CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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CLINICAL MICROBIOLOGY/VIROLOGY REVIEW(S)

CLINICAL MICROBIOLOGY NDA REVIEW

Food and Drug Administration
Center for Drug Evaluation and Research
Office of Drug Evaluation IV
Division on Nonprescription Drug Products

NDA 208-288 (original)

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DRUG PRODUCT NAMES:
Proprietary Name: SoluPrep™ Film-Forming Sterile Surgical Solution
Established Name: Chlorhexidine Gluconate (CHG) and Isopropyl Alcohol (IPA)
Structural Formula: CHG: 1,1'-hexamethylenebis[5-(4-chlorophenyl) bisguanide]di-D-gluconate
IPA: proan-2-ol

INDICATION:
- Patient preoperative skin preparation
- For preparation of the skin prior to surgery
- Helps reduce bacteria that potentially can cause skin infection

PHARMACOLOGICAL CATEGORY: Healthcare Antiseptic

DOSAGE FORM: Topical Solution containing 2% Chlorhexidine gluconate and 70% (v/v) Isopropyl alcohol in the following package configurations: 10.5 mL Tint, 10.5 mL Clear, and 26 mL Tint Sponge Applicators.

RELATED SUBMISSIONS: IND 76,549

MATERIALS REVIEWED:

Study No.	Title of Study
	Pre-Clinical Microbiology *In Vitro* Evaluations
EM-05-012700	Assessment of Microbicidal Activity of Tinted and Colorless 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation Formulations Using a Modified Time Kill Procedure
EM-05-012981	Assessment of Microbicidal Activity in the Presence of an Organic Challenge of Tinted and Colorless 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation Formulations Using a Modified Time Kill Procedure
EM-05-012758	Evaluation of Potential for Development of Antimicrobial Resistance to Tinted 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation Formulation
	Clinical *In Vivo* Microbiology Evaluations
EM-05-012760	Assessment of the Antimicrobial Efficacy of 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation against Resident Human Skin Flora on the Abdominal and Inguinal Regions (MBT 109-209)
EM-05-013260	Assessment of the Antimicrobial Efficacy of 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation against Resident Human Skin Flora on the Abdominal and Inguinal Regions (BSL 140537-103)
Report-05-256621	Assessment of Neutralization Sampling Solution for 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation
EM-05-012985	3M™ CHG/IPA Film-Forming Preoperative Skin Preparation 26-mL Applicator Area Coverage, Dry Time and Vapor Dissipation Study on Human Skin.
EM-05-013337	3M™ CHG/IPA Film-Forming Preoperative Skin Preparation 10.5-mL Applicator Area Coverage and Dry Time on Human Skin.

Table of Contents

Remarks: This review of NDA 208-288 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.

1. **Executive Summary:**

This NDA is submitted pursuant to section 505(b)(2) of the Federal Food Drug, and Cosmetic Act and in accordance with 21 CFR 314.50. The sponsor submits the application for 3M™ SoluPrep™ Film-Forming Sterile Surgical Solution (3M™ CHG/IPA Prep) for over-the-counter (OTC) healthcare professional use. 3M™ CHG/IPA Prep is an isopropyl alcohol (IPA) based topical antiseptic solution consisting of chlorhexidine gluconate (CHG) and a copolymer, acrylate. The IPA content of 3M™ CHG/IPA Prep is 70% v/v and the CHG content is 2% w/w. Both IPA and CHG are active ingredients. IPA provides rapid but transient antimicrobial activity. CHG is a highly effective antimicrobial agent providing persistent broad-spectrum antimicrobial activity. 3M™ CHG/IPA Prep is formulated with acrylate, a copolymer within which the CHG is contained. This polymeric barrier remains once the alcohol evaporates on the skin to prolong the presence of CHG at the application site.

The sponsor is proposing both 10.5 mL applicator (colorless and tinted) and 26 mL applicator (tinted) packaged for single use. The primary container closure for the finished drug product solution is a (b) (4) sealed glass ampule containing either 10.5 mL (tined and clolorless) or 26 mL (tinted) of the sterile drug solution. Each unit dose (b) (4) sealed glass ampule of solution is housed in a (b) (4) plastic applicator, with a (b) (4) sponge at the end through which the solution is dispensed for topical application to a patient's skin prior to surgery. Each applicator is sealed in a plastic peelable pouch. The 10.5 mL applicator (colorless and tinted) is labeled for the treatment area of the skin up to 13 in. x 13 in. The 26 mL applicator (tinted) is labeled for the treatment area of the skin up to 19.5 in. x 19.5 in. The sponsor is seeking the following indications for 3M™ CHG/IPA Prep: 1) for patient preoperative skin preparation; 2) for preparation of the skin prior to surgery; and 3) helps reduce bacteria that potentially can cause skin infection.

Conclusions:

***In vitro* Studies**

We have evaluated numerous broad spectrum antimicrobial activities (minimum inhibitory concentration (MIC)) of chlorhexidine gluconate drug product applications and decided that the full battery of organisms described in the 1994 TFM was not needed. Currently, we no longer recommend MIC studies against the organisms described in the 1994 TFM for chlorhexidine gluconate products. We have enough information regarding the spectrum of antimicrobial activity. Therefore, MIC testing is not necessary to support approval for chlorhexidine gluconate drug products. Instead, we recommend a modified *in vitro* time-kill study.

Study EM-05-012770: Assessment of Microbiocidal Activity of Tinted and Colorless 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation Formulation Using a Modified Time Kill Procedure

The time-kill study showed that SoluPrep™ Film-Forming Prep Tinted (full strength-1X), SoluPrep™ Film-Forming Prep Colorless (full strength-1X), and the active control ChloraPrep® produced ≥ 5 log reduction (>99.9%) killing effect in 3 minutes and 5 minutes all the organisms tested. When SoluPrep™ Film-Forming Prep Tint and Colorless was diluted to half its strength (0.5X) it produced ≥ 5 log reduction (>99.9%) killing effect in 3 minutes and 5 minutes all the organisms tested. The killing effect or antimicrobial activity of a drug needs to be ≥ 3 log reduction to be considered an active ingredient. When SoluPrep™ Film-Forming Prep Tint and Colorless was diluted to 0.01% (0.0001X) it produced ≤ 0.70 log reduction killing effect in 3 minutes and 5 minutes all the organisms tested. This is considered to be an inactive concentration. Overall, the results of the time-kill studies provided by the sponsor indicate that the test products SoluPrep™ Film-Forming Prep Tint and SoluPrep™ Film-Forming Prep Colorless achieved a >99.9% reduction in viable microbial cells in 3 and 5 minutes. These results are comparable to those achieved with the active control ChloraPrep® with Tint.

Study EM-05-012981: Assessment of Microbiocidal Activity in the Presence of an Organic Challenge of Tinted and Colorless 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation Formulation Using a Modified Time Kill Procedure

The results showed that SoluPrep™ Film-Forming Prep Tinted (full strength-1X) and SoluPrep™ Film-Forming Prep Colorless (full strength-1X) demonstrated >5 $\log_{10}$ reductions in the presence of a serum challenge at both the 3-minute and 5-minute time points for all organisms tested, thus meeting the 3 $\log_{10}$ reduction to define antimicrobial activity of a time kill. Chlorhexidine gluconate is not largely inactivated by organic material such as blood or the skin protein. This residual activity makes it an attractive choice for skin antisepsis where long-term reduction of microbial flora is desired.

The neutralization validation study results for EM-05-012770 and EM-05-012981 showed that the neutralization solution used in the test was not non-toxic and effectively neutralized the activity of SoluPrep™ Film-Forming Prep Tinted and SoluPrep™ Film-Forming Prep Colorless at various strengthens.

Chlorhexidine Gluconate Resistance Studies

The issue of antiseptic resistance has been a subject of concern by FDA. During recent years, there are concerns regarding the emergence of resistance to chlorhexidine gluconate and cross-resistance to clinically significant antibiotics. Review of the literature suggests lack of definitive evidence to show that there is an increase in the rate of resistance to chlorhexidine gluconate in the clinical setting. Results of studies in attempt to demonstrate development of resistance are contradictory because of varying methodologies conducted and the amount of chlorhexidine gluconate used in the studies. Although the development of reduced susceptibility to antiseptics caused by continuous exposure to chlorhexidine gluconate may occur, the level of resistance is reported to be low and the chlorhexidine gluconate concentration used in antiseptics is much higher than the level of resistance. For now, researchers recommend that susceptibility and resistance of microorganisms to

chlorhexidine gluconate should be closely monitored. We continue to request sponsors literature updates and to conduct resistance and cross-resistance to antibiotics on chlorhexidine gluconate drug products.

Study EM-050-012758: Evaluation of Potential for Development of Antimicrobial Resistance to Tinted 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation Formulation

The endpoints for both the SoluPrep™ Film-Forming Prep Tint and the aqueous chlorhexidine gluconate solution were the same or varied slightly by one doubling dilution in this study. This study did not show any trend toward higher MIC values with clinical isolates compared to ATCC laboratory strains. Overall the emergence of resistance, the MIC did not increase for any of the strains evaluated; therefore, the product is not considered to have the potential for the development of resistance.

An evaluation of the potential for cross-resistance was done by comparing the MIC of several antibiotics, both before and after extended exposure to sublethal levels of the antiseptic. Overall the cross-resistance to antibiotics study showed no indication of a change in MIC related to cross-resistance observed for any of the organism/antibiotic combination tested.

Coverage Area, Dry Time and Vapor Dissipation Studies

The study was designed to assess the area coverage and dry time with vapor dissipation when applied to healthy volunteers. Dry time was measured after application and vapor collection was used to confirm the dryness.

EM-0512985: 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation 26 mL Applicator Area Coverage, Dry Time and Vapor Dissipation Study on Human Skin

The area coverage results for the 3M™ CHG/IPA Prep with Tint 26 mL applicator was 2499 cm^2 and 387 in^2. The labeling coverage area states "19.5 in. x 19.5 in. area (approximate from the shoulder to groin in an average full size adult)." The coverage area size 19.5 in. x 19.5 in. is approximately 2451 cm^2 and 380 in^2. In addition, the labeling for the 26 mL applicator also states "discard the applicator after a single use along with any portion of the solution not required to cover the prepped area. It is not necessary to use the entire amount available." The coverage area study for the 3M™ CHG/IPA Prep 26 ml applicator is acceptable.

The 3M™ CHG/IPA Prep was considered dried on the average of 1.54 minutes (92 seconds). Excluding one subject who had a pooled area of solution that was not noticed during the application, the dry time on average was 1.35 minutes (81 seconds). Overall the drying time is well under the required class flammability labeling of "3 minutes". The labeling states "wait until solution is completely dry (minimum of 3 minutes on hairless skin; up to 1 hour in hair)." The dry time is acceptable for the 3M™ CHG/IPA Prep 26 mL applicator.

EM-05-013337: 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation 10.5 mL Applicator Area Coverage, Dry Time and Vapor Dissipation Study on Human Skin

The area coverage results for the 3M™ CHG/IPA Prep with Tint 10.5 mL applicator was 1153 cm^2 and 178 in^2. The labeling coverage area states "13 in. x 13 in. area." The coverage area size 13 in. x 13 in. is approximately 1090 cm^2 and 169 in^2. In addition, the labeling for the 10.5 mL applicator also states "discard the applicator after a single use along with any portion of the solution not required to cover the prepped area. It is not necessary to use the entire amount available." The coverage area study for the 3M™ CHG/IPA Prep 10.5 ml applicator is acceptable.

The 3M™ CHG/IPA Prep was considered dried on the average of 1.80 minutes (108 seconds). Overall the drying time is well under the required class flammability labeling of "3 minutes". The labeling states "wait until solution is completely dry (minimum of 3 minutes on hairless skin; up to 1 hour in hair)." The dry time is acceptable for the 3M™ CHG/IPA Prep 10.5 mL applicator.

Clinical Simulation Studies

Two pivotal clinical simulation studies (EM-05-012760: MicroBiotest and EM-05-01360: BioScience Laboratories) were designed to evaluate the antimicrobial efficacy and safety of SoluPrep™ Film-Forming Sterile Surgical Solution (Tinted and Clear), placebo saline control, and active control ChloraPrep® with Tint on the abdominal and inguinal regions. The procedures used in these pivotal studies were based on the American Society for Testing and Materials (ASTM) E1173-01 (reapproved 2009): Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations and the FDA 1994 Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Tentative final monograph (TFM) for Health Care Antiseptic Drug Products (59 FR 31402).

Study EM-05-012760: Assessment of the Antimicrobial Efficacy of 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation against Resident Human Skin Flora on the Abdominal and Inguinal Regions

1. Primary Analysis Responder Rates

   For the abdominal region modified intent-to-treat population, the lower bound of the 95% CI for responder rate was 76.1%, 82.4%, and 85.7% for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep 26 mL, and ChloraPrep®, respectively. The corresponding responder rates were 81.5%, 87.1, and 89.9, respectively. See Table 1 below.

   For the inguinal region modified intent-to-treat population, the lower bound of the 95% CI for responder rate was 77.1%, 74.3%, and 78.9% for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep 26 mL, and ChloraPre4p, respectively. The corresponding responder rates were 82.4%, 79.8%, and 84.0%, respectively. See Table 1 below.

   For both abdominal and inguinal regions, responder rates for all active products were statistically significantly higher than for the saline control ($p<0.001$). The responder rate following saline treatment was 0% for the abdominal region and 6.8% for the inguinal region. Therefore, this study has met the primary endpoint recommended requirement for this clinical simulation study. See Table 1 below.

Table 1. Responder rate (mITT population) Study EM-05-012760

	3M CHG/IPA Prep, 10.5 mL	3M CHG/IPA Prep, 26 mL	ChloraPrep	Saline
Abdominal Region				
N	205	201	198	62
Responder[a], n (%)	167 (81.5)	175 (87.1)	178 (89.9)	0
95% CI for rate[b]	76.1%, 86.8%	82.4%, 91.7%	85.7%, 94.1%	NA
P-value relative to negative control[c]	<0.001	<0.001	<0.001	NA
Inguinal Region				
N	204	203	200	59
Responder[a], n (%)	168 (82.4)	162 (79.8)	168 (84.0)	4 (6.8)
95% CI for rate[b]	77.1%, 87.6%	74.3%, 85.3%	78.9%, 89.1%	0.4%, 13.2%
P-value relative to negative control[c]	<0.001	<0.001	<0.001	NA

2. Secondary Analysis Efficacy Mean Log Reduction

For the abdominal region, the baseline mean bacterial (skin flora) count was approximately 3.3 $\log_{10}$ per cm^2 across study products. The mean (standard deviation, SD) reduction from baseline at 10 minutes following treatment was similar among active treatments: 2.66 (0.705) $\log_{10}$ per cm^2, 2.74 (0.644) $\log_{10}$ per cm^2, and 2.79 (0.565) $\log_{10}$ per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep 26 mL, and ChloraPrep®, respectively. Therefore, all the test products demonstrated ≥ 2 $\log_{10}$ reduction at the abdomen site. See Table 2 below.

At 6 hours following treatment, the mean (SD) reduction from baseline was similar among active treatments: 1.48 (0.866) $\log_{10}$ per cm^2, 1.64 (0.864) $\log_{10}$ per cm^2, and 1.77 (0.899) $\log_{10}$ per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep 26 mL, and ChloraPrep®, respectively. The Mean (SD) reduction from baseline following saline treatment was 0.64 (0.487) $\log_{10}$ per cm^2 at 6 hours. Therefore, all the test products did not exceed the baseline counts at 6 hours. See Table 2 below.

For the inguinal region, the baseline mean bacterial (skin flora) count was approximately 5.4 $\log_{10}$ per cm^2 across study products. The mean (SD) reduction from baseline at 10 minutes following treatment was similar among active treatments: 3.98 (1.034) $\log_{10}$ per cm^2, 3.89 (0.929) $\log_{10}$ per cm^2, and 4.03 (0.965) $\log_{10}$ per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep 26 mL, and ChloraPrep®, respectively. Therefore, all the test products demonstrated ≥ 3 $\log_{10}$ reduction at the inguinal site. See Table 2 below.

At 6 hours following treatment, the mean (SD) reduction from baseline was similar among active treatments: 2.24 (1.103) $\log_{10}$ per cm^2, 2.35 (1.046) $\log_{10}$ per cm^2, and 2.63 (1.047) $\log_{10}$ per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep 26 mL, and ChloraPrep®, respectively. The mean (SD) reduction from baseline following saline treatment was 1.34 (1.095) $\log_{10}$ per cm^2 at 10 minutes and 1.08 (0.834) $\log_{10}$ per cm^2 at 6 hours. Therefore, all the test products did not exceed the baseline counts at 6 hours. See Table 2 below.

Table 2. Summary statistics of log transformed bacterial (skin flora) endpoints (mITT population)

Time Point	Endpoint	Statistics	3M CHG/IPA Prep, 10.5 mL	3M CHG/IPA Prep, 26 mL	ChloraPrep	Saline
Abdominal Region						
Baseline	Log_{10}/cm^2 Skin Flora	N	205	201	198	62
		Mean (SD)	3.35 (0.427)	3.31 (0.370)	3.31 (0.391)	3.26 (0.342)
		Median	3.15	3.14	3.16	3.15
		Min-Max	3.01 - 5.32	3.02 - 4.74	3.01 - 5.21	3.01 - 4.96
10 Minutes	Log_{10} Recovery	N	205	201	198	62
		Mean (SD)	0.69 (0.604)	0.57 (0.575)	0.53 (0.499)	2.63 (0.582)
		Median	0.37	0.20	0.20	2.65
		Min-Max	0.20 - 3.33	0.20 - 2.91	0.20 - 2.65	1.49 - 4.52
	Log_{10} Reduction	N	205	201	198	62
		Mean (SD)	2.66 (0.705)	2.74 (0.644)	2.79 (0.565)	0.64 (0.487)
		Median	2.84	2.86	2.85	0.51
		Min-Max	0.91 - 5.12	0.23 - 4.54	1.16 - 4.54	-0.38 - 1.55
6 Hours	Log_{10} Recovery	N	205	201	198	62
		Mean (SD)	1.87 (0.804)	1.66 (0.804)	1.54 (0.807)	2.80 (0.726)
		Median	1.89	1.72	1.62	2.78
		Min-Max	0.20 - 3.61	0.20 - 3.74	0.20 - 3.51	1.04 - 4.63
	Log_{10} Reduction	N	205	201	198	62
		Mean (SD)	1.48 (0.866)	1.64 (0.864)	1.77 (0.899)	0.46 (0.651)
		Median	1.42	1.55	1.71	0.41
		Min-Max	-0.17 - 4.18	-0.37 - 3.97	-0.28 - 4.62	-0.86 - 2.06
Inguinal Region						
Baseline	Log_{10}/cm^2 Skin Flora	N	204	203	200	59
		Mean (SD)	5.44 (0.490)	5.42 (0.469)	5.44 (0.479)	5.39 (0.447)
		Median	5.22	5.21	5.22	5.14
		Min-Max	5.01 - 6.93	5.01 - 6.90	5.00 - 6.83	5.00 - 6.57
10 Minutes	Log_{10} Recovery	N	204	203	200	59
		Mean (SD)	1.47 (1.119)	1.53 (1.039)	1.40 (1.089)	4.06 (1.211)
		Median	1.45	1.50	1.44	4.19
		Min-Max	0.20 - 4.88	0.20 - 4.56	0.20 - 4.28	0.20 - 6.54
	Log_{10} Reduction	N	204	203	200	59
		Mean (SD)	3.98 (1.034)	3.89 (0.929)	4.03 (0.965)	1.34 (1.095)
		Median	4.09	3.82	4.03	1.23
		Min-Max	0.51 - 6.60	1.55 - 6.17	1.30 - 5.90	-0.66 - 4.85
6 Hours	Log_{10} Recovery	N	204	203	200	59
		Mean (SD)	3.20 (1.037)	3.07 (1.032)	2.80 (1.007)	4.31 (0.941)
		Median	3.35	3.23	2.98	4.49
		Min-Max	0.20 - 5.10	0.20 - 5.59	0.20 - 4.91	1.39 - 5.62
	Log_{10} Reduction	N	204	203	200	59
		Mean (SD)	2.24 (1.103)	2.35 (1.046)	2.63 (1.047)	1.08 (0.834)
		Median	2.16	2.20	2.48	0.99
		Min-Max	0.09 - 6.43	-0.42 - 5.30	0.38 - 6.08	-0.22 - 4.05

Abbreviations: 3M CHG/IPA Prep=3M™ Chlorhexidine Gluconate/Isopropyl Alcohol Film-Forming Preoperative Skin Preparation; ChloraPrep= ChloraPrep® Patient Preoperative Skin Preparation; Max = maximum; Min = minimum; mITT=modified intent-to-treat; SD = standard deviation

Study EM-05-013260: Assessment of the Antimicrobial Efficacy of 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation against Resident Human Skin Flora on the Abdominal and Inguinal Regions

1. Primary Analysis Responder Rates

   The primary efficacy endpoint of achieving ≥ 70% for the lower bound of the 95% CI for responder rate was met by all active study products on the abdominal region. For the abdominal region modified intent-to-treat population, the lower bound of the 95% CI for responder rate was 75.6%, 76.3%, and 77.9% for 3M™ CHG/IPA Prep, 3M™ CHG/IPA Prep with HETDA, and ChloraPrep®, respectively. The corresponding responder rates were 81.1%, 81.7%, and 83.2%, respectively, and were all higher and statistically significantly different ($p<0.001$) compared to the saline control (8.5%).

The primary efficacy endpoint of achieving ≥ 70% for the lower bound of the 95% CI for responder rate was not met by any active product on the inguinal region. For the inguinal region modified intent-to-treat population, the lower bound of the 95 CI for responder rate was 32.3%, 40.1%, and 46.4% for 3M™ CHG/IPA Prep, 3M™ CHG/IPA Prep with HEDTA, and ChloraPrep®, respectively. The corresponding responder rates were 38.9%, 46.9%, and 53.0%, respectively, and were all higher and statically significant different ($p<0.001$) compared to the saline control (1.6%). However, all the active test products did not make the FDA recommended ≥ 70% responder rate for the inguinal region. See Table 3 below.

Table 3. Responder rate (mITT population) Study EM-05-013260

	3M CHG/IPA Prep	3M CHG/IPA Prep with HEDTA	ChloraPrep	Saline
Abdominal Region				
N	196	202	196	59
Responder[a], n (%)	159 (81.1)	165 (81.7)	163 (83.2)	5 (8.5)
95% CI for rate[b]	(75.6, 86.6)	(76.3, 87.0)	(77.9, 88.4)	NA
P-value relative to negative control[c]	<0.001	<0.001	<0.001	NA
Inguinal Region				
N	208	209	219	61
Responder[a], n (%)	81 (38.9)	98 (46.9)	116 (53.0)	1 (1.6)
95% CI for rate[b]	(32.3, 45.6)	(40.1, 53.7)	(46.4, 59.6)	NA
P-value relative to negative control[c]	<0.001	<0.001	<0.001	NA

1. Secondary Analysis Efficacy Mean Log Reduction

For the abdominal region, the baseline mean bacterial (skin flora) count was approximately 3.6 $\log_{10}$ per cm^2 across study products. The mean (standard deviation, SD) reduction from baseline at 10 minutes following treatment was similar among active treatments: 2.78 (0935) $\log_{10}$ per cm^2, 2.73 (1.002) $\log_{10}$ per cm^2, and 2.75 (0.973) $\log_{10}$ per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep with HEDTA 10.5 mL, and ChloraPrep®, respectively. Therefore, all the active test products demonstrated ≥ 2 $\log_{10}$ reduction at the abdomen site. See Table 4 below.

At 6 hours following treatment, the mean (SD) reduction from baseline was similar among active treatments: 2.83 (0.851) $\log_{10}$ per cm^2, 2.86 (0.899) $\log_{10}$ per cm^2, and 2.83 (0.971) $\log_{10}$ per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep with HEDTA 10.5 mL, and ChloraPrep®, respectively. The Mean (SD) reduction from baseline following saline treatment was 1.00 (0.970) $\log_{10}$ per cm^2 at 6 hours. Therefore, all the test products did not exceed the baseline counts at 6 hours. See Table 4 below.

For the inguinal region, the baseline mean bacterial (skin flora) count was approximately 6.2 $\log_{10}$ per cm^2 across study products. The mean (SD) reduction from baseline at 10 minutes following treatment was similar among active treatments: 2.84 (1.191) $\log_{10}$ per

cm^2, 2.99 (1.125) log_{10} per cm^2, and 3.17 (1.243) log_{10} per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep with HEDTA 10.5 mL, and ChloraPrep®, respectively. Apparently, only the active control ChloraPrep® (3.17 (1.243) log_{10} per cm^2) made the secondary endpoint ≥ 3 log_{10} reduction at the inguinal site. See Table 4 below.
At 6 hours following treatment, the mean (SD) reduction from baseline was similar among active treatments: 3.46 (1.312) log_{10} per cm^2, 3.49 (1.329) log_{10} per cm^2, and 3.69 (1.286) log_{10} per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep with HEDTA 10.5 mL, and ChloraPrep®, respectively. The mean (SD) reduction from baseline following saline treatment was 1.01 (0.668) log_{10} per cm^2 at 10 minutes and 1.23 (0.732) log_{10} per cm^2 at 6 hours. Therefore, all the test products did not exceed the baseline counts at 6 hours. See Table 4 below.

Table 4. Summary statistics of log-transformed bacterial (skin flora) endpoints (mITT population, study EM-05-013260).

Time Point	Endpoint	Statistics	3M CHG/IPA Prep	3M CHG/IPA Prep with HEDTA	ChloraPrep	Saline
Abdominal Region						
Baseline	Log_{10}/cm^2 Skin Flora	N	196	202	196	59
		Mean (SD)	3.63 (0.460)	3.61 (0.479)	3.64 (0.487)	3.64 (0.533)
		Median	3.51	3.48	3.54	3.47
		Min-Max	3.00 - 5.26	3.00 - 5.13	3.01 - 5.39	3.00 - 5.00
10 Minutes	Log_{10} Recovery	N	196	201	196	59
		Mean (SD)	0.85 (0.947)	0.88 (0.984)	0.90 (0.938)	2.88 (0.703)
		Median	0.42	0.42	0.54	2.93
		Min-Max	0.24 - 4.55	0.24 - 4.22	0.24 - 4.92	1.43 - 4.57
	Log_{10} Reduction	N	196	202	196	59
		Mean (SD)	2.78 (0.935)	2.73 (1.002)	2.75 (0.973)	0.76 (0.814)
		Median	2.96	2.94	2.90	0.72
		Min-Max	-0.95 - 4.44	-0.22 - 4.89	-0.71 - 5.15	-0.82 - 2.54
6 Hours	Log_{10} Recovery	N	196	201	196	59
		Mean (SD)	0.80 (0.840)	0.74 (0.789)	0.81 (0.935)	2.65 (0.886)
		Median	0.33	0.24	0.42	2.58
		Min-Max	0.24 - 3.43	0.24 - 3.37	0.24 - 4.30	0.42 - 4.99
	Log_{10} Reduction	N	196	202	196	59
		Mean (SD)	2.83 (0.851)	2.86 (0.899)	2.83 (0.971)	1.00 (0.970)
		Median	2.95	2.99	2.98	0.86
		Min-Max	-0.03 - 4.59	0.00 - 4.69	-0.67 - 4.81	-0.88 - 3.56
Inguinal Region						
Baseline	Log_{10}/cm^2 Skin Flora	N	208	209	219	61
		Mean (SD)	6.23 (0.604)	6.21 (0.563)	6.27 (0.618)	6.09 (0.626)
		Median	6.28	6.33	6.34	6.16
		Min-Max	5.00 - 7.49	5.08 - 7.37	5.00 - 7.47	5.00 - 7.45
10 Minutes	Log_{10} Recovery	N	208	208	218	60
		Mean (SD)	3.39 (1.096)	3.21 (1.042)	3.08 (1.137)	5.08 (0.676)
		Median	3.61	3.36	3.26	5.28
		Min-Max	0.24 - 5.61	0.24 - 5.72	0.24 - 4.90	2.55 - 6.21
	Log_{10} Reduction	N	208	209	219	61
		Mean (SD)	2.84 (1.191)	2.99 (1.125)	3.17 (1.243)	1.01 (0.668)
		Median	2.74	2.93	3.13	1.04
		Min-Max	-0.25 - 6.70	0.00 - 6.15	0.00 - 6.36	-0.87 - 3.30
6 Hours	Log_{10} Recovery	N	208	209	219	60
		Mean (SD)	2.76 (1.202)	2.73 (1.220)	2.58 (1.182)	4.85 (0.749)
		Median	2.95	3.03	2.76	4.83
		Min-Max	0.24 - 5.44	0.24 - 5.13	0.24 - 5.17	2.59 - 6.50
	Log_{10} Reduction	N	208	209	219	61
		Mean (SD)	3.46 (1.312)	3.49 (1.329)	3.69 (1.286)	1.23 (0.732)
		Median	3.44	3.29	3.57	1.23
		Min-Max	0.35 - 6.84	0.14 - 6.49	0.61 - 6.77	-0.13 - 3.03

Abbreviations: 3M CHG/IPA Prep=3M™ Chlorhexidine Gluconate/Isopropyl Alcohol Film-Forming Preoperative Skin Preparation; ChloraPrep= ChloraPrep® Patient Preoperative Skin Preparation; HEDTA=(hydroxyethyl)ethylenediaminetriacetic acid; Max = maximum; Min = minimum; mITT=modified intent-to-treat; SD = standard deviation

2. Alternative Primary Analysis Assessment

Post hoc analysis demonstrated that the responder rates of the 3M™ CHG/IPA Prep and 3M™ CHG/IPA Prep with HEDTA groups were not significantly different from either body regions (p=0.898 for the abdominal region and p=0.114 for the inguinal region). See Table 5 below. The primary, secondary, and alternative primary efficacy results demonstrated the efficacy of 3M™ CHG/IPA Prep and 3M™ CHG/IPA Prep with HEDTA on the abdominal region and of 3M™ CHG/IPA Prep with HEDTA on the inguinal region.

Table 5. Alternative primary efficacy results for inguinal region in Study EM-05-01360: difference in log reduction at 10 minutes and difference in Return to baseline at 6 hours (mITT population)

	3M CHG/IPA Prep	3M CHG/IPA Prep with HEDTA	ChloraPrep
Inguinal Region			
N	208	209	219
95% CI for the difference in log_{10} reduction (ChloraPrep minus 3M CHG/IPA Prep) at the 10-minute time point[a]	(0.103, 0.567)	(-0.041, 0.410)	NA
97.5% CI for the difference in log_{10} reduction (ChloraPrep minus 3M CHG/IPA Prep) at the 10-minute time point[a]	NA	(-0.074, 0.443)	NA
N(%) of subjects with skin flora returning to baseline values at the 6-hour time point	0	0	0
P-value for the difference in the percentage of subjects with skin flora returning to baseline values at the 6-hour time point[b]	1.000	1.000	NA

Abbreviations: 3M CHG/IPA Prep=3M™ Chlorhexidine Gluconate/Isopropyl Alcohol Film-Forming Preoperative Skin Preparation; ChloraPrep= ChloraPrep® Patient Preoperative Skin Preparation; CI=confidence interval; CSR=clinical study report; HEDTA=(hydroxyethyl)ethylenediaminetriacetic acid; mITT=modified intent-to-treat; NA=not applicable

Neutralization Validation Studies for EM-05-012760 and EM-05-01360

The results from the neutralization validation study performed during the clinical simulation study showed that the neutralizer was effective in neutralizing the test product and was not toxic to the test organism. These results indicate that effective neutralization of the antimicrobial agent took place at the sampling time points. The results of the toxicity test indicate that the neutralizer does not contribute to the observed effectiveness of the antimicrobial.

Overall Assessment

Although study EM-05-013260 (BioScience Laboratories) containing the 3M™ CHG/IPA Prep 10.5 mL (38.9%), 3M™ CHG/IPA Prep with HEDTA 10.5 mL (46.9%) and ChloraPrep® (53%) did not pass the ≥ 70% responder rate (primary endpoint), the mean log reduction criteria (secondary endpoint) comes close to the recommended 3 $\log_{10}$ reduction in the inguinal site: 3M™ CHG/IPA Prep 10.5 mL (2.84; CI= 2.68, 3.00), 3M™ CHG/IPA Prep with HEDTA 10.5 mL (2.99; CI= 2.84, 3.14) and ChloraPrep® (3.17; CI= 3.01, 3.33). The mean log reduction for 3M™ CHG/IPA Prep with HEDTA in the inguinal site was 2.99 with CI of 2.84-3.14 and is very close to the recommended 3 $\log_{10}$ reduction. The 3M™ CHG/IPA Prep with HEDTA is the formulation that will be the marketed product.

We have experienced in the past the issue of antiseptic products having a difficult time not meeting the 3 $\log_{10}$ reduction point estimate efficacy and meeting the 70% responder rate for the 10 minute post treatment reductions at the groin site. It is difficult to determine why some antiseptic products do not perform consistently at the groin site. The test results for some antiseptic products and whether it meets the specified point estimate as an active control and/or antiseptic product, appears to vary by the laboratory doing the testing. One lab consistently report meeting the specified point estimate criteria, while the other lab does not report similar findings. The reasons for these differences are not clear. Some of the labs may conduct their study trials differently (e.g., the amount of pressure applied when scrubbing (sampling) the skin).

- The protocols for EM-05-012760 (MicroBiotest) and EM-05-013260 (BioScience Laboratories) are identical except for the glass cylinder sampling scrub cup size used between the two lab facilities. Table 6 shows a comparison between areas of the scrub cup used by the two pivotal studies.

Table 6. Scrubbing cup comparison

Clinical Trial Location	Internal Area of Scrubbing Cup
MicroBiotest	3.80 cm^2
BioScience Laboratories	3.46 cm^2

The average number of microorganisms recovered per square centimeter of skin is determined and reported:

MicroBiotest $CFU/cm^2 = \frac{CFU/mL \times 6\ mL}{3.80\ cm^2}$

BioScience Laboratories $CFU/cm^2 = \frac{CFU/mL \times 6\ mL}{3.46\ cm^2}$

The difference in formula is due to difference in cylinder size. The 1994 TFM does not specify the diameter of the sampling scrub cup used to sample the microorganisms. The TFM describes the following "Sterile glass cylinders, height approximately 2.5 centimeter, inside diameter of convenient size to place on anatomical area to be sampled. Useful sizes range from approximately 2.5 to 4.0 centimeters." The test method ASTM E1173 "Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations" describes

similar *in vivo* procedures and does not specify a size of the sampling cup but a range of 0.5 inches to 1.5 inches. It appears that if you use a cylinder scrub cup size of 3.80 cm^2 versus a cylinder scrub cup size of 3.46 cm^2, you will obtain a larger surface area. This means a possibility of getting a higher bacterial count.

- Another possible inconsistency is the amount of pressure or how hard you are scrubbing the skin using the hollow cylinder with the scrub solution and policeman. Here you are really scraping the epithelial layer of the skin to retrieve a high number of bacteria count in order to get a high log reduction count. This has been an issue of speculation for a number of years. You don't know if one person is the designated scrubber or if several different technicians are scrubbers. Although the directions states scrub with moderate pressure it is difficult to determine what is moderate pressure.

- In regards to the 3 log reduction in the groin site, forging ahead, we may wish to also consider a final threshold level that would provide some measure of confidence that a reduction in surgical rate infection rate would occur. However, a definitive link of decreasing bacterial count on the skin of any order of magnitude to clinical efficacy (reduction in incidence of post-operative infections), has not been made.

MicroBiotest for study EM-05-012760 and BioScience Laboratories for study EM-05-013760 have both passed clinical inspection with a "No Action Indicated" (NAI). Both of these laboratory facilities have always passed FDA inspection (NAI) since they have been in business (since late 1990's) conducting these types of studies. These laboratory facilities have never received an "Official Action Indicated" (OAI) a fail. Currently, these are the only two microbiological laboratory facilities that conduct clinical simulation studies for patient preoperative skin preparation drug products in the United States.

Although, we have been recommending IND/NDA sponsors the new statistical criteria and also have published our recommended statistical criteria in Safety and Effectiveness of Health Care Antiseptics Proposed Rule (80 FR 25166), we are currently still evaluating and assessing this new statistical criteria from comments and data received in the dockets. We will have a final decision on the proposed statistical criteria in the final rule on Health Care Antiseptic to be published on January 15, 2018.

Recommendations:

1. Based on the above discussion, this reviewer recommends that the *in vitro* and clinical simulation studies in this application be **approved** for the indication "patient preoperative skin preparation."

2. Due to the discrepancy in the cylinder scrubbing cup area size, we recommend that you use the same size cylinder scrubbing cup in all laboratory facilities in any future efficacy studies that will be used to demonstrate efficacy and to rule out any possibilities of lab-to-lab variability.

2. INTRODUCTION

3M™ SoluPrep™ Film-Forming Sterile Surgical Solution (3M™ CHG/IPA Prep) is a topical over-the-counter (OTC) combination drug product for skin antisepsis applications. The drug product contains an inactive copolymer (acrylate) and two antimicrobial active ingredients 2% (w/v) chlorhexidine gluconate (CHG) and 70% (v/v) isopropyl alcohol. When the solution is applied to the skin, the CHG-containing copolymer dries to form a protective water-insoluble film as the alcohol evaporates. The sponsor states that it is this antimicrobial film that makes the 3M™ CHG/IPA Prep unique form other CHG-containing skin preparations.

(b) (4)

The intended use of this drug product is for use in preparation of the patient's skin prior to surgery to help reduce the bacteria that can potentially cause skin infection. Reduction of the bacterial load on a patient's skin with topical antiseptics is an important part of the skin preparation prior to invasive surgical procedures. The goal of the sterile preoperative skin preparation solution is to create an operative field that is as close to sterile as possible by reducing the patient's own skin flora and to do so efficiently with minimal irritation to the skin at the site of proposed incision. Though skin sterility is impossible to achieve, the sterile skin preparation is intended to affect the highest possible reduction of skin flora, suppress the growth of resident skin flora, and suppress the growth of transient organisms that enter into the operative field. The ideal case is that the preoperative skin preparation continues to maintain antimicrobial activity at and around the incision site for the duration of the surgical procedure.

The 3M™ CHG/IPA Prep has the fast-acting, persistent (6-hour), broad-spectrum activity required for this class of products. It is intended to be used as a single-use, topically applied, patient preoperative skin preparation for use by healthcare professional. The sponsor states that the potential benefits of this product include the presence of a durable antimicrobial film on prepped skin that resists removal by irrigation and exposure to body fluids and improved adhesion of incise drapes to prepped skin. The sponsor's product has been developed in both a colorless and a tinted solution in sponge applicator sizes of 10.5 mL (colorless and tinted "Brite Green") and 26 mL (tinted "Brite Green") for different types of surgical procedures. A bright green tint provides improved visualization of the surgical area.

The formulation of 3M™ CHG/IPA Prep with (hydroxyethyl)-ethylenediaminetriacetic acid (HEDTA) was used in this study to confirm that the efficacy and safety of 3M™ CHG/IPA was unchanged with trace amount of HEDTA added to the formulation (b) (4) . This pivotal study incorporated 3M™ CHG/IPA Prep with HEDTA as a test product along with 3M™ CHG/IPA Prep without HEDTA to directly compare efficacy and safety results. The primary objective of the pivotal clinical efficacy studies was to evaluate the antimicrobial efficacy of various configurations of 3M™ CHG/IPA Prep when applied to the abdominal and inguinal regions of healthy subjects. ChloraPrep® was used as an active control, and sterile 0.9 saline was used as a negative

control. The 3M™ CHG/IP Prep configurations examined in the two completed pivotal studies (EM-05-012760 – MicroBiotest) and EM-05-013260 – BioScience Laboratories) included the tinted preparation in 10.5 mL and 26 mL applicators and the colorless preparation in 10.5 mL applicators, all without HEDTA, and the colorless preparation in 10.5 mL applicators with HEDTA. The 3M™ CHG/IPA Prep with HEDTA is the final formulation. Since all other studies in the program (non-clinical, *in vitro*, and *in vivo*) were conducted using the formulation without HEDTA, the sponsor opted to include formulations with and without HEDTA in the last pivotal study to compare the efficacy and safety to confirm that the addition of HEDTA does not impact efficacy or safety, and to bridge to all studies conducted using the formulation without HEDTA.

FDA has agreed to the following clinical development plan for the sponsor's drug application: 1) clinical trial comparing efficacy of 3M™ CHG/IPA Prep (colorless, tinted, with and without HEDTA) to an active control ChloraPrep® and negative control saline solution; 2) modified time kill studies; 3) antimicrobial resistance studies; 4) coverage area, dry time, and vapor dissipation studies; 5) drape adhesion studies; 6) flammability studies; 7) cumulative irritation patch test studies; and phototoxicity and photoallergy studies.

The sponsor provided a persistence efficacy study entitled "Assessment of the Persistent Antimicrobial Efficacy of 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation against Resident Human Skin Flora on the Abdominal and Inguinal Regions." The study was to evaluate the antimicrobial persistence of 3M™ CHG/IPA with HEDTA on the abdomen and groin at 48 hours and 72 hours post-treatment. In order to demonstrate persistence for a patient preoperative skin preparation claim, FDA requires that the level of resident microorganisms remain below baseline for the 6-hour time point. We have been advising sponsors if it wishes to obtain a claim with an associated time-frame greater than 6 hours, then it is necessary to show clinical benefit with clinically meaningful outcomes, not just log reductions. Therefore, the persistence efficacy study the sponsor submitted in the submission will not be reviewed. This was also noted in a memorandum to the IND file dated May 26, 2015 in DARRTS.

3. PRECLINICAL MICROBIOLOGY

3.1 Mechanism of Action of CHG

The 3M™ SoluPrep™ Film-Forming Sterile Surgical Solution contains CHG as one of its active ingredients (the other is IPA). CHG is an aqueous solution of 1,1'-hexamethylenebis[5-(4-chlorophenyl) bisguanide]di-D-gluconate. Woodcock[1] has reviewed the mechanism of action of CHG and related biguanides. The author stated that at relatively low concentrations, the action of CHG is bacteriostatic; whereas, at higher concentrations the action is rapidly bactericidal. The lethal mechanism has been shown to consist of a sequence of changes that result in cell death. Denton[2] has described the sequence as follows: 1) rapid attraction of CHG to the bacterial cell; 2) specific and strong adsorption to certain phosphate-containing compounds on the bacterial cell wall; 3) overcoming bacterial cell wall exclusion mechanisms; 4) attraction to the cytoplasmic membrane; 5) leakage of low molecular weight cytoplasmic components, e.g., potassium ions and inhibition of certain membrane-

bound enzymes such as adenosyl triphosphatase; 6) precipitation of the cytoplasm by formation of complexes with phosphate entities such as adenosine triphosphate and nucleic acids.

Bacterial cells normally carry a net negative charge on their surface. CHG, being positively charged, alters the surface charge of the bacterial cell wall, first by neutralizing it and then by reversing the charge[2]. The degree of charge reversal is proportional to the CHG concentration. The rapid electrostatic attraction of the positively charged CHG molecules and the negatively charged bacterial cell contribute to the rapid rate of lethality[3]. Several changes indicating damage to the cytoplasmic membrane have been observed in bacterial populations treated with bacteriostatic and bactericidal levels of CHG[2]. Leakage of cytoplasmic contents is an indication of damage to the membrane beginning with the loss of low molecular weight molecules. Electron micrographs of sub-lethally treated cells show plasmolysis of the protoplast. Cells treated with bacteriostatic levels of CHG can recover viability despite having lost up to 50 percent of their K^+ ions. As the CHG concentration is increased, higher molecular weight cell contents, such as nucleotides, appear in the supernatant fluid around the cell. Bacterial cells showing more than a 15 percent increase in nucleotide leakage are irreversibly damaged. The rate of membrane disruption and cell leakage increases with CHG concentration up to a maximum and then fall back. At bactericidal concentrations of 100 to 500 mg/L, leakage no longer occurs instead precipitation of cytoplasm contents caused by interaction between CHG and phosphate elements in the cytoplasm takes place. As a result, the antimicrobial activity of CHG is immediate as well as persistent and cumulative[2].

CHG has microbiocidal activity against vegetative gram-positive and gram-negative bacteria, yeast and fungi, and lipid-enveloped viruses[2]. Uptake of CHG into bacteria and yeasts is extremely rapid, with a maximum effect occurring within 20 seconds[4,5]. Although CHG is not sporicidal, it has also been shown to inhibit outgrowth of bacterial spores[6]. See section 3.3 of this review on "Mechanism of Chlorhexidine Gluconate Resistance."

Reviewer's comments: It is also noteworthy to discuss the mechanism of action of CHG in viruses. CHG is not always considered a particularly effective antiviral agent, and its activity is restricted to the lipid-enveloped viruses. This appears to be due to disruption of the lipid viral envelops, which can render the virus noninfectious. CHG does not inactivate non-enveloped viruses, such as rotaviruses, hepatitis A virus, or polioviruses. Its activity appears to be restricted to the nucleic acid core or the outer coat.

Mechanism of Action of IPA

The 3M™ SoluPrep™ Film-Forming Sterile Surgical Solution also contains IPA as one of its active ingredients. The most widely used alcohol as an antiseptic agent IPA. When used in concentrations of 60% to 90%, it has a broad spectrum of activity, showing killing effects on bacteria, mycobacteria, fungi, and large, lipid-containing

viruses but not against hydrophilic viruses[7]. Alcohols in healthcare personnel hand antiseptic, surgical scrub hand antiseptic, patient preoperative skin preparation and preinjections provide the most rapid and greatest reduction of microbial counts on the skin. The antimicrobial mechanism of action of IPA is nonspecific, primarily caused by denaturation and coagulation of proteins[8, 9, 10, 11, 12]. It is believed that IPA dehydrates the bacterial cell and denatures its proteins, particularly those that function as membrane-bound enzymes[13]. Protein coagulation occurs within concentration limits around an optimum alcohol level. In the absence of water, proteins are not denatured as readily as when water is present. This explains why absolute IPA, a dehydrating agent, is less bactericidal than mixture of alcohol and water. High concentrations of IPA are toxic to most microorganisms due to its high oxygen demand and membrane-disruptive characteristics[14]. Because of IPA's speed of action and multiple, nonspecific toxic effects, microorganisms have a difficult time developing resistance to it. A recent study identified and IPA-tolerate strain of *Paracoccus denitrificans* that could grow in IPA at a concentration of 1.6%[15], and a strain of *Bacillus pallidus* has been shown to grow in IPA up to 2.4%[16]. Thus, even IPA-tolerate strains could not survive in health care antiseptic products, which would contain at least 70% IPA[17]. Furthermore IPA evaporates readily after topical application, so no antiseptic residue is left on the skin that could contribute to the development of resistance[8, 9, 10, 11]. Consequently, the development of resistance as result of health care antiseptic use is unlikely.

Reviewer's comments*: It is also noteworthy to discuss the current status of the active ingredient, IPA. FDA published a proposed rule on "Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use" (80 FR 25166). FDA has identified some important scientific data gaps for active ingredients used in certain OTC health care antiseptic products and is requesting additional data to support the ingredients' safety and effectiveness. One of these ingredients includes IPA. In the 1994 TFM, IPA (70-91.3%) was proposed as Category I. This ingredient is now being proposed as Category III- need more data. While health care antiseptic products are an important component of infection control, more data are required to support a finding that they are generally recognized as safe and effective (GRASE). Emerging science suggests that for at least some active ingredients (including IPA), systemic exposure is higher than previously though. FDA is proposing that all active ingredients used in health care antiseptic products marketed under the OTC monograph need additional safety and effectiveness data. FDA does not believe that monograph health care antiseptic products containing these ingredients are ineffective or unsafe, but data are needed to support a GRASE designation.

3.2 MODIFIED TIME KILL STUDIES

The drug product 3M™ CHG/IPA Prep (colorless and tinted), an OTC topical antiseptic, was evaluated by various *in vitro* studies for antimicrobial effectiveness. These topical antiseptic products are intended for use in health care settings such as hospitals where the likely hood of transmission of nosocomial and community acquired pathogens is high.

The purpose of this study is to demonstrate that products intended for antiseptic skin preparation use have a satisfactory spectrum of activity against pathogens that are likely to be encountered in the healthcare setting. Thus, products used in these settings should be formulated with broad spectrum antimicrobials. The *in vitro* spectrum of activity studies are performed with organisms that are known to be nosocomial pathogens to assess whether the product is a broad spectrum antimicrobial[17]. These studies consisted of modified time kill studies for chlorhexidine gluconate drug products. These studies will assess whether the antiseptic is fast-acting as for an indication. The modified time-kill kinetic study is designed to measure the rate of kill by the antiseptic under controlled conditions. The modified time-kill *in vitro* studies allow us to gain insights into the potential utility of the antiseptic, insights that cannot be gained through the clinical simulation studies alone.

The sponsor's and FDA's agreements regarding *in vitro* studies include the following:

Date	Agreements
May 2012	• The FDA identified specific requirements including exposure times, formulations, and test concentrations for the *in vitro* time kill study and requested an evaluation of the effect of serum on antimicrobial activity. An emergence of resistance study was recommended. The FDA requested that these studies be done with both the tinted and colorless formulations. • Additional microbiology comments were provided to address the needs for a proper neutralization validation testing protocol using ASTM E1054-08.
June 2012	• The full clinical hold was removed. It was stated in this letter that the FDA does not consider the 3M™ acrylate copolymer to be a novel excipient. The IND was accepted by the FDA. • The FDA agreed that proposed concentrations for *in vitro* time kill study were acceptable and that 3M™ did not need to test the active ingredient alone (2% CHG) in the time kill assay.
June 2013	End-of-Phase-2 meeting was conducted via teleconference. Critical items: • For the emergence of resistance study, 3M™ provided an updated list of organisms including antibiotics to be tested for cross-resistance and stated plans to evaluate 1 ATCC strain and 1 clinical strain for each organism on the list. The FDA agreed with the plan but noted that 4 clinical isolates for each organism will be required. The FDA agreed that 3M™ could use the agar dilution.

	• The FDA agreed with the 3M™ approach to use a modified sampling solution due to the polymer and stated that 3M should ensure that the Crodamol™ GTCC does not have any antimicrobial activity. • The FDA stated that from a microbiology perspective, the studies are appropriate to support the NDA. The FDA recommended that 3M™ provide information about whether there is systemic exposure from the drug product/polymer. The FDA stated that 3M™ should perform an adhesion study with the final-to-be-marketed formulation. The FDA stated that the formulation used in the proposed Phase 3 clinical study protocols and dermal safety studies should be the final-to-be marketed formulation.
October 2013	3M™ submitted a revised Emergence of Resistance protocol and requested review and comment from the FDA. The FDA responded that the updated protocol is generally acceptable but had additional comments on clarification of testing 4 clinical isolates of each organism and confirmed that 3M™ intended to use a CHG control arm in the cross-resistance study as they recommended this for both the cross-resistance and emergence of resistance studies.
January 2014	The FDA agreed to 3M's proposal that only the tinted formulation be evaluated since the *in vitro* time kill study showed no meaningful difference between the formulations.

3.2.1 Assessment of Microbiocidal Activity of Tinted and Colorless 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation Formulations Using a Modified Time Kill Procedure (EM-05-012700)

This study was designed to supply basic antimicrobial data and to determine how rapidly and effectively the test product kills a variety of microorganisms. It incorporates the recommendations described in the "Manual of Clinical Microbiology," 5th ed., edited by A.B. Balows et al., ASM, Washington: ACM, 1991. The procedure is based on the ASTM E2315 – 03(2008) Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure.

Reviewer's comment: FDA currently does not have a standard time kill testing method for topical antiseptics products. The sponsor has incorporated methods described in the Manual of Clinical Microbiology and the ASTM E2315. This is acceptable.

A single lot each of 2 complete test formulations (test Products) and 1 reference product was tested against a variety of repository and clinical isolate strains of gram-negative and gram-positive bacteria, and yeast. The test products will be evaluated at 3 concentrations (actual use concentration, full-strength; a secondary concentration within the active range, 50% strength; and an inactive concentration, 0.01% strength). The reference product was evaluated at the actual use concentration (full strength) only.

Forty-eight (48) repository isolates (12 species, 4 isolates per species) and 144 clinical isolates (12 species, 12 isolates per species) was evaluated using each of the three products, at the required concentrations and two contact times to yield 2,688 evaluations. See Table 7 below for list of challenge microorganisms to be tested. To minimize buffer interference and to minimize reduction of the antimicrobial concentration, the volume of inoculum added to each test product was kept at less than or equal to 1% of the total volume of the test. Samples were removed at two selected contact times (3 minutes and 5 minutes) and neutralized. Serial dilutions, as required were performed and triplicate aliquots of the appropriate dilutions were plated. The plates were incubated and the average colony-forming units (CFU) recovered per mL were determined.

Table 7. List of challenge microorganisms

Challenge Microorganisms	
1. *Burkholderia cepacia,* ATCC 25416	25. *Pseudomonas aeruginosa,* ATCC 9027
2. *Burkholderia cepacia,* ATCC 25608	26. *Pseudomonas aeruginosa,* ATCC 15442
3. MDR *Burkholderia cepacia,* ATCC 55792	27. *Pseudomonas aeruginosa,* ATCC 27315
4. MDR *Burkholderia cepacia,* ATCC 700070	28. *Pseudomonas aeruginosa,* ATCC 27853
5. *Candida albicans,* ATCC 18804	29. *Serratia marcescens,* ATCC 8100
6. *Candida albicans,* ATCC 66027	30. *Serratia marcescens,* ATCC 13880
7. AR *Candida albicans,* ATCC 64124	31. *Serratia marcescens,* ATCC 14756
8. AR *Candida albicans,* ATCC 64550	32. MDR *Serratia marcescens,* ATCC 43297
9. *Enterococcus faecalis,* ATCC 19433	33. *Staphylococcus aureus,* ATCC 29213
10. *Enterococcus faecalis,* ATCC 29212	34. *Staphylococcus aureus,* ATCC 6538
11. VRE *Enterococcus faecalis,* ATCC 51299	35. MRSA *Staphylococcus aureus,* ATCC 33591
12. VRE *Enterococcus faecalis,* ATCC 51575	36. MRSA *Staphylococcus aureus,* ATCC 33592
13. *Enterococcus faecium,* ATCC 19434	37. *Staphylococcus epidermidis,* ATCC 12228
14. *Enterococcus faecium,* ATCC 25307	38. *Staphylococcus epidermidis,* ATCC 14990
15. VRE *Enterococcus faecium,* ATCC 700221	39. MRSE *Staphylococcus epidermidis,* ATCC 51625
16. MDR *Enterococcus faecium,* ATCC 51559	40. MRSE *Staphylococcus epidermidis,* ATCC 700563
17. *Escherichia coli,* ATCC 11775	41. *Streptococcus pneumoniae,* ATCC 6303
18. *Escherichia coli,* A TCC 25922	42. *Streptococcus pneumoniae,* ATCC 49619
19. MDR *Escherichia coli,* ATCC BAA-197	43. MDR *Streptococcus pneumoniae,* ATCC 51936
20. MDR *Escherichia coli,* ATCC BAA-200	44. MDR *Streptococcus pneumoniae,* ATCC 700671
21. *Klebsiella pneumoniae,* ATCC 13883	45. *Streptococcus pyogenes,* ATCC 14289
22. *Klebsiella pneumoniae,* ATCC 27736	46. *Streptococcus pyogenes,* ATCC 19615
23. MDR *Klebsiella pneumoniae,* ATCC 51503	47. MR *Streptococcus pyogenes,* ATCC BAA-1411
24. MDR *Klebsiella pneumoniae,* ATCC 700603	48. MR *Streptococcus pyogenes,* ATCC BAA-1413

MDR- Multidrug-resistant; AR- Azole-resistant; MRSA- Methicillin-resistant *Staphylococcus aureus*; MRSE- Methicillin-resistant *Staphylococcus epidermidis*; VRE- Vancomycin-resistant *Enterococcus*; MR- Macrolide-resistant

The results are presented in Tables 1-14 and Appendix II in Modular 5.3.5.1. Results are summarized in Tables 1-12. Summarized results (CFU/mL, Percent Reduction, and Log Reduction) are presented as the average values of the non-resistant isolates and the average values of the resistant isolates. Absolute values were used to calculate average values. Results for each individual isolates are presented in Appendix II in Modular 5.3.5.1. Table 8, 9, and 10 represent example of gram negative organism *Burkholderia cepacia*, gram positive organism *Entercoccus faecalis* and a fungus *Candida albicans* summary results.

Table 8. Results summary of *Burkholderia cepacia*. Results expressed as average CFU/mL, average percent reduction, and average log reduction

Microorganism	Product	Concentration	Contact Time	Average CFU/mL	Average Percent Reduction	Average Log Reduction
Burkholderia cepacia	Film-Forming Skin Prep - Tinted	1X	3 min.	1.0E+01	99.99986	5.87
			5 min.	1.0E+01	99.99984	5.84
		0.5X	3 min.	1.0E+01	99.99986	5.87
			5 min.	1.0E+01	99.99984	5.84
		0.0001X	3 min.	2.7E+06	62.80229	0.45
			5 min.	2.0E+06	70.26336	0.55
	Film-Forming Skin Prep - Colorless	1X	3 min.	1.0E+01	99.99986	5.87
			5 min.	1.0E+01	99.99984	5.84
		0.5X	3 min.	1.0E+01	99.99986	5.87
			5 min.	1.0E+01	99.99984	5.84
		0.0001X	3 min.	2.8E+06	59.61426	0.42
			5 min.	2.3E+06	64.27332	0.49
	ChloraPrep	1X	3 min.	1.0E+01	99.99986	5.87
			5 min.	1.0E+01	99.99984	5.84
Drug-resistant *Burkholderia cepacia*	Film-Forming Skin Prep - Tinted	1X	3 min.	1.0E+01	99.99978	5.67
			5 min.	1.0E+01	99.99981	5.74
		0.5X	3 min.	1.0E+01	99.99978	5.67
			5 min.	1.0E+01	99.99981	5.74
		0.0001X	3 min.	2.5E+06	46.73718	0.28
			5 min.	2.2E+06	59.14915	0.42
	Film-Forming Skin Prep - Colorless	1X	3 min.	1.0E+01	99.99978	5.67
			5 min.	1.0E+01	99.99981	5.74
		0.5X	3 min.	1.0E+01	99.99978	5.67
			5 min.	1.0E+01	99.99981	5.74
		0.0001X	3 min.	2.4E+06	46.87674	0.30
			5 min.	1.9E+06	62.89646	0.49
	ChloraPrep	1X	3 min.	1.0E+01	99.99978	5.67
			5 min.	1.0E+01	99.99981	5.74

Table 9. Results summary of *Entercoccus faecalis*. Results expressed as average CFU/mL, average percent reduction, and average log reduction

Microorganism	Product	Concentration	Contact Time	Average CFU/mL	Average Percent Reduction	Average Log Reduction
Enterococcus faecalis	Film-Forming Skin Prep - Tinted	1X	3 min.	1.0E+01	99.99980	5.70
			5 min.	1.0E+01	99.99979	5.69
		0.5X	3 min.	1.0E+01	99.99980	5.70
			5 min.	1.0E+01	99.99979	5.69
		0.0001X	3 min.	2.1E+06	58.60475	0.41
			5 min.	1.8E+06	63.09482	0.47
	Film-Forming Skin Prep - Colorless	1X	3 min.	1.0E+01	99.99980	5.70
			5 min.	1.0E+01	99.99979	5.69
		0.5X	3 min.	1.0E+01	99.99980	5.70
			5 min.	1.0E+01	99.99979	5.69
		0.0001X	3 min.	2.3E+06	55.43388	0.37
			5 min.	2.0E+06	59.56375	0.42
	ChloraPrep	1X	3 min.	1.0E+01	99.99980	5.70
			5 min.	1.0E+01	99.99979	5.69
Drug-resistant *Enterococcus faecalis*	Film-Forming Skin Prep - Tinted	1X	3 min.	1.0E+01	99.99974	5.70
			5 min.	1.0E+01	99.99976	5.73
		0.5X	3 min.	1.0E+01	99.99974	5.70
			5 min.	1.0E+01	99.99976	5.73
		0.0001X	3 min.	2.2E+06	58.00658	0.40
			5 min.	2.1E+06	63.02293	0.45
	Film-Forming Skin Prep - Colorless	1X	3 min.	1.0E+01	99.99974	5.70
			5 min.	1.0E+01	99.99976	5.73
		0.5X	3 min.	1.0E+01	99.99974	5.70
			5 min.	1.0E+01	99.99976	5.73
		0.0001X	3 min.	2.2E+06	58.08392	0.40
			5 min.	2.1E+06	63.04797	0.45
	ChloraPrep	1X	3 min.	1.0E+01	99.99974	5.70
			5 min.	1.0E+01	99.99976	5.73

Table 10. Results summary of *Candida albicans*. Results expressed as average CFU/mL, average percent reduction, and average log reduction

Microorganism	Product	Concentration	Contact Time	Average CFU/mL	Average Percent Reduction	Average Log Reduction
Candida albicans	Film-Forming Skin Prep - Tinted	1X	3 min.	1.0E+01	99.99978	5.75
			5 min.	1.0E+01	99.99977	5.75
		0.5X	3 min.	1.0E+01	99.99978	5.75
			5 min.	1.0E+01	99.99977	5.75
		0.0001X	3 min.	2.1E+06	54.00473	0.44
			5 min.	1.7E+06	64.75010	0.55
	Film-Forming Skin Prep - Colorless	1X	3 min.	1.0E+01	99.99978	5.75
			5 min.	1.0E+01	99.99977	5.75
		0.5X	3 min.	1.0E+01	99.99978	5.75
			5 min.	1.0E+01	99.99977	5.75
		0.0001X	3 min.	2.3E+06	51.73240	0.40
			5 min.	2.0E+06	61.40838	0.49
	ChloraPrep	1X	3 min.	1.0E+01	99.99978	5.75
			5 min.	1.0E+01	99.99977	5.75
Drug-resistant *Candida albicans*	Film-Forming Skin Prep - Tinted	1X	3 min.	1.0E+01	99.99941	5.27
			5 min.	1.0E+01	99.99938	5.25
		0.5X	3 min.	1.0E+01	99.99941	5.27
			5 min.	1.0E+01	99.99938	5.25
		0.0001X	3 min.	1.3E+06	28.25447	0.17
			5 min.	1.1E+06	32.83629	0.20
	Film-Forming Skin Prep - Colorless	1X	3 min.	1.0E+01	99.99941	5.27
			5 min.	1.0E+01	99.99938	5.25
		0.5X	3 min.	1.0E+01	99.99941	5.27
			5 min.	1.0E+01	99.99938	5.25
		0.0001X	3 min.	1.3E+06	29.46119	0.18
			5 min.	1.1E+06	33.52158	0.20
	ChloraPrep	1X	3 min.	1.0E+01	99.99941	5.27
			5 min.	1.0E+01	99.99938	5.25

***Reviewer's comment*: DNDP has revised the in vitro testing requirements for healthcare antiseptics. We currently recommend a modified time-kill assay as a means of assessing of how rapidly an antiseptic drug product produces a bactericidal effect and defining the spectrum of activity of the antiseptic drug product. Since we have evaluated numerous chlorhexidine gluconate in vitro studies, we currently no longer recommend minimum inhibitory concentration studies against the organisms described in the 1994 TFM for chlorhexidine gluconate drug products. Therefore, MIC testing is not necessary to support approval for chlorhexidine gluconate drug products. Instead, we recommend a modified in vitro time-kill study.**

The time-kill study showed that SoluPrep Film-Forming Prep Tinted (full strength-1X), SoluPrep Film-Forming Prep Colorless (full strength-1X), and the active control ChloraPrep® produced ≥ 5 log reduction (>99.9%) killing effect in 3 minutes and 5 minutes all the organisms tested. When SoluPrep Film-Forming Prep Tint and Colorless was diluted to half its strength (0.5X) it produced ≥ 5 log reduction (>99.9%) killing effect in 3 minutes and 5 minutes all the organisms tested. The killing effect or antimicrobial activity of a drug needs to be ≥ 3 log reduction to be considered an active ingredient. When SoluPrep Film-Forming Prep Tint and Colorless was diluted to 0.01% (0.0001X) it produced ≤ 0.70 log reduction killing effect in 3 minutes and 5 minutes all the organisms tested. This is considered to be an inactive concentration. Overall, the results of the time-kill studies provided by the sponsor indicate that the test products SoluPrep Film-Forming Prep Tint and SoluPrep Film-Forming Prep Colorless achieved a >99.9% reduction in viable microbial cells in 3 and 5 minutes. These results are comparable to those achieved with the active control ChloraPrep® with Tint.

Validation of the Neutralization System

The neutralization study was done to ensure that the neutralizing solution employed was effective in neutralizing the antimicrobial properties of the test and reference products. The neutralizers selected for performing these evaluations should not only be able to completely inactivate all bactericidial activity of the residual antimicrobial agent but must also be inherently non-toxic to the test organisms. The neutralizer system must be validated to make certain that the neutralizing solutions function accordingly.

The neutralization followed the guidelines set forth in the ASTM E 1054-08 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents." This control assay determined the neutralizer effectiveness by recovering and quantifying microorganism populations using agar media and is appropriate for antimicrobial agents that can be chemically inactivated or diluted to sub-inhibitory levels. The procedure was performed using one gram-negative (*Escherichia coli*, ATCC 11229) and one gram-positive (methicillin-resistant *Staphylococcus aureus*, ATCC 33591), whereas the gram-positive strain was antibiotic resistant. The neutralizing buffer used in this study is Butterfield's Phosphate Buffered Dilution Water (PBDW) containing 0.3% lecithin, 1.0% Tween 80, and 1.0 Tamol. Viability of test strains and product effectiveness to inhibit microorganisms were set up as growth control and effectiveness control, respectively. Neutralization of SoluPrep™ Tint Solution, SoluPrep™ Clear Solution, and ChloraPrep® Tint Solution were verified at MicroBiotest, Inc.

Neutralizer effectiveness (Test 1): Diluted inoculum was added into a tube containing 9 mL of sampling solution with neutralizers yielded a final inoculum concentration of approximately 30-100 CFU/mL. One mL aliquot of the test (SoluPrep™ Tint and Clear) or control (ChloraPrep® Tint) product was added to the sample. Triplicate plates containing 1 mL aliquots of immediate (< 30 seconds) and 30 minutes (± 2 minutes) post

inoculation were performed as described in Appendix II "Neutralizer Effectiveness Evaluation" section.

Neutralizer toxicity (Test 2): Diluted inoculum was added into a tube containing 9 mL of sampling solution with neutralizers yielded a final inoculum concentration of approximately 30-100 CFU/mL. Triplicate plates containing 1 mL aliquots of immediate (< 30 seconds) and 30 minutes (± 2 minutes) post inoculation were performed as described in Appendix II "Neutralizer Effectiveness Evaluation" section.

Test microorganism viability control (Test 3): Diluted inoculum was added into a tube containing 10 mL of sampling solution without neutralizers yielded a final inoculum concentration of approximately 30-100 CFU/mL. Triplicate plates containing 1 mL aliquots of immediate (< 30 seconds) and 30 minutes (± 2 minutes) post inoculation were performed as described in Appendix II "Neutralizer Effectiveness Evaluation" section.

Test product control (Test 4): Diluted inoculum was added into a tube containing 10 mL of test (SoluPrep™ Tint and Clear) or control (ChloraPrep® Tint) product yielded a final inoculum concentration of approximately 30-100 CFU/mL. Triplicate plates containing 1 mL aliquots of immediate (< 30 seconds) and 30 minutes (± 2 minutes) post inoculation were performed as described in Appendix II "Neutralizer Effectiveness Evaluation" section.

The following two tables (Table 11 and 12) below provide summaries of the data generated during the study.

Table 11. Neutralizer effectiveness control results. Results expressed as log_{10} colony forming units (CFU) per mL

Microorganism	*Escherichia coli*, ATCC 11229				*Staphylococcus aureus*, ATCC 33591			
Test 1 - Neutralizer Effectiveness (3M CHG/IPA Film-Forming Skin Prep Tinted)								
Contact Time	< 30 seconds		30 minutes		< 30 seconds		30 minutes	
Results	Log_{10} CFU/mL	Difference from Test 3	Log_{10} CFU/mL	Difference from Test 3	Log_{10} CFU/mL	Difference from Test 3	Log_{10} CFU/mL	Difference from Test 3
Rep. 1	1.87		1.83		1.76		1.75	
Rep. 2	1.90		1.80		1.71		1.76	
Rep. 3	1.88		1.93		1.73		1.78	
Average	1.88	0.07	1.85	0.06	1.73	0.06	1.76	0.09
Test 1 - Neutralizer Effectiveness (3M CHG/IPA Film-Forming Skin Prep Colorless)								
Contact Time	< 30 seconds		30 minutes		< 30 seconds		30 minutes	
Results	Log_{10} CFU/mL	Difference from Test 3	Log_{10} CFU/mL	Difference from Test 3	Log_{10} CFU/mL	Difference from Test 3	Log_{10} CFU/mL	Difference from Test 3
Rep. 1	1.92		1.76		1.84		1.79	
Rep. 2	1.92		1.84		1.76		1.74	
Rep. 3	1.92		1.95		1.81		1.75	
Average	1.92	0.03	1.85	0.07	1.80	-0.01	1.76	0.09
Test 1 - Neutralizer Effectiveness (Chloraprep with Tint)								
Contact Time	< 30 seconds		30 minutes		< 30 seconds		30 minutes	
Results	Log_{10} CFU/mL	Difference from Test 3	Log_{10} CFU/mL	Difference from Test 3	Log_{10} CFU/mL	Difference from Test 3	Log_{10} CFU/mL	Difference from Test 3
Rep. 1	1.85		1.83		1.76		1.72	
Rep. 2	1.83		1.87		1.71		1.76	
Rep. 3	1.87		1.83		1.69		1.75	
Average	1.85	0.10	1.84	0.07	1.72	0.08	1.74	0.10

Table 12. Neutralizer effectiveness control results. Results expressed as log_{10} colony forming units (CFU) per mL

Microorganism	*Escherichia coli*, ATCC 11229				*Staphylococcus aureus*, ATCC 33591			
Test 2 - Neutralizer Toxicity								
Contact Time	< 30 seconds		30 minutes		< 30 seconds		30 minutes	
Results	Log_{10} CFU/mL	Difference from Test 3	Log_{10} CFU/mL	Difference from Test 3	Log_{10} CFU/mL	Difference from Test 3	Log_{10} CFU/mL	Difference from Test 3
Rep. 1	1.95		1.88		1.87		1.90	
Rep. 2	1.95		1.92		1.85		1.77	
Rep. 3	1.89		1.87		1.77		1.83	
Average	1.93	0.02	1.89	0.03	1.83	-0.03	1.83	0.02
Test 3 – Test Microorganism Viability Control								
Contact Time	< 30 seconds		30 minutes		< 30 seconds		30 minutes	
Results	Log_{10} CFU/mL		Log_{10} CFU/mL		Log_{10} CFU/mL		Log_{10} CFU/mL	
Rep. 1	1.96		1.92		1.86		1.80	
Rep. 2	1.92		1.92		1.77		1.86	
Rep. 3	1.97		1.91		1.77		1.88	
Average	1.95		1.92		1.80		1.85	
Test 4 – Test Product Control (representative of all three products)								
Contact Time	3 minutes							
Results	Log_{10} CFU/mL				Log_{10} CFU/mL			
Rep. 1	0.00				0.00			
Rep. 2	0.00				0.00			
Rep. 3	0.00				0.00			
Average	0.00				0.00			

***Reviewer's comment**: For Escherichia coli and Staphylococcus aureus, no significant statistical difference was found between the average log_{10} values of the numbers control and the average log_{10} values for the toxicity control, test products (3M™ CHG/IPA tint and clear) or active control (Chloraprep®). These observations are made based upon the guidelines for neutralization validation in ASTM E1054-08 (reapproved 2013) – "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents." This document states that a log_{10} difference of 0.2 has been previously used for neutralization assays and that a difference determined between two samples of 0.2 log_{10} is considered a significant statistical difference. Here the sponsor has used a value of 0.3 log_{10} rather than the 0.2 log_{10} as a measure of significant statistical difference. We had found the sponsor's protocol for the neutralization validation acceptable; however, we did not noticed that the sponsor proposed to use a value of 0.3 log_{10} rather than the 0.2 log_{10} as a measure of significant statistical difference. This reviewer used both the 0.2 log_{10} and 0.3 log_{10} values and found that the statistical difference was well below the 0.2 log_{10} value. The sponsor's use of the 0.3 log_{10} value is acceptable. We will need to observe more carefully sponsor's proposal on the statistical value difference and inquire why its statistical value is different from what is proposed in the ASTM E1054-08.*

In this study the neutralization is considered effective if the post-preparation sample recovered is not more than 0.3 log_{10} less than the Viability Control sample. The sampling solutions are considered non-toxic if the Toxicity Control sample is not more than 0.3 log_{10} less than the Viability Control sample. The sponsor stated that for the neutralizer effectiveness (Test 1), if the log_{10} CFU/mL of the test sample was not more than 0.3 log_{10} less than the test microorganism viability control sample (Test 3), the neutralizer was considered effective.

Neutralizer effectiveness was calculated using the following equation: [Log_{10} CFU/mL from the Viability Control] – [Log_{10} CFU/mL from the test sample]. The sponsor also stated that for the neutralizer toxicity (Test 2), if the neutralizer toxicity control was not more than 0.3 log_{10} less than the test microorganism viability control sample (Test 3), the neutralizer was considered nontoxic. Neutralizer toxicity was calculated using the following equation: [Log_{10} CFU/mL from the Viability Control] – [Log_{10} CFU/mL from the toxicity control]. Overall, these results indicate that the neutralizer was effective and non-toxic to the test organisms.

Sterility Control:
Triplicate plates of each type of agar medium employed for a testing session were incubated with the test. In addition, triplicate 1 mL aliquots of Phosphate Buffer Dilution Water and neutralizer was plated using the appropriate plating technique in at least one of the agar media used for a particular test date. All plates were incubated with the test at 36±1°C for 48±2 hours.

Reviewer's comment: The sponsor stated that all sterility controls exhibited no growth. This is acceptable.

Antibiotic Resistance:
On the day of the test, an individual Mueller-Hilton agar plate was streaked with the prepared test culture in a crosshatch pattern and an appropriate antibiotic disk was added to the center of the plate. The plates were incubated for 24±2 hours at 36±1°C. Upon completion of incubation, the plate was observed and the zone of inhibition (the area immediately surrounding the antibiotic disk) was measured and documented. A Zone Diameter Interpretive Standards was used to measure the zone of inhibition that would determine if the organism was considered resistant, intermediate, or susceptible.

Table 13. Antibiotic resistance confirmation results.

Microorganism	Antibiotic disk	Zone of Inhibition (mm)	Interpretation[1]
Multidrug-resistant *E. coli*, ATCC BAA-197	Ceftazidime	0	Resistant
	Penicillin	0	Resistant
Multidrug-resistant *E. coli*, ATCC BAA-200	Ceftazidime	0	Resistant
	Penicillin	0	Resistant
Methicillin-resistant *S. aureus*, ATCC 33591	Oxacillin	0	Resistant
Methicillin-resistant *S.aureus*, ATCC 33592	Oxacillin	0	Resistant
Methicillin-resistant *S. epidermidis*, ATCC 51625	Oxacillin	0	Resistant
Multidrug-resistant *S. epidermidis*, ATCC 700563	Cefazolin	0	Resistant
	Oxacillin	0	Resistant
	Erythromycin	0	Resistant
Multidrug-resistant *B. cepacia*, ATCC 55792	Oxacillin	0	Resistant
	Ampicillin	0	Resistant
	Penicillin	0	Resistant
Multidrug-resistant *B. cepacia*, ATCC 700070	Ciprofloxacin	0	Resistant
	Chloramphenicol	0	Resistant
Vancomycin-resistant *E. faecalis*, ATCC 51299	Vancomycin	0	Resistant
Vancomycin-resistant *E. faecalis*, ATCC 51575	Vancomycin	0	Resistant
Vancomycin-resistant *E. faecium*, ATCC 700221	Vancomycin	8	Resistant
Multidrug-resistant *E. faecium*, ATCC 51559	Ampicillin	0	Resistant
	Ciprofloxacin	0	Resistant
	Gentamycin	0	Resistant
Multidrug-resistant *S. pneumoniae*, ATCC 51936	Penicillin	0	Resistant
	Trimethoprim/ Sulfamethoxazole	0	Resistant

[1]Based on Zone Diameter Interpretive Standards provided by the antibiotic disk manufacturer

Table 14. Antibiotic resistance confirmation results.

Microorganism	Antibiotic disk	Zone of Inhibition (mm)	Interpretation[1]
Multidrug-resistant *K. pneumoniae*, ATCC 51503	Ampicillin/ Sulbactam	0	Resistant
	Ceftazidime	0	Resistant
	Piperacillin	0	Resistant
Multidrug-resistant *K. pneumoniae*, ATCC 700603	Ampicillin	0	Resistant
	Ceftazidime	11	Resistant
	Piperacillin	0	Resistant
Multidrug-resistant *S. marcescens*, ATCC 43297	Amikacin	0	Resistant
	Ciprofloxacin	0	Resistant
	Piperacillin	0	Resistant
Multidrug-resistant *S. pneumoniae*, ATCC 700671	Penicillin	0	Resistant
	Trimethoprim/ Sulfamethoxazole	0	Resistant
Macrolide-resistant *S. pyogenes*, ATCC BAA-1411	Erythromycin	0	Resistant
Macrolide-resistant *S. pyogenes*, ATCC BAA-1413	Erythromycin	0	Resistant

[1]Based on Zone Diameter Interpretive Standards provided by the antibiotic disk manufacturer

***Reviewer's comment**: The antibiotic resistance testing was to confirm that the organisms listed above were considered resistant. See Tables 13 and 14. The zone of inhibition on agar plates containing the appropriate antibiotic disks were all considered 0 except for vancomycin-resistant E. faecium (zone of 8 mm) and multidrug-resistant K. pneumonia (zone of 11 mm). According to the Clinical and Laboratory Standards Institute (CLSI) MIC breakpoints: susceptible ≤ 16 mm; intermediate 13 to 15 mm; and resistant ≤ 12 mm. Since both the vancomycin-resistant E. faecium and multidrug-resistant K. pneumonia zone of inhibition are under 12 mm, they are considered resistant. This is acceptable.*

3.1.2 Assessment of Microbiocidal Activity in the Presence of an Organic Challenge of Tinted and Colorless 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation Formulations Using a Modified Time Kill Procedure (EM-05-012981)

This study was designed to supply basic antimicrobial data and to determine how rapidly and effectively the test product kills a variety of microorganisms in the presence of serum. It incorporates the recommendations described in the "Manual of Clinical Microbiology," 5th ed., edited by A.B. Balows et al., ASM, Washington: ACM, 1991. The procedure is based on the ASTM E2315 – 03(2008) Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure.

A single lot each of two complete test formulations (test Products) was tested against a select panel of clinically relevant microorganisms, in the presence of a 5% organic load. The test products will be evaluated using each of the challenge microorganisms at two contact times to yield 24 evaluations. To minimize buffer interference and to minimize reduction of the antimicrobial concentration, the volume of inoculum added to each test product was kept at less than or equal to 1% of the total volume of the test. Samples were removed at two selected contact times (3 minutes and 5 minutes) and neutralized. Serial dilutions, as required were performed and triplicate aliquots of the appropriate dilutions were plated. The plates were incubated and the average colony-forming units (CFU) recovered per mL were determined.

Table 15. List of challenge microorgamisms

Challenge Microorganisms
1. Multidrug-resistant *Escherichia coli*, ATCC BAA-197
2. Multidrug-resistant *Escherichia coli*, ATCC BAA-200
3. Methicillin-resistant *Staphylococcus aureus,* ATCC 33591
4. Methicillin-resistant *Staphylococcus aureus,* ATCC 33592
5. Methicillin-resistant *Staphylococcus epidermidis,* ATCC 51625
6. Multidrug-resistant *Staphylococcus epidermidis,* ATCC 700563

Table 16. Test and initial counts control results. Results expressed as CFU/mL, percent reduction, and log reduction

Microorganism	ATCC No.	Contact Time	Initial Count	Film-Forming Skin Prep - Tinted			Film-Forming Skin Prep - Colorless		
			Avg. CFU/mL	Avg. CFU/mL	Percent Reduction	Log Reduction	Avg. CFU/mL	Percent Reduction	Log Reduction
Multidrug-resistant *Escherichia coli*	BAA-197	3 min.	7.8E+06	< 1.0E+01	> 99.99987	> 5.89	< 1.0E+01	> 99.99987	> 5.89
		5 min.	7.8E+06	< 1.0E+01	> 99.99987	> 5.89	< 1.0E+01	> 99.99987	> 5.89
Multidrug-resistant *Escherichia coli*	BAA-200	3 min.	1.4E+07	< 1.0E+01	> 99.99993	> 6.13	< 1.0E+01	> 99.99993	> 6.13
		5 min.	1.2E+07	< 1.0E+01	> 99.99992	> 6.08	< 1.0E+01	> 99.99992	> 6.08
Methicillin-resistant *Staphylococcus aureus*	33591	3 min.	4.6E+06	< 1.0E+01	> 99.99978	> 5.66	< 1.0E+01	> 99.99978	> 5.66
		5 min.	4.8E+06	< 1.0E+01	> 99.99979	> 5.68	< 1.0E+01	> 99.99979	> 5.68
Methicillin-resistant *Staphylococcus aureus*	33592	3 min.	9.0E+06	< 1.0E+01	> 99.99989	> 5.95	< 1.0E+01	> 99.99989	> 5.95
		5 min.	1.3E+07	< 1.0E+01	> 99.99992	> 6.10	< 1.0E+01	> 99.99992	> 6.10
Methicillin-resistant *Staphylococcus epidermidis*	51625	3 min.	8.5E+06	< 1.0E+01	> 99.99988	> 5.93	< 1.0E+01	> 99.99988	> 5.93
		5 min.	1.3E+07	< 1.0E+01	> 99.99992	> 6.10	< 1.0E+01	> 99.99992	> 6.10
Multidrug-resistant *Staphylococcus epidermidis*	700563	3 min.	7.1E+06	< 1.0E+01	> 99.99986	> 5.85	< 1.0E+01	> 99.99986	> 5.85
		5 min.	6.6E+06	< 1.0E+01	> 99.99985	> 5.82	< 1.0E+01	> 99.99985	> 5.82

***Reviewer's comment*: The results showed that all test material demonstrated >5 log_{10} reductions in the presence of a serum challenge at both the 3-minute and 5-minute time points for all organisms tested, thus meeting the 3 log_{10} reduction to define antimicrobial activity of a time kill. Chlorhexidine gluconate is not largely inactivated by organic material such as blood or the skin protein. This residual activity makes it an attractive choice for skin antisepsis where long-term reduction of microbial flora is desired.**

Validation of the Neutralization System

The neutralization study was done to ensure that the neutralizing solution employed was effective in neutralizing the antimicrobial properties of the test and reference products. The neutralizers selected for performing these evaluations should not only be able to completely inactivate all bactericidial activity of the residual antimicrobial agent but must also be inherently non-toxic to the test organisms. The neutralizer system must be validated to make certain that the neutralizing solutions function accordingly.

The neutralization followed the guidelines set forth in the ASTM E 1054-08 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents." This control assay determined the neutralizer effectiveness by recovering and quantifying microorganism populations using agar media and is appropriate for antimicrobial agents that can be chemically inactivated or diluted to sub-inhibitory levels. The procedure was performed using one gram-negative (*Esherichia coli*, ATCC BAA-197) and one gram-positive (methicillin-resistant *Staphylococcus aureus*, ATCC 33591), whereas the gram-positive strain was antibiotic resistant. The procedure for neutralizer effectiveness (Test 1), neutralizer toxicity (Test 2), test microorganism viability control (Test 3), and test product control (Test 4) are the same as the procedures described above under the study "Assessment of Microbiocidal Activity of Tinted and Colorless 3M™ CHG/IPA Film-

Forming Preoperative Skin Preparation Formulations Using a Modified Time Kill Procedure (EM-05-012700)" on page 20.

Table 17. Neutralizer effectiveness control results for 3M™ CHG/IPA Film-Forming Skin Prep Tinted. Results expressed as mean log_{10} CFU/mL recovered.

Microorganism	Contact Time	Viability Control (Test 3)	Log_{10} CFU/mL Recovered	Difference
Escherichia coli, ATCC BAA-197	< 30 seconds	1.84	1.73	0.11
	30 minutes	1.79	1.79	0.00
Staphylococcus aureus, ATCC 33591	< 30 seconds	1.83	1.74	0.09
	30 minutes	1.79	1.65	0.14

Table 18. Neutralizer effectiveness control results for 3M™ CHG/IPA Film-Forming Skin Prep Colorless. Results expressed as mean log_{10} CFU/mL recovered.

Microorganism	Contact Time	Viability Control (Test 3)	Log_{10} CFU/mL Recovered	Difference
Escherichia coli, ATCC BAA-197	< 30 seconds	1.84	1.74	0.10
	30 minutes	1.79	1.76	0.03
Staphylococcus aureus, ATCC 33591	< 30 seconds	1.83	1.74	0.09
	30 minutes	1.79	1.65	0.14

Table 19. Neutralizer toxicity control results. Results expressed as mean log_{10} CFU/mL recovered.

Microorganism	Contact Time	Viability Control (Test 3)	Log_{10} CFU/mL Recovered	Difference
Escherichia coli, ATCC BAA-197	< 30 seconds	1.84	1.87	-0.03
	30 minutes	1.79	1.78	0.01
Staphylococcus aureus, ATCC 33591	< 30 seconds	1.83	1.77	0.06
	30 minutes	1.79	1.76	0.03

Table 20. Test product control results for 3M™ CHG/IPA Film-Forming Skin Prep Tinted and Colorless. Results expressed as mean log_{10} CFU/mL recovered.

Microorganism	Test Material	Log_{10} CFU/mL Recovered
Escherichia coli, ATCC BAA-197	3M CHG/IPA Film-Forming Skin Prep Tinted	0.00
	3M CHG/IPA Film-Forming Skin Prep Colorless	0.00
Staphylococcus aureus, ATCC 33591	3M CHG/IPA Film-Forming Skin Prep Tinted	0.00
	3M CHG/IPA Film-Forming Skin Prep Colorless	0.00

***Reviewer's comment*: The neutralization is considered effective if the post-preparation sample recovered is not more than 0.3 log_{10} less than the Viability Control sample. The sampling solutions are considered non-toxic if the Toxicity Control sample is not more than 0.3 log_{10} less than the Viability Control sample. The sponsor stated that for the neutralizer effectiveness (Test 1), if the log_{10} CFU/mL of the test sample was not more than 0.3 log_{10} less than the test microorganism viability control sample (Test 3), the neutralizer was considered effective.**

Neutralizer effectiveness was calculated using the following equation: [Log_{10} CFU/mL from the Viability Control] – [Log_{10} CFU/mL from the test sample]. The sponsor also stated that for the neutralizer toxicity (Test2), if the neutralizer toxicity control was not more than 0.3 log_{10} less than the test microorganism viability control sample (Test 3), the neutralizer was considered nontoxic. Neutralizer toxicity was calculated using the following equation: [Log_{10} CFU/mL from the Viability Control] – [Log_{10} CFU/mL from the toxicity control]. Overall, these results indicate that the neutralizer was effective and non-toxic to the test organisms.

Antibiotic Resistance:
On the day of the test, an individual Mueller-Hilton agar plate was streaked with the prepared test culture in a crosshatch pattern and an appropriate antibiotic disk was added to the center of the plate. The plates were incubated for 24±2 hours at 36±1°C. Upon completion of incubation, the plate was observed and the zone of inhibition (the area immediately surrounding the antibiotic disk) was measured and documented. A Zone Diameter Interpretive Standards was used to measure the zone of inhibition that would determine if the organism was considered resistant, intermediate, or susceptible.

Table 21. Antibiotic resistance confirmation results.

Microorganism	Antibiotic disk	Zone of Inhibition (mm)	Interpretation[1]
Multidrug-resistant *E. coli*, ATCC BAA-197	Ceftazidime	0	Resistant
	Penicillin	0	Resistant
Multidrug-resistant *E. coli*, ATCC BAA-200	Ceftazidime	0	Resistant
	Penicillin	0	Resistant
Methicillin-resistant *S. aureus*, ATCC 33591	Oxacillin	0	Resistant
Methicillin-resistant *S.aureus*, ATCC 33592	Oxacillin	0	Resistant
Methicillin-resistant *S. epidermidis*, ATCC 51625	Oxacillin	0	Resistant
Multidrug-resistant *Staphylococcus epidermidis*, ATCC 700563	Cefazolin	0	Resistant
	Oxacillin	0	Resistant
	Erythromycin	0	Resistant

[1]Based on Zone Diameter Interpretive Standards provided by the antibiotic disk manufacturer

Reviewer's comment: The antibiotic resistance testing was to confirm that the following organisms were considered resistant: multidrug-resistant E. coli (ATCC BAA-197), multidrug-resistant E.coil (ATCC BBA-200), methicillin-resistant S. aureus (ATCC 33591), methicillin-resistant S. aureus (ATCC 33592), methicillin-resistant S. epidermidis (ATCC 51625), and multidrug-resistant S. epidermidis (ATCC 700563). The zone of inhibition on agar plates containing the appropriate antibiotic disks were all considered 0. This is acceptable.

3.3 Antimicrobial Resistance

3.3.1 Mechanism of Chlorhexidine Gluconate Resistance

CHG resistance has been studied extensively. CHG is a widely used antiseptic, disinfectant, and preservative with broad-spectrum antimicrobial activity that has been in clinical use for several decades. According to McDonnel and Russell, "Chlorhexidine is probably the most widely used biocide in antiseptic products, in particular in hand washing and oral products but also as a disinfectant and preservative.[14]" It is active against many gram-positive and gram-negative bacteria and fungi, including yeasts. Its lethal action is primarily at the cytoplasmic membrane where disruption of the lipid bilayer occurs[2]. Low-level plasmid resistance has been shown in strains of *Staphylococcus aureus*. This resistance is conferred by the *qacA* gene[20]. Non-plasmid acquired resistance has been induced in strains of *Pseudomonas mirabilis*, *Pseudomonas aeruginosa*, and *Serratia marcescens* by exposing the organisms to increasing concentrations of CHG, although the stability of this phenotype is variable[21, 22, 23, 24].

Since the initial report on the antimicrobial activity of CHG in 1954, there has been no convincing evidence of the development of absolute resistance to CHG despite its widespread use[2, 13, 25]. Instead, researchers have shown that a low level of resistance occurs in some microorganisms, and several different mechanisms for that resistance

have been documented. However, none of these studies reported microorganism that were resistant to currently accepted, clinically relevant concentrations (0.5% to 4% in commercially available formulations) of CHG.

Early reports of resistance to CHG at clinical use concentrations occurred primarily because clinically relevant concentrations in use in the 1960s and 1970s were in the range of 0.05% to 0.1% (500 to 1,000 ppm). Stickler[26, 27] reported isolating gram-negative rods from paraplegic patients requiring intermittent urinary catheterization where the site was cleansed with 0.06% (600 ppm) aqueous CHG prior to the procedure. Kahan[28] reported that six patients developed infections with *Pseudomonas pickettii*, which was also isolated from a 0.05% aqueous solution of CHG.

Since that time, higher levels of CHG have been employed for skin and mucous membrane antisepsis. Commercially available formulations of CHG now range from 0.5% to 4.0%. Up to 0.1% CHG is generally regarded as a preservative level of the antimicrobial, and an oral rinse intended for treating periodontal disease contains 0.12% of the antimicrobial.

The issue of resistance to CHG is one part of the larger question of resistance to biocides and particularly antibiotics. Generally, antibiotics have been shown to act at one or at most, a few specific sites or metabolic pathways in the target microorganism. In contrast, biocides have been shown to have multiple sites of activity. CHG, as stated previously, acts on the cell membrane to disrupt cell integrity causing the loss of cytoplasmic compounds. It interferes with the activity of membrane-bound enzymes, and, when it enters the cell cytoplasm, it inhibits and precipitates intracellular molecules including proteins and nucleotides[2].

Microorganisms have intrinsic resistance mechanisms (the naturally occurring resistance to an antimicrobial that is normal for that organism) to increased levels of CHG. Except for mycobacteria and bacterial endospores (specialized resistance structures of some bacteria), no bacterium or fungus has been found that has absolute resistance to levels of CHG found routinely in topical antiseptics (e.g., 2% CHG in SoluPrep™). Unlike the situation with antibiotics, resistance has not been found to be due to acquisition of plasmids containing specific "resistance genes" from other microorganisms. The various mechanisms of resistance are due to structures and functions already present in the microorganism or metabolic pathways that can be activated in response to the antimicrobial. The structures and functions responsible for increased resistance to CHG include the outer membrane of gram-negative bacteria; the cell wall; cell membrane; efflux pumps, which actively remove CHG from the cell; and biofilms, which act as semi-permeable membranes to isolate the microorganism from the antimicrobial agent[13, 29]. Intrinsic resistance is generally associated with gram-negative bacteria, particularly of the genera *Klebsiella spp*., *Proteus spp*., and *Providencia spp*.[30] However, some gram-negative bacteria (e.g., *E. coli*) are only slightly less sensitive than gram-positive bacteria (e.g., *S. aureus*), which has been reported to be highly sensitive to CHG[29].

It is of interest that resistant organisms found in survey studies were already in the environment, and the use of low levels of the biocide (below clinically effective levels) selected for these organisms allowed them to expand clonally[29, 31]. Martin[32] and Simpson, et al[33] found that microorganisms isolated from an environment where CHG was routinely present have susceptibilities to CHG that were similar to strains of the same micro-organisms isolated from an environment where little or no CHG was present in that environment.

Brooks, et al.[31] found that strains of gram-negative bacteria isolated from around soap dispenser outlets (which dispensed 2% CHG-containing hand soap) were no more resistant than stock cultures of the same species obtained from the ATCC. The investigators concluded that the resistance to CHG was inherent, not acquired, and that continued exposure to the CHG did not allow development of increased resistance to the antimicrobial in the soap.

The issue of antiseptic resistance has been a subject of concern by FDA[34, 35, 36]. Overall, some laboratory studies have shown that exposure to nonlethal amounts of CHG can result in reduced susceptibility, particularly in gram-negative bacteria. Reduced susceptibility in these cases is thought to result from intrinsic mechanisms. The transmission of plasmid-encoded resistance determinants such as *qacA* is possible. Protocols to evaluate the risk for potential biocide resistance and antibiotic cross-resistance have been developed, and chlorhexidine does not pose a threat when evaluated by these methods[37, 38]. Tambe et al., described serial passage of a gram-positive skin flora organism, *Staphylococcus epidermidis* through nonlethal concentrations of CHG did not result in an increased MIC[37]. Knapp et al., recently published the results of their study, which evaluated several formulated products and active ingredients, including CHG, against a range of relevant gram-negative bacteria including *Salmonella enterica*, *Burkholderia cepacia*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*[38]. These authors also used an approach involving exposure to nonlethal concentrations of the microbicides, along with pre- and post-exposure antiseptic and antibiotic susceptibility profiling. They found no significant increases in the MIC, MBC, or antibiotic resistance for any strain. The continued surveillance of clinical and environmental isolates for increased chlorhexidine tolerance is warranted. Surveillance and monitoring pathogen antimicrobial susceptibility studies provide important information. For now, researchers recommend that susceptibility and resistance of microorganisms to CHG should be closely monitored. FDA should continue to request sponsor literature updates and conduct resistance and cross-resistance to antibiotics on chlorhexidine gluconate drug products.

3.3.2 Evaluation of Potential for Development of Antimicrobial Resistance to Tinted 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation Formulation

This study was designed to detect the potential for development of resistance to the chemical test product by sequential passage of a microorganism through increasing concentrations of the antimicrobial included in the culture medium. Potential development of antibiotic cross-resistance was also evaluated.

Reviewer's comment*: On June 4, 2013, we had a face-to-face End-of-Phase 2 Type B meeting with the sponsor. We had informed the sponsor that with regards to in vitro emergence of resistance, they would also need to study the potential for the development of cross-resistance to antibiotics. This information was requested at the April 14, 2011 meeting** (b) (4) ***We informed the sponsor that an evaluation of the potential for cross-resistance can be done by comparing the MICs of several antibiotics for a representative group of organisms, both before and after extended exposure to sublethal levels of the antiseptic. We also asked that sponsor to include Burkholderia cepacia and Acinetobacter in addition to the list of organisms they have proposed and to evaluate clinical isolates in addition to the laboratory strains. We had recommended that the sponsor include chlorhexidine gluconate-only as a control arm in the study.

On November 25, 2013, FDA sent an advice letter to the sponsor in regards to the protocol: EM-05-01258 "Evaluation of Potential for Development of Antimicrobial Resistance to Tinted and Colorless 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation." We had informed the sponsor that it was not clear if it intended to test four clinical isolates of each organism in the cross-resistance procedure. As discussed at the end-of-phase 2 meeting, we recommended that the sponsor test four clinical isolates of each organism for both the cross-resistance and emergence of resistance studies.

We also stated that it was not clear if the sponsor intend to include a chlorhexidine gluconate-only control arm in the cross-resistance study. We had recommended that the sponsor include a chlorhexidine gluconate-arm as a control arm in both the cross-resistance and emergence of resistance studies.

This *in vitro* method determined the potential for development of resistance to chlorhexidine gluconate by sequential passage of several clinically relevant microorganisms through increasing concentrations of the antimicrobial included in the culture medium. Ten repository isolates from eight species and four clinical isolates (2 resistant and 2 non-resistant) from the same eight species were evaluated for a total of 42 isolates. If the microorganisms are able to acclimate to at least a 4-fold increase in the concentration of the test or control product and maintain that increase after three serial passages on media that does not contain the antimicrobial, resistance to the product may have been established.

Table 22. List of challenge microorganisms

Organism	Strain Identification		Clinical Isolates Received from:
	ATCC Number	Clinical Isolate Number	
Acinetobacter baumannii	17904	14002 [1] 10058 [1] 10057 [1] 10059 [1]	(b) (4) University of Louisville

Organism	Strain Identification		Clinical Isolates Received from:
	ATCC Number	Clinical Isolate Number	
Burkholderia cepacia	25608	13052 [1] 13054 [1] 13053 [1] 13055 [1]	Center for Disease Control

Organism	Strain Identification		Clinical Isolates Received from:
	ATCC Number	Clinical Isolate Number	
Enterococcus faecalis	52199 [2]	99824 13046 [2] 99825 13047 [2]	(b) (4) University of Iowa

Organism	Strain Identification		Clinical Isolates Received from:
	ATCC Number	Clinical Isolate Number	
Escherichia coli	11229	99903 10100 [1] 99904 10101 [1]	(b) (4)

Organism	Strain Identification		Clinical Isolates Received from:
	ATCC Number	Clinical Isolate Number	
Pseudomonas aeruginosa	15442	99791 13015 [1] 99792 13016 [1]	(b) (4) University of Louisville

Organism	Strain Identification		Clinical Isolates Received from:
	ATCC Number	Clinical Isolate Number	
Serratia marcescens	14756 43297 [1]	99413 13026 [1] 99452 13027 [1]	Department of Veterans Affairs Medical Center; University of Louisville

Table 22 (continued). List of challenge microorganisms

Organism	Strain Identification			Clinical Isolates Received from:
	ATCC Number	Clinical Isolate Number		
Staphylococcus aureus	33591 [1] 25923 [2]	99510 99511	10113 [1] 10114 [1]	Department of Veterans Affairs (b) (4)

Organism	Strain Identification			Clinical Isolates Received from:
	ATCC Number	Clinical Isolate Number		
Staphylococcus epidermidis	51625 [1]	99530 99532	13031 [3] 13032 [3]	Department of Veterans Affairs Medical Center; University of Louisville

***Reviewer's comment*: The sponsor provided an updated list of organisms to be tested for FDA's consideration, including the antibiotics that would be evaluated for cross-resistance just prior to the June 4, 2013 End-of-Phase 2 Type B meeting. At that meeting, we informed the sponsor that at least four clinical isolates for each organism on the list would be required for testing. The list of organisms for resistance testing is acceptable.**

For each microorganism, per product, the agar surface of 10 plates containing the dilutions of the test and control products and the control plates containing no antimicrobial agent was spot inoculated with a pipet to deliver 0.01 mL. Approximately 10^4 CFU was delivered to an area 5 to 8 mm in diameter. Inoculated plates were allowed to stand undisturbed until the inoculum spots were completely absorbed and then incubated at 36±1°C for 18 to 20 hours.

Greater than or equal to 2 CFU present in an inoculated area was considered positive. Surviving organisms from the maximum noninhibitory concentration (MNC) were passaged twice in medium containing that same concentration of product. Two to five colonies were transferred from the appropriate plate to broth. The suspension was adjusted to approximately 1-2 x 10^6 using spectrophotometric methods extant in the laboratory. Once prepared, the inoculum was used within 30 minutes. Approximately 1.0 x 10^4 CFU (0.01 mL) was applied to 5-8 mm diameter areas as described above.

***Reviewer's comment**: A the June 4, 2013 meeting, we asked the sponsor why they preferred to use the agar dilution rather than the broth dilution. The sponsor explained that MIC values are used to determine susceptibilities of bacteria to evaluate the activity of antimicrobial agents. Agar dilution involves the incorporation of different concentrations of the antimicrobial substance into an agar medium followed by the application of standardized number of cells to the surface of the agar plate. Broth dilutions involves using a 96-well microtiter plate assay where bacteria are inoculated into a liquid growth medium in the presence of varying concentrations of an antimicrobial agent. The sponsor chose to use agar dilution procedure vs. a broth dilution procedure for this assay. We stated that the use of the agar dilution procedure was acceptable.*

A subsequent 2-fold dilution series of the product was prepared with the lowest concentration being equivalent to the MNC observed in the previous step and the testing was repeated using the new dilution series. If the MIC from the new dilution series does not increase compared to the initial MIC, testing was terminated and the product was not considered to have the potential for development of resistance. If the MIC increases, testing was continued in a step-wise fashion until at least 2 rounds of testing result in no change in MIC. If the MIC is 4 or more times the concentration of the initial MIC, the organism was transferred 3 times in fresh medium that does not contain the product, then subjected to the same concentrations of the product that were evaluated in the initial test step.

Table 23. Test results: emergence of resistance

Organism	ID	Minimum Inhibitory Concentration (mg/L)			
		Film-Forming Skin Prep Tinted		2% Aqueous CHG Solution	
		Initial	Post	Initial	Post
Acinetobacter baumannii	ATCC 17904	64	64	64	64
	CI 14002 [1]	64	64	64	64
	CI 10057 [1]	64	64	64	64
	CI 10058 [1]	64	64	64	64
	CI 10059 [1]	32	32	64	64
Burkholderia cepacia	ATCC 25608	513	513	513	513
	CI 13052 [1]	256	256	256	256
	CI 13053 [1]	32	32	32	32
	CI 13054 [1]	128	128	128	128
	CI 13055 [1]	32	32	32	32
Enterococcus faecalis	ATCC 51299 [2]	32	32	32	32
	CI 99824	32	32	16	32
	CI 99825	8	8	8	8
	CI 13046 [2]	8	8	16	16
	CI 13047 [2]	8	8	8	8
Escherichia coli	ATCC 11229	2	2	2	2
	CI 99903	2	2	2	2
	CI 99904	2	2	2	2
	CI 10100 [1]	2	2	2	2
	CI 10101 [1]	2	2	2	2
Pseudomonas aeruginosa	ATCC 15442	128	128	256	256
	CI 99791	64	64	64	64
	CI 99792	32	32	32	32
	CI 13015 [1]	128	128	256	256
	CI 13016 [1]	128	128	128	128
Serratia marcescens	ATCC 14756	128	128	128	128
	ATCC 43297 [1]	128	128	128	128
	CI 99413	32	32	32	32
	CI 99452	64	64	64	64
	CI 13026 [1]	64	64	128	128
	CI 13027 [1]	513	513	513	513

Table 23 (continued). Test results: emergence of resistance

Organism	ID	Minimum Inhibitory Concentration (mg/L)			
		Film-Forming Skin Prep Tinted		2% Aqueous CHG Solution	
		Initial	Post	Initial	Post
Staphylococcus aureus	ATCC 33591 [3]	2	2	2	2
	ATCC 25923 [4]	2	2	2	2
	CI 99510	2	2	2	2
	CI 99511	1	1	2	2
	CI 10113 [3]	1	1	1	1
	CI 10114 [3]	2	2	1	1
Staphylococcus epidermidis	ATCC 51625 [3]	16	16	16	16
	CI 99530	2	2	2	2
	CI 99532	2	2	2	2
	CI 13031 [1]	2	2	2	2
	CI 13032 [1]	2	2	2	2

[1] Multidrug-resistant; [2] Vancomycin-resistant; [3] Methicillin-resistant; [4] Methicillin-sensitive

***Reviewer's comment*: The sponsor confirmed that each challenge organism was done through a comparison of colonies from the inoculum control and test plates. Gram stains were performed on an isolated colony from the positive control and any suspicious colonies noted in the test plates. This procedure was conducted to ensure the purity of each challenge microorganism. The SoluPrep chlorhexidine gluconate was supplied at a chlorhexidine gluconate concentration of 20,000 µg/mL, which is approximately 80 times the MIC of the most resistant isolate tested in this study.**

The endpoints for both the SoluPrep and the aqueous chlorhexidine gluconate solution were the same or varied slightly by one doubling dilution in this study. This study did not show any trend toward higher MIC values with clinical isolates compared to ATCC laboratory strains. Overall the emergence of resistance, the MIC did not increase for any of the strains evaluated; therefore, the product is not considered to have the potential for the development of resistance.

Cross-Resistance to Antibiotics

An evaluation of the potential for cross-resistance was done by comparing the MIC of several antibiotics, both before and after extended exposure to sublethal levels of the antiseptic. The antimicrobial resistance of each microorganism (before and after exposure to the test and control products) to the antibiotics Clindamycin, Oxacillin, Vancomycin, Ampicillin, Ceftazidime, Imipenen, Piperacillin or Tobramycin as appropriate (see table below) was determined by Etest®. The MIC of Penicillin was determined by the well-established broth dilution method.

Table 24. Cross-resistance isolates and antibiotics.

Isolates		Antibiotic
Repository Isolate	Clinical Isolate	
Methicillin-resistant *Staphylococcus aureus* (MRSA), ATCC 33591	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Clindamycin, Oxacillin, Penicillin, Vancomycin
Methicillin-sensitive *Staphylococcus aureus* (MSSA), ATCC 25923	Methicillin-sensitive *Staphylococcus aureus* (MSSA)	Clindamycin, Oxacillin, Penicillin, Vancomycin
Methicillin-resistant *Staphylococcus epidermidis* (MRSE), ATCC 51625	Methicillin-resistant *Staphylococcus epidermidis* (MRSE)	Clindamycin, Oxacillin, Penicillin, Vancomycin
Vancomycin-resistant *Enterococcus faecalis* (VRE), ATCC 51299	Vancomycin-resistant *Enterococcus faecalis* (VRE)	Ampicillin, Vancomycin
Acinetobacter baumannii, ATCC 17904	*Acinetobacter baumannii*	Ceftazidime, Imipenem, Piperacillin, Tobramycin
Burkholderia cepacia, ATCC 25608	*Burkholderia cepacia*	Ceftazidime, Imipenem, Piperacillin, Tobramycin
Escherichia coli, ATCC 11229	*Escherichia coli*	Ceftazidime, Imipenem, Piperacillin, Tobramycin
Pseudomonas aeruginosa, ATCC 15442	*Pseudomonas aeruginosa*	Ceftazidime, Imipenem, Piperacillin, Tobramycin
Serratia marcescens, ATCC 14756	*Serratia marcescens*	Ceftazidime, Imipenem, Piperacillin, Tobramycin
Multi-Drug Resistant (MDR) *Serratia marcescens*, ATCC 43297	Multi-Drug Resistant (MDR) *Serratia marcescens*	Ceftazidime, Imipenem, Piperacillin, Tobramycin

Bacteria were subcultured from stock cultures onto agar and incubated overnight at 36±1°C in ambient air. At least 5 colonies from the overnight cultures were inoculated into 4 mL broth and thoroughly mixed. One-tenth mL of this suspension was transferred into 10 mL broth and incubated on a shaking incubator at 36±1°C for 2 to 6 hours. The suspension of the challenge organism was adjusted with phosphate buffer dilution water to contain approximately 1-2 x 10^6 CFU/mL using spectrophotometric methods. The inoculum was used within 30 minutes.

Etest® strips were used in the cross-resistance study which consist of a predefined gradient of antibiotic concentrations on a plastic strip and are used to determine the MICs of antibiotics. A single Muller-Hinton agar plate for each organism was inoculated in a cross hatch pattern. The appropriate Etest® strips were added to each plate in accordance with the manufacturer's directions. The plates were incubated at 36±1°C for 20 to 24 hours and observed for growth. The zones of inhibition was measured and reported.

For cross-resistance MIC broth dilution, 2 mL for each dilution was placed into sterile tubes. Each tube was inoculated with 0.05 mL of a 1:10 dilution of one of the challenge organisms. The micro-pipet tip was inserted below the surface of the antibiotic/broth solution avoiding any contact between the tip and the walls of the tube. The tip was rinsed in the solution. The tubes were incubated at 36±1°C for 20 to 24 hours and observed for growth. Tubes exhibiting growth at the most concentrated level of the antibiotic was streaked onto the Muller-Hinton agar and incubated at 36±1°C for 20 to 24 hours along with the corresponding viability control tube.

Table 25. Test results: development of cross-resistance

Organism	ID	Minimum Inhibitory Concentration (µg/mL)							
		Ceftazidime		Imipenem		Piperacillin		Tobramycin	
		Initial	Post	Initial	Post	Initial	Post	Initial	Post
Acinetobacter baumannii	ATCC 17904	8	8	0.38	0.38	24	24	0.75	0.75
	CI 14002 [1]	≥256	≥256	1.5	1.5	≥256	≥256	≥256	≥256
	CI 10057 [1]	≥256	≥256	≥256	≥256	≥256	≥256	256	256
	CI 10058 [1]	≥256	≥256	≥256	≥256	≥256	≥256	1.5	1.5
	CI 10059 [1]	≥256	≥256	≥256	≥256	≥256	≥256	192	192
Burkholderia cepacia	ATCC 25608	4	2	8	8	3	3	128	192
	CI 13052 [1]	24	16	≥256	≥256	≥256	≥256	≥256	≥256
	CI 13053 [1]	2	1	0.38	0.25	0.75	0.75	6	6
	CI 13054 [1]	3	3	12	24	8	8	0.38	0.38
	CI 13055 [1]	2	2	8	8	12	8	0.5	0.5

Table 25 (continued). Test results: development of cross-resistance

Organism	ID	Minimum Inhibitory Concentration (µg/mL)							
		Ceftazidime		Imipenem		Piperacillin		Tobramycin	
		Initial	Post	Initial	Post	Initial	Post	Initial	Post
Escherichia coli	ATCC 11229	0.38	0.38	0.25	0.25	3	3	0.5	0.5
	CI 99903	0.19	0.19	0.25	0.25	1.5	1.5	1	1
	CI 99904	0.047	0.047	0.19	0.19	0.38	0.38	0.25	0.25
	CI 10100 [1]	0.25	0.25	0.19	0.19	≥256	≥256	≥256	≥256
	CI 10101 [1]	1	1	0.25	0.25	≥256	≥256	64	64
Pseudomonas aeruginosa	ATCC 15442	3	1.5	1	1	12	3	1.5	1
	CI 99791	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256
	CI 99792	3	3	1.5	1.5	12	12	0.38	0.38
	CI 13015 [1]	2	1	≥256	≥256	24	2	0.38	0.125
	CI 13016 [1]	3	3	≥256	≥256	24	12	0.38	0.38
Serratia marcescens	ATCC 14756	0.094	0.19	0.5	0.5	1	2	3	3
	ATCC 43297 [1]	1.5	1	1	0.38	64	6	8	6
	CI 99413	0.19	0.125	1	0.75	1.5	1	3	1.5
	CI 99452	0.5	0.38	3	0.016	4	2	0.25	0.25
	CI 13026 [1]	0.25	0.125	1.5	0.75	3	3	2	1.5
	CI 13027 [1]	1	0.25	3	0.25	8	2	4	3

Table 25 (continued). Test results: development of cross-resistance

Organism	ID	Minimum Inhibitory Concentration (µg/mL)							
		Clindamycin		Oxacillin		Vancomycin		Penicillin	
		Initial	Post	Initial	Post	Initial	Post	Initial	Post
Staphylococcus aureus	ATCC 33591 [1]	≥256	≥256	≥256	≥256	1	1	250	250
	ATCC 25923 [2]	0.047	0.047	0.38	0.38	≥256	≥256	1	1
	CI 99510	≥256	≥256	16	16	1.5	1.5	2	2
	CI 99511	0.032	0.032	0.5	0.5	1	1	7.8	7.8
	CI 10113 [1]	0.016	0.016	48	48	0.75	0.75	250	250
	CI 10114 [1]	0.047	0.047	32	32	0.75	0.75	125	125
Staphylococcus epidermidis	ATCC 51625 [1]	96	96	≥256	≥256	≥256	≥256	< 0.5	< 0.5
	CI 99530	48	48	≥256	≥256	≥256	≥256	500	500
	CI 99532	≥256	≥256	≥256	≥256	3	3	7.8	7.8
	CI 13031 [3]	0.125	0.125	≥256	≥256	2	2	2	2
	CI 13032 [3]	≥256	≥256	≥256	≥256	3	3	1	1

[1] Methicillin-resistant; [2] Methicillin-sensitive; [3] Multidrug-resistant

Table 25 (continued). Test results: development of cross-resistance

Organism	ID	Minimum Inhibitory Concentration (µg/mL)			
		Ampicillin		Vancomycin	
		Initial	Post	Initial	Post
Enterococcus faecalis	ATCC 51299 [1]	≥256	≥256	≥256	≥256
	CI 99824	≥256	≥256	≥256	≥256
	CI 99825	4	2	1.5	1.5
	CI 13046 [1]	1.5	1	≥256	≥256
	CI 13047 [1]	1	1	≥256	≥256

[1] Vancomycin-resistant

***Reviewer's comment**: The sponsor confirmed that each challenge organism was done through a comparison of colonies from the inoculum control and test plates. Gram stains were performed from the Etest® strip plate and on the viability control plate streak. This procedure was conducted to ensure the purity of each challenge microorganism. Overall the cross-resistance to antibiotics study showed no indication of a change in MIC related to cross-resistance observed for any of the organism/antibiotic combination tested.*

4. CLINICAL SIMULATION STUDIES

OTC patient preoperative skin preparation antiseptics are considered an integral part of hospital infection control strategies. While the benefit of these products is a basic tenet of infection control, data from clinical trials demonstrating the impact of these products on infection rates are lacking. Isolating the contribution of antiseptics to infection control is difficult because these products are part of a multifaceted approach to infection prevention and is further complicated by numerous factors beyond hospital infection control measures, such as patient health status. While direct evidence of the clinical benefit of OTC patient preoperative skin preparation antiseptics is limited, the use of these products remains a standard of care.

FDA was challenged to regulate these OTC patient preoperative skin preparations without methods to directly assess their clinical effect. In response, FDA designated surrogate endpoints, as provided by current regulation. The experience with early NDAs for chlorhexidine gluconate was translated into a series of test methods as described in the 1994 TFM for health care antiseptics and proposed performance criteria as described in the 2015 proposed rule for health care antiseptics. A final rule for health care antiseptic drug products is scheduled to be published on January 15, 2018.

In vivo test methods and evaluation criteria are based on the premise that bacteria reductions translate to a reduced potential for infection and that bacterial reduction can be adequately demonstrated using tests that simulate conditions of actual use for patient preoperative skin preparation. For example, preoperative skin preps are tested against resident skin microflora. Preoperative skin prep testing tests a single application of the product on a dry skin site (abdomen) and a moist skin site (groin) with higher numbers of resident bacteria. Preoperative skin preps are also required to suppress bacterial growth for 6 hours.

4.1 Pivotal Studies

4.1.1 Study EM-05-012760 (MicroBioTest) and Study EM-05-01360 (BioScience Laboratories)

Two pivotal clinical simulation studies (EM-05-012760: MicroBioTest and EM-05-01360: BioScience Laboratories) were designed to evaluate the antimicrobial efficacy and safety of SoluPrep Film-Forming Sterile Surgical Solution (Tinted and Clear), placebo saline control, and active control ChloraPrep® with Tint on the abdominal and inguinal regions. The procedures used in these pivotal studies were based on the American Society for Testing and Materials (ASTM) E1173-01 (reapproved 2009): Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations and the FDA 1994 Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Tentative final monograph (TFM) for Health Care Antiseptic Drug Products (59 FR 31402).

Reviewer's comment: On June 29, 2012, we had informed the sponsor that a full clinical hold would be removed. After reconsideration from the

CMC point of view the acrylate copolymer in the CHG/IPA formulation was highly similar to the polymer complex that is an inactive ingredient in the sponsor's previously approved DuraPrep Surgical Solution product. Adequate information for the polymer has been provided to support the IND studies. We decided that the copolymer in this submission was not considered to be a novel excipient. Therefore, the sponsor would not need to be qualified as per the Guidance for Industry: Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients.

On February 20, 2014, FDA had a teleconference meeting with the sponsor. The sponsor asked FDA that if one of the pivotal studies generated data where the 3M™ test products and ChloraPrep® showed similar efficacy and were significantly better than saline, yet none of the active products met the current efficacy requirements defined by FDA, would the study results be accepted for demonstration of efficacy? We said no, for the study to be valid the test product and active control must meet the performance criteria and the test and active control performances must be superior to the vehicle control. The primary study outcome should be the percentage of subjects who meet the effectiveness criteria. Evaluate the mean log reduction as a secondary endpoint.

There was one additional pivotal study (EM-05-012759) that was conducted at BioScience Laboratories that was discontinued prematurely due to technical and data quality issues; thus, efficacy data were not evaluable, and only safety data were reported from this study.

Reviewer's comment: On February 20, 2014, FDA had a teleconference meeting with the sponsor regarding the stalled development program. The sponsor notified FDA via email on January 14, 2014 that they recently uncovered multiple compliance and data integrity issues at BioScience Laboratories and that they wanted to obtain FDA's advice on how to proceed. The issues involved non-compliance with GCP/GLP and numerous errors with bacteria enumeration and documentation for the first 27 subjects. The sponsor was first made aware of these issues on November 21, 2013 by BioScience Laboratories. The sponsor placed the clinical efficacy study at BioScience Laboratories on hold. FDA agreed that the sponsor officially stop the study. We also agreed that the sponsor may conduct a completely new study at BioScience Laboratories if BioScience Laboratories can address and correct any audit issues.

Two pivotal clinical simulation studies (EM-05-012760: MicroBioTest and EM-05-01360: BioScience Laboratories) both were entitled: Assessment of the Antimicrobial Efficacy of 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation against Resident Human Skin Flora on the Abdominal and Inguinal Regions.

Study Objectives

EM-05-012760: MicroBioTest – The primary objective of this study was to evaluate the antimicrobial efficacy of 3M™ CHG/IPA Prep (10.5 mL and 26 mL tinted configurations) and demonstrate that the product provided a lower bound of the 95% confidence interval of percent responders that was greater than or equal to 70%. On the abdomen, a responder was defined as a subject with a 2 $\log_{10}/cm^2$ bacterial reduction at 10 minutes and for whom the skin flora did not return to baseline at 6 hours. On the groin, a responder was a subject with a 3 $\log_{10}/cm^2$ bacterial reduction at 10 minutes and for whom the skin flora did not return to baseline at 6 hours.

Table 26. Description of test products used in Study EM-05-012760

Treatment Code	Study Product	Description
10.5	3M CHG/IPA Prep	Contains 2% w/v chlorhexidine gluconate (CHG) and 70% v/v isopropyl alcohol (IPA) in combination with an acrylate copolymer. 10.5-mL applicator, tinted.
26	3M CHG/IPA Prep	Contains 2% w/v chlorhexidine gluconate (CHG) and 70% v/v isopropyl alcohol (IPA) in combination with an acrylate copolymer. 26-mL applicator, tinted.
CP	ChloraPrep	Marketed active control. Contains 2% w/v chlorhexidine gluconate (CHG) and 70% v/v isopropyl alcohol (IPA). 26-mL applicator, Hi-Lite Orange® Tint.
S	Saline	Negative control. Sterile 0.9% saline. 20 mL bottle.

Reviewer's comment: On February 20, 2014, FDA had a teleconference type B meeting (End-of-Phase 2) with the sponsor. We had informed the sponsor that a responder should be defined as achieving the success criteria at both the 10 minute and 6 hour sample collection times. The success criteria at 10 minutes are a 2-$\log_{10}$ per cm^2 reduction of bacterial counts on the abdominal test area and a 3-$\log_{10}$ per cm^2 reduction on the inguinal test areas. The success criteria at 6 hours are having bacterial counts below the baseline at the 6-hour sample collection time.

Additional statistical comment was request by FDA (emailed on February 19, 2014): Provide the following details on the analyses performed at the MicroBiotes site: 1) Was the study completed and the database locked for the MicroBiotest site prior to conducting this analysis? 2) If not, what procedures were in place to prevent the introduction of operational bias into the study? The sponsor's response to FDA questions:

- ***The MicroBiotest study is still ongoing (enrollment is at approximately 50%).***
- ***The data provided to the 3M™ statistician was blinded to the actual treatment assignment provided to each treatment group. Therefore, it is possible to identify three active groups (different***

sample size than negative control), but it was not possible to identify which active treatment group is which.

- ***The final analysis for the study was being performed by the CRO, who remains fully blinded. The analysis was performed according to the Statistical Analysis Plan, which was approved before the studies started.***
- ***No results have been communicated to either study site.***
- ***No results were communicated to 3M™ study personnel outside the biostatistics group until three months after the studies had started. Results shared with the 3M™ clinical personnel typically included verbal summary of overall response rate for all actives.***
- ***No new instructions have been given to either site. Both sites were given the same initial training and no changes have been made to that instruction. 3M™ stated that it was confident that no operational bias has been introduced into either study.***

We stated that any unplanned interim analysis at any time during an active study is not recommended due to concerns of potential operational biases and study misconduct. We expressed concerns and asked the sponsor why the data from MicroBiotest were analyzed prior to data lock. The sponsor replied that it wanted to ensure it would be a valid study, and that it did not unblind the study. We stated that interim analyses should be pre-specified and documented in the statistical analysis plan.

EM-05-013260: BioScience Laboratories - The primary objective of this study was to evaluate the antimicrobial efficacy of 3M™ CHG/IPA Prep (10.5 mL colorless) and 3M™ CHG/IPA Prep with HEDTA (10.5 mL colorless) and demonstrate that the product provided a lower bound of the 95% confidence interval of percent responders that was greater than or equal to 70%. On the abdomen, a responder was defined as a subject with a 2 $\log_{10}/cm^2$ bacterial reduction at 10 minutes and for whom the skin flora did not return to baseline at 6 hours. On the groin, a responder was a subject with a 3 $\log_{10}/cm^2$ bacterial reduction at 10 minutes and for whom the skin flora did not return to baseline at 6 hours.

Table 27. Description of test products used in Study EM-05-013260

Treatment Code	Study Product	Description
Clear	3M CHG/IPA Prep	Contains 2% w/v chlorhexidine gluconate (CHG) and 70% v/v isopropyl alcohol (IPA) in combination with an acrylate copolymer. 10.5-mL applicator, colorless.
Clear-H	3M CHG/IPA Prep with HEDTA	Contains 2% w/v chlorhexidine gluconate (CHG) and 70% v/v isopropyl alcohol (IPA) in combination with an acrylate copolymer and (hydroxyethyl)ethylenediaminetriacetic acid (HEDTA). 10.5-mL applicator, colorless.
CP	ChloraPrep	Marketed active control. Contains 2% w/v chlorhexidine gluconate (CHG) and 70% v/v isopropyl alcohol (IPA). 10.5-mL applicator, Clear.
S	Saline	Negative control. Sterile 0.9% saline. 20-mL bottle.

***Reviewer's comment**: In an e-mail communication date July 2, 2014, the sponsor stated that the precedent for use of HEDTA is documented in the FDA inactive ingredient database where it cites an approved topical drug solution formulated with 40,000 ppm (0.4%) HEDTA well above the level proposed for the sponsor's product which is* (b) (4)*. The sponsor provided detailed information to FDA to justify that HEDTA is not soluble in alcohol and that the activity of isopropanol would not be affected by the addition of HEDTA. The sponsor also stated that the intention to provide data to support that CHG effectiveness is not affected by minor changes in ionic environment. The sponsor also provided additional information on the concentration of CHG versus HEDTA and NDA precedent with analogous molecules.*

On January 20, 2015, FDA had a face-to-face Pre-NDA meeting with the sponsor. The sponsor added one arm to its pivotal clinical study (EM-05-013260) that included formulation containing a trace amount of HEDTA in order to directly compare it to the sponsor's non-HEDTA solution. The sponsor stated that trisodium HEDTA (b) (4) ***We stated that it appears reasonable form a nonclinical perspective. However, final determination of the nonclinical studies required to support the sponsor's NDA submission will only be determined after completion of review of all data in the NDA submission. FDA also stated that it appeared reasonable not to conduct additional clinical safety trials prior to NDA submission but whether the product with HEDTA is safe for use in the nonprescription drug setting is a matter for NDA review. See Pharmacology/Toxicology Reviewer, Dr. Wafa Harrouk's review in DARRTS.***

Methodology (EM-05-012760 & EM-05-013260)
This was a randomized (within subject), controlled, third-party blind, single-center study in healthy volunteers where each subject receives 2 of the 4 possible study products on the groin and/or 2 of the 4 possible study products on the abdomen. The study staff performing the bacterial enumeration and the statistician performing the analysis was blinded to the study products.

Randomization and Blinding (EM-05-012760 & EM-05-013260)
The left and right test areas on each subject's abdominal and inguinal regions were assigned according to a computer-generated randomization schedule to receive 1 of the 4 study products. Randomization was balanced between left and right sides. There were 6 different combinations of treatments. To provide an ample number of subjects, the treatment groups were randomized into 12 blocks of 18-18-18-4-4-4 for each of groups 1 through 6, respectively. Baseline and post-prep samplings were randomized to sites

within each test area. The same test site randomization was used on each side of a subject's body to reduce the possibility of sampling errors.

***Reviewer's comment*: On May 25, 2012, in an advice letter sent to sponsor, we request the sponsor to include along with randomization of the test sites, that sampling within the sites also be randomized.**

Pretreatment Phase (washout) (EM-05-012760 & EM-05-013260)
Healthy subjects who met the inclusion/exclusion criteria were entered into a minimum 14-day pretreatment phase to allow for the removal of any antimicrobial agents from the subject's skin. Subjects were refrain from the use of products containing antibacterial agents, antibiotics, contact with chemically treated swimming pools or hot tubs, hot waxes, and depilatories on the test areas during this phase. They were given personal hygiene kits containing non-antimicrobial products for use during the study. In addition, subjects were not to shower or bathe for at least 72 hours prior to their scheduled screening appointment; sponge baths were allowed as long as the abdominal and inguinal areas were avoided. The site scheduled all subjects for a hair removal (clipping) and skin evaluation visit at 72 hours prior to the screening visit.

***Reviewer's comment*: We had previously recommended sponsors that the protocol should include the fact that, even though the subjects are not allowed to shower or bathe the test site for at least 48 to 72 hours of being sampled, they are allowed to take sponge baths, assuring that no sponging the test site area occurs. The washout period is standard and is acceptable.**

Screening Phase (EM-05-012760 & EM-05-013260)
During the screening phase, prior to collection of the screening baseline samples, the investigator completed the screening inclusion/exclusion criteria form and performed a visual inspection of the skin test areas. Screening baseline samples were collected from the center of each contralateral test areas within each anatomical region using a cup scrub technique. Samples from both the left and right sides of the abdominal and/or inguinal regions were to meet the inclusion criteria values to be enrolled in the Treatment Phase. Subjects who qualified were notified and scheduled for a treatment appointment at least 72 hours after the screening appointment. Subjects were not allowed to shower or bathe for at least 72 hours prior to the treatment appointment; sponge baths were allowed as long as the abdominal and inguinal areas were avoided.

***Reviewer's comment*: The screening phase procedure is standard and is acceptable.**

Table 28. Schedule of study procedures

Study Procedure	14-Day Pretreatment Phase	Screening Phase	Treatment Day			
			Baseline	Skin Prep	Post-prep: 10 minutes ± 30 seconds	Post-prep: 6 hours ± 30 minutes
Informed consent	X					
Inclusion/exclusion criteria	X	X	X			
Demographics			X			
Assign screening number	X					
Dispense personal hygiene kit	X					
Perform clipping (if necessary)	X[a]	X[a]				
Perform visual skin assessment		X	X			
Assign subject number			X			
Skin irritation rating			X		X	X
Apply study products				X		
Collect microbial samples		X	X		X	X
Protocol compliance verification[b]						X

[a] If clipping was needed, subjects were to return to the study site 72 hours prior to their scheduled Screening Day and Treatment Day.
[b] Verification was to confirm that subjects complied with the study instructions during the 6 hours post-prep.

A sufficient number of who met the inclusion criteria were enrolled into the Treatment Phase such that approximately 660 abdominal and 660 inguinal regions (randomized between left and right sides) were evaluable for efficacy at study completion. On treatment day, subjects were assigned a subject number and were randomized into 1 of the 6 treatment groups shown in the tables below.

Table 29. Subject treatment groups (EM-05-012760)

Treatment Group	Left and Right Treatments
1	3M CHG/IPA Prep 10.5 mL and 3M CHG/IPA Prep 26 mL
2	3M CHG/IPA Prep 10.5 mL and ChloraPrep
3	3M CHG/IPA Prep 26 mL and ChloraPrep
4	3M CHG/IPA Prep 10.5 mL and Saline
5	3M CHG/IPA Prep 26 mL and Saline
6	ChloraPrep and Saline

Abbreviations: 3M CHG/IPA Prep=3M™ Chlorhexidine Gluconate/Isopropyl Alcohol Film-Forming Preoperative Skin Preparation; ChloraPrep= ChloraPrep® Patient Preoperative Skin Preparation

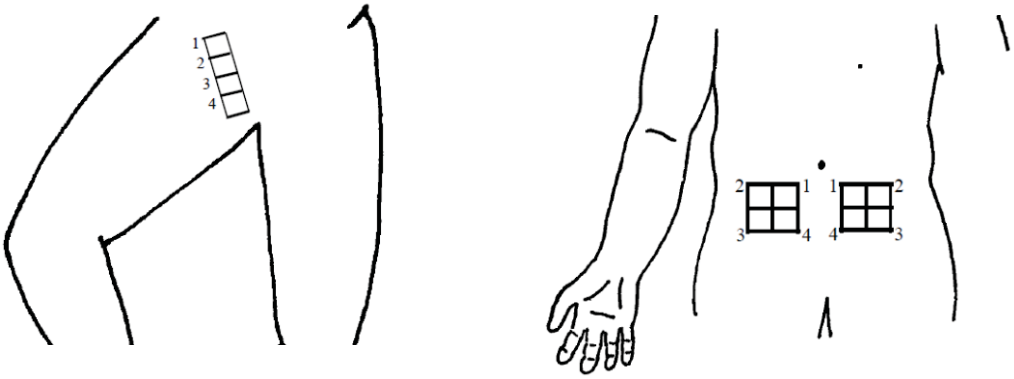
Table 30. Subject treatment groups (EM-05-013260)

Treatment Group	Left and Right Treatments
1	3M CHG/IPA Prep and 3M CHG/IPA Prep with HEDTA
2	3M CHG/IPA Prep and ChloraPrep
3	3M CHG/IPA Prep with HEDTA and ChloraPrep
4	3M CHG/IPA Prep and saline
5	3M CHG/IPA Prep with HEDTA and saline
6	ChloraPrep and saline

Abbreviations: 3M CHG/IPA Prep=3M™ Chlorhexidine Gluconate/Isopropyl Alcohol Film-Forming Preoperative Skin Preparation; ChloraPrep= ChloraPrep® Patient Preoperative Skin Preparation; HEDTA=(hydroxyethyl)ethylenediaminetriacetic acid

***Reviewer's comment**: The sponsor stated that the sample size was determined through simulation using R 2.1.8.0, Excel 2007 and SAS® 9.2, Proc Means. See detailed description in Dr. Mushfiqur Rashid's review in DARRTS.*

After completing the treatment inclusion/exclusion criteria form and visually inspecting the skin test areas, a sterile template to mark 4 sampling sites (for baseline pre-prep, 10-minute post-prep, 6-hour post-prep, and unused) within each abdominal test area. Baseline samples were collected using a cup scrub technique from the right and left test areas per the sampling randomization schedule; for the abdominal region, sampling was randomized to Sites 1, 3, and 4 (Site 2 was always the unused site). Similarly, the inguinal test areas were marked and baseline samples were collected per the sampling randomization schedule; for the inguinal region, sampling was randomized to Sites 2, 3, and 4 (Site 1 was always the unused site). Following baseline sample collection, contralateral abdominal and inguinal test areas were prepped with 1 of the 4 study products according to the treatment randomization code assigned to each subject. The duration of each prep procedure was recorded on the appropriate form. Post-prep timing began after study product application, including the 3-minute drying time.



Treatment Code:

- 3M™ CHG/IPA Prep, 10.5 mL tinted
- 3M™ CHG/IPA Prep, 10.5 mL colorless
- 3M™ CHG/IPA Prep with HEDTA, 10.5 mL colorless
- 3M™ CHG/IPA Prep, 26 mL tinted

1. Aseptically remove investigational product from sterile packaging and maintain sample in a sterile environment prior to sample application.
2. With the sponge in a downward position, snap the lever on the applicator allowing the fluid to flow into the sponge. Wait until the sponge fills completely.
3. Apply the product with repeated back-and forth strokes for 30 seconds on the abdomen or 2 minutes on the groin.
4. Do not allow solution to pool. Use sponge applicator to absorb excess solution and continue to apply.
5. Allow the test area to dry for 3 minutes.
6. Use a fresh applicator for each test area.
7. Contact time begins after step 5.
8. Residue product should be removed from test areas after the final sample collection is complete using alcohol wipes.
9. Used applicators should be returned to packaging and labeled with the subject number.

Treatment Code:

- ChloraPrep®, 10.5 mL clear
- ChloraPrep®, 26 mL Hi-Lite Orange Tint

1. Aseptically remove marketed active control product from sterile packaging and maintain sample in a sterile environment prior to sample application.
2. Hold the applicator with the sponge down. Pinch the wings only once to activate the ampoule and release the product.
3. Wet the sponge by pressing and releasing the sponge against the test area until liquid is visible on the skin.
4. Completely wet the area with product using gentle repeated back-and –forth strokes for 30 seconds on the abdomen or 2 minutes on the groin. (MicroBiotest)
4. Completely wet the area with product using repeated back-and –forth strokes for 30 seconds on the abdomen or 2 minutes on the groin. (BioScience Laboratories)
5. Do not allow solution to pool.
6. Allow the test area to air dry for 3 minutes.
7. Use a fresh applicator for each test area.
8. Contact time begins after step 6.
9. Residual product should be removed from test areas after the final sample collection is complete using alcohol wipes.
10. Used applicators should be returned to packaging and labeled with the subject number.

Reviewer's comment: On February 20, 2014, FDA had a teleconference type B meeting (End-of-Phase 2) with the sponsor. The sponsor asked FDA if we have any comment on ChloraPrep® 's application instructions. The sponsor wanted to confirm from FDA on the instructions that should be included for applying ChloraPrep® in their pivotal study. This information was needed to finalize a new clinical protocol that was going to be submitted to FDA for review prior to starting an additional pivotal study. FDA responded that the sponsor could use either the current label directions (that includes the term "gentle"), or the original instructions (that does not have the term "gentle") for the ChloraPrep® 10.5 mL Applicator that was relayed in the February 11, 2014 e-mail response. However, we stated that we do not recommend that the sponsor change the MicroBiotest study that was currently in progress.

Treatment Code:

- **Saline (Sterile 0.9% saline – 20 mL bottle, with sponge applicator)**

1. Remove petri plate from sleeve. Place lid sterile side up on bench.
2. Pour 20 mL sterile saline into lid.
3. Aseptically remove applicator from sterile packaging.
4. Holding applicator handle place one side of sponge into saline, allowing fluid to flow into sponge. Gently press sponge against lid until saturated. Flip sponge and repeat, ensuring both sides of sponge are completely saturated with fluid.
5. Apply the product with repeated back-and-forth strokes for 30 seconds on the abdomen or 2 minutes on the groin, flipping applicator as needed to ensure the test area remains wet during product application.
6. Allow the test area to dry for 3 minutes.
7. Use a fresh applicator for each test area.
8. Contact time begins after step 6.
9. Used applicators should be returned to packaging and labeled with the subject number.

Reviewer's comment: The study product application was applied following the baseline sample collection and randomly assigned contralateral abdominal and inguinal test areas were prepped with one of the four study products. The treatments were randomized between left and right test areas and post-prep sampling times were randomized amongst the sampling sites with a test area.

Microbial samples were collected using a cup scrub technique at 10 minutes post-prep from each test area. After the 10-minute samples were collected, a sterile non-occlusive dressing was secured over the remaining sample sites to allow subjects restricted mobility and to protect the sites from contamination between sampling times. Subjects were allowed to leave the study site as long as they returned within 6 hours for the next sample collection and met the compliance criteria upon their return. After the 6-hour post-prep collection, residual study products were removed from the skin with alcohol wipes. Each test area was evaluated for skin irritation prior to collection of baseline, 10-minute, and 6-hour samples using the Skin Irritation Rating form.

***Reviewer's comment*: The post application sample collection is standard and is acceptable. In the past, we have been recommending sponsors to allow subjects some degree of mobility between the time of treatment and the 6-hour post-treatment sampling by loosely draping the treated skin area with a sterile non-occlusive dressing. Subjects may leave the test facility as long as they return for the 6-hour time point.**

We had informed the sponsor in an advice letter dated May 25, 2012, that they would need to describe how the test formulation material containing the polymer will be removed from the subject's skin after the subject has completed the study. The sponsor stated that the residual study products will be removed from the subject's skin with alcohol wipes. This is also described in the direction for use. This is acceptable.

Sample Collection and Processing (EM-05-012760 & EM-05-01360)
Quantitative cultures were obtained from the test sites using a modification of the cup scrub method of Williamson and Kligman. Due to the film-forming component of 3M™ CHG/IPA Prep, a modification of the standard sampling solution (SSS) was required to dissolve the film for bacterial recovery in the two studies; all microbial samples were collected using modified sampling solution that contained triacetin (glyceryl triacetate).

***Reviewer's comment*: We informed to the sponsor in an advice letter dated May 25, 2012, that we had concerns that the sponsor's product may have physical properties that could interfere with microbiological sampling during the clinical simulation studies, and that their testing approach may need to be modified. The sponsor would need to address whether or not the product can be removed completely to allow sampling of the bacteria trapped in the polymer remaining on the skin during the clinical simulation studies. If trapped bacteria remain viable the sponsor would need to describe how they will recover them. The sponsor stated that due to the film-forming component of the 3M™ CHG/IPA Prep, a modification of the sampling solution is required to dissolve the film for bacterial recovery. The agent used to accomplish this is Crodamol GTCC, a fully saturated triglyceride. The sponsor stated that in order to alleviate concerns that the product may have physical properties that could interfere with microbiological sampling during clinical simulation studies, evaluations were performed using bacterial endospores to ensure that microbial recovery is not impacted by the presence of the film. The sponsor conducted similar type of studies with their previously approved product "DuraPrep Surgical Solution" that also had a film-forming solution. In a follow-up to the June 4, 2013 Type B End-of-Phase 2 meeting, the sponsor submitted an updated protocol (October 9, 2013) for FDA review and comment. The sponsor has changed the composition of the modified sampling solution required to dissolve the film for bacterial recovery from "Crodamol GTCC" to "Triacetin". We informed the sponsor in an advice letter dated February 7, 2014, that it will need to ensure that the**

"Triacetin" does not have any antimicrobial activity. The sponsor submitted the study and it's located in section4.3.2 of this review.

A sterile scrub cup was placed and held firmly on each site. For the first scrub, 1.0 mL of triacetin and 2.0 mL of SSS was aseptically pipetted into the scrub cup. The skin was scrubbed in a circular motion with moderate pressure for 1 minute using a sterile policeman. Using a sterile transfer pipette, the scrub solution was transferred to a sterile collection tube. For the second scrub, an additional 3.0 mL of fresh SSS was pipetted into the scrub cup, and the scrub procedure was repeated. This solution was pooled with the solution from the first scrub for a total sample volume of approximately 6 mL per site.

Reviewer's comment: The microbial sample collection and the scrub cup techniques are standard and are acceptable. However, the MicroBiotest facility used a scrub cup size 2.20 cm I.D., 3.80 cm^2 and the BioScience Laboratories facility used a scrub cup size 2.10 cm I.D., 3.46 cm^2. The TFM does not specify the diameter of the sampling cup used to sample the microorganisms. The TFM describes the following: "Sterile glass cylinders, height approximately 2.5 centimeters, inside diameter of convenient size to place on anatomical area to be sampled. Useful sizes range from approximately 2.5 to 4.0 centimeters." We have approved patient preoperative skin preparation NDA efficacy studies containing studies that have used scrub cup in various sizes. Ultimately, it would be up to the sponsor to choose the scrub cup size they feel that they would get the best results from their studies.

Following collection, each sample tube was capped tightly and pulsed 3 times on a vortex mixer followed by a 2-minute sonication and 30-second vortex. A 1.0 mL aliquot of each sample was diluted into sterile tubes containing 9.0 mL Butterfield's sterile phosphate buffered water (PBW). Serial 10-fold dilutions were performed in PBW. Aliquots (1 mL) of selected dilutions were pour-plated in duplicate using trypticase soy agar containing 0.5% Tween 80 and 0.07 lecithin (TSA+N). Samples were to be plated within 30 minutes of collection. After 72 (± 4) hours of incubation at 30 ± 2°C, colonies were counted, and viable cells in the original sample were calculated using standard methods.

Reviewer's comment: We had been recommending sponsors to include the type of neutralizers they will be using when incorporated into the sampling solution. The sponsor has described in the standard sampling solution (SSS) to contain 75mM phosphate buffer (0.04% monobasic potassium phosphate, and 1.01% dibasic sodium phosphate) containing 0.1% Triton® X-100, 0.3% lecithin, 3.0% polyoxyethylene sorbitan monooleate (Tween® 80), and 1.0% Tamol™ SN; pH 7.9 + 0.1, sterile. This is used for the ChloraPrep® and saline control applicators. The sponsor describes the modified sampling solution (MSS) containing a ratio 1 to 12 of Triacetin SSS. The Triacetin is a fully saturated triglyceride, commonly used as an emollient and is required to dissolve the film formed by the 3M™ CHG/IPA Prep. SSS is used to

neutralize ChloraPrep® and MSS is used to neutralize 3M™ CHG/IPA Prep. This is acceptable.

On February 20, 2014, FDA had a teleconference type B meeting (End-of-Phase 2) with the sponsor. The sponsor stated that it required both testing sites to use manual bacterial plating and enumeration methods. BioScience Laboratories has requested the use of automated methods (i.e., spiral plating and Q-Count), however, the MicroBiotest site does not have this capability. The sponsor asked if FDA would accept data generated using different plating and enumeration methods for each of the pivotal studies. We responded that validated automatic plating and enumeration methods are acceptable.

Selection of Study Population (EM-05-012760 & EM-05-01360)
Each subject identified by a screening identification number and a subject treatment number. Subject treatment numbers were not assigned until a subject passed the screening criteria, including screening baseline bacterial counts. Both the right and left sides of the abdomen and/or inguinal were to meet the screening baseline requirements stated in the Inclusion Criteria to qualify for treatment of that anatomical region in the study. A blinded review meeting took place between the study monitor and the statistician prior to breaking the blind, during which decisions about subjects' population categories were to be made and documented in meeting minutes. The study monitor assessed the data collected for all subjects for protocol violations prior to breaking of the study blind. There were 4 subject populations defined for the study analyses:

- All available subjects (AAS) population: All subjects who consented for the study, including screening visit failures, were termed the AAS population. This population was used for the subject enrollment table and disposition listing.
- Safety population/ Intent-to-treat (ITT) population: All subjects who were randomized and received at least 1 study treatment in the study. The ITT population was used for disposition, demographics, protocol deviation, and safety tables, and all listings except the disposition listing.
- Modified ITT (mITT) population: The mITT data set was used as the primary efficacy population. All subjects who met baseline requirements on at least 1 side of the abdominal region on Treatment Day were included in the mITT analysis for that side of the abdominal region, and all subjects who met baseline requirements on at least 1 side of the inguinal region were included in the mITT analysis for that side of the inguinal region. The exclusion of subjects who did not meet baseline requirements made this data set a modified ITT data set. A treatment side of a subject was excluded from the mITT population when the baseline $\log_{10}$ CFU/cm^2 was outside the range of 3.00 to 5.50 for the abdomen and the range of 5.00 to 7.50 for the inguinal region.

Reviewer's comment: On April 17, 2015, FDA sent an advice letter to the sponsor regarding statistical analysis of the clinical simulation studies. The sponsor implied that a subject had to pass the baseline criteria bilaterally (e.g., both the left and right sides of the abdomen) to

be included in the analysis. FDA previously pointed out, the experimental units are treatment sides. The sponsor asked if FDA agrees that all individual treatment sides that meet treatment day baseline requirements be included in the analysis? We agreed, as long as there is not an attempt to replace non-qualifying sides, we would consider it reasonable to qualify a treatment side for inclusion based on the test day baseline of that treatment side in the primary analysis. Therefore, if an ITT subject has one qualifying side and one non-qualifying side at the test day baseline, the qualifying side could still be used in the primary analysis.

- Per-protocol (PP) population: the PP population was used as a secondary efficacy population. A treatment side was excluded from the PP population when 1 of the following deviation occurred:
  - A laboratory accident resulted in no bacterial count data at 10 minutes.
  - A laboratory accident resulted in no bacterial count data at 6 hours, and the 10 minute $\log_{10}$ reduction was 2.00 or greater on the abdomen or 3.00 or greater on the inguinal.
  - A treatment side had a comprised dressing at 6 hours.
  - A subject was not compliant to study requirements during the time between the 10 minute and 6 hour sample collection.

<u>Reviewer's comment</u>: On April 17, 2015, FDA sent an advice letter on the sponsor's amendment dated March 13, 2014 containing the draft replacement pivotal abdomen/groin clinical study protocol entitled "Assessment of the Antimicrobial Efficacy of 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation Against Resident Human Skin Flora on the Abdominal and Inguinal Region." FDA asked the sponsor to clarify why subjects who do not meet the baseline requirements are randomized and then excluded in the proposed analysis population (modified intent-to-treat). In general, the ITT population consisting of all randomized patients is considered the primary analysis population. We also stated not to exclude or replace patients with missing data in the ITT analysis; rather, count these as non-responders. It was stated in section 8.3 of the protocol that the analysis data may exclude subjects if there are no data available for 10 minutes or 6 hours, due to the occurrence of laboratory accidents or site contamination. Such exclusions can introduce bias and impact the interpretability of the results. We recommended that the sponsor classify such subjects as non-responders.

Inclusion Criteria

Subjects to whom all of these conditions apply will be eligible for enrollment in this study:

1. Subjects of either gender and any race who are at least 18 years of age,
2. Subjects must be in good general health,

3. Subjects who satisfy all Inclusion/Exclusion Criteria and will voluntarily read and sign the Inform Consent Form including authorization to use and disclose protected health information,
4. Subjects who are cooperative and willing to follow Subject Instructions for the study,
5. Subjects who are willing to report to the study facility approximately 72 hours prior to the Screening or Treatment Day sampling for clipping, if needed,
6. Subjects who are willing to avoid showering and tub-bathing within 72 hours prior to Screening and Treatment Days (sponge-baths may be taken; however the lower abdomen and upper thigh regions must be avoided), Subjects who are willing to avoid showering, tub-bathing, swimming, and vigorous physical activity that may cause sweating during the 6 hour ± 30 minute period before the final sampling,
7. Subjects who are willing to return to the study facility within 6 hours of treatment for the final sampling, and
8. Subjects who have Screening Day baseline counts of ≥ 3.00 $\log_{10}$ per cm^2 bilaterally on the abdominal region and/or ≥ 5.00 $\log_{10}$ per cm^2 bilaterally on the inguinal region. (MicroBiotest)
8. Subjects who have Screening Day baseline counts of ≥ 2.5 $\log_{10}$ per cm^2 bilaterally on the abdominal region and/or ≥ 5.00 $\log_{10}$ per cm^2 bilaterally on the inguinal region. (BioScience Laboratories)

<u>Reviewer's comment</u>: In previous discussions with FDA, sponsors were asked to allow subjects over 65 years of age to participate in the study. In these current studies there is no upper age limit for study participations and this is acceptable.

In the previous protocol submitted by the sponsor, the inclusion "Subjects who have Screening and Treatment Day baseline counts in the ranges of 3.0 log_{10} per cm^2 to 6.0 log_{10} per cm^2 per abdominal site and/or 5.0 log_{10} per cm^2 to 8.0 log_{10} per cm^2 per inguinal site" has been revised to read "Subjects who have Screening and Treatment Day baseline counts of ≥ 2.5 log_{10} per cm^2 for BioScience Laboratories and ≥ 3.00 log_{10} per cm^2 for MicroBiotest bilaterally on the abdominal region and/or ≥ 5.0 log_{10} per cm^2 bilaterally on the inguinal region." The inclusion criteria for both BioScience Laboratories and MicroBiotest are standard for this type of study and are acceptable.

Exclusion Criteria

1. Participation in another clinical study in the past 30 days, current participation in another clinical study, or previous participation in this study,
2. Any tattoos, scars, breaks in the skin, or any form of dermatitis, or other skin disorders (including acne) on the applicable test areas,
3. A history of skin allergies,
4. A history of skin cancer within 6 inches of the test areas,
5. Known sensitivity to acrylate-, chlorhexidine gluconate-, or alcohol-containing products, or to medical tape, metals, natural rubber latex, vinyl, or skin-marking inks,

6. A medical diagnosis with physical condition that may put the subject at risk, such as a current or recent severe illness, hepatitis, organ transplant, congestive heart disease, or any immunocompromised conditions, such as AIDS (or HIV positive),
7. Any medical condition or use of any medications that, in the opinion of the Investigator, should preclude participation,
8. Pregnancy, possible pregnancy, attempting pregnancy, or nursing,
9. Topical antimicrobial exposure with 14 days prior to Screening and Treatment Days. Restrictions include, but are not limited to antimicrobial soaps, medicated shampoos, medicated lotions, antiperspirants/deodorants, perfumes, after shaves, and colognes,
10. Use of systemic or topical antibiotic medications, steroid medications (other than for hormonal contraception or postmenopausal reasons), or any other product known to affect the normal microbial flora of the skin within 14 days prior to Screening and Treatment Days,
11. Exposure of the test areas to solvents, acids, bases, strong detergents, fabric softener-treated clothing, or other household chemicals within 14 days prior to Screening and Treatment Days,
12. Swimming chemically treated pools or bathing in hot tubs, spas, or whirlpools within 14 days prior to Screening and Treatment Days,
13. Use of tanning beds, hot waxes, or depilatories (in the applicable test areas) within 14 days prior to Screening and Treatment Days,
14. Bathing and showering within 72 hours prior to Screening and Treatment Days.

Reviewer's comment: We had recommended that the protocol should specify that subjects who have qualified, began the treatment phase, and have withdrawn may not be re-entered into the study and subjects that have completed the study may not be re-entered into the study. The sponsor has included "Participation in another clinical study in the past 30 days, current participation in another clinical study, or previous participation in this study" for these exclusion criteria. The sponsor's inclusion and exclusion criteria are acceptable and in accordance with recommendations in the 1994 TFM for patient preoperative skin preparation studies.

Study Subjects

MicroBiotest: A total of 510 subjects provided informed consent; of these 489 (95.9%) subjects had screening data for microbial counts for both abdominal and inguinal regions. For the abdominal region, 67 (13.7%) subjects did not pass the microbial criteria and 16 (3.3%) subjects passed but did not attend the Treatment Day visit; thus 406 (79.6%) subjects were randomized to 6 treatment groups for the abdominal region. For the inguinal region, 86 (17.6%) subjects did not pass the microbial criteria and 12 (2.5%) subjects passed but did not attend the Treatment Day visit; thus 391 (76.7%) subjects were randomized to 6 treatment groups for the inguinal region.

Table 31. Subject disposition by treatment group (ITT/safety population)

Disposition	3M CHG/IPA Prep, 10.5 mL, and 3M CHG/IPA Prep, 26 mL	3M CHG/IPA Prep, 10.5 mL, and ChloraPrep	3M CHG/IPA Prep, 26 mL, and ChloraPrep	3M CHG/IPA Prep, 10.5 mL, and Saline	3M CHG/IPA Prep, 26 mL, and Saline	ChloraPrep and Saline	Total
Abdominal Region							
Randomized, N	110	111	111	25	24	25	406
Subjects not meeting Treatment Day baseline, n (%)	21 (19.1)	23 (20.7)	29 (26.1)	4 (16.0)	6 (25.0)	3 (12.0)	86 (21.2)
Modified ITT population, n (%)	96 (87.3)	94 (84.7)	90 (81.1)	25 (100.0)	19 (79.2)	22 (88.0)	346 (85.2)
PP population, n (%)	96 (87.3)	93 (83.8)	90 (81.1)	25 (100.0)	19 (79.2)	22 (88.0)	345 (85.0)
Completed study, n (%)	110 (100.0)	111 (100.0)	111 (100.0)	25 (100.0)	24 (100.0)	25 (100.0)	406 (100.0)
Discontinued early from study	0	0	0	0	0	0	0
Inguinal Region							
Randomized, N	108	107	106	23	24	23	391
Subjects not meeting Treatment Day baseline, n (%)	17 (15.7)	15 (14.0)	20 (18.9)	4 (17.4)	2 (8.3)	6 (26.1)	64 (16.4)
Modified ITT population, n (%)	94 (87.0)	95 (88.8)	89 (84.0)	20 (87.0)	22 (91.7)	19 (82.6)	339 (86.7)
PP population, n (%)	93 (86.1)	95 (88.8)	89 (84.0)	20 (87.0)	22 (91.7)	19 (82.6)	338 (86.4)
Completed study, n (%)	108 (100.0)	107 (100.0)	106 (100.0)	23 (100.0)	24 (100.0)	23 (100.0)	391 (100.0)
Discontinued early from study	0	0	0	0	0	0	0

Abbreviations: 3M CHG/IPA Prep=3M™ Chlorhexidine Gluconate/Isopropyl Alcohol Film-Forming Preoperative Skin Preparation; ChloraPrep= ChloraPrep® Patient Preoperative Skin Preparation; ITT=intent-to-treat; PP=per-protocol
Note: Percentages are based on the number of randomized subjects in each group. If only 1 side met baseline criteria, a subject appears in 2 rows of the table (Subjects not meeting Treatment Day baseline, Modified ITT population).

As shown in the table below, on the abdominal region, 246 subjects received 3M™ CHG/IPA Prep 10.5 mL, 245 subjects received 3M™ CHG/IPA Prep 26 mL, 247 subjects received ChloraPrep®, and 74 subjects received saline. On the inguinal region, 238 subjects received 3M™ CHG/IPA Prep 10.5 mL, 238 subjects received 3M™ CHG/IPA Prep 26 mL, 236 subjects received ChloraPrep®, and 70 subjects received saline.

Table 32. Subject disposition by study product (ITT/safety population)

Disposition, n (%)	3M CHG/IPA Prep, 10.5 mL	3M CHG/IPA Prep, 26 mL	ChloraPrep	Saline
Abdominal Region				
Randomized sides	246 (100.0)	245 (100.0)	247 (100.0)	74 (100.0)
mITT population	205 (83.3)	201 (82.0)	198 (80.2)	62 (83.8)
PP population	204 (82.9)	201 (82.0)	197 (79.8)	62 (83.8)
Completed study	246 (100.0)	245 (100.0)	247 (100.0)	74 (100.0)
Discontinued early from study	0	0	0	0
Inguinal Region				
Randomized sides	238 (100.0)	238 (100.0)	236 (100.0)	70 (100.0)
mITT population	204 (85.7)	203 (85.3)	200 (84.7)	59 (84.3)
PP population	203 (85.3)	202 (84.9)	199 (84.3)	59 (84.3)
Completed study	238 (100.0)	238 (100.0)	236 (100.0)	70 (100.0)
Discontinued early from study	0	0	0	0

Abbreviations: 3M CHG/IPA Prep=3M™ Chlorhexidine Gluconate/Isopropyl Alcohol Film-Forming Preoperative Skin Preparation; ChloraPrep= ChloraPrep® Patient Preoperative Skin Preparation; mITT= modified intent-to-treat; PP=per-protocol
Note: Percentages are based on the number of randomized treatment sides. Each subject is summarized in more than 1 treatment arm as he/she was treated with more than 1 treatment.

***Reviewer's comment*: The sponsor stated that all subjects who were randomized for the abdominal and/or inguinal regions received their treatments and completed the study. This is acceptable.**

BioScience Laboratories: A total of 1035 subjects provided inform consent for the abdominal region; of these 823 (79.5%) subjects had screening data for microbial counts. For the abdominal region, subjects not treated included 233 (28.3%) subjects who did not pass the microbial criteria, 3 (0.4%) subjects who did not meet inclusion/ exclusion criteria on Treatment Day, and 18 (2.2%) subjects who passed the microbial criteria, but did not attend the Treatment Day visit; 1 (0.1%) subject did not pass the microbial criteria, but was randomized and treated. Thus, 569 (55.0%) subjects were randomized to 6 treatment groups for the abdominal region.

A total of 715 subjects provided inform consent for the inguinal region; of these 579 (81%) subjects had screening data for microbial counts. For the inguinal region, subjects not treated included 138 (23.9) subjects who did not pass the microbial criteria, 4 (0.7%) subjects who did not meet inclusion/ exclusion criteria on Treatment Day, and 33 (5.7%) subjects who passed the microbial criteria, but not attend the Treatment Day visit; 1 (0.2%) subject did not meet inclusion/ exclusion criteria on Treatment Day, but was randomized and treated. Thus, 404 (56.5%) subjects were randomized to 6 treatment groups for the inguinal region.

Table 33. Subject disposition by treatment group (ITT/safety population)

Disposition	3M CHG/IPA Prep and 3M CHG/IPA Prep with HEDTA	3M CHG/IPA Prep and ChloraPrep	3M CHG/IPA Prep with HEDTA and ChloraPrep	3M CHG/IPA Prep and Saline	3M CHG/IPA Prep with HEDTA and Saline	ChloraPrep and Saline	Total
Abdominal Region							
Randomized, N	154	156	155	33	35	36	569
Subjects not meeting Treatment Day baseline, n (%)	86 (55.8)	83 (53.2)	74 (47.7)	16 (48.5)	17 (48.6)	25 (69.4)	301 (52.9)
Modified ITT population, n (%)	105 (68.2)	106 (67.9)	105 (67.7)	26 (78.8)	25 (71.4)	18 (50.0)	385 (67.7)
PP population, n (%)	104 (67.5)	105 (67.3)	102 (65.8)	26 (78.8)	25 (71.4)	17 (47.2)	379 (66.6)
Completed study, n (%)	154 (100.0)	156 (100.0)	154 (99.4)	33 (100.0)	35 (100.0)	36 (100.0)	568 (99.8)
Discontinued early from study	0	0	1 (0.6)[a]	0	0	0	1 (0.2)
Inguinal Region							
Randomized, N	106	111	113	25	25	24	404
Subjects not meeting Treatment Day baseline, n (%)	21 (19.8)	21 (18.9)	14 (12.4)	12 (48.0)	5 (20.0)	3 (12.5)	76 (18.8)
Modified ITT population, n (%)	95 (89.6)	99 (89.2)	105 (92.9)	24 (96.0)	23 (92.0)	23 (95.8)	369 (91.3)
PP population, n (%)	94 (88.7)	99 (89.2)	101 (89.4)	23 (92.0)	21 (84.0)	23 (95.8)	361 (89.4)
Completed study, n (%)	106 (100.0)	111 (100.0)	113 (100.0)	24 (96.0)	25 (100.0)	24 (100.0)	403 (99.8)
Discontinued early from study	0	0	0	1 (4.0)[a]	0	0	1 (0.2)

Abbreviations: 3M CHG/IPA Prep=3M™ Chlorhexidine Gluconate/Isopropyl Alcohol Film-Forming Preoperative Skin Preparation; ChloraPrep= ChloraPrep® Patient Preoperative Skin Preparation; HEDTA=(hydroxyethyl)ethylenediaminetriacetic acid; ITT=intent-to-treat; PP=per-protocol
Note: Percentages are based on the number of randomized subjects in each group. If only 1 side met baseline criteria, a subject appears in 2 rows of the table (Subjects not meeting Treatment Day baseline, Modified ITT population).

As shown in the table below, on the abdominal region, 343 subjects were randomized to receive 3M™ CHG/IPA Prep, 344 subjects were randomized to receive 3M™ CHG/IPA Prep with HEDTA, 347 subjects were randomized to receive ChloraPrep®, and 104 subjects were randomized to receive saline. On the inguinal region, 242 subjects were randomized to receive 3M™ CHG/IPA Prep, 244 subjects were randomized to receive 3M™ CHG/IPA with HEDTA, 248 subjects were randomized to receive ChloraPrep®, and 74 subjects were randomized to receive saline.

Table 34. Subject disposition by study product (ITT/safety population)

Disposition, n (%)	3M CHG/IPA Prep	3M CHG/IPA Prep with HEDTA	ChloraPrep	Saline
Abdominal Region				
Randomized sides	343	344	347	104
mITT population	196 (57.1)	202 (58.7)	196 (56.5)	59 (56.7)
PP population	191 (55.7)	197 (57.3)	191 (55.0)	59 (56.7)
Completed study	343 (100.0)	343 (99.7)	346 (99.7)	104 (100.0)
Discontinued early from study	0	1 (0.3)[a]	1 (0.3)[a]	0
Inguinal Region				
Randomized sides	242	244	248	74
mITT population	208 (86.0)	209 (85.7)	219 (88.3)	61 (82.4)
PP population	204 (84.3)	199 (81.6)	213 (85.9)	56 (75.7)
Completed study	241 (99.6)	244 (100.0)	248 (100.0)	73 (98.6)
Discontinued early from study	1 (0.4)[a]	0	0	1 (1.4)[a]

Abbreviations: 3M CHG/IPA Prep=3M™ Chlorhexidine Gluconate/Isopropyl Alcohol Film-Forming Preoperative Skin Preparation; ChloraPrep= ChloraPrep® Patient Preoperative Skin Preparation; HEDTA=(hydroxyethyl)ethylenediaminetriacetic acid; mITT= modified intent-to-treat; PP=per-protocol
Note: Percentages are based on the number of randomized treatment sides. Each subject is summarized in more than 1 treatment arm as he/she was treated with more than 1 treatment.

***Reviewer's comment*: The sponsor stated that all subjects who were randomized for the abdominal and/or inguinal regions received their treatments and completed the study. This is acceptable. Pending the statistician, Dr. Mushfiqur Rashid's review in DARRTS.**

Demographic and Other Baseline Characteristics

MicroBiotest: Subject demographics are summarized for the ITT/safety population by body region and treatment group in the table below. For the body regions, demographics were similar across treatment groups. Overall, the mean age was 36 years, there were approximately 50% women and 50% men, and the main race/ethnicities were approximately 38% white, 32% Asian, 17% black, and 12% Hispanic/Latino.

Table 35. Subject demographics (ITT/safety population) for Study EM-05-012760

Characteristic	3M CHG/IPA Prep, 10.5 mL, and 3M CHG/IPA Prep, 26 mL	3M CHG/IPA Prep, 10.5 mL, and ChloraPrep	3M CHG/IPA Prep, 26 mL, and ChloraPrep	3M CHG/IPA Prep, 10.5 mL, and Saline	3M CHG/IPA Prep, 26 mL, and Saline	ChloraPrep and Saline	Total
Abdominal Region							
Randomized N	110	111	111	25	24	25	406
Age (years)							
N	110	111	111	25	24	25	406
Mean (SD)	36.5 (14.49)	35.4 (14.93)	36.9 (14.35)	33.4 (13.94)	34.8 (14.46)	33.9 (12.85)	35.9 (14.4)
Median	30.5	31	32	28	30.5	29	31
Min–Max	18-72	18-80	18-73	18-68	20-72	18-58	18-80
Sex [n (%)]							
Male	57 (51.8)	57 (51.4)	53 (47.7)	14 (56.0)	11 (45.8)	10 (40.0)	202 (49.8)
Female	53 (48.2)	54 (48.6)	58 (52.3)	11 (44.0)	13 (54.2)	15 (60.0)	204 (50.2)
Race/Ethnicity [n (%)]							
American Indian or Alaska native	1 (0.9)	1 (0.9)	0	0	1 (4.2)	0	3 (0.7)
Asian	35 (31.8)	33 (29.7)	36 (32.4)	7 (28.0)	6 (25.0)	12 (48.0)	129 (31.8)
Black or African American	22 (20.0)	16 (14.4)	21 (18.9)	5 (20.0)	2 (8.3)	3 (12.0)	69 (17.0)
Hispanic/Latino	13 (11.8)	14 (12.6)	11 (9.9)	1 (4.0)	5 (20.8)	3 (12.0)	47 (11.6)
Native Hawaiian or other Pacific islander	1 (0.9)	0	0	0	0	0	1 (0.2)
White	38 (34.5)	46 (41.4)	40 (36.0)	12 (48.0)	10 (41.7)	7 (28.0)	153 (37.7)
Other	0	2 (1.8)	3 (2.7)	0	0	0	5 (1.2)
Inguinal Region							
Randomized N	108	107	106	23	24	23	391
Age (years)							
N	108	107	106	23	24	23	391
Mean (SD)	35.5 (14.79)	37.8 (14.74)	34.2 (13.8)	33.4 (11.72)	40.5 (14.64)	37.7 (17.74)	36.1 (14.56)
Median	30	37	30	29	41.5	29	31
Min–Max	18-80	18-72	18-71	18-59	18-73	18-69	18-80
Sex [n (%)]							
Male	64 (59.3)	58 (54.2)	54 (50.9)	11 (47.8)	10 (41.7)	11 (47.8)	208 (53.2)
Female	44 (40.7)	49 (45.8)	52 (49.1)	12 (52.2)	14 (58.3)	12 (52.2)	183 (46.8)
Race/Ethnicity [n (%)]							
American Indian or Alaska native	0	1 (0.9)	1 (0.9)	1 (4.3)	0	0	3 (0.8)
Asian	36 (33.3)	34 (31.8)	32 (30.2)	7 (30.4)	6 (25.0)	11 (47.8)	126 (32.2)
Black or African American	17 (15.7)	17 (15.9)	18 (17.0)	4 (17.4)	3 (12.5)	5 (21.7)	64 (16.4)
Hispanic/Latino	10 (9.3)	10 (9.3)	18 (17.0)	3 (13.0)	4 (16.7)	1 (4.3)	46 (11.8)
Native Hawaiian or other Pacific islander	1 (0.9)	1 (0.9)	0	0	0	0	2 (0.5)
White	42 (38.9)	44 (41.1)	36 (34.0)	8 (34.8)	11 (45.8)	6 (26.1)	147 (37.6)
Other	3 (2.8)	1 (0.9)	1 (0.9)	0	0	0	5 (1.3)

BioScience Laboratories: Subject demographics are summarized for the ITT/safety population by body region and treatment group in the table below. For both body regions, demographics were similar across treatment groups. Overall, the mean age was 31 years. For the abdominal region (569 subjects), there were approximately 71% men and 29% women, and the main races/ethnicities were approximately 91% white, 4% Hispanic/Latino, 2% Asian, 1% black, 1% American Indian or Alaska native, 1% other, and <1% native Hawaiian or other Pacific islander. For the inguinal region (404) subjects), there were approximately 74% men and 26% women, and the main races/ethnicities were approximately 91% white, 4% Hispanic/Latino, 3% Asian, 2%

other, 1% American Indian or Alaska native, <1% black, and <1% native Hawaiian or other Pacific islander.

Table 36. Subject demographics (ITT/safety population) for Study EM-05-013260

Characteristic	3M CHG/IPA Prep and 3M CHG/IPA Prep with HEDTA	3M CHG/IPA Prep and ChloraPrep	3M CHG/IPA Prep with HEDTA and ChloraPrep	3M CHG/IPA Prep and Saline	3M CHG/IPA Prep with HEDTA and Saline	ChloraPrep and Saline	Total
Abdominal Region							
Randomized N	154	156	155	33	35	36	569
Age (years)							
N	154	156	155	33	35	36	569
Mean (SD)	29.9 (13.69)	31.7 (15.9)	31.6 (15.85)	29.8 (14.97)	27.1 (12.73)	31.5 (13.87)	30.8 (14.94)
Median	23	24	24	23	21	26	23
Min–Max	18-74	18-79	18-80	18-64	18-65	18-66	18-80
Sex [n (%)]							
Male	112 (72.7)	115 (73.7)	106 (68.4)	24 (72.7)	27 (77.1)	21 (58.3)	405 (71.2)
Female	42 (27.3)	41 (26.3)	49 (31.6)	9 (27.3)	8 (22.9)	15 (41.7)	164 (28.8)
Race/Ethnicity [n (%)]							
American Indian or Alaska native	3 (1.9)	1 (0.6)	2 (1.3)	1 (3.0)	1 (2.9)	0	8 (1.4)
Asian	3 (1.9)	5 (3.2)	4 (2.6)	0	1 (2.9)	0	13 (2.3)
Black or African American	1 (0.6)	5 (3.2)	1 (0.6)	0	0	1 (2.8)	8 (1.4)
Hispanic/Latino	2 (1.3)	8 (5.1)	6 (3.9)	3 (9.1)	1 (2.9)	1 (2.8)	21 (3.7)
Native Hawaiian or other Pacific islander	2 (1.3)	1 (0.6)	0	0	0	0	3 (0.5)
White	142 (92.2)	137 (87.8)	145 (93.5)	29 (87.9)	33 (94.3)	34 (94.4)	520 (91.4)
Other	3 (1.9)	3 (1.9)	0	1 (3.0)	0	0	7 (1.2)
Inguinal Region							
Randomized N	106	111	113	25	25	24	404
Age (years)							
N	106	111	113	25	25	24	404
Mean (SD)	30.1 (15.21)	30.5 (13.75)	32.3 (15.4)	31.3 (15.84)	29.5 (12.49)	25.5 (10.19)	30.6 (14.51)
Median	23	24	24	23	24	20.5	24
Min–Max	18-76	18-64	18-80	18-66	18-65	18-60	18-80
Sex [n (%)]							
Male	84 (79.2)	85 (76.6)	79 (69.9)	17 (68.0)	17 (68.0)	17 (70.8)	299 (74.0)
Female	22 (20.8)	26 (23.4)	34 (30.1)	8 (32.0)	8 (32.0)	7 (29.2)	105 (26.0)
Race/Ethnicity [n (%)]							
American Indian or Alaska native	1 (0.9)	2 (1.8)	3 (2.7)	0	0	0	6 (1.5)
Asian	3 (2.8)	1 (0.9)	5 (4.4)	1 (4.0)	0	1 (4.2)	11 (2.7)
Black or African American	1 (0.9)	0	0	0	0	0	1 (0.2)
Hispanic/Latino	3 (2.8)	6 (5.4)	8 (7.1)	0	0	0	17 (4.2)
Native Hawaiian or other Pacific islander	0	2 (1.8)	0	0	0	0	2 (0.5)
White	99 (93.4)	99 (89.2)	99 (87.6)	24 (96.0)	24 (96.0)	23 (95.8)	368 (91.1)
Other	0	2 (1.8)	3 (2.7)	0	1 (4.0)	1 (4.2)	7 (1.7)

***Reviewer's comment**: We encourage sponsors to select study subjects that represent the range of patient populations that will be using the product. With the exception of Caucasian subjects (91%), treatment experience among other races is limited at the BioScience Laboratories testing site. The MicroBiotest testing sites were made up of diverse group of races: 38% Caucasian, 32% Asian, 17% black, and 12% Hispanic/Latino. However, we do not have any evidence that race makes a difference in the efficacy of topical antiseptics. These types of products (chlorhexidine gluconate and alcohol) has been marketed in the United States for a number of years and there are no reports in AERS or the literature to*

suggest that efficacy is affected by specific demographic factors. Also pending Medical Officer, Dr. Teresa Podruchny's review, see in DARRTS.

Efficacy Results

Results of Study EM-05-012760 (MicroBiotest)

On the abdominal region, 246 subjects received 3M™ CHG/IPA Prep 10.5 mL, 245 subjects received 3M™ CHG/IPA Prep 26 mL, 247 subjects received ChloraPrep®, and 74 subjects received saline. On the inguinal region, 238 subjects received 3M ™ CHG/IPA Prep 26 mL, 236 subjects received ChloraPrep®, and 70 subjects received saline. Based upon the study, all randomized subjects received their treatments and completed the study; all were in the safety/intent-to-treat population. All subjects who met baseline requirements on at least 1 side of a body region on Treatment Day were included in the modified intent-to-treat analysis for that side of the body region. As demonstrated in the Table 37 for the modified intent-to-treat population, the primary efficacy endpoint of achieving ≥ 70% for the lower bound of the 95% CI for responder rate was met by all active study products which included the 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep 26 mL, and ChloraPrep® in the abdominal and inguinal regions.

Table 37. Responder rate (mITT population, study EM-05-012760)

Abdominal Region				
	3M CHG/IPA Prep, 10.5 mL	3M CHG/IPA Prep, 26 mL	ChloraPrep®	Saline
N	205	201	198	62
Responder, n (%)	167 (81.5)	175 (87.1)	178 (89.9)	0
95% CI for rate	76.1%, 86.8%	82.4%, 91.7%	85.7%, 94.1%	NA
P-value relative to negative control	<0.001	<0.001	<0.001	NA
Inguinal Region				
	3M CHG/IPA Prep, 10.5 mL	3M CHG/IPA Prep, 26 mL	ChloraPrep®	Saline
N	204	203	200	59
Responder, n (%)	168 (82.4)	162 (79.8)	168 (84.0)	4 (6.8)
95% CI for rate	77.1%, 87.6%	74.3%, 85.3%	78.9%, 89.1%	0.4%, 13.2%
P-value relative to negative control	<0.001	<0.001	<0.001	NA

Reviewer's comment: On August 14, 2013, FDA sent an advice letter to the sponsor regarding statistical analysis of the clinical simulation studies. The sponsor asked if it can use logistic analysis to test for superiority. We stated that a logistic analysis approach would be appropriate. Regardless of the testing approach used, we would expect highly robust findings of superiority since responder rates in the negative control are expected to be very low (i.e., near 0%).

On February 20, 2014, FDA had a teleconference meeting with the sponsor. We informed the sponsor the following regarding the primary endpoint of the study:

- ***Base the primary analysis on the proportion of patient successes (responders) as a binomial endpoint. Success for a patient is defined as meeting the required 3-log$_{10}$ reduction form baseline at the groin site and 2-log$_{10}$ reduction at the abdomen site at 10 minutes (immediate effect) and the bacterial counts cannot exceed baseline at 6 hours (persistent effect) after application. We recommended the sponsor to establish adequate effectiveness of the test product and the appropriateness of the active control as an internal standard based on the following:***
  - ***The lower bound of a two-sided 95% CI for the responder rate of the test product ≥ 70%.***
  - ***The lower bound of a two-sided 95% CI for the responder rate of the active control ≥ 70%.***
- ***We expect the primary analyses to demonstrate efficacy for both dry sites (abdomen) and moist sites (groin) based on both immediate and persistent success rates demonstrating the following:***
  - ***Superiority of test vs. vehicle control based on the lower bound of a two-sided 95% CI for the difference in responder rates exceeding zero.***
  - ***Superiority of active control vs. vehicle control based on the lower bound of a two-sided 95% CI for the difference in responder rates exceeding zero.***

For the abdominal region modified intent-to-treat population, the lower bound of the 95% CI for responder rate was 76.1%, 82.4%, and 85.7% for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep 26 mL, and ChloraPrep®, respectively. The corresponding responder rates were 81.5%, 87.1, and 89.9, respectively.

For the inguinal region modified intent-to-treat population, the lower bound of the 95% CI for responder rate was 77.1%, 74.3%, and 78.9% for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep 26 mL, and ChloraPrep®, respectively. The corresponding responder rates were 82.4%, 79.8%, and 84.0%, respectively.

For both abdominal and inguinal regions, responder rates for all active products were statistically significantly higher than for the saline control ($p<0.001$). The responder rate following saline treatment was 0% for the abdominal region and 6.8% for the inguinal region. Therefore, the sponsor has met the primary endpoint recommended requirement for this clinical simulation study.

Summary statistics of log-transformed bacterial (skin flora) counts at each time point and reduction from baseline to each follow-up time point are presented by study product and body region in Table 38 for the mITT population.

Table 38. Summary statistics of log transformed bacterial (skin flora) endpoints (mITT population, study EM-05-012760)

Time Point	Endpoint	Statistics	3M CHG/IPA Prep, 10.5 mL	3M CHG/IPA Prep, 26 mL	ChloraPrep	Saline
Abdominal Region						
Baseline	Log_{10}/cm^2 Skin Flora	N	205	201	198	62
		Mean (SD)	3.35 (0.427)	3.31 (0.370)	3.31 (0.391)	3.26 (0.342)
		Median	3.15	3.14	3.16	3.15
		Min-Max	3.01 - 5.32	3.02 - 4.74	3.01 - 5.21	3.01 - 4.96
10 Minutes	Log_{10} Recovery	N	205	201	198	62
		Mean (SD)	0.69 (0.604)	0.57 (0.575)	0.53 (0.499)	2.63 (0.582)
		Median	0.37	0.20	0.20	2.65
		Min-Max	0.20 - 3.33	0.20 - 2.91	0.20 - 2.65	1.49 - 4.52
	Log_{10} Reduction	N	205	201	198	62
		Mean (SD)	2.66 (0.705)	2.74 (0.644)	2.79 (0.565)	0.64 (0.487)
		Median	2.84	2.86	2.85	0.51
		Min-Max	0.91 - 5.12	0.23 - 4.54	1.16 - 4.54	-0.38 - 1.55
6 Hours	Log_{10} Recovery	N	205	201	198	62
		Mean (SD)	1.87 (0.804)	1.66 (0.804)	1.54 (0.807)	2.80 (0.726)
		Median	1.89	1.72	1.62	2.78
		Min-Max	0.20 - 3.61	0.20 - 3.74	0.20 - 3.51	1.04 - 4.63
	Log_{10} Reduction	N	205	201	198	62
		Mean (SD)	1.48 (0.866)	1.64 (0.864)	1.77 (0.899)	0.46 (0.651)
		Median	1.42	1.55	1.71	0.41
		Min-Max	-0.17 - 4.18	-0.37 - 3.97	-0.28 - 4.62	-0.86 - 2.06
Inguinal Region						
Baseline	Log_{10}/cm^2 Skin Flora	N	204	203	200	59
		Mean (SD)	5.44 (0.490)	5.42 (0.469)	5.44 (0.479)	5.39 (0.447)
		Median	5.22	5.21	5.22	5.14
		Min-Max	5.01 - 6.93	5.01 - 6.90	5.00 - 6.83	5.00 - 6.57
10 Minutes	Log_{10} Recovery	N	204	203	200	59
		Mean (SD)	1.47 (1.119)	1.53 (1.039)	1.40 (1.089)	4.06 (1.211)
		Median	1.45	1.50	1.44	4.19
		Min-Max	0.20 - 4.88	0.20 - 4.56	0.20 - 4.28	0.20 - 6.54
	Log_{10} Reduction	N	204	203	200	59
		Mean (SD)	3.98 (1.034)	3.89 (0.929)	4.03 (0.965)	1.34 (1.095)
		Median	4.09	3.82	4.03	1.23
		Min-Max	0.51 - 6.60	1.55 - 6.17	1.30 - 5.90	-0.66 - 4.85
6 Hours	Log_{10} Recovery	N	204	203	200	59
		Mean (SD)	3.20 (1.037)	3.07 (1.032)	2.80 (1.007)	4.31 (0.941)
		Median	3.35	3.23	2.98	4.49
		Min-Max	0.20 - 5.10	0.20 - 5.59	0.20 - 4.91	1.39 - 5.62
	Log_{10} Reduction	N	204	203	200	59
		Mean (SD)	2.24 (1.103)	2.35 (1.046)	2.63 (1.047)	1.08 (0.834)
		Median	2.16	2.20	2.48	0.99
		Min-Max	0.09 - 6.43	-0.42 - 5.30	0.38 - 6.08	-0.22 - 4.05

Abbreviations: 3M CHG/IPA Prep=3M™ Chlorhexidine Gluconate/Isopropyl Alcohol Film-Forming Preoperative Skin Preparation; ChloraPrep= ChloraPrep® Patient Preoperative Skin Preparation; Max = maximum; Min = minimum; mITT=modified intent-to-treat; SD = standard deviation

***Reviewer's comment*: On February 20, 2014, FDA had a teleconference meeting with the sponsor. We informed the sponsor that in order to demonstrate effectiveness for the secondary endpoint (mean log reduction), we recommend that the lower bound of a 2-sided 95% CI be ≥ 2 log_{10} reduction on the abdomen and ≥ 3 log_{10} reduction on the groin and the bacterial counts not exceed baseline at 6 hours.**

For the abdominal region, the baseline mean bacterial (skin flora) count was approximately 3.3 log_{10} per cm^2 across study products. The mean (standard deviation, SD) reduction from baseline at 10 minutes following treatment was similar among active treatments: 2.66 (0.705) log_{10} per cm^2, 2.74 (0.644) log_{10} per cm^2, and 2.79 (0.565) log_{10} per cm^2 for 3M ™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep 26 mL, and ChloraPrep®, respectively. Therefore, all the test products demonstrated ≥ 2 log_{10} reduction at the abdomen site.

At 6 hours following treatment, the mean (SD) reduction from baseline was similar among active treatments: 1.48 (0.866) log_{10} per cm^2, 1.64 (0.864) log_{10} per cm^2, and 1.77 (0.899) log_{10} per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep 26 mL, and ChloraPrep®, respectively. The Mean (SD) reduction from baseline following saline treatment was 0.64 (0.487) log_{10} per cm^2 at 6 hours. Therefore, all the test products did not exceed the baseline counts at 6 hours.

For the inguinal region, the baseline mean bacterial (skin flora) count was approximately 5.4 log_{10} per cm^2 across study products. The mean (SD) reduction from baseline at 10 minutes following treatment was similar among active treatments: 3.98 (1.034) log_{10} per cm^2, 3.89 (0.929) log_{10} per cm^2, and 4.03 (0.965) log_{10} per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep 26 mL, and ChloraPrep®, respectively. Therefore, all the test products demonstrated ≥ 3 log_{10} reduction at the inguinal site.

At 6 hours following treatment, the mean (SD) reduction from baseline was similar among active treatments: 2.24 (1.103) log_{10} per cm^2, 2.35 (1.046) log_{10} per cm^2, and 2.63 (1.047) log_{10} per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep 26 mL, and ChloraPrep®, respectively. The mean (SD) reduction from baseline following saline treatment was 1.34 (1.095) log_{10} per cm^2 at 10 minutes and 1.08 (0.834) log_{10} per cm^2 at 6 hours. Therefore, all the test products did not exceed the baseline counts at 6 hours.

Efficacy Results of Study EM-05-013260 (BioScience Laboratories)

On the abdominal region, 343 subjects were randomized to receive 3M™ CHG/IPA Prep, 344 subjects were randomized to receive 3M™ CHG/IPA Prep with HEDTA, 347 subjects were randomized to receive ChloraPrep®, and 104 subjects were randomized to receive saline. On the inguinal region, 242 subjects were randomized to receive 3M™ CHG/IPA Prep, 244 subjects were randomized to receive 3M CHG/IPA Prep with HEDTA, 248 subjects were randomized to receive ChloraPrep®, and 74 subjects were randomized to receive saline. Based upon the study, all randomized subjects received treatments; however, one subject received 3M™ CHG/IPA Prep with HEDTA instead of the treatment randomized (3M™ CHG/IPA Pre) on the left groin, and one subject did not receive any study product on the right abdomen (randomized to 3M™ CHG/IPA Prep

with HEDTA) or the right groin (randomized to 3M™ CHG/IPA Prep). All but one treated subject completed the study; one subject was prematurely discontinued from the study due to sites being comprised during testing.

Table 39. Responder rate (mITT population, study EM-050013260)

Abdominal Region				
	3M CHG/IPA Prep, 10.5 mL	3M CHG/IPA Prep with HEDTA, 10.5 mL	ChloraPrep®	Saline
N	196	202	196	59
Responder, n (%)	159 (81.1)	165 (81.7)	163 (83.2)	5 (8.5)
95% CI for rate	75.6%, 86.6%	76.3%, 87.0%	77.9%, 88.4%	NA
P-value relative to negative control	<0.001	<0.001	<0.001	NA
Inguinal Region				
	3M CHG/IPA Prep, 10.5 mL	3M CHG/IPA Prep with HEDTA, 10.5 mL	ChloraPrep®	Saline
N	208	209	219	61
Responder, n (%)	81 (38.9)	98 (46.9)	116 (53.0)	1 (1.6)
95% CI for rate	32.3%, 45.6%	40.1%, 53.7%	46.4%, 59.6%	NA
P-value relative to negative control	<0.001	<0.001	<0.001	NA

***Reviewer's comment**: As demonstrated in Table 39, the primary efficacy endpoint of achieving ≥ 70% for the lower bound of the 95% CI for responder rate was met by all active study products on the abdominal region. For the abdominal region mITT population, the lower bound of the 95% CI for responder rate was 75.6%, 76.3%, and 77.9% for 3M™ CHG/IPA Prep, 3M™ CHG/IPA Prep with HETDA, and ChloraPrep®, respectively. The corresponding responder rates were 81.1%, 81.7%, and 83.2%, respectively, and were all higher and statistically significantly different (p<0.001) compared to the saline control (8.5%).*

The primary efficacy endpoint of achieving ≥ 70% for the lower bound of the 95% CI for responder rate was not met by any active product on the inguinal region. For the inguinal region mITT population, the lower bound of the 95 CI for responder rate was 32.3%, 40.1%, and 46.4% for 3M™ CHG/IPA Prep, 3M™ CHG/IPA Prep with HEDTA, and ChloraPrep®, respectively. The corresponding responder rates were 38.9%, 46.9%, and 53.0%, respectively, and were all higher and statically significant different (p<0.001) compared to the saline control (1.6%). However, all the active test products did not make the FDA recommended ≥ 70% responder rate for the inguinal region.

Table 40. Summary statistics of log-transformed bacterial (skin flora) endpoints (mITT population, study EM-05-013260)

Time Point	Endpoint	Statistics	3M CHG/IPA Prep	3M CHG/IPA Prep with HEDTA	ChloraPrep	Saline
Abdominal Region						
Baseline	Log_{10}/cm^2 Skin Flora	N	196	202	196	59
		Mean (SD)	3.63 (0.460)	3.61 (0.479)	3.64 (0.487)	3.64 (0.533)
		Median	3.51	3.48	3.54	3.47
		Min-Max	3.00 - 5.26	3.00 - 5.13	3.01 - 5.39	3.00 - 5.00
10 Minutes	Log_{10} Recovery	N	196	201	196	59
		Mean (SD)	0.85 (0.947)	0.88 (0.984)	0.90 (0.938)	2.88 (0.703)
		Median	0.42	0.42	0.54	2.93
		Min-Max	0.24 - 4.55	0.24 - 4.22	0.24 - 4.92	1.43 - 4.57
	Log_{10} Reduction	N	196	202	196	59
		Mean (SD)	2.78 (0.935)	2.73 (1.002)	2.75 (0.973)	0.76 (0.814)
		Median	2.96	2.94	2.90	0.72
		Min-Max	-0.95 - 4.44	-0.22 - 4.89	-0.71 - 5.15	-0.82 - 2.54
6 Hours	Log_{10} Recovery	N	196	201	196	59
		Mean (SD)	0.80 (0.840)	0.74 (0.789)	0.81 (0.935)	2.65 (0.886)
		Median	0.33	0.24	0.42	2.58
		Min-Max	0.24 - 3.43	0.24 - 3.37	0.24 - 4.30	0.42 - 4.99
	Log_{10} Reduction	N	196	202	196	59
		Mean (SD)	2.83 (0.851)	2.86 (0.899)	2.83 (0.971)	1.00 (0.970)
		Median	2.95	2.99	2.98	0.86
		Min-Max	-0.03 - 4.59	0.00 - 4.69	-0.67 - 4.81	-0.88 - 3.56
Inguinal Region						
Baseline	Log_{10}/cm^2 Skin Flora	N	208	209	219	61
		Mean (SD)	6.23 (0.604)	6.21 (0.563)	6.27 (0.618)	6.09 (0.626)
		Median	6.28	6.33	6.34	6.16
		Min-Max	5.00 - 7.49	5.08 - 7.37	5.00 - 7.47	5.00 - 7.45
10 Minutes	Log_{10} Recovery	N	208	208	218	60
		Mean (SD)	3.39 (1.096)	3.21 (1.042)	3.08 (1.137)	5.08 (0.676)
		Median	3.61	3.36	3.26	5.28
		Min-Max	0.24 - 5.61	0.24 - 5.72	0.24 - 4.90	2.55 - 6.21
	Log_{10} Reduction	N	208	209	219	61
		Mean (SD)	2.84 (1.191)	2.99 (1.125)	3.17 (1.243)	1.01 (0.668)
		Median	2.74	2.93	3.13	1.04
		Min-Max	-0.25 - 6.70	0.00 - 6.15	0.00 - 6.36	-0.87 - 3.30
6 Hours	Log_{10} Recovery	N	208	209	219	60
		Mean (SD)	2.76 (1.202)	2.73 (1.220)	2.58 (1.182)	4.85 (0.749)
		Median	2.95	3.03	2.76	4.83
		Min-Max	0.24 - 5.44	0.24 - 5.13	0.24 - 5.17	2.59 - 6.50
	Log_{10} Reduction	N	208	209	219	61
		Mean (SD)	3.46 (1.312)	3.49 (1.329)	3.69 (1.286)	1.23 (0.732)
		Median	3.44	3.29	3.57	1.23
		Min-Max	0.35 - 6.84	0.14 - 6.49	0.61 - 6.77	-0.13 - 3.03

Abbreviations: 3M CHG/IPA Prep=3M™ Chlorhexidine Gluconate/Isopropyl Alcohol Film-Forming Preoperative Skin Preparation; ChloraPrep= ChloraPrep® Patient Preoperative Skin Preparation; HEDTA=(hydroxyethyl)ethylenediaminetriacetic acid; Max = maximum; Min = minimum; mITT=modified intent-to-treat; SD = standard deviation

***Reviewer's comment**: As demonstrated in Table 40, for the abdominal region, the baseline mean bacterial (skin flora) count was approximately 3.6 log_{10} per cm^2 across study products. The mean (standard deviation, SD) reduction from baseline at 10 minutes following treatment was similar among active treatments: 2.78 (0935) log_{10} per cm^2, 2.73 (1.002) log_{10} per cm^2, and 2.75 (0.973) log_{10} per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep with HEDTA 10.5 mL, and ChloraPrep®, respectively. Therefore, all the active test products demonstrated ≥ 2 log_{10} reduction at the abdomen site.*

At 6 hours following treatment, the mean (SD) reduction from baseline was similar among active treatments: 2.83 (0.851) log_{10} per cm^2, 2.86

(0.899) log_{10} per cm^2, and 2.83 (0.971) log_{10} per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep with HEDTA 10.5 mL, and ChloraPrep®, respectively. The Mean (SD) reduction from baseline following saline treatment was 1.00 (0.970) log_{10} per cm^2 at 6 hours. Therefore, all the test products did not exceed the baseline counts at 6 hours.

For the inguinal region, the baseline mean bacterial (skin flora) count was approximately 6.2 log_{10} per cm^2 across study products. The mean (SD) reduction from baseline at 10 minutes following treatment was similar among active treatments: 2.84 (1.191) log_{10} per cm^2, 2.99 (1.125) log_{10} per cm^2, and 3.17 (1.243) log_{10} per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™ CHG/IPA Prep with HEDTA 10.5 mL, and ChloraPrep®, respectively. Apparently, only the active control ChloraPrep® (3.17 (1.243) log_{10} per cm^2) made the secondary endpoint ≥ 3 log_{10} reduction at the inguinal site.

At 6 hours following treatment, the mean (SD) reduction from baseline was similar among active treatments: 3.46 (1.312) log_{10} per cm^2, 3.49 (1.329) log_{10} per cm^2, and 3.69 (1.286) log_{10} per cm^2 for 3M™ CHG/IPA Prep 10.5 mL, 3M™CHG/IPA Prep with HEDTA 10.5 mL, and ChloraPrep®, respectively. The mean (SD) reduction from baseline following saline treatment was 1.01 (0.668) log_{10} per cm^2 at 10 minutes and 1.23 (0.732) log_{10} per cm^2 at 6 hours. Therefore, all the test products did not exceed the baseline counts at 6 hours.

The sponsor provided an alternative assessment (See Table 41) on the primary efficacy requirements that were met for 3M™ CHG/IPA Prep with HEDTA: non-inferiority to the 10 minute log reduction of ChloraPrep®, no difference in the return to baseline versus ChloraPrep® at 6 hours, and superiority to saline in responder rate. 3M™ CHG/IPA without HEDTA did not meet the non-inferiority portion of the alternative primary efficacy requirements.

Table 41. Alternative primary efficacy results for inguinal region: difference in log reduction at 10 minutes and difference in return to baseline at 6 hours (mITT population, study EM-05-013260).

	3M CHG/IPA Prep	3M CHG/IPA Prep with HEDTA	ChloraPrep
Inguinal Region			
N	208	209	219
95% CI for the difference in log_{10} reduction (ChloraPrep minus 3M CHG/IPA Prep) at the 10-minute time point[a]	(0.103, 0.567)	(-0.041, 0.410)	NA
97.5% CI for the difference in log_{10} reduction (ChloraPrep minus 3M CHG/IPA Prep) at the 10-minute time point[a]	NA	(-0.074, 0.443)	NA
N(%) of subjects with skin flora returning to baseline values at the 6-hour time point	0	0	0
P-value for the difference in the percentage of subjects with skin flora returning to baseline values at the 6-hour time point[b]	1.000	1.000	NA

Abbreviations: 3M CHG/IPA Prep=3M™ Chlorhexidine Gluconate/Isopropyl Alcohol Film-Forming Preoperative Skin Preparation; ChloraPrep= ChloraPrep® Patient Preoperative Skin Preparation; CI=confidence interval; CSR=clinical study report; HEDTA=(hydroxyethyl)ethylenediaminetriacetic acid; mITT=modified intent-to-treat; NA=not applicable

The sponsor suggested that a post hoc analysis demonstrated that the responder rates of the 3M™ CHG/IPA Prep and 3M™ CHG/IPA Prep with HEDTA groups were not statistically significantly different from either body regions (p=0.898 for the abdominal region and p=0.114 for the inguinal region). The primary, secondary, and alternative primary efficacy results demonstrated the efficacy of 3M™ CHG/IPA Prep and 3M™ CHG/IPA Prep with HEDTA on the abdominal region and of 3M™ CHG/IPA Prep with HEDTA on the inguinal region.

***Reviewer's comment*: On February 20, 2014, FDA had a teleconference meeting with the sponsor. The sponsor asked if FDA would ever allow a repeat study (failed study) at the same lab? FDA responded "no" and the reason we ask for multiple labs is to evaluate the lab-to-lab variability to test the robustness of the study. This reviewer feels that there are two options we have for the sponsor regarding the clinical simulation studies:**

1) ***Approval on submitted studies – We currently have precedence on approving two clinical simulation studies passing the abdomen site and one clinical simulation study passing the groin site last original NDA (21-586) antiseptic drug product. Although study EM-05-013260 (BioScience Laboratories) containing the 3M™ CHG/IPA Prep 10.5 mL(38.9%), 3M™ CHG/IPA Prep with HEDTA 10.5 mL (46.9%) and ChloraPrep® (53%) did not pass the ≥ 70% responder rate (primary endpoint), the mean log reduction criteria (secondary endpoint) comes***

close to the recommended 3 log_{10} reduction in the inguinal site: 3M™ CHG/IPA Prep 10.5 mL(2.87), 3M™ CHG/IPA Prep with HEDTA 10.5 mL (2.99) and ChloraPrep® (3.17). Although, we have been recommending IND/NDA sponsors the new statistical criteria and also have published our recommended statistical criteria in Safety and Effectiveness of Health Care Antiseptics Proposed Rule (80 FR 25166) requesting comments and data on this issue, we are currently still evaluating and assessing this new statistical criteria. We will have a final decision on the proposed statistical criteria in the final rule on Health Care Antiseptic to be published on January 15, 2018.

2) ***Non-approval on submitted studies - On February 20, 2014, FDA had a tele-conference meeting with the sponsor. The sponsor stated that it has experience conducting patient preoperative preparation clinical studies at*** (b) (4) ***a test laboratory in the country of Romania. The sponsor has found the site to be fully competent and compliant in conducting these types of studies. The sponsor asked if it were to start a new pivotal clinical study at his site, would FDA allow the study to begin under the IND prior to being FDA inspected, and would FDA inspection be conducted as part of the NDA review process? What specific action and/or information would be required by FDA to accept the filing of a clinical study at this site? We responded that FDA does not inspect clinical trial sites prior to NDA submission; however, it is likely that an FDA inspection would be conducted as part of the NDA review process. Foreign studies performed under an IND must meet the same requirements of 21 CFR Part 312 that apply to U.S. studies conducted under an IND. We also stated that the sponsor does not need to conduct foreign studies under the IND, although we encourage they do so (see 21 CFR 312.120 for foreign clinical studies not conducted under an IND).***

The sponsor provided a pilot study on the groin region only that was conducted in (b) (4) ***in Romania. The study was entitled: "Preoperative Skin Preparation Study Following ASTM E1173 Methods to Evaluate the Antimicrobial Properties of Five Test Products." A total of 180 subjects were admitted into the testing portion of the study and 161 subjects were treated. The test product was tested on four different directions for use.***

Test Products	Log_{10} reduction 30 seconds	Log_{10} reduction 10 minutes	Responder Rate
ChloraPrep® Product A	3.40	3.83	73%
Test Product B applied 2 min/ dry 3 min	3.39	3.64	71%

Test Product C applied 1 min/ dry 3 min	3.10	3.46	61%
Test Product D applied 2 min/ dry 3 min	3.65	4.00	71%
Test Product E Applied single coat/ dry 3 min	3.28	3.04	57%

The sponsor stated that no efficacy conclusions could be made from the study because there was a change made to the formulation, based on results obtained during the drape adhesion testing. (b) (4) ***The sponsor stated that while working with the new formulation, it was determined through spore recovery studies that the material used to dissolve the film in this study, Crodamol GTCC, may not have been adequate for that purpose. The antimicrobial efficacy data from this study could not be validated.***

Overall, this reviewer has concerns having the sponsor repeat the groin region efficacy testing at a new foreign site where there has never been an FDA inspection at the facility and no efficacy conclusions could be made from the study due to numerous flaws.

We have experienced in the past the issue of antiseptic products not meeting the 3 log_{10} reduction point estimate efficacy and meeting the 70% responder rate for the 10 minute post treatment reductions at the groin site (See detailed discussion in Clinical Overview in Module 2 on page 9 of the submission). It is difficult to determine why some antiseptic products do not perform consistently at the groin site. The test results for some antiseptic products and whether it meets the specified point estimate as an active control and/or antiseptic product, appears to vary by the laboratory doing the testing. One lab consistently report meeting the specified point estimate criteria, while the other lab does not report similar findings. The reasons for these differences are not clear. Some of the labs may conduct their study trials differently (e.g., the amount of pressure applied when scrubbing (sampling) the skin).

The protocols for EM-05-012760 (MicroBiotest) and EM-05-013260 (BioScience Laboratories) are identical except for the glass cylinder sampling scrub cup size used between the two lab facilities. After the baseline sample is taken the designated product are applied to the skin according to the directions for use. Once the product has dried and adjacent samples have been taken, the site designated for the 6 hour sampling time is covered with a sterile gauge held in place with a sterile non-occlusive dressing. This allows the subject to be mobile during the time 6-hour sampling time without compromising the test site.

Using the cylinder sampling (scrub cup) technique, a sample is taken 10 minutes after the product has dried on the skin. A sterile scrubbing cylinder is held firmly to the skin over the site to be sampled. Scrub solution with validated antimicrobial neutralizers specific to the product formulation is added to the cylinder. Both lab facilities used the exact same scrub solution with the exact same neutralizers. The area inside the cylinder is scrubbed for one minute using a sterile rubber policeman (spatula). The scrub solution is aspirated into a sterile test tube using a sterile pipette. The method is repeated using a second aliquot of scrub solution with validated antimicrobial neutralizers. The two aliquots are pooled. This technique is repeated for the 6-hour sample time. Table 42 shows a comparison between areas of the scrub cup used by the two pivotal studies.

Table 42. Scrubbing cup comparison

Clinical Trial Location	Internal Area of Scrubbing Cup	Volume of Scrub Solution Added
MicroBiotest	3.80cm^2	3.0 + 3.0 = 6mL
BioScience Laboratories	3.46 cm^2	3.0 +3.0 = 6mL

The pooled aliquots are diluted in 10-fold steps using Butterfield's Phosphate Buffer and appropriate validated antimicrobial neutralizers. The dilutions are plated on agar containing validated product neutralizers. The plates are incubated aerobically for 72 ± 4 hours at 30 ± 2^{o}C. This procedure is the exactly the same for both lab facilities. After incubation of the media, colonies are counted. On February 20, 2014, the sponsor requested that BioScience Laboratories would like to use an automated method (i.e., spiral plating and Q count) for plating and counting colonies that MicroBiotest does not have. We responded that this was appropriate; however, this is not mentioned in the protocol. The average number of microorganisms recovered per square centimeter of skin is determined and reported:

MicroBiotest $CFU/cm^2 = \frac{CFU/mL \times 6\ mL}{3.80\ cm^2}$

BioScience Laboratories $CFU/cm^2 = \frac{CFU/mL \times 6\ mL}{3.46\ cm^2}$

The difference in formula is due to difference in cylinder size. The CFU/mL data are converted to log_{10} and evaluated using statistics. The log_{10} calculated from the post-application sample is subtracted from the log_{10} calculated from the baseline sample, giving the result of log_{10} reduction from the baseline count. An average count reduction from baseline count is calculated using data from all qualified subjects. The data gathered are evaluated by using descriptive statistics to determine the

mean, standard deviation, 95% confidence interval and log_{10} reduction from baseline.

Overall, this reviewer finds one main inconsistency in the procedure. This inconsistency pertains to the variation in scrub cup diameter as described above. The 1994 TFM does not specify the diameter of the sampling scrub cup used to sample the microorganisms. The TFM describes the following "Sterile glass cylinders, height approximately 2.5 centimeter, inside diameter of convenient size to place on anatomical area to be sampled. Useful sizes range from approximately 2.5 to 4.0 centimeters." The test method ASTM E1173 "Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations" describes similar in vivo procedures and does not specify a size of the sampling cup but a range of 0.5 inches to 1.5 inches. It appears that if you use a cylinder scrub cup size of 3.80 cm^2 versus a cylinder scrub cup size of 3.46 cm^2, you will obtain a larger surface area. This means a possibility of getting a higher bacterial count.

Another possible inconsistency is the amount of pressure or how hard you are scrubbing the skin using the hollow cylinder with the scrub solution and policeman. Here you are really scraping the epithelial layer of the skin to retrieve a high number of bacteria count in order to get a high log reduction count. This has been an issue of speculation for a number of years. You don't know if one person is the designated scrubber or if several different technicians are scrubbers. Although the directions states scrub with moderate pressure it is difficult to determine what is moderate pressure.

MicroBiotest was inspected between November 23 and December 2, 2015 on the efficacy study EM-05-012760. The field investigator audited 47 subject files for this study. Data audits were done by comparing source data in the subject's files with data listing provided with the assignment. The records reviewed included protocol deviations, adverse events, eligibility criteria, physical assessments, subject demographics and efficacy assessments. Overall the assessments were acceptable.

The field investigator asked Dr. Bashir at MicroBiotest the reason for such a high responder rate in the groin area. According to Dr. Bashir, the main reason was because staff is given step by step training on techniques. When asked why their study had fewer screen failures (20-24% fewer) compared to the other site, Dr. Bashir stated subjects are compensated up to $500 for adherence to protocol. Other sites may offer only $50 resulting in less incentive to follow protocol. If a subject missed protocol adherence they would lose $200. He also attributed lower screen failures to population diversity in the Washington DC metropolitan region and humid weather. In contrast, other sites may enroll only Caucasians and have a

dry climate. Please see field investigator, Sharon Gershon's full report in DARRTS.

This reviewer agrees that climate conditions may have effect on baseline sampling counts. Higher versus lower baseline screening failures may be attributed to the weather conditions. BioScience Laboratories located in Bozeman, Montana has more of a drier climate than MicroBiotest located in Sterling, Virginia which has more of a humid climate. The climate conditions may have a rate at which a person perspires leading to a higher baseline counts and lower screen failures.

BioScience Laboratories was inspected between November 30 and December 10, 2015 on the efficacy study EM-05-13260. The results of the report showed that the field investigator interview staff and provided a detailed written review of the processes, procedures and techniques used for the microbial sample collection including scrubbing the test sites where test product was applied. All records were organized and complete. Review of records revealed that there was adequate documentation to assure that all study subjects did exist and were alive and available for the duration of their participation in the study. There were no discrepancies observed with respect to the data listings or information reported to the sponsor. Please see field investigator, Sharon Gershon's full report in DARRTS.

MicroBiotest for study EM-05-012760 and BioScience Laboratories for study EM-05-013260 have both passed inspection with a "No Action Indicated" (NAI). Both of these laboratory facilities have always passed FDA inspection (NAI) since they have been in business (since late 1990's) conducting these types of studies. These laboratory facilities have never received an "Official Action Indicated" (OAI) a fail. Currently, these are the only two microbiological laboratory facilities that conduct clinical simulation studies for patient preoperative skin preparation drug products in the United States.

4.1.2 Protocol Deviations

Study EM-05-012760 (MicroBiotest) Protocol Deviations

The sponsor reported a total of 4 (1%) subjects had a protocol deviation in the abdominal region ITT/safety population: 2(1.8%) in the 3M™ CHG/IPA Prep 10.5 mL and ChloraPrep® group, 1 (0.9%) in the 3M™ CHG/IPA Prep 26 mL and ChloraPrep® group, and 1 (4.2%) in the 3M™ CHG/IPA Prep 26 and saline group (See Table 43). All 4 subjects had a deviation of having a required sample collected or plated beyond the defined time interval; however, based on the results of the neutralization validation, it was determined that the delays would not have any negative impact on the study. One of these subjects (Subject (b) (6) in the 3M CHG/IPA Prep 10.5 mL and ChloraPrep® group) also had a deviation at baseline of having 1 mL triacetin and 4 mL of SSS in the first scrub instead of 1 mL triacetin and 2 mL SSS, then the second scrub was 1 mL SSS;

the sponsor stated that in the opinion of the Investigator, this deviation did not have a negative impact since the total volume of the sample was 6 mL.

Table 43. Protocol deviations intent-to-treat population/safety population abdominal region.

Protocol Deviation	3M CHG/IPA Prep, 10.5-mL and 3M CHG/IPA Prep, 26-mL (N=110)	3M CHG/IPA Prep, 10.5-mL and ChloraPrep (N=111)	3M CHG/IPA Prep, 26-mL and ChloraPrep (N=111)	3M CHG/IPA Prep, 10.5-mL and Saline (N=25)	3M CHG/IPA Prep, 26-mL and Saline (N=24)	ChloraPrep and Saline (N=25)	Total (N=406)
Subjects with any Protocol Deviation	0	2 (1.8%)	1 (0.9%)	0	1 (4.2%)	0	4 (1.0%)
Inadequate consent process	0	0	0	0	0	0	0
Subject did not meet inclusion / exclusion criteria	0	0	0	0	0	0	0
Randomization not followed	0	0	0	0	0	0	0
Required task not completed	0	0	0	0	0	0	0
Required task completed outside of defined time intervals	0	2 (1.8%)	1 (0.9%)	0	1 (4.2%)	0	4 (1.0%)
Use of excluded medications or therapies during the study	0	0	0	0	0	0	0
Other	0	1 (0.9%)	0	0	0	0	1 (0.2%)

***Reviewer's comment**: As mention above, the four subjects had a deviation of having a required sample collected or plated beyond the defined time interval. I agree with the sponsor that based on the results of the neutralization validation that the delays would not have any negative impact on the study. Once the samples are collected, the neutralizers essentially stop the action of any antimicrobial activity immediately. This was verified in the neutralization validation study. The one subject that had a deviation at the baseline of having 1 mL triacetin and 4 mL of SSS in the first scrub instead of the 1 mL triacetin and 2 mL SSS, then the second scrub was 1 mL SSS; this review agrees with the sponsor that this deviation did not have a negative impact since the total volume of the sample was 6 mL. See appendix 16.1.1 on page 23 for the detailed description of the modified sampling solution procedure.*

The sponsor reported a total of 8 (2.0%) subjects had a protocol deviation in the inguinal region ITT/safety population: 1 (0.9%) in the 3M™ CHG/IPA Prep 10.5 mL and 3M™ CHG/IPA Prep 26 mL group, 2 (1.9%) in the 3M™ CHG/IPA Prep 10.5 mL and ChloraPrep® group, 3 (2.8%) in the 3M™ CHG/IPA Prep 26 mL and ChloraPrep® group, 1 (4.3%) in the 3M™ CHG/IPA Prep 10.5 mL and saline group, and 1 (4.2%) in the 3M™ CHG/IPA Prep 26 mL and saline group (See Table 44). All of these subjects had a deviation of having a required sample collected or plated beyond the defined time interval; however, the sponsor stated that in the opinion of the Investigator, the delays did not have any negative impact on the study.

Table 44. Protocol deviations intent-to-treat population/safety population inguinal region.

Protocol Deviation	3M CHG/IPA Prep, 10.5-mL and 3M CHG/IPA Prep, 26-mL (N=108)	3M CHG/IPA Prep, 10.5-mL and ChloraPrep (N=107)	3M CHG/IPA Prep, 26-mL and ChloraPrep (N=106)	3M CHG/IPA Prep, 10.5-mL and Saline (N=23)	3M CHG/IPA Prep, 26-mL and Saline (N=24)	ChloraPrep and Saline (N=23)	Total (N=391)
Subjects with any Protocol Deviation	1 (0.9%)	2 (1.9%)	3 (2.8%)	1 (4.3%)	1 (4.2%)	0	8 (2.0%)
Inadequate consent process	0	0	0	0	0	0	0
Subject did not meet inclusion / exclusion criteria	0	0	0	0	0	0	0
Randomization not followed	0	0	0	0	0	0	0
Required task not completed	0	0	0	0	0	0	0
Required task completed outside of defined time intervals	1 (0.9%)	2 (1.9%)	3 (2.8%)	1 (4.3%)	1 (4.2%)	0	8 (2.0%)
Use of excluded medications or therapies during the study	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0

***Reviewer's comment**: As mention above, the eight subjects had a deviation of having a required sample collected or plated beyond the defined time interval. I agree with the sponsor that based on the results of the neutralization validation that the delays would not have any negative impact on the study. Once the samples are collected, the neutralizers essentially stop the action of any antimicrobial activity immediately. This was verified in the neutralization validation study.*

The sponsor reported that two subjects had sides that were not evaluated for per-protocol analyses due to their protocol deviations. For subject (b) (6), all samples from baseline and 10 minutes post-treatment for the left and right abdomen (ChloraPrep® and 3M™ CHG/IPA Prep 10.5 mL) and for the left and right groin (3M™ CHG/IPA Prep 10.5 mL and 26 mL) were plated beyond the defined interval by approximately 30 minutes; all 4 sides were excluded from the pre-protocol analyses. For subject (b) (6), the 6-hour sample from the right groin (ChloraPrep®) was taken at 5 hours, 29 minutes, 19 seconds; this side was excluded from the pre-protocol analyses. See Table45 for detailed description on all of the subjects.

Table 45. Protocol deviation description on intent-to-treat population/safety population abdominal and inguinal region.

Site-Screening Number	Site-Subject Number	Age(yr)/Sex	Deviation Type	Protocol Deviation
(b) (6)		46/M	Required task completed outside of defined time intervals	Samples of 10 minutes contact time for left abdomen, 10 minutes contact time for right groin and 6 hours contactime for right groin were plated beyoned 30 minutes. In the opinion of Principal Investigator approximately 1 - 5 minutes delay for plating did not have any negative impact on the study.
			Other (specify)	First scrub for the baseline from left abdomen was taken with one mL of Triacetin and 4 mL of SSS instead of one mL of Triacetin and 2 mL of SSS. Second scrub was taken with one mL of SSS. In the opinion of Principal Investigator this deviation did not have any negative impact since the total volume of the sample was 6 mL.
		31/F	Required task completed outside of defined time intervals	Baseline samples for left abdomen and right abdomen were plated beyoned 30 minutes. In the opinion of Principal Investigator approximately 6-7 minutes delay in plating did not not have any negative impact on the study.
		18/F	Required task completed outside of defined time intervals	Baseline sample for right groin was plated beyoned 3o minutes. In the opinion of Principal Investigator, one minute delay in plating did not have any negative impact on the study.
		52/F	Required task completed outside of defined time intervals	Baseline sample for right groin was plated beyoned 30 minutes. In the opinion of the Principal Investigator, a delay of less than one minute did not have any negative impact on the study.
		21/M	Required task completed outside of defined time intervals	6 hour sample for right groin was taken at 6 hours and 37 minutes. In the opinion of Principal Investigator, approximately 7 minutes delay for collection of 6 hour sample did not have any impact on the study.
		22/M	Required task completed outside of defined time intervals	6 hour sample from right groin was taken at 5 hours 29 minutes and 19 seconds, stead of at least 5 hours and 30 minutes. In the opionion of the Principal Investigator, 41 seconds early for collection of 6 hour collection time did not have any negative impact on the study.
		24/M	Required task completed outside of defined time intervals	Samples of baselines and 10 minutes contact time for left and right abdomen, left and right groin were plated beyoned 30 minutes. In the opinion of Principal Investigator approximately 30 minutes delay for plating did not have any negative impact on the study.
		56/M	Required task completed outside of defined time intervals	10 minutes contact time sample from right groin was collected at 11 minutes. In the opinion of PI, 30 seconds delay for sample collection did not have any negative impact on the study.
		21/M	Required task completed outside of defined time intervals	6 hour sample for left abdomen was collected beyond 6 hour and 30 minutes. In the opionion of the PI, 4 seconds delay for sample collection at 6 hour contact time did not have any negative impact on the study. 10 minute sample for right groin was plated beyond 30 minutes. In the opinion of PI, approximately 3 minutes delay for plating did not have any negative impact on the study.

***Reviewer's comment**: As mention above, the sponsor excluded two subjects for the study. For subject (b) (6), all samples from baseline and 10 minutes post-treatment for the left and right abdomen and for the left and right groin were plated beyond the defined interval by approximately 30 minutes; all 4 sides were excluded from the pre-protocol analyses. For subject (b) (6), the 6-hour sample from the right groin was not taken at the designated sample time therefore this subject was excluded from the pre-protocol analyses. This reviewer agrees with the sponsor's decision to exclude the subjects from the study.*

Study EM-05-013260 (BioScience Laboratories) Protocol Deviations

The sponsor reported a total of 18 (3.2%) subjects had a protocol deviation in the abdominal region ITT/safety population; 5 (3.2%) in the 3M™ CHG/IPA Prep and 3M™ CHG/IPA Prep with HEDTA group, 6 (3.8%) in the 3M™ CHG/IPA Prep and ChloraPrep® group, 5 (3.2%) in the 3M™ CHG/IPA Prep with HEDTA and ChloraPrep® group, 1 (2.9%) in the 3M™ CHG/IPA Prep with HEDTA and saline group, and 1 (2.8%) in the ChloraPrep® and saline group (See Table 46). For the abdominal region, deviations included protocol-specified procedures (sampling and/or data recording procedures) not being followed (11 subjects), required tasks being completed outside a defined time interval (3 subjects), subjects not being compliant with the 6-hour ban on exercising/sweating (2 subjects), required tasks not being completed (1 subject), and subjects not meeting the inclusion/exclusion criteria (1 subject).

Table 46. Protocol deviations for intent-to-treat population/safety population for the abdominal region.

Protocol Deviation	3M CHG/IPA Prep, Clear and 3M CHG/IPA Prep, Clear-H (N=154)	3M CHG/IPA Prep, Clear and ChloraPrep (N=156)	3M CHG/IPA Prep, Clear-H and ChloraPrep (N=155)	3M CHG/IPA Prep, Clear and Saline (N=33)	3M CHG/IPA Prep, Clear-H and Saline (N=35)	ChloraPrep and Saline (N=36)	Total (N=569)
Subjects with any Protocol Deviation	5 (3.2%)	6 (3.8%)	5 (3.2%)	0	1 (2.9%)	1 (2.8%)	18 (3.2%)
Inadequate consent process	0	0	0	0	0	0	0
Subject did not meet inclusion / exclusion criteria	0	1 (0.6%)	0	0	0	0	1 (0.2%)
Randomization not followed	0	0	0	0	0	0	0
Required task not completed	0	0	1 (0.6%)	0	0	0	1 (0.2%)
Required task completed outside of defined time intervals	2 (1.3%)	0	1 (0.6%)	0	0	0	3 (0.5%)
Use of excluded medications or therapies during the study	0	0	0	0	0	0	0
Other	3 (1.9%)	5 (3.2%)	3 (1.9%)	0	1 (2.9%)	1 (2.8%)	13 (2.3%)

***Reviewer's comment**: As mention above, for the abdominal region, deviations included protocol-specified procedures (sampling and/or data recording procedures) not being followed, required tasks being completed outside a defined time interval, subjects not being compliant with the 6-hour ban on exercising/sweating, required tasks not being completed, and subjects not meeting the inclusion/exclusion criteria. This reviewer agrees with the sponsor that all of these deviations described would have the potential to affect the study results and these data were not used in the analyses.*

The sponsor reported a total of 16 (4%) subjects had at least 1 protocol deviation in the inguinal region ITT/safety population: 3 (2.8%) in the 3M™ CHG/IPA Prep and 3M™ CHG/IPA Prep with HEDTA group, 2 (1.8%) in the 3M™ CHG/IPA Prep and ChloraPrep® group, 7 (6.2%) in the 3M™ CHG/IPA Prep with HEDTA and ChloraPrep® group, 1 (4%) in the 3M™ CHG/IPA Prep and saline group, 2 (8.0%) in the 3M™ CHG/IPA Prep with HEDTA and saline group, and 1 (4.2%) in the ChloraPrep® and saline group (See Table 47). For the inguinal region, deviations included protocol-specified procedures (sampling and/or data recording procedures) not being followed (8 subjects), required tasks being completed outside a define time interval (4 subjects), subjects not being compliant with the 6-hour ban on exercising/sweating (2 subjects), required tasks not being completed (1 subject), randomization not being followed (1 subject), and subjects not meeting the inclusion/exclusion criteria (1 subject).

Table 47. Protocol deviations for intent-to-treat population/safety population for the inguinal region.

Protocol Deviation	3M CHG/IPA Prep, Clear and 3M CHG/IPA Prep, Clear-H (N=106)	3M CHG/IPA Prep, Clear and ChloraPrep (N=111)	3M CHG/IPA Prep, Clear-H and ChloraPrep (N=113)	3M CHG/IPA Prep, Clear and Saline (N=25)	3M CHG/IPA Prep, Clear-H and Saline (N=25)	ChloraPrep and Saline (N=24)	Total (N=404)
Subjects with any Protocol Deviation	3 (2.8%)	2 (1.8%)	7 (6.2%)	1 (4.0%)	2 (8.0%)	1 (4.2%)	16 (4.0%)
Inadequate consent process	0	0	0	0	0	0	0
Subject did not meet inclusion / exclusion criteria	0	1 (0.9%)	0	0	0	0	1 (0.2%)
Randomization not followed	0	1 (0.9%)	0	0	0	0	1 (0.2%)
Required task not completed	0	0	0	1 (4.0%)	0	0	1 (0.2%)
Required task completed outside of defined time intervals	1 (0.9%)	1 (0.9%)	2 (1.8%)	0	0	0	4 (1.0%)
Use of excluded medications or therapies during the study	0	0	0	0	0	0	0
Other	2 (1.9%)	0	5 (4.4%)	0	2 (8.0%)	1 (4.2%)	10 (2.5%)

<u>Reviewer's comment</u>: As mentioned above, for the inguinal region deviations included protocol-specified procedures (sampling and/or data recording procedures) not being followed, required tasks being completed outside a define time interval, subjects not being compliant with the 6-hour ban on exercising/sweating, required tasks not being completed, randomization not being followed, and subjects not meeting the inclusion/ exclusion criteria. This reviewer agrees with the sponsor that all of these deviations described would have the potential to affect the study results and these data were not used in the analyses.

The sponsor reported that the treatment sides were excluded from the pro-protocol analyses due to the following protocol deviations that resulted in "microbial count potentially affected", "not all data collected", "wrong product applied", "no product applied", and "wrong location sampled": randomization not followed, no product applied, wrong location sampled, sampling not done, plating time recorded incorrectly, pour-plated with incorrect solution, solution volume recorded incorrectly, tasks performed outside defined time interval, subject noncompliance with ban on exercise, and technician wiping site between scrubs. See Table 48.

Table 48. Detailed description of protocol deviation.

Site-Screening Number	Site-Subject Number	Age(yr)/Sex	Deviation Type	Protocol Deviation
(b) (6)		34/M	Randomization not followed	Groin site randomization number 6 has the left side to receive treatment clear. However, clear H was applied to the left side in error.
		21/M	Procedure defined in protocol not performed correctly.	Section 5.3.4:1 details the procedure to be followed to perform sampling using a modification of the cup scrub method. The technician performing the 10 minute post treatment sampling of the left abdomen site deviated from the procedure by wiping the site with sterile gauze between scrub 1 and scrub 2.
		62/M	Incorrect time recorded	The plating time recorded for the left groin site 10 minute sample was 13:42. However, the collection time for that sample was 13:50:25 which is eight minutes after the recorded plating time. The actual plating time for this sample is unknown.
		75/F	Deviation from protocol methodology	Section 5.3.4.1 of the protocol specifies that 1.0 mL of selected dilutions of samples be pour-plated in duplicate using TSA+N (TSA+). The samples taken 6 hours post-prep from both the abdomen and groin sites were pour-plated using TSA.
		30/F	Deviation from protocol methodology	Section 5.3.4.1 of the protocol specifies that 1.0 mL of selected dilutions of samples be pour-plated in duplicate using TSA+N (TSA+). The samples taken 6 hours post-prep from the abdomen sites were pour-plated using TSA.
		32/M	Required task completed outside of defined time intervals	The left abdomen 10 minute sample was plated 31 minutes after the sample was taken (one minute outside the protocol window).

Site-Screening Number	Site-Subject Number	Age(yr)/Sex	Deviation Type	Protocol Deviation
(b) (6)		20/M	Required task completed outside of defined time intervals	The left groin 10 minute sample was plated 34 minutes after the sample was taken (four minutes outside of protocol window).
		30/M	Required task completed outside of defined time intervals	The right groin baseline sample was plated 31 minutes after the sample was taken (one minute outside the protocol window).
		26/M	Required task completed outside of defined time intervals	Product application timing deviation: Product was applied to subject's left abdomen for 42 seconds rather than 30 seconds (as stated in Section 14.2 of the protocol).
		19/F	Procedure defined in protocol section 5.3.4.1 not performed correctly.	Right inguinal (groin) 10 minute plating time was recorded on the Raw Data Collection Sheet as occurring 2 minutes after the collection time, which is not possible if all steps were followed as per protocol.
		24/M	Procedure defined in protocol not performed correctly.	Section 5.3.4.1 details the procedure to be followed to perform the sampling using a modification of the cup scrub method. It was noted the sample collected at 10 minutes on the left abdomen appeared to have the expected total volume, but not the expected 1:5 ratio of Triacetin to SSS. The Triacetin volume was greater than 1.0 mL.
		50/M	Procedure defined in protocol section 5.3.4.1 not performed correctly.	Right abdomen 10 minute plating time was not recorded on the Raw Data Collection Sheet at the time plated. The technician noted the error at 12:30 pm, but the exact time plated is not known.

Site-Screening Number	Site-Subject Number	Age(yr)/Sex	Deviation Type	Protocol Deviation
(b) (6)		20/F	Procedure defined in protocol not performed correctly.	Section 5.3.4.1 details the procedure to be followed to perform the sampling using a modification of the cup scrub method. It was noted the sample collected at 10 minutes on the left groin appeared to have the expected total volume, but not the expected 1:5 ratio of Triacetin to SSS. The Triacetin volume was greater than 1.0 mL.
		25/M	Procedure defined in protocol not performed correctly.	Section 5.3.4.1 details the procedure to be followed to perform the sampling using a modification of the cup scrub method. It was noted the sample collected at 10 minutes on the right groin appeared to have the expected total volume, but not the expected 1:5 ratio of Triacetin to SSS. The Triacetin volume was greater than 1.0 mL.
		23/F	Procedure complication that resulted in deviation to protocol 5.3.4.	Total fluid sample volume recovered was 4 mL and not the expected sample volume of 6 mL, from the left groin 10 minute collected sample.
		23/M	Subject was non-compliant to protocol section 4.1.	The subject reported riding his bicycle to the testing facility for the six-hour return and appeared to be sweating. Therefore, the subject did not follow test day instructions to avoid vigorous physical activity and is a deviation from protocol section 4.1, as recorded as DCS page #10.
		18/F	Subject did not meet inclusion / exclusion criteria	The subject was enrolled in the study with a history of a skin allergy. The subject reported an allergy to some sunscreens resulting in a reaction of hives. Therefore, the subject did not meet exclusion criteria #3 and is a deviation from protocol section 4.2.
			Required task completed outside of defined time intervals	The 10-minute left groin microbial sample was collected 30 seconds too early and not within the protocol time frame of 10 minutes +/- 30 seconds as defined in protocol section 5.3.3.4.

Table 48 (continued). Detailed description of protocol deviation.

Site-Screening Number	Site-Subject Number	Age(yr)/Sex	Deviation Type	Protocol Deviation
	(b) (6)	20/M	Subject was non-compliant to protocol section 4.1	The subject reported skateboarding activities that may have resulted in sweating after leaving treatment and prior to the six-hour return. Therefore, the subject did not follow test day instructions to avoid rigorous physical activity and thus is a deviation from protocol section 4.1, as is recorded on DCS page #10.
		57/F	Subject did not meet inclusion / exclusion criteria	Subject #384 did not meet screening day baseline counts of > 2.5 log10 per cm2 on the bilateral abdominal sites, as in accordance to protocol section 4.1. However, the subject was treated on the abdominal sites on treatment day, which is not in accordance to protocol section 5.3.2.
		18/M	Procedure complication that resulted in deviation to protocol 5.3.4.	Total fluid sample recovered was 3.9 mL and not the expected sample volume of 6.0 mL from the 6-hour right groin collected sample.
		26/M	Procedure complication that resulted in deviation to protocol 5.3.4.	Total fluid sample recovered was 2.8 mL and not the expected sample volume of 6.0 mL from the 6-hour right groin collected sample.
		20/M	Procedure defined in protocol section 5.3.4.1 not performed correctly.	The technician performing the 6-hour left abdomen sampling procedure inadvertently removed the cylinder and wiped the sampling site following the first 3 mL sampling aliquot scrub.
		62/F	Procedure defined in protocol section 5.3.4.1 not followed.	Triacetin volume dispensed during the 6-hour left abdomen sampling procedure was less than 1.0 mL, resulting in 5.1 mL of fluid recovered.

Site-Screening Number	Site-Subject Number	Age(yr)/Sex	Deviation Type	Protocol Deviation
	(b) (6)	19/M	Procedure deviation from Protocol section 5.3.4.	Total fluid sample recovered was 7.0 mL and not the expected sample volume of 6.0 mL from the left abdomen baseline sample collection. The technician reported a total of 3 mL SSS was added to 1 mL of Triacetin on the first aliquot and the second aliquot was performed per protocol.
		20/M	Required task completed outside of defined time intervals	The 10-minute right abdomen microbial sample was collected five minutes late and not within the protocol time frame of 10 minutes +/- 30 seconds as defined in protocol section 5.3.3.4. The 10-minute right groin microbial sample was collected four minutes after 3-minute dry time and not per the protocol time frame of 10 minutes +/- 30 seconds as defined in protocol section 5.3.3.4.
		28/M	Procedure defined in protocol section 5.3.4.1 not performed correctly.	The Left Abdomen baseline plating time was recorded on the Raw Data Collection Sheet as occurring four minutes after the collection time, which is not possible if all steps were followed as per protocol.
		60/M	Required task not completed (e.g., data not collected, missed visit)	On Treatment Day, baseline samples were collected on all but the left groin site. Testing product had been applied to the let groin and left abdomen testing areas. Procedures not done were: right inguinal baseline sample not done, product not applied to right inguinal or right abdomen test areas, 10-minute samples not done and 6-hour samples not completed.
		18/M	Pooling of samples resulting in deviation to protocol section 5.3.4.1.	The first aliquot for the right abdomen 10-minute sample was pooled with the full left abdomen 10-minute sample, resulting in the loss of both samples.

Site-Screening Number	Site-Subject Number	Age(yr)/Sex	Deviation Type	Protocol Deviation
	(b) (6)	20/M	Sampling site's randomization not followed	The right abdomen 10-minute site (randomized site #1) and the 6-hour site (randomized site #4) were inadvertently swapped during testing thus resulting in the 10-minute right abdomen being taken from site #4 and the 6-hour site being taken from site #1. Therefore, this is a deviation from the protocol section 5.3.3.1.

***Reviewer's comment**: As mention above, the sponsor excluded the treatment sides from the pro-protocol analyses due to "microbial count potentially affected", "not all data collected", "wrong product applied", "no product applied", and "wrong location sampled": randomization not followed, no product applied, wrong location sampled, sampling not done, plating time recorded incorrectly, pour-plated with incorrect solution, solution volume recorded incorrectly, tasks performed outside defined time interval, subject noncompliance with ban on exercise, and technician wiping site between scrubs. This reviewer agrees with the sponsor that all of these deviations described would have the potential to affect the study results and excluded certain treatment sides from the data analyses.*

4.1.3 Neutralization Validation for Study EM-05-012760 (MicroBiotest) and Study EM-05-013260 (BioScience Laboratories)

In order to accurately assess the efficacy of an antimicrobial product, it is necessary to completely inactivate the antimicrobial agent at the time point being evaluated. Inadequate neutralization would allow killing or inhibition of the microorganisms to continue beyond the specified contact time, resulting in an overestimation of antimicrobial activity[39, 40, 41]. In a 6-subject substudy prior to the start of the main study, the ability of the MSS to completely neutralize the active ingredients contained in 3M™ CHG/IPA Prep and ChloraPrep® when applied to the abdomen was examined using methods based on ASTM E1054-08 (reapproved 2013), Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents.

The entry criteria were similar to the main study, except that there was no requirement for a minimum baseline bacterial count, there were no restrictions on showering during the 72 hours prior to sampling, and subjects needed to avoid topical and systemic antimicrobials for only 7 days prior to the substudy Treatment Day. Subjects received all 3 active study products, which were applied to 3 separate test areas on the abdomen (each area was divided into 2 test sites). Microbial sampling of each of the 6 sites was performed, then 1 sample form each test area was inoculated with methicillin-resistant *Staphylococcus epidermidis* (MRSE), ATCC 51625 and 1 sample was inoculated with methicillin-sensitive *Staphylococcus epidermidis* (MSSE), ATCC 12228. Immediately (<1 minute) and at 40 ± 2 minutes post-inoculation, aliquots (750 µL) of the inoculated samples were pour-plated in triplicate using TSA+N. Plates were incubated inverted at 35 ± 2°C for 48 ± 4 hours.

***Reviewer's comment**: On June 4, 2013, FDA had a teleconference End-of-Phase 2 meeting with the sponsor. We informed the sponsor that Staphylococcus epidermidis (ATCC 12228) would need to be included in the study. We also recommended that the study include triplicate plating instead of duplicate plating. These recommendations were included in the studies.*

A numbers control and a toxicity control were prepared (in triplicate) from the appropriately diluted inoculum to provide assurance that the test organisms were not adversely affected by the treatments or the sampling procedures. Bacterial counts were performed, and data were converted to $\log_{10}$ CFU/mL. The MSS was considered effective in neutralizing the active ingredients if the mean $\log_{10}$ CFU/mL of the sample was not more than 0.3 $\log_{10}$ less than the mean $\log_{10}$ CFU/mL of the numbers control at each time point. The MSS was considered nontoxic if the mean $\log_{10}$ CFU/mL of the toxicity control was not more than 0.3 $\log_{10}$ less than the mean $\log_{10}$ CFU/mL of the numbers control at each time point.

Table 49. Results of the neutralization validation

Numbers Control								
Time	Immediate				40 minutes			
Results	Plate 1 CFU	Plate 2 CFU	Plate 3 CFU	Log CFU/mL*	Plate 1 CFU	Plate 2 CFU	Plate 3 CFU	Log CFU/mL*
Group 1 - Rep. 1	154	147	140	2.29	130	156	145	2.28
Group 1 - Rep. 2	139	152	144	2.29	142	148	153	2.29
Group 1 - Rep. 3	145	139	155	2.29	127	150	152	2.28
Group 2 - Rep. 1	155	146	140	2.29	129	158	142	2.28
Group 2 - Rep. 2	139	156	141	2.29	141	149	150	2.29
Group 2 - Rep. 3	147	138	154	2.29	126	156	147	2.28
Mean				**2.29**				**2.28**

Toxicity Control								
Time	Immediate				40 minutes			
Results	Plate 1 CFU	Plate 2 CFU	Plate 3 CFU	Log CFU/mL*	Plate 1 CFU	Plate 2 CFU	Plate 3 CFU	Log CFU/mL*
Group 1 - Rep. 1	145	137	131	2.26	137	153	146	2.29
Group 1 - Rep. 2	152	129	132	2.26	142	141	148	2.28
Group 1 - Rep. 3	137	147	152	2.29	136	145	142	2.27
Group 2 - Rep. 1	146	136	129	2.26	136	154	142	2.28
Group 2 - Rep. 2	153	128	139	2.27	146	145	149	2.29
Group 2 - Rep. 3	136	146	152	2.29	136	148	141	2.28
Mean				**2.27**				**2.28**
Diff. from NC				**0.02**				**0.00**

Neutralizer Effectiveness - 3M CHG/IPA Prep 10.5 mL Tinted								
Time	Immediate				40 minutes			
Results	Plate 1 CFU	Plate 2 CFU	Plate 3 CFU	Log CFU/mL*	Plate 1 CFU	Plate 2 CFU	Plate 3 CFU	Log CFU/mL*
Subject 1	139	128	139	2.26	143	135	134	2.26
Subject 2	146	136	132	2.26	140	141	136	2.27
Subject 3	134	146	148	2.28	119	150	141	2.26
Subject 4	141	139	129	2.26	140	136	130	2.26
Subject 5	123	151	140	2.26	139	141	134	2.26
Subject 6	125	139	149	2.26	129	138	137	2.25
Mean				**2.26**				**2.26**
Diff. from NC				**0.03**				**0.02**

Table 49 (continued). Results of the neutralization validation

Neutralizer Effectiveness - 3M CHG/IPA Prep 26 mL Tinted								
Time	Immediate				40 minutes			
Results	Plate 1 CFU	Plate 2 CFU	Plate 3 CFU	Log CFU/mL*	Plate 1 CFU	Plate 2 CFU	Plate 3 CFU	Log CFU/mL*
Subject 1	127	141	135	2.25	133	140	138	2.26
Subject 2	136	129	139	2.25	142	132	136	2.26
Subject 3	141	139	143	2.27	154	126	138	2.27
Subject 4	143	138	130	2.26	133	144	131	2.26
Subject 5	141	146	150	2.29	139	147	142	2.28
Subject 6	146	118	136	2.25	132	143	144	2.27
Mean				**2.26**				**2.27**
Diff. from NC				**0.03**				**0.01**

Neutralizer Effectiveness - ChloraPrep 26 mL Tinted								
Time	Immediate				40 minutes			
Results	Plate 1 CFU	Plate 2 CFU	Plate 3 CFU	Log CFU/mL*	Plate 1 CFU	Plate 2 CFU	Plate 3 CFU	Log CFU/mL*
Subject 1	142	136	134	2.26	141	139	138	2.27
Subject 2	126	141	132	2.25	132	136	140	2.26
Subject 3	129	141	145	2.27	136	139	143	2.27
Subject 4	151	120	132	2.25	119	148	136	2.25
Subject 5	145	139	135	2.27	134	147	138	2.27
Subject 6	137	147	130	2.26	128	134	143	2.26
Mean				**2.26**				**2.26**
Diff. from NC				**0.03**				**0.02**

***Reviewer's comment**: Table 49 represents the neutralization validation for study EM-05-012760 (MicroBiotest) using Staphylococcus epidermidis (MRSE), ATCC 51625. The results for Staphylococcus epidermidis (MSSE), ATCC 12228 is presented in Appendix 16.1.10. The neutralization validation results for study EM-05-013260 (BioScience Laboratories) presented in Appendix 16.1.10. For both studies, since the mean log_{10} CFU/mL of each of the active study products was not more than 0.3 log_{10} less than the mean log_{10} CFU/mL of the Numbers Control, the neutralization was considered effective. For both studies, since the mean log_{10} CFU/mL of each of the Toxicity Control was not more than 0.3 log_{10} less than the mean log_{10} CFU/mL of Numbers Control, the sampling solution was considered non-toxic. Overall, this reviewer finds the neutralization validation studies for both study EM-05-012760 (Microbiotest) and EM-05-013260 (BioScience Laboratories) acceptable.*

4.3.2 Assessment of Neutralization Sampling Solution for 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation

The objective of this study was to assess the sampling solution 1) capability of dissolving the dried polymer film formed by 3M™ CHG/IPA Prep and allowing recovery of organisms immobilized beneath or within the antiseptic film and 2) that is does not demonstrate antimicrobial activity against the organisms targeted for recovery.

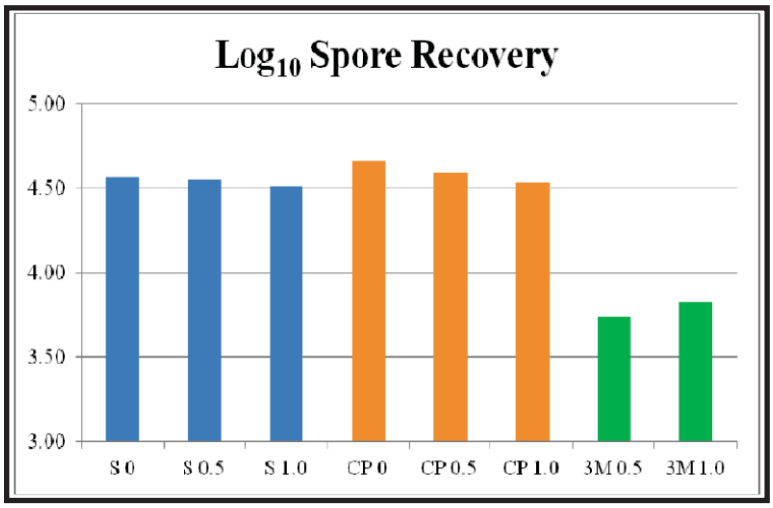
> ***Reviewer's comment: On June 4, 2013, FDA had a teleconference End-of-Phase 2 meeting with the sponsor. The sponsor stated that in evaluations to identify the appropriate sampling solution, it first evaluated spore recovery to select a sampling solution that was capable of dissolving the polymer to release any viable organisms trapped within the film. After selection of the sampling solution, additional evaluation was done using vegetative organisms to confirm that the sampling solution did not demonstrate antimicrobial activity. The sponsor asked FDA if a description and summary of this work be included in the clinical summary of efficacy. We stated that this was acceptable.***

Ex vivo porcine skin was stretched onto a board, clipped to remove hair, thoroughly disinfected with 70% IPA, and allowed to air dry. Sampling sites were marked on the skin using a marker and scrub cup template. The *Bacillus subtilis* spore inoculum was diluted to approximately 10^6 CFU/mL and a 25 µL aliquot was deposited onto each sampling site. The inoculum was spread over the sampling area using a sterile disposable inoculating loop and allowed to air dry for 20 minutes. Packaged 10.5 mL applicators were used to apply the study material and control products. For negative controls, the sponges on unactivated applicators were saturated with saline solution. Prior to treating the sampling sites, applicators were primed once in an empty sterile Petri dish to evenly distribute the test solution and blotted against the lid of the dish. Each sampling site was treated by pressing the applicator firmly against the inoculated porcine skin and the sites were allowed to air dry for 3 minutes before samples were collected.

A sterile scrub cup was placed over the previously delineated sampling area and held firmly. Microbial samples were collected by aseptically pipetting 3 mL of a combination of the film dissolving material being tested and sampling solution (SS) into the scrub cup and scrubbing the skin in a circular motion with moderate pressure for 1 minute a sterile policeman (scrub 1). The scrub solution was transferred to a sterile collection tube. An additional 3.0 mL of fresh SS was then pipetted into the scrub sup and the scrubbing procedure was repeated (scrub 2). This solution was pooled with the solution from the previous step for a total sample volume of approximately 6 mL per site. The test samples were then diluted, pour-plated with trypticase soy agar, and incubated according to standard enumeration techniques.

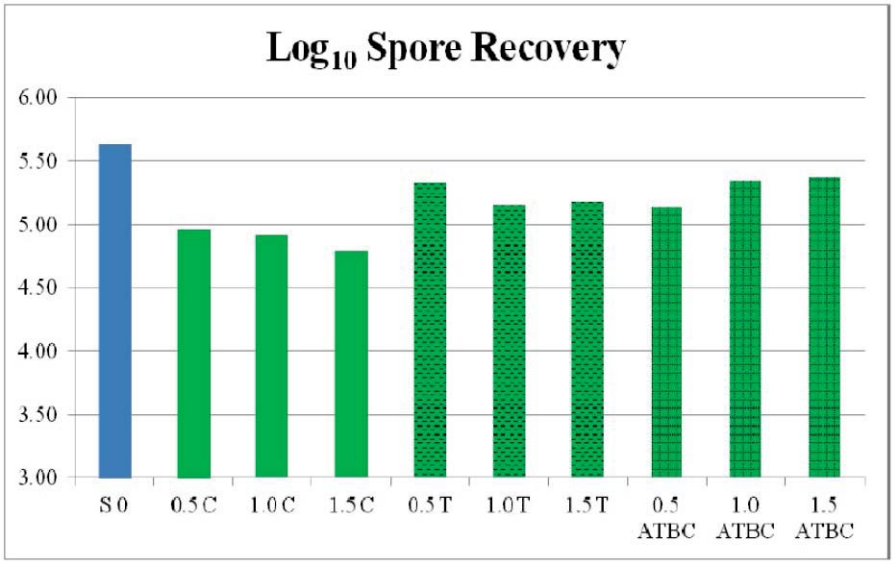
Toxicity: Following the spore recovery studies, the film-dissolver chosen was evaluated to ensure that it did not demonstrate any antimicrobial activity. This evaluation was performed by comparing recovery of *Staphylococcus epidermidis* from a numbers control to recovery from the SS containing the film dissolver shortly after inoculation and again 30 minutes later. Two different levels of film dissolver to SS were tested, one twelfth and one sixth of the total volume.

Table 50. Log_{10} spore recovery for Crodamol



The results showed that in the graph above that there was significant reduction in spore recovery with both levels of Crodamol compared to both saline and ChloraPrep®. The sponsor decided to increase the levels of Crodamol and sonication of the 3M™ CHG/IPA Prep samples was added to determine if spore recovery could be improved. However, the results were still the same. The sponsor decided to try two additional film dissolving chemistries, "triacetin" and "acetyl tributyl citrate (ATBC).

Table 51. Log_{10} spore recovery for triacetin



The results showed that spore recoveries for both triacetin and ATBC were higher than those for Crodamol. However, addition of these chemistries caused the SS to turn milky white after the sonicating. This effect was more pronounced with use of ATBC, which also increased viscosity of the SS significantly. The sponsor decided that using ATBC to dissolve the film would render the lowest dilution pour plates almost impossible to see through for enumeration. Therefore, triacetin was used as the film dissolver. The sponsor concluded that based on the spore recovery results from the studies performed, it was determined that using 1.0 mL of triacetin and 2.0 mL of SS in Scrub 1 and a 2-minute sonication following sample collection yielded recoveries that were within approximately 0.1 $\log_{10}$ of the results obtained for the saline control using 0 mL of film dissolver. The study showed that the addition of 1.0 mL of triacetin to Scrub 1 did not have a negative impact on spore recoveries for saline or ChloraPrep® and the toxicity study showed that there was no antimicrobial activity associated with the use of triacetin at this level.

Reviewer's comment: On February 7, 2014, FDA sent an advice letter to the sponsor. We noticed that the sponsor had changed the composition of the modified sampling solution required to dissolve the film for bacterial recovery from "Crodamol GTCC" to "triacetin". We informed the sponsor that it would need to ensure that the triacetin does not have any antimicrobial activity. The sponsor have justified using triacetin as the film dissolver and provided data to show that it does not have any antimicrobial activity. This review finds this acceptable.

5. AREA COVERAGE, DRY TIME AND VAPOR DISSIPATION

5.1 26 mL and 10.5 mL Applicator Stdueis

3M™ CHG/IPA Prep is an investigational antimicrobial skin preparation containing 2% (w/v) CHG and 70% (v/v) IPA in combination with an acrylate copolymer. When the solution is applied to the skin, it dries to form a water-soluble film.

Reviewer's comment: Because CHG and alcohol combination antiseptic products has ignited when electrocautery was used during surgical procedures when it is not permitted to dry (or is permitted to pool under the patient), it is critical that it be completely dry before an electrical spark is permitted in the operating field. We have implemented class flammability warning that all antiseptics products containing alcohol and used as a patient preoperative skin preparation, the labeling states "completely dry a minimum of 3 minutes on hairless skin."

On June 4, 2013, FDA had a teleconference End-of-Phase 2 meeting with the sponsor. We provided comments to the sponsor on its general study design of the treatment area coverage and dry time label claims. The product should be applied in the same manner as in the clinical simulation study. The

application method used during this study should duplicate that which appears on the labeling. The sponsor need to ensure that all applications are performed by the same person in order to minimize differences in pressure applied during application. The study should include subjects of varying weights and weights in the study. The sponsor has incorporated FDA's recommendations.

We had agreed with the general study design for the vapor dissipation study. We asked the sponsor to include a positive and negative control to the study. The sponsor stated that that they intend to use 70% IPA for the positive control and 0.9% saline solution for the negative control. We agreed with this proposal.

5.1.1 Study EM-05-012985: 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation 26-mL Applicator Area Coverage, Dry Time and Vapor Dissipation Study on Human Skin.

This study was designed to assess the area coverage and dry time with vapor dissipation, and the safety of 3M™ CHG/IPA Prep 26 mL tinted configuration when applied to healthy volunteers. The primary objective of this study was to assess the area coverage of 3M™ CHG/IPA Film forming Preoperative Skin Preparation with tint in the 26 mL applicator. Dry time was measured after application and vapor collection was used to confirm the dryness. The secondary objective of this study was removability of the prep with 70% IPA.

This is an open-label, controlled study in healthy volunteers to evaluate 3M™ CHG/IPA Prep 26 mL (tinted configuration) in three primary endpoints: average area coverage in cm^2, average dry time and vapor dissipation. The secondary endpoint is the removability of the prep with 70% IPA and gauze assessed as easy, moderate, or difficult to remove. Calibration was performed that consisted of collecting IPA vapors during prep drying. These vapor collection data were used to quantify the 70% IPA concentration during the time that the investigational prep solution and positive / negative controls were drying. The same trained professional applied the preps for all subjects. One person assessed the dryness and removability for all subjects.

<u>Reviewer's comment</u>: We have recommended in the past that the same person perform all applications, in order to minimize differences in pressure used.

This controlled study was not randomized. The first subject enrolled in the study was assigned to the vapor collection device calibration with positive control. The second and third subjects were assigned to the vapor collection device calibration with investigation product. The fourth subject enrolled was assigned the vapor collection with negative control. The next 16 subjects were assigned to the area coverage and dry time portion of the study. The study consisted of two phases: 1) the Vapor Collection Device Calibration (4 subjects) and 2) the Area Coverage and Dry Time (16 subjects). Healthy male subjects who met the inclusion/exclusion criteria were entered into the study.

During the Vapor Collection Device Calibration Phase, sample air was drawn through the collection device and transported through Teflon tubing to the spectrometer. A vacuum pump attached to the exhaust side of the spectrometer drew the sample air through the system at a determine rate. Prior to collection of vapors, the study coordinator completed the screening inclusion/exclusion criteria form and performed a visual inspection of the skin test areas. When necessary, the hair was clipped in potential treatment areas.

The product to be applied (the 26 mL applicator investigational product or a container with 26 mL of 70% IPA, or 26 mL of saline with a package of sterile gauze sponges in a tray) was weighed on a calibrated balance. The pre-weight was recorded on the CRF in grams. A 40 cm x 40 cm section of the subject's back was marked. For each application, the study monitor applied surgical prep for all subjects. The vapor collection device was place and moved over the prepped section of the subject's back. The vapor was collected for 5 minutes. The post-weight of the applied product (the investigational product used applicator or the 70% IPA empty container and used applicator or saline empty container, sponges and tray) was collected using a calibrated balance. The weight was reported on the CRF in grams.

For subjects treated with the investigational product, the study coordinator removed the prep using the 70% IPA and gauze. The removability of the prep was assessed by the study coordinator and recorded on the CRF. Skin irritation assessment using the Skin Irritation Scoring System (Modified Draize) were observed at the test site 30 minutes after the prep was removed from the 2 subjects receiving the investigational product and 30 minutes after vapor collection was complete for the control subjects. The skin irritation for each subject was recorded in the CRF.

Area coverage in cm^2 is summarized in Table 51, including the 95% CI for mean and standard deviation. Area coverage in in^2 is summarized in Table 52, including the 95% CI for mean and standard deviation.

Table 51. Area coverage cm^2

Area Coverage cm^2									
N	Mean	Std Dev	Std Err	Minimum	Maximum	95% CL Mean		95% CL Std Dev	
16	2499.8	206.2	51.5424	2150.0	2923.0	2389.9	2609.6	152.3	319.1

Table 52. Area coverage in^2

Area Coverage in^2									
N	Mean	Std Dev	Std Err	Minimum	Maximum	95% CL Mean		95% CL Std Dev	
16	387.5	31.96	7.9891	333.3	453.1	370.4	404.5	23.61	49.46

Dry time is summarized in Table 53, including the 95% CI for mean and standard deviation. The analysis was repeated excluding dry time for subject 9 and shown in Table 54. This subject had a pooled area of solution that was not noticed during application, which falsely extended the dry time.

***Reviewer's comment**: The area coverage results for the 3M™ CHG/IPA Prep with Tint 26 mL applicator was 2499 cm^2 and 387 in^2. The labeling coverage area states "19.5 in. x 19.5 in. area (approximate from the shoulder to groin in an average full size adult)." The coverage area size 19.5 in. x 19.5 in. is approximately 2451 cm^2 and 380 in^2. In addition, the labeling for the 26 mL applicator also states "discard the applicator after a single use along with any portion of the solution not required to cover the prepped area. It is not necessary to use the entire amount available." The coverage area study for the 3M™ CHG/IPA Prep 26 ml applicator is acceptable.*

Table 53. Dry time in minutes.

Dry Time Minutes									
N	Mean	Std Dev	Std Err	Minimum	Maximum	95% CL Mean		95% CL Std Dev	
16	1.5385	0.7867	0.1967	1.0833	4.3333	1.1194	1.9577	0.5811	1.2175

Table 54. Dry time in minutes excluding Subject 9.

Dry Time – Excluding Subject 9 Minutes									
N	Mean	Std Dev	Std Err	Minimum	Maximum	95% CL Mean		95% CL Std Dev	
15	1.3522	0.2606	0.0673	1.0833	1.9667	1.2079	1.4966	0.1908	0.4110

***Reviewer's comment**: The 3M™ CHG/IPA Prep was considered dried on the average of 1.54 minutes (92 seconds). Excluding one subject who had a pooled area of solution that was not noticed during the application, the dry time on average was 1.35 minutes (81 seconds). Overall the drying time is well under the required class flammability labeling of "3 minutes". The labeling states "wait until solution is completely dry (minimum of 3 minutes on hairless skin; up to 1 hour in hair)." See this reviewer's labeling review in DARRTS. The dry time is acceptable for the 3M™ CHG/IPA Prep 26 mL applicator.*

Table 55 Prep removability.

Prep Removability				
Rating	Frequency	Percent	Cumulative Frequency	Cumulative Percent
Moderate	18	100.00	18	100.00

***Reviewer's comment*: Table 55 describes that the removability of the 3M™ CHG/IPA Prep 26 mL applicator is at 100% with moderate level of removal. The Drug Facts labeling describes the prep removability under Other information "the tint will slowly fade from the skin. Alcohol may be used to remove the tint if desired." It would have been interesting to see the sponsor test various concentration of alcohol, since the concentration of alcohol is not specified in the labeling and there are various OTC alcohol concentrations available on the market. It would have been also interesting to see if just using plain soap and water would have removed the film coating on the skin. Overall, this reviewer finds the prep removability from the skin acceptable.**

5.1.2 Study EM-05-013337: 3M™ CHG/IPA Film-Forming Preoperative Skin Preparation 10.5-mL Applicator Area Coverage, Dry Time and Vapor Dissipation Study on Human Skin.

This study was designed to assess the area coverage and dry time with vapor dissipation, and the safety of 3M™ CHG/IPA Prep 10.5 mL tinted configuration when applied to healthy volunteers. The primary objective of this study was to assess the area coverage of 3M™ CHG/IPA Film forming Preoperative Skin Preparation with tint in the 10.5 mL applicator. Dry time was measured after application and vapor collection was used to confirm the dryness. The secondary objective of this study was removability of the prep with 70% IPA.

This is an open-label, controlled study in healthy volunteers to evaluate 3M™ CHG/IPA Prep 10.5 mL (tinted configuration) in two primary endpoints: average area coverage in cm^2 and average dry time. The secondary endpoint is the removability of the prep with 70% IPA and gauze assessed as easy, moderate, or difficult to remove. The same trained professional applied the preps for all subjects. One person assessed the dryness and removability for all subjects.

This controlled study was not randomized. Sixteen healthy male subjects who met the inclusion/exclusion criteria were entered into the study. The study coordinator completed the screening inclusion/exclusion criteria form and performed a visual inspection of the skin test areas. When necessary, the hair was clipped in potential treatment areas. The product to be applied (the 10.5 mL applicator investigational product) was weighed on a calibrated balance. The pre-weight was recorded on the CRF in grams.

The product was applied using repeated back and forth strokes covering a rectangular area on the subject's back. The rectangular area was extended until there was no visual difference in coating intensity. If needed, the rectangular area was extended to legs and arms until there was a visual difference in the coating intensity. Once the test product application was completed, the study coordinator measured the dry time until there was a visual difference in the coating intensity and recorded the dry time in the CRF. After the completion of product application, the used 10.5 mL applicator was weighed on a calibrated balance and this post-application weight was recorded on the CRF. The study coordinator removed the prep using the 70% IPA and gauze. The removability of the

prep was assessed by the study coordinator as easy, moderate or difficult to remove and recorded on the CRF. Skin irritation assessment using the Skin Irritation Scoring System (Modified Draize) were observed at the test site 30 minutes after the prep was removed. The skin irritation for each subject was recorded in the CRF.

Area coverage in cm^2 is summarized in Table 56, including the 95% CI for mean and standard deviation. Area coverage in in^2 is summarized in Table 57, including the 95% CI for mean and standard deviation.

Table 56. Area coverage cm^2

Area Coverage cm^2									
N	Mean	Std Dev	Std Err	Minimum	Maximum	95% CL Mean		95% CL Std Dev	
16	1153.7	108.7	27.2	966.0	1397.3	1095.8	1211.7	80.3	168.2

Table 57. Area coverage in^2

Area Coverage in^2									
N	Mean	Std Dev	Std Err	Minimum	Maximum	95% CL Mean		95% CL Std Dev	
16	178.8	16.8	4.2	149.7	216.6	169.9	187.8	12.4	26.1

***Reviewer's comment**: The area coverage results for the 3M™ CHG/IPA Prep with Tint 10.5 mL applicator was 1153 cm^2 and 178 in^2. The labeling coverage area states "13 in. x 13 in. area." The coverage area size 13 in. x 13 in. is approximately 1090 cm^2 and 169 in^2. In addition, the labeling for the 10.5 mL applicator also states "discard the applicator after a single use along with any portion of the solution not required to cover the prepped area. It is not necessary to use the entire amount available." The coverage area study for the 3M™ CHG/IPA Prep 10.5 ml applicator is acceptable.*

Dry time is summarized in Table 58, including the 95% CI for mean and standard deviation.

Table 58. Dry time in minutes.

Dry Time Minutes									
N	Mean	Std Dev	Std Err	Minimum	Maximum	95% CL Mean		95% CL Std Dev	
16	1.80	0.48	0.12	0.92	2.90	1.54	2.06	0.36	0.74

***Reviewer's comment**: The 3M™ CHG/IPA Prep was considered dried on the average of 1.80 minutes (108 seconds). Overall the drying time is well under the required class flammability labeling of "3 minutes". The labeling states "wait until solution is completely dry (minimum of 3 minutes on hairless skin; up to 1 hour in hair)." See this reviewer's labeling review in DARRTS. The dry time is acceptable for the 3M™ CHG/IPA Prep 10.5 mL applicator.*

Table 59. Prep removability.

Prep Removability				
Rating	Frequency	Percent	Cumulative Frequency	Cumulative Percent
Moderate	7	43.75	7	43.75
Difficult	9	56.25	9	56.25

***Reviewer's comment**: Table 59 describes that the removability of the 3M™ CHG/IPA Prep 10.5 mL applicator is at 44% with moderate level of removal on 7 subjects and 56% with difficult level of removal on 9 subjects participating in the study. I cannot really identify the difference between the two studies conducted on the coverage, dry time, and vapor dissipation but the applicator size difference. If you were to combine the two study results, the results would be 72% with moderate level of removal on 24 subjects and 28% with difficult level of removal on 9 subjects participating in both studies together. The Drug Facts labeling describes the prep removability under Other information "the tint will slowly fade from the skin. Alcohol may be used to remove the tint if desired." Overall, this reviewer finds the prep removability from the skin acceptable.*

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