CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

207964Orig1s000

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 207964 (original)

Sponsor Package Submission: October 20, 2017
Received by Reviewer: October 25, 2017
Review Completed: September 10, 2018

REVIEWER: Michelle M. Jackson, PhD

TEAM LEADER: Francisco Martínez-Murillo, PhD

PROJECT MANAGER: Celia Peacock, RDN, MPH

NAME AND ADDRESS OF APPLICANT
Medline Industries, Inc.
One Medline Place
Mundelein, IL 60060

CONTACT PERSON
Bill Parthun
Director of Research and Development
(847) 643-3839

DRUG PRODUCT NAMES:
Proprietary Name: ReadyPrep™ CHG
Established Name: Chlorhexidine Gluconate (CHG)
Structural Formula: CHG: 1,1'-hexamethylenebis[5-(4-chlorophenyl) bisguanide]di-D-gluconate

INDICATION: Patient preoperative skin preparation

PHARMACOLOGICAL CATEGORY: Health Care Antiseptic

DOSAGE FORM: Topical solution containing 2% chlorhexidine gluconate in a two-cloth (9 in x 10.5 in) per pack configuration and is for single use only.

RELATED SUBMISSION: IND 107899

MATERIALS REVIEWED:

Study No.	Title of Study
	Nonclinical Microbiology In Vitro Evaluations
R14-013	Study R14-013 Microbiological Time-Kill Study on Medline 2% Chlorhexidine Gluconate Solution
R17-004	Study R17-004 Comparative In Vitro Time-Kill Study on Medline 2% Chlorhexidine Gluconate Solution
R14-012	Study R14-012 Evaluation of Potential for Development of Antimicrobial Resistance Study
	Clinical In Vivo Microbiology Evaluations
R13-053	Assessment of the Antimicrobial Efficacy of Medline 2% Chlorhexidine Gluconate Cloth Preoperative Skin Preparation (MicroBioTest)
R15-029	Assessment of the Antimicrobial Efficacy of Medline 2% Chlorhexidine Gluconate Cloth Preoperative Skin Preparation (Evic Romania)
R13-042	Pilot Trial Assessment of the Antimicrobial Efficacy of Medline 2% Chlorhexidine Gluconate Cloth Preoperative Skin Preparation (MicroBioTest)
R14-015	Pilot Trial II Assessment of the Antimicrobial Efficacy of Medline 2% Chlorhexidine Gluconate Cloth Preoperative Skin Preparation (BioScience)
R15-028	Pilot Trial III Assessment of the Antimicrobial Efficacy of Medline 2% Chlorhexidine Gluconate Cloth Preoperative Skin Preparation (Evic Romania)
R16-034	Evaluation of the Area Covered by Medline 2% Chlorhexidine Gluconate Cloth Preoperative Skin Preparation

Table of Contents

1. EXECUTIVE SUMMARY

1.1 Recommended Regulatory Action

Remarks: This review of NDA 207964 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.

This NDA was 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 submitted the application for ReadyPrep™ CHG Patient Preoperative Skin Preparation Cloth (ReadyPrep™ CHG and/or Medline 2% CHG) for over-the-counter (OTC) health care professional use. ReadyPrep™ CHG is an antiseptic solution consisting of chlorhexidine gluconate (CHG) absorbed within a cloth. The ReadyPrep™ CHG content is 2% w/w CHG. The CHG is the only active ingredient. CHG is a highly effective antimicrobial agent providing persistent broad-spectrum antimicrobial activity. The Sponsor is proposing a two-cloth (9 in x 10.5 in) per pack configuration for single use. The ReadyPrep™ CHG cloth is labeled for the treatment area of the skin up to 5 in. x 5 in. in the dry site and 2 in. x 5 in. in the moist site. The Sponsor is seeking a patient preoperative skin preparation indication for this ReadyPrep™ CHG cloth product.

1.1.1. Studies Conducted and Conclusions:

In vitro Studies

To this date, we have evaluated numerous broad spectrum antimicrobial activity studies (minimum inhibitory concentration (MIC)) for Chlorhexidine Gluconate (CHG) drug product applications and we have accumulated a body of evidence, information, and knowledge that helps us better understand the spectrum of antimicrobial activity for CHG products. Because CHG is a well-known antimicrobial agent with broad spectrum activity, FDA accepts a modified in vitro testing scheme. This acceptable in vitro time-kill study includes the following modifications: a limited number of organisms, rather than requiring the full battery of organisms (four ATCC strains instead of 25, and 12 representative clinical isolates instead of 25); an specification to test three concentrations of the final formulation (actual use concentration, another concentration in the active range, and an inactive concentration); and the concept that minimum inhibitory concentration is no longer required.

Study R14-013: Microbiological Time-Kill Study on Medline 2% Chlorhexidine Gluconate Solution

The time-kill study showed that Medline 2% CHG solution (full strength-1X), secondary concentration within the active range (0.5X), and the active control, Dyna-Hex 2®, produced ≥3 $\log_{10}$ reduction (>99.9%) killing effect in 6 minutes and 10 minutes in all the organisms tested. When Medline 2% CHG Solution was diluted to half its strength (0.5X) it still produced ≥5 $\log_{10}$ reduction (>99.9%) killing effect in 6 minutes and 10 minutes in most of the organisms tested. The killing effect or antimicrobial activity of a drug for a particular

microorganism needs to be ≥3 log_{10} reduction to be considered an active ingredient. When Medline 2% CHG solution was diluted to 0.01% (0.0001X) it produced ≤1 log_{10} reduction killing effect in 6 minutes and 10 minutes in most of the organisms tested. This is an inactive concentration. Overall, the results of the time-kill studies provided by the Sponsor indicate that the test product Medline 2% CHG solution achieved a >99.9% reduction in viable microbial cells in 6 and 10 minutes. These results are comparable to those achieved with the active control, Dyna-Hex 2®.

The neutralization validation study results for R14-013 showed that the neutralization solution used in the test was non-toxic and effectively neutralized the activity of Medline 2% CHG solution at various strengths.

R17-004: Assessment of Microbial Activity of Two Medline ReadyPrep™ CHG Solution Formulations Using a Modified Time-Kill Procedure

Per agreement with the Agency during the Type A meeting discussion on May 23, 2016, the Sponsor planned to demonstrate the similarity in effectiveness of ReadyPrep™ CHG as an antimicrobial cloth between its proposed New formulation (b) (4) and the Old formulation (b) (4) to support the scientific bridge to the clinical safety and efficacy data and to the quality data supporting the prior information. The Sponsor employed the modified in vitro time-kill study to evaluate the susceptibility of bacteria to the "New" and "Old" ReadyPrep™ CHG formulations. The time-kill study showed that both ReadyPrep™ CHG products ("Old" and "New" formulation) produced ≥3 log_{10} reduction (>99.9%) killing effect in 6 minutes and 10 minutes for most organisms tested. In addition, the testing showed less than 3 log_{10} reduction for some specific organism, such as *Enterococcus faecalis* and *Staphylococcus aureus*. Overall, the results of the time-kill studies provided by the Sponsor indicate that the (b) (4) has no impact on the antiseptic effectiveness of the "New" ReadyPrep™ CHG formulation.

Chlorhexidine Gluconate Resistance Studies

The issue of antiseptic resistance has been a subject of concern for FDA and industry. During recent years, there are concerns regarding the emergence of resistance to CHG 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 CHG in the clinical setting. Results of studies attempting to demonstrate development of resistance have been contradictory because of the varying methodologies conducted and the amount of CHG used in the studies. Although the development of reduced susceptibility to antiseptics caused by continuous exposure to CHG may occur, the level of resistance is reported to be low and the CHG concentrations used in antiseptics are much higher than the concentration potentially associated to resistance. For the time being, researchers recommend that susceptibility and resistance of microorganisms to CHG should be closely monitored. We continue to request sponsors to provide literature updates and to conduct resistance and cross-resistance to antibiotics on CHG drug products.

Study R14-012: Evaluation of Potential for Development of Antimicrobial Resistance to ReadyPrep™ CHG Solution

The endpoints for ReadyPrep™ CHG 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, in relation to 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.

Assessment of the Vehicle (inactive) Control for ReadyPrep™ CHG

A vehicle control (b) (4) was evaluated against ATCC strains. See Table 1 below for the composition of the vehicle excluding the CHG solution. As this vehicle solution was utilized to (b) (4) (b) (4) microorganisms in Sage CHG Cloth[1]. The time-kill testing of the vehicle was incorporated in the "Assessment of Microbicidal Activity of ReadyPrep™ CHG Solution Using a Modified Time-Kill Procedure (R14-013)." Benzalkonium chloride (b) (4) is used as a (b) (4) in this formulation, (b) (4) Nevertheless, similarly to isopropyl alcohol, based on the study results using the product vehicle, it seems that benzalkonium chloride does not significantly contribute to the activity of this product. According to the FDA inactive ingredient database for approved drug products, benzalkonium chloride (b) (4) has also been used as an excipient in at least one approved topical lotion[2] product.

Table 1. Composition of ReadyPrep™ 2% Chlorhexidine Gluconate Solution.

Component	Quality Standard	Function	Amount (% w/w)
Purified Water	USP	(b) (4)	(b) (4)
Chlorhexidine Gluconate Solution	(b) (4) USP	Drug Substance	
Glycerin	USP	(b) (4	
Propylene Glycol	USP		
(b) (4) Dimethicone NF Emulsion	(b) (4)		
Isopropyl Alcohol	USP		
(b) (4) Benzalkonium Chloride Solution	NF		

The vehicle demonstrated some antimicrobial activity, although less than the 2% CHG containing products. ReadyPrep™ CHG and Dyna-Hex 2® produced comparable $\log_{10}$ reductions on the same microorganisms tested. These two CHG containing products had generally $\log_{10}$ reductions greater than 5 $\log_{10}$. The activity observed with the vehicle did not

affect the antimicrobial effectiveness of the ReadyPrep™ CHG, when compared to Dyna-Hex 2® on the same microorganisms evaluated. The log_{10} reductions for the vehicle solution were mostly ≤ 3 log_{10} reduction, indicating no significant activity. There were two microorganisms *Serratia marcescens* and *Streptococcus pneumoniae* that showed a 3 log_{10} reduction at 6 and 10 minutes. This is not surprising, due to the inactive ingredients such as isopropyl alcohol and benzalkonium chloride, which are otherwise commonly used as antimicrobial preservatives in topical products to prevent bacterial growth. Benzalkonium chloride, like alcohol, is also used as (b) (4) Overall, the ReadyPrep™ CHG formulation was efficacious at reducing the level of ATCC repository and clinical isolate organisms within the 6- and 10-minute evaluations. Log_{10} reductions observed with the ReadyPrep™ CHG were similar to the comparator, Dyna-Hex 2®. The vehicle did not significantly contribute to the overall antimicrobial activity of ReadyPrep™ CHG formulation.

Coverage Area and Drying Time Studies

A study was designed to assess the coverage area of Medline 2% CHG cloth as well as the drying time when applied to 30 healthy volunteers. Dry time was measured after application. Drying times were recorded by three different technicians, independently. The amount of product applied was determined by subtracting the final weight of the cloth plus packaging from the initial weight.

R16-034: Evaluation of the Area Covered by Medline 2% Chlorhexidine Gluconate Cloth Preoperative Skin Preparation

The area coverage results for the Medline 2% CHG cloth was 3.66 g / 0.0081 g/cm^2 = 451 cm^2. The average coverage in square inches is 70 in^2 (10 x 7 inches). The labeling coverage area for the dry site (i.e., abdomen) states, "use one cloth to cleanse each 161 cm^2 area (approximately 5 x 5 inches) of skin to be prepared." and for the moist site (i.e., groin), the labeling states, "use one cloth to cleanse each 65 cm^2 area (approximately 2 x 5 inches) of skin to be prepared." In addition, the labeling for the Medline 2% CHG cloth also states, "After package has been opened discard any unused cloths." The coverage area study for the Medline 2% CHG cloth is acceptable.

The Medline 2% CHG cloth was considered dried on the average of 1.10 minutes (70 seconds), excluding one subject who had a 6.15 minutes (369 seconds) dry time on average. The Sponsor stated that this outlier was considered extreme enough that it would make the numerical results of the drying time analyses suspect or invalid if it were included. This is an unusually high drying time that can be considered an error with an undetermined root cause, therefore, the drying time from this subject was excluded from further analyses. The drying time on the label states, "Allow area to dry for one (1) minute." Since the active ingredient is only CHG (b) (4) flammability labeling is not required. The drying time of one minute is acceptable for the Medline 2% CHG cloth labeling.

Clinical Simulation Studies

The Sponsor included an 8-hour time point in three of its phase II pilot studies. The pilot studies were used to determine the test article application procedure and to evaluate the

efficacy level at endpoints of 10-minutes, 6-hours and 8-hours posttreatment using the test and positive control articles. The data of the pilot studies were used to determine the appropriate application time and determine if the 8-hour endpoint time was achievable. The results would then be used to calculate the number of subjects required to meet the FDA criteria for efficacy. If the 8-hour endpoint remains below the treatment day baseline, the Sponsor proposed, this endpoint would be included in the pivotal studies, in addition to the 10-minutes and 6-hours posttreatment endpoints. The Sponsor included the 8-hour time point in the pivotal studies (b) (4) (b) (4) (b) (4) also noted in microbiologist Dr. Pranvera Ikonomi's review to the IND file dated December 8, 2014 in DARRTS.

Two pivotal clinical simulation studies (R13-053: MicroBioTest and R15-029: Evic Romania) were designed to evaluate the antimicrobial efficacy and safety of Medline 2% CHG Cloth, Vehicle Cloth control, and active control Dyna-Hex 2® 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 R13-053 (MicroBioTest): Assessment of the Antimicrobial Efficacy of Medline 2% CHG Cloth Preoperative Skin Preparation

1. Primary Analysis Responder Rates

   For the abdominal region modified intent-to-treat population, the responder rate 95% CI lower bounds were 89.4% and 80.0% for Medline 2% CHG Cloth and Dyna-Hex 2®, respectively. The corresponding responder rate point estimates were 93.2% and 85.0, respectively. See Table 2 below.

   For the inguinal region modified intent-to-treat population, the responder rate 95% CI lower bounds were 80.9% and 58.7% for Medline 2% CHG Cloth and Dyna-Hex 2®, respectively. The corresponding responder rate point estimates were 85.8% and 65.0%, respectively. See Table 2 below.

   For both abdominal and inguinal regions, responder rates for all active products were significantly higher (more effective) than for the Vehicle Cloth Control. The responder rate following Vehicle Cloth Control treatment was 50% for the abdominal region and 25% for the inguinal region. At 6 hours, Medline 2% CHG Cloth and Dyna-Hex 2® had 100% responder rates (all values below baseline) for both the abdominal and inguinal sites. See Table 2 below. Therefore, the Medline 2% CHG Cloth has met the primary

endpoint recommended requirement for this clinical simulation study. See Table 2 below.

Table 2. Responder Rate at 10 Minutes and 6 Hours (mITT Population) Study R13-053 MicroBioTest).

Body Area	Treatment	10 Minute Responder Rates	
		Rate (%) (counts)	95% Exact Confidence Interval
Abdomen	Dyna-Hex 2	85.04% (216 of 254)	0.8005 to 0.8919
Abdomen	Medline Cloth	93.25% (235 of 252)	0.8942 to 0.9602
Abdomen	Vehicle Cloth	50.00% (24 of 48)	0.3523 to 0.6477
Groin	Dyna-Hex 2	65.06% (162 of 249)	0.5879 to 0.7097
Groin	Medline Cloth	85.83% (218 of 254)	0.8092 to 0.8987
Groin	Vehicle Cloth	25.00% (12 of 48)	0.1364 to 0.3960
Body Area	**Treatment**	**6 Hour Responder Rates**	
		Rate (%) (counts)	95% Exact Confidence Interval
Abdomen	Dyna-Hex 2	100.00% (254 of 254)	0.9856 to 1.0000
Abdomen	Medline Cloth	100.00% (252 of 252)	0.9855 to 1.0000
Abdomen	Vehicle Cloth	97.92% (47 of 48)	0.8893 to 0.9995
Groin	Dyna-Hex 2	100.00% (249 of 249)	0.9853 to 1.0000
Groin	Medline Cloth	100.00% (254 of 254)	0.9856 to 1.0000
Groin	Vehicle Cloth	100.00% (48 of 48)	0.9260 to 1.0000

2. Secondary Analysis Efficacy Mean Log_{10} Reduction

For the abdominal region, the baseline mean bacterial (skin flora) count was approximately 3.4 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: 3.17 (0.2812) log_{10} per cm^2 and 2.91 (0.525) log_{10} per cm^2 for Medline 2% CHG Cloth and Dyna-Hex 2®, respectively. Therefore, all the test products demonstrated ≥2 log_{10} reduction at the abdomen site. See Table 3 below.

At 6 hours following treatment, the mean (SD) reduction from baseline was similar among active treatments: 2.51 (0.945) log_{10} per cm^2 and 2.23 (1.207) log_{10} per cm^2 for Medline 2% CHG Cloth and Dyna-Hex 2®, respectively. The Mean (SD) reduction from baseline following Vehicle Cloth Control treatment was 1.50 (1.98) log_{10} per cm^2 at 6 hours. Therefore, all the test products did not exceed the baseline counts at 6 hours. See Table 3 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: 4.27 (1.175) log_{10} per cm^2 and 3.67 (1.790) log_{10} per cm^2 for Medline 2% CHG Cloth and Dyna-Hex 2®, respectively. Therefore, all the test products demonstrated ≥3 log_{10} reduction at the inguinal site. See Table 3 below.

At 6 hours following treatment, the mean (SD) reduction from baseline was similar among active treatments: 3.10 (2.348) log_{10} per cm^2 and 2.66 (2.801) log_{10} per cm^2 for

Medline 2% CHG Cloth and Dyna-Hex 2®, respectively. The mean (SD) reduction from baseline following Vehicle Cloth Control treatment was 2.47 (2.935) log_{10} per cm^2 at 10 minutes and 2.06 (3.353) log_{10} per cm^2 at 6 hours. Therefore, all the test products did not exceed the baseline counts at 6 hours. See Table 3 below.

Table 3. Summary Statistics of Log Transformed Bacterial (Skin Flora) Endpoints Mean Log_{10} CFU Counts and Changes from Baseline (mITT Population, Study R13-053 MicroBioTest).

Body Area	Treatment	Baseline	10 Minutes		6 Hours	
			Value	Change	Value	Change
Abdomen	Dyna-Hex 2	3.4383	0.5255	2.9128	1.2072	2.2311
Abdomen	Medline Cloth	3.4586	0.2812	3.1774	0.9456	2.5131
Abdomen	Vehicle Cloth	3.4852	1.5232	1.9620	1.9811	1.5041
Groin	Dyna-Hex 2	5.4634	1.7908	3.6726	2.8017	2.6617
Groin	Medline Cloth	5.4510	1.1753	4.2757	2.3489	3.1021
Groin	Vehicle Cloth	5.4148	2.9351	2.4797	3.3531	2.0617

Study R15-029 (Evic Romania): Assessment of the Antimicrobial Efficacy of Medline 2% Chlorhexidine Gluconate Cloth Preoperative Skin Preparation

1. Primary Analysis Responder Rates

The primary efficacy endpoint of achieving a responder rate 95% CI lower bound ≥70% was met by the Medline 2% CHG Cloth and was borderline for the Dyna-Hex 2® on the abdominal region. For the abdominal region modified intent-to-treat population, the lower bounds of the 95% CI for responder rate were 74.9% and 65.5% for Medline 2% CHG Cloth and Dyna-Hex 2®, respectively. The corresponding responder rate point estimates were 80.5% and 71.5%, respectively, and were all higher and significantly effective when compared to the Vehicle Cloth control (50%).

The primary efficacy endpoint of achieving a responder rate 95% CI lower bound ≥70% was met by the Medline 2% CHG Cloth and was borderline for the Dyna-Hex 2® on the inguinal region. For the inguinal region modified intent-to-treat population, the lower bounds of the 95 CI for responder rate were 79.4% and 67.1% for Medline 2% CHG Cloth and Dyna-Hex 2®, respectively. The corresponding responder rate point estimates were 84.5% and 72.9%, respectively, and were all higher and significantly effective when compared to the Vehicle Cloth control (55%). Dyna-Hex 2® did make (72.9%) the FDA recommended ≥70% responder rate for the inguinal region; however, did not quite make the lower bound CI (67.1%). See Table 4 below.

For both abdominal and inguinal regions, responder rates for all active products were significantly more effective than the Vehicle Cloth Control. The responder rate following Vehicle Cloth Control treatment was 50% for the abdominal region and 55% for the inguinal region. At 6 hours, Medline 2% CHG Cloth showed 100% responder rates (all values below baseline) for both abdominal and inguinal sites. At 6 hours, Dyna-Hex 2® had a 100% responder rate (all values below baseline) for the inguinal site and a 98.81% responder rate (values below the baseline) for the abdominal site. It appears that

3 out of 253 subjects had values above the baseline. This may be attributed to subjects leaving the lab during the 6-hour period and moving around more than normal, sweating, and the gauze adhesive pad accidently not adhering to the skin.

Table 4. Responder Rate at 10 Minutes and 6 Hours (mITT Population) Study R15-029 Evic Romania.

Body Area	Treatment	10 Minute Responder Rates	
		Rate (%) (counts)	95% Exact Confidence Interval
Abdomen	Dyna-Hex 2	71.54% (181 of 253)	0.6555 to 0.7702
Abdomen	Medline Cloth	80.50% (194 of 241)	0.7492 to 0.8530
Abdomen	Vehicle Cloth	50.00% (25 of 50)	0.3553 to 0.6447
Groin	Dyna-Hex 2	72.97% (189 of 259)	0.6713 to 0.7828
Groin	Medline Cloth	84.52% (213 of 252)	0.7946 to 0.8876
Groin	Vehicle Cloth	55.77% (29 of 52)	0.4133 to 0.6953
Body Area	**Treatment**	**6 Hour Responder Rates**	
		Rate (%) (counts)	95% Exact Confidence Interval
Abdomen	Dyna-Hex 2	98.81% (250 of 253)	0.9657 to 0.9975
Abdomen	Medline Cloth	100.00% (241 of 241)	0.9848 to 1.0000
Abdomen	Vehicle Cloth	96.00% (48 of 50)	0.8629 to 0.9951
Groin	Dyna-Hex 2	100.00% (259 of 259)	0.9859 to 1.0000
Groin	Medline Cloth	100.00% (252 of 252)	0.9855 to 1.0000
Groin	Vehicle Cloth	100.00% (52 of 52)	0.9315 to 1.0000

2. Secondary Analysis Efficacy Mean Log_{10} Reduction

For the abdominal region, the baseline mean bacterial (skin flora) count was approximately 3.7 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: 2.89 (0.887) log_{10} per cm^2 and 2.55 (1.220) log_{10} per cm^2 for Medline 2% CHG Cloth and Dyna-Hex 2®, respectively. Therefore, all the active test products demonstrated ≥2 log_{10} reduction at the abdomen site. See Table 5 below.

At 6 hours following treatment, the mean (SD) reduction from baseline was similar among active treatments: 3.08 (0.699) log_{10} per cm^2, and 2.69 (1.073) log_{10} per cm^2 for Medline 2% CHG Cloth and Dyna-Hex 2®, respectively. The Mean (SD) reduction from baseline following Vehicle Cloth Control treatment was 2.24 (1.460) log_{10} per cm^2 at 6 hours. Therefore, all the test products did not exceed the baseline counts at 6 hours. See Table 5 below.

For the inguinal region, the baseline mean bacterial (skin flora) count was approximately 6.1 log_{10} per cm^2 across study products. The mean (SD) reduction from baseline at 10 minutes following treatment was similar among active treatments: 4.58 (1.536) log_{10} per cm^2 and 3.66 (2.433) log_{10} per cm^2 for Medline 2% CHG Cloth and Dyna-Hex 2®, respectively. See Table 5 below.

At 6 hours following treatment, the mean (SD) reduction from baseline was similar among active treatments: 4.98 (1.140) log_{10} per cm^2 and 3.96 (2.132) log_{10} per cm^2 for Medline 2% CHG Cloth and Dyna-Hex 2®, respectively. The mean (SD) reduction from

baseline following Vehicle Cloth Control treatment was 3.66 (2.480) log_{10} per cm^2 at 10 minutes and 3.76 (2.374) log_{10} per cm^2 at 6 hours. Therefore, all the test products did not exceed the baseline counts at 6 hours. See Table 5 below.

Table 5. Summary Statistics of Log-Transformed Bacterial (Skin Flora) Endpoints Mean Log_{10} CFU Counts and Changes from Baseline (mITT Population, Study R15-029 Evic Romania).

Body Area	Treatment	Baseline	10 Minutes		6 Hours	
			Value	Change	Value	Change
Abdomen	Dyna-Hex 2	3.7734	1.2200	2.5534	1.0736	2.6998
Abdomen	Medline Cloth	3.7865	0.8879	2.8987	0.6990	3.0875
Abdomen	Vehicle Cloth	3.7050	1.6605	2.0445	1.4601	2.2449
Groin	Dyna-Hex 2	6.1000	2.4330	3.6670	2.1325	3.9675
Groin	Medline Cloth	6.1210	1.5369	4.5842	1.1404	4.9806
Groin	Vehicle Cloth	6.1436	2.4806	3.6630	2.3741	3.7695

Neutralization Validation Studies for R13-053 and R15-029

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

Test Product: In study R13-053 (MicroBioTest), the test product had a mean log_{10} reduction of 4.27 for the groin site and a mean log_{10} reduction of 3.18 for the abdomen site. The test product met the 70% responder rate at the 10-minutes time point for both the groin site (85.98%, with lower bound CI of 78.34%) and abdomen site (93.28%, with lower bound CI of 88.16%) and remained below baseline (100%) at the 6-hour time point for both the groin and abdomen site. In study R15-029 (Romania), the test product had a mean log_{10} reduction of 4.58 for the groin site and a mean log_{10} reduction of 2.86 for the abdomen site. The test product met the 70% responder rate at the 10-minutes time point for both the groin site (84.25% with lower bound CI of 77.74%) and abdomen site (78.99% with lower bound CI of 71.66%) and remained below baseline (100%) at the 6-hour time point for both the groin and abdomen site. The test product at both laboratory facilities passed the required mean log reduction and the 70% responder rate.

Active control: In study R13-053 (MicroBioTest), for the groin site the active control Dyna-Hex 2® did not pass the ≥70% responder rate primary endpoint (65%) and the lower bound of the CI (58%), however, the mean log reduction criteria met the recommended 3 log_{10} reduction (3.67 log_{10} reduction). In study R15-029 (Evic Romania), the active control Dyna-Hex 2® did pass the ≥70% responder rate primary endpoint (71% for the abdomen and 72% for the groin), however, it did not pass the lower bound of the CI (65% for the abdomen and

67% for the groin). For the mean log reduction criteria Dyna-Hex 2® met the recommended 2 $\log_{10}$ reduction in the abdomen site (2.55 $\log_{10}$ reduction) and 3 $\log_{10}$ reduction in the groin site (3.66 $\log_{10}$ reduction).

The Vehicle Cloth Control showed efficacy at both the abdomen (2.0 $\log_{10}$ reduction) and groin (3.66 $\log_{10}$ reduction) for the Evic Romania study (also shown in the pilot study conducted by BioScience Laboratories). This is not surprising, due to the mechanical action of scrubbing with the vehicle cloth. The in vitro time-kill test demonstrated that the vehicle (with no active ingredient and no cloth) showed no antimicrobial activity, and the saline solution also showed no antimicrobial activity, therefore, there is no value in repeating the in vivo clinical simulation study with a negative control (saline).

The responder rates of the FDA-approved and marketed positive control (Dyna-Hex 2®) fail to confirm reproducibility of responder rate outcomes between the two laboratories. MicroBioTest failed at the groin site with a responder rate of 65% (58% lower bound 95% CI). The Evic Romania was borderline for the abdomen and groin sites, 71.54% (65% lower bound 95% CI) and 72.97% (67% lower bound 95% CI), respectively. The differences in demographics, climate and microbiomes of available subjects between the two testing laboratories may account for these differences in responder rates (b) (4) Although Dyna-Hex 2® is a solution in a bottle, the direction for use requires the use of a sterile gauze pad. The gauze pad is made of a woven cotton material, whereas the Medline cloth is made of an (b) (4) 100% polyester material. We provided the Sponsor advice on using a similar FDA-approved antiseptic cloth product, however the Sponsor opted for using Dyna-Hex 2® as its active control.

We have experienced in the past the issue of antiseptic products having a difficult time not meeting the 70% responder rate for the 10-minute post treatment reductions either at the abdomen or the groin site. It is difficult to determine why some antiseptic products do not perform consistently at these sites. The test results for some antiseptic products and whether they meet the specified point estimate statistical criteria for effectiveness, appears to vary by the laboratory doing the testing. One lab consistently reports meeting the specified point estimate criteria, while the other lab does not report similar findings. The reasons for these differences are not clear. Below are comments on the possible contributing factors:

- The labs may conduct their study trials differently (e.g., the amount of pressure applied when scrubbing (sampling) the skin).

- The protocols for R13-053 (MicroBioTest) and R15-029 (Evic Romania) 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
Evic Romania	3.46 cm^2

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

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

Evic Romania $CFU/cm^2 = \frac{CFU/mL \times 6\ mL}{3.46\ cm^2}$

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 1994 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 could suggest a possibility of getting a higher bacterial count, however, the fact that the sampling is normalized per subject, and that it is the site with lower cylinder area for sampling the one that shows higher performance rates, does not clarify the contribution of this factor to the variability observed.

- Another possible inconsistency is the amount of pressure or how hard the technician is scrubbing the skin using the hollow cylinder with the scrub solution and policeman. The outcome is to scrape the epithelial layer of the skin to retrieve a high number of bacteria count in order to get a high $\log_{10}$ reduction count. This has been an issue of speculation for several years. You don't know if one person is the designated scrubber for the whole study or if several different technicians are scrubbers. Although the directions state to scrub with moderate pressure, it is difficult to determine what is "moderate pressure".

- Regarding the 3 $\log_{10}$ 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.

Evic Romania was inspected between March 26 and April 5, 2018 on the efficacy study R13-029. The results of the report showed that the field investigator interviewed the laboratory manager (Dr. Olsavszky) and 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. The Office of Scientific Investigations (OSI) judged that the deficiencies noted and discussed could be considered regulatory violations, and OSI classified the inspection outcome as Voluntary Action Indicated (VAI). The main deficiencies were as follows:

- Discrepancies between source records and data listings with respect to bacterial sample collection times and scrub application times.
- Microbial sample collections were outside the protocol specified timeframes.
- Enrollment of subjects who did not meet the baseline CFU bacterial counts.

Overall the field investigator concluded that the findings were unlikely to have a significant impact on the efficacy evaluation. The investigator also stated the following: "The data from the clinical investigator site submitted by the Sponsor in support of the pending application are acceptable and the study was conducted adequately to support approval."

Although, we had recommended IND/NDA sponsors to use the percent responder rate statistical criteria and had published our recommended statistical criteria in the Safety and Effectiveness of Health Care Antiseptics Proposed Rule (80 FR 25166), we have since then evaluated and assessed these statistical criteria from comments regarding how unattainable it is to achieve these criteria. We have made a final decision on the proposed statistical criteria in the final rule on Health Care Antiseptic published on December 20, 2017, and we are now also accepting the use of the average treatment effects (ATE) across subjects meeting indication-specific conditions of superiority and non-inferiority, rather than the significance of the percentage of subjects who met an indication-specific threshold, to exceed 70 percent. In this submission, we are focusing on the responder rate analysis rather than any other analysis methodology, such as the average treatment effect, because we realize that a non-inferiority analysis can be confounded by the fact that the vehicle uses a cloth, which introduces a level of effectiveness to the study results that is difficult to extricate from the product. Please refer to the statistician, Dr. Elande Baro's review in DARRTS.

1.1.2 Recommendation:

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. INTRODUCTION

The Sponsor submits an NDA for ReadyPrep™ CHG, a 2% CHG cloth, under Section 505(b)(2) of the FD&C Act. CHG is an established antimicrobial agent and ReadyPrep™ CHG is indicated for use as a preoperative skin preparation. ReadyPrep™ CHG is composed of a 2% CHG solution (equivalent to 500 mg chlorhexidine gluconate per cloth) on single fiber, polyester cloth in a two-cloth per pack configuration and is intended solely for topical use. The solution is designed to dry on the skin and not be washed off.

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 preoperative skin preparation solution is to create an operative field that is as close to sterile as possible, by reducing the patient's 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 preoperative 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 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.

FDA has agreed to the following clinical development plan for the Sponsor's drug application: 1) clinical trial comparing efficacy of ReadyPrep™ CHG to an active control Dyna-Hex 2® and vehicle control; 2) modified time-kill studies; 3) antimicrobial resistance studies; 4) coverage area, and drying time studies; 5) cumulative irritation and contact sensitizing test studies; and 6) pharmacokinetic study.

The drug product contains CHG as the main active ingredient. Most of the clinical studies were conducted with a ReadyPrep™ CHG Cloth product that contained the following ingredients: glycerin, propylene glycol, dimethicone, isopropyl alcohol, benzalkonium chloride, (b) (4). Many of these ingredients, propylene glycol, dimethicone, isopropyl alcohol, and benzalkonium chloride, have been extensively evaluated or used on other topical drug products. (b) (4) (b) (4) (b) (4) The two formulations (b) (4) were bridged in an in vitro time-kill study (see section 3.2.2. of this review).

3. PRECLINICAL MICROBIOLOGY

3.1 Mechanism of Action of ReadyPrep™ CHG

The main active ingredient for ReadyPrep™ CHG is 2% CHG. CHG is an aqueous solution of 1,1'-hexamethylenebis[5-(4-chlorophenyl) bisguanide]di-D-gluconate. Woodcock[3] 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 results in cell death. Denton[4] 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; and 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[4]. 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[5]. Several changes indicating damage to the cytoplasmic membrane have been observed in bacterial populations treated with bacteriostatic and bactericidal levels of CHG[4]. 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[4].

CHG has microbicidal activity against vegetative gram-positive and gram-negative bacteria, yeast and fungi, and lipid-enveloped viruses[4]. Uptake of CHG into bacteria and yeasts is extremely rapid, with a maximum effect occurring within 20 seconds[6,7]. Although CHG is not sporicidal, it has also been shown to inhibit outgrowth of bacterial spores[8]. 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 nonenveloped viruses, such as rotaviruses, hepatitis A virus, or polioviruses. Its activity appears to be restricted to the nucleic acid core or the outer coat.***

3.2 Time-Kill Studies

The drug product ReadyPrep™ CHG, 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 likelihood 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 health care setting. Thus, products used in these settings should be formulated as 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[9]. These studies consist of modified time-kill studies for CHG drug products. These studies are designed to measure

the rate of kill by the antiseptic under controlled conditions, and assess whether the antiseptic is fast-acting for an indication. The modified time-kill in vitro studies allow us to gain insight into the potential utility of the antiseptic, insight 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
December 2011	(b) (4)
September 2012	(b) (4)
May 2016	• In the Refuse to file letter dated April 8, 2016, the Agency stated that the application was incomplete because it did not include the assessmen (b) (4) to support short term dermal use. • In a Type A meeting on May 23, 2016, the Agency agreed that it would be acceptable to remove (b) (4)

	(b) (4). However, the Sponsor needs to bridge the new formulation (b) (4) to the clinical safety and efficacy data and to the quality data supporting the previous product formulation.
December 2016	• FDA agrees that the Sponsor can employ in vitro time-kill studies to demonstrate the similarity of antimicrobial activity between the new and previous ReadyPrep™ CHG formulations. The Agency recommended testing of 48 repository (ATCC) isolates and 24 clinical isolates to yield a total of 864 evaluations.
March 2017	• FDA agrees that the comparative in vitro time-kill study is sufficient to establish a bridge between ReadyPrep™ Old and New formulations and pivotal studies, sensitization/irritation and antimicrobial resistance studies do not need to be repeated with the new formulation.

3.2.1 Assessment of Microbicidal Activity of ReadyPrep™ 2% CHG Solution Using a Modified Time-Kill Procedure (R14-013)

This study was designed to supply basic antimicrobial data and to determine how rapidly and effectively the test product kills a variety of microorganisms. The study was conducted to characterize the antimicrobial effect of the ReadyPrep™ CHG formulation against a variety of ATCC repository and clinical isolate strains of gram-positive and gram-negative bacteria and yeast. 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 comments: 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 the test formulation (test product) and one reference product was tested against a variety of repository and clinical isolate strains of gram-negative and gram-positive bacteria, and yeast. The test product was evaluated at three concentrations: actual use concentration, 1X; a secondary concentration within the active range, 0.5X; and an inactive concentration, 0.0001X. 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) were evaluated using each of the two

products, at the required concentrations and two contact times per evaluation to yield 1,536 evaluations. See Table 7 below for list of challenge microorganisms to be tested. For the vehicle, 12 repository isolates (comprised of 12 species and one isolate per species) were evaluated using two contact times per evaluation to yield 24 additional evaluations to ensure that the vehicle of the test article has no antimicrobial activity of its own. 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 (6 minutes and 10 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 ATCC 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

Table 8. List of Clinical Isolate Microorganisms.

Challenge Microorganisms	
Clinical Isolates	**Location**
1. *Burkholderia cepacia,* 2002, 13052, 13053, 13054, 13055, 13056	
2. MDR *Burkholderia cepacia,* 13057, 13058, 13059, 13060, 13061, 13062	(b) (4)
3. *Candida albicans,* 99580, 99581, 99582, 99586, 99587, 99585	(b) (4)
4. AR *Candida albicans,* 13040, 13041, 13042, 13043, 13044, 13045	(b) (4)
5. *Enterococcus faecalis,* 99824, 99825, 99826, 99827, 99828, 99829	(b) (4)
6. VRE *Enterococcus faecalis,* 13046, 13047, 13048, 13049, 13050, 13051	
7. *Enterococcus faecium,* 99855, 99856, 99857, 99858, 99859, 99860	(b) (4)
8. VRE *Enterococcus faecium,* 99640, 99641, 99642, 99643, 99644, 99645	
9. *Escherichia coli,* 99903, 99904, 99905, 99906, 99907, 99908	(b) (4)
10. MDR *Escherichia coli,* 10100, 10101, 10102, 10103, 10104, 10105	
11. *Klebsiella pneumoniae,* 99490, 99491, 99492, 99493, 99494, 99495	(b) (4)
12. MDR *Klebsiella pneumoniae,* 13011, 13012, 13013, 13014, 2004, 10002	
13. *Pseudomonas aeruginosa,* 99791, 99792, 99793, 99794, 99795, 99796	(b) (4)
14. MDR *Pseudomonas aeruginosa,* 2012, 13015, 13016, 13017, 13018, 13019	
15. *Serratia marcescens,* 99788, 99655, 99413, 99452, 13020, 13021	(b) (4)
16. MDR *Serratia marcescens,* 13022, 13023, 13024, 13025, 13026, 13027	(b) (4)
17. *Staphylococcus aureus,* 99510, 99511, 99512, 99513, 99514, 99515	(b) (4)
18. MRSA *Staphylococcus aureus,* 10113, 10114, 10115, 10116, 10117, 10118	(b) (4)
19. *Staphylococcus epidermidis,* 99530, 99532, 99524, 99525, 99526, 99527	(b) (4) (b) (4) (b) (4)
20. MRSE *Staphylococcus epidermidis,* 13031, 13032, 13033, 13034, 99289, 99288	
21. *Streptococcus pneumoniae,* 99370, 99371, 99372, 99373, 99374, 99375	(b) (4)
22. MDR *Streptococcus pneumoniae,* 13035, 13036, 13037, 13038, 13039, 2011	(b) (4)
23. *Streptococcus pyogenes,* 99890, 99891, 99892, 99893, 99894, 99895	(b) (4)
24. MR *Streptococcus pyogenes,* 13063, 13064, 13065, 13066, 13067, 130689	(b) (4)

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 5.3.5.4. R14-013. Results are summarized in Table 1. Summarized results (CFU/mL, Percent Reduction, and Log Reduction) are presented as the average values of the nonresistant isolates and the average values of the resistant isolates. Absolute values were used to calculate average values. Results for each individual isolate are presented in Appendix II in Modular 5.3.5.4. Tables 9, 10, and 11 below represent example of gram-negative organism *Burkholderia cepacia*, gram-positive organism *Enterococcus faecalis*, and fungus *Candida albicans* summary results.

Table 9. Results Summary of *Burkholderia cepacian* Clinical Isolates. Results Expressed as Average CFU/mL, Average Percent Reduction, and Average Log_{10} Reduction.

Microorganism	CI No.	Product	Concentration	Contact Time	Initial Count Avg. CFU/mL	CFU/mL	Percent Reduction	Log Reduction
Burkholderia cepacia	2002	Medline 2% CHG Solution	1X	6 min	6.6E+06	< 1.0E+01	> 99.99985	> 5.82
				10 min	6.6E+06	< 1.0E+01	> 99.99985	> 5.82
			0.5X	6 min	6.6E+06	< 1.0E+01	> 99.99985	> 5.82
				10 min	6.6E+06	< 1.0E+01	> 99.99985	> 5.82
			0.0001X	6 min	6.6E+06	4.0E+06	39.89899	0.22
				10 min	6.6E+06	1.9E+06	71.11111	0.54
		Dyna-Hex 2	1X	6 min	6.6E+06	< 1.0E+01	> 99.99985	> 5.82
				10 min	6.6E+06	< 1.0E+01	> 99.99985	> 5.82
Burkholderia cepacia	13052	Medline 2% CHG Solution	1X	6 min	3.5E+06	< 1.0E+01	> 99.99971	> 5.54
				10 min	2.3E+06	< 1.0E+01	> 99.99956	> 5.36
			0.5X	6 min	3.5E+06	< 1.0E+01	> 99.99971	> 5.54
				10 min	2.3E+06	< 1.0E+01	> 99.99956	> 5.36
			0.0001X	6 min	3.5E+06	2.4E+06	31.25000	0.16
				10 min	2.3E+06	2.2E+06	3.64964	0.02
		Dyna-Hex 2	1X	6 min	3.5E+06	< 1.0E+01	> 99.99971	> 5.54
				10 min	2.3E+06	< 1.0E+01	> 99.99956	> 5.36
Burkholderia cepacia	13053	Medline 2% CHG Solution	1X	6 min	1.6E+06	< 1.0E+01	> 99.99936	> 5.19
				10 min	1.5E+06	< 1.0E+01	> 99.99933	> 5.18
			0.5X	6 min	1.6E+06	< 1.0E+01	> 99.99936	> 5.19
				10 min	1.5E+06	< 1.0E+01	> 99.99933	> 5.18
			0.0001X	6 min	1.6E+06	1.1E+06	27.25322	0.14
				10 min	1.5E+06	8.6E+05	42.79379	0.24
		Dyna-Hex 2	1X	6 min	1.6E+06	< 1.0E+01	> 99.99936	> 5.19
				10 min	1.5E+06	< 1.0E+01	> 99.99933	> 5.18
Burkholderia cepacia	13054	Medline 2% CHG Solution	1X	6 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.03
				10 min	9.0E+06	< 1.0E+01	> 99.99989	> 5.95
			0.5X	6 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.03
				10 min	9.0E+06	< 1.0E+01	> 99.99989	> 5.95
			0.0001X	6 min	1.1E+07	3.4E+06	68.01242	0.50
				10 min	9.0E+06	1.8E+06	80.37175	0.71
		Dyna-Hex 2	1X	6 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.03
				10 min	9.0E+06	< 1.0E+01	> 99.99989	> 5.95

Table 10. Results Summary of *Entercoccus faecalis*. Results Expressed as Average CFU/mL, Average Percent Reduction, and Average Log_{10} Reduction.

Microorganism	CI No.	Product	Concentration	Contact Time	Initial Count Avg. CFU/mL	CFU/mL	Percent Reduction	Log Reduction
Enterococcus faecalis	99824	Medline 2% CHG Solution	1X	6 min	4.1E+06	< 1.0E+01	> 99.99975	> 5.61
				10 min	3.7E+06	< 1.0E+01	> 99.99973	> 5.57
			0.5X	6 min	4.1E+06	< 1.0E+01	> 99.99975	> 5.61
				10 min	3.7E+06	< 1.0E+01	> 99.99973	> 5.57
			0.0001X	6 min	4.1E+06	3.1E+06	22.95082	0.11
				10 min	3.7E+06	1.6E+06	56.93694	0.37
		Dyna-Hex 2	1X	6 min	4.1E+06	9.6E+03	99.76393	2.63
				10 min	3.7E+06	< 1.0E+01	> 99.99973	> 5.57
Enterococcus faecalis	99825	Medline 2% CHG Solution	1X	6 min	7.2E+06	< 1.0E+01	> 99.99986	> 5.86
				10 min	8.3E+06	< 1.0E+01	> 99.99988	> 5.92
			0.5X	6 min	7.2E+06	< 1.0E+01	> 99.99986	> 5.86
				10 min	8.3E+06	< 1.0E+01	> 99.99988	> 5.92
			0.0001X	6 min	7.2E+06	3.4E+05	95.27778	1.33
				10 min	8.3E+06	3.3E+05	96.02410	1.40
		Dyna-Hex 2	1X	6 min	7.2E+06	< 1.0E+01	> 99.99986	> 5.86
				10 min	8.3E+06	< 1.0E+01	> 99.99988	> 5.92
Enterococcus faecalis	99826	Medline 2% CHG Solution	1X	6 min	8.4E+06	< 1.0E+01	> 99.99988	> 5.92
				10 min	9.2E+06	< 1.0E+01	> 99.99989	> 5.96
			0.5X	6 min	8.4E+06	1.1E+02	99.99873	4.90
				10 min	9.2E+06	3.7E+01	99.99960	5.40
			0.0001X	6 min	8.4E+06	9.7E+05	88.45238	0.94
				10 min	9.2E+06	8.0E+05	91.23636	1.06
		Dyna-Hex 2	1X	6 min	8.4E+06	1.6E+03	99.98151	3.73
				10 min	9.2E+06	< 1.0E+01	> 99.99985	> 5.96
Enterococcus faecalis	99827	Medline 2% CHG Solution	1X	6 min	6.3E+06	1.9E+02	99.99591	4.51
				10 min	5.3E+06	< 1.0E+01	> 99.99981	> 5.72
			0.5X	6 min	6.3E+06	3.3E+02	99.99479	4.28
				10 min	5.3E+06	< 1.0E+01	> 99.99981	> 5.72
			0.0001X	6 min	6.3E+06	4.6E+06	26.59574	0.13
				10 min	5.3E+06	2.4E+06	54.55696	0.34
		Dyna-Hex 2	1X	6 min	6.3E+06	7.0E+02	99.98883	3.95
				10 min	5.3E+06	< 1.0E+01	> 99.99981	> 5.72

Table 11. Results Summary of *Candida albicans*. Results Expressed as Average CFU/mL, Average Percent Reduction, and Average Log_{10} Reduction.

Microorganism	CI No.	Product	Concentration	Contact Time	Initial Count Avg. CFU/mL	CFU/mL	Percent Reduction	Log Reduction
Candida albicans	99580	Medline 2% CHG Solution	1X	6 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.03
				10 min	9.8E+06	< 1.0E+01	> 99.99990	> 5.98
			0.5X	6 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.03
				10 min	9.8E+06	< 1.0E+01	> 99.99990	> 5.98
			0.0001X	6 min	1.1E+07	6.1E+06	43.47826	0.25
				10 min	9.8E+06	3.5E+06	63.06620	0.43
		Dyna-Hex 2	1X	6 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.03
				10 min	9.8E+06	< 1.0E+01	> 99.99990	> 5.98
Candida albicans	99581	Medline 2% CHG Solution	1X	6 min	6.1E+06	< 1.0E+01	> 99.99984	> 5.78
				10 min	6.6E+06	< 1.0E+01	> 99.99985	> 5.82
			0.5X	6 min	6.1E+06	< 1.0E+01	> 99.99984	> 5.78
				10 min	6.6E+06	< 1.0E+01	> 99.99985	> 5.82
			0.0001X	6 min	6.1E+06	3.5E+06	42.30769	0.24
				10 min	6.6E+06	1.4E+06	79.14573	0.68
		Dyna-Hex 2	1X	6 min	6.1E+06	< 1.0E+01	> 99.99984	> 5.78
				10 min	6.6E+06	< 1.0E+01	> 99.99985	> 5.82
Candida albicans	99582	Medline 2% CHG Solution	1X	6 min	5.2E+06	< 1.0E+01	> 99.99981	> 5.72
				10 min	5.1E+06	< 1.0E+01	> 99.99980	> 5.71
			0.5X	6 min	5.2E+06	2.2E+02	99.99586	4.38
				10 min	5.1E+06	< 1.0E+01	> 99.99980	> 5.71
			0.0001X	6 min	5.2E+06	3.6E+06	31.21019	0.16
				10 min	5.1E+06	1.9E+06	62.35294	0.42
		Dyna-Hex 2	1X	6 min	5.2E+06	2.9E+02	99.99452	4.26
				10 min	5.1E+06	1.5E+02	99.99706	4.53
Candida albicans	99586	Medline 2% CHG Solution	1X	6 min	3.4E+06	< 1.0E+01	> 99.99970	> 5.53
				10 min	3.6E+06	< 1.0E+01	> 99.99972	> 5.55
			0.5X	6 min	3.4E+06	< 1.0E+01	> 99.99970	> 5.53
				10 min	3.6E+06	< 1.0E+01	> 99.99972	> 5.55
			0.0001X	6 min	3.4E+06	2.5E+06	27.12871	0.14
				10 min	3.6E+06	1.8E+06	49.81308	0.30
		Dyna-Hex 2	1X	6 min	3.4E+06	< 1.0E+01	> 99.99970	> 5.53
				10 min	3.6E+06	< 1.0E+01	> 99.99972	> 5.55

<u>Reviewer's comments</u>: DNDP has revised the in vitro testing requirements for health care 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. Because CHG is a well-known antimicrobial agent with broad spectrum activity, FDA accepts a modified in vitro testing scheme that includes the following: a limited number of organisms rather than requiring a full battery of organisms (4 ATCC strains instead of 25 and 12 representative clinical isolates instead of 25); and testing three concentrations of the final formulation (actual use concentration, another concentration in the active range, and an inactive concentration), and the concept that minimum inhibitory concentration is no longer required. Since we have evaluated numerous CHG in vitro studies, we currently no longer recommend minimum inhibitory concentration (MIC) studies against the organisms described in the 1994 TFM for CHG drug products. Therefore, MIC testing is not necessary to support approval for CHG drug products. Instead, we recommend a modified in vitro time-kill study.

The time-kill study showed that Medline 2% CHG (full strength-1X), and the active control Dyna-Hex 2® produced ≥3 log_{10} reduction (>99.9%) killing effect in 6 minutes and 10 minutes for most organisms tested. When Medline 2% CHG was diluted to half its strength (0.5X) it produced ≥3 log_{10} reduction (>99.9%) killing effect in 6 minutes and 10 minutes in the majority of the organisms tested. The killing effect or antimicrobial activity of a drug needs to be ≥3 log_{10} reduction to be considered an active ingredient.

Some of the organisms, such as Staphylococcus aureus and Streptococcus pneumoniae, showed less than 3 log_{10} reduction (<99.9%) at both 6 and 10 minutes (see Tables 12 and 13 below). These results are comparable to those achieved with the active control Dyna-Hex 2®. This is acceptable since majority of the organisms tested showed greater than 3 log_{10} reduction (>99.9%) at both 6 and 10 minutes using 1X and 0.5X concentrations of the test product.

When Medline 2% CHG was diluted to 0.01% (0.0001X) it produced ≤0.70 log_{10} reduction killing effect in 6 minutes and 10 minutes for 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 product, Medline 2% CHG, achieved a >99.9% reduction in most viable microbial cells in 6 and 10 minutes.

Table 12. R14-013 Comparison of Log_{10} Reductions of CHG on Multiresistance Organisms.

ATCC Repository Strains	Medline 2% CHG Log_{10} Reduction* (10 min)		2% CHG (Dyna-Hex 2) Log_{10} Reduction* (10 min)	
	Non-Resistant	Resistant	Non-Resistant	Resistant
Burkholderia cepacia	>3.27	>5.64	>3.47	>5.64
Candida albicans	>5.08	>5.26	>5.08	>5.26
Enterococcus faecailis	>5.28	>5.23	>3.23	>5.23
Enterococcus faecium	>5.56	>3.90	>4.24	>3.54
Escherichia coli	>6.19	>5.74	>6.19	>5.74
Klebsiella pneumonia	>5.56	>5.68	>5.56	>5.68
Pseudomonas aeruginosa	>3.81	>6.01	>5.78	>6.01
Serratia marcescens	>5.80	>5.56	>5.80	>6.56
Staphylococcus aureus	>2.39	>5.62	>2.93	>3.46
Staphylococcus epidermidis	>5.06	>6.03	>5.06	>6.03
Streptococcus pneumonia	>5.75	>5.06	>4.38	>4.93
Streptococcus pyogenes	>4.66	>5.06	>4.77	>5.06

*Represents lowest Log_{10} reduction at use concentration (1X) for all isolates evaluated

Table 13. R14-013 Log_{10} Reductions for Clinical Isolate Strains.

Clinical Isolate Strains	Log_{10} Reduction* Medline 2% CHG (10 min)	Log_{10} Reduction* 2% CHG (Dyna-Hex 2) (10 min)
	Non-Resistant	Non-Resistant
Burkholderia cepacia	>5.18	>5.18
Candida albicans	>5.06	>4.53
Enterococcus faecailis	>3.49	>3.09
Enterococcus faecium	>5.18	>5.18
Escherichia coli	>5.74	>5.19
Klebsiella pneumoniae	>5.75	>5.75
Pseudomonas aeruginosa	>5.27	>5.27
Serratia marcescens	>5.17	>3.84
Staphylococcus aureus	>1.72	>3.84
Staphylococcus epidermidis	>5.20	>5.20
Streptococcus pneumonia	>1.77	>3.64
Streptococcus pyogenes	>5.35	>5.16

*Represents lowest Log_{10} reduction at full strength for all isolates evaluated

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 bactericidal activity of the residual antimicrobial agent but must also be inherently nontoxic 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 (non-resistant *Escherichia coli*, ATCC BAA-197) and one gram-positive (methicillin-resistant *Staphylococcus aureus*, ATCC 33591). The neutralizing buffer used in this study is (b) (4) 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 Medline 2% CHG was verified at MicroBioTest, Inc.

Neutralizer effectiveness (Test 1): Diluted inoculum was added into a tube containing 9 mL of sampling solution with neutralizers yielding a final inoculum concentration of approximately 30-100 CFU/mL. One mL aliquot of the test (Medline 2% CHG solution) or control (Dyna-Hex 2®) 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 yielding 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 yielding 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 (Medline 2% CHG solution) or control (Dyna-Hex 2®) product yielding 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 14 and 15) provide summaries of the data generated during the study.

Table 14. Neutralizer Effectiveness Control Results. Results Expressed as Log_{10} Colony Forming Units (CFU) per mL.

Microorganism	*Escherichia coli*, ATCC BAA-197				*Staphylococcus aureus*, ATCC 33591			
Test 1 - Neutralizer Effectiveness (Medline 2% CHG Solution)								
Contact Time	< 1 minute		30 minutes		< 1 minute		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		1.90		1.73		1.71	
Rep. 2	1.87		1.86		1.57		1.47	
Rep. 3	1.82		1.83		1.59		1.63	
Average	1.85	0.08	1.86	0.03	1.63	0.19	1.60	0.21
Test 1 - Neutralizer Effectiveness (Medline Placebo Solution)								
Contact Time	< 1 minute		30 minutes		< 1 minute		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	1.77		1.71		1.64		1.78	
Rep. 2	1.66		1.64		1.60		1.56	
Rep. 3	1.63		1.69		1.66		1.59	
Average	1.69	0.24	1.68	0.21	1.63	0.19	1.64	0.17
Test 1 - Neutralizer Effectiveness (Dyna-Hex 2)								
Contact Time	< 1 minute		30 minutes		< 1 minute		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	1.82		1.83		1.74		1.68	
Rep. 2	1.86		1.84		1.54		1.77	
Rep. 3	1.79		1.88		1.61		1.76	
Average	1.82	0.10	1.85	0.05	1.63	0.19	1.74	0.07

Table 15. Neutralizer Effectiveness Control Results. Results Expressed as Log_{10} Colony Forming Units (CFU) per mL.

Microorganism	*Escherichia coli*, ATCC BAA-197				*Staphylococcus aureus*, ATCC 33591			
Test 2 - Neutralizer Toxicity								
Contact Time	< 1 minute		30 minutes		< 1 minute		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	1.79		1.76		1.80		1.76	
Rep. 2	1.80		1.86		1.81		1.73	
Rep. 3	1.86		1.88		1.76		1.76	
Average	1.82	0.11	1.83	0.06	1.79	0.03	1.75	0.06
Test 3 – Test Microorganism Viability Control								
Contact Time	< 1 minute		30 minutes		< 1 minute		30 minutes	
Results	Log_{10} CFU/mL		Log_{10} CFU/mL		Log_{10} CFU/mL		Log_{10} CFU/mL	
Rep. 1	1.93		1.91		1.79		1.80	
Rep. 2	1.94		1.92		1.82		1.79	
Rep. 3	1.91		1.86		1.85		1.83	
Average	1.93		1.90		1.82		1.81	
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 comments: ***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 (Medline 2% CHG), or active control (Dyna-Hex 2®). These observations are made based upon the guidelines for neutralization validation in ASTM E1054-08 "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. This reviewer finds the Sponsor's protocol for the neutralization validation acceptable.***

In this study, the neutralization is considered effective (Test 1) if the post preparation sample recovered is not more than 0.2 log_{10} less than the Viability Control sample (Test 3). 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 sampling solutions are considered nontoxic (Test 2) if the Toxicity Control sample is not more than 0.2 log_{10} less than the Viability Control sample. 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 nontoxic 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 test date. All plates were incubated with the test at 36±1°C for 48±2 hours.

Reviewer's comments: ***The Sponsor stated that all sterility controls exhibited no growth. This is acceptable.***

Antibiotic Resistance:
On the day of the test, an individual (b) (4) 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 16. 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	4	Resistant
Multidrug-resistant *E. faecium*, ATCC 51559	Ampicillin	0	Resistant
	Ciprofloxacin	0	Resistant
	Gentamicin	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 17. 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	Ceftazidime	10	Resistant
	Piperacillin	0	Resistant
Multidrug-resistant *S. marcescens*, ATCC 43297	Amikacin	6	Resistant
	Ciprofloxacin	5	Resistant
	Piperacillin	2	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

<u>Reviewer's comments</u>: The antibiotic resistance testing was to confirm that the organisms listed above were considered resistant. See Tables 16 and 17 above. The zone of inhibition on agar plates containing the appropriate antibiotic disks were all considered 0 except for vancomycin-resistant Enterococcus faecium

(zone of 4 mm), multidrug resistant Klebsiella pneumoniae (zone of 10 mm) and multidrug-resistant Serratia marcescens (zone of 6 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 the vancomycin-resistant Enterococcus faecium, the multidrug-resistant Serratia marcescens and multidrug-resistant Klebsiella pneumonia zone of inhibition are under 12 mm, they are considered resistant. This is acceptable.

3.2.2 Assessment of Microbicidal Activity of Two Medline ReadyPrep™ CHG Solution Formulations Using a Modified Time-Kill Procedure (R17-004)

This study was designed to supply basic antimicrobial data and to determine how rapidly and effectively the test product kills a variety of microorganisms. The study was conducted to characterize the antimicrobial effects of the new ReadyPrep™ CHG formulation (b) (4) (b) (4) in comparison to the old ReadyPrep™ CHG formulation (b) (4) against a variety of ATCC repository and clinical isolate strains of gram-positive and gram-negative bacteria and yeast. 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 formulations: "Old" formulation (b) (4) and (b) (4)) and "New" formulation ((b) (4) and (b) (4)), the vehicle of the test article (for New formulation) and a saline solution was tested against a variety of repository and clinical isolate strains of gram-negative and gram-positive bacteria and yeast. Each formulation of the test article was evaluated at three concentrations (use concentration, a secondary concentration within the active range, and an inactive concentration).

Forty-eight (48) repository isolates (comprised of 12 species and four isolates per species) and 24 clinical isolates (comprised of 12 species and 2 isolates per species) were evaluated using each of the two test article formulations, per required concentration as outlined above, using two contact times per evaluation to yield 864 evaluations. For the vehicle and saline solution, twelve repository isolates (comprised of 12 species and one isolate per species) were evaluated using two contact times per evaluation to yield 48 additional 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 (6 minutes and 10 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 18. List of ATCC 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 Appendix II in Modular 5.3.5.4. Summarized results (CFU/mL, Percent Reduction, and Log Reduction) are presented as the average values of the nonresistant isolates and the average values of the resistant isolates. Absolute values were used to calculate average values. Tables 19, 20, and 21 below represent examples of results from a fungus, *Candida albicans;* gram-positive organism, *Enterococcus faecalis;* and a gram-negative organism, *Escherichia coli*.

Table 19. Results Summary of *Candida albicans*. Results Expressed as Average CFU/mL, Average Percent Reduction, and Average Log_{10} Reduction.

Microorganism	ATCC No.	Product	Concentration	Contact Time	Initial Count Avg. CFU/mL	CFU/mL	Percent Reduction	Log Reduction
Candida albicans	18804	2% CHG Solution (Old Formulation)	1X	6 min	5.0E+05	< 1.0E+01	> 99.99799	> 4.70
				10 min	4.5E+05	< 1.0E+01	> 99.99776	> 4.65
			0.5X	6 min	5.0E+05	< 1.0E+01	> 99.99799	> 4.70
				10 min	4.5E+05	< 1.0E+01	> 99.99776	> 4.65
			0.0001X	6 min	5.0E+05	3.8E+05	24.16107	0.12
				10 min	4.5E+05	2.6E+05	40.74627	0.23
		2% CHG Solution (New Formulation)	1X	6 min	5.0E+05	< 1.0E+01	> 99.99799	> 4.70
				10 min	4.5E+05	< 1.0E+01	> 99.99776	> 4.65
			0.5X	6 min	5.0E+05	< 1.0E+01	> 99.99799	> 4.70
				10 min	4.5E+05	< 1.0E+01	> 99.99776	> 4.65
			0.0001X	6 min	5.0E+05	3.4E+05	31.54362	> 0.16
				10 min	4.5E+05	2.8E+05	37.61194	0.20
		2% CHG Solution (New Formulation) vehicle	1X	6 min	5.0E+05	< 1.0E+01	> 99.99799	> 4.70
				10 min	4.5E+05	< 1.0E+01	> 99.99776	> 4.65
		0.85% NaCl	1X	6 min	5.0E+05	3.5E+05	28.85906	0.15
				10 min	4.5E+05	4.0E+05	11.19403	0.05
Candida albicans	66027	2% CHG Solution (Old Formulation)	1X	6 min	7.1E+06	< 1.0E+01	> 99.99986	> 5.85
				10 min	6.7E+06	< 1.0E+01	> 99.99985	> 5.83
			0.5X	6 min	7.1E+06	< 1.0E+01	> 99.99986	> 5.85
				10 min	6.7E+06	< 1.0E+01	> 99.99985	> 5.83
			0.0001X	6 min	7.1E+06	4.7E+06	33.01887	0.17
				10 min	6.7E+06	4.1E+06	39.60396	0.22
		2% CHG Solution (New Formulation)	1X	6 min	7.1E+06	< 1.0E+01	> 99.99986	> 5.85
				10 min	6.7E+06	< 1.0E+01	> 99.99985	> 5.83
			0.5X	6 min	7.1E+06	< 1.0E+01	> 99.99986	> 5.85
				10 min	6.7E+06	< 1.0E+01	> 99.99985	> 5.83
			0.0001X	6 min	7.1E+06	5.5E+06	21.69811	0.11
				10 min	6.7E+06	4.5E+06	33.16832	0.18
Azole-resistant *Candida albicans*	64124	2% CHG Solution (Old Formulation)	1X	6 min	1.3E+06	< 1.0E+01	> 99.99924	> 5.12
				10 min	1.3E+06	< 1.0E+01	> 99.99924	> 5.12
			0.5X	6 min	1.3E+06	< 1.0E+01	> 99.99924	> 5.12
				10 min	1.3E+06	< 1.0E+01	> 99.99924	> 5.12
			0.0001X	6 min	1.3E+06	2.3E+05	82.28426	0.75
				10 min	1.3E+06	2.0E+05	84.62121	0.81
		2% CHG Solution (New Formulation)	1X	6 min	1.3E+06	< 1.0E+01	> 99.99924	> 5.12
				10 min	1.3E+06	< 1.0E+01	> 99.99924	> 5.12
			0.5X	6 min	1.3E+06	8.3E+01	99.99365	4.20
				10 min	1.3E+06	< 1.0E+01	> 99.99924	> 5.12
			0.0001X	6 min	1.3E+06	2.3E+05	82.51269	0.76
				10 min	1.3E+06	2.2E+05	83.63636	0.79
Azole-resistant *Candida albicans*	64550	2% CHG Solution (Old Formulation)	1X	6 min	4.3E+06	< 1.0E+01	> 99.99977	> 5.64
				10 min	4.4E+06	< 1.0E+01	> 99.99977	> 5.64
			0.5X	6 min	4.3E+06	< 1.0E+01	> 99.99977	> 5.64
				10 min	4.4E+06	< 1.0E+01	> 99.99977	> 5.64
			0.0001X	6 min	4.3E+06	1.3E+06	69.53846	0.52
				10 min	4.4E+06	7.9E+05	82.12121	0.75
		2% CHG Solution (New Formulation)	1X	6 min	4.3E+06	< 1.0E+01	> 99.99977	> 5.64
				10 min	4.4E+06	< 1.0E+01	> 99.99977	> 5.64
			0.5X	6 min	4.3E+06	< 1.0E+01	> 99.99977	> 5.64
				10 min	4.4E+06	< 1.0E+01	> 99.99977	> 5.64

Table 20. Results Summary of *Entercoccus faecalis*. Results Expressed as Average CFU/mL, Average Percent Reduction, and Average Log_{10} reduction.

Microorganism	ATCC No.	Product	Concentration	Contact Time	Initial Count Avg. CFU/mL	CFU/mL	Percent Reduction	Log Reduction
Enterococcus faecalis	29212	2% CHG Solution (Old Formulation)	1X	6 min	1.2E+07	< 1.0E+01	> 99.99992	> 6.07
				10 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.05
			0.5X	6 min	1.2E+07	< 1.0E+01	> 99.99992	> 6.07
				10 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.05
			0.0001X	6 min	1.2E+07	3.4E+06	71.62921	0.55
				10 min	1.1E+07	7.5E+06	32.73810	0.17
		2% CHG Solution (New Formulation)	1X	6 min	1.2E+07	< 1.0E+01	> 99.99992	> 6.07
				10 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.05
			0.5X	6 min	1.2E+07	< 1.0E+01	> 99.99992	> 6.07
				10 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.05
			0.0001X	6 min	1.2E+07	4.0E+06	66.57303	0.48
				10 min	1.1E+07	3.5E+06	68.45238	0.50
		2% CHG Solution (New Formulation) vehicle	1X	6 min	5.1E+06	< 1.0E+01	> 99.99980	> 5.71
				10 min	5.1E+06	< 1.0E+01	> 99.99980	> 5.71
		0.85% NaCl	1X	6 min	5.1E+06	4.8E+06	5.22876	0.02
				10 min	5.1E+06	4.8E+06	5.22876	0.02
Enterococcus faecalis	19433	2% CHG Solution (Old Formulation)	1X	6 min	1.6E+06	5.7E+01	99.99641	4.44
				10 min	2.0E+06	< 1.0E+01	> 99.99950	> 5.30
			0.5X	6 min	1.6E+06	5.9E+03	99.62368	2.42
				10 min	2.0E+06	4.5E+02	99.97767	3.65
			0.0001X	6 min	1.6E+06	1.2E+06	25.36998	0.13
				10 min	2.0E+06	1.1E+06	47.33333	0.28
		2% CHG Solution (New Formulation)	1X	6 min	1.6E+06	3.3E+03	99.79281	2.68
				10 min	2.0E+06	2.8E+02	99.98600	3.85
			0.5X	6 min	1.6E+06	1.1E+04	99.30655	2.16
				10 min	2.0E+06	2.0E+03	99.90000	3.00
			0.0001X	6 min	1.6E+06	8.5E+05	46.30021	0.27
				10 min	2.0E+06	1.5E+06	24.66667	0.12
Vancomycin-resistant *Enterococcus faecalis*	51299	2% CHG Solution (Old Formulation)	1X	6 min	1.1E+07	6.1E+03	99.94647	3.27
				10 min	1.2E+07	1.1E+03	99.99058	4.03
			0.5X	6 min	1.1E+07	8.3E+03	99.92706	3.14
				10 min	1.2E+07	1.7E+03	99.98632	3.86
			0.0001X	6 min	1.1E+07	7.7E+06	32.05882	0.17
				10 min	1.2E+07	8.2E+06	48.62637	0.29
		2% CHG Solution (New Formulation)	1X	6 min	1.1E+07	9.0E+03	99.92029	3.10
				10 min	1.2E+07	1.1E+03	99.99104	4.05
			0.5X	6 min	1.1E+07	1.1E+04	99.89912	3.00
				10 min	1.2E+07	2.3E+03	99.98110	3.72
			0.0001X	6 min	1.1E+07	8.7E+06	23.52941	0.12
				10 min	1.2E+07	8.8E+06	27.47253	0.14
Vancomycin-resistant *Enterococcus faecalis*	51575	2% CHG Solution (Old Formulation)	1X	6 min	4.5E+06	3.9E+02	99.99134	4.06
				10 min	5.5E+06	< 1.0E+01	> 99.99982	> 5.74
			0.5X	6 min	4.5E+06	8.4E+02	99.98112	3.72
				10 min	5.5E+06	2.3E+01	99.99957	5.37
			0.0001X	6 min	4.5E+06	2.7E+06	40.59701	0.23
				10 min	5.5E+06	3.4E+06	37.19512	0.20
		2% CHG Solution (New Formulation)	1X	6 min	4.5E+06	4.5E+03	99.90000	3.00
				10 min	5.5E+06	3.6E+02	99.99341	4.18
			0.5X	6 min	4.5E+06	6.6E+03	99.85224	2.83
				10 min	5.5E+06	5.9E+02	99.98927	3.97
			0.0001X	6 min	4.5E+06	3.6E+06	19.40299	0.09
				10 min	5.5E+06	3.9E+06	28.65854	0.15

Table 21. Results Summary of *Escherichia coli*. Results Expressed as Average CFU/mL, Average Percent Reduction, and Average Log_{10} Reduction.

Microorganism	ATCC No.	Product	Concentration	Contact Time	Initial Count Avg. CFU/mL	CFU/mL	Percent Reduction	Log Reduction
Multidrug-resistant *Escherichia coli*	BAA-197	2% CHG Solution (Old Formulation)	1X	6 min	1.2E+07	< 1.0E+01	> 99.99992	> 6.08
				10 min	1.2E+07	< 1.0E+01	> 99.99992	> 6.07
			0.5X	6 min	1.2E+07	9.2E+03	99.92312	3.11
				10 min	1.2E+07	4.6E+02	99.99610	4.41
			0.0001X	6 min	1.2E+07	6.6E+06	44.84680	0.26
				10 min	1.2E+07	5.1E+06	95.70225	1.37
		2% CHG Solution (New Formulation)	1X	6 min	1.2E+07	5.2E+03	99.95655	3.36
				10 min	1.2E+07	2.3E+02	99.99803	4.71
			0.5X	6 min	1.2E+07	1.3E+04	99.89554	2.98
				10 min	1.2E+07	6.0E+02	99.99497	4.30
			0.0001X	6 min	1.2E+07	8.3E+06	30.91922	0.16
				10 min	1.2E+07	7.5E+06	37.07865	0.20
		2% CHG Solution (New Formulation) vehicle	1X	6 min	1.2E+07	8.9E+03	99.92563	3.13
				10 min	1.2E+07	2.5E+02	99.99792	4.68
		0.85% NaCl	1X	6 min	1.2E+07	9.9E+06	16.89164	0.08
				10 min	1.2E+07	8.4E+06	28.93258	0.15
Escherichia coli	11775	2% CHG Solution (Old Formulation)	1X	6 min	9.3E+06	< 1.0E+01	> 99.99989	> 5.97
				10 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.06
			0.5X	6 min	9.3E+06	9.7E+01	99.99896	4.98
				10 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.06
			0.0001X	6 min	9.3E+06	5.8E+06	37.27599	0.20
				10 min	1.1E+07	5.0E+06	55.84795	0.36
		2% CHG Solution (New Formulation)	1X	6 min	9.3E+06	3.1E+02	99.99667	4.48
				10 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.06
			0.5X	6 min	9.3E+06	3.9E+02	99.99581	4.38
				10 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.06
			0.0001X	6 min	9.3E+06	4.7E+06	49.10394	0.29
				10 min	1.1E+07	4.7E+06	58.47953	0.38
Escherichia coli	25922	2% CHG Solution (Old Formulation)	1X	6 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.05
				10 min	1.2E+07	< 1.0E+01	> 99.99992	> 6.07
			0.5X	6 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.05
				10 min	1.2E+07	< 1.0E+01	> 99.99992	> 6.07
			0.0001X	6 min	1.1E+07	6.7E+06	40.47619	0.23
				10 min	1.2E+07	4.2E+06	64.32584	0.45
		2% CHG Solution (New Formulation)	1X	6 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.05
				10 min	1.2E+07	< 1.0E+01	> 99.99992	> 6.07
			0.5X	6 min	1.1E+07	< 1.0E+01	> 99.99991	> 6.05
				10 min	1.2E+07	< 1.0E+01	> 99.99992	> 6.07
			0.0001X	6 min	1.1E+07	8.2E+06	26.78571	0.14
				10 min	1.2E+07	3.3E+06	72.47191	0.56
Multidrug-resistant *Escherichia coli*	BAA-200	2% CHG Solution (Old Formulation)	1X	6 min	7.3E+06	< 1.0E+01	> 99.99986	> 5.87
				10 min	7.7E+06	< 1.0E+01	> 99.99987	> 5.89
			0.5X	6 min	7.3E+06	4.3E+01	99.99941	5.23
				10 min	7.7E+06	< 1.0E+01	> 99.99987	> 5.89
			0.0001X	6 min	7.3E+06	2.5E+06	66.18182	0.47
				10 min	7.7E+06	3.2E+06	58.62069	0.38
		2% CHG Solution (New Formulation)	1X	6 min	7.3E+06	7.0E+01	99.99905	5.02
				10 min	7.7E+06	< 1.0E+01	> 99.99987	> 5.89
			0.5X	6 min	7.3E+06	1.7E+02	99.99768	4.63
				10 min	7.7E+06	< 1.0E+01	> 99.99987	> 5.89
			0.0001X	6 min	7.3E+06	3.8E+06	47.72727	0.28
				10 min	7.7E+06	3.9E+06	49.56897	0.30

***Reviewer's comments**: Per agreement with the Agency during the Type A meeting discussion on May 23, 2016, the Sponsor planned to demonstrate the similarity in effectiveness of ReadyPrep™ CHG as an antimicrobial wipe between its proposed New formulation (b) (4) and the Old formulation (b) (4) to support the scientific bridge to the clinical safety and efficacy data and to the quality data supporting the prior information. The Sponsor employed the modified in vitro time-kill study to evaluate the susceptibility of bacteria to the new and old ReadyPrep™ 2% CHG formulations. The time-kill study showed that both ReadyPrep™ 2% CHG products (Old and New formulation) produced ≥3 log_{10} reduction (>99.9%) killing effect in 6 minutes and 10 minutes for most organisms tested, some organism, such as Enterococcus faecalis and Staphylococcus aureus showed less than 3 log_{10} reduction. Overall, the results of the time-kill studies provided by the Sponsor indicate that (b) (4) has no impact on the antiseptic effectiveness of the new ReadyPrep™ CHG 2% formulation.*

3.2.3 Assessment of the Vehicle (inactive) Control for ReadyPrep™ CHG

ReadyPrep™ CHG is a cloth dosage form which delivers 2% chlorhexidine gluconate topical solution USP to the site of administration. The composition of one unit of the finished dosage form is provided in Table 22 below.

Table 22. Composition of 2% Chlorhexidine Gluconate Solution.

Component	Quality Standard	Function	Amount (% w/w)
Purified Water	USP	(b) (4)	(b) (4)
Chlorhexidine Gluconate Solution	(b) (4) USP	Drug Substance	
Glycerin	USP	(b) (4)	
Propylene Glycol	USP		
(b) (4) Dimethicone NF Emulsion	(b)		
Isopropyl Alcohol	USP		
(b) (4) Benzalkonium Chloride Solution	NF		

FDA had a face-to-face Type C meeting with the Sponsor on December 13, 2011 to

(b) (4)

(b) (4)

(b) (4)

In an advice letter to the Sponsor dated September 2, 2014, we requested the Sponsor to clarify the final concentration of benzalkonium chloride in the 2% chlorhexidine gluconate cloth. It was unclear whether benzalkonium chloride will be used at a final concentration of (b) (4)% or (b) (4)%. We also stated the following:

> *"You must demonstrate that these ingredients do not contribute to the antimicrobial activity of the final formulation or your product may be subject to the Agency's combination policy as provided for in 21 CFR 330.10((a)(4)(iv): An over-the-counter drug may combine two or more safe and effective active ingredients and may be generally recognized as safe and effective when each active ingredient makes a contribution to the claimed effect(s); when combining of the active ingredients does not decrease the safety or effectiveness of any of the individual active ingredients; and when the combination, when used under adequate directions for use and warnings against unsafe use, provides rational concurrent therapy for a significant proportion of the target population. It may be possible to do this through in vitro studies."*

Reviewer's comments: ***The Sponsor states that benzalkonium chloride (b) (4)) is used as (b) (4) in this formulation. Benzalkonium chloride is also considered an antiseptic under the 1994 TFM for health care topical antiseptics in the range of (b) (4)% to (b) (4)%. However, and similarly to isopropyl alcohol, based on the study results using the product vehicle, it seems that benzalkonium chloride does not significantly contribute to the activity of this product. Additionally, the FDA inactive ingredient database for approved drug products, includes benzalkonium chloride up to (b) (4)% when used as an excipient for a (b) (4) product.***

A vehicle control (b) (4) was also evaluated against ATCC strains. As this vehicle solution was utilized to (b) (4) The time-kill testing of the vehicle was incorporated in the "Assessment of Microbicidal Activity of ReadyPrep™ 2% CHG Solution Using a Modified Time-Kill Procedure (R14-013)." The results are provided in Table 23 below.

Table 23. R14-013 Log_{10} Reduction for ATCC Strains with Vehicle Formulation.

ATCC Strains	Log_{10} Reduction Vehicle (6 min)	Log_{10} Reduction Vehicle (10 min)
Burkholderia cepacia	2.54	2.54
Candida albicans	2.74	2.75
Enterococcus faecalis	2.38	2.28
Enterococcus faecium	2.61	2.57
Escherichia coli	2.78	2.74
Klebsiella pneumoniae	2.60	2.56
Pseudomonas aeruginosa	2.34	2.35
Serratia marcescens	3.07	3.10
Staphylococcus aureus	0.58	0.81
Staphylococcus epidermidis	2.04	2.06
Streptococcus pneumonia	3.15	3.20
Streptococcus pyogenes	2.80	2.80

Reviewer's comments: ***The vehicle demonstrated some antimicrobial activity, although less than the 2% CHG containing products. ReadyPrep™ CHG and Dyna-Hex 2® produced comparable log_{10} reductions on the same microorganisms tested. These two CHG containing products had log_{10} reduction greater than 5 log_{10}. The activity observed with the vehicle did not affect the antimicrobial effectiveness of the ReadyPrep™ CHG compared to Dyna-Hex 2® on the same microorganisms evaluated.***

Generally, in a time-kill test, a 3 log_{10} reduction is considered the minimum level that would indicate a product has significant killing activity against a particular test microorganism. The log_{10} reductions for the vehicle solution were mostly ≤3 log_{10}, indicating no significant killing activity. There were two

microorganisms Serratia marcescens and Streptococcus pneumoniae that showed over a 3 log_{10} reduction at 6 and 10 minutes. (b) (4) ***It was unfortunate that during the IND phase, we should have requested a protocol on how the Sponsor was going to evaluate the inactive ingredients. It would have been interesting to see what the individual results for each inactive ingredient would have produced versus the vehicle.***

This reviewer concludes that the ReadyPrep™ CHG formulation was efficacious at reducing the level of ATCC repository and clinical isolate organisms within the 6 and 10-minute evaluations. Log_{10} reductions observed with the ReadyPrep™ CHG were similar to those from the comparator, Dyna-Hex 2®. The vehicle did not significantly contribute to the antimicrobial activity of ReadyPrep™ CHG.

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.[5]" 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[4]. Low-level plasmid resistance has been shown in strains of *Staphylococcus aureus*. This resistance is conferred by the *qacA* gene[20]. Nonplasmid 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[11, 12, 13, 14].

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[4, 15]. 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 microorganisms that were resistant to currently accepted, clinically relevant concentrations (b) (4) 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 (b) (4). Stickler[16, 17] reported isolating gram-negative rods from paraplegic patients requiring intermittent urinary catheterization where the site was

cleansed with (b) (4) aqueous CHG prior to the procedure. Kahan[18] reported that six patients developed infections with *Pseudomonas pickettii*, which was also isolated from a (b) (4) 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 (b) (4) Up to (b) (4) CHG is generally regarded as a preservative level of the antimicrobial, and an oral rinse intended for treating periodontal disease contains (b) (4) 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[4].

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 semipermeable membranes to isolate the microorganism from the antimicrobial agent[19, 20]. Intrinsic resistance is generally associated with gram-negative bacteria, particularly of the genera *Klebsiella spp*., *Proteus spp*., and *Providencia spp*.[21] 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[19].

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[19, 22]. Martin[23] and Simpson, et al.[24] found that microorganisms isolated from an environment where CHG was routinely present have susceptibilities to CHG that were similar to strains of the same microorganisms isolated from an environment where little or no CHG was present in that environment. Brooks, et al.[22] 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[25, 26, 27]. 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[28, 29]. 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[28]. 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 pneumoniae,* and *Pseudomonas aeruginosa*[29]. 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.

A recent review of the literature on the impact of CHG bathing on health care associated infections (HAIs) reports conflicting information on the development of resistance to clinical use of CHG. Soma et al. (2012) found that the frequency of antimicrobial nonsusceptibility was significantly higher among coagulase negative *Staphylococci* with the use of higher CHG minimum inhibitory concentrations[30]. These finding suggests that there might be an association between CHG minimum concentrations and resistance in coagulase negative *Staphylococci.* Others have also found resistance to prolonged use of CHG in daily bathing application to mucous membranes in gram-negative *Bacilli* and a selection of hospital MRSA isolates[31]. These studies highlight concerns about the potential for CHG resistance in horizontal bathing.

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 sponsors to submit 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 ReadyPrep™ CHG Solution

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 comments**: On December 13, 2011, we had a Type C Pre-IND meeting with the Sponsor.* (b) (4)

(b) (4)

In the resistance study, ten repository isolates from eight species and four clinical isolates (2 resistant and 2 nonresistant) from the same eight species were evaluated, for a total of 42 isolates. If the microorganisms can 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. One test article, ReadyPrep™ 2% CHG solution and one control article, the active ingredient only 2% CHG solution were evaluated.

Table 24. List of Challenge Microorganisms.

Organism	Strain Identification			Clinical Isolates Received from: (b) (4)
	ATCC Number	Clinical Isolate Number		
Acinetobacter baumannii	17904	14002 10057	10058 10059	
Burkholderia cepacia	25608	13052** 13053**	13054** 13055**	
Enterococcus faecalis	52199*	99824 99825	13046* 13047*	

Table 25. List of Challenge Microorganisms (cont.).

Organism	Strain Identification		Clinical Isolates Received from: (b) (4)
	ATCC Number	Clinical Isolate Number	
Escherichia coli	11229	99903 10100** 99904 10101**	
Pseudomonas aeruginosa	15442	99791 13015* 99792 13016*	
Serratia marcescens	14756 43297**	99413 13026** 99452 13027**	
Staphylococcus aureus	33591* 25923**	99510 10113* 99511 10114*	
Staphylococcus epidermidis	51625*	99530 13031* 99532 13032*	

*Methicillin-resistant
**Methicillin-sensitive

Reviewer's comment*: *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 were 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*: *The agar dilution procedure is 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 did 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 increased, testing was continued in a step-wise fashion until at least 2 rounds of testing resulted in no change in MIC. If the MIC was 4 or more times the concentration of the initial MIC, the organism was transferred 3 times in fresh medium that did not contain the product, then subjected to the same concentrations of the product that were evaluated in the initial test step. The results are presented in Appendix III in Module 5.3.5.4.

Table 26. Test Results: Emergence of Resistance.

Results Expressed as Minimum Inhibitory Concentration (mg/L)

Organism	ID	Minimum Inhibitory Concentration of CHG (mg/L)			
		Medline 2% CHG Solution		2% CHG Solution Only	
		Initial	Final	Initial	Final
Pseudomonas aeruginosa	ATCC 15442	128	128	128	128
	CI 99791	128	128	128	128
	CI 99792	64	64	64	64
	CI 13015*	64	64	64	64
	CI 13016*	128	128	128	128
Serratia marcescens	ATCC 14756	64	64	64	64
	ATCC 43297*	256	256	256	256
	CI 99413	128	128	128	128
	CI 99452	64	64	64	64
	CI 13026*	128	128	128	128
	CI 13027*	513	513	513	513
Staphylococcus aureus	ATCC 33591**	2	2	2	2
	ATCC 25923***	2	2	2	2
	CI 99510	2	2	2	2
	CI 99511	2	2	2	2
	CI 10113**	2	2	2	2
	CI 10114**	1	1	2	2
Staphylococcus epidermidis	ATCC 51625**	2	2	2	2
	CI 99530	2	2	2	2
	CI 99532	4	4	4	4
	CI 13031*	4	4	4	4
	CI 13032*	4	4	4	4

*Multidrug-resistant; **Methicillin-resistant; ***Methicillin-sensitive

Table 27. Test Results: Emergence of Resistance (continued).

Results Expressed as Minimum Inhibitory Concentration (mg/L)

Organism	ID	Minimum Inhibitory Concentration of CHG (mg/L)			
		Medline 2% CHG Solution		2% CHG Solution Only	
		Initial	Final	Initial	Final
Acinetobacter baumannii	ATCC 17904	64	64	64	64
	CI 14002*	64	64	64	64
	CI 10057*	64	64	64	64
	CI 10058*	64	64	64	64
	CI 10059*	32	32	32	32
Burkholderia cepacia	ATCC 25608	513	513	513	513
	CI 13052*	256	256	256	256
	CI 13053*	32	32	32	32
	CI 13054*	32	32	32	32
	CI 13055*	32	32	32	32
Enterococcus faecalis	ATCC 51299**	8	8	16	16
	CI 99824	8	8	16	16
	CI 99825	128	128	128	128
	CI 13046**	16	16	16	16
	CI 13047**	16	16	16	16
Escherichia coli	ATCC 11229	4	4	4	4
	CI 99903	2	2	4	4
	CI 99904	16	16	16	16
	CI 10100*	4	4	8	8
	CI 10101*	4	4	8	8

*Multidrug-resistant; **Vancomycin-resistant

***Reviewer's comments*: 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. 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 28 below) was determined by (b) (4). The MIC of Penicillin was determined by the well-established broth dilution method.

Table 28. Cross-Resistance Isolates and Antibiotics.

Table 1: 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.

(b) (4) 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 (b) (4) agar plate for each organism was inoculated in a cross-hatch pattern. The appropriate (b) (4) 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 were 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 micropipette 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 were streaked onto the (b) (4) agar and incubated at 36±1°C for 20 to 24 hours along with the corresponding viability control tube. The results are presented in Appendix II in Module 5.3.5.4.

Table 29. Test Results: Development of Cross-Resistance.

Results Expressed as Zone of Inhibition (µg/mL)

Organism	ID	Zone of Inhibition (µg/mL)							
		Ceftazidime		Imipenem		Piperacillin		Tobramycin	
		Initial	Post	Initial	Post	Initial	Post	Initial	Post
Acinetobacter baumannii	ATCC 17904	4	8	0.38	0.25	32	64	1	0.75
	CI 14002*	No zone	No zone	3	1	No zone	No zone	No zone	No zone
	CI 10057*	No zone	No zone	No zone	No zone	No zone	No zone	No zone	No zone
	CI 10058*	No zone	No zone	No zone	No zone	No zone	No zone	1	1
	CI 10059*	No zone	No zone	No zone	No zone	No zone	No zone	No zone	128
Burkholderia cepacia	ATCC 25608	4	2	4	4	1.5	1	32	96
	CI 13052*	32	32	No zone	No zone	No zone	No zone	No zone	No zone
	CI 13053*	2	1.5	0.25	0.125	0.75	0.5	6	4
	CI 13054*	6	6	8	8	6	6	1	1
	CI 13055*	4	4	6	6	2	8	0.75	0.75
Escherichia coli	ATCC 11229	0.38	0.38	0.38	0.25	3	2	0.25	0.38
	CI 99903	0.38	0.38	0.5	0.25	1.5	2	0.25	0.38
	CI 99904	0.125	0.125	0.25	0.25	0.38	0.5	0.25	0.19
	CI 10100*	2	1.5	0.19	0.125	No zone	No zone	0.125	0.25
	CI 10101*	1.5	1.5	0.38	0.25	No zone	No zone	24	16
Pseudomonas aeruginosa	ATCC 15442	8	4	3	1.5	32	24	2	1.5
	CI 99791	No zone	No zone	No zone	No zone	No zone	No zone	No zone	No zone
	CI 99792	No zone	No zone	No zone	No zone	No zone	No zone	No zone	No zone
	CI 13015*	8	2	No zone	No zone	64	24	12	6
	CI 13016*	12	8	No zone	No zone	32	32	32	24
Serratia marcescens	ATCC 14756	0.19	0.19	0.5	0.75	1.5	1.5	2	2
	ATCC 43297*	1.5	1	1.5	1.5	32	32	16	16
	CI 99413	0.19	0.19	1	1	2	2	2	2
	CI 99452	0.5	0.38	1	1	3	3	1.5	1.5
	CI 13026*	0.25	0.25	0.75	0.75	3	3	2	2
	CI 13027*	1	0.38	2	2	3	1.5	3	3

*Multidrug-resistant

Table 30. Test Results: Development of Cross-Resistance.

Results Expressed as Zone of Inhibition (µg/mL)

Organism	ID	Zone of Inhibition (µg/mL)							
		Clindamycin		Oxacillin		Vancomycin		Penicillin	
		Initial	Post	Initial	Post	Initial	Post	Initial	Post
Staphylococcus aureus	ATCC 33591*	No zone	No zone	No zone	No zone	1.5	1.5	250	250
	ATCC 25923**	0.064	0.125	1	0.5	2	2	1	1
	CI 99510	No zone	No zone	64	64	2	1.5	2	2
	CI 99511	0.023	0.032	1.5	2	2	1.5	7.8	7.8
	CI 10113*	0.032	0.016	64	48	1	1	250	250
	CI 10114*	0.094	0.064	No zone	No zone	1.5	1.5	125	125
Staphylococcus epidermidis	ATCC 51625*	48	24	32	No zone	192	192	> 1000	> 1000
	CI 99530	128	48	No zone	No zone	No zone	128	500	500
	CI 99532	No zone	No zone	No zone	No zone	2	3	7.8	7.8
	CI 13031***	0.064	0.064	4	No zone	3	2	2	2
	CI 13032***	No zone	No zone	4	No zone	3	3	1	1

*Methicillin-resistant; **Methicillin-sensitive; ***Multidrug-resistant

Table 31. Test Results: Development of Cross-Resistance.

Results Expressed as Zone of Inhibition (µg/mL)

Organism	ID	Zone of Inhibition (µg/mL)			
		Ampicillin		Vancomycin	
		Initial	Post	Initial	Post
Enterococcus faecalis	ATCC 51299*	0.75	0.75	8	8
	CI 99824	1.5	0.75	1.5	1
	CI 99825	No zone	No zone	No zone	No zone
	CI 13046*	No zone	No zone	No zone	No zone
	CI 13047*	1.5	0.5	No zone	No zone

*Vancomycin-resistant

Reviewer's comments: ***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*** (b) (4) ***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 combinations 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 CHG was translated into a series of test methods as described in the 1994 TFM for health care antiseptics (59 FR 31402), proposed performance criteria as described in the 2015 proposed rule for health care antiseptics (80 FR 25166), and revised performance criteria and statistical study design described in the final rule for health care antiseptics (82 FR 60474).

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 R13-053 (MicroBioTest) and Study R15-029 (Evic Romania)

Two pivotal clinical simulation studies (R13-053: MicroBioTest and R15-029: Evic Romania) were designed to evaluate the antimicrobial efficacy and safety of Medline 2% CHG Cloth 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).

There was one additional pivotal study (R13-052) that was conducted at BioScience Laboratories that was discontinued prematurely due to low enrollment issues; thus, efficacy data were not evaluable, and only safety data were reported from this study.

> ***Reviewer's comments: On July 31, 2015, FDA sent the Sponsor an advice letter regarding a question: "Would it be possible to end pivotal study #2 at BioScience Laboratories early and submit the data we have available in our NDA with it being acceptable to the Agency?" FDA responded that stopping the trial early was at the Sponsor's own risk. Based on obtaining only 68% enrollment of planned subjects, the abdomen site study was unlikely to serve as a pivotal study.***

The two pivotal clinical simulation studies used (R13-053: MicroBioTest and R15-029: Evic Romania) were both entitled: Assessment of the Antimicrobial Efficacy of Medline 2% CHG Cloth Preoperative Skin Preparation.

Study Objectives

R13-053: MicroBioTest and R15-029: Evic Romania – The primary objective of the study was to measure the antimicrobial effectiveness of a single investigational test article, Medline 2% CHG cloth, as specified by the TFM using the procedures specified by FDA. At 10-minutes post prep the test article would achieve a mean 2 $\log_{10}$ per cm^2 reduction on the abdomen site and a mean 3 $\log_{10}$ per cm^2 reduction on the groin site. Samples taken at 6-hours (and 8-hours) post prep may not exceed the test day baselines. The vehicle formulation of the test article as well as a positive control was evaluated using the same methodology.

Reviewer's comments: ***On December 13, 2011, we had a face-to-face type C meeting to discuss the Sponsor's proposed drug product development program in support of the 2% CHG cloth product.*** (b) (4)

(b) (4)

(b) (4)

[redacted]

Study Design

This was a randomized, paired-comparison design where each subject receives two of the planned treatments (or one, if used for replacement purposes).

Table 32. Treatments, Anatomical Sites of Evaluation, Application and Dry Times and Coverage Areas.

Treatment (Quantity/Volume)	Body Site	Application Time	Dry Time	Area of Coverage
Medline 2% CHG cloth (one cloth per body site)	Abdomen (sebaceous poor)	3 minutes total	1 minute	5" x 5"
	Groin (sebaceous rich)	3 minutes total	1 minute	2" x 5"
Medline placebo solution cloth (one cloth per body site)	Abdomen (sebaceous poor)	3 minutes total	1 minute	5" x 5"
	Groin (sebaceous rich)	3 minutes total	1 minute	2" x 5"
Dyna-Hex 2	Abdomen (sebaceous poor)	2 x 2 minutes (4 minutes total)	After wiping off second application	5" x 5"
	Groin (sebaceous rich)	2 x 2 minutes (4 minutes total)	After wiping off second application	2" x 5"

Randomization and Blinding (R13-053 & R15-029)

The subjects were randomized to treatment using the following block design:
Treatment Balance:
Each subject received two different treatments, one on the right side of the body and one on the left. This means there are three possible combinations of treatments:

- Medline 2% CHG and Medline placebo solution
- Medline 2% CHG and Dyna-Hex 2®
- Medline placebo solution and Dyna-Hex 2®

The treatment assignments were balanced such that the number of readings per anatomical site matches the calculated requirements. The two active treatments were applied to an equal number of anatomical sites. The Medline placebo solution was applied to the number of anatomical sites necessary to generate a baseline for comparison

to the active substances with that number being determined by the investigative site and statistical consultant based on experience, prior data, and data from the pilot study.

The investigator was responsible for ensuring that the randomization was followed. The final randomization schedule was prepared before the initial treatment. The test and control articles were labeled with the appropriate codes as designated by the study randomization. Subjects whose abdominal and groin regions qualified for testing were assigned a subject number. Therefore, for each of the study materials, a participating subject was assigned two identification numbers.

- Screening subjects were assigned numbers ranging from 9001 to 9999.
- Subjects were assigned numbers ranging from 0001 to a four-digit number equal to the total number of test subjects needed (0275 for the estimated numbers).

The study materials were not blinded from the Investigator or other study staff performing the study material application or bacterial sample collections. The staff member(s) performing bacterial enumeration was blinded from the identification of treatment assignment. The study staff performing the bacterial enumeration was not involved in the study material application or the collection of samples. The Raw Data Sheet sections of the case report form were maintained separately (from the pages within the case report form which include study treatment identifications) during the conduct phase of the study. The study staff performing the bacterial enumeration recorded counts directly onto the Raw Data Sheet pages of the case report form without accessing the subject study documentation folder containing the other case report form pages. The Raw Data Sheets were compiled with the entire case report form after all data recording had been completed.

Study Materials

The materials identified in the table below were used in the study. Specific product identification codes and lot numbers were also included on the form titled "Confirmation of Release and Receipt of Study Materials" at the time the clinical supplies were shipped to the study site.

Table 33. Study Materials.

Study Arm	Name	Description	Lot No.	Exp.
Test Article	Medline 2% CHG cloth	(b) (4)	TBD	TBD
Vehicle Article	Medline placebo solution cloth		TBD	TBD
Positive Control Article	Dyna-Hex 2		TBD	TBD

The Investigator had the choice to discontinue individual subjects from the study at any time. Subjects could voluntarily withdraw from the study at any time without reason or consequence. The subject was asked to report the reason for withdrawal. The

Investigator would provide a written report on the appropriate case report form (CRF) including the date and reason for discontinuance. Subjects who qualified on Screening Day and begun the treatment phase could not be reentered into the study, regardless of whether they completed the study. Any enrolled subject would be replaced for the following reasons:

1. Treatment Day baseline counts less than the minimum baseline values, that is, 1.3 x 10^3 CFU/cm^2 per abdominal site (left or right) or less than 1.0 x 10^5 CFU/cm^2 per groin site (left or right).
2. Missing Treatment Day baseline the 10 minutes, 6-hour, or the 8-hour sampling interval which may be due to subject discontinuation, early withdrawal, missed appointment or a lab accident.
3. A skin irritation rating of 3 for any individual skin condition at any evaluation following the application of study treatment (A skin irritation rating of 2 for any individual skin condition at any evaluation following the application of study material may also be the cause for subject discontinuation at the discretion of the Investigator.)
4. Experiencing a serious protocol deviation that compromises the data results, for example, using a topical antibiotic at a test site during the study.

Discontinued subjects requiring replacement were to be replaced with another qualified subject as soon as reasonably possible. The replacement subject would follow the same treatment (randomization) schedule as the disqualified subject.

R13-053 MicroBioTest
Replacement subjects were assigned a subject number starting with 1xxx (or 2xxx if needed), and the randomization schedule from the disqualified subject was reassigned to the replacement subject. (For example, if Subject 0003 needed to be replaced, the replacement subject's number would be 1003. If Subject 1003 then needed to be replaced, the replacement subject's number would be 2003).

R15-029 Evic Romania
For both abdomen and groin the number of possible treatment combinations between products and sampling sites was 24 (Appendix 14.4). These combinations were repeated until the number of readings per anatomical site (abdomen and groin), per treatment, per sampling interval (10-minutes, 6-hours and 8-hours post application) was completed.

The leftover treatment combinations due to Treatment Day Baseline criteria failure are given to replacement subjects but making sure to have the same product on either of the sides (left or right). Replacement subjects were assigned a subject number and would receive one combination on abdomen and another one for groin, having the same product on either of the sides. For example, a subject included to complete the leftover combinations could receive the combination 10 on abdomen and combination 9 or 10 or 11 or 12 on groin.

Inclusion Criteria
Subjects to whom all of these conditions apply were eligible for enrollment in this study:

1. Males and/or females, at least 16 years or older. Subjects less than 18 must have written custodial consent. (MicroBioTest)
2. Males and/or females, at least 18 years or older. (Evic Romania)
3. Are in good general health.
4. Have skin within 6 inches of the test sites that is free of tattoos, dermatoses, abrasions, cuts, lesions or other skin disorders.
5. Cooperative and willing to follow Subject Instructions (appendix 14.6).
6. Cooperative and willing to sign Consent Form and HIPAA Authorization Form.
7. Have Screening Day baseline counts of at least 1.3×10^3 CFU/cm^2 per abdominal site (left and right) and 1.0×10^5 CFU/cm^2 per groin site (left and right). For replacement subjects, have Screening Day baseline counts of at least 1.3×10^3 CFU/cm^2 per abdominal site (left and right) and/or 1.0×10^5 CFU/cm^2 per groin site (left and right).
8. Negative urine pregnancy test for women at the Treatment Day (Evic Romania)

Reviewer's comments: ***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.***

On May 16, 2018, FDA sent an information request to the Sponsor requesting to clarify the prespecified treatment day baseline criteria for R13-053, section 8.1 of the protocol states that "The baseline bacterial count requirements are in the range of 3.00-5.00 $\log_{10}$ /cm^{2}on the abdomen and 5.00-7.50 $\log_{10}$ /cm^{2}on the groin." However, section 3.6.2 of the protocol states that "Treatment Day baseline counts less than the minimum baseline values, that is 1.3×10^3 CFU/cm^2 per abdominal site (left and right) and/or 1.0×10^5 CFU/cm^2 per groin site (left and right)." The Sponsor responded that there was an inconsistency in the abdominal baseline CFU criteria. The intended minimum treatment day baseline for the abdomen was 1.3×10^3 CFU/cm^2, which is approximately 3.11 $\log_{10}$ CFU/cm^2. The value 1.3×10^3 CFU/cm^2 was used for the reanalysis. This is acceptable.

Exclusion Criteria

1. Topical or systemic antimicrobial exposure within 14 days prior to Screening Day Restrictions include, but are not limited to antimicrobial soaps, antiperspirants/deodorants, shampoos, lotions, perfumes, after shaves, colognes, and topical or systemic antibiotics.
2. Swimming in chemically treated pools or bathing in hot tubs, spas and whirlpools within 14 days prior to Screening Day.
3. Use of tanning beds, hot waxes, or depilatories, including shaving (in the applicable test areas) within 14 days prior to Screening Day.
4. Contact with solvents, acids, bases, fabric softener-treated clothing or other household chemicals in the applicable test areas within 14 days of the Screening Day. Subjects who have a history of sensitivity to natural rubber latex, adhesive skin products (e.g., Band-Aids, medical tapes), or chlorhexidine gluconate products.

5. Subjects who have a history of diabetes.
6. Subjects who have a history of skin allergies.
7. Subjects who have a history of skin cancer within 6 inches of the applicable test areas.
8. Subjects who are pregnant, attempting pregnancy or nursing.
9. Subjects who have showered or bathed within 48 hours of the Screening Day or Treatment Day (sponge baths may be taken; however, the lower abdomen and upper thigh region must be avoided).
10. Subjects who receive an irritation score of 1 for any individual skin condition prior to the Screening Day Baseline or Treatment Day baseline sample collection.

> ***Reviewer's comments:*** ***We recommended that the protocol should specify that subjects who withdrew from the study after qualifying and having started the treatment phase, may not be reentered into the study, and subjects that completed the study may not be reentered into the study. The Sponsor included this statement under the "Subject Discontinuation and Replacement" section. This is acceptable. The Sponsor's inclusion and exclusion criteria are acceptable and in accordance with recommendations in the 1994 TFM for patient preoperative skin preparation studies.***

Pretreatment Phase (washout) (R13-053 & R13-029)
Subjects were provided a kit with nonantimicrobial personal care products for exclusive use during the study. Subjects were also provided with written instructions regarding the use of these products. A visual skin assessment of the test areas was performed. If subjects required hair removal to facilitate sample collection, the subject was asked to return to the test facility at least 48 hours before the Screening Day. Subjects were required to refrain from bathing or showering for 48 hours prior to both the Screening Day and Treatment Day. Sponge bathing was allowed; however, the subject had to avoid the lower abdomen and upper thigh region.

> ***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 (R13-053 & R15-029)
A baseline screening sample was collected from each test area using the Williamson-Kligman cup scrub technique. Baseline samples were taken from the center of each contralateral test area within each anatomical region. Samples from both the left and right sides of a body region must meet the minimum value indicated in the Inclusion Criteria for the subject to be enrolled into the treatment phase of the study for that region. Subjects must qualify for both the abdominal portion and the groin portion of the study, unless they are replacement subjects. Subjects who qualified for the study were notified and would continue to follow the subject instructions until completion of the scheduled Treatment Day. Subjects again were required to refrain from bathing or showering 48

hours prior to Treatment Day and hair was clipped at least 48 hours prior to Treatment Day.

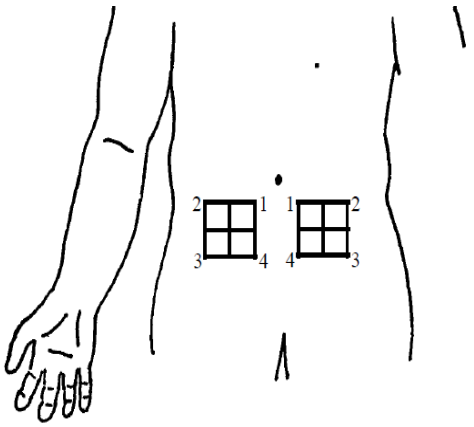
Reviewer's comment: ***The screening phase procedure is standard and is acceptable.***

Treatment Phase (R13-053 & R15-029)

A sufficient number of subjects who met the entrance criteria were enrolled into the treatment phase of the study for each region, such that the total number of abdominal regions and the total number of groin regions met or exceeded the number determined from analysis of the pilot (544 abdominal regions and 544 groin regions, with 248 of each region for each active treatment and 48 of reach region for the placebo. The randomization schedule designated the treatment to each side of the abdomen and groin.

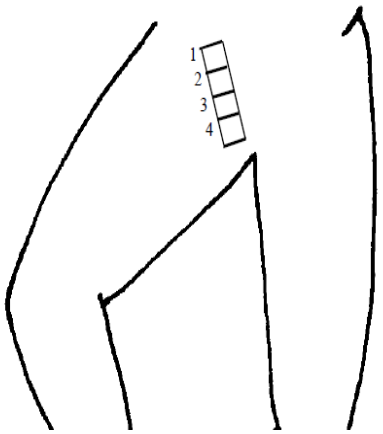
Preparation of Abdominal Test Area

The test site within the abdominal region (abdominal test area) was defined as the area below the umbilicus and above the groin. Using a 5" x 5" sterile template, the corners of each abdominal test area were marked directly on the skin using a nontoxic skin marker. Four sampling sites were numbered within each abdominal test area, on each side of the abdominal region. The positioning and numbering of the abdominal sampling sites were standard for all subjects. Sampling sites on the contra-lateral side of the abdomen were numbered in a mirror-image orientation. The four sampling sites within each abdominal test area represented one baseline (preprep) site, and two or three postprep samples sites (10-minutes, 6-hours and 8-hours).



Preparation of the Groin Test Area

The test site within the groin region (groin test area) was defined as the inner aspect of the upper thigh within and parallel to the inguinal crease below the groin. Using a 2" x 5" sterile template, the corners of each groin test area were marked directly on the skin using a nontoxic skin marker. Four sampling sites were numbered within each groin test area, on each side of the groin region. The positioning and numbering of the groin sampling sites are standard for all subjects. Sampling sites on the contralateral side of the groin were numbered in a mirror-image orientation. The four sampling sites within each groin test area represented one baseline (preprep) site, and two or three postprep sample sites (10-minutes, 6-hours and 8-hours).



Treatment Materials Application

Medline 2% CHG Cloth and Medline Placebo Solution Cloth
On Abdomen and Groin

1. Using a single cloth, vigorously scrub skin in a back and forth motion for 3 minutes completely wetting the treatment area (5" x 5" for the abdomen or 2" x 5" for the groin). Approximately halfway through the 3-minute application, the cloth would be turned over. If necessary, the subject's skin should be held taut to ensure that the maximum amount of the cloth contacts the area being prepped.
   Note: product handling, when opening the packaging, a single cloth (single unit) will be used for each anatomical region. Contact between the cloth and the outside of the packaging will be avoided to reduce risk of cloth contamination.
2. At the completion of the 3-minute application, the area is allowed to air-dry for one minute prior to the initiation of the contact times.

Dyna-Hex 2® (positive control)
On Abdomen and Groin

1. Based on the manufacturer's instructions, five mL of the reference product is applied onto a sterile gauze pad.
2. The product is applied to the treatment area using the same area used for the test product for two minutes. The area is dried by wiping with a sterile towel or sterile gauze.
3. Steps 1-2 is repeated.
4. Contact time begins after the site has been wiped dry a second time.

Reviewer's comments: ***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 three study products. The treatments were randomized between left and right test areas and postprep sampling times were randomized amongst the sampling sites with a test area. For the Medline 2% CHG Cloth and Medline Placebo Solution Cloth the directions above state the following: "Using a single cloth, vigorously scrub skin in a back and forth motion for 3 minutes completely wetting the treatment area (5" x 5" for the abdomen). Approximately halfway through the 3-minute application, the cloth would be turned over." However, on the labeled directions it states the following: "Use one cloth to cleanse each 161 cm^2 area approximately 5 x 5 inches) of skin to be prepared. Vigorously scrub skin back and forth for 3 minutes, completely wetting treatment area, then discard." This reviewer finds this acceptable based on the pilot studies conducted using the directions vigorously scrub skin back and forth for 3 minutes and then dry for one minute. The results showed achieving the required log_{10} reduction at the abdomen and groin site and meeting the 70% responder rate at the abdomen and groin site. Also a similar product, Sage CHG Cloth, has the same directions vigorously scrub skin back and forth for 3 minutes and then dry for one minute.***

Timing of Post Application Sample Collection

Microbial samples were collected at 10 minutes (±30 seconds), 6 hours (±30 minutes) and 8 hours (±30 minutes) post treatment application for both the abdomen and the groin regions. Post application timing begins upon completion of the treatment material application, including drying time. Microbial samples were collected using the scrub cup technique. After the 10-minute samples have been collected, a piece of sterile gauze and a nonocclusive dressing was secured over the remaining sample sites to allow subjects restricted mobility and to protect the sites from contamination between sampling times. The subjects were allowed to leave the clinical test facility but had to return 6 hours (±30 minutes) post treatment application, for post application sample collection. A skin irritation assessment was performed.

Reviewer's comments: 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 posttreatment sampling by loosely draping the treated skin area with a sterile nonocclusive dressing. Subjects may leave the test facility if they return for the 6-hour time point.

We had informed the Sponsor in an advice letter dated May 25, 2012, that it 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.

Microbial Sample Collection / Scrub Cup Technique (R13-053 & R15-029)

Quantitative cultures were obtained from the test sites using a modification of the cup scrub method of Williamson and Kligman. To collect the samples, a sterile scrub cup (2.20 cm I.D. for MicroBioTest and 2.10 cm I.D. for Evic Romania) was placed on the site and held firmly to the skin. Sampling solution (3.0 mL) was pipetted into the cup and the skin scrubbed in a circular motion with moderate pressure for 1 minute using a sterile rubber policeman. Using a sterile transfer pipette, the sampling solution was removed and placed in a sterile test tube. An additional 3.0 mL of fresh sampling solution was pipetted into the cup and the scrub procedure was repeated. This solution was pooled with the first solution collected.

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 Evic Romania 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 is up to the sponsors to choose the scrub cup size they feel would give the best results for their studies.

Bacterial Enumeration Methods

Following sample collection, 10-fold serial dilutions (1 mL sample +9 mL (b) (4) sterile phosphate buffered water (b) (4) were prepared. One mL aliquots of appropriate dilutions were pour-plated in triplicate using trypticase soy agar containing neutralizers (TSA+N). Samples were plated within 30 minutes of collection. After 72±4 hours of aerobic incubation at 30±2°C, colonies were counted and viable cells in the original sample were calculated according to Standard Operating Procedures. After incubation, plates could be refrigerated up to 48 hours prior to counting.

Reviewer's comment: We had been recommending sponsors to include the type of neutralizers they will be using when incorporating into the sampling solution. The Sponsor described the sampling solution (SS) to contain 75mM phosphate buffer (b) (4) ***with 0.1% Triton® X-100, 0.3% lecithin, 1.0% polyoxyethylene sorbitan monooleate*** (b) (4) ***and 1.0%*** (b) (4) ***SN; pH 7.9 + 0.1, sterile. This is acceptable.***

Selection of Study Population (R13-053 & R15-029)

Healthy male and female volunteers, 16 years of age or older (subject less than 18 must have written custodial consent) (MicroBioTest), 18 years of age or older (Evic Romania), with no dermatological conditions or known history of sensitivity to natural rubber latex, adhesive skin products (e.g., Band-Aids, medical tapes), or CHG were enrolled into the study. The number of volunteers enrolled were based on the results of analysis of the pilot study, with the goal of meeting the FDA TFM guidelines with a statistical power of at least 80%. This required a sufficient number of volunteers in the screening phase such that at least a total of 544 abdominal regions and 544 groin regions were evaluable at completion of the study, balanced as indicated in Table 34 below. Subjects must satisfy all Screening Day and Treatment Day procedures. If the required numbers of subjects did not qualify from the initial screening group, additional volunteers were to be recruited.

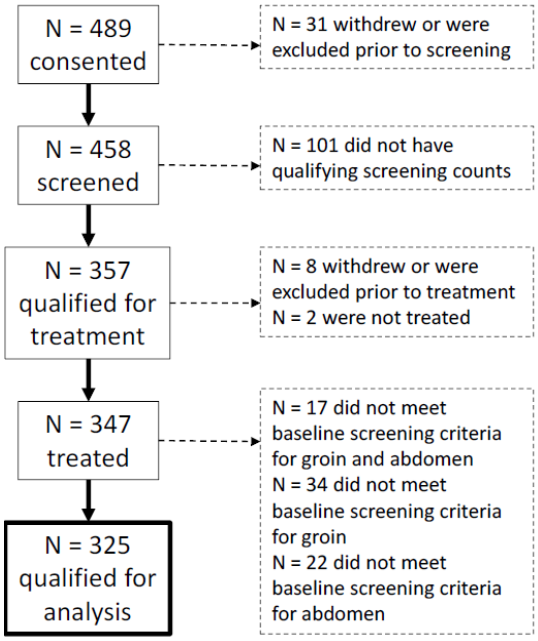
Reviewer's comments: MicroBioTest has started to enroll subjects as low as 16 years of age with written custodial consent. This laboratory must be having a hard time enrolling subjects into studies and decided to lower the age to have a more robust enrollment. This is acceptable.

Study Subjects

R13-053 MicroBioTest: Subjects who met the minimum baseline inclusion criteria on the Screening and Treatment Day of the study on both sites of the body (groin and abdomen) were considered evaluable for efficacy for that region. The subject disposition is diagrammed in Figure 1. A total of 489 subjects consented to the study, and screening samples were collected from 458 subjects. Only subjects with qualifying screening

counts of at least 1.3 x 10^3 CFU/cm^2 per abdominal site and 1.0 x 10^5 CFU/cm^2 per groin site were treated in the study.

Figure 1. Flow Chart of Subject Disposition for Study R13-053.



Per study protocol, subjects (N=347) were treated prior to baseline bacterial enumeration, and samples from subjects that did not exhibit "Treatment Day baseline" counts of at least 1.3 x 10^3 CFU/cm^2 per groin site were not analyzed. Three hundred twenty-five (325) subjects qualified met baseline criteria for further analysis. The number of treatments is presented in the table below.

Table 34. Study R13-053 Treatments and Number of Applications.

Treatment	Number of Applications	
	Abdomen	Groin
Medline 2% CHG cloth	253	254
Dyna-Hex 2	253	250
Medline vehicle cloth	48	48

***Reviewer's comments*: On May 16, 2018, FDA sent an information request to the Sponsor requesting the following information: "Submit the results of analyses for R13-053 based on a modified intent-to-treat population (mITT), after correction of all errors that were identified after submission of the clinical study reports. The mITT population, all subjects who were randomized and meet the prespecified treatment day baseline requirements on at least one side of a body area are included (regardless of protocol deviations) and are analyzed in the groups to which they were randomized. In particular, correct the following errors that affect your study results:**

- ***Subject (b) (6) right inguinal region should have been excluded from the primary analysis as a treatment day baseline failure."***

On May 25, 2018, the Sponsor had a question where the previous FDA instructions were ambiguous: "Subject (b) (6) had a passing abdominal day baseline values but also had a deviation noting that inguinal data may have been mixed with abdominal data. By the instruction in the May 16, 2018 letter, the subject should be included in the mITT population regardless of the deviation, but the deviation call into question the validity of all of the subject's data, which would normally lead to exclusion. Please indicate whether this subject should be included or excluded from the MITT population." On June 1, 2018, FDA responded to the Sponsor's question: "Include subject (b) (6) with passing abdominal day baseline values in the mITT analysis, despite the deviation noting that inguinal data may have been mixed with abdominal data."

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. Also refer to the statistician, Dr. Elande Baro's review in DARRTS.

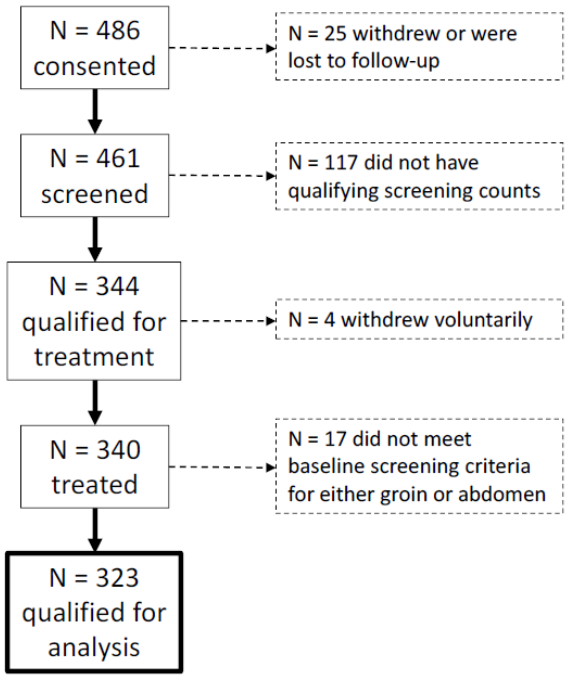
Reanalysis of Data
When a subject failed to meet the required treatment baseline values, a replacement subject was tested. The replacement subject received the same treatments, even if the baseline failure only affected one treatment. Therefore, the number of actual treatments was higher in some cases than the design minimum. The treatments and number of subjects that were used for the study are shown in the table below.

Table 35. Reanalysis of Group Treatments and Number of Subjects - R13-053.

Treatment	Role	Number of Applications	
		Abdomen	Groin
Medline 2% CHG cloth	test article	254	249
Dyna-Hex 2	comparator	252	254
Placebo	placebo control	48	48

R15-029 Evic Romania: Subjects who met the minimum baseline inclusion criteria on the Screening and Treatment Day of the study on both sides of the body (groin and abdomen) were considered evaluable for efficacy for that region. The subject disposition is diagrammed in Figure 2. A total of 486 subjects consented to the study, and screening samples were collected from 461 subjects. Only subjects with qualifying screening counts of at least 1.3×10^3 CFU/cm^2 per abdominal site and 1.0×10^5 CFU/cm^2 per groin site were treated in the study.

Figure 2. Flow Chart of Subject Disposition for Study R15-029.



Per study protocol, subjects (N = 344) were treated prior to baseline bacterial enumeration, and samples from subjects that did not exhibit "Treatment Day baseline" counts of at least 1.3 x 10^3 CFU/cm^2 per abdominal site and 1.0 x 10^5 CFU/cm^2 per groin site were not analyzed. Three hundred twenty-three (323) qualified subjects met baseline criteria for further analysis for the abdominal and/or inguinal sites.

***Reviewer's comments**: On May 16, 2018, FDA sent an information request to the Sponsor requesting the following information: "Clarify whether treatments were correctly recorded in the submitted datasets for subjects (b) (6) in R15-029. We noted the following inconsistencies between treatment randomized (see randomization scheme in Appendix 16.1.5 in R15-029) and treatment recorded in the data set. For One subject ((b) (6)) on the left side of the groin and four subjects ((b) (6)) on the left side of the abdomen were randomized to Medline Cloth but the data suggests that these subjects were treated with Medline Vehicle." The Sponsor clarified that subject (b) (6) on the left side of the groin and subjects (b) (6) on the left side of the abdomen had incorrect treatment listings in the prior data set. The treatments for the indicated sides were changed to "Medline Cloth" in the updated data set, which was used for this analysis.*

FDA indicated that 33 subjects were treated who should have been excluded as screening failures, based on the FDA inspection. The Sponsor and the site independently reviewed the screening CFU values and identified 17 subjects who had screening baseline CFU values below the required minimums but were treated. The Sponsor also investigated deviations but did not identify any

additional treated subjects who failed screening day requirements. The Sponsor does not have access to the FDA inspection and therefore cannot use it to identify the remaining 16 subjects who failed the screening day requirements but were not excluded. The site was also queried but was unsure of the identity of the other subjects. The Sponsor stated that it will exclude the 17 identified subjects from the analysis. The Sponsor inquired how it should proceed for the other 16 subjects?

FDA responded that the Sponsor should conduct the mITT analysis without excluding any of the subjects with screening failures identified from the analysis. In other words, do not exclude from the mITT analysis the 33 subjects that the FDA inspection identified or the 17 subjects that the Sponsor identified. Therefore, the Sponsor stated that the 17 subjects who had screening day baseline failures but were treated are now included in the analysis. This is acceptable.

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. Also refer to the statistician, Dr. Elande Baro's review in DARRTS.

Reanalysis of Data
When a subject failed to meet the required treatment baseline values, a replacement subject was tested. The replacement subject received the same treatments, even if the baseline failure only affected one treatment. Therefore, the number of actual treatments was higher in some cases than the design. There was also a protocol deviation for one subject that affected treatment counts. The treatments and number of subjects in the mITT population are shown in Table 36 below.

Table 36. Reanalysis of Group Treatments and Number of Subjects - R15-029.

Treatment	Role	Number of Applications	
		Abdomen	Groin
Dyna-Hex 2	comparator	253	259
Medline 2% CHG cloth	test article	241	252
Medline vehicle	vehicle control	50	52

Demographics and Other Baseline Characteristics
R13-053 MicroBioTest: The study population age ranged from 16-79 years of age with a mean age of 35 years. A higher percentage of males (60%) were enrolled. Although the racial distribution was predominantly Caucasian (40%), other racial groups (Asian, Black, and Hispanic) were comparably represented. The study demographics are presented in the table below.

Table 37. Study R13-053 Subject Demographics for Study.

T

Demographic Category		
Age (Years)	Mean	35
	Minimum	16
	Maximum	79
Sex [Number of Subjects (%)]	Males	209 (60%)
	Females	138 (40%)
Race [Number of Subjects (%)]	White/Caucasian	139 (40%)
	Black/African American	66 (19%)
	Hispanic	45 (13%)
	Asian	87 (25%)
	Other	10 (3%)

R15-029 Evic Romania: The study population ranged from 18-69 years of age with a mean age of 51 years. A higher percentage of females (54%) were enrolled. The entire study population was Caucasian. The study demographics are presented in the table below.

Table 38. Study R15-029 Subject Demographics for Study.

Demographic Category		
Age (Years)	Mean	51
	Minimum	18
	Maximum	69
Sex [Number of Subjects (%)]	Males	158 (46%)
	Females	182 (54%)
Race [Number of Subjects (%)]	White/Caucasian	340 (100%)

Reviewer's comments: ***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 (100%), treatment experience among other races is limited at the Evic Romania testing site. The MicroBioTest testing sites were made up of a diverse group of races: 40% Caucasian, 25% Asian, 19% black, and 13% Hispanic/Latino. However, we do not have any evidence that race makes a difference in the efficacy of topical antiseptics. These type of products (CHG) has been marketed in the United States for several years and there are no reports in AERS or the literature to suggest that efficacy is affected by specific demographic factors. Also refer to Medical Officer, Dr. Martha Lenhart's review in DARRTS.***

Efficacy Results

Efficacy Results of Study R13-053 (MicroBioTest)

On the abdominal region, 252 subjects received Medline 2% CHG Cloth, 254 subjects received Dyna-Hex 2®, and 48 subjects received Vehicle Cloth. On the inguinal region, 254 subjects received Medline 2% CHG Cloth, 249 subjects received Dyna-Hex 2®, and 48 subjects received Vehicle Cloth. 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 one 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 Table 39 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 test product Medline 2% CHG Cloth in the abdominal and inguinal regions. The responder rate for Dyna-Hex 2® at 10 minutes was significantly higher than 70% for the abdomen but not for the groin. The Responder rate for the vehicle at 10 minutes was significantly lower than 70% for both abdomen and groin.

Table 39. Responder Rate at 10 Minutes (mITT Population, Study R13-053 MicroBioTest).

Body Area	Treatment	10 Minute Responder Rates	
		Rate (%) (counts)	95% Exact Confidence Interval
Abdomen	Dyna-Hex 2	85.04% (216 of 254)	0.8005 to 0.8919
Abdomen	Medline Cloth	93.25% (235 of 252)	0.8942 to 0.9602
Abdomen	Vehicle Cloth	50.00% (24 of 48)	0.3523 to 0.6477
Groin	Dyna-Hex 2	65.06% (162 of 249)	0.5879 to 0.7097
Groin	Medline Cloth	85.83% (218 of 254)	0.8092 to 0.8987
Groin	Vehicle Cloth	25.00% (12 of 48)	0.1364 to 0.3960

Table 40. Responder Rate at 6 Hours (mITT Population, Study R13-053 MicroBioTest).

Body Area	Treatment	6 Hour Responder Rates	
		Rate (%) (counts)	95% Exact Confidence Interval
Abdomen	Dyna-Hex 2	100.00% (254 of 254)	0.9856 to 1.0000
Abdomen	Medline Cloth	100.00% (252 of 252)	0.9855 to 1.0000
Abdomen	Vehicle Cloth	97.92% (47 of 48)	0.8893 to 0.9995
Groin	Dyna-Hex 2	100.00% (249 of 249)	0.9853 to 1.0000
Groin	Medline Cloth	100.00% (254 of 254)	0.9856 to 1.0000
Groin	Vehicle Cloth	100.00% (48 of 48)	0.9260 to 1.0000

***Reviewer's comments*: On May 16, 2018, FDA sent an advice letter to the Sponsor regarding statistical analysis of the clinical simulation studies. FDA requested that the Sponsor conduct the result analysis using the 95% exact confidence interval instead of the 99% exact confidence interval.**

For the 10-minute time point for the abdominal region modified intent-to-treat population, the lower bound of the 95% CI for responder rate was 80.0%, 89.4%, and 35.2% for Dyna-Hex 2®, Medline CHG Cloth, and Vehicle Cloth, respectively. The corresponding responder rate point estimates were 85.0%, 93.2%, and 50.0%, respectively.

For the 10-minute time point for the inguinal region modified intent-to-treat population, the lower bound of the 95% CI for responder rate was 58.8%, 80.9%, and 13.6% for Dyna-Hex 2®, Medline CHG Cloth, and Vehicle Cloth,

respectively. The corresponding responder rate point estimates were 65.0%, 85.8%, and 25.0%, respectively.

For both abdominal and inguinal regions, responder rates for all active products were significantly higher than for the Vehicle Cloth Control. The responder rate following Vehicle Cloth Control treatment was 50% for the abdominal region and 25% for the inguinal region.

The Medline CHG Cloth was statistically significantly better than Dyna-Hex 2® and the Vehicle Cloth Control for log_{10} CFU/cm^2 changes from baseline for both body areas and all post application sampled times. At 10 minutes Medline CHG Cloth had mean responder rates and responder rate confidence intervals above 70% for both the abdomen and the groin. At 6 hours Medline CHG Cloth had 100% responder rates (all values below baseline) for both body areas.

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 41 for the mITT population.

Table 41. Summary Statistics of Log_{10} Transformed Bacterial (Skin Flora) Endpoints Mean Log_{10} CFU Counts and Changes from Baseline (mITT Population, study R13-053).

Body Area	Treatment	Baseline	10 Minutes		6 Hours	
			Value	Change	Value	Change
Abdomen	Dyna-Hex 2	3.4383	0.5255	2.9128	1.2072	2.2311
Abdomen	Medline Cloth	3.4586	0.2812	3.1774	0.9456	2.5131
Abdomen	Vehicle Cloth	3.4852	1.5232	1.9620	1.9811	1.5041
Groin	Dyna-Hex 2	5.4634	1.7908	3.6726	2.8017	2.6617
Groin	Medline Cloth	5.4510	1.1753	4.2757	2.3489	3.1021
Groin	Vehicle Cloth	5.4148	2.9351	2.4797	3.3531	2.0617

<u>Reviewer's comments</u>: On May 16, 2018, FDA sent an advice letter to the Sponsor regarding statistical analysis of the clinical simulation studies. FDA requested that the Sponsor conduct the result analysis to include the mITT subjects.

We informed the Sponsor that, in order to demonstrate effectiveness for the secondary endpoint (mean log_{10} 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 means bacterial (skin flora) count was approximately 3.0 log_{10} per cm^2 across the active study products. The mean (standard deviation, SD) reduction from baseline at 10 minutes following treatment was similar among active treatments: 2.91 (0.525)

log_{10} per cm^2 and 3.17 (0.281) log_{10} per cm^2 for Dyna-Hex 2® and Medline CHG Cloth, 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: 2.23 (1.207) log_{10} per cm^2 and 2.51 (0.945) log_{10} per cm^2 for Dyna-Hex 2® and Medline CHG Cloth, respectively. The Mean (SD) reduction from baseline following Vehicle Cloth Control treatment was 1.96 (1.523) log_{10} per cm^2 at 10 minutes and 1.50 (1.962) 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 means bacterial (skin flora) count was approximately 3.9 log_{10} per cm^2 across active study products. The mean (SD) reduction from baseline at 10 minutes following treatment was similar among active treatments: 3.67 (1.790) log_{10} per cm^2 and 4.27 (1.175) log_{10} per cm^2 for Dyna-Hex 2® and Medline CHG Cloth, 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: 2.66 (2.801) log_{10} per cm^2 and 3.10 (2.348) log_{10} per cm^2 for Dyna-Hex 2® and Medline CHG Cloth, respectively. The mean (SD) reduction from baseline following Vehicle Cloth Control treatment was 2.47 (2.935) log_{10} per cm^2 at 10 minutes and 2.06 (3.353) log_{10} per cm^2 at 6 hours. Therefore, all the test products did not exceed the baseline counts at 6 hours.

It is not surprising that the results of the Vehicle Cloth Control showed some effectiveness results. The Vehicle Cloth contained the following excipients: purified water (b) (4)%, glycerin (b) (4)%, propylene glycol (b) (4)%, dimethicone NF emulsion (b) (4)%, isopropyl alcohol (b) (4)%, and benzalkonium chloride (b) (4)%. These excipients showed limited activity in the in vitro assay testing results. Additionally, the application of the vehicle cloth itself may cause the mechanical elimination of bacterial cells, with a corresponding observation of bacterial log reduction.

Efficacy Results of Study R15-029 (Evic Romania)

On the abdominal region, 241 subjects received Medline 2% CHG Cloth, 253 subjects received Dyna-Hex 2®, and 50 subjects received Vehicle Cloth. On the inguinal region, 252 subjects received Medline 2% CHG Cloth, 252 subjects received Dyna-Hex 2®, and 52 subjects received Vehicle Cloth. Based on the study records, 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 shown in tables 42 and 43 below, 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 the test product, Medline 2% CHG Cloth, in the abdominal and inguinal regions. The responder rate for Dyna-Hex 2® at 10 minutes was significantly higher than 70% for the abdomen and the groin. The Responder rate for the vehicle at 10 minutes was significantly lower than 70% for both abdomen and groin.

Table 42. Responder Rate at 10 Minutes (mITT Population, Study R15-029).

Body Area	Treatment	10 Minute Responder Rates	
		Rate (%) (counts)	95% Exact Confidence Interval
Abdomen	Dyna-Hex 2	71.54% (181 of 253)	0.6555 to 0.7702
Abdomen	Medline Cloth	80.50% (194 of 241)	0.7492 to 0.8530
Abdomen	Vehicle Cloth	50.00% (25 of 50)	0.3553 to 0.6447
Groin	Dyna-Hex 2	72.97% (189 of 259)	0.6713 to 0.7828
Groin	Medline Cloth	84.52% (213 of 252)	0.7946 to 0.8876
Groin	Vehicle Cloth	55.77% (29 of 52)	0.4133 to 0.6953

Table 43. Responder Rate at 6 Hours (mITT Population, Study R15-029 Evic Romania).

Body Area	Treatment	6 Hour Responder Rates	
		Rate (%) (counts)	95% Exact Confidence Interval
Abdomen	Dyna-Hex 2	98.81% (250 of 253)	0.9657 to 0.9975
Abdomen	Medline Cloth	100.00% (241 of 241)	0.9848 to 1.0000
Abdomen	Vehicle Cloth	96.00% (48 of 50)	0.8629 to 0.9951
Groin	Dyna-Hex 2	100.00% (259 of 259)	0.9859 to 1.0000
Groin	Medline Cloth	100.00% (252 of 252)	0.9855 to 1.0000
Groin	Vehicle Cloth	100.00% (52 of 52)	0.9315 to 1.0000

***Reviewer's comment*: As demonstrated in Table 42, the primary efficacy endpoint of achieving ≥70% for the lower bound of the 95% CI for responder rate was met by the Medline CHG Cloth on the abdominal region. For the abdominal region mITT population, the lower bound of the 95% CI for responder rate was 74.9% for the Medline CHG Cloth. However, the Dyna-Hex 2® achieved <70% for the lower bound of the 95% CI for responder rate with the 65.5% which is close to 70%. The corresponding responder rates were 71.5% and 80.5% for Dyna-Hex 2® and Medline CHG Cloth, and were all higher compared to the Vehicle Cloth Control (50.0%).**

The primary efficacy endpoint of achieving ≥70% for the lower bound of the 95% CI for responder rate was met by the Medline CHG Cloth on the inguinal region. For the inguinal region mITT population, the lower bound of the 95 CI for responder rate was 74.9% for the Medline CHG Cloth. However, the Dyna-Hex 2® achieved <70% for the lower bound of the 95% CI for responder rate with 67.1%, which is close to 70%. The corresponding responder rates were 84.5% and 72.9% for Medline CHG Cloth and Dyna-Hex 2® and were all higher compared to the Vehicle Cloth Control (55.7%).

For both abdominal and inguinal regions, responder rates for all active products were significantly higher than for the Vehicle Cloth Control. The responder rate following Vehicle Cloth Control treatment was 50.0% for the abdominal region and 55.7% for the inguinal region.

The Medline CHG Cloth was statistically significantly better than Dyna-Hex 2® and the Vehicle Cloth Control for log_{10} CFU/cm^2 changes from baseline for both body areas and all post application sampled times. At 10 minutes Medline CHG Cloth had mean responder rates and responder rate confidence intervals above 70% for both the abdomen and the groin. At 6 hours Medline CHG Cloth had 100% responder rates (all values below baseline) for both body areas.

Table 44. Summary Statistics of Log_{10}-Transformed Bacterial (Skin Flora) Endpoints Mean log_{10} CFU Counts and Changes from Baseline (mITT Population, Study R15-029).

Body Area	Treatment	Baseline	10 Minutes		6 Hours	
			Value	Change	Value	Change
Abdomen	Dyna-Hex 2	3.7734	1.2200	2.5534	1.0736	2.6998
Abdomen	Medline Cloth	3.7865	0.8879	2.8987	0.6990	3.0875
Abdomen	Vehicle Cloth	3.7050	1.6605	2.0445	1.4601	2.2449
Groin	Dyna-Hex 2	6.1000	2.4330	3.6670	2.1325	3.9675
Groin	Medline Cloth	6.1210	1.5369	4.5842	1.1404	4.9806
Groin	Vehicle Cloth	6.1436	2.4806	3.6630	2.3741	3.7695

<u>Reviewer's comments</u>: On May 16, 2018, FDA sent an advice letter to the Sponsor regarding statistical analysis of the clinical simulation studies. FDA requested that the Sponsor conduct the R15-029 result analysis to include the mITT subjects. We had previously informed the Sponsor that, in order to demonstrate effectiveness for the secondary endpoint (mean log_{10} 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 of bacterial (skin flora) count was approximately 2.7 log_{10} per cm^2 across the active study products. The mean (standard deviation, SD) reduction from baseline at 10 minutes following treatment was similar among active treatments: 2.55 (1.220) log_{10} per cm^2 and 2.89 (0.887) log_{10} per cm^2 for Dyna-Hex 2® and Medline CHG Cloth, respectively. Therefore, all the test products demonstrated ≥2 log_{10} reduction at the abdomen site.

At 6 hours following treatment, the mean (SD) reduction form baseline was similar among active treatments: 2.67 (1.073) log_{10} per cm^2 and 3.08 (0.699) log_{10} per cm^2 for Dyna-Hex 2® and Medline CHG Cloth, respectively. The mean (SD) reduction from baseline following Vehicle Cloth Control treatment was 2.044 (1.660) log_{10} per cm^2 at 10 minutes and 2.244 (1.460) 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 of bacterial (skin flora) count was approximately 4.12 log_{10} per cm^2 across active study products. The mean (SD) reduction from baseline at 10 minutes flowing treatment was similar among active treatments: 3.66 (2.433) log_{10} per cm^2 and 4.58 (1.536) log_{10} per cm^2 for Dyna-Hex 2® and Medline CHG Cloth, 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: 2.69 (1.073) log_{10} per cm^2 and 3.08 (0.699) log_{10} per cm^2 for Dyna-Hex 2® and Medline CHG Cloth, respectively. The mean (SD) reduction from baseline following Vehicle Cloth Control treatment was 3.66 (2.480) log_{10} per cm^2 at 10 minutes and 3.76 (2.374) log_{10} per cm^2 at 6 hours. Therefore, all the test products did not exceed the baseline counts at 6 hours.

Vehicle Cloth Control
It is not surprising that the results of the Vehicle Cloth Control showed some effectiveness, as described above. In the Sage CHG Cloth (NDA 21-669) review assessment, there was no negative or vehicle control cloth. Microbiologist reviewer, Dr. Peter Coderre stated in his review that, since the test product is a CHG solution applied with a cloth, there is a device component to the product. Thus, there are two possible mechanisms for the removal of bacteria from the skin: the chemical action of the CHG and the physical action of the cloth. Just the physical action of the cloth may produce log_{10} reduction on the skin. Therefore, the results of the Vehicle Cloth Control showing mean log_{10} reduction of 2 log_{10} reduction at the abdominal site and 3 log_{10} reduction at the groin site is not surprising.

Dyna-Hex 2® (FDA-approved positive control)
The responder rates of the FDA-approved and marketed positive control (Dyna-Hex 2®) fail to confirm reproducibility of responder rate outcomes between the two laboratories. MicroBioTest failed at the groin site responder rate 65% (58% CI). The Evic Romania was borderline for the abdomen and groin site, 71.54% (65% CI) and 72.97% (67% CI). The differences in demographics, climate and microbiomes of available subjects between the two testing laboratories may account for this variability in responder rates (b) (4)

In pilot study R13-042 (MicroBioTest), Dyna-Hex 2® passed the mean log_{10} reduction and acceptable percent responder rate for both the abdomen and groin site. However, in pilot study R13-052 (BioScience Laboratory), Dyna-Hex 2® passed the mean log_{10} reduction and percent responder rate for the abdomen and was borderline for the groin site.

In the Safety and Effectiveness of Health Care Antiseptics Final Rule (82 FR 60474: December 20, 2017), based on comments submitted on the 2015 Health Care Antiseptic proposed rule and the Agency's further evaluation of additional data, we have updated the underlying statistical analysis related to the log_{10} reduction criteria. We no longer require an analysis of the proportion of subjects who meet the recommended log_{10} reduction criteria based on superiority to a negative control and a two-sided 95% CI statistical approach. We also no longer recommend that the success rate or responder rate of the test product be significantly higher than 70%. The comments argued about the difficulty of the number of subjects meeting the 70% responder rate. The current, updated analysis is designed to assess whether the average treatment effects (ATE) across subjects meet indication-specific conditions of superiority and noninferiority, rather than whether the percentage of subjects who meet an indication-specific threshold significantly exceeds 70%.

Inspection
MicroBioTest was inspected between (b) (4) (b) (4) (b) (4) ***Overall the assessments were acceptable. They were not required to be inspected again for NDA 207964, since this inspection was considered recent (every three years).***

Evic Romania was inspected between March 26 and April 5, 2018 on the efficacy study R13-029. The results of the report showed that the field investigator interviewed laboratory manager (Dr. Olsavszky) and 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. The Office of Scientific Investigations (OSI) judged that the deficiencies noted and discussed could be considered regulatory violations, and OSI classified the inspection outcome as Voluntary Action Indicated (VAI). The main deficiencies were as follows:

- ***Discrepancies between source records and data listings with respect to bacterial sample collection times and scrub application times.***
- ***Microbial sample collections were outside the protocol specified timeframes.***
- ***Enrollment of subjects who did not meet the baseline CFU bacterial counts.***

Overall the field investigator concluded that the findings were unlikely to have a significant impact on the efficacy evaluation. The investigator also stated the following: "The data from the clinical investigator site submitted by the Sponsor in support of the pending application are acceptable and the study was

conducted adequately to support approval." Please see field investigator, Sharon Gershon's full report in DARRTS dated August 27, 2018.

4.1.2 Protocol Deviations

Study R13-053 (MicroBioTest) Protocol Deviations

Subject no. (b) (6) Placebo was inadvertently used in place of Medline CHG Cloth on the right side of the abdomen.

Subject no. (b) (6) and (b) (6): Medline CHG Cloth was inadvertently used in place of placebo on the right side of the abdomen.

Reviewer's comment: The deviation regarding the randomization schedule for Subject (b) (6), (b) (6), and (b) (6) have no effect on the integrity of the study because the application of the test products was randomly performed, and the efficacy data were not affected.

Subject no. (b) (6): The protocol required the Screening Day baseline counts of at least 1.3 x 10^3 CFU/cm^2 on each abdominal site. The subject had 1.2 x 10^3 CFU/cm^2 on left and 1.1 x 10^3 CFU/cm^2 on right. However, this deviation had no negative impact on the study as the baseline values were reevaluated on the Treatment Day. The data was included in the analysis.

Reviewer's comments: The deviation of the baseline counts of 1.2 x 10^3 CFU/cm^2 on left and 1.1 x 10^3 CFU/cm^2 on right is still equivalent to 3 log_{10}. This reviewer agrees with the Sponsor that this has no negative impact on the study.

Subject no. (b) (6): The results of groin were inadvertently recorded on the results page for abdomen. This deviation had no negative impact on the study since this subject will be replaced with new screened subject. The data was not included in the analysis.

Reviewer's comments: The deviation of the data for the groin site was inadvertently recorded on the results page for the abdomen. The Sponsor replaced the subject with a new screened subject and the data was not included in the analysis. This is acceptable.

Subjects no. (b) (6), (b) (6), and (b) (6): The pregnancy test was not performed on the day of treatment. This deviation had no negative impact on the study since the subjects were positive that they were not pregnant.

Reviewer's comments: The deviation regarding not performing the pregnancy test on the day of treatment on the subjects (b) (6), (b) (6), and (b) (6) have no effect on the integrity of the study because the subjects were positive that they were not pregnant. This reviewer agrees with the Sponsor that there was no negative impact of the study.

Study R15-029 (Evic Romania) Protocol Deviations

Sampling time deviations: Section 5.1.2.3 of the study protocol states "Microbial samples will be collected at 10-minutes (±30 sec.), 6-hours (±30 min.), and 8-hours (±30 min.) post treatment application for both the abdomen and the groin regions."

Table 45. Deviations of Passed Baseline Subjects on Abdomen at 10 Minutes Sampling Time.

Subject reference	Side	Actual sampling time	Subject reference	Side	Actual sampling time
(b) (6)	right	(b) (6)	(b) (6)	right	(b) (6)
	left			left	
	right			left	
	left			right	
	right			left	
	left			left	
	right			left	
	right			right	
	left			left	
	right			right	
	left			right	
	right			right	
	right			left	
	left			right	
	left			right	
	left			left	
	right			left	
	left			left	
	left			left	
	left			left	
	right			left	
	left			left	
	left			right	
	right			right	
	right			left	
	left			right	
	right			right	
	left			right	
	left			right	
	right			right	

Table 46. Deviations of Passed Baseline Subjects on Groin at 10 Minutes Sampling Time.

Subject reference	Side	Actual sampling time	Subject reference	Side	Actual sampling time
(b) (6)	left	(b) (6)	(b) (6)	left	(b) (6)
	right			right	
	left			right	
	left			left	
	left			left	
	right			left	
	left			right	
	right			left	
	left			right	
	right			left	
	left			right	
	right			left	
	right			right	
	left			left	
	right			right	
	right			left	
	left			left	
	left			right	
	right			left	
	left			right	
	left			right	
	right			right	
	right			left	
	right			left	
	left			right	

***Reviewer's comments**: For the abdomen, 60 subjects had a deviation of having a required 10-minute (±30 seconds) sample collected beyond the defined time interval. The deviation range was from 10:31 to 11:23 minutes. For the groin, 49 subjects had a deviation of having a required 10-minute (±30 seconds) sample collected beyond the defined time interval. The deviation range was from 9:17 to 11:49 minutes. I agree with the Sponsor that these are considered minor and have no impact on the study. The field investigator from Office of Scientific Investigations (OSI) assessed the data and concluded there was no impact on the efficacy of the study. Based on the results of the neutralization validation, 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.*

Table 47. Deviations of Passed Baseline Subjects on Abdomen at 6 Hours Sampling Time.

Subject reference	Side	Actual sampling time
(b) (6)	left	(b) (6)
	left	
	left	
	left	

Table 48. Deviations of Passed Baseline Subjects on Groin at 6 Hours Sampling Time.

Subject reference	Side	Actual sampling time
(b) (6)	left	(b) (6)
	left	
	left	
	right	
	left	

***Reviewer's comments**: For the abdomen site, four subjects had a deviation of having a required 6-hour (±30 minutes) sample collected beyond the defined time interval. The range was from 6:33:40 to 6:41:09 minutes. For the groin, five subjects had a deviation of having a required 6-hour (±30 minutes) sample collected beyond the defined time interval. The range was from 5:29:09 to 6:39:16 minutes. I agree with the Sponsor that these are considered minor and have no impact on the study. Based on the results of the neutralization validation, 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.

Product application deviations: Section 5.1.2.2 of the study protocol states "The treatment materials will be applied, and the sampling configurations will be performed per the Randomization Schedule (Appendix 14.4)". For subject (b) (6) combination #8 (product Medline 2% CHG cloth on left side and product Dyna-Hex 2® on right side) was assigned, but on the groin left side the product Medline placebo solution cloth was applied. The deviation was due to technical execution error. The subject (b) (6) was replaced on groin by subject (b) (6). Therefore, the Sponsor concludes that there was no adverse impact on the study outcome.

Reviewer's comments: As mention above, subject (b) (6) combination #8 (product Medline 2% CHG cloth on left side and product Dyna-Hex 2® on right side) was assigned, but on the groin left side the product Medline placebo solution cloth was applied. Since this was considered a technical error, subject (b) (6) was replaced by subject (b) (6) I agree with the Sponsor that there was no adverse impact on the study outcome.

Product application deviation: Section 5.1.2.2 of study protocol states "The treatment materials will be applied, and the sampling configurations will be performed per the Randomization Schedule (Appendix 14.4)". For subjects (b) (6) (b) (6) and (b) (6) the sampling sites provided by the study protocol are site 1 for Baseline, site 2 for 10 minutes, site 3 for 6 hours, site 4 for 8 hours. However, the sampling was performed as follows: Baseline sample from site 2, 10 minutes sample from site 3, 6 hours sample from site 4, 8 hours sample from site 1. The deviation was due to a technical error on creating the randomizations. These subjects were replaced by subjects (b) (6), (b) (6) and (b) (6). Therefore, the Sponsor concludes that there was no adverse impact on the study outcome.

Reviewer's comments: As mention above, for subjects (b) (6), (b) (6) and (b) (6) the sampling sites provided by the study protocol are site 1 for Baseline, site 2 for 10 minutes, site 3 for 6 hours, site 4 for 8 hours. However, the sampling was performed as follows: Baseline sample from site 2, 10 minutes sample from site 3, 6 hours sample from site 4, 8 hours sample from site 1. Since this was considered a technical error, these subjects were replaced by subjects (b) (6), (b) (6), and (b) (6). I agree with the Sponsor that there was no adverse impact on the study outcome.

Bacterial counting entry data deviation: Section 5.2.3 of the study protocol states "Raw colony counts from each dilution will be recorded on the appropriate CRFs for each subject". For the groin right, 8-hour sample from subject (b) (6) the raw colony count was recorded in the CRF only for the first dilution (10^0) and the rest of the plates were disposed without recording the counts. The deviation was due to microbiologist entry data error. The average CFU/cm^2 was calculated using the counts from the first dilution. Therefore, the Sponsor concludes that there was no adverse impact on the study outcome.

Reviewer's comments: ***As mentioned above, for subject*** (b) (6) ***the raw colony count was recorded in the CRF only for the first dilution (10^0) and the rest of the plates were disposed without recording the counts. The Sponsor stated that this was due to data entry error and the average CFU/cm^2 was calculated using the counts from the first dilution. This is acceptable. I agree with the Sponsor that there was no adverse impact on the study outcome.***

4.1.3 Neutralization Validation for Study R13-053 (MicroBioTest) and Study R15-029 (Evic Romania)

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 9-subject sub study prior to the start of the main study, the ability of the sampling solution (SS) to completely neutralize the active ingredients contained in Medline CHG Cloth and Dyna-Hex 2® 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 48 hours prior to sampling, and subjects needed to avoid topical and systemic antimicrobials for only 7 days prior to the sub study Treatment Day. Subjects received all three study products (Medline CHG Cloth, Dyna-Hex 2®, and Vehicle Cloth control), which were applied to the abdomen regions using bilateral applications so that six applicators are performed for each treatment using bilateral application (a total of nine subjects). Microbial sampling of each of the sites was performed, then one sample form each test area was inoculated with methicillin-resistant *Staphylococcus epidermidis* (MRSE), ATCC 51625 and one 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.

(b) (4)

(b) (4)

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 SS was considered effective in neutralizing the active ingredients if the mean $\log_{10}$ CFU/mL of the sample was not more than 0.2 $\log_{10}$ less than the mean $\log_{10}$ CFU/mL of the numbers control at each time point. The SS was considered nontoxic if the mean $\log_{10}$ CFU/mL of the toxicity control was not more than 0.2 $\log_{10}$ less than the mean $\log_{10}$ CFU/mL of the numbers control at each time point.

Table 49. Results of the Neutralization Validation.

Test Article Control
Medline CHG Cloth
Results Expressed as Log_{10} CFU/mL

Time	**Test 3	***Test 4	Difference from Test 3
MSSE			
<1 minute	*1.68	*0.00	1.68
30 minutes	*1.66	*0.00	1.66
MRSE			
<1 minute	*1.82	*0.00	1.82
30 minutes	*1.81	*0.00	1.81

* All results are average of Replicate 1, 2 and 3
** Test Microorganism Viability Control
*** Test Article Control

Test Article Control
Placebo
Results Expressed as Log_{10} CFU/mL

Time	**Test 3	***Test 4	Difference from Test 3
MSSE			
<1 minute	*1.68	*0.00	1.68
30 minutes	*1.66	*0.00	1.66

Test Article Control
Dyna-Hex 2
Results Expressed as Log_{10} CFU/mL

Time	**Test 3	***Test 4	Difference from Test 3
MSSE			
<1 minute	*1.68	*0.00	1.68
30 minutes	*1.66	*0.00	1.66
MRSE			
<1 minute	*1.82	*0.00	1.82
30 minutes	*1.81	*0.00	1.81

* All results are average of Replicate 1, 2 and 3
** Test Microorganism Viability Control
*** Test Article Control

Neutralizer Toxicity Control
Results Expressed as Log_{10} CFU/mL
MSSE

Time	**Test 3	***Test 2	Difference from Test 3
<1 minute	*1.68	*1.60	0.08
30 minutes	*1.66	*1.62	0.04

* All results are average of Replicate 1, 2 and 3
** Test Microorganism Viability Control
*** Neutralizer Toxicity Control

Neutralizer Toxicity Control
Results Expressed as Log_{10} CFU/mL
MRSE

Time	**Test 3	***Test 2	Difference from Test 3
<1 minute	*1.82	*1.81	0.01
30 minutes	*1.81	*1.79	0.02

* All results are average of Replicate 1, 2 and 3
** Test Microorganism Viability Control
*** Neutralizer Toxicity Control

Neutralizer Effectiveness
Medline 2% CHG cloth
Results Expressed as Log_{10} CFU/mL
MSSE

Randomization No.	Time	**Test 3	***Test 1	Difference from Test 3
01	<1 minute	*1.68	1.56	0.12
	30 minutes	*1.66	1.56	0.10
02	<1 minute	*1.68	1.54	0.14
	30 minutes	*1.66	1.57	0.09
04	<1 minute	*1.68	1.54	0.14
	30 minutes	*1.66	1.51	0.15
05	<1 minute	*1.68	1.54	0.14
	30 minutes	*1.66	1.54	0.12
07	<1 minute	*1.68	1.59	0.09
	30 minutes	*1.66	1.52	0.14
08	<1 minute	*1.68	1.54	0.14
	30 minutes	*1.66	1.57	0.09

* All results are average of Replicate 1, 2 and 3
** Test Microorganism Viability Control
*** Neutralizer Effectiveness

Neutralizer Effectiveness
Medline placebo solution cloth
Results Expressed as Log_{10} CFU/mL
MSSE

Randomization No.	Time	**Test 3	***Test 1	Difference from Test 3
01	<1 minute	*1.68	1.51	0.17
	30 minutes	*1.66	1.49	0.17
03	<1 minute	*1.68	1.60	0.08
	30 minutes	*1.66	1.59	0.07
04	<1 minute	*1.68	1.54	0.14
	30 minutes	*1.66	1.52	0.14
06	<1 minute	*1.68	1.54	0.14
	30 minutes	*1.66	1.54	0.12
07	<1 minute	*1.68	1.56	0.12
	30 minutes	*1.66	1.56	0.10
09	<1 minute	*1.68	1.56	0.12
	30 minutes	*1.66	1.57	0.09

* All results are average of Replicate 1, 2 and 3
** Test Microorganism Viability Control
*** Neutralizer Effectiveness

Neutralizer Effectiveness
Dyna-Hex 2
Results Expressed as Log_{10} CFU/mL
MSSE

Randomization No.	Time	**Test 3	***Test 1	Difference from Test 3
02	<1 minute	*1.68	1.54	0.14
	30 minutes	*1.66	1.52	0.14
03	<1 minute	*1.68	1.59	0.09
	30 minutes	*1.66	1.57	0.09
05	<1 minute	*1.68	1.56	0.12
	30 minutes	*1.66	1.56	0.10
06	<1 minute	*1.68	1.56	0.12
	30 minutes	*1.66	1.51	0.15
08	<1 minute	*1.68	1.59	0.09
	30 minutes	*1.66	1.56	0.10
09	<1 minute	*1.68	1.54	0.14
	30 minutes	*1.66	1.57	0.09

* All results are average of Replicate 1, 2 and 3
** Test Microorganism Viability Control
*** Neutralizer Effectiveness

Reviewer's comments: ***Table 49 represents the neutralization validation for study R13-053 (MicroBioTest) using Staphylococcus epidermidis (MSSE), ATCC 51625. The results for Staphylococcus epidermidis (MRSE), ATCC 12228 is presented in Appendix 16.4. The neutralization validation results for study R15-029 (Evic Romania) is presented in Appendix 16.4. For both studies, since the mean log_{10} CFU/mL of each of the active study products was not more than 0.2 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 0.2 log_{10} less than the mean log_{10} CFU/mL of Numbers Control, the sampling solution was considered nontoxic. Overall, this reviewer finds the neutralization validation studies for both study R13-053 (MicroBioTest) and R15-029 (Evic Romania) acceptable.***

4.4 Pilot Studies

4.4.1 Pilot Trial Assessment of the Antimicrobial Efficacy of Medline 2% CHG Cloth Preoperative Skin Preparation (R13-042: MicroBioTest and R14-015: BioScience)

The two pilot studies are reported together because they have similar designs and objectives and because the statistical report was conducted for both studies together. The primary objectives of both pilot studies were to measure the safety and antimicrobial properties of the Medline 2% CHG cloth to be used in the pivotal studies, to determine if the label claim could be extended to 8 hours, and to determine if there was a difference in efficacy based on length of application time.

Study R13-042 was performed at MicroBioTest in Sterling, Virginia, and Study R14-015 was performed at BioScience Laboratories in Bozeman, Montana. These studies were performed according design listed in the sections above, except that Medline 2% CHG cloth application times varied. In Study R13-042, Medline 2% CHG cloth was applied for 1, 2, or 3 minutes; in Study R14-015, Medline 2% CHG cloth was applied for either 1 or 2 minutes. In addition, Study R14-015 examined the use of automated microbiological dilution and plating techniques instead of traditional dilution and plating methods.

For both studies, the planned size for each treatment group was 12 subjects. Thirty-four (34) subjects were enrolled into Study R13-042. Twenty-seven (27) were treated on groin and abdomen sites and twenty-four (24) of these subjects who achieved required baseline levels on day of treatment were included in the efficacy analysis. Sixty-seven (67) subjects were enrolled into the second pilot Study R14-015. Thirty-three (33) were treated on abdomen and groin regions and twenty-four (24) subjects achieved baseline levels on day of treatment which were included in the efficacy analysis. The demographic information of subjects receiving treatments in both pilot studies are presented in the table below.

Table 50. Subject Demographics for Studies R13-042 and R14-015.

Demographic Category		Study R13-042	Study R14-015
Age (Years)	Mean	36	50
	Minimum	17	19
	Maximum	72	74
Sex [Number of Subjects (%)]	Males	18 (53%)	21 (64%)
	Females	16 (47%)	12 (36%)
Race [Number of Subjects (%)]	White/Caucasian	10 (29%)	30 (91%)
	Black/African American	2 (6%)	0 (0%)
	Hispanic	0 (0%)	1 (3%)
	Asian	18 (53%)	1 (3%)
	Hawaiian Islander	4 (12%)	0 (0%)
	Other	0 (0%)	1 (3%)

The Sponsor stated that due to treatment day baseline failures and replacements, the actual number of treatment applications was higher in some cases. The baseline bacterial count requirements for this study were 1.3 x 10^3 CFU/cm^2 for the abdomen and 1.0 x 10^5 CFU/cm^2 for the groin. The treatments and actual group sizes are presented in the table below.

Table 51. Treatments and Number of Applications for Studies R13-042 and R14-015.

Treatment	Number of Applications (R13-042 / R14-015)	
	Abdomen	Groin
Medline 2% CHG cloth for 1 minute	12 / 14	12 / 15
Medline 2% CHG cloth for 2 minutes	12 / 15	12 / 15
Medline 2% CHG cloth for 3 minutes	12 / NA	12 / NA
Dyna-Hex 2	12 / 14	12 / 14

This study calculated responder rates and log_{10} CFU/cm^2 changes from baseline, but confidence intervals for these values were not calculated. Instead, both studies were included in a mixed ANOVA statistical model with subject being considered a random variable and study site, group (product plus application time), and sample time considered fixed variables. Two-variable interactions were considered and included in the final model if they were both significant and improved the results. The primary result of this analysis was a determination of which effects were significant; α of 0.05 was considered significant. Tests using the same model were made to determine the differences between application times and the 6- and 8-hour time points. The log_{10} CFU/cm^2 reductions from baseline and the corresponding responder rate results are summarized in the tables below.

Table 52. Log_{10} CFU/cm^2 Changes from Baseline for Studies R13-042 and R14-015.

Site	Product	Application Time	Study	Comparing Baseline To:		
				10 minutes	6 Hours	8 Hours
				Mean Log_{10} CFU Reduction (std. dev.)		
Abdomen	Medline 2% CHG cloth	1 minute	R13-042	2.65 (0.73)	2.03 (0.86)	1.95 (0.84)
			R14-015	1.95 (1.57)	2.59 (1.25)	2.59 (0.98)
		2 minutes	R13-042	2.93 (0.77)	2.55 (0.95)	2.51 (1.12)
			R14-015	2.02 (1.48)	2.46 (1.37)	2.20 (1.57)
		3 minutes	R13-042	3.11 (0.64)	2.46 (0.87)	2.00 (0.82)
	Dyna-Hex 2	2 minutes, twice	R13-042	2.61 (0.76)	1.91 (0.64)	1.58 (0.95)
			R14-015	1.29 (1.84)	1.71 (1.61)	1.39 (1.17)
Groin	Medline 2% CHG cloth	1 minute	R13-042	3.44 (0.94)	2.70 (0.67)	2.98 (0.38)
			R14-015	1.91 (0.64)	2.31 (0.67)	2.48 (0.91)
		2 minutes	R13-042	4.31 (1.43)	2.85 (1.13)	3.05 (0.83)
			R14-015	2.39 (1.14)	2.82 (1.24)	2.67 (0.83)
		3 minutes	R13-042	4.41 (1.23)	3.45 (0.95)	3.21 (0.80)
	Dyna-Hex 2	2 minutes, twice	R13-042	3.14 (0.79)	2.66 (1.09)	2.52 (0.94)
			R14-015	2.07 (1.07)	1.88 (0.80)	2.16 (1.14)

Table 53. Responder Rates for Studies R13-042 and R14-015.

Site	Product	Application Time	Study	Comparing Baseline To:		
				10 minutes	6 Hours	8 Hours
				Responder Rate % (proportion)		
Abdomen	Medline 2% CHG cloth	1 minute	R13-042	0.75 (9/12)	1.00 (12/12)	1.00 (12/12)
			R14-015	0.64 (8/14)	0.93 (14/15)	1.00 (15/15)
		2 minutes	R13-042	0.92 (11/12)	1.00 (12/12)	1.00 (12/12)
			R14-015	0.47 (7/15)	0.93 (14/15)	0.87 (13/15)
		3 minutes	R13-042	0.92 (11/12)	1.00 (12/12)	1.00 (12/12)
	Dyna-Hex 2	2 minutes, twice	R13-042	0.75 (9/12)	1.00 (12/12)	1.00 (12/12)
			R14-015	0.50 (7/14)	0.93 (13/14)	0.92 (12/13)
Groin	Medline 2% CHG cloth	1 minute	R13-042	0.75 (9/12)	1.00 (12/12)	1.00 (12/12)
			R14-015	0.00 (0/15)	1.00 (12/12)	1.00 (15/15)
		2 minutes	R13-042	0.75 (9/12)	1.00 (12/12)	1.00 (12/12)
			R14-015	0.20 (3/15)	1.00 (14/14)	1.00 (15/15)
		3 minutes	R13-042	0.92 (11/12)	1.00 (12/12)	1.00 (12/12)
	Dyna-Hex 2	2 minutes, twice	R13-042	0.75 (9/12)	1.00 (12/12)	1.00 (12/12)
			R14-015	0.14 (2/14)	0.93 (13/14)	1.00 (14/14)

The Sponsor stated that the significance differences found for site and sample time were expected and help confirm the model, see table below. The difference in studies indicates that the studies did produce significantly different results. The significance found for the Study Sample Time and Study Site interactions means that not only were the overall study results different, but also the pattern of results over time was different between studies, and the pattern of abdominal and groin results was different between studies. The Sponsor concluded that, taken together, this means that the differences in labs were significant.

The Sponsor stated that a review of differences in application times using the same model indicated that there was a significant difference between 1- and 2-minute application times (0.2931 higher $\log_{10}$ CFU/cm^2 counts at 1 minute, P = 0.0027) but there was not a significant difference between 2- and 3-minute application times (0.09459 higher $\log_{10}$ CFU/cm^2 counts for 3 minutes, P = 0.4809). The comparison between 1 and 3 minutes was not performed. The Sponsor also stated that the differences between 6 and 8 hours using the same model indicated that there was no significant difference between them: the estimated mean difference in CFU/cm^2 counts was 0.07501, which was not statistically significant (P = 0.4198). The Sponsor concluded that, based on the results obtained from these two pilot studies, the 3-minute application time data from Study R13-042 were selected for sample size calculations for pivotal efficacy studies.

Table 54. Significant Factors in the Statistical Model.

Factor	Significance (P > F)
Study (R13-042 or R14-015)	0.0131
Site (abdomen or groin)	<0.0001
Application Time (1, 2, or 3 minutes, N/A for Dyna-Hex 2)	<0.0001
Sample Time (baseline, 10 minutes, 6 hours, or 8 hours)	<0.0001
Study * Sample Time interaction	<0.0001
Study * Site interaction	0.0069
Site * Sample Time interaction	0.0004

4.4.2 Pilot Trial III Assessment of the Antimicrobial Efficacy of Medline 2% CHG Cloth Preoperative Skin Preparation (R15-028: Evic Romania)

The Sponsor stated that due to slow subject enrollment into Study R13-052 (BioScience), Study R15-028 was designed as a pilot study to determine if a pivotal study could be placed at the Evic Romania site located in Bucharest, Romania to evaluate the site's compliance, safety, and study conduct. The study was performed according to the general design as described above. In addition to evaluating the potential to conduct a pivotal study at Evic Romania, the study objective was to measure the safety and antimicrobial properties of a single investigational test product: Medline 2% CHG Cloth, in circumstances similar to the pilot trials at other sites. The study was conducted to confirm the 3-minute application of Medline 2% CHG cloth, Dyna-Hex 2® treatment, and microbiological techniques in a geographically distinct population.

A total of 15 subjects were enrolled into the study. Fourteen (14) were treated on abdomen and groin sites and 14 subjects achieved baseline levels on day of treatment

were included in the efficacy analysis. The demographic information of subjects receiving treatments is presented in the table below. The study population age ranged from 24-67 years of age with a mean age of 56 years. A higher percentage of females (79%) were enrolled. All subjects were Caucasian (100%).

Table 55. Study R15-028 Subject Demographics for Study.

Demographic Category		
Age (Years)	Mean	56
	Minimum	24
	Maximum	67
Sex [Number of Subjects (%)]	Males	3 (21%)
	Females	11 (79%)
Race [Number of Subjects (%)]	White/Caucasian	14 (100%)
	Black/ African American	0 (0%)
	Hispanic	0 (0%)
	Asian	0 (0%)
	Other	0 (0%)

The Sponsor reported responder rates (2 $\log_{10}$ and 3 $\log_{10}$ reductions on abdomen and groin, respectively, within 10 minutes, 6 hours, and 8 hours of application) are described in the table below. The Medline 2% CHG cloth treatment (3-minute application time) was effective in achieving responder rates of 70% or greater at all time points for abdomen regions. The responder rate for the groin region at 10 minutes was 64.3% at 10 minutes. Dyna-Hex 2® was not effective on either the abdomen or groin regions (66.7% and 50% respectively) at 10 minutes. Responder rates of 70% were achieved for both abdomen and groin regions 6 and 8 hours after application.

Table 56. Responder Rates for Study R15-028.

Site	**Treatment**	**Responder Rate**		
		10 Minute (95% Exact CI)	**6 Hour (95% Exact CI)**	**8 Hour (95% Exact CI)**
Abdomen	Medline 2% CHG cloth	0.8333 (0.5159 to 0.9791)	1.0000 (0.7354 to 1.0000)	1.0000 (0.7354 to 1.0000)
	Dyna-Hex 2	0.6667 (0.3489 to 0.9008)	1.0000 (0.7354 to 1.0000)	1.0000 (0.7354 to 1.0000)
Groin	Medline 2% CHG cloth	0.6429 (0.3514 to 0.8724)	1.0000 (0.7684 to 1.0000)	1.0000 (0.7684 to 1.0000)
	Dyna-Hex 2	0.5000 (0.2304 to 0.7696)	1.0000 (0.7684 to 1.0000)	1.0000 (0.7684 to 1.0000)

The Sponsor noted that because responder rates are highly variable in low-group-count studies, the $\log_{10}$ CFU/cm^2 reductions from baseline were also examined in the table below. Medline 2% CHG cloth treatment, using a 3-minute vigorous rub, achieved consistent $\log_{10}$ reductions within the required 1994 TFM guidelines at 10 minutes, 6 hours, and 8 hours. The comparator, Dyna-Hex 2®, was slightly less effective but was also able to achieve comparable 2 $\log_{10}$ and 3 $\log_{10}$ reductions in abdomen and groin areas, respectively.

Table 57. Log_{10} CFU/cm^2 Reductions from Baseline for Study R15-028.

Site	Treatment	Log_{10} CFU/cm^2 Reduction from Baseline		
		10 Minute (95% Exact CI)	6 Hour (95% Exact CI)	8 Hour (95% Exact CI)
Abdomen	Medline 2% CHG cloth	2.9038 (2.1431 to 3.6645)	2.8694 (2.0823 to 3.6566)	2.8847 (2.1545 to 3.6149)
	Dyna-Hex 2	2.2995 (1.5388 to 3.0602)	2.4802 (1.6931 to 3.2674)	2.2764 (1.5462 to 3.0066)
Groin	Medline 2% CHG cloth	3.6719 (2.9676 to 4.3762)	3.9763 3.2478 to 4.7047)	4.6384 (3.9657 to 5.3112)
	Dyna-Hex 2	3.0457 (2.3414 to 3.7499)	3.3365 (2.6081 to 4.0650)	3.3416 (2.6689 to 4.0144)

The Sponsor concluded that Medline 2% CHG cloth met all the responder rate efficacy endpoints except for the endpoint for the groin at 10 minutes. Dyna-hex 2® did not meet the 10-minute endpoints but did meet the 6-hour and 8-hour endpoints. The log_{10} CFU/cm^2 reductions from baseline and their confidence intervals were sufficient to indicate that a pivotal study with similar results should be able to meet the efficacy requirements. Therefore, the pivotal study was initiated at the Evic Romania study site.

5. AREA COVERAGE AND DRYING TIME

5.1.1 Evaluation of the Area Covered by Medline 2% Chlorhexidine Gluconate Cloth Preoperative Skin Preparation (R16-034)

This study was designed to assess the dosage of the coverage area of Medline 2% CHG cloth was well as the drying time. Safety was also assessed for all subjects who signed the informed consent. This was an open-label design, controlled study in 30 healthy volunteers using appropriate inclusion and exclusion criteria and each subject received the single study treatment: Medline 2% CHG cloth. Subjects not meeting inclusion and exclusion criteria were excluded from the study. The treatment application instructions were:

1. Weigh the unopened cloth package using a calibrated balance and record the weight in grams.
2. Open the package and remove a single cloth.
3. Using the single cloth, vigorously scrub skin in a back and forth motion for 3 minutes completely wetting the treatment area (7” x 10” area). Approximately halfway through the 3-minute application, the cloth will be turned over. If necessary, the subject’s skin will be held taut to ensure that the maximum amount of the cloth contacts the area being prepped. Note: product handling, when opening the packaging, a single cloth (single unit) will be used. Contact between the cloth and the outside of the packaging will be avoided to reduce risk of cloth contamination.
4. At the completion of the 3-minute application, start a calibrated stopwatch to initiate the drying time. Perform drying time observations in accordance with the protocol.
5. Return the used cloth to the original opened packaging. Reweigh using a calibrated balance and record the weight in grams.

Drying times were recorded by three different technicians, independently. The amount of product applied was determined by subtracting the final weight of the cloth plus packaging, from the initial weight. Skin irritation was determined upon screening, pre-treatment, and post treatment. The mean drying time (in seconds), the amount of product used (in grams), dose per area (gram of product per cm^2 covered), and the coverage (cm^2 covered per gram of product) were analyzed numerically. The results are shown in the table below; the column labeled difference is later referred to as dose. Dose per area was calculated as dose / 451.612 cm^2; coverage was calculated as 1 / (dose per area).

Table 58. Reported Drying Time and Coverage Results.

Subject Number (b) (6)	Drying Times (seconds)				Product Weights (g)			Dose per Area (g/cm^2)	Coverage (cm^2/g)
	Tech 1	Tech 2	Tech 3	Mean	Pre-Tmt.	Post-Tmt.	Difference		
	107	108	103	106	78.24	74.40	3.84	0.0085	118
	65	66	63	65	77.41	73.60	3.81	0.0084	119
	83	83	85	84	77.06	73.41	3.65	0.0081	124
	87	86	86	86	78.28	73.65	4.63	0.0103	98
	38	38	37	38	77.61	72.21	5.40	0.0120	84
	87	86	83	85	78.81	73.61	5.20	0.0115	87
	85	85	84	85	76.14	72.25	3.89	0.0086	116
	76	76	76	76	76.91	73.26	3.65	0.0081	124
	370	369	368	369	76.85	72.95	3.90	0.0086	116
	105	103	104	104	76.69	73.01	3.68	0.0081	123
	66	57	68	64	78.56	75.11	3.45	0.0076	131
	92	92	92	92	77.84	74.41	3.43	0.0076	132
	69	68	68	68	77.12	73.64	3.48	0.0077	130
	58	56	60	58	77.28	73.45	3.83	0.0085	118
	45	43	45	44	76.45	73.14	3.31	0.0073	136
	57	53	60	57	76.65	73.27	3.38	0.0075	134
	70	72	70	71	76.81	72.63	4.18	0.0093	108
	88	86	90	88	77.53	74.24	3.29	0.0073	137
	76	76	77	76	77.94	74.05	3.89	0.0086	116
	55	55	50	53	77.96	74.63	3.33	0.0074	136
	63	63	63	63	77.91	74.62	3.29	0.0073	137
	29	29	29	29	77.36	74.25	3.11	0.0069	145
	59	59	59	59	77.16	73.53	3.63	0.0080	124
	66	67	65	66	79.01	75.78	3.23	0.0072	140
	56	56	56	56	76.82	73.71	3.11	0.0069	145
	60	62	58	60	79.12	76.01	3.11	0.0069	145
	70	70	70	70	76.59	72.92	3.67	0.0081	123
	64	64	64	64	78.34	75.08	3.26	0.0072	139
	78	78	78	78	78.19	75.02	3.17	0.0070	142
	80	78	80	79	75.68	72.55	3.13	0.0069	144

The Sponsor provided four types of analyses. First, the individual technician results were examined to determine if their readings were consistent with each other. This examination was useful to demonstrate that the mean drying times were valid and usable for later analyses. Second, the relationships between the results were reviewed using graphs and some numerical analysis. Third, summary statistics were produced for the numerical results, and fourth, the numerical data were examined to determine which statistical distributions might be applicable to them.

The values that the technicians logged were examined to determine if they were consistent with each other. The calculated values are shown in Table 59 below:

Table 59. Technician Variability.

Subject Number	Drying Times (seconds)				
	Tech 1	Tech 2	Tech 3	Subject Mean	Subject CV (%)
(b) (6)	107	108	103	106.00	2.50%
	65	66	63	64.67	2.36%
	83	83	85	83.67	1.38%
	87	86	86	86.33	0.67%
	38	38	37	37.67	1.53%
	87	86	83	85.33	2.44%
	85	85	84	84.67	0.68%
	76	76	76	76.00	0.00%
	370	369	368	369.00	0.27%
	105	103	104	104.00	0.96%
	66	57	68	63.67	9.20%
	92	92	92	92.00	0.00%
	69	68	68	68.33	0.84%
	58	56	60	58.00	3.45%
	45	43	45	44.33	2.60%
	57	53	60	56.67	6.20%
	70	72	70	70.67	1.63%
	88	86	90	88.00	2.27%
	76	76	77	76.33	0.76%
	55	55	50	53.33	5.41%
	63	63	63	63.00	0.00%
	29	29	29	29.00	0.00%
	59	59	59	59.00	0.00%
	66	67	65	66.00	1.52%
	56	56	56	56.00	0.00%
	60	62	58	60.00	3.33%
	70	70	70	70.00	0.00%
	64	64	64	64.00	0.00%
	78	78	78	78.00	0.00%
	80	78	80	79.33	1.46%
Group Means:	**80.13**	**79.47**	**79.70**	**79.77**	**1.72%**

The Sponsor reported that the mean drying times for each technician across all subjects were less than 0.5 seconds different from the overall mean drying time. The coefficients of variation (mean divided by standard deviation, as a percent) were overall quite low. This indicated that the technician's measurements were highly consistent with each other: the mean drying times were not highly influenced by any one technician and could be used for further analyses.

From the graph below, the results showed that one drying time was over three times the value of any other drying time in the study. The Sponsor stated that this was the drying time for subject (b) (6) – the value was 369 seconds, while the closest other drying time was 106 seconds. This outlier was extreme enough that it would make the numerical results of drying time analyses suspect or invalid if it were included; therefore, the drying time from subject (b) (6) was excluded from further analyses. None of the other data from subject (b) (6) were clearly unusual, and the technicians performing the application did not notice anything about subject (b) (6) that was obviously different from the other subjects, but the other data from that subject were excluded from further analyses because the drying time itself was a clear outlier. If similar studies are conducted in the future, the Investigator and staff should be aware of the possibility of extreme drying time outliers and should investigate possible causes if they occur.

The Sponsor noted that there were some doses that were noticeably outside the main cluster of doses, but there were three to four of them and they were somewhat spread.

Table 60. Summary Statistics from Study R16-034.

Statistic	Drying Time (seconds)	Dose (g)	Dose per Area (g/cm^2)	Coverage (cm^2/g)
Mean	70	3.66	0.0081	126
Standard Deviation	18	0.58	0.0013	16
Median	68	3.48	0.0077	130
Minimum	29	3.11	0.0069	84
Maximum	106	5.40	0.0120	145
Skew	-0.07	1.80	1.80	-1.09
Kurtosis	0.15	3.26	3.26	0.98

***Reviewer's comments*: The area coverage results for the Medline 2% CHG cloth was 3.66 g / 0.0081 g/cm^2 = 451 cm^2. The average coverage in square inches is 70 in^2 (10 x 7 inches). The labeling coverage area for the dry site (i.e., abdomen) states "use one cloth to cleanse each 161 cm^2 area (approximately 5 x 5 inches) of skin to be prepared." and for moist site (i.e., groin) states "use one cloth to cleanse each 65 cm^2 area (approximately 2 x 5 inches) of skin to be prepared." In addition, the labeling for the Medline 2% CHG cloth also state "After package has been opened discard any unused cloths." The coverage area study for the Medline 2% CHG cloth is acceptable.**

The Medline 2% CHG cloth was considered dried on the average of 1.10 minutes (70 seconds). Excluding one subject who had a dry time average of 6.15 minutes (369 seconds). The Sponsor stated that this outlier was considered extreme enough that it would make the numerical results of the drying time analyses suspect or invalid if it were included; therefore, the drying time from this subject was excluded from further analyses. The drying time on the label states "Allow area to dry for one (1) minute." Since the active ingredient is only chlorhexidine gluconate (does not include an alcohol combination), flammability labeling is not required. The drying time of the one minute is acceptable for the Medline 2% CHG cloth labeling.

6. REFERENCES

1. FDA safety alert, 2016, Sage Products Expands Voluntary Worldwide Recall of Specific Lots of Topical Skin Products Due to Potential Microbial Contamination – Second Expansion, available at https://www.fda.gov/safety/recalls/ucm517547.htm

2. FDA inactive ingredient database, available at https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm?event=BasicSearch.page

3. Woodcock, PM, 1988, Chapter 2, Biguanides as Industrial Biocides, In: Payne KR, ed., Industrial Biocides, Chichester: John Wiley & Sons; p. 19-36.

4. Denton, GW, 2001, Chlorhexidine. In: Block SS, ed., Disinfection, Sterilization, and Preservation, 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; p. 321-36.

5. McDonnell, G and AD Russell, 2001, Antiseptics and Disinfectants: Activity, Action, and Resistance, Clinical Microbiology Review, 12(1):147-79.

6. Fitzgerald, KA, A Davies, and AD Russell, 1989, Uptake of ^{14}C-Chlorhexidine diacetate to *Escherichia coli* and *Pseudomonas aeruginosa* and its Release by Azolectin, FEMS Microbiology Letters, 51:327-32.

7. Hiom, SJ, JR Furr, AD Russell, and JR Dickinson, 1992, Effects of Chlorhexidine Diacetate on *Candida albicans*, *C. glabrata* and *Saccharomyces cerevisiae*, Journal of Applied Bacteriology, 72:335-340.

8. Russell, AD, D Berys, and P Milburn, 1985, Reversal of the Inhibition of Bacterial Spore Germination and Outgrowth by Antibacterial Agents, International Journal of Pharmacology, 25:105-12.

9. Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Tentative Final Monograph for Health Care Antiseptic Drug Products; Proposed Rule, 1994, In: Federal Register, 59 FR 31402 at 31441-31452.

10. Littlejohn, TG, IT Paulsen, MT Gillespie, JM Tennent, M Midgley, IG Jones, AS Purewal and RA Skurray, 1992, Substrate Specificity and Energetics of Antiseptic and Disinfectant Resistance in *Staphylococcus aureus*, FEMS Microbiology Letters, 95:259-265.

11. Prince, HN, WS Nonemaker, RC Norgard, and DL Prince, 1978, Drug Resistance Studies with Topical Antiseptics, Journal of Pharmacology Science, 67:1629-1631.

12. Uyeda, M, A Miura, M Shibata, and T Hitoshi, 1981, In vitro Isolation of Chlorhexidine-Resistant Strain from *Serratia marcescens* and its Countermeasure, Chemotherapy (Tokyo), 29:733-737.

13. Thomas, L, JY Maillard, RJ Lambert, and AD Russell, 2000, Development of Resistance to Chlorhexidine Diacetate in *Pseudomonas aeruginosa* and the Effect of a “Residual” Concentration, Journal of Hospital Infection, 46:297-303.

14. Michel, G, J Olle, and C Lalaurette, 1979, Emergence of Resistance to Chlorhexidine and to Povidone-Iodine Studied In Vitro, Microbia, 5:15-26.

15. Davies, GE, J Francis, AR Martin, FL Rose, and G Swain, 1954, 1:6-Di-4'-Chlorophenyldiguanidohexane (Hibitane); Laboratory Investigation of a New Antimicrobial Agent of High Potency, British Journal of Pharmacology, 9:192-196.

16. Stickler, DJ, 1974, Chlorhexidine Resistance in *Proteus mirabilis*, Journal of Clinical Pathology, 27:284-287.

17. Stickler, DJ, B Thomas, CL Clayton, and JC Chawla, 1983, Studies on the Genetic Basis of Chlorhexidine Resistance, British Journal of Clinical Practice, 25:23-30.

18. Kahan, A, A Philippon, G Paul, S Weber, C Richard, G Hazebroucq, and M Degeorges, 1983, Nosocomial Infections by Chlorhexidine Solution Contaminated with *Pseudomonas pickettii* (Biovar VA-I), Journal of Infection, 7:256-263.

19. Gilbert, P, DG Allison, and AJ McBain, 2002, Biofilms In vitro and In vivo: Do Singular Mechanisms Imply Cross-Resistance? Journal of Applied Microbiology, Symposium Supplement (92):98S-110S.

20. Rutala, WA, DJ Weber, and Healthcare Infection Control Practices Advisory Committee (HICPAC), 2008, Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008, available at https://www.cdc.gov/infectioncontrol/pdf/guidelines/disinfection-guidelines.pdf

21. Russell, AD and I Chopra, 1996, Chapter 6, Genetic and Biochemical Basis of Resistance to Antiseptics, Disinfectants and Preservatives, In Understanding Antibacterial Action and Resistance, 2nd ed. London: Ellis Horwood; 207-242.

22. Brooks, SE, MA Walczak, R Hameed, and P Coonan, 2002, Chlorhexidine Resistance in Antibiotic-Resistant Bacteria Isolated from the Surfaces of Dispensers of Soap Containing Chlorhexidine, Infection Control of Hospital Epidemiology, 23:692-695.

23. Martin, TDM, 1968, Sensitivity of the Genus Proteus to Chlorhexidine, Journal of Medical Microbiology, 2:101-108.

24. Simpson, RA, PM Hawkey, and PM Woodcock, 1989, Antibiotic Resistance and Chlorhexidine Susceptibility of Gram-Negative Bacteria in the Hospital and Community, Paper presented at: American Society for Microbiology Conference, September 17-20, 1989; Houston, TX. In: Programs and Abstracts of the Twenty-Ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington DC: American Society for Microbiology:212.

25. Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Tentative Final Monograph for Health Care Antiseptic Drug Products; Proposed Rule, 1994, In: Federal Register, 59 FR 31402 at 31441-31452.

26. Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record; Proposed Rule, 2015, In: Federal Register, 80 FR 25166-25205.

27. FDA, Transcript for the September 3, 2014 Meeting of the Nonprescription Drugs Advisory Committee (NDAC), 2014, Silver Spring, MD.

28. Tambe, SM, L Sampath, and SM Modak, 2001, In vitro Evaluation of the Risk of Developing Bacterial Resistance to Antiseptics and Antibiotics Used in Medical Devices, Journal Antimicrobial Chemotherapy, 47:589-598.

29. Knapp, L, A Amezquita, P McClure, S Stewart, and JY Maillard, 2015, Development of a Protocol for Predicting Bacterial Resistance to Microbicides," Applied Environmental Microbiology, 81:2652-2659.

30. Soma, VL, X Qin, C Zhou, A Adler, JE Berry, and DM Zerr, 2012, The Effects of Daily Chorhexidine Bathing on Cutaneous Bacterial Isolates: A Pilot Study, Infectious Drug Resistance, 5:75-78.

31. Denny, J and CL Murno, 2017, Chlorhexidine Bathing Effects on Health-Care-Associated Infections, Biological Research for Nursing, 19:123-136.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

MICHELLE M JACKSON
10/16/2018

FRANCISCO MARTINEZ-MURILLO
10/16/2018

CLINICAL MICROBIOLOGY REVIEW

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Nonprescription Drug Products, ODE IV

NDA 207964

Sponsor Package Submission: January 27, 2017
Received by CDER White Oak: January 27, 2017
Received by Reviewer: January 27, 2017
Review Completed: February 14, 2017

REVIEWER: Michelle M. Jackson, PhD

TEAM LEADER: Francisco Martínez-Murillo, PhD

PROJECT MANAGER: Celia Peacock, MPH, RD, CAPT

NAME AND ADDRESS OF APPLICANT
Medline Industries, Inc.
One Medline Place
Mundelein, IL 60060

CONTACT PERSON
Bill Parthun
Director, Research & Development
(847) 643-3839

DRUG PRODUCT NAMES:
Proprietary Name: ReadyPrep™ CHG (2% Chlorhexidine Gluconate Cloth)
Established Name: 2% chlorhexidine gluconate

INDICATION: Patient Preoperative Skin Preparation

PHARMACOLOGICAL CATEGORY: Healthcare Antiseptic

DOSAGE FORM: Topical Solution

MATERIAL REVIEWED: 1) Meeting Package with Questions
2) Protocol: "Evaluation of the Area Covered by Medline 2% Chlorhexidine Gluconate Cloth Preoperative Skin Preparation"
3) Statistical Analysis for: Evaluation of the Area Covered by Medline 2% Chlorhexidine Gluconate Cloth Preoperative Skin Preparation

Executive Summary:
Medline Industries, Inc. (Medline) submitted a 505(b)(2) New Drug Application (NDA) on February 9, 2016 for ReadyPrep™ (chlorhexidine gluconate cloth, 2%). The indication for this drug product is patient preoperative skin preparation. The application was not sufficiently complete to permit a substantive review, and the FDA refused to file (RTF) the application on April 8, 2016. The Sponsor failed to address the following issues: the safety of the (b) (4) the Clinical Study Reports in module 5 of the Electronic Common Technical Document (eCTD) did not contain a subgroup analysis; and the application did not contain an appropriate patent certification as required under 21 CFR 314.50(i). Medline submitted a Type A meeting request on April 22, 2016. According to the meeting request, the meeting objective was to discuss the items determined by the Agency to be incomplete in the April 8, 2016 RTF letter. The meeting was held on May 23, 2016. On January 27, 2017, Medline submitted a type C written response only meeting request to discuss issues related to the refiling of their NDA.

Recommendations to the Sponsor:
We are only reviewing and responding to microbiology related questions: 2 and 8.

Question 2
We appreciate the response the Agency provided in the Type C meeting minutes on December 6th, 2016 regarding the skin coverage and drying study (Reference ID: 4023755, Question 5). In that response, the Agency encouraged Medline to submit the protocol for review prior to the conduct of the study. After receipt of the Refusal to File for NDA 207964 on April 8th, 2016, Medline had planned to run the skin coverage and drying study. Medline designed and conducted this study and it has recently been completed. We have provided the protocol and the summary of study results in the Type C meeting package (see appendix 2 for protocol and appendix 3 for summary of study results). We propose that the study that we conducted is adequate to address the Agency's issues. Does the Agency agree?

Response to Question 2
Yes, we agree with the skin coverage and drying time protocol "Evaluation of the Area Covered by Medline 2% Chlorhexidine Gluconate Cloth Preoperative Skin Preparation" and have no comments. However, the acceptability of the study results for the "Statistical Analysis for: Evaluation of the Area Covered by Medline 2% Chlorhexidine Gluconate Preoperative Skin Preparation" will be an NDA review issue once you resubmit/refile your NDA.

Question 8
Based on the establishment of the bridge through the conduct of the Comparative In Vitro Time-Kill study, we intend to rely on the following studies conducted with the old formulation of ReadyPrep™ CHG in the NDA:

i. Pivotal Studies: R13-053 and R15-029
ii. Sensitization/Irritation Study: R13-051
iii. Antimicrobial Resistance Study: R14-012

Does the Agency agree that with the establishment of the scientific bridge through the conduct of the Comparative In Vitro Time-Kill study, it will allow us to use these studies in the NDA and these studies do not need to be repeated with the new formulation?

Response to Question 8

Yes, we find this approach acceptable. We also recommend that you include a vehicle control (b) (4) *in the new formulation bridging study.*

Background

The Sponsor is preparing an NDA for ReadyPrep™ CHG, under Section 505(b)(2) of the FD&C Act. The proposed ReadyPrep™ CHG product, a 2% CHG cloth, is indicated for use as a preoperative skin preparation. The product is formulated as 2% CHG (equivalent to 500 mg of the active moiety, CHG, per cloth), an inactive excipient profile, and a polyester cloth. CHG is applied through a single application, consisting of a 3-minute vigorous rub followed by a 1-minute dry time, at the therapeutic site of action.

On February 9 2016, the Sponsor submitted a 505(b)(2) NDA for ReadyPrep™ CHG. However, in the Refuse-to-File letter dated April 8, 2016, among other issues, the Agency stated that the application was incomplete because it did not include (b) (4) In addition, in a Type A meeting on May 23, 2016 (meeting minutes in DARRTS), the Agency agreed that it would be acceptable to (b) (4) However, the Sponsor needs to bridge the new formulation (b) (4) to the clinical safety and efficacy data and to the quality data (i.e., stability data) supporting the previous product formulation. The Agency stated that the Sponsor will need to provide adequate scientific justification for how the new, to-be-marketed formulation can rely upon previously conducted studies supporting the prior product formulation. The Agency also stated that the Sponsor will need to provide all chemistry data on the new product formulation and demonstrate that (b) (4) does not affect stability. Lastly, the Sponsor will need to address whether such change has any effect on the microbiological profile and identify any issues related to biopharmaceutics.

Per agreement with the Agency during the Type A meeting discussion, the Sponsor plans to demonstrate the similarity in effectiveness of ReadyPrep™ CHG as an antimicrobial wipe between its proposed new product (b) (4) and the prior product (b) (4) to support the scientific bridge to the clinical safety and efficacy data and to the quality data supporting the prior formulation.

(b) (4)

(b) (4)

The Sponsor predicts that (b) (4) The new formulation and prior formulation will provide the same drug exposure at the site of action as both are formulations of a locally acting drug, CHG. The Sponsor submitted protocols to provide rationale for the scientific bridge to show the similarity in stability, antimicrobial activity and skin-penetration capability between the new formulation of ReadyPrep™ CHG and prior formulation. This reviewer will only address the protocol on skin coverage by Medline's new formulation of ReadyPrep™ CHG.

Reviewer's Comments on Protocol

Medline Protocol #R16-034: MicroBioTest Project No. 721-122 "Evaluation of the Area Covered by Medline 2% Chlorhexidine Gluconate Cloth Preoperative Skin Preparation"

Study Objective and Purpose

The Sponsor stated that the objective of this study is to assess the dosage of the coverage area of Medline 2% CHG cloth. Drying time will be assessed post-application and safety will also be assessed for all subjects who sign the informed consent.

> ***Reviewer's comment: On April 8, 2016, FDA sent a Refuse to File letter to the Sponsor for its NDA 207-964 submission that was submitted on February 9, 2016. We informed the Sponsor under the clinical microbiology section of the letter that it would need to provide drying time and skin coverage studies for the Medline 2% CHG Cloth. On December 6, 2016, FDA sent a Type C Written Response letter to the Sponsor in reference to the background package containing the Type C written response only meeting request that was sent on September 15, 2016.*** (b) (4) ***The Sponsor asked if the Agency agrees that this fulfills the request from Question 2 under section "Clinical Microbiology" in the Refuse to File letter dated April 8, 2016. We responded, in a written response only letter sent to the Sponsor on December 6, 2016, that this was acceptable. We encouraged the Sponsor to submit its protocol for our review and comment before conducting the study.***

Study Design

This study is an open-label study. The two primary endpoints of this study are coverage area dosage, calculated in grams per square centimeter, and the average dry time. This controlled study is not randomized and will not be blinded. Each subject will receive the single study treatment. To reduce application variability, a single technician will perform the study treatment application for all subjects. Subjects will be identified by their initials and a subject number.

- Subjects to be treated will be assigned numbers ranging from 0001 to a four digit number equal to the total number of test subjects needed to complete the study (30 subjects).

***Reviewer's comment**: Normally, randomization and blinding is not incorporated or not needed in the skin coverage and drying time study because only one test product is being used. This is acceptable. We have recommended in the past that the same person perform all applications in order to minimize differences in pressure applied during the process. The Sponsor stated that a single trained technician will apply all products. The Sponsor will be using 30 subjects per single cloth application. This is acceptable.*

The materials identified in the table below will be used in the study. Specific product identification codes and lot numbers will also be included on the form titled "Confirmation of Release and Receipt of Study Materials" at the time the clinical supplies are shipped to the study site.

Table 3.4: Study Materials

Study Arm	Name	Description	Lot No.	Exp.
Test Article	Medline 2% CHG cloth	(b) (4)	TBD	TBD

Medline Industries, Inc. will label, package and ship the study materials required for Medline 2% CHG cloth to the research facility. The following study supplies will be provided by the study site:

- Treatment Material Disposition forms
- Consent/Authorization forms, IRB-approved
- Case report forms
- Gloves, sterile
- Marking templates, 7"x10", sterile (for marking test sites)
- Non-toxic marking pen (Sharpie or equivalent)
- Rubber policemen, sterile
- Timers or stopwatches
- Surgical Clipper & clipper blades
- Balance, calibrated

***Reviewer's comment**: The materials proposed in this study are standard for skin coverage and drying time studies and are acceptable.*

The expected duration of this study for each subject is up to one week. Regarding the study termination, Medline Industries, Inc. or the Investigator has the right to discontinue the study at any time for medical and/or administrative reasons. As far as possible, this should occur after mutual consultation. The Investigator may discontinue individual subjects from the study at any time. Subjects may voluntarily withdraw from the study at any time without reason or consequence. The subject will be asked to report the reason for withdrawal. The Investigator will provide a written report on the appropriate case repost form (CRF) including the date and reason for discontinuance.

In order to implement a valid revocation of authorization, the subject or their representative must make the request in writing to MicroBioTest, 105 Carpenter Drive, Sterling, VA, 20164. The revocation cannot stop the use or disclosure of information that has been collected prior to the revocation, or is needed to ensure complete and accurate study results, and/or is required by law or government regulation (e.g., reporting adverse events, etc.). Revocation of an authorization may not be used to withhold normal medical care from the subject, but may [or will] make the subject ineligible to receive the study treatment or care.

> ***Reviewer's comment: The study termination or the investigator's right to discontinue the study at any time for medical and/or administrative reasons are standard for this study and are acceptable. This reviewer also defers to the Medical Officer's clinical assessment.***

Medline Industries, Inc. requires Investigators to maintain accountability and adequate inventory security of the study material at all times. The Investigator or designee will:

- complete the Confirmation of Release and Receipt of Study Materials form upon receipt of the shipment and maintain and account for inventory on the Study Material Disposition form.
- keep study materials in a secure storage area, accessible only to authorized individuals.
- dispense study material only to subjects properly enrolled into the study.
- return all unused study materials to Medline Industries, Inc. at the end of the study or dispose of unused study materials as agreed upon.

Protocol Amendments: The party initiating an amendment must confirm it clearly in writing using the Amendment/Administrative Revision form. It must be signed and dated by Medline Industries, Inc. and, in the case of a significant amendment, the Investigator. A significant amendment means one that affects the safety, rights or welfare of subjects, the scope of the investigation or the scientific quality of the study. Medline Industries, Inc. will submit significant protocol amendments to the Investigator for submission to the IRB. Medline Industries, Inc. will also notify the Investigator when a protocol amendment may be implemented.

> ***Reviewer's comment: The investigator's maintenance of accountability and adequate inventory security of the study materials are considered standard and acceptable. The protocol amendments are also considered standard and are acceptable. This reviewer also defers to the Medical Officer's clinical assessment.***

Protocol Deviations: Sponsor notifications are deviations that potentially affect 1) subject safety, rights or welfare, 2) data integrity or 3) compromise the statistical analysis of the study require immediate communication to Medline Industries, Inc. The Investigator must contact the Medline Industries, Inc. study Sponsor within 24 hours of occurrence via phone and email using the contact information listed on the title page of this document. A Protocol Deviation Form must be completed by the Investigator and include a description of the circumstances surrounding and the reason t=for the deviation, any actions taken, and whether or not the subject was allowed to continue in the study. A copy must be sent to the Medline Industries, Inc. sponsor representative within 24 hours of identifying the occurrence.

IRB Notification: Deviations which are made to protect the life or physical well-being of a subject in an emergency must be reported by the Investigator to the IRB as soon as possible, or no later than 5 working days after the investigative site learns of the occurrence.

Reviewer's comment: The protocol deviations are considered standard and are acceptable. This reviewer also defers to the Medical Officer's clinical assessment.

Subject Selection

Healthy male volunteers, at least 18 years of age, with no dermatological conditions or known history of sensitivity to chlorhexidine gluconate will be enrolled into this study. A sufficient number of volunteers will be enrolled to achieve the required number of study treatments (30). Subjects must satisfy all Inclusion/Exclusion Criteria prior to study treatment. Subjects will be identified by their initials and subject number. All volunteers will be given verbal and written information about the study procedures. The following Inclusion/Exclusion Criteria will be reviewed on Treatment Day to establish eligibility for participation.

Reviewer's comment: The Sponsor used only male volunteers for the skin coverage and drying time study. We have had similar experience with other sponsors using all male subjects in the skin coverage and drying time study due to the fact that the subjects will need to remove their shirt and lay on the table in a prone position (on their stomach) for at least 30 minutes. This is acceptable.

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

- Healthy male volunteers, 18 years of age or older.
- Are in good general health.
- Have skin within 6 inches of the test sites that is free of tattoos, dermatoses, abrasions, cuts, lesions or other skin disorders.
- Cooperative and willing to sign Consent Form and HIPAA Authorization Form.
- Possess the ability to lie prone for up to 30 minutes.

Reviewer's comment: The inclusion criteria used in this study are standard for skin coverage and drying time studies and are acceptable. This reviewer also defers to the Medical Officer's clinical assessment.

Subject Exclusion Criteria: Subjects to whom any of these conditions apply will be excluded from this study:

- Subjects who have a history of sensitivity to chlorhexidine gluconate products.
- Subjects who have a history of skin allergies.
- Subjects who have a history of skin cancer within 6 inches of the applicable test areas.
- Subjects who receive an irritation score of 1 for any individual skin condition prior to the treatment day.
- Participation in another clinical trial in the 30 days prior to Treatment Day of this study (treatment with test materials in this study), or be currently enrolled in another clinical trial, or had previously participated in this study.

- Subjects who have used moisturizers or lotions on the test sites within 24 hours of the Treatment Day of this study.

***Reviewer's comment*: The exclusion criteria used in this study are standard for skin coverage and drying time studies and are acceptable. This reviewer also defers to the Medical Officer's clinical assessment.**

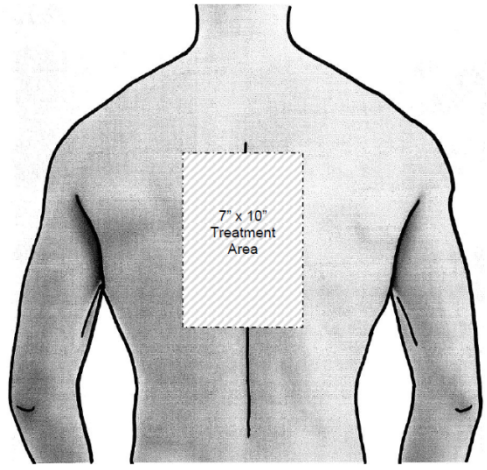
Study Treatment

The Treatment Day Inclusion/Exclusion Criteria CRF will be completed. If these criteria are satisfied, a visual skin assessment will be performed to evaluate the condition of the test area. If an irritation score of 1 for any individual skin condition is assigned, the subject will be excluded from the treatment phase of the study. The subject will be instructed to remove his shirt and lie in a prone position on the procedure table. If required, hair will be clipped in the potential treatment area. The test site will be marked on the subject's back using a 7" x 10" sterile template. The corners of the test area will be marked directly on the skin using a non-toxic skin marker. A Test Site Diagram for the back area is shown below.

***Reviewer's comment*: The Sponsor has proposed a single treatment area coverage using one 9 x 10.5 inch single disposable cloth. The directions for use state the following:**

- ***Use first cloth to prepare the skin area indicated for a moist or dry site, making certain to keep the second cloth where it will not be contaminated. Use second cloth to prepare larger areas.***
- ***Discard each cloth after a single use***
- ***After package has been opened, discard any unused cloths***

Note: each package container contains two 9 x 10.5 inch disposable cloths.



Back (posterior): 7" x 10" Treatment Area

The study material will be weighed pre-application and post-application using a calibrated balance. The 7" x 10" test site will be prepped with the study material for three minutes following the Treatment Application Instructions:

Medline 2% CHG Cloth

1. Weigh the unopened cloth package using a calibrated balance and record the weight in grams.
2. Open the package and remove a single cloth.
3. Using the single cloth, vigorously scrub skin in a back and forth motion for 3 minutes completely wetting the treatment area (7" x 10" area). Approximately halfway through the 3 minute application, the cloth will be turned over. If necessary, the subject's skin will be taut to ensure that the maximum amount of the cloth contacts the area being prepped.

   Note: product handling: when opening the packaging, a single cloth (single unit) will be used. Contact between the cloth and the outside of the packaging will be avoided to reduce risk of cloth contamination.
4. At the completion of the 3 minute application, start a calibrated stopwatch to initiate the drying time. Perform drying time observations in accordance with the protocol.
5. Return the used cloth to the original opened packaging. Re-weigh using a calibrated balance and record the weight in grams.

Reviewer's comment: The directions described above for the application of the drug product are similar to the directions on the labeled drug product.

Labeled directions:

- ***Dry surgical sites (such a as abdomen or arm): Use one cloth to cleanse each 161 cm^2 area (approximately 5 x 5 inches) of skin to be prepared. Vigorously scrub skin back and forth for 3 minutes, completely wetting treatment area, then discard. Allow area to dry for one (1) minute. Do not rinse.***
- ***Moist surgical sites (such as inguinal fold): Use one cloth to cleanse each 65 cm^2 area (approximately 2 x 5 inches) of skin to be prepared. Vigorously scrub skin back and forth for 3 minutes, completely wetting treatment area, then discard. Allow area to dry for one (1) minute. Do not rinse.***

This is acceptable. The test methods used in this study are standard for skin coverage and drying time study and are acceptable. However, how the cloth is applied in the direction for use described in the skin coverage testing and the clinical simulation testing will need to be reflected in the labeling. This will be an NDA review issue.

At the completion of the 3 minute application, a calibrated stopwatch will be started to initiate the drying time observations. Drying time is defined as when the entire treatment area appears visibly dry. Drying time observations will be performed simultaneously by three technicians, including at least one technician with operating room experience, and the three dry times will be averaged. At the completion of the drying time observations, a post-treatment visual skin assessment will be performed to evaluate the condition of the test area.

Reviewer's comment: Since the chlorhexidine gluconate is the only active ingredient in the ReadyPrep CHG patient preoperative skin preparation cloth, there is no need for the class flammability warning. We have implemented class flammability warning for all antiseptic products containing alcohol and used as a patient preoperative skin preparation. Their labeling states: (b) (4)

(b) (4) ***Because chlorhexidine gluconate and alcohol combination antiseptic products have ignited when electrocautery was used during surgical procedures when they were not permitted to dry (or were permitted to pool under the patient), it is critical that the product be completely dry before an electrical spark is permitted in the operating field.***

If an irritation score of 3 for any individual skin condition at any observation period is assigned, the subject will be discontinued from the study and an adverse event will be recorded. Following the skin assessment, residual study material will be removed from the subject's skin using tap water and paper towel.

Subjects will be asked to refrain from the use of lotion or moisturizing products on their backs for 24 hours prior to the treatment visit. Answers to the inclusion/exclusion questions asked at the beginning of the screening and treatment phases will determine compliance to the subject Instructions provided to each subject upon participation. Documentation of the Inclusion/ Exclusion criteria shall serve as confirmation of subject compliance with the Subject Instructions.

Reviewer's comment: Parameters such as discontinuation of subjects based on a particular irritation score; asking subjects to refrain from using lotion on their backs; and using the answers to the inclusion/exclusion questions at the beginning of the screening and treatment phases to determine subject's compliance are all standard practice for these types of studies, and are acceptable.

Adverse Events

The Investigator is responsible for identifying adverse events that occur to each subject throughout the study. An adverse event can occur at any time during the conduct of the study. Adverse events will be captured for all subjects from the time of screening baselines are taken until the time of discharge from the study. An adverse event can be identified by the Investigator or reported by the subject. An Adverse Event/Experience is any unexpected or undesirable experience occurring to a subject during a study, which may or may not be related to the test article. All adverse event/experiences will be recorded and reported according to the Standard Operating Procedures of the laboratory. All adverse events, regardless of severity or the causal/effect relationship, will be recorded.

Reviewer's comment: The Adverse Event Form proposed is standard for skin coverage and drying time studies and is acceptable. This reviewer also defers to the Medical Officer's clinical assessment.

Statistical Methods

The coverage area treatment dose and dry time analyses will include all treated subjects who have calculable dose or dry time information. All subjects who have signed the informed consent will be included in the safety analyses. Efficacy analyses will include summary tables that will be produced for all responses. For prep weight used, coverage area treatment dose (grams per cm^2), and dry time, the tables will include n, mean, standard deviation, minimum and maximum. Ninety-five percent confidence intervals for the mean dose and mean dry time will

be generated using Student's t distribution. Safety analysis will be all subjects who have signed the informed consent will be considered evaluable for safety. Skin irritation scores assessed in accordance with Appendix 13.7 will be reported for any subject who is scored with a 1 or more at any observation (screening day, pre-treatment, and post-treatment) in any category. Adverse events (including post treatment skin irritation scores of 3 in accordance with Appendix 13.7) will also be summarized. Summary tables will present incidence rates of adverse events for all subjects who enter the treatment period. Listings of adverse events will be provided.

Sample Size Justification

Assuming *s* represents the standard deviation, and 30 subjects complete the study, the width of the confidence intervals will be $2*t_{1-\alpha/2,30-1}*s/\text{sqrt}(30)$, or approximately $0.7468s$. In other words, 30 subjects will allow estimation of the mean values of the dose and dry time within a width of about 0.75 of the standard deviations of the data.

> ***Reviewer's comment: Normally, sample size justification is not incorporated or not needed in the skin coverage study because only one test product is generally used for a group of 10-20 subjects. However, the Sponsor is enrolling 30 subjects and has, therefore, incorporated sample size justification in this study. This is acceptable.***

Medline Study #R16-034: MicroBioTest Project No. 721-122 "Statistical Analysis for: Evaluation of the Area Covered by Medline 2% Chlorhexidine Gluconate Preoperative Skin Preparation"

> ***Reviewer's comment: The Sponsor provided summary results of the coverage area and drying time studies. The acceptability of this data will be an NDA review issue once the Sponsor resubmits its NDA.***

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MICHELLE M JACKSON
04/18/2017

FRANCISCO MARTINEZ-MURILLO
04/18/2017

CLINICAL MICROBIOLOGY REVIEW

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Nonprescription Drug Products, ODE IV

NDA 207964

Sponsor Package Submission: October 31, 2016
Received by CDER White Oak: November 1, 2016
Received by Reviewer: November 1, 2016
Review Completed: November 10, 2016

REVIEWER: Michelle M. Jackson, PhD

TEAM LEADER: Francisco Martínez-Murillo, PhD

PROJECT MANAGER: Celia Peacock, MPH, RD, CAPT

NAME AND ADDRESS OF APPLICANT
Medline Industries, Inc.
One Medline Place
Mundelein, IL 60060

CONTACT PERSON
Bill Parthun
Director, Research & Development
(847) 643-3839

DRUG PRODUCT NAMES:
Proprietary Name: ReadyPrep™ CHG (2% Chlorhexidine Gluconate Cloth)
Established Name: 2% chlorhexidine gluconate

INDICATION: Patient Preoperative Skin Preparation

PHARMACOLOGICAL CATEGORY: Healthcare Antiseptic

DOSAGE FORM: Topical Solution

MATERIAL REVIEWED: 1) Meeting Package with Questions
2) Protocol: "Comparative In Vitro Pharmacodynamics Employing Time-Kill Study between New and Prior ReadyPrep CHG 2% Products"

Executive Summary:
Medline Industries, Inc. (Medline) submitted a 505(b)(2) New Drug Application (NDA) on February 9, 2016 for ReadyPrep™ (chlorhexidine gluconate cloth), 2%. The indication for this drug product is patient preoperative skin preparation. The application was not sufficiently complete to permit a substantive review, and the FDA refused to file (RTF) the application on April 8, 2016. The Sponsor failed to address the following issues: the safety of the (b) (4) the Clinical Study Reports in module 5 of the Electronic Common Technical Document (eCTD) did not contain a subgroup analysis; and the application did not contain an appropriate patent certification as required under 21 CFR 314.50(i). Medline submitted a Type A meeting request on April 22, 2016. According to the meeting request, the meeting objective was to discuss the items determined by the Agency to be incomplete in the April 8, 2016 RTF letter. The meeting was held on May 23, 2016. On September 15, 2016, Medline submitted a type C written response only meeting request to discuss issues related to the refiling of their NDA.

Recommendations to the Sponsor:
We are only reviewing and responding to microbiology related questions: 1, 4, and 5.

(b) (4)

Response to Question 1
(Microbiology)
Comparative In Vitro Pharmacodynamics Employing Time-Kill Study between New and Prior ReadyPrep CHG 2% Products
Yes, we agree from a microbiological perspective to employ the in vitro time-kill study approach. However, we disagree that the comparison between the new and prior ReadyPrep™ CHG formulations will only be performed on three organisms Escherichia coli, Staphylococcus aureus and Candida albicans. Since we agreed that it would be acceptable to (b) (4) you would need to bridge the new formulation (b) (4) to the clinical efficacy data supporting the previous product formulation. In regards to the microbiological in vitro effectiveness data, we recommend that you conduct a robust time-kill study comparing (bridging) the new and prior ReadyPrep™ CHG formulations. We recommend that you test 48 repository (ATCC) isolates (12 species and four isolates per species) and 24 clinical isolates (12 species and two isolates per species) as described in the Table below. The 48 repository isolates should be the same isolates used in the Medline Study R14-013 and you may select any two clinical isolates out of

the 12 that were used from the same study. You will have a total of 864 evaluations compared to 1,536 evaluations in the Medline Study R14-013.

Organism	Strain Identification		Clinical Isolates Received from:
	ATCC Strains	Clinical Isolates	
Burkholderia cepacia	ATCC 25416, ATCC 25608	One isolate	(b) (4)
Multidrug-resistant *Burkholderia cepacia*	ATCC 55792, ATCC 700070	One isolate	
Candida albicans	ATCC 18804, ATCC 66027	One isolate	
Azole-resistant *Candida albicans*	ATCC 64124, ATCC 64550	One isolate	
Enterococcus faecalis	ATCC 19433, ATCC 29212	One isolate	
Vancomycin-resistant *Enterococcus faecalis*	ATCC 51299 ATCC 51575	One isolate	
Enterococcus faecium	ATCC 19434, ATCC 25307	One isolate	
Vancomycin-resistant *Enterococcus faecium*	ATCC 51559, ATCC 700211	One isolate	
Escherichia coli	ATCC 25922, ATCC 11775	One isolate	
Multidrug-resistant *Escherichia coli*	BAA-197, BAA-200	One isolate	
Klebsiella pneumoniae	ATCC 13883, ATCC 27736	One isolate	
Multidrug-resistant *Klebsiella pneumoniae*	ATCC 51503, ATCC 700603	One isolate	
Pseudomonas aeruginosa	ATCC 9027, ATCC 27853, ATCC 27315, ATCC 15442	One isolate	
Multidrug-resistant *Pseudomonas aeruginosa*	Not applicable	One isolate	
Serratia marcescens	ATCC 8100, ATCC 13880, ATCC 14756	One isolate	
Multidrug-resistant *Serratia marcescens*	ATCC 43297	One isolate	
Staphylococcus aureus	ATCC 6538, ATCC 29213	One isolate	
Methicillin-resistant *Staphylococcus aureus*	ATCC 33591, ATCC 33592	One isolate	

			(b) (4)
Staphylococcus epidermidis	ATCC 12228, ATCC 14990	One isolate	
Methicillin-resistant *Staphylococcus epidermidis*	ATCC 51625, ATCC 700563	One isolate	
Streptococcus pneumoniae	ATCC 6303, ATCC 49619	One isolate	
Multidrug-resistant *Streptococcus pneumoniae*	ATCC 51936, ATCC 700671	One isolate	
Streptococcus pyogenes	ATCC 14289, ATCC 19615	One isolate	
Macrolide-resistant *Streptococcus pyogenes*	BAA-1411, BAA-1413	One isolate	

Question 4 (Raw Plate Count Data)
Medline intends to provide raw plate count data for the following studies R14-013, R14-012, R13-053 and R15-029. The raw plate count data will be provided in the form of Case Report Forms for clinical studies and comparable documents for non-clinical studies from which the data was originally captured. Medline will not provide raw plate count data for study R13-052 since we do not intend to rely on this study for efficacy in the resubmission. Does the agency agree this approach is acceptable?

Response to Question 4
Yes, we find this approach acceptable.

Question 5 (Skin Coverage and Drying Time Study)
Medline intends to perform the requested skin coverage and drying study on the new formulation (b) (4) rather than on the original formulation. Does the agency agree that this fulfills the request from Question 2 under section "Clinical Microbiology" in the Refuse to File letter dated April 8, 2016?

Response to Question 5
Yes, this acceptable. We encourage you to submit your protocol for our review and comment before conducting the study.

Background

The Sponsor is preparing an NDA for ReadyPrep™ CHG, under Section 505(b)(2) of the FD&C Act. The proposed ReadyPrep™ CHG product, a 2% CHG cloth, is indicated for use as a preoperative skin preparation. The product is formulated as 2% CHG (equivalent to 500 mg of the active moiety, CHG, per cloth), an inactive excipient profile, and a polyester cloth. CHG is applied through a single application, consisting of a 3-minute vigorous rub followed by a 1-minute dry time, at the therapeutic site of action.

On February 9 2016, the Sponsor submitted a 505(b)(2) NDA for ReadyPrep™ CHG. However, in the Refuse-to-File letter dated April 8, 2016, among other issues, the Agency stated that the application was incomplete because it did not include the assessment of safety (including genetic toxicity, general toxicity, and reproductive toxicity) (b) (4) In addition, in a Type A meeting on May 23, 2016 (meeting minutes in DARRTS), the Agency agreed that it would be acceptable to (b) (4) However, the Sponsor needs to bridge the new formulation (b) (4) to the clinical safety and efficacy data and to the quality data (i.e., stability data) supporting the previous product formulation. The Agency stated that the Sponsor will need to provide adequate scientific justification for how the new, to-be-marketed formulation can rely upon previously conducted studies supporting the prior product formulation. The Agency also stated that the Sponsor will need to provide all chemistry data on the new product formulation and demonstrate (b) (4) does not affect stability. Lastly, the Sponsor will need to address whether such change has any effect on the microbiological profile and identify any issues related to biopharmaceutics.

Per agreement with the Agency during the Type A meeting discussion, the Sponsor plans to demonstrate the similarity in effectiveness of ReadyPrep™ CHG as an antimicrobial wipe between its proposed new product (b) (4) and the prior product (b) (4) to support the scientific bridge to the clinical safety and efficacy data and to the quality data supporting the prior formulation.

(b) (4)

(b) (4)

The Sponsor predicts that (b) (4) The new formulation and prior formulation will provide the same drug exposure at the site of action as both are formulations of a locally acting drug, CHG. The Sponsor submitted protocols to provide rationale for the scientific bridge to show the similarity in stability, antimicrobial activity and skin-penetration capability between the new formulation of ReadyPrep™ CHG and prior formulation. This reviewer will only address the protocol on antimicrobial activity between the new formulation of ReadyPrep™ CHG and prior formulation.

Reviewer's Comments on Protocol
"Comparative In Vitro Pharmacodynamics Employing Time-Kill Study between New and Prior ReadyPrep™ CHG 2% Products"

The Sponsor stated that in order to demonstrate the similarity in antimicrobial activity between the new and prior ReadyPrep™ CHG 2% formulations, the in vitro time-kill study will be employed to evaluate the susceptibility of bacteria to the new and prior ReadyPrep™ 2% CHG formulations. The results from the comparison of the time-kill rate curves will be used to validate that (b) (4) will have no impact on the antiseptic effectiveness of the new formulation.

- Reference Product: Chlorhexidine gluconate, 2%-prior formulation (b) (4)
- Test Product: Chlorhexidine gluconate, 2%-new formulation (b) (4)

Studies will be performed according to standard procedure at a certified laboratory. All sampling will be performed in triplicate.

> ***Reviewer's comment: The Sponsor should use similar protocol that's described in its Medline Study R14-013. This reviewer finds it acceptable to use the Reference Product: Chlorhexidine gluconate, 2%-prior formulation*** (b) (4) ***and Test Product: Chlorhexidine gluconate, 2%-new formulation*** (b) (4) ***for the time kill study to show that*** (b) (4) ***will have no impact on the antiseptic effectiveness of the new formulation.***

With the prior ReadyPrep™ CHG 2% formulation, Sponsor has performed 1,536 antimicrobial evaluations on both antibiotic resistant and non-resistant strains of repository and clinical isolates such as *Escherichia coli, Staphylococcus aureus, Candida albicans, Enterococcus faecailis, Enterococcus faecium, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus epidermidis, Streptococcus pneumonia, Streptococcus pyogenes, Serratia marcescens, Burkholderia cepacia* (Medline Study R14-013). The Sponsor stated that the results also showed that the vehicle did not contribute to antimicrobial activity of the Medline prior ReadyPrep™

CHG 2% formulation. It is, therefore, predicted that the [redacted] (b) (4) will have no impact on the antiseptic effectiveness of the new ReadyPrep™ CHG 2% formulation.

To strengthen the previous findings in Medline Study R14-013 regarding the no effects of vehicle on the new ReadyPrep™ CHG 2% formulation, the time-killing study will be conducted to show the similarity in antimicrobial activity between the new and prior ReadyPrep™ CHG 2% formulations on representative bacteria. As antimicrobial activity was done thoroughly in Medline Study R14-013 and the Sponsor proposed product is indicated for Patient Preoperative Surgical Preparation, the time-kill comparison between the new and prior ReadyPrep™ CHG 2% formulations will only be performed on *Escherichia coli, Staphylococcus aureus* and *Candida albicans*. In addition, antimicrobial resistant (e.g., clinical isolates, n = 3) and non-resistant strains (e.g., repository isolates, n = 3) of *Escherichia coli, Staphylococcus aureus* and *Candida albicans* will be tested.

***Reviewer's comment**: This reviewer disagrees that the comparison between the new and prior ReadyPrep™ CHG 2% formulations will only be performed on three organisms Escherichia coli, Staphylococcus aureus and Candida albicans. Since we agreed that it would be acceptable to* [redacted] (b) (4) *the Sponsor would need to bridge the new formulation* [redacted] (b) (4) *to the clinical safety and efficacy data and to the quality data supporting the previous product formulation. In regards to the microbiological in vitro effectiveness data, this reviewer recommends that the Sponsor conducts a robust time-kill study comparing (bridging) the new and prior ReadyPrep™ CHG 2% formulations. This reviewer recommends that the Sponsor tests 48 repository (ATCC) isolates (12 species and four isolates per species) and 24 clinical isolates (12 species and two isolates per species) as described in the Table below. The 48 repository isolates should be the same isolates used in the Medline Study R14-013 and the Sponsor may select any two clinical isolates out of the 12 that were used from the same study. The Sponsor will have a total of 864 evaluations compared to 1,536 evaluations in the Medline Study R14-013.*

48 repository [12 species x 4 isolates per species] + 24 clinical isolates [12 species x 2 isolates per species] = 72 organisms
72 organisms x 6 (3 concentrations for current formulation and 3 concentrations for prior formulation) = 432 evaluations
432 evaluations x 2 (6 minute and 10 minute time points) = 864 total evaluations

Organism	Strain Identification		Clinical Isolates Received from:
	ATCC Strains	Clinical Isolates	
Burkholderia cepacia	ATCC 25416, ATCC 25608	One isolate	[redacted] (b) (4)
Multidrug-resistant *Burkholderia cepacia*	ATCC 55792, ATCC 700070	One isolate	

(b) (4)

Candida albicans	ATCC 18804, ATCC 66027	One isolate
Azole-resistant *Candida albicans*	ATCC 64124, ATCC 64550	One isolate
Enterococcus faecalis	ATCC 19433, ATCC 29212	One isolate
Vancomycin-resistant *Enterococcus faecalis*	ATCC 51299 ATCC 51575	One isolate
Enterococcus faecium	ATCC 19434, ATCC 25307	One isolate
Vancomycin-resistant *Enterococcus faecium*	ATCC 51559, ATCC 700211	One isolate
Escherichia coli	ATCC 25922, ATCC 11775	One isolate
Multidrug-resistant *Escherichia coli*	BAA-197, BAA-200	One isolate
Klebsiella pneumoniae	ATCC 13883, ATCC 27736	One isolate
Multidrug-resistant *Klebsiella pneumoniae*	ATCC 51503, ATCC 700603	One isolate
Pseudomonas aeruginosa	ATCC 9027, ATCC 27853, ATCC 27315, ATCC 15442	One isolate
Multidrug-resistant *Pseudomonas aeruginosa*	Not applicable	One isolate
Serratia marcescens	ATCC 8100, ATCC 13880, ATCC 14756	One isolate
Multidrug-resistant *Serratia marcescens*	ATCC 43297	One isolate
Staphylococcus aureus	ATCC 6538, ATCC 29213	One isolate
Methicillin-resistant *Staphylococcus aureus*	ATCC 33591, ATCC 33592	One isolate
Staphylococcus epidermidis	ATCC 12228, ATCC 14990	One isolate
Methicillin-resistant *Staphylococcus epidermidis*	ATCC 51625, ATCC 700563	One isolate
Streptococcus pneumoniae	ATCC 6303, ATCC 49619	One isolate
Multidrug-resistant *Streptococcus pneumoniae*	ATCC 51936, ATCC 700671	One isolate

			(b) (4)
Streptococcus pyogenes	ATCC 14289, ATCC 19615	One isolate	
Macrolide-resistant *Streptococcus pyogenes*	BAA-1411, BAA-1413	One isolate	

Due to the nature of the in vitro test methods, testing will not be evaluated on the Medline ReadyPrep™ CHG cloth 2% products. Instead, the test will be conducted on the solution of the 2% CHG cloth new and prior formulations that used to manufacture the final cloth products. By conducting the study in this manner, it will remove the potential for technical deviations in trying to extract the solution from the cloth product prior to testing.

Reviewer's comment: This reviewer finds this acceptable.

In the time-kill study, antimicrobial effectiveness will be assessed by determining the $\log_{10}$ reductions after exposure to the new and prior ReadyPrep™ CHG 2% formulation solutions for 6 and 10 minutes. In addition, 3 concentrations of the new and prior ReadyPrep™ CHG 2% formulation will be evaluated ranging from full (2%) concentration, partial concentration within the effective range (1%), and an ineffective concentration (0.0002%). Neutralization effectiveness will be verified prior to study conduct for all treatments.

Reviewer's comment: This reviewer finds this acceptable. We have been recommending to Sponsors that the test article should be evaluated at three concentrations (use concentration, a secondary concentration within the active range, and an inactive concentration).

Statistical Analysis
The test products will be considered bactericidal at the concentration and the contact time that demonstrates a 3 $\log_{10}$ (99.9%) or greater reduction on bacterial viability compared to the initial counts. Percent reduction will be calculated for time points 6 minutes and 10 minutes of exposure. A non-parametric hypothesis statistical test such as Wilcoxon Rank Sign Test will be utilized for the comparisons of percent reduction between the new formulation and prior formulation for both time points 6 minutes and 10 minutes of exposure. A p-value greater than 0.05 will be considered no statistical significance.
• Percent reduction (colony-forming unit/mL) will be calculated as follow:

$$\text{Percent reduction} = \frac{\text{Average initial count} - \text{Test results}}{\text{Average initial count}} \times 100$$

• Log_{10} reduction will be calculated using the following equations:

$$\text{Log}_{10}\text{ reduction} = \text{Log}_{10}(\text{average initial count}) - \text{Log}_{10}(\text{test results})$$

Reviewer's comment: The test products will be considered bactericidal at the concentration and the contact time that demonstrates a 3 $\log_{10}$ (99.9%) or greater reduction on bacterial viability compared to the initial counts and percent reduction will be calculated for time points 6 minutes and 10 minutes of exposure. This reviewer

finds these testing measurement points acceptable. However, the evaluation of the use of the non-parametric hypothesis statistical test such as Wilcoxon Rank Sign Test for the comparisons of percent reduction between the new formulation and prior formulation for both time points 6 minutes and 10 minutes of exposure will depend on the statistician's assessment (see review in DARRTS).

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MICHELLE M JACKSON
02/03/2017

FRANCISCO MARTINEZ-MURILLO
02/03/2017