

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**210872Orig1s000**

**CLINICAL MICROBIOLOGY/VIROLOGY**  
**REVIEW(S)**



# CLINICAL MICROBIOLOGY NDA REVIEW

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

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NDA 210872 (Original)

Sponsor Package Submission: June 29, 2018  
Review Completed: March 3, 2019

REVIEWER: Anita Kumar, PhD

TEAM LEADER: Francisco Martínez-Murillo, PhD

PROJECT MANAGER: Sherry Stewart, PharmD.

NAME AND ADDRESS OF APPLICANT

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DRUG PRODUCT NAMES:

Proprietary Name: ZuraGard™ Surgical Solution  
Established Name: Isopropyl Alcohol, 70% v/v

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

PHARMACOLOGICAL CATEGORY: Health Care Antiseptic

DOSAGE FORM: Topical solution containing 70% isopropyl alcohol in a 10.5 mL applicator.

RELATED SUBMISSIONS: (b) (4)

MATERIALS REVIEWED:

Study No.	Title of Study
<b>Clinical In Vitro Microbiology Evaluations</b>	
(b) (4) 130734-202 (ZX-ZP-0014)	Determination of the Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of Two Test Products, One Active Ingredient, One Reference Product, and One Negative Control When Challenged With Various Microorganism Strains
(b) (4) 130733-201 (ZX-ZP-0015)	An In Vitro Time-Kill Evaluation of Two Test Products, One Active Ingredient, One Reference Product, and One Negative Control for Their Antimicrobial Properties When Challenged With Various Microorganism Strains
(b) (4) 130548-201	Determination of the Dose-Response of Various Microorganism Strains to One Test Product, Five Active Ingredients, and Two Controls Using an In Vitro Time-Kill Procedure
(b) (4) 865-102 (ZX-ZP-0043)	Evaluation of Potential for Development of Antimicrobial Resistance
<b>Clinical In Vivo Microbiology Studies</b>	
MBT 865-104 (ZX-ZP-0068)	Pilot Clinical Evaluation to Characterize the In Vivo Effects of Topically Applied ZuraPrep™ and ZuraPrep™ Vehicle (March 18, 2016)
MBT 865-105 (ZX-ZP-0073)	Pivotal Clinical Evaluation of the Antimicrobial Effectiveness of Topically Applied ZuraPrep™
BSLI 150316-103 (ZX-ZP-0074)	Pivotal Clinical Evaluation of ZuraPrep™, a Patient Preoperative Skin Preparation
(b) (4) 865-106 (ZX-ZP-0083)	Evaluation of the Skin Area Covered and Dry Time of a Preoperative Skin Preparation

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## 1. Executive Summary:

### 1.1. Recommended Regulatory Action

#### Remarks:

This review of NDA 210872 describes the findings and recommendations of the Clinical Microbiology Reviewer for this file. 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 under the provisions of section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act in accordance with 21 CFR 314.50. The Sponsor submitted application for ZuraGard™ Surgical Solution (Isopropyl alcohol 70% v/v) for a patient preoperative skin preparation indication that relied on the Agency's previous findings on the safety of isopropyl alcohol for ChloraPrep (NDA 20832), since it contains the same active ingredient and has the same dosage form, route of administration, and indication for use. Isopropyl alcohol is a wide spectrum antimicrobial ingredient that provides a rapid antimicrobial effect while it evaporates from the skin.

The Sponsor is proposing a 10.5 mL applicator packaged for single use. The to-be-marketed dosage form comprises a single-use 10.5-mL plastic applicator with a sponge tip containing ZuraGard™ Surgical Solution. The container closure system is comprised (b) (4)

To highlight the coverage area once applied to the skin, the ZuraGard™ formulation includes an excipient (b) (4) methylene blue (b) (4). The Sponsor is seeking the following indication for its ZuraGard™ Surgical Solution: for patient preoperative skin preparation; for preparation of the skin prior to surgery; helps reduce bacteria that potentially can cause skin infection. (b) (4)

This NDA was submitted with proposed proprietary name ZuraPrep™ on June 29, 2018. On September 26, 2018, the Agency denied the proprietary name ZuraPrep™. On December 6, 2018, the Sponsor submitted the new proprietary name "ZuraGard™," which was approved on March 1, 2019 by the Agency. Since the Sponsor performed the effectiveness studies with the product's name as "ZuraPrep," this review may, on occasion, address the test product as "ZuraPrep" for review purposes only, with the understanding that is referring to the approved name "ZuraGard".

#### 1.1.1. Studies Conducted and Conclusions:

##### Clinical In Vitro Microbiology Studies:

Study ZX-ZP-0014 ( (b) (4) 130734-202): Determination of the Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of Two Test Products, One Active Ingredient, One Reference Product, and One Negative Control When Challenged With Various Microorganism Strains

The in vitro antimicrobial spectrum and minimum bactericidal concentration (MBC) of ZuraGard solution was determined against 180 different microorganism strains (2 laboratory strains and 10 fresh clinical isolates of 15 different microorganism species) in the pivotal study

130734-202. These organisms included both Gram-positive bacteria, Gram-negative bacteria, and yeast. Test product ZuraGard was bactericidal for 155 of the 180 strains when diluted 1:16. On the other hand, ZuraGard's vehicle was bactericidal for 33 of the 180 strains when diluted 1:16, suggesting the vehicle has weak subtherapeutic activity. The MBC range for ZuraGard was >4,297 µg/ml to 137,500 µg/mL, and 17,188 µg/mL to 275,000 µg/mL for 70% IPA. These MBC values are well below the actual use concentration of the active ingredient 70% IPA ( (b) (4) µg/mL) in the ZuraGard.

Study ZX-ZP-0015 ( (b) (4) 130733-201): An In Vitro Time-Kill Evaluation of Two Test Products, One Active Ingredient, One Reference Product, and One Negative Control for Their Antimicrobial Properties When Challenged With Various Microorganism Strains

The time-kill study performed at full strength concentration for ZuraGard final product, 70% v/v isopropyl alcohol independently, and ChloroPrep product, showed a >3.0 log<sub>10</sub> (>99.9%) reduction in viable microbial cells within 30 seconds for all 148 challenge strains tested, in the three test products. The killing effect or antimicrobial activity of a drug needs to reach ≥3 log reduction to be considered active. The minimum log<sub>10</sub> reduction observed was 5.1 for ZuraGard, 4.7 for 70% v/v isopropyl alcohol, and 5.1 for ChloroPrep.

ZuraGard's vehicle showed activity against 51 of the 148 organisms tested. However, the Sponsor demonstrated, through pilot clinical simulation study ZX-ZP-0068, that the log reduction achieved by ZuraGard's vehicle and a normal saline negative control were similar, indicating that ZuraGard's excipients do not significantly contribute towards the effectiveness of the test product. Overall, the results of this time-kill study showed that ZuraGard provides immediate killing of the tested microorganisms at exposure times of 30, 60, and 120 seconds, and is an effective bactericidal agent.

Study ZX-ZP-0043 ( (b) (4) 865-102): Evaluation of Potential for Development Antimicrobial Resistance of ZuraPrep

The study ZX-ZP-0043 was intended to determine the potential for development of resistance to ZuraGard and 70% v/v isopropyl alcohol by sequential passage of several clinically relevant microorganisms through increasing concentrations of an antimicrobial/antibiotic included in the culture medium. Ten repository isolates and 4 clinical isolates from 8 species were evaluated for a total of 42 isolates. The study results did not show any higher MIC values with clinical isolates compared to ATCC laboratory strains and the baseline. These results suggest that the product (ZuraGard) does not have significant potential for the development of resistance.

An evaluation of the potential for antibiotic cross-resistance due to isopropyl alcohol was performed by comparing the MICs of several antibiotics both before and after extended exposure to sublethal concentrations of isopropyl alcohol. Similar to the final product testing, no changes to MICs were observed for isopropyl alcohol.

In conclusion, this study results indicate that ZuraGard and isopropyl alcohol do not induce or select for resistance in clinically relevant bacteria and do not mediate cross-resistance with clinically useful antibiotics.

### **Clinical In Vivo Microbiology Studies**

One pilot clinical evaluation study (ZX-ZP-0068) and two pivotal clinical simulation studies, MicroBioTest ZX-ZP-0073, and BioScience Laboratories ZX-XP-0074, were designed to evaluate the antimicrobial efficacy and safety of ZuraGard, active control ChloroPrep, and ZuraGard's vehicle on the abdominal and groin/inguinal regions of the body. The procedures

used in these pivotal studies were based on the American Society for Testing and Materials standards (ASTM E1173-01, reapproved 2009: Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations) and the FDA’s 1994 Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Tentative Final Monograph (TFM) for Health Care Antiseptic Drug Products (59 FR 31402). Prepping procedure consisted of 30 seconds of product application time on abdomen and 2 minutes on the groin site, followed by 3 minutes of drying time. Sampling was performed at 30 seconds, 10 minutes, and 6 hours after the post-application drying time.

Analyses Results:

1. Primary Analysis by Responder Rate and 95% CI lower bound at 10 Minutes

ZuraGard met the primary efficacy criteria of having a responder rate 95% CI lower bound  $\geq 70\%$  at 10 minutes on the abdomen and groin sites (see Table 1 below):

- Study ZX-ZP-0073: At 10 minutes, the responder rate 95% CI lower bound for the abdominal region was 94.0% for ZuraGard, and 94.3% for ChloroPrep. The corresponding responder rate point estimates were 96.5% and 96.8%, respectively. The responder rate 95% CI lower bound for the groin region was 89.4% for ZuraGard, and 87.5% for ChloroPrep, with corresponding responder rate point estimates of 92.7% and 91.1%, respectively.
- Study ZX-ZP-0074: At 10 minutes, the responder rate 95% CI lower bound for the abdominal region was 76.2% for ZuraGard, and 74.5% for ChloroPrep. The corresponding responder rate point estimates were 80.9% and 79.4%, respectively. The responder rate 95% CI lower bound for the groin region was 70.3% for ZuraGard, and 67.5% for ChloroPrep, with corresponding responder rate point estimates of 75.2% and 72.4%, respectively.

For both, abdominal and inguinal regions, the responder rates of the test product ZuraGard and the active control ChloroPrep at 10 minutes were significantly higher than that of the vehicle control. The responder rate point estimate for the vehicle control was 17.4% and 11.8% for the abdominal region and 16.2% and 1.4% for the inguinal region for studies ZX-ZP-0073 and ZX-ZP-0074, respectively. Therefore, both studies met the responder rate primary endpoint recommended for the clinical simulation study.

**Table 1. Responder Rate (mITT population) Study ZX-ZP-0073 and ZX-ZP-0074 (Source: Table 2.7.3-11, module 2 summary of clinical efficacy)**

Study	Abdomen			Groin		
	Vehicle Rate (%) (95% CI)	ZuraPrep Rate (%) (95% CI)	ChloroPrep Rate (%) (95% CI)	Vehicle Rate (%) (95% CI)	ZuraPrep Rate (%) (95% CI)	ChloroPrep Rate (%) (95% CI)
ZX-ZP-0074	11.8 (5.2, 21.9)	80.9 (76.2, 85.0)	79.4 (74.5, 83.7)	1.4 (0.0, 7.3)	75.2 (70.3, 79.7)	72.4 (67.5, 77.0)
ZX-ZP-0073	17.4 (9.3, 28.4)	96.5 (94.0, 98.2)	96.8 (94.3, 98.4)	16.2 (8.4, 27.1)	92.7 (89.4, 95.3)	91.1 (87.5, 94.0)

2. Primary Analysis by Average Treatment Effect (Superiority and Noninferiority) at 10 Minutes

In both, Study ZX-ZP-0073 and Study ZX-ZP-0074, ZuraGard met the expected ATE analysis criteria. The upper limit of the 95% confidence interval for the non-inferiority of ZuraGard vs. ChloroPrep was below 0.5, and the lower limit of the 95% confidence interval for the superiority of ZuraGard vs. its vehicle was above 1.2.

- Study ZX-ZP-0073: At 10 minutes, the ATE noninferiority point estimate of ZuraGard to ChloroPrep was 0.039 (95% CI: -0.18 to 0.10), and 0.021 (95% CI: 0.09 to 0.05) for the groin and abdominal sites, respectively. The ATE superiority point estimate of ZuraGard to its vehicle control was 2.595 (95% CI: 2.34 to 2.84), and 1.87 (95% CI: 1.74 to 1.99) for the groin and abdominal sites, respectively (see Table 2 below).
- Study ZX-ZP-0074: At 10 minutes, the ATE noninferiority point estimate of ZuraGard to ChloroPrep was -0.020 (95% CI: -0.21 to 0.17), and -0.045 (95% CI: -0.20 to 0.11) for the groin and abdominal sites, respectively. The ATE superiority point estimate of ZuraGard to its vehicle control was 2.54 (95% CI: 2.1 to 2.77), and 1.97 (CI 1.69 to 2.24) on the groin and abdominal sites, respectively (see Table 3 below).

**Table 2. Study ZX-ZP-0073 Analysis by Average Treatment Effect (Source: IR response dated October 24, 2018)**

Body Area	Treatments	30 Seconds		10 Minutes	
		ATE Difference	95% CI	ATE Difference	95% CI
Groin	Non-inferiority (ChloroPrep vs ZuraPrep)	-0.078	(-0.264 to 0.108)	-0.039	(-0.184 to 0.106)
	Superiority – ZuraPrep vs Vehicle	2.300	(1.983 to 2.618)	2.595	(2.347 to 2.843)
	Superiority – ChloroPrep vs Vehicle	2.222	(1.904 to 2.540)	2.556	(2.308 to 2.804)
Abdomen	Non-inferiority - ChloroPrep vs ZuraPrep	-0.111	(-0.238 to 0.016)	-0.021	(-0.096 to 0.054)
	Superiority – ZuraPrep vs Vehicle	1.892	(1.673 to 2.111)	1.870	(1.740 to 1.999)
	Superiority – ChloroPrep vs Vehicle	1.781	(1.562 to 2.000)	1.849	(1.719 to 1.979)

ATE = average treatment effect; CI = confidence interval.

**Table 3. Study ZX-ZP-0074 Analysis by Average Treatment Effect (Source: IR response dated October 24, 2018)**

Body Area	Treatments	30 Seconds		10 Minutes	
		ATE Difference	95% CI	ATE Difference	95% CI
Groin	Non-inferiority (ChloroPrep vs ZuraPrep)	-0.024	(-0.217 to 0.169)	-0.020	(-0.212 to 0.172)
	Superiority – ZuraPrep vs Vehicle	2.609	(2.283 to 2.934)	2.454	(2.129 to 2.778)
	Superiority – ChloroPrep vs Vehicle	2.584	(2.259 to 2.909)	2.434	(2.110 to 2.757)
Abdomen	Non-inferiority - ChloroPrep vs ZuraPrep	-0.023	(-0.196 to 0.150)	-0.045	(-0.208 to 0.117)
	Superiority – ZuraPrep vs Vehicle	2.048	(1.756 to 2.341)	1.972	(1.697 to 2.247)
	Superiority – ChloroPrep vs Vehicle	2.025	(1.733 to 2.318)	1.927	(1.651 to 2.202)

ATE = average treatment effect; CI = confidence interval.

3. Secondary Analysis by Responder Rate and 95% CI lower bound at 30 Seconds

The responder rate 95% confidence interval lower bound at 30-seconds for ZuraGard exceeded 70% for the abdomen; for the groin, it was slightly below 70% (see Table 4 below).

- Study ZX-ZP-0073: At 30 seconds, the responder rate 95% CI lower bound for the abdominal region was 79.9%, for ZuraGard, and 76.0% for ChloroPrep. The corresponding responder rate point estimates were 84.2% and 80.6%, respectively (see Table 4 below). The responder rate 95% CI lower bound for the groin region was 69.5% for ZuraGard, and 62.7% for ChloroPrep, with corresponding responder rate point estimates of 74.5% and 68.1%, respectively (see Table 4 below).
- Study ZX-ZP-0074: At 30 seconds, the responder rate 95% CI lower bound for the abdominal region was 71.5%, for ZuraGard, and 71.2% for ChloroPrep. The corresponding responder rate point estimates were 76.5% and 76.3%, respectively (see Table 4 below). The responder rate 95% CI lower bound for the groin region was 65.7% for ZuraGard, and 66.0% for ChloroPrep, with corresponding responder rate point estimates of 70.8% and 71.0%, respectively (see Table 4 below).

Both ZuraGard and ChloroPrep successfully met the primary efficacy goals in both studies: the 95% confidence interval lower bound for the responder rate was  $\geq 70\%$  at 10 minutes in both the groin and the abdomen sites. The responder rate at 30 seconds is an additional timepoint obtained for informational purposes.

**Table 4. Responder Rate (mITT population) at 30 Seconds Study ZX-ZP-0073 and ZX-ZP-0074 (Source: Table 2.7.3-13, module 2, summary of clinical efficacy)**

Study	Abdomen			Groin		
	Vehicle Rate (%) (95% CI)	ZuraPrep Rate (%) (95% CI)	ChloroPrep Rate (%) (95% CI)	Vehicle Rate (%) (95% CI)	ZuraPrep Rate (%) (95% CI)	ChloroPrep Rate (%) (95% CI)
ZX-ZP-0074	4.4 (0.9, 12.4)	76.5 (71.5, 81.1)	76.3 (71.2, 80.8)	1.4 (0.0, 7.3)	70.8 (65.7, 75.6)	71.0 (66.0, 75.7)
ZX-ZP-0073	4.4 (0.9, 12.2)	84.2 (79.9, 87.9)	80.6 (76.0, 84.7)	2.9 (0.4, 10.2)	74.6 (69.5, 79.2)	68.1 (62.7, 73.1)

4. Secondary Analysis by Average Treatment Effect (Superiority and Noninferiority) at 30 Seconds

In both studies, ZuraGard met the expected ATE analysis criteria. The upper limit of the 95% confidence interval for the non-inferiority of ZuraGard vs. ChloroPrep was below 0.5, and the lower limit of the 95% confidence interval for the superiority of ZuraGard vs. its vehicle was above 1.2.

- Study ZX-ZP-0073: At 30 seconds, the ATE noninferiority point estimate of ZuraGard vs. ChloroPrep was -0.078 (95% CI: 0.26 to 0.108), and -0.111 (95% CI: -0.23 to 0.016) for the groin and abdominal sites, respectively. The ATE superiority point estimate of ZuraGard to its vehicle control was 2.3 (95% CI: 1.98 to 2.68), and 1.89 (95% CI: 1.67-2.11) for the groin and abdominal sites, respectively (see Table 2 above).

- Study ZX-ZP-0074: At 30 seconds, the ATE noninferiority point estimate of ZuraGard vs. ChloroPrep was -0.024 (95% CI: -0.21 to 0.16), and -0.23 (95% CI: -0.19 to 0.015) for the groin and abdominal sites, respectively. The ATE superiority point estimate of ZuraGard to its vehicle control was 2.6 (95% CI: 2.28 to 2.93), and 2.04 (95% CI: 1.75-2.34) for the groin and abdominal sites, respectively (see Table 3 above).

### **Coverage Area and Dry Time Study**

#### **Study ZX-ZP-0083 (b) (4) 865-106): Evaluation of the Skin Area Covered and Dry Time of a Preoperative Skin Preparation:**

The objective of this study was to assess the coverage area and dosage of the coverage area for ZuraGard's 10.5 mL applicator, and the drying time post-application.

Overall the area coverage results for the ZuraGard 10.5 mL applicator indicate a coverage area of 455 cm<sup>2</sup> or 70.52 in<sup>2</sup>.

The labeling for ZuraGard 10.5 mL applicator specifies a coverage area of 8.4 X 8.4 inches, or 457 cm<sup>2</sup>. Also, the labeling states "discard the applicator after a single use along with any portion of the solution not required to cover the prepped area. It is not necessary to use the entire amount available", indicating that it is not necessary to use the full content of the applicator. The coverage area study for the ZuraGard 10.5 mL applicator is acceptable and satisfactory.

#### **1.1.2 Recommendations:**

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

Zurex Pharma’s ZuraGard™ Surgical Solution (IPA, 70% v/v) is a nonsterile, blue, topical solution over-the-counter (OTC) drug product containing a combination of excipient ingredients indicated for patient preoperative skin preparation and for use in presurgical settings as an antiseptic/antimicrobial agent to reduce bacteria that potentially can cause infection. The to-be-marketed dosage form comprises a single-use 10.5-mL plastic applicator containing ZuraGard Surgical Solution with a sterile barrier system to ensure that the applicator surfaces are sterile (while the solution product remains not sterile). The following table lists the final product’s ingredient composition. This review of NDA 210872 describes the findings and recommendations of the Clinical Microbiology Reviewer for this file.

**Table 5. Components of ZuraGard™ Solution (Source: Table 2.3.P.1.1, module 2)**

Component	Amount (per unit)	Type of Ingredient	Function	Reference to Quality Standards
Isopropyl alcohol, (b) (4)	70% (v/v)	Active ingredient	Antiseptic (b) (4)	USP
Citric acid, (b) (4)	(b) (4)	Excipient	(b) (4)	USP
(b) (4)		Excipient		USP
Methylparaben		Excipient		NF
Propylparaben		Excipient		NF
Methylene blue (b) (4)		Excipient		USP
Purified water		Excipient		USP
NF = National Formulary; USP = United States Pharmacopeia.				
(b) (4)				

## 3. PRECLINICAL MICROBIOLOGY

### 3.1. Mechanism of Action of ZuraGard™ Solution (70% Isopropyl Alcohol v/v)

ZuraGard contains 70% isopropyl alcohol v/v as the active ingredient with antiseptic activity properties. We describe below a summary of the mechanism of action for 70% isopropyl alcohol.

Alcohols (ethanol and isopropyl alcohol) are both considered antiseptics and intermediate-level disinfectants (1). The Agency has categorized isopropyl alcohol at concentrations from 71.3% to 91.3% (v/v in water) as an active ingredient deferred from final rulemaking in the Health Care Antiseptic Final Monograph (82 FR 60474) for the patient preoperative skin preparation indication. It is believed that isopropyl alcohol dehydrates the bacterial cell and denatures its proteins, particularly those that function as membrane-bound enzymes (1, 2). Protein coagulation occurs within concentration limits around an optimum alcohol level. In the absence of water, proteins are not denatured as readily as when water is present. Therefore, it is thought that absolute isopropyl ethanol, a dehydrating agent, is less bactericidal than certain mixtures of isopropyl alcohol and water.

Isopropyl alcohol-induced coagulation of proteins occurs at the cell wall, the cytoplasmic membrane and among the various plasma proteins. Coagulation of enzymatic proteins leads to

loss of cellular functions. The interaction of isopropyl alcohol with proteins raises the issue of interference between the antiseptic and serum proteins or proteinaceous soils. Isopropyl alcohol is known to have immediate activity against Gram-positive and Gram-negative bacteria (1, 2).

**3.2. Study ZX-ZP-0014 (b) (4) 130734-202). “Determination of the Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of Two Test Products, One Active Ingredient, One Reference Product, and One Negative Control When Challenged With Various Microorganism Strains”**

This study, a Minimum Bactericidal Concentration and Minimum Bactericidal Concentration evaluation of two test products (ZuraGard and ZuraGard’s vehicle), one active ingredient (70% Isopropyl alcohol), one reference product (ChloraPrep), and one negative control (0.9% sodium chloride), was performed using modifications of the Macrodilution Broth Method outlined in CLSI Document M07-A9, “Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Ninth Edition,” as well as CLSI Document M26-A, “Methods for Determining Bactericidal Activity of Antimicrobial Agents (September 1999).” Each test material was evaluated against a total of 180 different microorganism strains, 2 American Type Culture Collection (ATCC) strains and 10 clinical isolates of each of the following species:

*Burkholderia cepacia*,  
*Candida albicans*,  
*Enterococcus faecalis*,  
*Vancomycin-Resistant Enterococcus faecalis (VRE)*,  
*Enterococcus faecium*,  
*Escherichia coli*,  
*Klebsiella pneumoniae*,  
*Pseudomonas aeruginosa*,  
*Serratia marcescens*,  
*Staphylococcus aureus*,  
*Methicillin-Resistant Staphylococcus aureus (MRSA)*,  
*Staphylococcus epidermidis*,  
*Methicillin-Resistant Staphylococcus epidermidis (MRSE)*,  
*Streptococcus pneumoniae*,  
*Streptococcus pyogenes*.

Briefly, for each strain’s inoculum preparation, lyophilized cultures were grown on appropriate solid growth medium for 24 hours. Three consecutive subcultures were performed on solid growth media for each strain. A suspension of each challenge strain was prepared in saline and exposed to each of 13 doubling dilutions of each test product prepared in appropriate nutrient broth to yield a final concentration of (b) (4) CFU/mL. Following a 24-48-hour incubation period, the MIC of each product was determined visually and documented. The MIC of each test material against each of the microorganism strains was recorded as the highest dilution of test material that completely inhibits growth of the microorganism as detected by visual examination without any aid. Subsequently, aliquots of the three highest dilutions of each product that exhibited no visually detectable growth of the challenge strain were neutralized and subcultured using agar media. Following incubation, the agar subcultures were examined, and the MBC of each product was reported as the highest dilution (lowest product

concentration) resulting in a  $\geq 3.0$  Log<sub>10</sub> ( $\geq 99.9\%$ ) reduction in the population of the challenge strain.

A neutralization verification was performed to demonstrate that the neutralizing solution, Butterfield's Phosphate Buffer solution with product neutralizers including 1.17% lecithin and 10% polysorbate 80 (BBP++) effectively quenched the antimicrobial activity of the test materials (section 13 of study report in the submission).

***Reviewer's comments: For the Sponsor Type B meeting of April 16, 2013, FDA communicated that "Due to the short contact time of antiseptics, we no longer consider minimum inhibitory antimicrobial activity relevant to the use of these products. Instead, you will need to evaluate the spectrum of the bactericidal activity, and demonstrate the effective concentration range, i.e., dose response of your product. Minimum bactericidal concentrations (MBCs) should be determined for a variety of clinically relevant organisms (refer to the TFM as a guide)."***

***This reviewer notes that the Sponsor has included clinically important drug - resistant strains (e.g., methicillin resistant Staphylococcus aureus (MRSA), methicillin - resistant Staphylococcus epidermidis (MRSE), and vancomycin - resistant Enterococcus (VRE)) in its list of microorganisms to be tested, and two ATCC reference strains and ten representative clinical isolates for each organism. We provided comments on this protocol to the Sponsor on July 10, 2013 and determined that the selection of organisms is acceptable. The Sponsor has used clinical isolates obtained from*** (b) (4)

***This is acceptable.*** (b) (4)

***The Sponsor has subcultured each strain three times before preparing the inoculum. This reviewer finds this acceptable since subculturing the challenge organisms is an important step to bring the culture into log phase. The Sponsor has performed the neutralizer validation study in accordance with recommendations in the ASTM standard: "ASTM E 1054-08, Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents", this is acceptable.***

**MIC-MBC Results (Study ZX-ZP-0014, (b) (4) 130734-202):**

On October 29, 2018, the Agency sent an information request (IR) to the Sponsor regarding study 130734-202, asking the Sponsor to submit the MIC and MBC results for each organism in a tabular format, including the individual organism results obtained with each arm tested. FDA also asked for the submission of the MIC and MBC values expressed in concentration units (e.g.,  $\mu\text{g/mL}$ ) for each article tested, including individual organism results obtained with each arm tested.

The Sponsor responded on November 13, 2018, providing the results as requested. The results were presented in module 5, under study report 130734-202, and in module 2 under the "summary of clinical efficacy". Table 6 below represents an example of MIC and MBC results for *Candida albicans*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*.

**Table 6. MIC and MBC summary results of *Candida albicans*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae* and *Streptococcus pyogenes* (Source: IR response dated Nov 13, 2018)**

Organism	ZuraPrep (dilution)		Vehicle (dilution)		70% IPA (dilution)		ChloraPrep (dilution)		ZuraPrep (b) (4) ug/mL IPA		Vehicle (b) (4) ug/mL excipients		70% IPA (b) (4) ug/mL IPA		ChloraPrep (b) (4) ug/mL IPA		ChloraPrep (b) (4) ug/mL CHG	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>Candida albicans</i> (ATCC #10231)	1:16	1:16	1:8	1:2	1:8	1:8	1:32	1:32	34,375	34,375	6,375	25,500	68,750	68,750	17,188	17,188	625	625
<i>Candida albicans</i> (ATCC #14053)	1:4	1:4	1:4	1:2	1:8	1:8	1:32	1:32	137,500	137,500	12,750	25,500	68,750	68,750	17,188	17,188	625	625
<i>Candida albicans</i> (BSL1 #112613Ca1)	1:8	1:8	1:4	1:2	1:4	1:4	1:32	1:32	68,750	68,750	12,750	25,500	137,500	137,500	17,188	17,188	625	625
<i>Candida albicans</i> (BSL1 #112613Ca2)	1:8	1:8	1:4	1:2	1:4	1:4	1:32	1:32	68,750	68,750	12,750	25,500	137,500	137,500	17,188	17,188	625	625
<i>Candida albicans</i> (BSL1 #112613Ca4)	1:16	1:8	1:4	1:2	1:2	1:2	1:32	1:16	34,375	68,750	12,750	25,500	275,000	275,000	17,188	34,375	625	1,250
<i>Staphylococcus epidermidis</i> (BSL1 #112613MSE1) (b) (4)	1:32	1:16	1:64	<1:16	1:16	1:4	>1:8192	1:2048	17,188	34,375	797	>3188	34,375	137,500	<67	269	<2.4	9.8
<i>Staphylococcus epidermidis</i> (BSL1 #112613MSE4) (b) (4)	1:128	<1:32	1:512	<1:128	1:16	1:4	>1:8192	1:2048	4,297	>17,188	100	>398	34,375	137,500	<67	269	<2.4	9.8
<i>Staphylococcus epidermidis</i> (BSL1 #112613MSE5) (b) (4)	1:32	1:16	1:16	<1:4	1:16	1:8	>1:8192	<1:2048	17,188	34,375	3,188	>12,750	34,375	68,750	<67	>269	<2.4	>8.8
<i>Staphylococcus epidermidis</i> (BSL1 #112613MSE6) (b) (4)	1:512	<1:128	1:512	<1:128	1:32	<1:8	>1:8192	<1:2048	1,074	<4,297	100	>398	17,188	>68,750	<67	>269	<2.4	>8.8
<i>Staphylococcus epidermidis</i> (BSL1 #112613MSE7) (b) (4)	1:16	1:16	1:8	1:4	1:16	1:4	>1:8192	<1:2048	34,375	34,375	6,375	12,750	34,375	137,500	<67	>269	<2.4	>8.8
<i>Streptococcus pneumoniae</i> (BSL1 #112613Sp6) (b) (4)	1:64	1:64	1:32	1:32	1:32	1:16	>1:8192	1:4096	8,594	8,594	1,594	1,594	17,188	34,375	<67	134	<2.4	4.9
<i>Streptococcus pneumoniae</i> (BSL1 #112613Sp7) (b) (4)	1:32	1:32	1:64	1:64	>1:32	1:32	1:4096	1:4096	17,188	17,188	797	797	<17,188	17,188	134	134	4.9	4.9
<i>Streptococcus pneumoniae</i> (BSL1 #112613Sp8) (b) (4)	1:32	1:32	1:32	1:32	1:16	1:8	>1:8192	1:4096	17,188	17,188	1,594	1,594	34,375	68,750	<67	134	<2.4	4.9
<i>Streptococcus pneumoniae</i> (BSL1 #112613Sp9) (b) (4)	1:64	1:32	1:64	1:32	1:16	1:8	1:4096	1:4096	8,594	17,188	797	1,594	34,375	68,750	134	134	4.9	4.9
<i>Streptococcus pneumoniae</i> (BSL1 #112613Sp10) (b) (4)	1:32	1:32	1:64	1:64	>1:32	1:32	>1:8192	1:4096	17,188	17,188	797	797	<17,188	17,188	<67	134	<2.4	4.9
<i>Streptococcus pyogenes</i> (ATCC #12344) (b) (4)	1:64	1:64	1:64	1:32	1:16	1:8	1:2048	1:512	8,594	8,594	797	1,594	34,375	68,750	269	1,074	9.8	39
<i>Streptococcus pyogenes</i> (ATCC #15615) (b) (4)	1:64	1:64	1:64	1:32	1:16	1:8	1:2048	<1:512	8,594	8,594	797	1,594	34,375	68,750	269	>1,074	9.8	>39
<i>Streptococcus pyogenes</i> (BSL1 #060613Sp1) (b) (4)	1:64	1:32	1:64	1:32	1:16	1:8	1:2048	<1:512	8,594	17,188	797	1,594	34,375	68,750	269	>1,074	9.8	>39
<i>Streptococcus pyogenes</i> (BSL1 #060613Sp2) (b) (4)	1:64	1:64	1:64	1:32	1:16	1:8	1:2048	1:2048	8,594	8,594	797	1,594	34,375	68,750	269	269	9.8	9.8
<i>Streptococcus pyogenes</i> (BSL1 #060613Sp3) (b) (4)	1:64	1:64	1:32	1:32	1:16	1:8	1:2048	<1:512	8,594	8,594	1,594	1,594	34,375	68,750	269	>1,074	9.8	>39

**Table 7. Minimum Bactericidal Concentration Against 180 Different Microorganism Strains for ZuraGard, ZuraGard's Vehicle, Isopropyl Alcohol, and ChloraPrep (Source: Table 2.7.3-8, study 130734-202, module 2, summary of clinical efficacy)**

Compound	MBC	# Strains	Microorganism Strains
ZuraPrep	<1:128	2	<i>S. epidermidis</i> (b) (4) #112613MSE6), <i>S. epidermidis</i> MRSE (b) (4) #060613MRSE1)
	1:64	10	4 <i>S. pneumoniae</i> , 6 <i>S. pyogenes</i>
	1:32	26	<i>E. faecalis</i> VRE (b) (4) #112613VREfs2), 10 <i>P. aeruginosa</i> , <i>S. marcescens</i> (b) (4) #112613 Sm6), <i>S. aureus</i> (ATCC #6538), <i>S. epidermidis</i> (ATCC #12228), 8 <i>S. pneumoniae</i> , 4 <i>S. pyogenes</i>
	<1:32	2	2 <i>S. epidermidis</i>
	1:16	115	12 <i>B. cepacia</i> , <i>C. albicans</i> (ATCC #10231), 12 <i>E. faecalis</i> , 10 <i>E. faecalis</i> VRE, 9 <i>E. faecium</i> , 12 <i>E. coli</i> , 11 <i>K. pneumoniae</i> , 2 <i>P. aeruginosa</i> , 11 <i>S. marcescens</i> , 8 <i>S. aureus</i> , 11 <i>S. aureus</i> MRSA, 8 <i>S. epidermidis</i> , 6 <i>S. epidermidis</i> MRSE, 2 <i>S. pyogenes</i>
	1:8	20	9 <i>C. albicans</i> , <i>E. faecalis</i> VRE (ATCC #51299), 2 <i>E. faecium</i> , <i>K. pneumoniae</i> (b) (4) #060613Kpn7), 2 <i>S. aureus</i> , <i>S. aureus</i> MRSA (b) (4) #060613MRSA8), 4 <i>S. epidermidis</i> MRSE
	<1:8	1	<i>E. faecium</i> (b) (4) #112613VSEfm2)
	1:4	4	2 <i>C. albicans</i> , <i>S. aureus</i> (ATCC #29213), <i>S. epidermidis</i> MRSE (b) (4) #112613MRSE6)
ZuraPrep Vehicle	<1:128	3	2 <i>S. epidermidis</i> , <i>S. epidermidis</i> MRSE (b) (4) #060613MRSE1)
	1:64	2	2 <i>S. pneumoniae</i>
	1:32	18	6 <i>S. pneumoniae</i> , 12 <i>S. pyogenes</i>
	<1:32	1	<i>S. epidermidis</i> MRSE (b) (4) #060613MRSE5)
	1:16	9	<i>B. cepacia</i> (b) (4) #112613Bcl), 4 <i>P. aeruginosa</i> , <i>S. epidermidis</i> (ATCC #12228), 3 <i>S. pneumoniae</i>
	<1:16	1	<i>S. epidermidis</i> (b) (4) #112613MSE3)
1:8	8	<i>E. faecalis</i> (b) (4) #112613VSEfs3), <i>E. coli</i> (ATCC #25922), <i>P. aeruginosa</i> (b) (4) #060613Pa5), 2 <i>S. marcescens</i> , 2 <i>S. epidermidis</i> , <i>S. pneumoniae</i>	

**Table 7 (continued). Minimum Bactericidal Concentration Against 180 Different Microorganism Strains for ZuraGard, ZuraGard’s Vehicle, Isopropyl Alcohol, and ChloroPrep (Source: Table 2.7.3-8, study 130734-202, module 2, summary of clinical efficacy)**

Compound	MBC	# Strains	Microorganism Strains
	<1:8	9	(ATCC #49619) <i>E. faecalis</i> (b)(4)#112613VSEfs7), 5 <i>S. aureus</i> MRSA, 3 <i>S. epidermidis</i> MRSE
	1:4	68	10 <i>B. cepacia</i> , 5 <i>E. faecalis</i> , 7 <i>E. faecalis</i> VRE, 7 <i>E. faecium</i> , 6 <i>E. coli</i> , 3 <i>K. pneumoniae</i> , 3 <i>P. aeruginosa</i> , 8 <i>S. marcescens</i> , 4 <i>S. aureus</i> , 4 <i>S. aureus</i> MRSA, 5 <i>S. epidermidis</i> , 6 <i>S. epidermidis</i> MRSE
	<1:4	34	5 <i>E. faecalis</i> , 4 <i>E. faecalis</i> VRE, 5 <i>E. faecium</i> , 5 <i>E. coli</i> , 3 <i>K. pneumoniae</i> , 4 <i>P. aeruginosa</i> , 3 <i>S. aureus</i> , 3 <i>S. aureus</i> MRSA, <i>S. epidermidis</i> (b)(4)#112613MSSe5), <i>S. epidermidis</i> MRSE (b)(4)#112613MRSE6)
	1:2	19	10 <i>C. albicans</i> , <i>E. faecalis</i> VRE (b)(4)#112613VREfs4), 3 <i>K. pneumoniae</i> , 5 <i>S. aureus</i>
	<1:2	8	<i>B. cepacia</i> (ATCC #25416), 2 <i>C. albicans</i> , 3 <i>K. pneumoniae</i> , 2 <i>S. marcescens</i>
Isopropyl Alcohol	1:32	2	2 <i>S. pneumoniae</i>
	1:16	7	<i>E. coli</i> (ATCC #11229), <i>K. pneumoniae ozaenae</i> (ATCC #11296), <i>S. epidermidis</i> (ATCC #12228), 4 <i>S. pneumoniae</i>
	1:8	81	10 <i>B. cepacia</i> , 5 <i>C. albicans</i> , 11 <i>E. coli</i> , 11 <i>K. pneumoniae</i> , 11 <i>P. aeruginosa</i> , 12 <i>S. marcescens</i> , 2 <i>S. epidermidis</i> , <i>S. epidermidis</i> MRSE (b)(4)#060613MRSE5), 6 <i>S. pneumoniae</i> , 12 <i>S. pyogenes</i>
	<1:8	13	<i>B. cepacia</i> (ATCC #25416), 6 <i>E. faecium</i> , <i>P. aeruginosa</i> (b)(4)#060613Pa6), <i>S. epidermidis</i> (b)(4)#112613MSSe6), 4 <i>S. epidermidis</i> MRSE
	1:4	74	<i>B. cepacia</i> (BSLI #112613Bc1), 5 <i>C. albicans</i> , 11 <i>E. faecalis</i> , 12 <i>E. faecalis</i> VRE, 6 <i>E. faecium</i> , 12 <i>S. aureus</i> , 12 <i>S. aureus</i> MRSA, 8 <i>S. epidermidis</i> , 7 <i>S. epidermidis</i> MRSE
	1:2	3	2 <i>C. albicans</i> , <i>E. faecalis</i> (b)(4)#112613VSEfs7).

Compound	MBC	# Strains	Microorganism Strains
ChloroPrep	1:8192	2	<i>S. epidermidis</i> (ATCC #12228), <i>S. pneumoniae</i> (ATCC #49136)
	1:4096	17	2 <i>E. coli</i> , <i>K. pneumoniae</i> (b)(4)#060613Kpn3), 3 <i>S. aureus</i> , 2 <i>S. epidermidis</i> MRSE, 9 <i>S. pneumoniae</i>
	1:2048	25	3 <i>E. faecalis</i> , <i>E. faecalis</i> VRE (BSLI #112613VREfs9), <i>E. faecium</i> (b)(4)#112613VREfs10), 3 <i>E. coli</i> , <i>K. pneumoniae ozaenae</i> (ATCC #11296), 3 <i>S. aureus</i> , 3 <i>S. aureus</i> MRSA, 2 <i>S. epidermidis</i> , 5 <i>S. epidermidis</i> MRSE, 3 <i>S. pyogenes</i>
	<1:2048	62	<i>B. cepacia</i> (b)(4)#112613Bc1), 9 <i>E. faecalis</i> , 5 <i>E. faecalis</i> VRE, 11 <i>E. faecium</i> , 5 <i>E. coli</i> , 2 <i>K. pneumoniae</i> , 6 <i>S. aureus</i> , 9 <i>S. aureus</i> MRSA, 9 <i>S. epidermidis</i> , 5 <i>S. epidermidis</i> MRSE
	1:1024	7	5 <i>E. faecalis</i> VRE, <i>S. marcescens</i> (b)(4)#112613Sm2), <i>S. pyogenes</i> (b)(4)#060613Spy6)
	<1:1024	4	<i>E. faecalis</i> VRE (b)(4)#112613VREfs10), <i>E. coli</i> (b)(4)#060613Ec3), <i>K. pneumoniae</i> (b)(4)#060613Kpn2), <i>P. aeruginosa</i> (b)(4)#060613Pa9)
	1:512	12	<i>B. cepacia</i> (b)(4)#112613Bc3), <i>E. coli</i> (b)(4)#060613Ec9), <i>K. pneumoniae</i> (b)(4)#060613Kpn8), 3 <i>P. aeruginosa</i> , 4 <i>S. marcescens</i> , 2 <i>S. pyogenes</i>
	<1:512	16	3 <i>B. cepacia</i> , 3 <i>K. pneumoniae</i> , 3 <i>P. aeruginosa</i> , <i>S. marcescens</i> (b)(4)#112613Sm4), 6 <i>S. pyogenes</i>
	1:256	9	2 <i>B. cepacia</i> , <i>K. pneumoniae</i> (b)(4)#060613Kpn7), 2 <i>P. aeruginosa</i> , 4 <i>S. marcescens</i>
	<1:256	6	2 <i>B. cepacia</i> , 2 <i>K. pneumoniae</i> , 2 <i>P. aeruginosa</i>
	1:128	4	<i>B. cepacia</i> (b)(4)#120413Bc3), 2 <i>S. marcescens</i> , <i>S. pneumoniae</i> (ATCC #49619)
	<1:128	2	<i>B. cepacia</i> (b)(4)#120413Bc6), <i>P. aeruginosa</i> (b)(4)#060613Pa6)
	<1:64	1	<i>B. cepacia</i> (b)(4)#120413Bc4)
	1:32	8	7 <i>C. albicans</i> , <i>S. pneumoniae</i> (b)(4)#112613Spn1)
	1:16	4	4 <i>C. albicans</i>
	<1:8	1	<i>C. albicans</i> (b)(4)#112613Ca5)

Abbreviations: ATCC = American Type Culture Collection; (b)(4)MBC = minimum bactericidal concentration; MRSA – methicillin-resistant *S. aureus*; MRSE – methicillin-resistant *S. epidermidis*; VRE = vancomycin-resistant enterococcus

Source: Study 130734-202 Report Executive Summary

***Reviewer’s Comments:*** *The Sponsor provides in vitro susceptibility test results on the bacterial and yeast species listed in the 1994 TFM. The number of isolates for each species and ATCC strains to be tested to demonstrate in vitro effectiveness (per Agency’s July 10, 2013 advice letter) was met for the majority of the species. The test product ZuraGard was bactericidal for 155 of the 180 strains when diluted 1:16. On the other hand, ZuraGard’s Vehicle was bactericidal for 33 of the 180 strains when diluted 1:16, suggesting the vehicle may have weak subtherapeutic activity. Note that the time-kill and in vivo effectiveness results corroborate that the vehicle does not show significant activity on its own, whereas the final product shows a satisfactory activity level (see below). In conclusion, as shown above in Table 6 (source Sponsor’s IR response dated November 13, 2018), MBCs were obtained for all the isolates tested. The MBC range for ZuraGard was >4,297 µg/mL to 137,500 µg/mL; the MBC for 70% IPA ranged from 17,188 µg/mL to 275, 000 µg/mL. These MBC values are well below the actual use concentration of the active ingredient, 70% IPA ( (b) (4) µg/mL).*

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**Neutralization Validation Results (Study ZX-ZP-0014. (b) (4) 130734-202):**

A neutralization verification was performed to demonstrate that the neutralizing solution, Butterfield's Phosphate Buffer solution with product neutralizers including 1.17% lecithin and 10% polysorbate 80 (BBP++) effectively quenched the antimicrobial activity of the test materials. The challenge species used for the neutralization verification procedures included: *Burkholderia cepacia* (ATCC #25416), *Candida albicans* (ATCC #10231), *Enterococcus faecalis* (ATCC #29212), *Enterococcus faecalis* VRE, MDR (ATCC #51575), *Enterococcus faecium*, VRE, MDR (ATCC #51559), *Escherichia coli* (ATCC #11229), *Klebsiella pneumoniae ozaenae* (ATCC #11296), *Pseudomonas aeruginosa* (ATCC #15442), *Serratia marcescens* (ATCC #14756), *Staphylococcus aureus* (ATCC #6538), *Staphylococcus aureus* MRSA (ATCC #33592), *Staphylococcus epidermidis* (ATCC #12228), *Staphylococcus epidermidis* MRSE (ATCC #51625), *Streptococcus pneumoniae* (ATCC #49619), and *Streptococcus pyogenes* (ATCC #19615). Detailed results of the neutralization validation are presented in module 5 under study report of 130734-202. Table 8 below shows *Burkholderia cepacia* (ATCC #25416) as a representative example.

**Table 8. Results of neutralization validation of *Burkholderia cepacia* (ATCC #25416) (Source: Table 1, study report of 130734-202)**

Challenge Microorganism (ATCC #)	Test Description	Product Dilution	Log <sub>10</sub> (CFU)	Neutralization Effective? (Yes/No)❶
<i>Burkholderia cepacia</i> (ATCC #25416)	Growth Control	N/A	2.2095	N/A
	Test Product #1: ZuraPrep™ – Drug Product Lot Number ZP0004A	1:8	2.3424	Yes
		1:16	2.3502	Yes
		1:32	2.3424	Yes
	Test Product #2: ZuraPrep without Isopropyl Alcohol Lot Number XP0002	1:2	2.3979	Yes
		1:4	2.3502	Yes
		1:8	2.3674	Yes
	Active Ingredient #1: Isopropyl Alcohol (70%) Lot Number 09/18/2013	1:8	2.3444	Yes
		1:16	2.3032	Yes
		1:32	2.3096	Yes
	Reference Product: ChlorPrep® Lot Number 65404	1:128	2.3560	Yes
		1:256	2.3075	Yes
		1:512	2.3139	Yes
	Negative Control: 0.9% Sodium Chloride Irrigation, USP Lot Number 29-610-4B-01	1:2	2.3096	Yes
		1:4	2.3522	Yes
1:8		2.3345	Yes	

***Reviewer's comments:*** Similar to the case of *Burkholderia cepacia* (ATCC #25416), no significant statistical difference was found between the average log<sub>10</sub> values of the growth control and the average log<sub>10</sub> values for test products ZuraGard, ZuraGard's vehicle, active control (ChloroPrep), and active ingredient alone (70% IPA). This reviewer finds the Sponsor's neutralization validation results acceptable.

**3.3. Study (b) (4) 130548-201: Determination of the Dose-Response of Various Microorganism Strains to One Test Product, Five Active Ingredients, and Two Controls Using an In Vitro Time-Kill Procedure**

Study 130548-201 is an in vitro time-kill kinetic evaluation of ZuraGard test product, 5 ingredients (citrate (b) (4) solution, methylene blue solution, methylparaben solution, propylparaben solution, isopropyl alcohol), and 2 controls (0.9% sodium chloride irrigation, United States Pharmacopeia (USP), and purified water), versus suspensions of 15 different microorganism strains (15 American Type Culture Collection strains): *Burkholderia cepacia*, *Candida albicans*, *Enterococcus faecalis*, vancomycin-resistant *Enterococcus faecalis*, multidrug- and vancomycin-resistant *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae ozaenae*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*.

Test product ZuraGard and the 5 ingredients were evaluated at concentrations of 99% (v/v), 75% (v/v), 50% (v/v), and 25% (v/v); the controls were evaluated at a single concentration, 99% (v/v). The percent and log<sub>10</sub> reductions from the initial population of each challenge microorganism were determined following 30-second, 60-second, 120-second, and 5-minute exposures to each test material. All agar plating was performed in triplicate. Test materials were considered bactericidal at the concentration and contact time that demonstrated a 3 log<sub>10</sub> (99.9%) or greater reduction in bacterial viability as compared to the initial inoculum.

ZuraGard achieved a  $\geq 3$  log<sub>10</sub> reduction from baseline for all bacterial species evaluated, demonstrating a broad antimicrobial activity at all time points tested (source, Table 2.7.3-5 from submission). Every individual ingredient alone, with the exception of isopropyl alcohol, failed to achieve a 3 log<sub>10</sub> reduction from baseline that would be considered bactericidal. Thus, the results from this dose-response study confirm that ZuraGard contains only one therapeutically active ingredient, 70% v/v isopropyl alcohol.

**Table 9. Summary of Bactericidal Effectiveness Against 15 Different Microorganism Strains for ZuraGard, the Individual Components of ZuraGard, 0.9% Sodium Chloride, and Purified Water (In vitro Time-Kill Dose-Response Study) (Source: Table 2.7.3.5, study 130548-201, module 2)**

Compound	Exposure	Number of Strains With $\geq 3$ log <sub>10</sub> Reduction			
		99% (v/v)	75% (v/v)	50% (v/v)	25% (v/v)
ZuraPrep	30 seconds	15	15	15	12
	60 seconds	15	15	15	14
	120 seconds	15	15	15	14
	5 minutes	15	15	15	14
Citrate (b) (4)	30 seconds	1	1	1	1
	60 seconds	1	1	1	1
	120 seconds	3	3	2	2
	5 minutes	5	6	5	5
(b) (4) Methylene Blue	30 seconds	0	0	0	0
	60 seconds	0	0	0	0
	120 seconds	0	0	0	0
	5 minutes	1	0	0	0
(b) (4) Methylparaben	30 seconds	1	0	0	0
	60 seconds	1	0	0	0
	120 seconds	2	1	0	0
	5 minutes	3	1	2	0
(b) (4) Propylparaben	30 seconds	0	0	0	0
	60 seconds	1	0	0	0
	120 seconds	2	2	1	0
	5 minutes	3	2	1	0
70% v/v Isopropyl Alcohol	30 seconds	15	15	15	0
	60 seconds	15	15	15	0
	120 seconds	15	15	15	0
	5 minutes	15	15	15	2
(b) (4)	30 seconds	0			
	60 seconds	0			
	120 seconds	0			
	5 minutes	0			
(b) (4) Purified Water	30 seconds	0			
	60 seconds	0			
	120 seconds	0			
	5 minutes	1			
(b) (4)					

Source: Study 130548-201 Report Table 11 through Table 36.

***Reviewer’s comments: During April 16, 2013’s face-to-face meeting, we informed the Sponsor that, “Since it is not clear how many ingredients are contributing to the efficacy of the final formulation, we suggest that you perform a Time-Kill study similar to the proof-of concept study included in your briefing package in appendix D to assess the contribution of each component. All of the single ingredients in the study should be tested at the same concentration and at the same pH (b) (4) as in the to-be-marketed product. (b) (4)***

(b) (4)

(b) (4) *Based on the results of these Time-Kill studies, we can s should be considered active ingredients and discuss the in vivo study design at that time. We also remind you that you will need to satisfy the agency's combination policy (21 CFR 330.10(a)(4)(iv)) for all active ingredients,*

*As shown in Table 9 above, both ZuraGard test product and 70% v/v isopropyl alcohol produced a  $\geq 3$  log<sub>10</sub> CFU/cm<sup>2</sup> reduction in 15 of the 15-bacterial species at 30 seconds to 5 minutes at 99%, 75% and 50% v/v concentrations. However, excipients citrate (b) (4) and methylparaben showed antimicrobial activity in only one out of 15 microorganisms tested at 30 seconds. For the excipients, time kill activity was dependent on concentration and exposure time, and none of the excipients achieved the time-kill activity against all 15 bacterial strains tested. The results from this dose-response study demonstrate that the excipients of ZuraGard, although may show some antimicrobial activity against a few organisms, do not exhibit activity against all 15 strains tested, and that isopropyl alcohol is mainly responsible for the activity of the final product.*

**3.4. Study ZX-ZP-0015 ( (b) (4) 130733-201): An In Vitro Time-Kill Evaluation of Two Test Products, One Active Ingredient, One Reference Product, and One Negative Control for Their Antimicrobial Properties When Challenged With Various Microorganism Strains**

Time-kill studies were performed to demonstrate the in vitro bactericidal and fungicidal activity of the test product (ZuraGard), ZuraGard's Vehicle, reference active product (ChloroPrep), and a negative control (0.9% Sodium Chloride Irrigation. USP). Each product was evaluated at a 99% (v/v) concentration. The percent and log<sub>10</sub> reductions from the initial population of each challenge microorganism were determined following 30-second, 60-second, and 120-second exposures to each product. All agar-plating was performed in triplicate.

**Table 10. List of ATCC Challenge Microorganisms (Source: study report 130733-201)**

8.1	<i>Acinetobacter baumannii</i> (ATCC #19003)
8.2	<i>Acinetobacter baumannii</i> (ATCC #19606)
8.3	<i>Acinetobacter baumannii</i> MDR (ATCC #BAA-1789)
8.4	<i>Acinetobacter baumannii</i> MDR (ATCC #BAA-1790)
8.5	<i>Acinetobacter lwoffii</i> (ATCC #15309)
8.6	<i>Acinetobacter lwoffii</i> (ATCC #17925)
8.7	<i>Bacteroides fragilis</i> (ATCC #25285)
8.8	<i>Bacteroides fragilis</i> (ATCC #29762)
8.9	<i>Burkholderia cepacia</i> (ATCC #25416)
8.10	<i>Burkholderia cepacia</i> (ATCC #25608)
8.11	<i>Burkholderia cepacia</i> (Clinical Isolate; (b) (4) #112613Bc2)
8.12	<i>Burkholderia cepacia</i> (Clinical Isolate; (b) (4) #112613Bc3)
8.13	<i>Burkholderia cepacia</i> (Clinical Isolate; (b) (4) #120413Bc4)
8.14	<i>Burkholderia cepacia</i> (Clinical Isolate; (b) (4) #120413Bc6)
8.15	<i>Candida albicans</i> (ATCC #10231)
8.16	<i>Candida albicans</i> (ATCC #14053)
8.17	<i>Candida albicans</i> (Clinical Isolate; (b) (4) #112613Ca1)
8.18	<i>Candida albicans</i> (Clinical Isolate; (b) (4) #112613Ca2)
8.19	<i>Candida albicans</i> (Clinical Isolate; (b) (4) #112613Ca4)
8.20	<i>Candida albicans</i> (Clinical Isolate; (b) (4) #112613Ca5)
8.21	<i>Candida tropicalis</i> (ATCC #750)
8.22	<i>Candida tropicalis</i> (ATCC #13803)
8.23	<i>Corynebacterium jeikeium</i> (ATCC #43216)
8.24	<i>Corynebacterium jeikeium</i> (ATCC #43734)
8.25	<i>Enterobacter aerogenes</i> (ATCC #13048)
8.26	<i>Enterobacter aerogenes</i> (ATCC #51697)
8.27	<i>Enterobacter cloacae cloacae</i> (ATCC #13047)
8.28	<i>Enterobacter cloacae cloacae</i> (ATCC #35588)
8.29	<i>Enterococcus faecalis</i> (ATCC #19433)
8.30	<i>Enterococcus faecalis</i> (ATCC #29212)
8.31	<i>Enterococcus faecalis</i> VRE (ATCC #51299)
8.32	<i>Enterococcus faecalis</i> VRE, MDR (ATCC #51575)
8.33	<i>Enterococcus faecalis</i> VRE (Clinical Isolate; (b) (4) #112613VREfs1)
8.34	<i>Enterococcus faecalis</i> VRE (Clinical Isolate; (b) (4) #112613VREfs2)
8.35	<i>Enterococcus faecalis</i> VRE (Clinical Isolate; (b) (4) #112613VREfs3)
8.36	<i>Enterococcus faecalis</i> VRE (Clinical Isolate; (b) (4) #112613VREfs5)
8.37	<i>Enterococcus faecium</i> VRE, MDR (ATCC #51559)
8.38	<i>Enterococcus faecium</i> VRE (ATCC #700221)
8.39	<i>Enterococcus faecium</i> (Clinical Isolate; (b) (4) #112613VSEfm1)

MDR = Multi-Drug Resistant  
VRE = Vancomycin-Resistant *Enterococcus*

**Table 10 (continued). List of ATCC Challenge Microorganisms (Source: study report 130733-201)**

8.40	<i>Enterococcus faecium</i> (Clinical Isolate; (b) (4) #112613VSEfm2)
8.41	<i>Enterococcus faecium</i> (Clinical Isolate; (b) (4) #112613VSEfm3)
8.42	<i>Enterococcus faecium</i> (Clinical Isolate; (b) (4) #112613VSEfm5)
8.43	<i>Escherichia coli</i> (ATCC #8739)
8.44	<i>Escherichia coli</i> (ATCC #10798)
8.45	<i>Escherichia coli</i> (ATCC #11229)
8.46	<i>Escherichia coli</i> (ATCC #25922)
8.47	<i>Escherichia coli</i> MDR, ESBL (ATCC #BAA-196)
8.48	<i>Escherichia coli</i> MDR, ESBL (ATCC #BAA-200)
8.49	<i>Escherichia coli</i> serotype O157:H7 (ATCC #35150)
8.50	<i>Escherichia coli</i> serotype O157:H7 (ATCC #43888)
8.51	<i>Escherichia coli</i> (Clinical Isolate; (b) (4) #060613Ec1)
8.52	<i>Escherichia coli</i> (Clinical Isolate; (b) (4) #060613Ec2)
8.53	<i>Escherichia coli</i> (Clinical Isolate; (b) (4) #060613Ec3)
8.54	<i>Escherichia coli</i> (Clinical Isolate; (b) (4) #060613Ec4)
8.55	<i>Haemophilus influenzae</i> (ATCC #8149)
8.56	<i>Haemophilus influenzae</i> (ATCC #33930)
8.57	<i>Klebsiella oxytoca</i> (ATCC #13182)
8.58	<i>Klebsiella oxytoca</i> (ATCC #15764)
8.59	<i>Klebsiella pneumoniae ozaenae</i> (ATCC #11296)
8.60	<i>Klebsiella pneumoniae ozaenae</i> (ATCC #11297)
8.61	<i>Klebsiella pneumoniae</i> (Clinical Isolate; (b) (4) #060613Kpn1)
8.62	<i>Klebsiella pneumoniae</i> (Clinical Isolate; (b) (4) #060613Kpn2)
8.63	<i>Klebsiella pneumoniae</i> (Clinical Isolate; (b) (4) #060613Kpn3)
8.64	<i>Klebsiella pneumoniae</i> (Clinical Isolate; (b) (4) #060613Kpn4)
8.65	<i>Listeria monocytogenes</i> (ATCC #7644)
8.66	<i>Listeria monocytogenes</i> (ATCC #15313)
8.67	<i>Micrococcus luteus</i> (ATCC #4698)
8.68	<i>Micrococcus luteus</i> (ATCC #7468)
8.69	<i>Proteus mirabilis</i> (ATCC #7002)
8.70	<i>Proteus mirabilis</i> (ATCC #35659)
8.71	<i>Proteus vulgaris</i> (ATCC #6380)
8.72	<i>Proteus vulgaris</i> (ATCC #49132)
8.73	<i>Pseudomonas aeruginosa</i> (ATCC #9027)
8.74	<i>Pseudomonas aeruginosa</i> (ATCC #15442)
8.75	<i>Pseudomonas aeruginosa</i> (ATCC #25619)
8.76	<i>Pseudomonas aeruginosa</i> (ATCC #27853)
8.77	<i>Pseudomonas aeruginosa</i> (Clinical Isolate; (b) (4) #060613Pa1)
8.78	<i>Pseudomonas aeruginosa</i> (Clinical Isolate; (b) (4) #060613Pa2)
8.79	<i>Pseudomonas aeruginosa</i> (Clinical Isolate; (b) (4) #060613Pa3)
8.80	<i>Pseudomonas aeruginosa</i> (Clinical Isolate; (b) (4) #060613Pa4)
8.81	<i>Pseudomonas stutzeri</i> (ATCC #17588)
8.82	<i>Pseudomonas stutzeri</i> (ATCC #17591)
8.83	<i>Salmonella enterica enterica</i> serovar Adelaide (ATCC #10718)
8.84	<i>Salmonella enterica enterica</i> serovar Rubislaw (ATCC #10717)
8.85	<i>Salmonella enterica enterica</i> serovar Enteritidis (ATCC #13076)
8.86	<i>Salmonella enterica enterica</i> serovar Enteritidis (ATCC #31194)
8.87	<i>Salmonella enterica enterica</i> serovar Typhi (ATCC #6539)
8.88	<i>Salmonella enterica enterica</i> serovar Typhi (ATCC #19430)

**Table 10 (continued). List of ATCC Challenge Microorganisms (Source: study report 130733-201)**

8.89	<i>Salmonella enterica enterica</i> serovar Typhimurium (ATCC #14028)
8.90	<i>Salmonella enterica enterica</i> serovar Typhimurium (ATCC #13311)
8.91	<i>Serratia marcescens</i> (ATCC #8100)
8.92	<i>Serratia marcescens</i> (ATCC #14756)
8.93	<i>Serratia marcescens</i> (Clinical Isolate; (b) (4)) #112613Sm1
8.94	<i>Serratia marcescens</i> (Clinical Isolate; (b) (4)) #112613Sm2
8.95	<i>Serratia marcescens</i> (Clinical Isolate; (b) (4)) #112613Sm3
8.96	<i>Serratia marcescens</i> (Clinical Isolate; (b) (4)) #112613Sm4
8.97	<i>Shigella dysenteriae</i> (ATCC #13313)
8.98	<i>Shigella dysenteriae</i> (ATCC #49557)
8.99	<i>Shigella sonnei</i> (ATCC #11060)
8.100	<i>Shigella sonnei</i> (ATCC #25931)
8.101	<i>Staphylococcus aureus</i> MRSA (ATCC #BAA-811)
8.102	<i>Staphylococcus aureus aureus</i> (ATCC #6538)
8.103	<i>Staphylococcus aureus aureus</i> (ATCC #9144)
8.104	<i>Staphylococcus aureus aureus</i> (ATCC #19095)
8.105	<i>Staphylococcus aureus aureus</i> (ATCC #25923)
8.106	<i>Staphylococcus aureus aureus</i> (ATCC #29213)
8.107	<i>Staphylococcus aureus aureus</i> (ATCC #29737)
8.108	<i>Staphylococcus aureus aureus</i> MRSA (ATCC #33591)
8.109	<i>Staphylococcus aureus aureus</i> MRSA (ATCC #33592)
8.110	<i>Staphylococcus aureus aureus</i> MRSA (ATCC #43300)
8.111	<i>Staphylococcus aureus aureus</i> MRSA (ATCC #700698)
8.112	<i>Staphylococcus aureus aureus</i> MRSA (ATCC #700699)
8.113	<i>Staphylococcus aureus</i> MRSA (b) (4) #020714SaNRS123; NARSA Strain NRS123 [USA400]
8.114	<i>Staphylococcus aureus</i> MRSA (b) (4) #083012MRSA2; NARSA Strain NRS382 [USA100]
8.115	<i>Staphylococcus aureus</i> MRSA (b) (4) #020714SaNRS383; NARSA Strain NRS383 [USA200]
8.116	<i>Staphylococcus aureus</i> MRSA (b) (4) #083012MRSA1; NARSA Strain NRS384 [USA300]
8.117	<i>Staphylococcus aureus</i> VRSA (b) (4) #083012VRSA1; NARSA Strain VRS1)
8.118	<i>Staphylococcus aureus</i> VRSA (b) (4) #020714VRSA2; NARSA Strain VRS2)
8.119	<i>Staphylococcus epidermidis</i> (ATCC #12228)
8.120	<i>Staphylococcus epidermidis</i> (ATCC #14990)
8.121	<i>Staphylococcus epidermidis</i> MRSE (ATCC #51625)
8.122	<i>Staphylococcus epidermidis</i> MDR (ATCC #700562)
8.123	<i>Staphylococcus epidermidis</i> MRSE (Clinical Isolate; (b) (4)) #112613MRSE1)
8.124	<i>Staphylococcus epidermidis</i> MRSE (Clinical Isolate; (b) (4)) #112613MRSE2)
8.125	<i>Staphylococcus epidermidis</i> MRSE (Clinical Isolate; (b) (4)) #112613MRSE4)
8.126	<i>Staphylococcus epidermidis</i> MRSE (Clinical Isolate; (b) (4)) #112613MRSE5)
8.127	<i>Staphylococcus epidermidis</i> VISE (b) (4) #020714SeNRS7; NARSA Strain NRS7)
8.128	<i>Staphylococcus epidermidis</i> VISE (b) (4) #020714SeNRS8; NARSA Strain NRS8)
8.129	<i>Staphylococcus haemolyticus</i> (ATCC #29970)
8.130	<i>Staphylococcus haemolyticus</i> (ATCC #43252)
8.131	<i>Staphylococcus hominis hominis</i> (ATCC #27844)
8.132	<i>Staphylococcus hominis hominis</i> (ATCC #27845)
8.133	<i>Staphylococcus saprophyticus</i> (ATCC #35552)
8.134	<i>Staphylococcus saprophyticus</i> (ATCC #49453)

MDR = Multi-Drug Resistant

MRSA = Methicillin-Resistant *Staphylococcus aureus*

MRSE = Methicillin-Resistant *Staphylococcus epidermidis*

VRSA = Vancomycin-Resistant *Staphylococcus aureus*

WISE = Vancomycin-Intermediate *Staphylococcus epidermidis*

NARSA = Network on the Antimicrobial Resistance in *Staphylococcus aureus* (NARSA Program, Herndon, VA)

**Table 10 (continued). List of ATCC Challenge Microorganisms (Source: study report 130733-201)**

8.135	<i>Streptococcus pneumoniae</i> (ATCC #49136)	
8.136	<i>Streptococcus pneumoniae</i> (ATCC #49619)	
8.137	<i>Streptococcus pneumoniae</i> MDR, PRSP (ATCC #700904)	
8.138	<i>Streptococcus pneumoniae</i> MDR, PRSP (ATCC #700905)	
8.139	<i>Streptococcus pneumoniae</i> (Clinical Isolate; (b)(4)	#112613Spn1)
8.140	<i>Streptococcus pneumoniae</i> (Clinical Isolate; (b)(4)	#112613Spn2)
8.141	<i>Streptococcus pneumoniae</i> (Clinical Isolate; (b)(4)	#112613Spn3)
8.142	<i>Streptococcus pneumoniae</i> (Clinical Isolate; (b)(4)	#112613Spn4)
8.143	<i>Streptococcus pyogenes</i> (ATCC #12344)	
8.144	<i>Streptococcus pyogenes</i> (ATCC #19615)	
8.145	<i>Streptococcus pyogenes</i> (Clinical Isolate; (b)(4)	#060613Spy1)
8.146	<i>Streptococcus pyogenes</i> (Clinical Isolate; (b)(4)	#060613Spy2)
8.147	<i>Streptococcus pyogenes</i> (Clinical Isolate; (b)(4)	#060613Spy3)
8.148	<i>Streptococcus pyogenes</i> (Clinical Isolate; (b)(4)	#060613Spy4)

MDR = Multi-Drug Resistant

PRSP = Penicillin-Resistant *Streptococcus pneumoniae*

The study specifies that for inoculum preparation (challenge suspension) of each strain, three subcultures of each challenge strain were performed prior to use in testing. In general, 2-6 days prior to testing, an inoculum from a lyophilized or cryogenic culture was suspended in saline and inoculated onto the surface of the appropriate agar medium. The initial challenge suspension was  $10^9$  CFU per mL. After 1:100 dilution, a final concentration of  $10^7$  CFU per mL was achieved. After achieving the final challenge concentration  $10^7$  CFU, every microorganism was exposed to each test materials for 30, 60 or 120 seconds. After making 10-fold dilutions in neutralizing buffer, samples were plated in pour or spread plates in triplicate. Plates were incubated for the appropriate time and temperature, and then were counted manually. Plates showing 30-300 colonies were used for data collection.

***Reviewer's comments: FDA's current policy for in-vitro testing of an antiseptic new molecular entity is to follow the testing specified in the 1994 TFM (59 FR 31402) along with clinical isolates of the microorganisms to be tested. As shown in Table 10 above, the Sponsor has included an extensive representation of the microorganisms from the TFM list and has also included at least two ATCC strains for each microorganism. Clinical isolates (four) and multidrug resistant strains were also included (Table 10). This reviewer finds the spectrum of microorganisms for the time-kill assay acceptable. The final plated inoculum was defined as  $10^7$ , which is within the range specified in ASTM 2315-03 (at minimum of  $10^6$ /mL); this is acceptable. Also, the time-kill assay evaluated the instant killing of the microorganisms by the test product at the exposure times of 30, 60 and 120 seconds; this is acceptable. The study included triplicate plating for the final product/neutralizer/challenge suspension, which is consistent with our current policy and is acceptable.***

The results are presented in Module 5.3.5.4, 130733-201 study report. Results are summarized in Tables 6-10 of the report. Summarized results (CFU/mL, Percent Reduction,

and Log Reduction) are shown as the average values of all the organisms tested under this study. Results for each individual isolate are included in Addendum 5 in Module 5.3.5.4. Table 11 below shows summary results for a representative gram negative organism, *Burkholderia cepacia*, and a fungus, *Candida albicans*, against ZuraGard.

**Table 11. Summary Results of *Burkholderia cepacia* (clinical isolates) and *Candida albicans* (ATCC reference and clinical isolates) against ZuraGard. Results Expressed as Inoculum level CFU/mL, Average Percent Reduction, and Average Log<sub>10</sub> Reduction (Source: Table 6, study report 130733-201)**

No.	Microorganism Species (ATCC # or (b) (4) # (b) (4) #)	Challenge Suspension Population (CFU/mL)	Inoculum Level (CFU/mL)	Exposure Time	Post-Exposure Population (CFU/mL)	Percent Reduction	Log <sub>10</sub> Reduction
11	<i>Burkholderia cepacia</i> (b) (4) #112613Bc2) (b) (4) #	2.1133 x 10 <sup>9</sup>	2.1133 x 10 <sup>7</sup>	30 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.3250
				60 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.3250
				120 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.3250
12	<i>Burkholderia cepacia</i> (b) (4) #112613Bc3) (b) (4) #	1.9467 x 10 <sup>9</sup>	1.9467 x 10 <sup>7</sup>	30 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.2893
				60 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.2893
				120 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.2893
13	<i>Burkholderia cepacia</i> (b) (4) #120413Bc4) (b) (4) #	7.9667 x 10 <sup>8</sup>	7.9667 x 10 <sup>6</sup>	30 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	5.9013
				60 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	5.9013
				120 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	5.9013
14	<i>Burkholderia cepacia</i> (b) (4) #120413Bc6) (b) (4) #	4.0333 x 10 <sup>8</sup>	4.0333 x 10 <sup>6</sup>	30 seconds	< 1.00 x 10 <sup>1</sup>	99.9998%	5.6057
				60 seconds	< 1.00 x 10 <sup>1</sup>	99.9998%	5.6057
				120 seconds	< 1.00 x 10 <sup>1</sup>	99.9998%	5.6057
15	<i>Candida albicans</i> (ATCC #10231)	1.1467 x 10 <sup>9</sup>	1.1467 x 10 <sup>7</sup>	30 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.0594
				60 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.0594
				120 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.0594
16	<i>Candida albicans</i> (ATCC #14053)	1.270 x 10 <sup>9</sup>	1.270 x 10 <sup>7</sup>	30 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.1038
				60 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.1038
				120 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.1038
17	<i>Candida albicans</i> (b) (4) #112613Ca1) (b) (4) #	1.090 x 10 <sup>9</sup>	1.090 x 10 <sup>7</sup>	30 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.0374
				60 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.0374
				120 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.0374
18	<i>Candida albicans</i> (b) (4) #112613Ca2) (b) (4) #	1.420 x 10 <sup>9</sup>	1.420 x 10 <sup>7</sup>	30 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.1523
				60 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.1523
				120 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.1523
19	<i>Candida albicans</i> (b) (4) #112613Ca4) (b) (4) #	1.5133 x 10 <sup>9</sup>	1.5133 x 10 <sup>7</sup>	30 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.1799
				60 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.1799
				120 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.1799
20	<i>Candida albicans</i> (b) (4) #112613Ca5) (b) (4) #	1.5433 x 10 <sup>9</sup>	1.5433 x 10 <sup>7</sup>	30 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.1885
				60 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.1885
				120 seconds	< 1.00 x 10 <sup>1</sup>	99.9999%	6.1885

**Table 12. Summary of Log<sub>10</sub> Reductions for ZuraGard, 70% v/v Isopropyl Alcohol, ChloroPrep, and ZuraGard’s Vehicle (Source: Table 2.7.3.6, Time-Kill study 130733-201, module 2, summary of clinical efficacy)**

Exposure Time	Total Strains Reduced by ≥3.0 Log <sub>10</sub> (N=148)	Gram-negative Strains (N=72)	Gram-positive Strains (N=68)	Yeast (N=8)
<b>ZuraPrep, 70% v/v Isopropyl Alcohol, and ChloroPrep</b>				
<b>ZuraPrep</b>				
30 seconds	148	72	68	8
<b>70% v/v Isopropyl Alcohol</b>				
30 seconds	148	72	68	8
<b>ChloroPrep</b>				
30 seconds	148	72	68	8
Exposure Time	Total Strains Reduced by ≥3.0 Log <sub>10</sub>	Gram-negative Strains	Gram-positive Strains	Yeast
<b>ZuraPrep Vehicle</b>				
30 seconds	51	40	11	0
60 seconds	25	17	8	0
120 seconds	20	15	5	0
Source: <i>Study 130733-201 Report Executive Summary</i>				

***Reviewer’s comments:*** *The time-kill study showed that, at full strength concentration, ZuraGard, 70% v/v isopropyl alcohol, and ChloroPrep produced a >3.0 log<sub>10</sub> (>99.9%) reduction in viable microbial cells within 30 seconds for all 148 total challenge strains. The minimum log<sub>10</sub> reduction observed was 5.1 for ZuraGard, 4.7 for 70% v/v isopropyl alcohol, and 5.1 for ChloroPrep. Overall results of time-kill study showed that ZuraGard provides instant killing of the microorganisms, at exposure times of 30, 60, and 120 seconds, and is an effective bactericidal agent. These results are acceptable.*

Comparison of the time-kill kinetics of ZuraGard and ChloroPrep for *Acinetobacter* species (6 strains, including 2 multidrug resistant strains), *E. coli* (12 strains, including 2 multidrug resistant/extended-spectrum beta lactamase-positive strains), *E. faecalis* (8 strains, including 6 vancomycin-resistant strains), *E. faecium* (6 strains, including 2 vancomycin-resistant strains), *S. aureus* (18 strains, including 12 methicillin- or vancomycin-resistant strains), and *S. epidermidis* (10 strains, including 8 methicillin-resistant strains) suggest that the presence or absence of antibiotic resistance determinants do not influence antiseptic time-kill kinetics as both test products produced >5 log<sub>10</sub> reduction in 30 seconds for those strains (source module 2-summary of clinical efficacy).

ZuraGard’s vehicle exhibited varying degrees of antimicrobial activity, depending upon the challenge strain evaluated and the duration of exposure time investigated. ZuraGard’s vehicle reduced microbial populations of 51 of the 148 total challenge strains by >3.0 log<sub>10</sub> within 30 seconds (40 gram-negative strains and 11 gram-positive strains). The clinical significance of these results was investigated to determine the observed time-kill kinetic relationship to surgical site infection pathogens (source module 2-summary of clinical efficacy).

***Reviewer’s comments:*** *As shown in Table 12 above, ZuraGard’s vehicle was bactericidal against of 51 of 148 organisms. Some of this activity is expected*

because the ZuraGard final formulation contains citric acid and (b) (4) methylparaben and propylparaben, and these ingredients contain weak antimicrobial activity. Also, the results of study 130548-201, pilot time-kill dose response (specified above in section 3.3) show that the citrate (b) (4) and methylparaben were bactericidal (against one of 15 microorganisms tested), suggesting again that the vehicle provides some bactericidal activity. Therefore, bactericidal activity of the vehicle against a wider range of microorganisms was not surprising. However, the Sponsor showed, in pilot clinical simulation study (ZX-ZP-0068), that the log reduction achieved by ZuraGard’s vehicle and saline solution (as negative control) were similar, suggesting that ZuraGard’s excipients do not contribute towards the overall effectiveness of the test product. For these reasons, these time-kill results of the vehicle are considered acceptable.

**Validation of the Neutralizer System (Study ZX-ZP-0015, (b) (4) 130733-201):**

The neutralization procedure was based on guidelines set forth in ASTM E 1054-08, “Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents.” Neutralization studies of each product were performed against *Bacteroides fragilis* (ATCC #25285), *Candida albicans* (ATCC #10231), *Escherichia coli* (ATCC #25922), *Staphylococcus aureus* MRSA (ATCC #33592), and *Streptococcus pneumoniae* (ATCC #49619) (source, Section 12 of study protocol 130733-201). The neutralization procedure included verification of the effectiveness of the neutralizer and verification of the neutralizer’s lack of toxicity for the test organisms, and it included other controls such as viability and active controls. The initial inoculum for the challenge suspension was 10<sup>4</sup> CFU per mL in the neutralization validation assay (study protocol 130733-201, Section 12.8). The initial suspension was then diluted 1:90 for the neutralization effectiveness and toxicity tests, and 1:100 for the test organism viability and the test material controls. Details of the neutralization procedure are included in module 5.3.5.4. Table 13 below represents the example results of *Staphylococcus aureus* MRSA (ATCC #33592), and *Streptococcus pneumoniae* (ATCC #49619).

**Table 13. Neutralization Validation Results (Source: Table 1, study report 130733-201)**

Challenge Microorganism (ATCC #)	Test/Phase	Exposure/“Hold” Time	Replicate	Log <sub>10</sub> CFU	Average Log <sub>10</sub> CFU (n = 3)	Pass/Fail <sup>Ⓢ</sup>
<i>Staphylococcus aureus</i> MRSA (ATCC #33592)	Test Organism Viability (Test C)	15 minutes	1	2.6467	2.6229	N/A
			2	2.6163		
			3	2.6056		
	Neutralization Effectiveness Evaluation (Test A)	15 minutes	1	2.6021	2.6173	Pass
			2	2.6163		
			3	2.6335		
	Neutralizer Toxicity Evaluation (Test B)	15 minutes	1	2.7076	2.6620	Pass
			2	2.5911		
			3	2.6873		
	Test Material Control (Test D)	15 minutes	1	0.0000	0.0000	Pass
			2	0.0000		
			3	0.0000		
<i>Streptococcus pneumoniae</i> (ATCC #49619)	Test Organism Viability (Test C)	*	1	2.0057	2.3736	N/A
			2	2.5835		
			3	2.5315		
	Neutralization Effectiveness Evaluation (Test A)	*	1	2.5563	2.4586	Pass
			2	2.5228		
			3	2.2967		
	Neutralizer Toxicity Evaluation (Test B)	*	1	2.3483	2.2473	Pass
			2	2.1624		
			3	2.2313		
	Test Material Control (Test D)	15 minutes	1	0.0000	0.0000	Pass
			2	0.0000		
			3	0.0000		

Ⓢ = Reference Protocol #130733-201 Sections 12.58 and 12.59.  
 MRSA = Methicillin-Resistant *Staphylococcus aureus*  
 N/A = Not Applicable  
 \* Dilute and Plate Immediately

***Reviewer's comments:*** *As shown in Table 13 above for Staphylococcus aureus MRSA (ATCC #33592), and Streptococcus pneumoniae (ATCC #49619), no significant statistical difference was found between the average log<sub>10</sub> values of the controls and the average log<sub>10</sub> values for the toxicity control, test products (ZuraGard and 70% IPA), or active control (ChloroPrep). According to the guidelines for neutralization validation in ASTM E1054-08 (reapproved 2013), "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents," a log<sub>10</sub> difference of 0.2 has been previously used for neutralization assays and a difference determined between two samples of 0.2 log<sub>10</sub> is considered a significant statistical difference. The Sponsor has used a value of 0.2 log<sub>10</sub> as a measure of significant statistical difference. These results are acceptable.*

### 3.5. Antimicrobial Resistance

The Agency continues to believe that the development of bacteria that are resistant to antibiotics is an important public health issue, and additional data may tell us whether the continued use of antiseptics in healthcare settings may contribute to the selection of bacteria that are less susceptible to both antiseptics and antibiotics. However, the Health Care Antiseptic Proposed Rule (80 FR 25166 at 25187) states: "The antimicrobial mechanism of action of alcohol is considered nonspecific. It is believed that alcohol has multiple toxic effects on the structure and metabolism of microorganisms, primarily caused by denaturation and coagulation of proteins (Refs. 146 through 149). Alcohol's reactive hydroxyl (-OH) group readily forms hydrogen bonds with proteins, which leads to loss of structure and function, causing protein and other macromolecules to precipitate (Ref. 148). Alcohol also lyses the bacterial cytoplasmic membrane, which releases the cellular contents and leads to bacterial inactivation (Ref. 146). Because of alcohol's speed of action and multiple, nonspecific toxic effects, microorganisms have a difficult time developing resistance to alcohol." "Health care antiseptic products contain at least 60 percent alcohol (59 FR 31402 at 31442), and bacteria are unable to grow in this relatively high concentration of alcohol. Furthermore, alcohol evaporates readily after topical application, so no significant antiseptic residue is left on the skin that could contribute to the development of resistance (Refs. 146 and 148). Consequently, the development of resistance as a result of health care antiseptic use is unlikely, and additional data on the development of antimicrobial resistance to alcohol are not needed to support a GRAS determination."

***Reviewer's comments:*** *The Agency has previously recommended to assess the potential for the development of antiseptic resistance (Advice Letter, May 14, 2013). Specifically, we stated that the potential for the development of cross-resistance to antibiotics should be addressed by changes in antibiotic susceptibility to the test organisms as a result of exposure to ZuraGard™. Testing should include clinically relevant organisms of known susceptibilities to first line antibiotics. The Sponsor has performed the "Evaluation of Potential for Development Antimicrobial Resistance of ZuraGard" per Agency's recommendations (see below). This is acceptable.*

#### 3.5.1. Study ZX-ZP-0043 (b) (4) 865-102): Evaluation of Potential for Development Antimicrobial Resistance of ZuraGard

In vitro Study (ZX-ZP-0043) determined the potential for development of resistance to ZuraGard and 70% v/v isopropyl alcohol by sequential passage of several clinically relevant microorganisms through increasing concentrations of the antimicrobial included in the culture medium. A total of ten repository isolates from 8 species and 4 clinical isolates from each of the same 8 species were evaluated, tallying 42 isolates (see challenge organisms specified in the list below). Of the 4 clinical isolates, 2 were resistant and 2 were non-resistant for each of the species. If the microorganisms were able to stably maintain at least a 4-fold increase in the MIC of the test product for 3 serial passages on isopropyl alcohol-free media, resistance to the test product was considered established. ZuraGard test product and the active ingredient by itself (70% v/v isopropyl alcohol) were evaluated, and the results are described in Table 14 below.

**Table 14. List of Challenge Microorganisms (Source: study report ZX-ZP-0043)**

Organism	Strain Identification		Clinical Isolates Received from:
	ATCC Number	Clinical Isolate Number	
<i>Acinetobacter baumannii</i>	17904	14002 <sup>2</sup>	(b) (4)
		10057 <sup>2</sup>	
<i>Burkholderia cepacia</i>	25608	13052 <sup>2</sup>	(b) (4)
		13053 <sup>2</sup>	
<i>Enterococcus faecalis</i>	52199 <sup>1</sup>	99824	(b) (4)
		99825	
<i>Escherichia coli</i>	11229	99903	(b) (4)
		99904	
<i>Pseudomonas aeruginosa</i>	15442	99791	(b) (4)
		99792	
<i>Serratia marcescens</i>	14756	99413	(b) (4)
		99452	
<i>Staphylococcus aureus</i>	33591 <sup>1</sup>	99510	(b) (4)
		25923 <sup>2</sup>	
<i>Staphylococcus epidermidis</i>	51625 <sup>1</sup>	99530	(b) (4)
		00532	

<sup>1</sup> Methicillin-resistant  
<sup>2</sup> Methicillin-sensitive  
<sup>3</sup> Multidrug-resistant

***Reviewer's comments: The list of organisms for resistance testing is acceptable.***

### **Results of Emergence of Resistance (Study ZX-ZP-0043)**

The MICs of both ZuraGard test product and 70% v/v isopropyl alcohol did not increase for any of the strains evaluated. The results suggest that neither have the potential for development of resistance (source study ZX-ZP-0043 report, see Table 15 below).

***Reviewer’s comments: The study results did not show any higher MIC values with clinical isolates compared to ATCC laboratory strains, and MIC did not increase for any of the strains evaluated; therefore, the test product (ZuraGard) is not considered to have the potential for the development of resistance.***

**Table 15. Test Results: Emergence of Resistance (Source: Table 1, ZX-ZP-0043 study report)**

Organism	ID	Minimum Inhibitory Concentration (mg/L) (Represents Ten Replicates)			
		ZuraPrep™		70% IPA	
		Initial	Post	Initial	Post
<i>Acinetobacter baumannii</i>	ATCC 17904	21875	21875	87500	87500
	CI 14002 <sup>1</sup>	21875	21875	87500	87500
	CI 10057 <sup>1</sup>	21875	21875	87500	87500
	CI 10058 <sup>1</sup>	21875	21875	43750	43750
	CI 10059 <sup>1</sup>	21875	21875	43750	43750
<i>Burkholderia cepacia</i>	ATCC 25608	21875	21875	21875	21875
	CI 13052 <sup>1</sup>	21875	21875	21875	21875
	CI 13053 <sup>1</sup>	43750	43750	43750	43750
	CI 13054 <sup>1</sup>	43750	43750	21875	21875
	CI 13055 <sup>1</sup>	21875	21875	43750	43750
<i>Enterococcus faecalis</i>	ATCC 51299 <sup>2</sup>	43750	43750	87500	87500
	CI 99824	43750	43750	87500	87500
	CI 99825	43750	43750	87500	87500
	CI 13046 <sup>1</sup>	21875	21875	87500	87500
	CI 13047 <sup>1</sup>	21875	21875	87500	87500
<i>Escherichia coli</i>	ATCC 11229	43750	43750	87500	87500
	CI 99903	43750	43750	87500	87500
	CI 99904	43750	43750	87500	87500
	CI 10100 <sup>1</sup>	43750	43750	87500	87500
	CI 10101 <sup>1</sup>	43750	43750	87500	87500

<sup>1</sup> Multidrug-resistant  
<sup>2</sup> Vancomycin-resistant

Organism	ID	Minimum Inhibitory Concentration (mg/L) (Represents Ten Replicates)			
		ZuraPrep™		70% IPA	
		Initial	Post	Initial	Post
<i>Pseudomonas aeruginosa</i>	ATCC 15442	21875	21875	43750	43750
	CI 99791	21875	21875	43750	43750
	CI 99792	21875	21875	43750	43750
	CI 13015 <sup>1</sup>	21875	21875	43750	43750
	CI 13016 <sup>1</sup>	21875	21875	43750	43750
<i>Serratia marcescens</i>	ATCC 14756	43750	43750	87500	87500
	ATCC 43297 <sup>1</sup>	43750	43750	87500	87500
	CI 99413	43750	43750	87500	87500
	CI 99452	43750	43750	175000	175000
	CI 13026 <sup>1</sup>	43750	43750	87500	87500
<i>Staphylococcus aureus</i>	ATCC 33591 <sup>2</sup>	43750	43750	175000	175000
	ATCC 25923 <sup>3</sup>	43750	43750	87500	87500
	CI 99510	43750	43750	175000	175000
	CI 99511	43750	43750	175000	175000
	CI 10113 <sup>2</sup>	43750	43750	87500	87500
	CI 10114 <sup>2</sup>	43750	43750	87500	87500
<i>Staphylococcus epidermidis</i>	ATCC 51625 <sup>2</sup>	43750	43750	87500	87500
	CI 99530	43750	43750	87500	87500
	CI 99532	43750	43750	43750	43750
	CI 13031 <sup>1</sup>	43750	43750	87500	87500
	CI 13032 <sup>1</sup>	43750	43750	87500	87500

<sup>1</sup> Multidrug-resistant  
<sup>2</sup> Methicillin-resistant  
<sup>3</sup> Methicillin-sensitive

**Development of Antibiotic Cross-Resistance (Study ZX-ZP-0043)**

An evaluation of the potential for antibiotic cross-resistance to isopropyl alcohol was performed by comparing the MICs of several antibiotics (clindamycin, oxacillin, vancomycin, ampicillin, ceftazidime, imipenem, piperacillin, or tobramycin, as appropriate) both, before and after extended exposure to sublethal concentrations of isopropyl alcohol. If the microorganisms were able to stably maintain at least a 4-fold increase in the MIC of the test product for 3 serial passages on isopropyl alcohol-free media, resistance to the test product was considered established. The results are presented in study report of ZX-ZP-0043, in module 5.3.5.4. Table 16 below is a representative example of the results for *Acinetobacter baumannii* and *Burkholderia cepacia* ATCC strains.

**Table 16. Test Results: Development of Cross-Resistance (Source: Table 2, ZX-ZP-0043 study report)**

Organism	ID	Minimum Inhibitory Concentration (µg/mL)							
		Ceftazidime		Imipenem		Piperacillin		Tobramycin	
		Initial	Post	Initial	Post	Initial	Post	Initial	Post
<i>Acinetobacter baumannii</i>	ATCC 17904	3	2	6	6	48	32	3	1.5
	CI 14002 <sup>1</sup>	≥256	≥256	≥32	≥32	≥256	≥256	≥256	≥256
	CI 10057 <sup>1</sup>	128	128	≥32	≥32	≥256	≥256	64	64
	CI 10058 <sup>1</sup>	≥256	≥256	≥32	≥32	≥256	≥256	1.5	1.5
	CI 10059 <sup>1</sup>	≥256	≥256	≥32	≥32	≥256	≥256	≥256	≥256
<i>Burkholderia cepacia</i>	ATCC 25608	4	1.5	≥32	≥32	3	16	32	0.75
	CI 13052 <sup>1</sup>	24	1.5	≥32	≥32	≥256	≥256	≥256	≥256
	CI 13053 <sup>1</sup>	1.5	1.0	≥32	≥32	1.0	6	6	1.5
	CI 13054 <sup>1</sup>	2	2	≥32	≥32	16	12	1.0	1.0
	CI 13055 <sup>1</sup>	1.5	1.5	≥32	≥32	16	4	1.0	1.0

<sup>1</sup> Multidrug-resistant

***Reviewer’s comments:*** *The Summary of Clinical Efficacy (module 2.7.3) summarizes that for development of cross resistance after prolonged exposure to ZuraGard and 70% v/v isopropyl alcohol, 9 of the 42 organisms developed a decrease in their susceptibility to 1 or more of the antibiotics tested (Study ZX-ZP-0043 Report Table 2, Table 3, and Table 4). With only 3 exceptions, each of the increases in resistance were no greater than a 2-fold difference; therefore, the results were not considered significant once the sensitivity of the test was considered. For all remaining organisms, there was either a greater increase in the susceptibility of the strains to the antibiotics evaluated after exposure to isopropyl alcohol or no change in MICs related to antibiotic cross-resistance observed for any of the antibiotic combinations tested. Overall, ZuraGard test product and isopropyl alcohol do not seem to induce or select for resistance in clinically relevant bacteria and do not seem to mediate cross-resistance with clinically useful antibiotics. This reviewer finds these results acceptable.*

## 4. CLINICAL SIMULATION STUDIES

Based on the NDAC recommendations at its March 23, 2005 meeting, the Agency continues to rely on the use of bacterial log reduction as a means of demonstrating that health care antiseptics are GRAE (3). The test methods as described in the 1994 TFM for health care antiseptics (4) and the proposed performance criteria as described in the 2015 proposed rule for health care antiseptics were used (5). In the 2015 Health Care Antiseptics Proposed Rule, FDA made revisions to the effectiveness criteria set forth in the 1994 TFM, while continuing to recommend bacterial log reduction studies. FDA recommended that these bacterial log reduction studies: 1) include both a negative control and an active control; 2) have an adequate sample size to show that the test product is superior to its negative control; 3) incorporate the use of an appropriate neutralizer and a demonstration of neutralizer validation; and 4) include an analysis of the proportion of subjects who meet the recommended log reduction criteria based on a two-sided statistical test for superiority to negative control and a 95 percent confidence interval approach (80 FR 25166 at 25178 to 25179). FDA also recommended that the success rate or responder rate of the test product be significantly higher than 70 percent.

A final rule for health care antiseptic drug products was published on December 20, 2017 (82 FR 60474 at 60487) (6). This final rule includes a separate analysis criteria for patient preinjection skin preparations, to more accurately reflect the actual use of these products. The updated analysis applies the use of non-inferiority of test product to an active control by a margin of 0.5 and superiority of test product to a negative control by an indication-specific margin. Rather than using only a change from baseline, each criterion uses the average treatment effect, an estimated difference of the effect of two treatments correcting for baseline count. That is, the average treatment effect is estimated from a linear regression of post-treatment bacterial count ( $\log_{10}$  scale) correcting for baseline or pre-treatment measurement ( $\log_{10}$  scale). Superiority to negative control by a specific margin is needed because our evaluation suggests that application of a negative control, whether vehicle or saline, may exhibit some minimal antimicrobial properties. Thus, using superiority to negative control by the margins listed below will help ensure that we can appropriately assess the effectiveness of the antimicrobial products. Based upon prior agreement on the clinical simulation study design, the Sponsor has submitted two pivotal studies using the study design described in the 2015 Health Care Antiseptics proposed rule. Based on the most recent developments of the statistical analysis, our assessment of this submission includes also the performance of the studies according to the updated statistical analysis criteria described in the 2017 Health Care Antiseptics Final Rule.

### 4.1. Pilot Study

#### 4.1.1. Study ZX-ZP 0068 (MBT 865-104): Pilot Clinical Evaluation to Characterize the In Vivo Effects of Topically Applied ZuraGard and ZuraGard' Vehicle

A Phase 2, randomized, four arms, paired-comparisons, pilot trial (ZX-ZP-0068) was performed to evaluate that ZuraGard's vehicle (ZuraGard product without isopropyl alcohol) and saline solution are equally therapeutically inactive and are not substantially different in antimicrobial  $\log_{10}$  reduction from baseline at the time points described in the 1994 TFM (10 minutes and 6 hours after product application), as well as at the newly proposed 30 second time point (May 1, 2015 TFM, 80 FR 25166).

In this trial each subject received two of the planned treatments: ZuraGard 10.5 mL Applicator; ChloroPrep 10.5 mL Applicator; ZuraGard’s vehicle; and normal saline. The trial was conducted at MicroBioTest, Sterling, VA. The primary objective of this study was to characterize the in vivo effects of the ZuraGard’s vehicle in comparison to the normal saline control. The in vivo performance of the investigational products with the proposed sampling interval at 30 seconds and 10 minutes were evaluated. This study measured the antimicrobial activity of ZuraGard as compared to the positive product, ChloroPrep 10.5 mL Applicator (b) (4) Tint), and of the ZuraGard’s vehicle compared to a negative control, normal saline (see Table 17 below).

**Table 17. Treatments, Anatomical Sites of Evaluation, Application and Dry Times and Coverage Areas (Source: Table 2, ZX-ZP-0068 study report)**

Treatment (Quantity/Volume)	Body Site	Application Time	Dry Time	Area of Coverage
ZuraPrep™ 10.5 mL Applicator	Abdomen (sebaceous poor)	30 seconds	3 minutes	5" x 5"
	Groin (sebaceous rich)	2 minutes	3 minutes	1.5" x 5"
ChloroPrep® 10.5 mL Applicator (b) (4) Tint)	Abdomen (sebaceous poor)	30 seconds	3 minutes	5" x 5"
	Groin (sebaceous rich)	2 minutes	3 minutes	1.5" x 5"
ZuraPrep™ vehicle	Abdomen (sebaceous poor)	30 seconds	3 minutes	5" x 5"
	Groin (sebaceous rich)	2 minutes	3 minutes	1.5" x 5"
Normal saline (negative control)	Abdomen (sebaceous poor)	30 seconds	3 minutes	5" x 5"
	Groin (sebaceous rich)	2 minutes	3 minutes	1.5" x 5"

Four products (source Table 3.1 from study ZX-ZP 0068 protocol) were applied on the abdominal sites for 30 seconds and on the groin sites for 2 minutes, the drying time for both sites were 3 minutes. Healthy male or female volunteers of at least 18 years of age with no dermatological conditions or known history of sensitivity were enrolled into this study. On the screening day the baseline counts (source Table 3.2 study from ZX-ZP-0068 protocol) were at least  $1.0 \times 10^3$  CFU/cm<sup>2</sup> per abdominal site (left and right) and at least  $1.0 \times 10^5$  CFU/cm<sup>2</sup> per groin site (left and right). A total of 89 subjects were treated. 82 subjects were treated on both the abdomen and groin, 4 on the abdomen only, and 3 on the groin site only.

A list of 72 test product assignments was created in six blocks of 12 assignments each and the list was sorted by block and random number. The resulting group assignment order was assigned to subjects sequentially in the order accrued. Sample area versus timing of sampling was separately block-randomized using a list of the 24 sampling orders. Neutralization was separately randomized using a list of the six possible test product assignments

**Table 18. Minimum and Maximum Treatment Day Baseline and Expected Mean Log<sub>10</sub> Reduction per Anatomical site (Source: Table 3.2, ZX-ZP-0068 study protocol)**

Table 3.2: Minimum and Maximum Treatment Day Baseline and Expected Mean Log <sub>10</sub> Reduction per Anatomical Site		
Anatomical Site	Minimum and Maximum Treatment Day Baseline*	Expected Mean Log <sub>10</sub> Reduction
Abdomen	1.0 x 10 <sup>3</sup> – 3.2 x 10 <sup>5</sup> CFU/cm <sup>2</sup> (3.0 Log <sub>10</sub> – 5.5 Log <sub>10</sub> )	2.0 log <sub>10</sub> @ 30-seconds and 10-minutes
Groin	1.0 x 10 <sup>5</sup> – 3.2 x 10 <sup>7</sup> CFU/cm <sup>2</sup> (5.0 Log <sub>10</sub> – 7.5 Log <sub>10</sub> )	3.0 log <sub>10</sub> @ 30-seconds and 10-minutes

The primary goal of the efficacy study was the reduction of skin flora on the abdominal and groin sites 30-seconds and 10-minutes following application of the test treatments, relative to the treatment day baseline log<sub>10</sub> counts (source Table 3.2 study ZX-ZP 0068 protocol). The study was analyzed per the 1994 Tentative Final Monograph (TFM) standards for Effectiveness Testing of a Patient Preoperative Skin Preparation (59 FR 31402 at 31450-31452), and by the 2015 Health Care antiseptics Proposed Rule (80 FR 25166 at 25166). As the primary end point, the 1994 TFM indicates that the test product and the active control should achieve a 2 log<sub>10</sub> per cm<sup>2</sup> mean reduction on the abdomen site and a 3 log<sub>10</sub> per cm<sup>2</sup> mean reduction on the groin site at 10 minutes post application. The 2015 Proposed Rule (80 FR 25166 at 25178 to 25179) indicates 30 seconds as the primary efficacy time point and a >70% lower bound of the 95% confidence interval for the responder rate for test and active control.

**Results (Pilot Study ZX-ZP-0068, MBT 865-104)**

**Table 19. Log Reductions from baseline at 30 seconds, 10 Minutes, and 6 Hours (Source: Table 5, ZX-ZP-0068 study report)**

Population	Body Area	Treatment	Mean* Log <sub>10</sub> CFU/cm <sup>2</sup> Reductions from Baseline (95% confidence interval)		
			30 Seconds	10 Minutes	6 Hours
Intent-To-Treat	Abdomen	ZuraPrep™	2.45 (2.10 to 2.81)	3.17 (2.85 to 3.49)	2.20 (1.86 to 2.54)
		ChloroPrep®	2.67 (2.33 to 3.02)	3.06 (2.75 to 3.38)	2.04 (1.70 to 2.38)
		ZuraPrep™ vehicle	0.95 (0.67 to 1.23)	1.30 (1.04 to 1.56)	1.16 (0.88 to 1.44)
		Normal saline	0.56 (0.27 to 0.85)	0.70 (0.44 to 0.97)	0.81 (0.52 to 1.10)
	Groin	ZuraPrep™	3.40 (3.09 to 3.72)	4.20 (3.91 to 4.50)	2.81 (2.50 to 3.12)
		ChloroPrep®	3.22 (2.91 to 3.54)	4.02 (3.73 to 4.31)	2.51 (2.20 to 2.82)
		ZuraPrep™ vehicle	1.31 (0.94 to 1.68)	1.84 (1.50 to 2.18)	1.87 (1.51 to 2.24)
		Normal saline	1.17 (0.78 to 1.56)	1.35 (1.00 to 1.71)	1.50 (1.12 to 1.88)
No Replacements	Abdomen	ZuraPrep™	2.42 (2.03 to 2.80)	3.14 (2.79 to 3.50)	2.17 (1.79 to 2.54)
		ChloroPrep®	2.73 (2.34 to 3.12)	3.09 (2.73 to 3.44)	2.12 (1.74 to 2.50)
		ZuraPrep™ vehicle	0.90 (0.58 to 1.21)	1.21 (0.92 to 1.51)	1.09 (0.78 to 1.41)
		Normal saline	0.56 (0.23 to 0.89)	0.70 (0.39 to 1.00)	0.74 (0.41 to 1.06)
	Groin	ZuraPrep™	3.49 (3.15 to 3.83)	4.29 (3.97 to 4.60)	2.81 (2.47 to 3.15)
		ChloroPrep®	3.35 (3.00 to 3.70)	4.12 (3.80 to 4.44)	2.46 (2.11 to 2.80)
		ZuraPrep™ vehicle	1.32 (0.91 to 1.74)	1.86 (1.48 to 2.25)	1.87 (1.47 to 2.27)
		Normal saline	1.13 (0.69 to 1.57)	1.32 (0.92 to 1.72)	1.43 (1.00 to 1.85)
Per-Protocol	Abdomen	ZuraPrep™	2.66 (2.30 to 3.02)	3.38 (3.06 to 3.70)	2.42 (2.07 to 2.77)
		ChloroPrep®	2.91 (2.55 to 3.26)	3.35 (3.03 to 3.67)	2.23 (1.89 to 2.58)
		ZuraPrep™ vehicle	1.03 (0.74 to 1.33)	1.39 (1.12 to 1.65)	1.26 (0.97 to 1.54)
		Normal saline	0.64 (0.33 to 0.95)	0.78 (0.50 to 1.07)	0.94 (0.63 to 1.24)
	Groin	ZuraPrep™	3.56 (3.24 to 3.88)	4.28 (3.99 to 4.57)	3.01 (2.70 to 3.31)
		ChloroPrep®	3.36 (3.03 to 3.69)	4.20 (3.90 to 4.50)	2.72 (2.40 to 3.04)
		ZuraPrep™ vehicle	1.24 (0.85 to 1.63)	1.83 (1.48 to 2.18)	1.98 (1.61 to 2.36)
		Normal saline	1.20 (0.80 to 1.61)	1.43 (1.06 to 1.79)	1.60 (1.21 to 1.99)

\* Least-squares means. Light gray = mean value meets goal; dark gray = confidence interval meets goal.

***Reviewer’s comments:*** *In this phase-2 randomized, four arms (test product-ZuraGard, active control-ChloraPrep, ZuraGard’s vehicle, and normal saline) trial, results showed (source Table-5 study ZX-ZP 0068 report) that both ZuraGard and ChloraPrep met the primary effectiveness criteria at abdominal and groin sites at 30 seconds and 10 minutes time points: at least 2 log<sub>10</sub> per cm<sup>2</sup> reduction from the baseline on the abdominal site and 3 log<sub>10</sub> per cm<sup>2</sup> reduction from the baseline on the groin site; and count values below baseline at 6 hours.*

**Table 20. Differences Log Reductions from baseline at 30 seconds 10 Minutes and 6 Hours (Source: Table 7, ZX-ZP-0068 study report)**

Pop.	Body Area	Treatment <sup>#</sup>	Differences in Log <sub>10</sub> CFU/cm <sup>2</sup> Reductions from Baseline* (95% Adjusted CI)		
			30 Seconds	10 Minutes	6 Hours
Intent-To-Treat	Abdomen	ZP™-CP®	-0.22 (-0.79 to 0.35)	0.11 (-0.41 to 0.62)	0.16 (-0.38 to 0.70)
		Vehicle-Saline	0.39 (-0.18 to 0.96)	0.60 (0.08 to 1.12)	0.35 (-0.19 to 0.89)
		0±1/2 SD	-0.63 to 0.63	-0.70 to 0.70	-0.56 to 0.56
	Groin	ZP™-CP®	0.18 (-0.39 to 0.76)	0.18 (-0.34 to 0.71)	0.30 (-0.25 to 0.85)
		Vehicle-Saline	0.14 (-0.43 to 0.70)	0.49 (-0.02 to 1.00)	0.38 (-0.16 to 0.91)
		0±1/2 SD	-0.71 to 0.71	-0.81 to 0.81	-0.56 to 0.56
No Replacements	Abdomen	ZP™-CP®	-0.31 (-0.95 to 0.32)	0.06 (-0.53 to 0.64)	0.05 (-0.55 to 0.64)
		Vehicle-Saline	0.34 (-0.31 to 0.98)	0.52 (-0.08 to 1.11)	0.36 (-0.25 to 0.96)
		0±1/2 SD	-0.64 to 0.64	-0.71 to 0.71	-0.57 to 0.57
	Groin	ZP™-CP®	0.14 (-0.50 to 0.78)	0.17 (-0.42 to 0.75)	0.35 (-0.25 to 0.95)
		Vehicle-Saline	0.19 (-0.45 to 0.83)	0.54 (-0.04 to 1.13)	0.44 (-0.16 to 1.04)
		0±1/2 SD	-0.74 to 0.74	-0.84 to 0.84	-0.57 to 0.57
Per-Protocol	Abdomen	ZP™-CP®	-0.25 (-0.84 to 0.33)	0.03 (-0.49 to 0.56)	0.19 (-0.37 to 0.75)
		Vehicle-Saline	0.39 (-0.21 to 0.99)	0.60 (0.07 to 1.14)	0.32 (-0.25 to 0.90)
		0±1/2 SD	-0.63 to 0.63	-0.70 to 0.70	-0.54 to 0.54
	Groin	ZP™-CP®	0.20 (-0.38 to 0.79)	0.08 (-0.45 to 0.61)	0.29 (-0.28 to 0.85)
		Vehicle-Saline	0.03 (-0.55 to 0.62)	0.40 (-0.12 to 0.93)	0.38 (-0.18 to 0.95)
		0±1/2 SD	-0.72 to 0.72	-0.82 to 0.82	-0.53 to 0.53

<sup>#</sup> ZP™ = ZuraPrep™; CP® = ChloraPrep®; Vehicle = ZuraPrep™ vehicle; Saline = normal saline  
\* Light gray = within 1 standard deviation, i.e. within 0 ± 1/2 SD

***Reviewer’s comments:*** *Table 20 (source: ZX-ZP-0068 study report Table-7) shows the differences in the bacterial log<sub>10</sub> per cm<sup>2</sup> reductions from baseline between normal saline and ZuraGard’s vehicle, which was below 0.6 log<sub>10</sub> per cm<sup>2</sup>. These results are consistent with the standards provided in the FDA February 22, 2016 Advice Letter, which specifies that ZuraGard’s vehicle and normal saline are considered equivalent when the comparisons of mean log<sub>10</sub> per cm<sup>2</sup> reduction from baseline are below 1 log<sub>10</sub> per cm<sup>2</sup> for both body areas and all timepoints (30 seconds, 10 minutes and 6 hours). This reviewer finds these results acceptable.*

**Table 21. Responder Rate at 30 seconds, 10 Minutes, and 6 Hours (Source: Table 6, ZX-ZP-0068 study report)**

Pop.	Body Area	Treatment	Responder Rate (%) (95% CI*)		
			30 Seconds	10 Minutes	6 Hours
Intent-to-treat	Abdomen	ZuraPrep™	59 (42 to 74)	83 (68 to 93)	90 (77 to 97)
		ChloraPrep®	68 (52 to 81)	82 (67 to 92)	82 (67 to 92)
		ZuraPrep™ vehicle	10 (3 to 23)	14 (5 to 29)	83 (69 to 93)
		Normal saline	2 (0 to 12)	4 (1 to 15)	78 (63 to 90)
	Groin	ZuraPrep™	55 (38 to 71)	78 (62 to 89)	93 (80 to 98)
		ChloraPrep®	44 (29 to 60)	65 (49 to 79)	84 (69 to 93)
		ZuraPrep™ vehicle	2 (0 to 13)	5 (1 to 16)	86 (71 to 95)
		Normal saline	2 (0 to 12)	2 (0 to 12)	82 (68 to 92)
No-Replacements	Abdomen	ZuraPrep™	56 (38 to 72)	81 (64 to 92)	89 (74 to 97)
		ChloraPrep®	67 (49 to 81)	78 (61 to 90)	78 (61 to 90)
		ZuraPrep™ vehicle	3 (0 to 15)	9 (2 to 23)	86 (70 to 95)
		Normal saline	3 (0 to 14)	3 (0 to 14)	76 (59 to 88)
	Groin	ZuraPrep™	58 (41 to 74)	78 (61 to 90)	92 (78 to 98)
		ChloraPrep®	50 (33 to 67)	69 (52 to 84)	81 (64 to 92)
		ZuraPrep™ vehicle	3 (0 to 15)	6 (1 to 19)	86 (70 to 95)
		Normal saline	3 (0 to 14)	3 (0 to 14)	81 (65 to 92)
Per-Protocol	Abdomen	ZuraPrep™	65 (47 to 80)	92 (78 to 98)	100 (91 to 100)
		ChloraPrep®	83 (67 to 94)	100 (90 to 100)	100 (90 to 100)
		ZuraPrep™ vehicle	11 (3 to 26)	17 (6 to 33)	97 (85 to 100)
		Normal saline	3 (0 to 14)	5 (1 to 18)	95 (82 to 99)
	Groin	ZuraPrep™	59 (42 to 75)	84 (68 to 94)	100 (91 to 100)
		ChloraPrep®	53 (35 to 70)	78 (61 to 90)	100 (90 to 100)
		ZuraPrep™ vehicle	3 (0 to 15)	6 (1 to 19)	100 (90 to 100)
		Normal saline	3 (0 to 14)	3 (0 to 14)	100 (91 to 100)

\* Light gray = responder rate is above 70%; dark gray = confidence interval is above 70%.

***Reviewer’s comments:*** *The secondary efficacy goal for active products was to have the lower bound of the 95% confidence interval for the responder rate to be greater than or equal to 70% at 10 minutes. Both the test product (ZuraGard) and the active control (ChloraPrep) met the 70% lower bound responder rate at 10 minutes and 6 hours using per protocol analyses for the abdomen area. However, at the groin area, ZuraGard and ChloraPrep met the 70% responder rate at 6 hours but not at the 10 minutes timepoint (Table-6 study ZX-ZP 0068 report). At 30 seconds, ZuraGard and ChloraPrep could not achieve the 70% responder rate (neither on abdomen nor groin site). This is acceptable because this was a pilot study with the objective to evaluate and characterize the ZuraGard vehicle when compared to saline and given that the primary objective (per 1994 TFM log reduction criteria) was achieved as shown in Table 7 above (also considering the totality of evidence as described below).*

## 4.2. Pivotal Studies

### 4.2.1. Study ZX-ZP-0073 (MicroBioTest MBT 865-105) and Study ZX-ZP-0074 (BioScience Labs, BSLI 150316-103)

Two pivotal clinical simulation studies, ZX-ZP-0073 (MicroBioTest) and ZX-ZP-0074 (BioScience Labs) entitled: “Evaluate the antimicrobial effectiveness of a single application of ZuraGard” were designed to evaluate the immediate (30 seconds and 10 minutes) and persistent (6 hour) antimicrobial efficacy on abdomen and groin sites of patient preoperative skin preparations (ZuraGard test product, ChloroPrep control, and ZuraGard’s vehicle). The abdominal assessments were performed using a prepping procedure consisting of 30 seconds product application time and approximately 3 minutes drying time. The 30-second, 10-minute and 6-hour post application sampling times were performed after the conclusion of the post-application drying time. These pivotal studies used the standard method specified by 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 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 Objectives (Study ZX-ZP-0073 and ZX-ZP-0074)**

The primary objective of these studies was to assess antimicrobial efficacy of single investigational test product ZuraGard - preoperative skin preparation at 10 minutes on dry (abdomen) and wet (groin) sites. At 10 minutes post prep the test article should achieve at least a 2 log<sub>10</sub> per cm<sup>2</sup> mean reduction on the abdomen site and a 3 log<sub>10</sub> per cm<sup>2</sup> mean reduction on the groin site. In addition, the responder rate 95% confidence interval lower bound at 10 minutes should be equal or higher than 70%. For secondary objective the responder rates at 30 seconds and 6 hours were calculated identically to the 10-minute rates. At 6 hours, a site is considered a responder if it is below baseline. The secondary efficacy goals also had the 95% confidence intervals for the responder rates to be greater than or equal to 70%. The 30-second, 10-minute and 6-hour post application sampling times were performed after the conclusion of the post-application drying time.

**Table 22. Efficacy Endpoints Study ZX-ZP-0073 and ZX-ZP-0074 (Source: Table 2.7.3-4, module 2, summary of clinical efficacy)**

Study	Efficacy Endpoints	
	Primary	Secondary/Other
<b>Pivotal Studies</b>		
ZX-ZP-0074	<ul style="list-style-type: none"> <li>Percentage of responders<sup>a</sup> at 10 minutes post application</li> <li>ATE<sup>b</sup> at 10 minutes post application</li> </ul>	<ul style="list-style-type: none"> <li>Percentage of responders<sup>a</sup> at 30 seconds and 6 hours post application</li> <li>Log<sub>10</sub> CFU/cm<sup>2</sup> reduction at each post application sampling time</li> <li>ATE<sup>b</sup> at 30 seconds post application</li> </ul>
ZX-ZP-0073	<ul style="list-style-type: none"> <li>Percentage of responders<sup>a</sup> at 10 minutes post application</li> <li>ATE<sup>b</sup> at 10 minutes post application</li> </ul>	<ul style="list-style-type: none"> <li>Percentage of responders<sup>a</sup> at 30 seconds and 6 hours post application</li> <li>Log<sub>10</sub> CFU/cm<sup>2</sup> reduction at each post application sampling time</li> <li>ATE<sup>b</sup> at 30 seconds post application</li> </ul>
Abbreviations: ATE = average treatment effect; CFU = colony forming units; CSR = clinical study report <sup>a</sup> Responder at 30 seconds and 10 minutes was defined as reduction $\geq 2 \log_{10}$ CFU/cm <sup>2</sup> on the abdomen or $\geq 3 \log_{10}$ CFU/cm <sup>2</sup> on the groin. Responder at 6 hours for abdomen and groin was defined as below baseline value. <sup>b</sup> The ATE was estimated from a linear regression of posttreatment bacterial count (log <sub>10</sub> scale) at 30 seconds and 10 minutes on the additive effect of a treatment indicator and the baseline or pretreatment measurement (log <sub>10</sub> scale).		

***Reviewer's comments:*** *On June 17, 2016 in an End of Phase 2/Pre-phase 3 meeting, the FDA advised the Sponsor to perform three arm (test product, active control and vehicle control) pivotal trials based on the four-arm pilot study (ZX-ZP 0068) results (as discussed in section 4.1.1). Specifically, we recommended the following criteria: "As noted in the September 30, 2015 meeting minutes, the May 1, 2015 proposed rule (80 FR 25166) proposes various design and method considerations that are provisional and undergoing assessment pending a final rule. You can use responder rate at 10 minutes for your primary analyses; however, the responder rate at 30 seconds and 6 hours should then be secondary endpoints rather than exploratory endpoints. We will take into consideration all the data, and the results obtained will guide the labeling of your product, if approved. We recommend that efficacy data be collected at 30 seconds, 10 minutes and 6 hours after application and drying time are complete. Therefore, for your pivotal studies base the primary analysis on the proportion of patient successes (responders) as a binary endpoint. Success for a patient is defined as meeting the required 3 log reduction from baseline at the groin site and 2 log<sub>10</sub> per cm<sup>2</sup> reduction at the abdomen site. The primary efficacy criteria for the test product is that the lower bound of a 95% confidence interval for the responder rate is ≥70% at 10 minutes in both the groin and the abdomen sites. Important study validity goals are for the active control to meet ≥70% responder rate criterion at 10 minutes at groin and abdomen and that both the test product and active control are superior to the vehicle."*

*On July 10, 2017, FDA informed the Sponsor about the Agency's new effectiveness analysis for antiseptic products. Specifically, FDA stated: "in January 2017, FDA issued deferral letters requesting that ethyl alcohol, isopropyl alcohol, povidone iodine, benzalkonium chloride, benzethonium chloride, and chloroxylenol be deferred from the final rulemaking to fill safety and efficacy data gaps and establish that those active ingredients are generally recognized as safe and effective when used in health care settings. These deferral letters, an example available at <https://www.regulations.gov/document?D=FDA-2015-N-0101-1325>, recommend an updated effectiveness analysis for antiseptic products based on the Agency's current thinking. Thus, we recommend you add these effectiveness analyses to your primary statistical analysis and send a detailed statistical analysis plan for review reflecting these additions. More specifically, we recommend that, in addition to the responder rates, product effectiveness be measured using the average treatment effects (ATE). The ATE is estimated from a linear regression of posttreatment bacterial count (log<sub>10</sub> scale) on the additive effect of a treatment indicator and the baseline or pretreatment measurement (log<sub>10</sub> scale). To show effectiveness, we recommend that your test product be (1) non-inferior to the ChloroPrep® in your study with a 0.5 margin (log<sub>10</sub> scale) and (2) superior to the ZuraGard's vehicle control in your study by a 1.2 margin (log<sub>10</sub> scale). That is, we expect the upper bound of the 95 percent confidence interval of the ATE of the ChloroPrep® compared to the test product to be less than 0.5 (log<sub>10</sub> scale), and the lower bound of the 95 percent confidence interval of the ATE of the test product to be no less than 1.2 (log<sub>10</sub> scale). For both pivotal studies ZX-ZP-0073 and ZX-ZP-0074 primary and secondary objectives are satisfactory, consistent with FDA's June 17, 2016, and July 10, 2017 Advice Letters, and they are acceptable.*

**Study Design (study ZX-ZP-0073 and ZX-ZP-0074)**

These two trials were randomized, paired-comparison design, where each subject receives two of the planned treatments (ZuraGard 10.5 mL Applicator; ChloroPrep 10.5 mL Applicator (positive control) and ZuraGard’s vehicle (negative control).

**Table 23. Treatments, Anatomical Sites of Evaluation, Application and Dry Times, and Coverage Areas (Source: Table 3.1, ZX-ZP-0073 protocol)**

Treatment (Quantity/Volume)	Body Site	Application Time	Dry Time	Area of Coverage
ZuraPrep™ 10.5 mL Applicator	Abdomen (sebaceous poor)	30 seconds	3 minutes	5" x 5"
	Groin (sebaceous rich)	2 minutes	3 minutes	1.5" x 5"
ChloroPrep® 10.5 mL Applicator (b) (4) Tint, (positive control)	Abdomen (sebaceous poor)	30 seconds	3 minutes	5" x 5"
	Groin (sebaceous rich)	2 minutes	3 minutes	1.5" x 5"
ZuraPrep™ Vehicle, 10.5 mL application (negative control)	Abdomen (sebaceous poor)	30seconds	3 minutes	5" x 5"
	Groin (sebaceous rich)	2 minutes	3 minutes	1.5" x 5"

**Randomization and Blinding (Study ZX-ZP-0073 and ZX-ZP-0074)**

Subjects meeting treatment day baseline sampling criteria ( $1.0 \times 10^3$  CFU/cm<sup>2</sup> abdominal site and  $1.0 \times 10^5$  CFU/cm<sup>2</sup> groin site) were assigned numbers and randomized to treatment using the following block design:

- a. Treatment Balance: Each subject received two different treatments, one on the right side of the body and one on the left. This means there were three possible combinations of treatments per subject:
  - ZuraGard and ChloroPrep
  - ZuraGard and ZuraGard’s vehicle
  - ChloroPrep and ZuraGard’s vehicle
  
- b. Left/Right Balance: Treatment assignments were balanced so that the number of readings per anatomical site matched the calculated requirements. The applications were randomized so that each treatment was used on an equal number of left and right sides of the body. The two active treatments (ZuraGard and ChloroPrep) were applied to an equal number of anatomical sites. ZuraGard’s vehicle was applied to the number of anatomical sites necessary to generate a baseline for comparison.

**Reviewer’s comments: The randomization and blinding seem acceptable and in**

*accordance with FDA’s recommendations. Defer to Statistical Discipline review for additional comments.*

### 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 24. Description of Investigational Products (Source: Table 3, study ZX-ZP-0073 report)**

Investigational Products	Description	Lot No.	Expiry Date
ZuraPrep™ 10.5 mL Applicator (test product)	Active Ingredient: Isopropyl Alcohol (~70 %) Other Ingredients: Citric Acid (b) (4) Methylparaben (b) (4) mg/mL, Propylparaben (b) (4) mg/mL, Methylene Blue (b) (4) mg/mL, and purified water. pH range (b) (4)	ZP0007A	Oct. 2017
ChloraPrep® 10.5 mL Applicator (b) (4) Tint (reference/active control)	2% Chlorhexidine Gluconate (w/v) and 70% isopropyl alcohol (v/v)	96574	04/2018
ZuraPrep™ Vehicle (negative/inactive control)	ZuraPrep™ Vehicle solution: Active Ingredient: Not Applicable Other Ingredients: Purified Water, Citric Acid (b) (4) (b) (4) (b) (4) mg/mL, Methylparaben (b) (4) mg/mL, Propylparaben (b) (4) mg/mL, and Methylene Blue (b) (4) mg/mL. (b) (4) (b) (4)	XP0007A	Stability on file with Sponsor
	Empty ZuraPrep™ Applicators (b) (4)		
	Note: 10.5 mL of ZuraPrep™ Vehicle solution was (b) (4) added to each individual applicator just prior to treatment. A single applicator was used for each anatomical application.	AP0001C	N/A

### Study Subjects

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

- Male or female, at least 18 years or older.
- Were in good general health.
- Had skin within 6 inches of the test sites that were free of tattoos, dermatoses, abrasions, cuts, lesions or other skin disorders.
- Were cooperative and willing to follow Subject Instructions (Appendix 16.1.3).
- Were cooperative and willing to sign the Consent/HIPAA Authorization Form.
- Had Screening Day baseline counts of at least  $1.0 \times 10^3$  CFU/cm<sup>2</sup> per abdominal site (left and right) and at least  $1.0 \times 10^5$  CFU/cm<sup>2</sup> per groin site (left and right).

Exclusion Criteria: Subjects to whom any of these conditions applied were excluded from this study:

- Topical or systemic antimicrobial exposure from within 14 days prior to Screening Day

through the remainder of the study. Restrictions included, but were not limited to antimicrobial soaps, antiperspirants/deodorants, shampoos, lotions, perfumes, after shaves, colognes, and topical or systemic antibiotics.

- Swam in chemically treated pools or bathed in hot tubs, spas and whirlpools from within 14 days prior to Screening Day through the remainder of the study.
- Used tanning beds, hot waxes, or depilatories, including shaving (in the applicable test areas) from within 14 days prior to the Screening Day through the remainder of the study.
- Had contact with solvents, acids, bases, fabric softener-treated clothing or other household chemicals in the applicable test areas from within 14 days prior to Screening Day through the remainder of the study.
- Subjects who had a history of sensitivity to natural rubber latex, adhesive skin products (e.g., Band-Aids, medical tapes), isopropyl alcohol, citric acid, methylene blue, methylparaben, propylparaben, or chlorhexidine gluconate products.
- Subjects who had a history of skin allergies.
- Subjects who had a history of skin cancer within 6 inches of the applicable test areas.
- Subjects who were pregnant, attempting pregnancy or nursing.
- Subjects who had showered or bathed within 72 hours of the Screening Day or Treatment Day (sponge baths may have been taken, however, the lower abdomen and upper thigh region must have been avoided).
- Subjects who received an irritation score of 1 for any individual skin condition prior to the Screening Day baseline or Treatment Day baseline sample collection.
- Participated in another clinical trial in the 30 days prior to the Treatment Day of this study (treatment with test materials in this study), or were currently enrolled in another clinical trial, or had previously participated in this study.

***Reviewer's comments: The Sponsor's inclusion and exclusion criteria are acceptable and in accordance with recommendations in the 1994 TFM for patient preoperative skin preparation studies. Defer to Medical Officer's review for any additional comments.***

### **Screening Phase (Study ZX-ZP-0073 and ZX-ZP-0074)**

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 were again 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 comments: The screening phase procedure is standard and is acceptable.***

### **Treatment Phase (study ZX-ZP-0073 and ZX-ZP-0074)**

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 each region for the placebo (vehicle) arm. The randomization schedule designated the treatment to each side of the abdomen and groin.

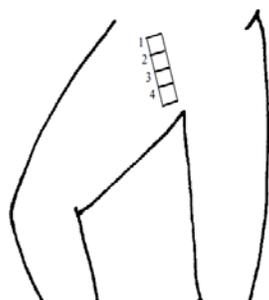
### **Preparation of Abdominal Test Area**

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 (pre-prep) site, and three post-prep samples sites (30 seconds, 10 minutes, 6 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 1.5" 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 (pre-prep) site, and two or three post-prep sample sites (30 seconds, 10-minutes, 6-hours).



### **Treatment Materials Application**

ZuraGard 10.5 mL Applicator, one pre-weighed applicator per applicable test site, was applied topically by scrubbing for 2 minutes over a 1.5" x 5" area on the groin or scrubbing for 30 seconds over 5" x 5" area on the abdomen. Application was performed using repeated back and forth strokes of the sponge. Each test site was air-dried for 3 minutes. Post treatment weight of the applicator was recorded.

ChloroPrep 10.5 mL Applicator (b) (4) Tint), one pre-weighed applicator per applicable test site, was applied topically by scrubbing for 2 minutes over a 1.5" x 5" area on the groin or scrubbing for 30 seconds over 5" x 5" area on the abdomen. Application was performed using repeated back and forth strokes of the sponge. Each test site was air-dried for 3 minutes. Post treatment weight of the applicator was recorded.

ZuraGard's vehicle, 0.5mL of ZuraGard's vehicle, was added aseptically to a pre-weighed empty applicator for use per applicable test site and applied topically by scrubbing for 2 minutes over a 1.5" x 5" area on the groin or scrubbing for 30 seconds over 5" x 5" area on the abdomen. Application was performed using repeated back and forth strokes of the sponge. Each test site was air-dried for 3 minutes. Post treatment weight of the applicator was recorded.

***Reviewer's comments: The application procedure is consistent with the labeling directions provided for ZuraGard and is acceptable.***

#### **Timing of Post Application Sample Collection**

Microbial samples were collected at 30 seconds ( $\pm$  5 seconds), 10 minutes ( $\pm$  30 seconds), and 6 hours ( $\pm$  30 minutes) post treatment application for both the abdomen and the groin regions. Post application timing began upon completion of the treatment material application, including drying time. Microbial samples were collected using the scrub cup technique.

A skin irritation assessment was performed prior to collection of the post treatment microbial sample collection (30 seconds, 10 minutes, and 6 hours) and a corresponding rating score for each individual skin condition was recorded in the subject's Case Report Form.

If an irritation score of 3 for any individual skin condition at any post treatment observation was assigned, the subject was discontinued from the study and an adverse event was recorded. Following final sample collection, residual study materials were wiped/cleansed.

***Reviewer's comments: On June 17, 2016, FDA advised the Sponsor to collect samples and analyze data at 30 seconds, 10 minutes, and 6 hours after drying time is complete. The sample collection timings are consistent with FDA's advice, and is acceptable.***

#### **Microbial Sample Collection/Scrub Cup Technique**

Quantitative cultures (screening baselines, treatment baselines, and post treatment application) were collected by using a modification of the cylinder sampling technique of Williamson-Kligman scrub cup technique. To collect the samples, a sterile scrub cup (2.20

cm I.D. for ZX-ZP-0073 study; 3.46 cm<sup>2</sup> for ZX-ZP-0074) was placed on the site and held firmly to the skin. Sampling solution (SS, 3.0 mL) was pipetted into the cup and the skin was scrubbed in a circular motion with moderate pressure for 1 minute using a sterile rubber policeman. With a sterile transfer pipette, the SS was then 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 comments: The microbial sample collection and the scrub cup techniques are standard methodology and are acceptable. We note that the study ZX-ZP-0073 performed at MicroBioTest facility consistently used a scrub cup size 2.20 cm I.D., (3.80 cm<sup>2</sup>), while study ZX-ZP-0074 performed at the BioScience Labs consistently used a scrub cup size 2.10 cm I.D., (3.46 cm<sup>2</sup>). Since the 1994 TFM (59 FR 31402 at 31450) does not specify the diameter of the sampling cup used to sample the microorganisms, and it provides a range from approximately 2.5 to 4.0 centimeters, these scrub sizes and methodology are acceptable.***

### **Bacterial Enumeration Methods**

Following sample collection, 10-fold serial dilutions (1 mL sample and 9 mL Butterfield's sterile phosphate buffered water (PBW)) 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 comments: The sampling solution (SS) contains 75mM phosphate buffer (0.04% monobasic potassium phosphate, and 1.01% dibasic sodium phosphate) with 0.1% Triton® X-100, 0.3% lecithin, 1.0% polyoxyethylene sorbitan monooleate (Tween® 80), and 1.0% Tamol™ SN; pH 7.9 ± 0.1, sterile. This sampling solution contains neutralizers, consistent with the Agency's recommendations to sponsors to include neutralizers in the sampling solution. This is acceptable.***

### **Selection of Study Subjects**

Healthy male and female volunteers, 18 years of age or older, 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%. Based on the pilot study ZX ZP 0068 study results this required a sufficient number of volunteers in the screening phase so that at least a total of 320 subjects per active treatment arm (ZuraGard and ChloroPrep) and 64 for the vehicle control arm (ZuraGard's vehicle) were available. 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. See following table for a representation of demographic characteristics of the study population.

**Table 25. Demographic Characteristics of Clinical Studies (Source: Table 2.7.3-10, module 2, summary of clinical efficacy)**

Study	Number of Subjects		
	ZX-ZP-0074 (N = 640)	ZX-ZP-0073 (N = 440)	ZX-ZP-0068 (N = 89)
<b>Age, years</b>			
Mean (standard deviation)	30 <sup>a</sup>	38.43 (15.32)	38.88 (14.31)
Minimum, maximum	18, 85	18, 80	19, 75
<b>Sex, n (%)</b>			
Female	164 (25.63)	190 (43.18)	32 (35.96)
Male	476 (74.38)	250 (56.82)	57 (64.04)
Study	Number of Subjects		
	ZX-ZP-0074 (N = 640)	ZX-ZP-0073 (N = 440)	ZX-ZP-0068 (N = 89)
<b>Race, n (%)</b>			
Asian	8 (1.25)	119 (27.05)	39 (43.82)
Black/African American	9 (1.41)	84 (19.09)	12 (13.48)
Hispanic/Latino	20 (3.13) <sup>b</sup>	45 (10.23)	7 (7.87)
Caucasian	576 (90.00)	176 (40.00)	30 (33.71)
Other	47 (7.34) <sup>c</sup>	16 (3.64)	1 (1.12)

Abbreviations: CSR = clinical study report

<sup>a</sup> Median is presented; mean and standard deviation were not reported.

<sup>b</sup> Subjects double-counted as another race.

<sup>c</sup> A total of 23 subjects who chose not to disclose race are included with 'other' race.

### **Study Subjects (Study ZX ZP-0073)**

A total of 440 subjects were treated on the abdomen and groin in Study ZX-ZP-0073. There were 344 subjects who had qualifying Treatment Day baseline counts on the groin (right and left) and the abdomen (right and left) and completed the study. Of the 440 treated subjects, 34 subjects had qualifying Treatment Day baseline counts only on the abdomen (right and left and 5 of them only on 1 side) and completed the study. A total of 19 subjects had qualifying Treatment Day baseline counts only on the groin (right and left and 2 of them only on 1 side) and completed the study. This resulted in 751 evaluable abdomen sites and 724 evaluable groin sites. Most subjects were male (56.82%) and the most common races ( $\geq 10\%$  of subjects) were Caucasian (40.00%), Asian (27.05%), Black/African-American (19.09%), and Hispanic (10.23%). Age ranged from 18 to 80 years, with a mean age of 38.43 years.

### **Study Subjects (Study ZX ZP-0074)**

A total of 641 subjects were randomized, 640 subjects were treated, and 639 subjects completed testing in Study ZX-ZP-0074. A total of 416 subjects were treated at both abdomen and groin sites, 69 subjects were treated at the groin site only, and 155 subjects were treated at the abdomen site only. Of the 640 treated subjects, 67 subjects were baseline failures at all sites; therefore, 573 subjects were used in the efficacy analysis. The majority of treated subjects were male (74.38%) and the most common race ( $\geq 10\%$  of subjects) was Caucasian (90.00%). Age ranged from 18 to 85 years, with a median age of 30 years.

***Reviewer’s comments:*** *During the End of Phase 2 June 17, 2016 meeting, the FDA stated that, for the primary analysis, the Sponsor could use the modified Intent-to-Treat (mITT) population. The mITT consists of all randomized subjects who have met all the inclusion criteria but none of the exclusion criteria at baseline (pre-treatment). Subjects who failed the baseline bacterial count criteria would be excluded from this analysis population and would not be considered non-responders. Post-randomization and post-treatment protocol violations should be adjudicated as failures in this primary analysis. The Sponsor states that for the study ZX-ZP 073, 440 subjects with treatment day baseline counts were treated. For study ZX ZP 0074, 641 randomized subjects 639 completed the study, although 67 subjects were baseline failures and were excluded from the analysis per FDA’s June 17, 2016 advise. This is acceptable. The table 26 below (source June 6, 2016 FDA statistician Yueqin Zhao review of IND 117045) specifies the sample size calculation for the pivotal studies, which was based on the pilot studies performed.*

**Table 26. Sample Size Estimation (Source: FDA statistician Yueqin Zhao review of IND 117045, dated June 6, 2016)**

Treatment	Number of Abdomen Evaluations	Number of Groin Evaluations
ZuraPrep™ 10.5 mL Applicator	190	190
ChloraPrep® 10.5 mL Applicator (b) (4) (int)	190	190
Normal saline (negative control)	60	60

*Also, refer to the statistician, Dr. Sai Dharmarajan’s review in DARRTS for additional comments. For subject selection for the evaluation of effectiveness studies, the Agency encourages the Sponsor’s to include the population more likely to use these products. Study ZX ZP 073 has representation from Caucasian (40.00%), Asian (27.05%), Black/African-American (19.09%), and Hispanic (10.23%) origin, which acceptable. However, study ZX ZP 074 had a predominant Caucasian population 90%, also 74.38% male. In our view, these differences in the demographic population in the study ZX-ZP-0074 should not impact the efficacy results. Also, refer to Medical Officer’s review in DARRTS. The Sponsor has used mITT population for the primary analysis, this is acceptable.*

#### 4.2.2. Efficacy Results

##### Analysis of Effectiveness Data

A Modified Intent-to-Treat (mITT) Population was used for analysis and consisted of all subjects who had at least one site (left or right for abdominal or inguinal) that passed the treatment day baseline and had CFU results for any other sample time for that site. Body

sites were included in the mITT population only if they met the treatment day baseline criteria. The mITT data set was evaluated for efficacy.

***Reviewer’s comments: During the End of Phase-2 June 17, 2016 meeting, we stated the following: “For the primary analysis, you can use the modified Intent-to-Treat (mITT) population. The mITT consists of all randomized subjects who have met all the inclusion criteria but none of the exclusion criteria at baseline (pre-treatment). Subjects who failed the baseline bacterial count criteria would be excluded from this analysis population and would not be considered non-responders. Adjudicate post-randomization and post-treatment protocol violations as failures in this primary analysis.” “The sample size for this study needs to account for the baseline failure rate. Treatment assignment should be randomized for all subjects in the study. Note that a deterministic assignment of treatment to a new site based on which treatments the failed sites were assigned to would violate randomization”.***  
***This reviewer notes that mITT population is used for the analysis, consistent with FDA’s recommendations. This is acceptable.***

#### 4.2.3. Efficacy Results of Study ZX-ZP 0073 and ZX-ZP 0074

In study ZX-ZP-0073, a total of 440 subjects were randomized and treated, 344 passed the treatment day baseline counts and were used in the study. Study ZX-ZP 0073 evaluated 342 (ZuraGard), and 340 (ChloraPrep) abdominal regions, and 330 (ZuraGard), and 326 (ChloraPrep) groin regions. For ZuraGard’s vehicle control, 69 abdominal and 68 groin regions were treated. In the study ZX-ZP-0074, a total of 641 subjects were randomized, 640 subjects were treated, and 573 subjects passed the treatment day baseline counts and completed testing. Overall, study ZX-ZP 0074 evaluated 324 (ZuraGard) and 320 (ChloraPrep) abdominal regions; and 343 (ZuraGard) and 352 (ChloraPrep) groin regions. For ZuraGard’s vehicle control, 68 abdominal and 74 groin regions were treated.

##### 1) Primary Analysis Responder Rates

**Table 27. Responder Rate at 10 Minutes (mITT population, study ZX-ZP 0073 and ZX-ZP-0074) (Source: Table 2.7.3-11 module 2, summary of clinical efficacy)**

Study	Abdomen			Groin		
	Vehicle Rate (%) (95% CI)	ZuraPrep Rate (%) (95% CI)	ChloraPrep Rate (%) (95% CI)	Vehicle Rate (%) (95% CI)	ZuraPrep Rate (%) (95% CI)	ChloraPrep Rate (%) (95% CI)
ZX-ZP-0074	11.8 (5.2, 21.9)	80.9 (76.2, 85.0)	79.4 (74.5, 83.7)	1.4 (0.0, 7.3)	75.2 (70.3, 79.7)	72.4 (67.5, 77.0)
ZX-ZP-0073	17.4 (9.3, 28.4)	96.5 (94.0, 98.2)	96.8 (94.3, 98.4)	16.2 (8.4, 27.1)	92.7 (89.4, 95.3)	91.1 (87.5, 94.0)

**Table 28. Responder rate at 30 Seconds (mITT population, study ZX-ZP-0073 and ZX-ZP-0074) (Source: Table 2.7.3-13 module 2, summary of clinical efficacy)**

Study	Abdomen			Groin		
	Vehicle Rate (%) (95% CI)	ZuraPrep Rate (%) (95% CI)	ChloraPrep Rate (%) (95% CI)	Vehicle Rate (%) (95% CI)	ZuraPrep Rate (%) (95% CI)	ChloraPrep Rate (%) (95% CI)
ZX-ZP-0074	4.4 (0.9, 12.4)	76.5 (71.5, 81.1)	76.3 (71.2, 80.8)	1.4 (0.0, 7.3)	70.8 (65.7, 75.6)	71.0 (66.0, 75.7)
ZX-ZP-0073	4.4 (0.9, 12.2)	84.2 (79.9, 87.9)	80.6 (76.0, 84.7)	2.9 (0.4, 10.2)	74.6 (69.5, 79.2)	68.1 (62.7, 73.1)

**Table 29. Responder rate at 6 Hour (mITT population, study ZX-ZP-0073 and ZX-ZP-0074) (Source: Table 2.7.3-15, module 2, summary of clinical efficacy)**

Study	Abdomen			Groin		
	Vehicle Rate (%) (95% CI)	ZuraPrep Rate (%) (95% CI)	ChloraPrep Rate (%) (95% CI)	Vehicle Rate (%) (95% CI)	ZuraPrep Rate (%) (95% CI)	ChloraPrep Rate (%) (95% CI)
ZX-ZP-0074	86.8 (76.4, 93.8)	99.1 (97.3, 99.8)	99.1 (97.3, 99.8)	100.0 (96.0, 100.0)	100.0 (99.1, 100.0)	99.4 (98.0, 99.9)
ZX-ZP-0073	97.1 (89.9, 99.7)	99.4 (97.9, 99.9)	100.0 (98.9, 100.0)	100.0 (94.7, 100.0)	100.0 (98.9, 100.0)	100.0 (98.9, 100.0)

***Reviewer’s comments:*** As per the End of Phase 2 June 17, 2016 meeting advice, using mITT population the responder rate at 10 minutes is used for the primary analysis, and the responder rate at 30 seconds and 6 hours are used as secondary endpoints. This is acceptable. The primary efficacy criteria for the test product is that the responder rate 95% confidence interval lower bound should be  $\geq 70\%$  at 10 minutes in both, the groin and the abdomen sites.

***Primary Analysis at 10 minute time point (Table 27):*** Both ZuraGard and ChloraPrep met the primary efficacy criteria, the responder rate is  $\geq 70\%$  at 10 minutes on the abdomen and groin sites.

- ***ZX-ZP-0073:*** The lower bound of the 95% confidence interval for the 10-minute responder rate for ZuraGard was above 70% for both the abdomen (point estimate: 96.5%, confidence interval: 94.0% to 98.2%) and the groin site (point estimate: 92.7%, confidence interval: 89.4% to 95.3%). The lower bound of the 95% confidence interval for the 10-minute responder rate for ChloraPrep was also above 70% (point estimate: 96.8%, confidence interval: 94.3% to 98.4%) and the groin (point estimate: 91.1%, confidence interval: 87.5% to 94%).
- ***ZX-ZP-0074-*** The lower bound of the 95% confidence interval for the 10-minute responder rate for ZuraGard was above 70% for both the abdomen (point estimate: 80.9%, confidence interval: 76.2% to 85.0%) and the groin site (point estimate: 75.2%, confidence interval: 70.3% to 79.7%). The lower bound of the 95% confidence interval for the 10-minute responder rates for ChloraPrep was slightly lower for the abdomen (point estimate: 79.4%, confidence interval: 74.5% to 83.7%) and the groin site (point estimate: 72.4%, confidence interval: 67.5% to 77.0%).

***For studies ZX-ZP-0073 and ZX-ZP-0074 on abdominal and groin sites, the***

*responder rate for all active products was significantly higher than that for the vehicle control. The responder rate point estimate for vehicle control was 17.4 and 11.8 for the abdominal region, and 16.2 and 1.4 for the groin region, for study ZX-ZP-0073 and ZX-ZP-0074, respectively.*

**Responder Rate at 30 second time point (Table 28):**

- *ZX-ZP-0073: On the abdominal site, for ZuraGard and ChloroPrep, the responder rate point estimate was 84.2% and 80.6%, respectively. At the groin site, the responder rate was 74.6% and 68.1% for ZuraGard and ChloroPrep, respectively.*
- *ZX-ZP-0074: On the abdominal site, for ZuraGard and ChloroPrep, the responder rate point estimate was 76.5% and 76.3%, respectively. At the groin site, the responder rate was 70.8% and 71.0% for ZuraGard and ChloroPrep, respectively.*

**Responder Rate at 6 hour time point (Table 29):**

- *ZX-ZP-0073: At the 6-hour time point, responder rate for ZuraGard was 99.4% at abdomen and 100% at groin site (all values below baseline). ChloroPrep showed a 100% responder rate for both the abdomen and the groin sites.*
- *ZX-ZP-0074: At the 6-hour time point, ZuraGard showed a 99.1% responder rate at the abdomen and a 100% responder rate at the groin site (all values below baseline). ChloroPrep showed a 99.1% responder rate on abdomen and 99.4% on groin.*

*Among subjects who received ZuraGard or ChloroPrep for studies ZX-ZP-0073 and ZX-ZP-0074, 8 assessments (5 ZuraGard, 3 ChloroPrep) exceeded baseline CFU/cm<sup>2</sup> for the abdomen and 2 assessments (ChloroPrep) exceeded baseline CFU/cm<sup>2</sup> for the groin. Sponsor states (source module-2 summary of Clinical efficacy) that most of these subjects were classified as non-responders. A few subjects showed values exceeding baseline at the abdomen while they still met the below baseline criteria at groin site at 6 hours.*

*For the secondary end point at 30 seconds analysis, both ZuraGard and ChloroPrep met the responder rate of  $\geq 70\%$  on abdominal site, this is acceptable. However, at 30 seconds, on the groin site ZuraGard was able to achieve 74.6% but ChloroPrep could only achieve 68.1% responder rate (Table 28). Since, per FDA advice of June 17, 2016, validity goals are for the active control to meet  $\geq 70\%$  responder rate criterion at 10 minutes at groin and abdomen, and for both the test product and the active control to be superior to the vehicle, this is acceptable. The results described above show that both ZuraGard and ChloroPrep successfully met primary efficacy goals (“lower bound of a 95% confidence interval for the responder rate  $\geq 70\%$  at 10 minutes in both the groin and the abdomen sites”). This reviewer finds this acceptable since ChloroPrep has achieved the primary efficacy end point.*

**2) Analysis of Efficacy Results by Average Treatment Effect for Study ZX-ZP 0073 and ZX-ZP 0074**

As described by FDA on July 10, 2017, the test product’s effectiveness was also assessed using the average treatment effect (ATE). The ATE was estimated from a linear regression of posttreatment bacterial count (log10 scale) at 10 minutes on the additive effect of a treatment indicator compared to the baseline or pretreatment measurement (log10 scale). To show effectiveness, the test product would have been: 1) non-inferior to ChloroPrep by a 0.5 margin (log<sub>10</sub> scale, upper bound of 95% confidence interval of the difference in ATE values ≤0.5) at 10 minutes; and 2) superior to the vehicle control by a margin of 1.2 (log<sub>10</sub> scale, lower bound of 95% confidence interval of the difference in ATE values ≥1.2) at 10 minutes.

**Table 30. Study ZX-ZP-0073 Analysis by Average Treatment Effect (Source: IR response dated October 24, 2018)**

Body Area	Treatments	30 Seconds		10 Minutes	
		ATE Difference	95% CI	ATE Difference	95% CI
Groin	Non-inferiority (ChloroPrep vs ZuraPrep)	-0.078	(-0.264 to 0.108)	-0.039	(-0.184 to 0.106)
	Superiority – ZuraPrep vs Vehicle	2.300	(1.983 to 2.618)	2.595	(2.347 to 2.843)
	Superiority – ChloroPrep vs Vehicle	2.222	(1.904 to 2.540)	2.556	(2.308 to 2.804)
Abdomen	Non-inferiority - ChloroPrep vs ZuraPrep	-0.111	(-0.238 to 0.016)	-0.021	(-0.096 to 0.054)
	Superiority – ZuraPrep vs Vehicle	1.892	(1.673 to 2.111)	1.870	(1.740 to 1.999)
	Superiority – ChloroPrep vs Vehicle	1.781	(1.562 to 2.000)	1.849	(1.719 to 1.979)

ATE = average treatment effect; CI = confidence interval.

**Table 31. Study ZX-ZP-0073 Analysis by Average Treatment Effect (Source: IR response dated October 24, 2018)**

Body Area	Treatments	30 Seconds		10 Minutes	
		ATE Difference	95% CI	ATE Difference	95% CI
Groin	Non-inferiority (ChloroPrep vs ZuraPrep)	-0.024	(-0.217 to 0.169)	-0.020	(-0.212 to 0.172)
	Superiority – ZuraPrep vs Vehicle	2.609	(2.283 to 2.934)	2.454	(2.129 to 2.778)
	Superiority – ChloroPrep vs Vehicle	2.584	(2.259 to 2.909)	2.434	(2.110 to 2.757)
Abdomen	Non-inferiority - ChloroPrep vs ZuraPrep	-0.023	(-0.196 to 0.150)	-0.045	(-0.208 to 0.117)
	Superiority – ZuraPrep vs Vehicle	2.048	(1.756 to 2.341)	1.972	(1.697 to 2.247)
	Superiority – ChloroPrep vs Vehicle	2.025	(1.733 to 2.318)	1.927	(1.651 to 2.202)

ATE = average treatment effect; CI = confidence interval.

***Reviewer’s comments: On September 7, 2018, FDA requested the Sponsor to***

*reanalyze the clinical efficacy data using Average Treatment Effect measures and 95% confidence intervals and assess non-inferiority and superiority of the two pivotal efficacy studies (ZX-ZP-0073 and ZX-ZP-0074). On October 18, 2018 the Sponsor submitted the reanalyzed data for both ZX-ZP-0073 and ZX-ZP-0074 studies. Table 30 and Table 31 above (source- IR response October 24, 2018) summarize the ATE analysis results.*

**Study ZX-ZP-0073:**

- *At 10 minutes, the noninferiority ATE point estimates for ZuraGard vs. ChloroPrep were -0.039 (CI: -0.18 to 0.106) and -0.211 (CI: -0.09-0.05) for groin and abdominal sites, respectively. The superiority ATE point estimates for ZuraGard vs. vehicle were 2.595 (CI: 2.34 to 2.84), and 1.87 (CI: 1.74 to 1.99) for the groin and abdominal sites, respectively.*
- *At 30 seconds, the noninferiority ATE point estimates for ZuraGard vs. ChloroPrep were -0.078 (CI: -0.26 to 0.108), and -0.11(CI: -0.23 to 0.016) for groin and abdominal sites, respectively. The superiority ATE point estimates for ZuraGard vs. vehicle were 2.3 (CI: 1.98 to 2.68), and 1.89 (CI: 1.67-2.11) for groin and abdominal sites, respectively.*

**Study ZX-ZP-0074:**

- *At 10 minutes, the noninferiority ATE point estimates for ZuraGard vs. ChloroPrep were -0.020 (CI: -0.21 to 0.17), and -0.045 (CI: -0.20 to 0.11) for groin and abdominal sites, respectively. ZuraGard was superior to vehicle control by a margin of 2.54 (CI: 2.1 to 2.77), and 1.97 (CI: 1.69 to 2.24) for the groin and abdominal sites, respectively.*
- *At 30 seconds, the noninferiority ATE point estimates for ZuraGard vs. ChloroPrep were -0.024 (CI: -0.21 to 0.16), and -0.23 (CI: -0.19 to 0.015) for groin and abdominal sites, respectively. ZuraGard was superior to vehicle control by a margin of 2.60 (CI: 2.28 to 2.93), and 2.04 (CI: 1.75 -2.34) on groin and abdominal sites, respectively.*

*In both studies, ZX-ZP-0073 and ZX-ZP-0074, the ATE analysis criteria was satisfactory in accordance with FDA's recommendations, the upper limit of the 95% confidence interval for the non-inferiority of ZuraGard vs. ChloroPrep was below 0.5, and the lower limit of the 95% confidence interval for the superiority of ZuraGard vs. vehicle was above 1.2.*

**3) Analysis of Study ZX-ZP-0073 and Study ZX-ZP-0074 Data by Mean Log<sub>10</sub> CFU/cm<sup>2</sup> Reduction from Baseline**

**Table 32. Mean log<sub>10</sub> CFU/cm<sup>2</sup> reductions from baseline and their 95% confidence intervals at 10 Minutes (mITT population, study ZX-ZP 0073 and ZX-ZP 0074) (Source: Table 2.7.3-17, module 2, summary of clinical efficacy)**

Study	Abdomen			Groin		
	Vehicle Mean (95% CI)	ZuraPrep Mean (95% CI)	ChloraPrep Mean (95% CI)	Vehicle Mean (95% CI)	ZuraPrep Mean (95% CI)	ChloraPrep Mean (95% CI)
ZX-ZP-0074	1.00 (0.78, 1.21)	2.99 (2.87, 3.11)	2.91 (2.79, 3.03)	1.60 (1.44, 1.76)	4.04 (3.90, 4.18)	4.01 (3.86, 4.16)
ZX-ZP-0073	1.47 (1.30, 1.65)	3.35 (3.27, 3.44)	3.34 (3.26, 3.43)	2.23 (2.05, 2.41)	4.83 (4.74, 4.91)	4.78 (4.69, 4.87)

**Table 33 Mean log<sub>10</sub> CFU/cm<sup>2</sup> reductions from baseline and their 95% confidence intervals at 30 Seconds (mITT population, study ZX-ZP 0073 and ZX-ZP 0074) (Source: Table 2.7.3-18, module 2, summary of clinical efficacy)**

Study	Abdomen			Groin		
	Vehicle Mean (95% CI)	ZuraPrep Mean (95% CI)	ChloraPrep Mean (95% CI)	Vehicle Mean (95% CI)	ZuraPrep Mean (95% CI)	ChloraPrep Mean (95% CI)
ZX-ZP-0074	0.71 (0.56, 0.86)	2.77 (2.64, 2.89)	2.73 (2.60, 2.86)	1.23 (1.07, 1.39)	3.82 (3.68, 3.97)	3.79 (3.64, 3.94)
ZX-ZP-0073	1.07 (0.84, 1.30)	2.99 (2.87, 3.10)	2.89 (2.78, 3.00)	1.59 (1.35, 1.82)	3.91 (3.80, 4.03)	3.83 (3.71, 3.94)

**Table 34. Mean log<sub>10</sub> CFU/cm<sup>2</sup> reductions from baseline and their 95% confidence intervals at 6 Hours (mITT population, study ZX-ZP 0073 and ZX-ZP 0074) (Source: Table 2.7.3-19, module 2, summary of clinical efficacy)**

Study	Abdomen			Groin		
	Vehicle Mean (95% CI)	ZuraPrep Mean (95% CI)	ChloraPrep Mean (95% CI)	Vehicle Mean (95% CI)	ZuraPrep Mean (95% CI)	ChloraPrep Mean (95% CI)
ZX-ZP-0074	1.37 (1.10, 1.64)	3.03 (2.91, 3.14)	2.96 (2.85, 3.06)	2.10 (1.91, 2.28)	3.96 (3.83, 4.10)	3.81 (3.67, 3.95)
ZX-ZP-0073	1.17 (0.96, 1.38)	2.45 (2.34, 2.55)	2.42 (2.32, 2.53)	2.03 (1.82, 2.24)	3.00 (2.90, 3.11)	2.99 (2.88, 3.09)

**Table 35. Mean Log<sub>10</sub> CFU/cm<sup>2</sup> values with Standard Deviation (SD) Study ZX-ZP-0073 (Source: Appendix Table 3 of Statistical Review dated March 19, 2019)**

Body Area		N	Baseline		30 Seconds		10 Minutes		6 Hours	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Abdomen	ChloraPrep	340	3.43	0.50	0.53	0.92	0.08	0.52	1.00	0.96
	Vehicle	69	3.37	0.50	2.30	0.67	1.91	0.67	2.19	0.69
	ZuraPrep	342	3.42	0.52	0.42	0.83	0.05	0.48	0.97	0.91
Groin	ChloraPrep	326	5.40	0.45	1.57	1.29	0.62	1.01	2.41	0.98
	Vehicle	68	5.36	0.37	3.77	0.71	3.15	0.83	3.34	0.93
	Zuraprep	330	5.43	0.48	1.51	1.27	0.59	0.98	2.43	0.99

**Table 36. Mean Log<sub>10</sub> CFU/cm<sup>2</sup> values with Standard Deviation (SD) Study ZX-ZP-0074 (Source: Appendix Table 4 of Statistical Review dated March 19, 2019)**

Body Area		N	Baseline		30 Seconds		10 Minutes		6 Hours	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Abdomen	ChloraPrep	320	3.63	0.49	0.90	1.23	0.72	1.09	0.68	0.98
	Vehicle	68	3.66	0.55	2.95	0.82	2.66	0.98	2.28	1.16
	ZuraPrep	324	3.69	0.48	0.92	1.19	0.70	1.09	0.66	0.96
Groin	ChloraPrep	352	5.85	0.50	2.06	1.38	1.84	1.38	2.04	1.28
	Vehicle	74	5.89	0.49	4.66	0.67	4.29	0.57	3.79	0.76
	Zuraprep	343	5.87	0.50	2.04	1.33	1.83	1.32	1.90	1.28

***Reviewer's comments:*** We had previously informed the Sponsor (June 17, 2016 during the End of Phase 2/Pre-phase 3 meeting) that, in order to demonstrate effectiveness for the secondary endpoint (mean log<sub>10</sub> reduction), we expected the lower bound of a 2-sided 95% CI to be ≥2 log<sub>10</sub> reduction on the abdomen, and ≥3 log<sub>10</sub> reduction on the groin, and the bacterial counts not to exceed the baseline at 6 hours.

Both ZuraGard and ChloraPrep met the secondary efficacy criteria at 10 minutes and 30 seconds (≥2 log<sub>10</sub> reduction on abdomen and ≥3 log<sub>10</sub> reduction on the groin from baseline). Tables 32 and 33 show the mean log reductions with 95% confidence intervals.

- ZX-ZP-0073: At 10-minutes; for ZuraGard on abdomen the mean log<sub>10</sub> reduction (confidence interval) was 3.35 (3.27 to 3.44) and for the groin 4.83 (4.74 to 4.91). For ChloraPrep on abdomen mean log<sub>10</sub> reduction (confidence interval) was 3.34 (3.26 to 3.43) and on groin 4.78 (4.69 to 4.87)
- ZX-ZP-0074: At 10-minutes; for ZuraGard on abdomen 2.99 (2.87 to 3.11) and on groin mean 4.04 (3.90 to 4.18). For ChloraPrep on abdomen 2.91 (2.79 to 3.03) and on groin mean 4.01 (3.86 to 4.16).

The mean log<sub>10</sub> reduction (confidence interval) at 30 seconds was similar for ZuraGard and ChloraPrep in both studies, ZX-ZP-0073 and ZX-ZP-0074; achieving ≥2 log<sub>10</sub> reduction on abdomen and ≥3 log<sub>10</sub> reduction on the groin.

For both, ZuraGard and ChloraPrep, and for the abdominal and groin sites, the log reductions at the 6 hour timepoint were similar to the log reductions achieved at 30 seconds, which were lower than the baseline mean Log<sub>10</sub> CFU/cm<sup>2</sup> values (Tables 35 and 36, source: appendix table 3 and 4 of statistical review dated March 19, 2019 in DARRTS), therefore, both ZuraGard and ChloraPrep did not exceed the baseline counts at 6 hours.

#### 4.3. Neutralization Validation for Study ZX-ZP 0073 (MicroBioTest) and Study ZX-ZP 0074 (BioScience Labs)

The purpose of the neutralization study is to ensure that neutralizers used in the recovery medium quench the antimicrobial activity of the test material, while not being toxic to the bacteria. The study comprised both an in vivo component performed using human subjects,

and an in vitro testing performed based on ASTM E1054-08 (2013), “Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents.” *Staphylococcus epidermidis* MRSE (ATCC #51625) and *Staphylococcus epidermidis* (ATCC #12228) were used as the challenge species in both components of the neutralizer validation study.

Nine subjects were used for the neutralization validation study. Each subject met the inclusion and exclusion criteria described above for the pivotal study, except for the minimum baseline bacterial counts. No minimum bacterial counts were required, and the washout period was only necessary for 7 days. Subjects received all three test materials (ZuraGard, ChloroPrep and ZuraGard’s vehicle). However, these treatments were assigned randomly so each subject received two of the three products on the abdomen. Details are provided in Section 14.10 of the ZX-ZP 0073 protocol and in Appendix 3 of the ZX-ZP 0074 protocol.

**Table 37. Results of the Neutralization Validation (Source: Tables 2-4, appendix 16.4, study report ZX-ZP-0073)**

Test Article Control  
 ZuraPrep™ 10.5 mL Applicator  
 Results Expressed as Log<sub>10</sub> CFU/mL  
 Methicillin Sensitive *Staphylococcus epidermidis*

Time	**Test 3	***Test 4	Difference from Test 3
≤1 minute	*1.72	*0.00	1.72
30 minutes	*1.78	*0.00	1.78

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

Test Article Control  
 ZuraPrep™ 10.5 mL Applicator  
 Results Expressed as Log<sub>10</sub> CFU/mL  
 Methicillin Resistant *Staphylococcus epidermidis*

Time	**Test 3	***Test 4	Difference from Test 3
≤1 minute	*1.90	*0.00	1.90
30 minutes	*1.94	*0.00	1.94

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

Test Article Control  
 ChloroPrep® 10.5 mL Applicator (b) (4) Tint  
 Results Expressed as Log<sub>10</sub> CFU/mL  
 Methicillin Sensitive *Staphylococcus epidermidis*

Time	**Test 3	***Test 4	Difference from Test 3
≤1 minute	*1.72	*0.00	1.72
30 minutes	*1.78	*0.00	1.78

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

**Table 37 (continued). Results of the Neutralization Validation (Source: Tables 2-4, appendix 16.4, study report ZX-ZP-0073)**

Test Article Control  
 Chloraprep® 10.5 mL Applicator (b) (4) Tint  
 Results Expressed as Log<sub>10</sub> CFU/mL  
 Methicillin Resistant *Staphylococcus epidermidis*

Time	**Test 3	***Test 4	Difference from Test 3
≤1 minute	*1.90	*0.00	1.90
30 minutes	*1.94	*0.00	1.94

\*All results are the average of Replicates 1, 2 and 3

\*\*Test Microorganism Viability Control

\*\*\*Test Article Control

Neutralizer Toxicity Control  
 Results Expressed as Log<sub>10</sub> CFU/mL  
 Methicillin Resistant *Staphylococcus epidermidis*

Time	**Test 3	***Test 2	Difference from Test 3
≤1 minute	*1.90	*1.88	0.02
30 minutes	*1.94	*1.90	0.04

\*All results are the average of Replicates 1, 2 and 3

\*\* Test Microorganism Viability Control

\*\*\* Neutralizer Toxicity Control

Neutralizer Toxicity Control  
 Results Expressed as Log<sub>10</sub> CFU/mL  
 Methicillin Sensitive *Staphylococcus epidermidis*

Time	**Test 3	***Test 2	Difference from Test 3
≤1 minute	*1.72	*1.69	0.03
30 minutes	*1.78	*1.74	0.04

\*All results are the average of Replicates 1, 2 and 3

\*\* Test Microorganism Viability Control

\*\*\* Neutralizer Toxicity Control

**Table 38. Neutralizer Effectiveness Control Results (Source: Table 9, appendix 16.4, study report ZX-ZP-0073)**

Neutralizer Effectiveness  
 ZuraPrep™ 10.5 mL Applicator  
 Results Expressed as Log<sub>10</sub> CFU/mL  
 Methicillin Resistant *Staphylococcus epidermidis*

Randomization No.	Time	**Test 3	***Test 1	Difference from Test 3
N02	≤1 minute	*1.90	1.81	0.09
	30 minutes	*1.94	1.88	0.06
N03	≤1 minute	*1.90	1.89	0.01
	30 minutes	*1.94	1.92	0.02
N04	≤1 minute	*1.90	1.89	0.01
	30 minutes	*1.94	1.93	0.01
N05	≤1 minute	*1.90	1.90	0.00
	30 minutes	*1.94	1.92	0.02
N06	≤1 minute	*1.90	1.87	0.03
	30 minutes	*1.94	1.90	0.04
N08	≤1 minute	*1.90	1.88	0.02
	30 minutes	*1.94	1.90	0.04

\* All results are the average of Replicates 1, 2 and 3

\*\* Test Microorganism Viability Control

\*\*\* Neutralizer Effectiveness

Neutralizer Effectiveness  
 ChloroPrep® 10.5 mL Applicator (b) (4) (int)  
 Results Expressed as Log<sub>10</sub> CFU/mL  
 Methicillin Resistant *Staphylococcus epidermidis*

Randomization No.	Time	**Test 3	***Test 1	Difference from Test 3
N01	≤1 minute	*1.90	1.89	0.01
	30 minutes	*1.94	1.90	0.04
N02	≤1 minute	*1.90	1.89	0.01
	30 minutes	*1.94	1.90	0.04
N03	≤1 minute	*1.90	1.86	0.04
	30 minutes	*1.94	1.90	0.04
N04	≤1 minute	*1.90	1.88	0.02
	30 minutes	*1.94	1.90	0.04
N07	≤1 minute	*1.90	1.89	0.01
	30 minutes	*1.94	1.93	0.01
N09	≤1 minute	*1.90	1.89	0.01
	30 minutes	*1.94	1.91	0.03

\* All results are the average of Replicates 1, 2 and 3

\*\* Test Microorganism Viability Control

\*\*\* Neutralizer Effectiveness

**Table 38 (continued). Neutralizer Effectiveness Control Results (Source: Table 9, appendix 16.4, study report ZX-ZP-0073)**

Neutralizer Effectiveness  
ZuraPrep™ Vehicle  
Results Expressed as Log<sub>10</sub> CFU/mL  
Methicillin Resistant *Staphylococcus epidermidis*

Randomization No.	Time	**Test 3	***Test 1	Difference from Test 3
N01	≤1 minute	*1.90	1.87	0.03
	30 minutes	*1.94	1.89	0.05
N05	≤1 minute	*1.90	1.88	0.02
	30 minutes	*1.94	1.92	0.02
N06	≤1 minute	*1.90	1.89	0.01
	30 minutes	*1.94	1.92	0.02
N07	≤1 minute	*1.90	1.89	0.01
	30 minutes	*1.94	1.91	0.03
N08	≤1 minute	*1.90	1.81	0.09
	30 minutes	*1.94	1.87	0.07
N09	≤1 minute	*1.90	1.89	0.01
	30 minutes	*1.94	1.93	0.01

\* All results are the average of Replicates 1, 2 and 3

\*\* Test Microorganism Viability Control

\*\*\* Neutralizer Effectiveness

***Reviewer's comments:*** On June 15, 2015, FDA asked Sponsor to include *Staphylococcus aureus* MRSA (ATCC 33591 or 33592), or *Staphylococcus epidermidis* MRSE (ATCC 51625), or *Enterococcus faecalis* VRE (ATCC 51299 or 51575) in the neutralization validation assays. Table 38 above represents the neutralization validation results for study ZX-ZP-0073 (MicroBioTest) using *Staphylococcus epidermidis* (MRSE), ATCC 12228. The results for *Staphylococcus epidermidis* (MSSE), ATCC 51625 are included in the Appendix 16.4. The neutralization validation results for study ZX-ZP -0074 (BioScience Labs) are included in Appendix 16.2.6 of the submission. For both studies, since the mean log<sub>10</sub> CFU/mL of each of the active study products (ZuraGard and ChloroPrep) was not more than 0.2 log<sub>10</sub> less than the mean log<sub>10</sub> CFU/mL of the Control Numbers (in accordance with the neutralization validation protocol criteria), the neutralization process was considered effective. Likewise, for both studies, since the mean log<sub>10</sub> CFU/mL of each of the Toxicity Control was not more than 0.2 log<sub>10</sub> less than the mean log<sub>10</sub> CFU/mL of the Control Numbers, sampling solution including neutralizers was considered nontoxic to the tested organisms. The sterility test control did not exhibit any growth and results indicate that the neutralizer was effective and non-toxic. Overall, this reviewer finds the neutralization validation studies for both studies, ZX-ZP -0073 (MicroBioTest) and ZX-ZP -0074 (BioScience Labs) acceptable.

#### 4.4. Protocol Deviations

##### Study ZX-ZP-0073 Protocol Deviations

Sponsor noted eleven protocol deviations during the conduct of the study. None, in the opinion of the Principal Investigator, had an impact on study results (source section 10.2 of study report).

##### Treatment application related deviations:

Subject No. (b) (6): The treatments used were not in accordance with the randomization scheme. On the left side, ZuraGard and on the right side, ChloroPrep were used instead of ZuraGard on the right side and ChloroPrep on the left side.

Subject No. (b) (6): The treatments used were not in accordance with the randomization scheme. On the left side ZuraGard and on the right side ChloroPrep were used instead of ZuraGard on the right side and ChloroPrep on the left side.

***Reviewer's comments: The deviations regarding the randomization schedule for Subjects (b) (6) have no effect on the study results because the application of the test products was randomly performed, and the efficacy data were not affected.***

##### Sampling related deviations:

Subject (b) (6): The sample sites for contact times 10 minutes and 6 hours on the abdomen were not followed per randomization. The 10 minutes contact time sample was collected from the sample site meant for 6 hours; and the 6 hour sample was collected from sample site meant for 10 minutes on abdomen.

Subject (b) (6): the 6 hour samples from the right groin and the left abdomen were collected about 5-9 minutes earlier than the required time.

***Reviewer's comments: The deviation regarding the sampling for subject (b) (6) is acceptable since samples were collected for both 6 hour and 10 minute time points. Subjects (b) (6), 5-9 minutes earlier sample collection did not reflect change in efficacy results since counts for all subjects were below the baseline counts.***

##### Skin Irritation related deviations

Subject No. (b) (6): Skin irritation scores were not documented before the 6 hours sample collection on the groin. No irritation was observed and documented for this subject at all other timepoints and sites.

Subject ID (b) (6): On the screening day, all baseline samples were collected from both the abdomen and the groin sites, however the skin irritation scores were not documented on the CRF. Since all samples were collected, it is assumed there was no skin irritation of all four sites.

***Reviewer's comments: Since the skin irritation scores were 0 (source CSR section 12.1) before and after the test product application for all subjects in the study, this is acceptable. Refer to Medical Officer's review for any additional comments.***

Other deviations:

Subject No. (b) (6): This subject was consented and treated a second time on (b) (6), after being previously treated on (b) (6) under subject no. (b) (6). More than 30 days elapsed between first treatment and second consent. Data from both subject numbers were evaluable.  
 Subject No. (b) (6): The screening baseline counts on the left abdomen were less than the required amount for treatment qualification; subject inadvertently proceeded to treatment. The average of the two plates on the left abdomen was inadvertently miscalculated. The treatment day baseline on both sides of the abdomen were within the required range.

***Reviewer’s comments: For Subject No. (b) (6) there was lapse of 30 days between two treatments, which will not impact study results. This reviewer agrees with the Sponsor and find it is acceptable. For subject (b) (6) since the baseline counts were within required range, this is acceptable.***

**Study ZX-ZP-0074 Protocol Deviations:**

Section 10.2 of the ZX-ZP-0074 study report specifies that a total of 49 individual instances of protocol deviations occurred during the study and have been combined into 12 categories or groups. The protocol deviations are summarized in the table below (descriptive narrative for each category by deviation reference number are included in the report of ZX-ZP-0074).

**Table 40. Summary of Protocol Deviations for Study ZX-ZP-0074 (Source: study report)**

Deviation Number	Protocol Section	Summary	Impact
01	Section 5.2 of Protocol Appendix 1	Neutralization Subjects enrolled before 30 days after previous clinical trial participation.	None
02	Section 8.0	Designated training applicator use in testing.	None
04	Section 13.3	Randomization deviations	None
08	Section 15.2	Irritation dismissal errors.	None
09	Section 13.5	30-second and 10-minute sampling exceptions	None
10	Section 13.5	6-hour sampling exceptions	Subject (b) (6) Abdomen 6-hour samples not performed; non-responders for Test Materials A and B.
18 & 21	Section 13.7	Incubation duration exception	None

**Table 40 (continued). Summary of Protocol Deviations for Study ZX-ZP-0074 (Source: study report)**

Protocol Deviation Summary Table (Continued)

Deviation Number	Protocol Section	Summary	Impact
20	Section 13.6	Cylinder Sampling (Scrub Cup) Technique errors	Subject (b) (6) 10-minute groin sample lost; non-responder; Test Material A. Subject (b) (6) 6-hour groin sample lost; non-responder; Test Material B.
22	Section 13.7	Treatment Day Samples plated beyond 30 minutes	None
27	Section 13.5	Product application error	None
32	Section 13.8	Plating Technician Blinding Compromised	None

**6-hour sampling time exceptions**

Sampling was to occur at 6 hours ± 30 minutes following completion of test material application. For this study, it was pre-determined that 6-hour samples taken within one hour of the exact 6 hour time would be used in analysis. The following exceptions occurred due to technician error or subject returned late:

Subject (b) (6) (Test #42): the left abdomen 6-hour sample was taken late; 6 hours, 51 minutes, and 52 seconds after the air-dry stop time – this is 21 minutes and 52 seconds outside of the ± 30 minutes window (ZuraGard™). Subject met the test day baseline criteria; therefore, data were used in analysis.

Subject (b) (6) (Test #180): the right groin 6-hour sample was taken early; 5 hours, 23 minutes, and 33 seconds after the air-dry stop time – this is 6 minutes and 27 seconds outside of the ± 30 minutes window (ChloraPrep®). Subject met the test day baseline criteria; therefore, data were used in analysis.

Subject (b) (6) (Test #452): the left abdomen 6-hour sample was taken late; 6 hours, 30 minutes, and 14 seconds after the air-dry stop time – this is 14 seconds outside of the ± 30 minutes window (ZuraGard™ Vehicle). Subject met the test day baseline criteria; therefore, data used in analysis.

Subject (b) (6) (Test #499): the left abdomen 6-hour sample was taken late; 6 hours, 42 minutes, and 22 seconds after the air-dry stop time – this is 12 minutes and 22 seconds outside of the ± 30 minutes window (ChloraPrep®). Subject met the test day baseline criteria; therefore, data were used in analysis.

***Reviewer’s comments: For the abdomen and groin sites site, four subjects had a deviation of having a required 6-hour (±30 minutes) sample collected before or beyond the defined time interval. The range was from 14 seconds to 21.52 minutes, and the outcome did not impact the difference from baseline. This reviewer finds it acceptable, these are considered minor deviations and have no effect on the study results.***

Subject (b) (6) (Test #557): the subject failed to appear for the 6-hour sampling; therefore, samples could not be taken (left side product was ChloroPrep®, right side product was ZuraGard™). Subject passed the test day baseline criteria on both sides of the abdomen; therefore, the 6-hour samples were treated as non-responders in the efficacy analysis.

**Reviewer's comment: This is acceptable.**

### **Cylinder Sampling (Scrub Cup) Technique errors**

Baseline samples were to use sterile Stripping Suspending Fluid (SSF), and post-test material application samples were to use sterile Stripping Suspending Fluid with product neutralizers (SS+). Two separate sampling aliquots of 3.0 mL of the appropriate sterile Stripping Suspending Fluid followed by a 1 minute scrub with a sterile rubber policeman were to be performed. The sampling fluid was then to be removed with a sterile pipette and transferred to a sterile test tube after each aliquot and pooled. According to the Sponsor, the following exceptions occurred due to technician error:

Subject (b) (6) (Test #138): only one 3 mL-aliquot/1-minute scrub was performed for the left groin 10-minute sample. The highest proportion of microorganisms are typically removed from the skin during the first 1-minute scrub. The data was used as this is considered a worst-case challenge to efficacy without the second aliquot. The calculations were adjusted to be performed with 3 mL. Subject (b) (6) passed test day baseline on this site and the data were used in analysis. A possible effect was a higher bacterial load resulting in the sample being a non-responder, however, the product achieved a 3.35 log<sub>10</sub> reduction from baseline.

Subject (b) (6) (Test #150): SS+ was used for the baseline sample on the right groin site. Although the site was wiped, residual neutralizer may remain on the skin to which product was applied, resulting in test material neutralization during product application. This is worst-case challenge for the test material. The subject passed test day baseline on this site and the data were used in analysis. The subject did not achieve a 3 log<sub>10</sub> reduction at the 10-minute sample time.

Subject (b) (6) (Test #237): the first aliquot of the right groin baseline sample was pooled into the completed left groin 10-minute sample. The sample was lost/unusable. The subject passed test day baseline on this site, therefore the sample was treated as a non-responder. On the right groin, a new baseline sample was taken next to the original baseline sample, and the remaining data for that side were used in analysis.

Subject (b) (6) (Test #356): only the second 3 mL-aliquot was recovered for the right groin 6-hour sample. The sample was lost/unusable. The subject passed test day baseline on this site, therefore the sample was treated as a non-responder.

Subject (b) (6) (Test #398): the 30-second sample was taken using SSF on the left groin. A total of approximately 5 minutes elapsed from the sample start time to the time the sample was exposed to neutralizers in the agar. The subject passed test day baseline on this site. When comparing the bacterial counts of the 30-second sample (approximately 5 minutes test material exposure) and the 10-minute sample (10 minutes test material exposure), the populations justify the use of the data; the 10-minute sample had greater bacterial counts than the 30-second sample. The data was used in analysis.

Subject (b) (6) (Test #419): Only one 3 mL-aliquot/1-minute scrub was performed for the right groin 6-hour sample. The highest proportion of microorganisms are typically removed from the skin during the first 1-minute scrub. The data was used as this is considered a worst-case challenge to efficacy without the second aliquot. The calculations were adjusted to be performed with 3 mL. The subject did not pass test day baseline on this site, so the data were not used in analysis. There was no adverse effect on the outcome of the study.

Subject (b) (6) (Test #406): The baseline sample was taken using SS+ on the right groin. A paper towel with tap water was used to wipe the baseline site and remove as much neutralizer as possible prior to product application which can overlap the sampling baseline area. The subject passed test day baseline and data were used in analysis.

***Reviewer's comments:*** *This reviewer finds these deviations acceptable since they do not impact the overall integrity of the study for the following reasons:*

- *For subjects (b) (6), where neutralizer was left on application site and product was applied, since subject passed test day baseline, it is acceptable to include these in the analysis. I concur with the Sponsor that it would be a worst-case challenge for the test material.*
- *Subject (b) (6), since new baseline sample was taken, it is acceptable to use the data for the analysis.*
- *Subject (b) (6), where only one 3 mL aliquot was used in calculations, and subject did not pass the test day baseline. Data were not used in the analysis, this is acceptable.*
- *Subject (b) (6), at 30 second time point sample was plated with neutralizers 5 minutes beyond the specified time but bacterial counts were lower than 10 minutes timepoint, data used in analysis. This is acceptable.*
- *Subject (b) (6) was considered non-responder due to recovery of only 3 mL 6 hour groin site sample. This is acceptable.*

## 5. AREA COVERAGE AND DRYING TIME

### 5.1. Study ZX-ZP-0083 ((b) (4) 865-106). Evaluation of the Skin Area Covered and Dry Time of a Preoperative Skin Preparation

The objective of this study was to assess the coverage area and dosage of the coverage area of ZuraGard 10.5 mL applicator, the drying time (post-application), and the safety of the procedures for all subjects who signed the informed consent.

***Reviewer's comments:*** *Because alcohol-containing antiseptic products that have pooled or not been able to dry can ignite when electrocautery is used during surgical procedures, on December 2014, FDA informed the Sponsor that, in order to support a patient preoperative skin preparation indication, drying time and skin coverage studies will need to be produced to help inform labeling. On March 13, 2017, we had agreed with the general study design for the drying time and skin coverage studies.*

**Method:**

1. **Study design:** This study was intended to establish the observed drying time and skin coverage for the ZuraGard 10.5 mL applicator. Twenty applicators were used on 20 test subjects with the distribution as described in the following table.

**Table 41. Summary of Treated Subjects Demographic Variables for Study ZX-ZP-083 (Source: Table 4, study report)**

Demographic Summary		
Age	Mean	35.40
	Standard Deviation	16.55
	Minimum	20
	Maximum	77
Sex Frequency (Percent)	Male	20 (100%)
	Females	0 (0%)
Race Frequency (Percent)	White/Caucasian	8 (40%)
	Black/African-American	1 (5%)
	Hispanic	1 (5%)
	Asian	9 (45%)
	Other	1 (5%)

2. **Testing parameters:** ZuraGard™ 10.5 mL Applicator (Active) – A single applicator was used per treatment area. The investigational product was applied topically using repeated back and forth strokes of the sponge for 30 seconds over the treatment area (8.4” x 8.4” of the subject’s back) and the skin was allowed to air dry.
3. The containers were weighed before and after the procedure to determine the volume used. The drying time was independently observed by three technicians.

**Reviewer’s comments:** *On March 13, 2017, we advised the following to the Sponsor for their ZX-ZP-0083 protocol:*

- *“Perform the skin coverage study on the flat side of the subject’s back, where the product does not pool.*
- *Test at least 20 individual subjects per applicator type.*
- *Adequately define this application time, which will be reflected in the directions for use of your product’s labeling.*
- *The application directions for use you establish during the skin coverage and drying time study should then be used in the clinical simulation studies and reflected in the final labeling for the proposed product.*
- *For your study, we expect you will define consistent time point(s) post-treatment in which the skin irritation scoring will be measured.”*

*The Sponsor used 20 subjects and applied the product on the back of the subjects per our advice, this is acceptable. However, at the time of study ZX-ZP-0083 protocol review, the Sponsor submitted (December 20, 2016) the draft directions for use for their proposed product ZuraGard, for informational purposes only, which specify the following:*

*“Completely wet the treatment area with antiseptic.  
 Dry surgical sites (e.g. abdomen or arm): use repeated back-and-forth strokes for 30 seconds. Moist Surgical sites (e.g. inguinal fold): use*

*repeated back-and-forth strokes for 2 minutes.”*  
*Although we recommended “adequately define this application time, which will be reflected in the directions for use of your product’s labeling,” this reviewer notes that the Sponsor has used the application for dry surgical site for 30 seconds and did not follow the directions for moist surgical sites for application for 2 minutes. Since, the application site was the back of the subjects, a dry surgical site, 30 seconds application time for skin coverage study is acceptable. Additionally, the Sponsor has used both dry and moist surgical sites application directions of use (as specified on the labeling) in the two pivotal effectiveness studies ZX-ZP 0073 (source-Table 3.1 of protocol) and ZX-ZP-0074 (source-Appendix 1 of protocol). This is acceptable.*

**Results (Study ZX-ZP-0083, (b) (4) 865-102)**

**Table 42. Coverage and Dry Time Results by Subject (Source: Table 5, study report ZX-ZP-0083)**

Subject Number	Drying Times (seconds)				Product Weights (g)			Dose per Area (g/cm <sup>2</sup> )	Coverage (cm <sup>2</sup> /g)
	Tech 1	Tech 2	Tech 3	Mean	Pre-Tmt	Post-Tmt	Dose		
0001	167	172	158	165.67	35.10	33.09	2.01	0.00442	226.5
0002	122	123	123	122.67	34.90	32.57	2.33	0.00512	195.4
0003	114	114	112	113.33	34.75	32.28	2.47	0.00543	184.3
0004	115	117	115	115.67	35.03	32.54	2.49	0.00547	182.8
0005	79	80	78	79.00	34.86	32.61	2.25	0.00494	202.3
0006	111	107	101	106.33	35.09	32.67	2.42	0.00532	188.1
0007	97	104	96	99.00	34.79	32.32	2.47	0.00543	184.3
0008	100	108	106	104.67	35.08	32.56	2.52	0.00554	180.6
0009	77	80	82	79.67	35.09	32.93	2.16	0.00474	210.8
0010	84	84	85	84.33	34.93	32.26	2.67	0.00587	170.5
0011	93	91	93	92.33	35.22	32.86	2.36	0.00518	192.9
0012	96	96	96	96.00	34.95	32.29	2.66	0.00584	171.1
0013	76	75	80	77.00	35.03	32.26	2.77	0.00608	164.3
0014	108	107	108	107.67	34.91	32.09	2.82	0.00619	161.4
0015	136	136	136	136.00	34.85	32.11	2.74	0.00602	166.1
0016	97	104	107	102.67	34.77	32.00	2.77	0.00608	164.3
0017	107	106	107	106.67	34.96	32.14	2.82	0.00619	161.4
0018	82	84	82	82.67	34.95	32.24	2.71	0.00595	168.0
0019	110	110	110	110.00	35.26	32.43	2.83	0.00622	160.9
0020	87	87	89	87.67	34.92	32.17	2.75	0.00604	165.5

**Table 43. Summary of Dry Time and Coverage per Dose (Source: Table 6, study report ZX-ZP-0083)**

	Excluding outlier			
	Coverage Area Dose (g/cm <sup>2</sup> )	Dry Time (sec)	Dose (g)	Coverage per Dose (cm <sup>2</sup> /g)
Mean	0.00567	100.2	2.58	178
Median	0.00584	102.7	2.66	171
Min	0.00474	77.0	2.16	161
Max	0.00622	136.0	2.83	211

**Reviewer’s comments:**

- Demographics:** *There were 20 males in the test subject population. There were 8 Caucasians and 9 Asian, the rest were from African-American, and Hispanic origin, with a mean age of 35.4 years (source Table 4 of study ZX-*

*ZP-0083 report).*

2. *Subject (b) (6) was determined to be an outlier and was not included in the summary of evaluation shown above.*
3. *Amount used: The average amount of product used was 2.58 g*
4. *Drying time: As shown in table 5 and summary table (source Synopsis of study ZX-ZP-0083 report), the average drying time was 100.2 seconds (ranged from 77-136 seconds).*
5. *Coverage area results: For the ZuraGard 10.5 mL applicator, the area is  $2.58 \text{ g}/0.00567 \text{ g/cm}^2 = 455 \text{ cm}^2$ . The average coverage in square inches is  $70.52 \text{ in}^2$ .*

*Overall, the area coverage results for the ZuraGard 10.5 mL applicator was  $455 \text{ cm}^2$ , or  $70.52 \text{ in}^2$ . The labeling for ZuraGard 10.5mL applicator specifies coverage area for is 8.4 X 8.4 inches or  $457 \text{ cm}^2$ . Also, the labeling states “discard the applicator after a single use along with any portion of the solution not required to cover the prepped area. It is not necessary to use the entire amount available.” The defined coverage area study for the ZuraGard 10.5 ml applicator is acceptable.*

6. *Skin irritation scores were 0 for all the observations at screening, prior to treatment and post treatment for all enrolled and treated subjects. There was no skin irritation and no adverse events for any subject. Refer also to the Medical Officer’s or other discipline’s review for any additional comments.*

## 6. References

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4. 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.
5. 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.
6. Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Final Rule 2017, In: *Federal Register* 82 FR 60474
7. FDA Deferral Letter for Isopropyl Alcohol in Health Care Antiseptics on January 19, 2017. Available at <https://www.regulations.gov/document?D=FDA-2015-N-0101-1325>

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/s/  
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