrep w/o I<sub>2</sub> showed no activity against the bacterial challenge under any of the test conditions. These results support the contribution of iodine to the persistence of the antimicrobial activity of DuraPrep solution.

**Table 58. Average Log Reduction (6 Hours Post-Preparation)**

Organism Contact Time	Preparation					
	Betadine Combination		DuraPrep Solution		DuraPrep w/o I <sub>2</sub>	
	MEAN	SD	MEAN	SD	MEAN	SD
5 minutes	5.67	0.69	5.67	0.69	-0.43	0.55
15 minutes	5.67	0.69	5.67	0.69	-0.36	0.42
30 minutes	5.67	0.69	5.55	0.89	-0.03	0.51

SD = Standard deviation.

Source: Table 2, LIMS 8089 CSR.

**Pilot Study to Evaluate the Persistent Antimicrobial Activity of DuraPrep™ Surgical Solution and Betadine® Solution Following Exposure to Blood and Saline using a Bacterial Challenge Method**

LIMS 8061 is a paired comparison pilot study designed to evaluate the bacterial challenge test methodology used to assess the antimicrobial persistence of DuraPrep solution following a wash with autologous blood and saline. The primary efficacy endpoint is the log reduction of organisms recovered from DuraPrep solution or Betadine combination treated test sites (calculated from the corresponding untreated recovery controls) immediately after the blood and saline wash and at six hours post-preparation.

Test areas on each subject's upper to mid back are prepped with DuraPrep solution and Betadine combination. After the preparations are allowed to dry, each test area is washed with autologous blood and saline to simulate exposure to fluids during surgery. Individual sites within each test area are inoculated with a challenge organism (tetracycline-resistant *S. aureus*) immediately following the blood and saline wash and at six hours post-preparation. Contact times for the challenge organism are 5, 15, and 30 minutes. The organisms are recovered using a cup scrub technique and quantified.

Five subjects were enrolled into the study. The paired difference in log reduction between the test products of interest was calculated for each subject. A paired t-test is used to compare the two treatments. The average log bacterial reductions seen immediately after the preparation was dry and at six hours post-preparation are shown in Table 59. The difference in activity, as measured by log reduction, is statistically significant at all times measured, with DuraPrep solution showing significantly higher log reductions compared with Betadine combination.

**Table 59. Log Reduction - All Treatment Conditions**

Time	Contact Time (minutes)	Preparation			
		Betadine Combination		DuraPrep Solution	
		Mean	SD	Mean	SD
0 hours	5	0.96	1.38	1.95	1.13
	15	0.96	0.85	2.24	0.84
	30	0.71	0.94	2.76	0.50
6 hours	5	-0.15	0.20	2.19	2.20
	15	-0.12	0.37	4.04	1.40
	30	-0.04	0.34	4.80	0.87

SD = Standard deviation.

Source: Table 2, LIMS 8061 CSR.

In conclusion, the test methodology used in this study shows the persistence of DuraPrep solution following a wash with autologous blood and saline. DuraPrep solution significantly reduces the bacterial challenge, compared with Betadine combination, at all time points and at all organism residence times (5, 15, and 30 minutes) and remains active at six hours. Betadine combination retains no activity at the 6-hour time point. DuraPrep solution, after a simulated blood and saline wash, remains persistent against transient organisms.

*Reviewer's comments:* The Applicant supplied SD values but did not supply p values thus the Applicant's statement regarding statistical significance is unclear.

### **Persistent Activity of DuraPrep™ Surgical Solution**

SRFE 1513 is designed to evaluate the antimicrobial persistence of DuraPrep solution using a tetracycline-resistant strain of *S. aureus* as a challenge organism.

Prior to Treatment Day, all eight subjects participate in a 7-day washout period in which no antimicrobial products are used. On Treatment Day, skin sites located on the upper back are treated with either DuraPrep solution or DuraPrep w/o I<sub>2</sub>. Test sites are inoculated with tetracycline-resistant *S. aureus* (10<sup>5</sup> to 10<sup>6</sup> CFU) immediately following application of the surgical prepping solution and at 12-hours post-application. Thirty minutes after each inoculation the sites are sampled using a cup scrub technique. The number of bacteria recovered from the site treated with DuraPrep solution is compared with the number recovered from the site treated with DuraPrep w/o I<sub>2</sub>.

Immediately after application and at 12-hours post-application, DuraPrep solution achieves greater than a 4-log reduction of the bacterial challenge. This log reduction is statistically significant (p<0.0001) at both time points compared with the control (DuraPrep w/o I<sub>2</sub>). The log reduction at T=12 hours is slightly lower than that at T=0. Since the number of bacteria recovered from the control at T=12 hours (6.28 log) is slightly lower than the number of bacteria recovered from the control at T=0 hours (6.58 log), the lower reduction is expected. The results of this study indicate the iodophor in DuraPrep solution retains its bactericidal activity for the duration of a 12-hour test period.

## **CONCLUSIONS**

The subject of this application is DuraPrep, a patient preoperative skin preparation containing an iodophor (0.7% available iodine) and isopropanol (IPA, 74% w/w). Each of these ingredients contributes different attributes to the function of the final product. The isopropanol is a wide spectrum antimicrobial providing the final product with a rapid antimicrobial effect as it evaporates from the skin. The iodine is a wide spectrum antimicrobial that acts to augment suppression of the resident skin flora and is believed to function as a protective barrier against the transient flora that may be acquired during surgical procedures. Since this product contains two active ingredients, the drug product must meet the drug combination policy. This policy requires the Applicant demonstrate the contribution of each active ingredient in adequate and well-controlled clinical studies. Thus, this NDA was reviewed with this regulatory perspective.

### ***In Vitro Studies***

***Spectrum of Activity.*** The *in vitro* antimicrobial spectrum and MBC of DuraPrep solution was determined against 50 different microbial isolates (25 laboratory strains and 25 fresh clinical isolates) of 21 different organisms in the pivotal study, LIMS 7720. These organisms included both Gram-positive bacteria, Gram-negative bacteria, and yeast. For all isolates tested, the MBCs are well below the use concentration of iodine in DuraPrep solution and Betadine solution. DuraPrep w/o I<sub>2</sub> is bactericidal against only a few isolates of five of the organisms tested and only at higher concentrations (7 \_\_\_\_\_).

The MBC study design for LIMS 7720 was based on the Tentative Final Monograph (TFM) for Topical Antimicrobial Drug Products for Over-the-Counter Human Use (Federal Register 59[116]:31444-31445; 17 Jun 94), using a modification of methodology established by the National Committee for Clinical Laboratory Standards (entitled "Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically," Document M7-A5, 5th edition, 20:2, 2000). This modification is explained on p28 of this review.

***Contribution of Iodine.*** Due to technical constraints on testing, the Applicant found it necessary to make some modifications to the methods in the TFM. Primarily, the formulation of DuraPrep solution puts inherent limits on the ability to isolate the separate contribution of alcohol. The copolymer in DuraPrep solution is soluble in a mixture of IPA and water as long as the concentration of IPA remains 70% or higher. Therefore, it is not possible to create a formulation of DuraPrep solution that contains a concentration of alcohol that is low enough to have no antimicrobial effect. To study the contribution of IPA to the antimicrobial activity of DuraPrep solution, the activity of DuraPrep solution was compared with that of the dried film (after the IPA had evaporated off). LIMS 7311 was a time-kill study using the dried film method and LIMS 8919 was a time-kill study conducted without evaporating off the alcohol. The contribution of alcohol to the antimicrobial activity of DuraPrep solution could be indirectly ascertained by comparing the results of these two studies.

***Time-kill Kinetics.*** The microbial kill rate of DuraPrep solution as measured by time-kill kinetics was determined against 15 different organisms in LIMS 8919. Time-kill kinetics was not conducted against all of the organisms listed in the TFM but several species responsible for surgical site infections were included. It was determined that the method used for this study was inappropriate for testing Betadine solution; therefore, the testing of Betadine solution was discontinued. After one minute of exposure to DuraPrep, a range of log<sub>10</sub> reduction of 4.0 or greater (4.0 to 7.1) in microbial counts was shown with 14 of the 15 test organisms. The microbial kill rate of the iodine from dried DuraPrep film was determined against 27 different organisms in LIMS 7311.

LIMS 7311 demonstrated the contribution of iodine in DuraPrep solution. LIMS 8919 demonstrated the effect of both iodine and alcohol in the formulation. The complete formulation in LIMS 8919 always had a higher log reduction of bacterial counts compared with the dried film in LIMS 7311, indicating the contribution of IPA to the microbial activity of the complete formulation.

The three *in vitro* pivotal studies showed that DuraPrep solution is an effective bactericidal agent. Furthermore, the dried films of DuraPrep solution and Betadine solution exhibited similar kill rates against the majority of the organisms tested.

**Pilot Studies.** In addition to these three pivotal studies, five pilot studies (LIMS 7215, SRFE 1623, SRFE 1624, SRFE 1625, and SRFE 1263) were conducted to examine the MBC and time-kill characteristics of DuraPrep solution, and to optimize the methods for completion of the pivotal studies.

**Microbial Resistance.** The development of resistance to DuraPrep solution was not examined through any specific *in vitro* studies but was instead evaluated by a review of the literature. Minimal information regarding resistance to iodine or IPA was found. No development of resistance has been determined for iodine in povidone-iodine.

**In Vivo Studies**

**Efficacy Against Resident Skin Flora.** Two pivotal studies, LIMS 8304 and LIMS 8918, demonstrated effectiveness of DuraPrep solution against resident skin flora on the abdomen *but not* the groin. Both studies confirmed that DuraPrep solution reduced resident skin flora and maintained counts below baseline for 24 hours. In LIMS 8304, only DuraPrep solution met the TFM reduction criteria of a 2-log reduction on the abdomen; neither Hibiclens nor DuraPrep met the 3-log reduction on the groin. In LIMS 8918, while both products met the 2-log reduction on the abdomen, neither product met the 3-log reduction on the groin, although both products performed equivalently. Results are shown in Table A (Table 5.13.1 of the NDA submission).

**Table A. LIMS 8304 and LIMS 8918 Data Summary: Mean Log Reduction of Bacterial Counts (CFU/cm<sup>2</sup>) (SD)**

	LIMS 8304		LIMS 8918	
	Hibiclens Cleanser (N=31)	DuraPrep Solution (N=31)	Hibiclens Cleanser (N=34)	DuraPrep Solution (N=34)
<b>Abdomen Data</b>				
Baseline Log Counts	3.83 (0.491)	3.84 (0.678)	3.51 (0.329)	3.52 (0.433)
Log Reductions:				
2 Minutes	2.52 (1.595)	2.45 (1.377)	2.16 (1.229)	2.42 (1.294)
10 Minutes	1.83 (1.647)	2.48 (1.444)	2.15 (1.302)	2.47 (1.146)
6 Hours	2.02 (1.522)	2.34 (1.520)	1.75 (1.149)	2.31 (1.266)
24 Hours	2.01 (1.456)	1.70 (1.669)	1.78 (0.883)	1.57 (1.154)
<b>Groin Data</b>				
Baseline Log Counts	6.39 (0.478)	6.40 (0.486)	5.89 (0.480)	5.82 (0.511)
Log Reductions:				
10 Minutes	2.93 (1.168)	2.95 (1.265)	1.94 (0.964)	2.37 (1.085)
6 Hours	3.36 (1.087)	2.70 (1.318)	2.31 (0.947)	2.29 (0.971)
24 Hours	2.92 (1.222)	2.51 (1.411)	2.69 (0.882)	2.13 (0.796)

SD = Standard deviation.

Note: Log Reduction = average of Screening and Treatment Day baseline log-transformed bacterial counts minus post-treatment log-transformed bacterial counts.

Note: Only subjects with data available from a treatment pair for a given sampling time point were included in this summary table.

Source: Tables 9 and 10, LIMS 8304 and Tables 7 and 8, LIMS 8918 Final CSRs.

In a teleconference dated June 12, 2003, the Applicant noted that the criteria outlined in the TFM for the groin site were not attained in LIMS 8918 but that the results of the product were equivalent to those of Hibiclens. The Division concluded that it was not necessary to repeat the study; the Division clarified that this does not mean that the application will be approved but only that repeating the study would not be beneficial. However the Division recommended that the Applicant submit the data in their NDA along with a *rationale for the acceptance of this data* by the Division. This Reviewer assumes that the rationale for the acceptance of this data by the Division is that the log reductions demonstrated by DuraPrep were greater than the log reductions demonstrated by the positive control, Hibiclens.

In either study, both the positive control (Hibiclens) and the test product (DuraPrep) failed to meet the TFM criteria for log reduction in the groin site. However, both the positive control and the test product did meet the 2-log reduction criterion for the abdominal site in LIMS 8918; only DuraPrep met the 2-log reduction criterion for the abdominal site in LIMS 8304. However, reexamination of this data (taken from the study reports) in a different format reveals a possible explanation for the log reduction data from the individual subjects as well as the mean log reduction. In addition, the new format identifies whether the log reductions for the *individual subjects* met the TFM criterion and the *percentage* of individual subjects who did meet the TFM criterion.

**Table B. Mean log reductions and percentage of individuals meeting the TFM log reduction threshold**

		mean log reduction	% meeting threshold
<b>LIMS 8304</b>			
abdomen			
	DuraPrep	2.52	67.90
	Hibiclens	2.09	52.50
inguinal			
	DuraPrep	2.78	35.71
	Hibiclens	2.93	51.28
<b>LIMS 8919</b>			
abdomen			
	DuraPrep	2.4	68.96
	Hibiclens	2.11	58.97
inguinal			
	DuraPrep	2.23	20.89
	Hibiclens	1.94	12.00

From Table B, it is clear that the *majority of individuals* as well as *the mean of those individuals* from both studies met the 2-log reduction at the abdominal site for both the test product and the positive control. In both studies, both DuraPrep and Hibiclens easily reached the 2-log reduction criterion for the abdominal site. In fact, DuraPrep outperformed Hibiclens in both the \_\_\_\_\_ and \_\_\_\_\_ studies with log reductions

of 2.52 and 2.4 log reductions, respectively. *Clearly, reaching the TFM 2-log reduction criterion for the abdominal site is readily achievable.*

It is also clear from Table B that the in both studies, neither the majority of individuals nor the mean of those individuals met the 3-log reduction at the inguinal site. DuraPrep only outperformed the positive control, Hibiclens, in one study, yet, in both studies neither DuraPrep nor Hibiclens meet the TFM 3-log reduction criterion for the inguinal site. *Clearly, reaching the TFM 3-log reduction criterion for the inguinal site is not readily achievable but indeed, difficult to achieve.*

This phenomenon, in which the TFM criteria for the abdominal but not the inguinal site is met by either test product or positive control (Hibiclens), is not unique to this product, DuraPrep. Therefore, there must be a reason common to most or all topical antiseptics which explains why topical antiseptics fail in the wet skin site (inguinal site) but not in the dry skin site (abdominal site).

The reasons may be difficult to determine due to the plethora of variables that could affect the success of a topical antiseptic in the dry skin sites versus the wet skin sites. These variables may include: different normal skin flora, different numbers of bacteria, and different immunological responses. For example, there are different varieties and numbers of bacteria that comprise the normal skin flora in dry and wet sites. Wet sites generally possess higher bacterial counts and the inguinal site would be expected to contain a higher percentage of Gram-negative bacteria due to the proximity to the perianal region. Most antiseptics are generally more effective against either Gram-negative or Gram-positive bacteria.

It is important to recognize that the *in vivo* studies for topical antiseptics rely on clinical simulations that measure the reduction in numbers of normal resident skin flora, not pathogens. In addition, there has been no direct correlation made between bacterial log reduction by the use of a topical antiseptic and the risk of infection via the skin during surgery. Studies that might be more useful in demonstrating the efficacy of topical antiseptics might include *in vivo* studies in animal models and studies that utilize bacterial challenge to the skin with organisms known to cause surgical infections. To their credit, the Applicant has supplied bacterial challenge data.

At the End of Phase 2 meeting held November 6, 2000, the Agency agreed with the Applicant that the bacterial challenge test method is acceptable to demonstrate the contribution of iodine if no difference is seen in standard TFM testing. However, the Agency stated that two studies would be required, at separate labs if the study was used to demonstrate the contribution of iodine.

The Applicant has supplied data from such studies in which surgical site pathogens such as tetracycline resistant *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* were used in bacterial challenge methodology in a pig skin model. LIMS 8626, LIMS 8676, and LIMS 8690 pilot studies tested DuraPrep solution and DuraPrep w/o I<sub>2</sub> with such methodology. Using this methodology in three pilot studies, the Applicant

shows that DuraPrep demonstrates log reductions against three different surgical site pathogens, *S. aureus*, *E. coli*, and *E. faecalis*. While the list of surgical site pathogens tested in the animal model is by no means comprehensive, it is a positive and innovative step in the direction of determining a correlation between microbial log reductions and risk of surgical site infection.

**Efficacy Against a Bacterial Challenge and Contribution of Iodine.** The contribution of iodine to the antimicrobial efficacy of the formulation was demonstrated by the greater reduction of a bacterial challenge using DuraPrep solution compared with DuraPrep w/o I<sub>2</sub> in studies LIMS 8197 and LIMS 9302. Both studies demonstrated significant mean log reductions for DuraPrep solution relative to DuraPrep w/o I<sub>2</sub> although the magnitude of the reductions differed somewhat. Results are summarized in Table C (Table 5.13.2 of the NDA submission).

**Table C. LIMS 8197 and LIMS 9302 Data Summary: Mean Log Reduction of a Bacterial Challenge (CFU/cm<sup>2</sup>) (SD)**

Inoculation Time/ Contact Time	LIMS 8197		LIMS 9302	
	DuraPrep w/o I <sub>2</sub> (N=30)	DuraPrep Solution (N=30)	DuraPrep w/o I <sub>2</sub> (N=24)	DuraPrep Solution (N=24)
<b>When Preparation is Dry</b>				
5 Minutes <sup>1</sup>	-0.05 (0.507)	1.45 (1.550)	-0.02 (0.136)	0.51 (1.346)
30 Minutes <sup>2</sup>	-0.67 (0.895)	2.82 (1.924)	-0.39 (0.701)	3.47 (1.905)
<b>2 Hours Post-Preparation</b>				
5 Minutes	0.22 (1.083)	1.26 (1.621)	-0.02 (0.139)	0.75 (1.485)
30 Minutes	-0.52 (0.804)	3.04 (1.782)	-0.03 (1.261)	3.39 (1.702)
<b>6 Hours Post-Preparation</b>				
5 Minutes	0.03 (0.194)	1.82 (1.781)	0.02 (0.111)	0.71 (1.146)
30 Minutes	-0.18 (0.841)	2.96 (1.761)	0.05 (0.612)	3.77 (1.699)

<sup>1</sup> Subject 011 was missing the assessment at 5-minute residence time when preparation was dry due to technician error.

<sup>2</sup> Subject 205 was missing the assessment at 30-minute residence time when preparation was dry, due to technician error.

SD = standard deviation.

Source: Table 7, LIMS 8197 and Table 7, LIMS 9302 Final CSRs.

*Despite the inability of both DuraPrep and Hibiclens to meet the 3-log reduction criterion in the TFM, DuraPrep had larger bacterial log reductions than the positive control (Hibiclens) at either the abdominal or inguinal sites in the clinical simulations. Coupled with the success of DuraPrep against three surgical site pathogens in a bacterial challenge method in a pig skin model and human clinical simulations, this Reviewer deems that this NDA application is approvable contingent upon compliance with the indicated changes to the Microbiology Section of the Package Insert.*

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