

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*  
**50-793**

**MICROBIOLOGY REVIEW**

**Division of Anti-Infective Drug Products**  
**Clinical Microbiology Acting Team Leader Memorandum for NDA 21- 669**  
**Sage Products, Inc.**

Sage Products Inc submitted NDA 21- 669 on September 4, 2003. The Microbiology Review of this Application was completed on 10 June 2004.

This memorandum is written to provide comments and address the concerns and deficiencies presented by the Primary Microbiology Reviewer, Dr. Peter Coderre, with regards to this application.

Background Information:

The product is a \_\_\_\_\_, polyester cloth impregnated with a 2% chlorhexidine gluconate (CHG) solution. Chlorhexidine gluconate is the active ingredient in this product. The product is designed to apply a 2% CHG solution to the skin as a pre-surgical skin preparation.

To determine the appropriate *in vitro* testing to support the claim of a preoperative skin preparation, the Agency recommended that the Applicant refer to the Tentative Final Monograph (TFM) for Topical Antimicrobial Drug Products for Over-the-Counter Human Use (Federal Register 59[116]:31444-31445; 17 Jun 94). Effectiveness testing of patient preoperative skin preparation is described in the TFM for Topical Antimicrobial Drug Products for Over-the-Counter Human Use (Federal Register 59[116]:31450-31451; 17 Jun 94).

Antimicrobial Spectrum of Activity

Chlorhexidine gluconate solution is an aqueous solution of 1,1'-hexamethylenebis[5-(4-chlorophenyl)biguanide] di-D-gluconate. The drug substance contains \_\_\_\_\_ of (CHG)  $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$  (w/v).

Chlorhexidine shows both inhibitory and lethal actions against vegetative Gram-positive and Gram-negative bacteria; however, bacterial spores are resistance to its killing action except at increased temperatures. It also shows activity against certain viruses and fungal species. The levels of activity differ from genera to genera and species to species. Chlorhexidine demonstrates bacteriostatic activity at low concentration and is bactericidal at higher concentrations.<sup>1</sup>

According to the Primary Reviewer's report, studies to determine the spectrum of activity of \_\_\_\_\_ were performed at \_\_\_\_\_ followed the recommendations listed in the TFM. The bulk drug product was tested against 1124 different microbial isolates. The Applicant provided data that showed the

<sup>1</sup> Ranganathan, NS. Chlorhexidine. A chapter in Handbook of Disinfectants and Antiseptics. Ascenzi, JM. Ed. Marcel Dekker, Inc. NY.1996. page-235-257

average minimum inhibitory concentrations (MIC) expressed in dilutions, of the bulk product, vehicle, and active ingredient. The Applicant reported that the active ingredient CHG is the chemical responsible for the antimicrobial activity of the drug product. The Primary Reviewer finds the data (Table 1, page 10 of the Reviewer's report) provided by the Applicant acceptable ( Reviewer's comment, page 11)

### Time-Kill Studies

Time-kill studies were done to demonstrate the *in vitro* bactericidal and fungicidal activity of the test product. According to the Primary Reviewer's report, performed the time-kill study in 2% CHG . The drug product was tested against 25 American Type Culture Collection (ATCC) bacteria and 26 clinical bacterial isolates as described in the TFM. Following the TFM, microbial log<sub>10</sub> reduction for each challenge microorganism was determined, following exposures to the product at 15 sec, 1 min, 3 min, 6 min. 6 min., 9 min., 12 min., 15 min., 20 min., and 30 min were completed. The Primary Reviewer noted that the time-kill studies showed 3 log<sub>10</sub> reduction occurred within 15 seconds among the Gram-negative bacteria, while the 3 log<sub>10</sub> reduction among Gram positive bacteria and yeasts required a longer time to achieve. The Primary Reviewer expressed concerns regarding these observations (page 15 of Reviewer's report) and efficacy of the product since Gram-positive organisms such as , *E. faecalis*, *E. faecium*, and *S. aureus*, are often indicated in post surgical infections Although the Reviewer's observations are notable, what follows offer some explanation for these concerns.

Several factors influence the degree of killing of microorganisms. These factors include the types of organisms, number of organisms present or microbial load, concentration of antiseptic agent, amount of organic material present, and the site or nature of the surface to which the material is applied. Organisms vary greatly in their ability to withstand the effects of chemical or physical agents. This variability which occurs between genera and from species to species, may be partly due to the biochemical composition of the target cells and the protective mechanisms afforded by the cellular constituents.<sup>2</sup> A linear reduction of bacterial numbers when plotted against exposure time may be expected; however, the demonstrated antibacterial activity of the agent will vary, depending on the ability of the organism to resist the action of the chemical agent. The Primary Reviewer's observation regarding a slower microbial load reduction among Gram positive organisms and yeast, based on the data provided by the Applicant, when compared with the rate of reduction among the Gram negative organisms is noted. However, similar observations have been reported previously<sup>3</sup>. These reports show a similar trend; the mean log<sub>10</sub> reductions among Gram positive cocci and fungi including yeasts were achieved at a slower rate than the Gram negative (GN) organisms. The enhanced activity of chlorhexidine against GN species may be attributed to the mechanism of action of chlorhexidine. Because chlorhexidine is rapidly absorbed by bacterial cells by virtue of the lipophilic groups of the drug molecules, cytological changes that induce permeability

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<sup>2</sup> Mahon, C. Sterilization and Disinfection, a chapter in Textbook of Diagnostic Microbiology, WB Saunders, Philadelphia, 2000. pages

<sup>3</sup> Block, Disinfection, Sterilization and Preservation pages 323-326

of the cells ensue. Electron microscopy and assays for characteristic outer membrane components demonstrated that sublethal concentrations of chlorhexidine bring about changes in the outer membrane integrity of GN bacteria.<sup>2</sup>

Neutralization Studies

Neutralization validation studies were performed to ensure that the neutralizing agent used in all post-exposure testing was non-toxic to the microorganisms and was effective in neutralizing the inhibitory effects of the antimicrobial agent. The Reviewer concluded that the results of the neutralization studies showed that the neutralizing agent was non-toxic and was effective.

Clinical Studies

Clinical simulation trials with [redacted] were performed at [redacted] facilities. Both laboratories followed the guidelines described in the TFM. These studies were performed to demonstrate the efficacy of [redacted] as a preoperative skin product. Body sites that were tested to demonstrate effectiveness of the product were the abdomen and inguinal areas, per TFM guideline. A mean 2 log<sub>10</sub> microbial load reduction from the initial baseline bacterial count within 10 minutes with counts not to exceed baseline counts in 6 hours should occur for the abdominal site; a mean 3 log<sub>10</sub> reduction within 10 minutes with counts not to exceed the baseline count in 6 hours for the inguinal site.

Results are shown in the Table shown below (taken from the Primary Reviewer’s Report, Table 20, page 28)

Results of Clinical Simulation Trials at the Three Sites

Protocol Number	Principal Investigators	Number of Subjects	Age	Sex	Location of Final Report	Result
01-109381-11	[redacted]	69 enrolled 51 qualified	18 - 69	M (19) F (32)	Appendix 6	Test product met required log reduction on abdominal sites but <i>did not</i> meet required log reduction on the inguinal sites
020125-103	[redacted]	88 enrolled 35 qualified	18-70	M (25) F (10)	Appendix 7	Test product met required log reduction on both anatomical sites
500-102	[redacted]	43 enrolled 32 qualified	18-61	M (14) F (18)	Appendix 8	The test product met the required log reduction on the inguinal site

Subjects tested at the [redacted] as reported by the Applicant, failed to meet the target microbial load reduction at the inguinal site. The Applicant discussed these findings with the Agency. Based on the communication with the Agency (Teleconference, 25 October 2002), the Applicant proposed a second efficacy trial and identified a third independent site to conduct the clinical simulation study for the inguinal site only. The third site, [redacted] met the mean 3 log<sub>10</sub> reduction for the inguinal site.

In summary, results of the studies at the three independent laboratories demonstrated that [REDACTED] meet the requirements set in the TFM for pre-operative skin preparations; [REDACTED] met the mean  $2\log_{10}$  reduction for the abdominal site and the mean  $3\log_{10}$  reduction for the inguinal site. This fulfilled the requirements for the preoperative skin preparation as described in the TFM. Results are shown on the Summary Table below. (Table 26, page 35. Reviewer's report).

Summary Table

Anatomical site	Requirement per Tentative Final Monograph	Study location	Result at 10-minutes post-prep for test product	Result at 6 hours post-prep for test product
Abdomen	<ul style="list-style-type: none"> <li><math>&gt; 2.0 \log_{10}</math> reduction from baseline within 10 minutes</li> <li><math>\log_{10}</math> does not exceed baseline at 6 hours</li> </ul>	[REDACTED]	$> 2.0 \log_{10}$ reduction from baseline	$\log_{10}$ does not exceed baseline
		[REDACTED]	$> 2.0 \log_{10}$ reduction from baseline	$\log_{10}$ does not exceed baseline
Inguinal	<ul style="list-style-type: none"> <li><math>&gt; 3.0 \log_{10}</math> reduction from baseline within 10 minutes</li> <li><math>\log_{10}</math> does not exceed baseline at 6 hours</li> </ul>	[REDACTED]	$> 3.0 \log_{10}$ reduction from baseline	$\log_{10}$ does not exceed baseline
		[REDACTED]	$> 3.0 \log_{10}$ reduction from baseline	$\log_{10}$ does not exceed baseline

Lack of Negative Controls

From the Primary Reviewer's perspective, the Applicant has not demonstrated the efficacy of the test product in the clinical simulations because of lack of negative controls. Negative controls were not incorporated in the design of the clinical simulation trials.

The Primary Reviewer's viewpoint regarding the issue of negative controls is a valid point. Standardized and reliable laboratory methodology is necessary in order to produce valid and accurate test results. Quality control programs are introduced into methodologies to assure that data collected from clinical and laboratory studies are reproducible, precise, and accurate. Each quality control parameter introduced in the methodology serves a specific purpose (i.e. detect the limitations of the procedure, procedure is followed correctly, reagents are working properly) and has specific and defined acceptable limits. The use of negative control in the clinical simulation trials of this product may have been helpful to determine the cause of bacterial reduction on the skin, and perhaps better ascertain the efficacy of the product, or increase the validity of the results. However, whether the lack of negative controls diminishes the validity of the results of studies to demonstrate the efficacy of the Sage product is not known. Negative

controls have not been used in clinical trials for the purpose of demonstrating efficacy in other approved products of this type.

Nevertheless, the value of negative controls cannot be underestimated and the significance of the data from such controls should probably be assessed through validation studies. Validation may be performed through demonstration or pilot studies. The inclusion of negative controls in clinical simulations protocols may be recommended to sponsors of similar products in future submissions.

### Conclusion

The concerns and deficiencies presented by the Microbiology Primary Reviewer of this NDA submission may be attributed to several variables inherent to the methodologies of the currently used in *in vitro* tests and the clinical simulation studies to determine the efficacy of antiseptic products. The significance of these variables cannot be assessed at this time. Nevertheless, these variables should be addressed in the review of future clinical simulation protocols submitted for pre-operative skin preparations. The use of additional test controls, for example, may be recommended to Sponsors during the review of their clinical trial protocols. Use of a standardized procedure may be recommended to reference laboratories that perform these studies. With regards to [REDACTED] from the microbiology perspective, this application may be approved.

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Connie R. Mahon, MS, CLS  
Acting Microbiology Team Leader  
HFD520  
23 June 2004

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HFD-520/Dept/Dir/L. Gavrilovich

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/s/  
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Connie Mahon

6/25/04 10:21:04 AM

MICROBIOLOGIST

Acting Microbiology Team Leader's Comments regarding Microbiology Primary R  
review.

Lillian Gavrilovich

6/25/04 02:29:39 PM

MEDICAL OFFICER

Sage Products, Inc.

Division of Anti-Infective Drug Products  
Clinical Microbiological Review

NDA: 21-669

Date Completed: June 23, 2004

**Applicant (NDA):**

Sage Products, Inc.  
3909 Three Oaks Road  
Cary, IL 60013  
(815) 455-4700

**Chem/Ther. Type:** Antimicrobial

**Submissions Reviewed:** NDA 21-669, N000(B2)

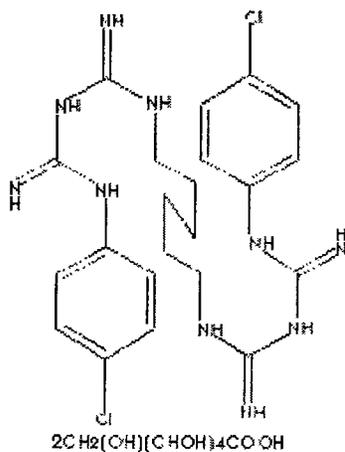
**Providing for:** \_\_\_\_\_ for preparation of skin  
prior to surgery.

**Product Name:**

Proprietary: \_\_\_\_\_  
Non-proprietary/USAN: Chlorhexidine Gluconate (CHG)  
Compendia: Chlorhexidine Gluconate

**Chemical name:** 2,4,11,13-Tetraazatetradecanediimidamide, *N,N''*-bis(4-chlorophenyl)-3,12-diimino-, di-D-gluconate

**Structural formula:**



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**Molecular formula:** C<sub>22</sub>H<sub>30</sub>Cl<sub>2</sub>N<sub>10</sub>•2C<sub>6</sub>H<sub>12</sub>O<sub>7</sub>

**Dosage form:** 2% CHG solution

**Route of administration:** Topical via [REDACTED] cloth

**Pharmacological Category:** Topical antiseptic/antimicrobial

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

**Initial Submission Dates**

Received by CDER: September 4, 2003

Received by Reviewer: September 22, 2003

Review Completed: June 10, 2004

**Related Documents:** IND 64,413

**Remarks:**

This review of NDA 21-669 describes the findings and recommendations of the Clinical Microbiology Reviewer. These recommendations are for evaluation by the Division Director for the determination of a decision whether to approve this drug application.

**Conclusions/Recommendations:**

This NDA submission is for a product containing a 2% CHG solution impregnated into a [REDACTED] polyester cloth intended for use as a patient preoperative skin preparation. The Applicant has demonstrated the preclinical (*in vitro*) efficacy of the product; **however, there is no clear evidence that the chemical properties of the CHG component of the product are responsible for efficacy *in vivo*.** What follows are comments and deficiencies noted by this Reviewer.

1. In general, the application is poorly organized and presented. In some cases, data is not easily available and required time-consuming searches. In some cases, data is presented in an incorrect format. For example, data from the time-kill studies required conversion from percent reduction to log<sub>10</sub> reduction.
2. Clearly, the product demonstrates efficacy against a broad spectrum of microorganisms *in vitro*. MICs were obtained from 25 fresh clinical isolates and 25 laboratory strains of the organisms listed in the TFM. In some cases, less than 25 laboratory strains or less than 25 clinical strains were tested; however, 50 total isolates were tested and thus the low number of clinical isolates or laboratory isolates is allowed.
3. The product exhibits rapid bactericidal action as demonstrated by time-kill kinetics. However, some organisms show a more rapid reduction in microbial numbers. All Gram-negative organisms exhibit at least a 5-log reduction within 15 sec. However, some organisms, particularly Gram-positive and yeast are slower to exhibit a 3-log reduction. Thus, this Reviewer is concerned about the [REDACTED] and efficacy

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of the product since these three organisms, *E. faecalis*, *E. faecium*, and *S. aureus*, are often indicated in post surgical infections.

4. A comparison of the MIC results with the time-kill kinetics results should demonstrate that the product kills organisms at higher dilutions, and therefore more sensitive to CHG, *more* rapidly. Conversely, one should observe that the product kills organisms with lower dilutions and therefore more resistant to CHG, *less* rapidly. Organisms killed at high dilutions but slower time-kill kinetics include: *Enterococcus faecalis*, *Enterococcus faecium*, and *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*. These observations suggest that neither spectrum of activity data nor time-kill kinetic data be used separately but in conjunction with one another in the determination of the efficacy of active ingredients and product performance.
5. From the neutralization validation studies for the time-kill kinetics, this Reviewer concludes that the neutralizer is both effective and not toxic. Although there are statistical differences between the numbers controls versus the toxicity and efficacy controls, the toxicity and efficacy controls are *higher*, not lower than the numbers controls. In addition, the toxicity and efficacy controls demonstrate greater than 50% recovery compared to the numbers control.
6. The Applicant presents MIC data for a number of antibiotic resistant organisms. Most but not all of these organisms have high dilution values suggesting sensitivity to CHG. The Applicant also presents time-kill kinetic data for several antibiotic resistant organisms, specifically, [REDACTED]. The time-kill kinetics for these organisms show slow killing indicated by less than 3-log reductions at 15 sec. However, from the data given, it is impossible to determine if the MIC values and the time-kill kinetics data are for the same organisms. Therefore, a correlation between MIC dilution values and the time-kill kinetics data are not possible. [REDACTED]  
[REDACTED]  
[REDACTED]
7. Since several antibiotic resistant organisms show lower MIC dilution values but slower time-kill kinetics for CHG, it would be prudent to be aware of any future changes in antibiotic resistance or CHG resistance patterns.
8. A number of procedural inconsistencies for the application of the product during the clinical simulations are noted and require mention. These inconsistencies include variation in cup scrub diameter, neutralization composition, processing of bacterial samples, and variation in formulas for the determination of CFU/cm<sup>2</sup>.
  - a. First, the Applicant notes that the internal diameters of the scrubbing cups used by the different contract laboratories i.e. [REDACTED] [REDACTED] for the sampling are different; the Applicant states that this variation in size does not affect the microbial count data obtained. However, the Applicant does not reference or supply data to support this statement.
  - b. It is unclear if the neutralizers used by all the contract laboratories are identical in composition or concentration. Both [REDACTED] state the neutralizer consists of 1% Polysorbate 80 and 0.3% lecithin; the composition and

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- concentration of the neutralizer used by [REDACTED] could not be located. While these are appropriate neutralizers for use against chlorhexidine according to the ASTM document E 1054-02 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents", consistency in the protocol is required to eliminate variability.
- c. Finally, the Applicant states that after incubation of the media, the colonies are counted. Due to the variation in the cup scrub diameter, different formulas are used by [REDACTED] to determine the CFU/cm<sup>2</sup> of skin. It is unclear as to which formula is used by
9. The preoperative preparation application procedures are very similar for the three contract laboratories, but some differences in the protocol are present. The significance of these differences is not immediately apparent.
  10. A second area of concern with the clinical simulations is the failure of the positive control, Hibiclens, to meet the TFM requirements. Hibiclens failed to meet the 2-log<sub>10</sub> reduction after 10 minutes in the abdominal site, performed at [REDACTED] and the 3-log<sub>10</sub> reduction in the inguinal site at both [REDACTED]. As Hibiclens is often a positive control for most clinical simulations for patient preoperative preparation products, these data may be reason for concern regarding the validity of the positive control.
  11. *However, the most flagrant deficiency in the design of the clinical simulation trials is the lack of negative controls.* Since the test product is a 2% CHG solution applied with a washcloth, there is a device component to the product. Thus, there are two possible mechanisms for the removal of bacteria from the skin: the chemical action of the CHG and the physical action of the washcloth. Since the mechanical action of washing with soap and water removes microbes from the skin, the responsibility for the bacterial reductions of both the chemical and mechanical action must be determined separately. Therefore, two negative controls are necessary to determine the cause of the bacterial reductions on the skin. One negative control is the application of the test product without mechanical action of the washcloth. This control may be achieved by layering the test product on the skin. A second negative control is to omit the active ingredient from the washcloth during the application. The washcloth here may only include vehicle. Without both negative controls, it is impossible to determine the contribution of the chemical component or the device component of the product. ***Therefore, the Applicant has not demonstrated the efficacy of the product in the clinical simulations.***
  12. Neutralization validation assays for the clinical simulations performed by all three laboratories demonstrate the neutralizer is effective and non-toxic. However, to be consistent and reduce the potential for variability, the test organisms should be the same for the neutralization validation assays for all three laboratories.
  13. **A package insert was not submitted by the Applicant.**

The Applicant presents data that demonstrate *in vitro* efficacy of the product by determining the antimicrobial spectrum of activity of the active ingredient, CHG. However, these MIC studies do have limitations since the concentration and duration of exposure are fixed and may not mimic actual preoperative conditions. This limitation is important because antimicrobial activity for antiseptics is time and concentration

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dependent for antiseptics. Therefore, time-kill kinetics and clinical simulations are necessary to determine the antimicrobial activity of the antiseptic in actual use conditions.

The purpose of the time-kill kinetic studies is to attempt to establish a relationship between the *in vitro* kill rates caused by the product and the *in vivo* kill rates during clinical simulation studies. These studies measure bacterial log<sub>10</sub> reductions at reference time points. The TFM requires a 1:10 dilution of the product to perform the studies in order to simulate the preoperative situation in which the product may become diluted by blood and other body fluids during surgery. The product was not diluted since the product is a leave-on product, that is, it is not intended to be used with water.

The time-kill kinetics studies indicate the product is fast-acting against most organisms tested. As an arbitrary time point, a 3-log reduction at 15 sec. is considered the criterion for a designation of fast-acting. Most organisms meet this criterion with the exception of: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus faecalis* and *Enterococcus faecium*. In addition, the following antibiotic resistant organisms failed the 3-log reduction including: [REDACTED]

[REDACTED] In a comparison between time-kill kinetics and MICs, all of these organisms demonstrate slow time-kill kinetics but high MICs. The apparent disconnect between the MICs and time-kill kinetics suggest that while the product may be effective against these organisms *in vitro*, the microbicidal action is slow and thus may not be as effective *in vivo* in a surgical environment for which the product is intended. These observations are additionally disconcerting since many of these organisms are responsible for most infections seen post-surgery.

Judgement of the efficacy of a patient preoperative preparation is based upon *in vitro* evidence that includes MIC studies and time-kill kinetics, and *in vivo* evidence that includes clinical simulations. While the spectrum of activity evidence is strong, the time-kill kinetics evidence wavers. But most notably, the *in vivo* clinical simulation evidence is weak.

It is important to recognize that the *in vivo* evidence relies upon clinical simulations in which healthy volunteers act as surrogates for surgical personnel. Thus, it is imperative that the evidence from the clinical simulations is strong in order to establish the connection between the *in vitro* evidence and the *in vivo* evidence.

The *in vivo* evidence from the clinical simulations is weak. There is no clear evidence that the efficacy of the product is due to the chemical properties of CHG and not to the mechanical application of the product. The Applicant's product is a mechanical device impregnated with a chemical that is purported to be antimicrobial. Proper controls were incorporated with the MIC data; the MIC data was derived for not just the bulk drug product, but also a negative control (vehicle) as well as a positive control (active ingredient). No such negative control was utilized in the clinical simulations, thus, it is not possible to determine whether the *chemical* action of the CHG contained in the

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product or the *mechanical* action of the cloth on the treated skin was responsible for the  $\log_{10}$  reductions.

**This Reviewer recommends that this application not be approved** until the clinical simulations are completed with the required negative controls to the satisfaction of the Agency.

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Sage Products, Inc.

## INTRODUCTION

Sage Products [REDACTED] is designed to apply a 2% chlorhexidine gluconate solution to the skin prior to a surgical procedure. Based on the recommendations in FDA's response to the pre-IND briefing document, the Tentative Final Monograph (TFM) for Topical Antimicrobial Drug Products for Over-the-Counter Human Use (Federal Register 59[116]:31444-31445; 17 Jun 94) was the basis for determining the appropriate testing to support the claim of a preoperative skin preparation.

[REDACTED]

[REDACTED]

The Microbiology Section of NDA 21-669 is intended to provide support for the product for its proposed indication as a preoperative skin preparation. The section utilizes data from *in vitro* studies performed on the bulk drug product, *in vivo* studies performed on the finished drug product, and references to the published literature. The study reports for the *in vitro* studies, *in vivo* pivotal efficacy studies, and the bibliography for the referenced articles are found in the appendices at the end of the Microbiology section of the NDA submission. Both the bulk drug product used in the *in vitro* studies and the finished drug product tested in the *in vivo* pivotal efficacy studies utilize the same formula. The tested formulation is the same formulation the Applicant plans to market as its [REDACTED]

[REDACTED]

## PRECLINICAL EFFICACY-IN VITRO

### Mechanism of Action

The [REDACTED] contain 2% chlorhexidine gluconate as its active ingredient. Chlorhexidine gluconate solution is an aqueous solution of 1,1'-hexamethylenebis[5-(4-chlorophenyl)biguanide] di-D-gluconate. The drug substance [REDACTED]

$C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$  (w/v).

The molecule has a symmetrical appearance. Each "half" of the molecule consists of a biguanide unit, which is substituted on the end by a *p*-chlorophenyl group. It is believed the presence of two of these aryl-biguanidine moieties give the compound its antibacterial properties.

The antibacterial activity of chlorhexidine is related to its physical properties whereby the di-cation binds to negatively-charged bacterial membranes. After binding, the hydrophobic portion of the molecule interacts with the cell wall, disrupting its integrity. At low concentrations, chlorhexidine interferes with cell

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membrane function and acts as a bacteriostatic agent. At high concentrations, the cell membrane becomes leaky, causing irreversible damage and cell death. Bactericidal activity does not occur with acid-fast bacilli and heat resistant bacterial spores.

#### **Antimicrobial Spectrum of Activity**

The MIC study was performed by \_\_\_\_\_

The bulk drug product was tested using 1124 different microbial strains. At least 50 strains of each species, as specified by the TFM, were evaluated. Most organisms were one of 25 American Type Culture Collection (ATCC) strains or one of 25 fresh clinical isolates of each of the 22 species listed in the TFM. Where there are insufficient ATCC strains available, additional clinical isolates are tested to achieve a total of at least 50 strains for each species. For *Staphylococcus haemolyticus*, and *Staphylococcus hominis*, for which insufficient clinical isolates are available, clinical isolates of Coagulase-Negative *Staphylococcus* (CNS) species are tested.

The product vehicle and the product active ingredient dilution series were challenged with 228 different microorganism strains. At least ten strains (five ATCC and five clinical isolates) of each of the 22 species listed in TFM were evaluated for each of these products. The product vehicle and active ingredient testing show that the active ingredient identified (CHG) is the chemical that provides the antimicrobial activity of the drug product.

The testing was performed using a modification of the Macrodilution Broth Method outlined in NCCLS Document M7-A5, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow Aerobically*, fifth edition.

Table 1 provides the average MIC results for each of the 22 species tested. The results of the testing for each isolate of each species can be found in Addendum II, which is included in APPENDIX 1 (Volume 7) of the NDA submission.

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**Table 1. MICs for 22 Species of Microorganisms Tested.**

Microorganism Species	Bulk drug product			Vehicle			Active Ingredient		
	Average MIC (Expressed as Product Dilution)	Minimum	Maximum	Average MIC (Expressed as Product Dilution)	Minimum	Maximum	Average MIC (Expressed as Product Dilution)	Minimum	Maximum
<i>Acinetobacter</i> species	1:16,384			1:4			1:16,384		
<i>Bacteroides</i> species	1:8,192			1:32			1:8,192		
<i>Candida</i> species	1:256			1:4			1:64		
<i>Candida albicans</i>	1:32			<1:4			<1:32		
<i>Enterobacter</i> species	1:8,192			1:4			1:8,192		
<i>Enterococcus faecalis</i>	1:8,192			1:4			1:4,096		
<i>Enterococcus faecium</i>	1:16,384			1:4			1:16,384		
<i>Escherichia coli</i>	1:65,536			1:4			1:32,768		
<i>Haemophilus influenzae</i>	1:32,768			1:64			1:32,768		
<i>Klebsiella</i> species	1:8,192			1:4			1:16,384		
<i>Klebsiella pneumoniae</i>	1:16,384			1:4			1:32,768		
<i>Micrococcus</i> species	1:8,192			1:4			1:8,192		
<i>Proteus mirabilis</i>	1:8,192			1:4			1:2,048		
<i>Pseudomonas aeruginosa</i>	1:4,096			1:4			1:4,096		
<i>Serratia marcescens</i>	1:2,048			1:4			1:2,048		

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Table 1. MICs for 22 Species of Microorganisms Tested. (continued)

Microorganism Species	Bulk drug product			Vehicle			Active Ingredient		
	Average MIC (Expressed as Product Dilution)	Minimum	Maximum	Average MIC (Expressed as Product Dilution)	Minimum	Maximum	Average MIC (Expressed as Product Dilution)	Minimum	Maximum
<i>Staphylococcus aureus</i>	1:32,768	[REDACTED]	[REDACTED]	1:4	[REDACTED]	[REDACTED]	1:32,768	[REDACTED]	[REDACTED]
<i>Staphylococcus epidermidis</i>	1:32,768			1:4			1:65,536		
<i>Staphylococcus haemolyticus</i>	1:65,536			1:4			1:32,768		
<i>Staphylococcus hominis</i>	1:65,536			1:4			1:65,536		
Coagulase-Negative <i>Staphylococcus</i> species	1:32,768			N/A			N/A		
<i>Staphylococcus saprophyticus</i>	1:65,536			1:4			1:65,536		
<i>Streptococcus pneumoniae</i>	1:16,384			1:4			1:16,384		
<i>Streptococcus pyogenes</i>	1:65,536			1:4			>1:65,536		

**Reviewer’s comments:** The Tentative Final Monograph states that the Applicant must determine the MICs using 25 fresh clinical isolates and 25 laboratory strains of the organisms from a provided list. The Applicant has provided such data for the majority of the organisms in this list. Table 2 shows the requisite number of isolates was not met for several organisms. However, in many of these cases, less than 25 laboratory strains (ATCC strains) exist. Because 50 total isolates were tested, this low number of laboratory strains will be allowed. *M. luteus*, is rarely a pathogen; as a result, only three clinical isolates were tested. Because 50 total isolates were tested, this low number of clinical isolates will be allowed.

Table 2. Numbers of Isolates of Select Organisms from the TFM List.

organism	# ATCC strains	laboratory strains	clinical isolates
<i>Bacteriodes fragilis</i>	21	19	25
<i>Micrococcus luteus</i>	25	25	3
<i>Staphylococcus epidermidis</i>	48	22	25
<i>Staphylococcus haemolyticus</i>	7	6	25
<i>Staphylococcus hominis</i>	12	8	12
<i>Staphylococcus saprophyticus</i>	7	5	25

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

The time-kill study was performed at \_\_\_\_\_

\_\_\_\_\_ The time-kill study tests the drug product, active ingredient and the product vehicle in order to demonstrate the broad-spectrum antimicrobial efficacy shown by the product. The product was not tested as a 10% aqueous solution as specified by the TFM, because it is provided in a ready-to-use form and does not require dilution prior to use. Instead, the product was tested at a \_\_\_\_\_ (v/v) concentration.

The drug product was evaluated using a total of 51 strains of microorganisms. The organisms consist of 25 ATCC strains and 26 fresh clinical isolates of the same species as described in the TFM. The active ingredient and product vehicle were tested with the 10 ATCC microorganism strains referenced in the TFM.

The percent and  $\log_{10}$  reductions from the initial populations were determined for each challenge microorganism following exposures to the appropriate products for 15 seconds, 1 minute, 3 minutes, 6 minutes, 9 minutes, 12 minutes, 15 minutes, 20 minutes, and 30 minutes.

Table 2 demonstrates the results of the time-kill studies. The  $\log_{-10}$  reduction data is presented for the test product against each of the bacterial and yeast strains designated in the TFM.

**Reviewer's comments:** Table 3 was constructed from Table III located in Appendix 2 (volume 10) of the NDA submission. This table demonstrates the mean  $\log_{10}$  reductions for the time kill studies for the various organisms. Table 3 consolidates the data from the figures for the time kill studies. The figures for time kill studies express the data in percent reduction rather than  $\log_{10}$  reduction.

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**Table 3. Test Product (Product 1)**

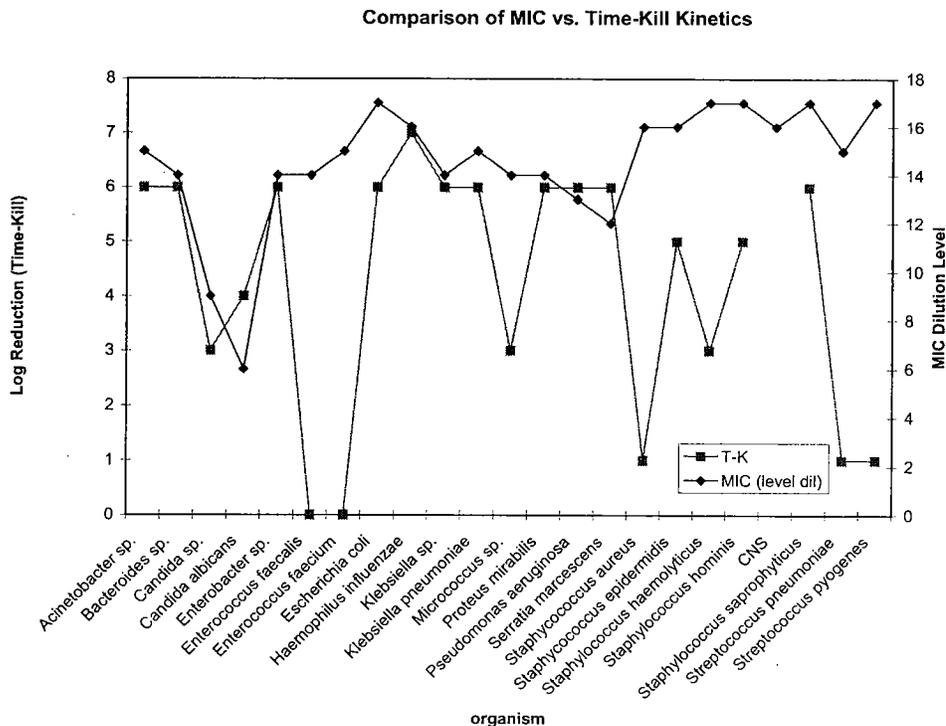
		log <sub>10</sub> reduction at various exposure times								
organism	ATCC strain or BSLI #	15 sec.	1 min.	3 min.	6 min.	9 min.	12 min.	15 min.	20 min.	30 min.
<i>E. faecalis</i>	29212									
<i>E. faecalis</i>	061700Efs1									
<i>E. faecium</i>	51559									
<i>E. faecium</i>	040400VREfm3									
<i>Micrococcus luteus</i>	7468									
<i>Micrococcus luteus</i>	061901MI1									
<i>S. aureus</i>	6538									
<i>S. aureus</i>	121699Sa2									
<i>S. aureus</i>	29213									
<i>S. aureus</i>	040400Sa3									
<i>S. epidermidis</i>	12228									
<i>S. epidermidis</i>	061700Se1									
<i>S. haemolyticus</i>	29970									
<i>S. haemolyticus</i>	060700Sha4									
<i>S. hominis</i>	27844									
<i>S. hominis</i>	060700Sh02									
<i>S. saprophyticus</i>	15305									
<i>S. saprophyticus</i>	081388Ss									
<i>S. pneumoniae</i>	33400									
<i>S. pneumoniae</i>	062900Spn3									
<i>S. pyogenes</i>	19615									
<i>S. pyogenes</i>	040400Spy1									
<b>Yeast</b>										
<i>Candida albicans</i>	10231									
<i>Candida albicans</i>	040400Ca3									
<i>Candida tropicalis</i>	750									
<i>Candida tropicalis</i>	121799Ct									

**Reviewer’s comments:** The TFM states that the product be diluted ten fold for evaluation in the time kill studies. This dilution may be problematic for CHG since it is a “leave-on” product, not intended for use with water. What is significant is that the time required for a 3-log reduction from the initial baseline. The time-kill studies are done as an *in vitro* comparison to the clinical simulations. All Gram-negative organisms exhibited at least a 5-log reduction within 15 sec. However, some organisms, particularly Gram-positive and yeast were slower to exhibit a 3-log reduction. Two clinical isolates of yeast, one clinical isolate of *S. aureus* and two ATCC strains of Streptococci exhibited 3-log or greater reductions within one minute. One clinical isolate of Streptococci required three min. to achieve a 3-log reduction. Two ATCC strains, one *E. faecalis* and one *S. aureus* as well as two clinical isolates, one *E. faecium* and one *S. aureus* required six min. to exhibit a 3-log reduction. Two ATCC strains, one *S. aureus*, one *E. faecium* and one clinical isolate, *E. faecalis* required nine min. to exhibit a 3-log reduction. Thus, *E. faecalis*, *E. faecium* and *S. aureus* strains were slowest to exhibit a 3-log reduction.

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A comparison of the MIC dilution results with the time-kill kinetics results should show that organisms killed at higher dilutions and therefore more sensitive to CHG, are killed more rapidly by the 2% CHG. Conversely, one should observe that organisms killed at lower dilutions and therefore more resistant to CHG, are killed less rapidly by the 2% CHG. However, the data do not entirely support this logic as indicated by the figure below.

Below is a comparison of the CHG MIC dilutions and time-kill kinetics for each organism tested. MICs are indicated by the dilution level e.g. a high dilution level is indicative of a high MIC. Time-kill kinetics are measured by the log<sub>10</sub> reduction for each organism. If the data follow the above logic, the data points for the log reductions and the MIC dilution level be close to one another e.g. the lines below should parallel one another. Organisms demonstrating a high dilution but slower time-kill kinetics include: *Enterococcus faecalis*, *Enterococcus faecium*, and *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*. One possible explanation for this observation is that all of these organisms are Gram-positive and thus may be related to the slow entry of CHG past the peptidoglycan layer of the cell wall. However, three species of *Staphylococcus* did not exhibit this behavior. These observations suggest that neither MIC data nor time-kill kinetic data be used separately but in conjunction with one another in the determination of the efficacy of active ingredients and product performance.



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**Validation of the Neutralization System: Time-Kill Studies**

A neutralization study was performed for the test product versus *Bacteriodes fragilis* (ATCC #25285), *Escherichia coli* (ATCC #11229), and *Streptococcus pneumoniae* (ATCC #33400), and for the reference product versus *Escherichia coli* (ATCC #11229), and *Staphylococcus aureus* (ATCC #6538). The neutralization study was done to ensure that the neutralizing solution employed was effective in neutralizing the antimicrobial properties of the test and reference products. The neutralization followed the guidelines set forth in ASTM E 1054-02 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents", and was performed at [REDACTED]

The following tables provide summaries of the data generated during the study. Raw data and statistical analysis can be found in Volume 10, pp 3869-3890 of the NDA submission. Tables 4-7 [pp 23-26 of the NDA amendment 21-669 N000(B2)] show the percent recovery for the test bacterium used for the neutralization study. Tables 8-11 [pp 27-28 of the NDA amendment 21-669 N000(B2)] show the average log<sub>10</sub> values and differences from the numbers control.

**Table 4:% Recovery, Challenge Strain: *Bacteriodes fragilis* (ATCC #25285)**

Article (Test Description)	Plating Time	Replicate 1	CFU/mL Replicate 2	Replicate 3	Average CFU/mL	% Recovery
Initial Population	>15 min.	2.56 x 10 <sup>5</sup>	3.85 x 10 <sup>5</sup>	4.25 x 10 <sup>5</sup>	3.55 x 10 <sup>5</sup>	NA
Toxicity Control	>15 min.	1.14 x 10 <sup>6</sup>	1.27 x 10 <sup>6</sup>	1.24 x 10 <sup>6</sup>	1.22 x 10 <sup>6</sup>	> 100
Product #1 Test Product Lot#201-2022-01	>15 min.	1.13 x 10 <sup>6</sup>	1.17 x 10 <sup>6</sup>	1.28 x 10 <sup>6</sup>	1.19 x 10 <sup>6</sup>	> 100
Product #3 Active Ingredient Lot # 202-01R&D-01	NA	NA	NA	NA	NA	NA
Product #2 Vehicle Lot# 203-2022-02	NA	NA	NA	NA	NA	NA

**Table 5:% Recovery, Challenge Strain: *Escherichia coli* (ATCC #11229)**

Article (Test Description)	Plating Time	Replicate 1	CFU/mL Replicate 2	Replicate 3	Average CFU/mL	% Recovery
Initial Population	>15 min.	1.34 x 10 <sup>5</sup>	1.18 x 10 <sup>5</sup>	1.17 x 10 <sup>5</sup>	1.23 x 10 <sup>5</sup>	NA
Toxicity Control	>15 min.	1.10 x 10 <sup>5</sup>	1.31 x 10 <sup>5</sup>	1.16 x 10 <sup>5</sup>	1.19 x 10 <sup>5</sup>	96.7
Product #1 Test Product Lot#201-2022-01	>15 min.	1.04 x 10 <sup>5</sup>	1.19 x 10 <sup>5</sup>	1.06 x 10 <sup>5</sup>	1.1 x 10 <sup>5</sup>	89.4
Product #3 Active Ingredient Lot # 202-01R&D-01	>15 min.	1.04 x 10 <sup>5</sup>	1.15 x 10 <sup>5</sup>	1.09 x 10 <sup>5</sup>	1.09 x 10 <sup>5</sup>	88.6
Product #2 Vehicle Lot# 203-2022-02	>15 min.	1.19 x 10 <sup>5</sup>	1.06 x 10 <sup>5</sup>	1.14 x 10 <sup>5</sup>	1.13 x 10 <sup>5</sup>	91.9

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**Table 6: % Recovery, Challenge Strain: *Staphylococcus aureus* (ATCC #6538)**

Article (Test Description)	Plating Time	Replicate 1	CFU/mL Replicate 2	Replicate 3	Average CFU/mL	% Recovery
Initial Population	>15 min.	$9.3 \times 10^4$	$9.1 \times 10^4$	$1.01 \times 10^5$	$9.5 \times 10^4$	NA
Toxicity Control	>15 min.	$1.1 \times 10^5$	$1.02 \times 10^5$	$1.04 \times 10^5$	$1.05 \times 10^5$	>100
Product #1 Test Product Lot#201-2022-01	NA	NA	NA	NA	NA	NA
Product #3 Active Ingredient Lot # 202-01R&D-01	>15 min.	$9.75 \times 10^4$	$8.55 \times 10^4$	$9.95 \times 10^4$	$9.42 \times 10^4$	99.2
Product #2 Vehicle Lot# 203-2022-02	>15 min.	$1.03 \times 10^5$	$8.95 \times 10^4$	$9.65 \times 10^4$	$9.63 \times 10^4$	>100

**Table 7: % Recovery, Challenge Strain: *Streptococcus pneumoniae* (ATCC #33400)**

Article (Test Description)	Plating Time	Replicate 1	CFU/mL Replicate 2	Replicate 3	Average CFU/mL	% Recovery
Initial Population	>15 min.	$1.18 \times 10^5$	$1.33 \times 10^5$	$1.7 \times 10^5$	$1.4 \times 10^5$	NA
Toxicity Control	>15 min.	$2.31 \times 10^5$	$2.72 \times 10^5$	$2.46 \times 10^5$	$2.5 \times 10^5$	>100
Product #1 Test Product Lot#201-2022-01	>15 min.	$1.94 \times 10^5$	$1.14 \times 10^5$	$2.22 \times 10^5$	$1.77 \times 10^5$	>100
Product #3 Active Ingredient Lot # 202-01R&D-01	NA	NA	NA	NA	NA	NA
Product #2 Vehicle Lot# 203-2022-02	NA	NA	NA	NA	NA	NA

For Tables 4-7: Final Population  
 % Recovery =  $\frac{\text{(Toxicity Control, or post-exposure Products \#1, \#2, \#3)}}{\text{Initial Population}} \times 100$

**Table 8: Average Log<sub>10</sub> values and differences-Challenge Strain: *Bacteroides fragilis* (ATCC #25285)**

Test Procedure	Average Log <sub>10</sub> Value	Difference from Numbers Control	Within 0.2 of Numbers Control*
Numbers Control	5.54	NA	NA
Toxicity Control	6.09	0.54	No
Product #1 Test Product Lot #201-2022-01	6.08	0.53	No
Product #3 Active Ingredient Lot # 202-01R&D-01	NA	NA	NA
Product #2 Vehicle Lot# 203-2022-02	NA	NA	NA

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**Table 9: Average Log<sub>10</sub> values and differences-Challenge Strain: *Escherichia coli* (ATCC #11229)**

Test Procedure	Average Log <sub>10</sub> Value	Difference from Numbers Control	Within 0.25 of Numbers Control*
Numbers Control	5.09	NA	NA
Toxicity Control	5.07	0.02	Yes
Product #1 Test Product Lot#201-2022-01	5.04	0.05	Yes
Product #3 Active Ingredient Lot # 202-01R&D-01	5.04	0.05	Yes
Product #2 Vehicle Lot # 203-2022-02	5.05	0.04	Yes

**Table 10: Average Log<sub>10</sub> values and differences-Challenge Strain: *Staphylococcus aureus* (ATCC #6538)**

Test Procedure	Average Log <sub>10</sub> Value	Difference from Numbers Control	Within 0.2 of Numbers Control*
Numbers Control	4.98	NA	NA
Toxicity Control	5.02	0.04	Yes
Product #1 Test Product Lot#201-2022-01	NA	NA	NA
Product #3 Active Ingredient Lot # 202-01R&D-01	4.97	0	Yes
Product #2 Vehicle Lot# 203-2022-02	4.98	0.01	Yes

**Table 11: Average Log<sub>10</sub> values and differences-Challenge Strain: *Streptococcus pneumoniae* (ATCC #33400)**

Test Procedure	Average Log <sub>10</sub> Value	Difference from Numbers Control	Within 0.2 of Numbers Control*
Numbers Control	5.14	NA	NA
Toxicity Control	5.4	0.25	No
Product #1 Test Product Lot#201-2022-01	5.23	0.09	Yes
Product #3 Active Ingredient Lot # 202-01R&D-01	NA	NA	NA
Product #2 Vehicle Lot # 203-2022-02	NA	NA	NA

For Tables 8-11:

\* If "Yes", then no difference from Numbers Control; If "No", then difference from Numbers Control.

The data was evaluated according to ASTM Standard E 1054-02 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents". The average number of challenge microorganisms was determined. The number of survivors was converted to log<sub>10</sub> values. An ANOVA test was performed to statistically compare the results of the test to the Numbers control.

Results of the statistical analyses indicate that:

- For *B. fragilis*, the toxicity control and test product log<sub>10</sub> averages are not 0.2 log<sub>10</sub> lower than the log<sub>10</sub> averages for the numbers control.

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- For *E. coli*, no differences are found between the average log<sub>10</sub> values of the numbers control and the average log<sub>10</sub> values for the toxicity control, test product, active ingredient or vehicle.

**Reviewer's comments:** For *Staphylococcus aureus* and *Escherichia coli* strains, no significant statistical difference was found between the average log<sub>10</sub> values of the numbers control and the average log<sub>10</sub> values for the toxicity control, test product, active ingredient or vehicle. However, for *Bacteriodes fragilis* there was a significant statistical difference between the average log<sub>10</sub> values of the numbers control and the average log<sub>10</sub> values for the toxicity control and the test product. For *Streptococcus pneumoniae*, there was a significant statistical difference between the average log<sub>10</sub> values of the numbers control and the average log<sub>10</sub> values for the toxicity control. These observations are made based upon the guidelines for neutralization in ASTM E 1054-02 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents". This document states that a log<sub>10</sub> difference of 0.2 has been previously used for neutralization assays and that a difference determined between two samples of 0.2 log<sub>10</sub> is considered a significant statistical difference. Here, the Applicant has used a value of 0.25 log<sub>10</sub> rather than the stated 0.2 log<sub>10</sub> as a measure of significant statistical difference.

Although the Applicant states there are statistical differences between the numbers controls versus the toxicity and efficacy controls, the toxicity and efficacy controls are *higher*, not lower than the numbers controls. In addition, the toxicity and efficacy controls demonstrate greater than 50% recovery compared to the numbers control. Thus, this Reviewer concludes that the neutralizer was both efficacious and not toxic.

### Activity Against Selected Resistant Organisms

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\_\_\_\_\_ were tested in the MIC study described in Section 7.3.1 of the briefing package. APPENDIX 3 (volume 11) of the briefing package contains a summary of resistant organisms that were tested in the first MIC study and the drugs to which they are resistant.

Table 12 summarizes the results of the resistant organisms tested in the MIC study described in Section 7.3.1 of the briefing package.

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**Table 12. Aerobic Resistant Organism Minimum Inhibitory Concentration Data**

Microorganism Species	Bulk drug product		
	Number of Organisms Tested	Average MIC (Expressed as Product Dilution)	Minimum Maximum
<i>Acinetobacter</i> species	16	1:4,096	
<i>Bacteroides</i> species	6	1:8,192	
<i>Candida</i> species	5	1:38	
<i>Enterobacter</i> species	26	1:8,192	
<i>Enterococcus faecalis</i>	21	1:8,192	
<i>Enterococcus faecium</i>	26	1:8,192	
<i>Escherichia coli</i>	5	1:32,768	
<i>Haemophilus influenzae</i>	2	1:16,384	
<i>Klebsiella</i> species	7	1:8,192	
<i>Klebsiella pneumoniae</i>	25	1:16,384	
<i>Micrococcus</i> species	2	1:32,768	
<i>Proteus mirabilis</i>	6	1:1,024	
<i>Pseudomonas aeruginosa</i>	14	1:2,048	
<i>Serratia marcescens</i>	3	1:2,048	
<i>Staphylococcus aureus</i>	30	1:32,768	
<i>Staphylococcus epidermidis</i>	7	1:32,768	
<i>Staphylococcus haemolyticus</i>	1	1:16,384	
<i>Staphylococcus hominis</i>	3	1:65,536	
Coagulase-Negative <i>Staphylococcus</i> species	17	1:65,536	
<i>Staphylococcus saprophyticus</i>	7	1:65,536	
<i>Streptococcus pneumoniae</i>	2	1:16,384	
<i>Streptococcus pyogenes</i>	0	N/A	

The results of the testing for each isolate listed in table 3 above can be found in Addendum II of the final report for the MIC study, which is included in APPENDIX 1 of the briefing package.

An additional MIC study was performed at [redacted]. One organism not included in the original MIC study is [redacted]. Because control of this organism is of particular interest in the hospital environment, the Applicant elected to perform an additional MIC study against [redacted] utilizing the same methodology that was used for the previous MIC, with two exceptions.

1. Only the bulk drug product was tested. The previous MIC tested the vehicle and active in addition to the bulk drug product in order to demonstrate that the active ingredient, and not the vehicle, was responsible for the antimicrobial activity of the bulk product. This was shown to be the case and therefore was not repeated here.

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2. The growth conditions and materials required adjustment to accommodate this anaerobic organism. The previous study tested only aerobes.

The results of this study show a MIC equivalent to those seen with the previously tested organisms that is, [REDACTED] demonstrates a MIC of 1:4096. A copy of the final report from this study can be found in APPENDIX 4 (volume 11) of the NDA briefing package.

An additional time-kill study was performed at [REDACTED]. The time-kill study tested the drug product in order to demonstrate the product's antimicrobial efficacy against selected antibiotic resistant organisms. The product was not tested as a 10% aqueous solution, as specified by the TFM, because it is provided in a ready-to-use form and does not require dilution prior to use. Instead, the product is tested at a [REDACTED] (v/v) concentration.

The drug product was evaluated using a total of 21 microorganisms. The list of organisms consists of eight ATCC strains and 13 fresh clinical isolates.

The percent and  $\log_{10}$  reductions from the initial populations were determined for each challenge microorganism following exposures to the appropriate product for 15 seconds, 1 minute, 3 minutes, 6 minutes, 9 minutes, 12 minutes, and 15 minutes.

The test utilized the same methodology used for the previously described time-kill studies, with three exceptions:

1. Only the bulk drug product was tested. The previous time-kill study tested the vehicle and active ingredient in addition to the bulk drug product in order to demonstrate that the active ingredient, and not the vehicle, causes the antimicrobial activity of the bulk product. This was shown to be the case and therefore is not repeated here.
2. The growth conditions and materials used were adjusted to accommodate an anaerobic organism. The previous study tested only aerobes.
3. The 20-minute and 30-minute time points were eliminated from this study because the previously described study demonstrates the product had greater than a 99% kill in less than 20 minutes. Therefore those two data points were not repeated.

Table 13 shows the  $\log_{10}$  reduction results of the time-kill study. The  $\log_{10}$  results and the percent reductions can be found in Table IV (volume 11) located in APPENDIX 5 of the briefing package.

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*Staphylococcus aureus* (ATTC #33591) to ensure that the neutralizing solution employed is effective in neutralizing the antimicrobial properties of the test product. The neutralization followed the guidelines set forth in ASTM E 1054-02 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents", and was performed at \_\_\_\_\_

The following tables provide summaries of the data generated during the study. Raw data and statistical analysis can be found in Volume 11, pp 4388-4404 of the NDA submission. Tables 14-16 [pp 32-33 of the NDA amendment 21-669 N000(B2)] show the percent recovery for the test bacterium used for the neutralization study. Tables 17-19 [pp 33-34 of the NDA amendment 21-669 N000(B2)] show the average log<sub>10</sub> values and differences from the numbers control.

**Table 14: % Recovery, Challenge Strain: \_\_\_\_\_**

Article (Test Description)	Plating Time	Replicate 1	CFU/mL Replicate 2	Replicate 3	Average CFU/mL	% Recovery
Initial Population	immediate	$3.7 \times 10^5$	$4.9 \times 10^5$	$6.5 \times 10^5$	$5.05 \times 10^5$	NA
Toxicity Control	immediate	$5.9 \times 10^5$	$4.15 \times 10^5$	$6.85 \times 10^5$	$5.63 \times 10^5$	>100
Product #1 Test Product Lot#125-1	immediate	$4.05 \times 10^5$	$4.75 \times 10^5$	$6.75 \times 10^5$	$5.18 \times 10^5$	>100

**Table 15: % Recovery, Challenge Strain: *Enterococcus faecalis* (ATCC #51575)**

Article (Test Description)	Plating Time	Replicate 1	CFU/mL Replicate 2	Replicate 3	Average CFU/mL	% Recovery
Initial Population	>15 min.	$1.81 \times 10^5$	$1.82 \times 10^5$	$1.62 \times 10^5$	$1.75 \times 10^5$	NA
Toxicity Control	>15 min.	$1.58 \times 10^5$	$1.76 \times 10^5$	$1.76 \times 10^5$	$1.7 \times 10^5$	97.1
Product #1 Test Product Lot#125-1	>15 min.	$1.58 \times 10^5$	$1.77 \times 10^5$	$1.28 \times 10^5$	$1.54 \times 10^5$	88

**Table 16: % Recovery, Challenge Strain: *Staphylococcus aureus* (ATCC #33591)**

Article (Test Description)	Plating Time	Replicate 1	CFU/mL Replicate 2	Replicate 3	Average CFU/mL	% Recovery
Initial Population	>15 min.	$1.34 \times 10^5$	$1.33 \times 10^5$	$1.36 \times 10^5$	$1.34 \times 10^5$	NA
Toxicity Control	>15 min.	$1.39 \times 10^5$	$1.61 \times 10^5$	$1.44 \times 10^5$	$1.48 \times 10^5$	>100
Product #1 Test Product Lot#125-1	>15 min.	$1.31 \times 10^5$	$1.31 \times 10^5$	$1.2 \times 10^5$	$1.27 \times 10^5$	94.8

Final Population

$$\% \text{ Recovery} = \frac{\text{(Toxicity Control, or post-exposure Products \#1, \#2, \#3)}}{\text{Initial Population}} \times 100$$

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**Table 17: Average Log<sub>10</sub> values and differences-**

Test Procedure	Average Log <sub>10</sub> Value	Difference from Numbers Control	Within 0.2 of Numbers Control*
Numbers Control	5.7	NA	NA
Toxicity Control	5.74	0.05	Yes
Product #1 Test Product Lot # 125-1	5.71	0.01	Yes

**Table 18: Average Log<sub>10</sub> values and differences-*Enterococcus faecalis* (ATCC #51575)**

Test Procedure	Average Log <sub>10</sub> Value	Difference from Numbers Control	Within 0.2 of Numbers Control*
Numbers Control	5.24	NA	NA
Toxicity Control	5.23	0.01	Yes
Product #1 Test Product Lot # 125-1	5.18	0.06	Yes

**Table 19: Average Log<sub>10</sub> values and differences-*Staphylococcus aureus* (ATCC #33591)**

Test Procedure	Average Log <sub>10</sub> Value	Difference from Numbers Control	Within 0.2 of Numbers Control*
Numbers Control	5.13	NA	NA
Toxicity Control	5.17	0.04	Yes
Product #1 Test Product Lot # 125-1	5.1	0.02	Yes

For Tables 17-19:

\* If "Yes", then no difference from Numbers Control;

If "No", then difference from Numbers Control.

The data was evaluated according to ASTM Standard E 1054-02 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents". The average number of challenge microorganisms was determined. The number of survivors was converted to log<sub>10</sub> values. An ANOVA test was performed to statistically compare the results of the test product to the numbers control.

For , *E. faecalis* and *S. aureus*, no significant difference exists between the average log<sub>10</sub> values of the numbers control and the average log<sub>10</sub> values for the toxicity control or test product.

These results indicate the neutralizer is both effective and non-toxic.

### Mechanism of Resistance

**Introduction.** Chlorhexidine is a cationic bisbiguanide antimicrobial agent that disrupts cell membranes and causes denaturation and precipitation of cellular



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difference does not appear to be clinically significant.<sup>16</sup> The reason for impaired susceptibility of \_\_\_\_\_ to chlorhexidine is not fully understood. A plasmid pSAJ1 has been described which confers resistance to antibiotics as well as chlorhexidine.<sup>17</sup> Another plasmid, pWG115, encoding resistance to gentamicin and cationic surface-active compounds, has been identified in \_\_\_\_\_. The general role of plasmids in antiseptic resistance is not fully understood.<sup>18,19</sup> Acquired resistance does not appear to be transferable between bacterial species. Also, there remains some doubt as to whether organisms can be trained to become highly resistant to the antiseptic.<sup>20</sup> Cookson et al.<sup>21</sup> failed to increase resistance of \_\_\_\_\_ strains to chlorhexidine after repeated exposure *in vivo* or serial passage *in vitro*.

**Summary.** The potential for bacterial resistance to CHG appears low, but resistance has been reported.<sup>4,5</sup> In certain instances, reduced susceptibility to chlorhexidine has been found to be associated with factors other than intrinsic or acquired resistance. For example, in an investigation into the occurrence of chlorhexidine resistant organisms among bacteria causing urinary tract infections, Baillie<sup>4</sup> concluded that the *in vivo* activity of chlorhexidine was impaired by the presence of urine. This study found that charged particles present in urine may compete with chlorhexidine for membrane binding sites or may bind directly with chlorhexidine thereby reducing its activity. In summary, primary bacterial resistance to chlorhexidine is rare and acquired resistance is detected only when diluted aqueous solutions are used.

**Reviewer's comments:** Resistance to CHG among Gram-positive organisms appears to be due to acquired resistance which is mediated by transferable elements such as plasmids. The Applicant notes that acquired resistance does not appear to be transferable among bacterial species. It is worth noting that the time-kill studies show that the time required for 3-log reductions among Gram-positives were often longer than time required for 3-log reductions among Gram-negatives. These observations suggest that acquired resistance may be increasing among Gram-positive organisms and perhaps should be monitored over time in the future. An increase in acquired resistance is particularly important to Gram-positive organisms since these organisms are more commonly responsible for surgical site infections. Since several antibiotic resistant organisms show higher MIC values and slower time-kill kinetics for CHG, it would be prudent to be aware of any future changes in antibiotic resistance or CHG resistance patterns.

### **Clinical Pharmacology**

Minimal, if any, absorption of chlorhexidine gluconate occurs through the skin<sup>23</sup>. Furthermore, results of clinical percutaneous absorption studies have demonstrated there is virtually no penetration of chlorhexidine through intact human skin<sup>21,22</sup>. Therefore, human pharmacology studies were not completed for this product.

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Sage's \_\_\_\_\_ are intended to be applied to the skin of patients prior to surgery. Results of clinical percutaneous absorption studies found virtually no penetration of chlorhexidine through intact human skin<sup>21,22,23</sup>.

Chlorhexidine gluconate, CHG, was developed in the early 1950's in England and was introduced in the USA in the 1970's. There are a number of NDA's approved which contain chlorhexidine gluconate in amounts ranging from 0.5% to 4.0%. CHG is recognized to have "a good safety record"<sup>23</sup>.

The biopharmaceutics of chlorhexidine gluconate is well established in the literature and numerous products containing this active ingredient are currently available on the market. Sage's \_\_\_\_\_ contain the same active ingredient and concentration as other currently available products.

Based on the literature and marketing history of chlorhexidine gluconate, the Applicant has requested a waiver of the bioavailability requirement.

### **CLINICAL SIMULATION STUDIES-*IN VIVO***

#### **Description of Controlled Efficacy Studies**

Based on the results of the uncontrolled pilot studies, the Applicant chose an application time of three minutes for the \_\_\_\_\_. The controlled preoperative skin preparation studies described in Tables 6 and 7 utilize the three minute application time. These studies were used to demonstrate the effectiveness of Sage's \_\_\_\_\_ product as a preoperative skin preparation.

All studies are designed utilizing several common traits. All studies concern preoperative skin preparation. All studies are designed to be completely blocked, blinded, and randomized. In each study, the finished dosage form is applied for three minutes and used Hibiclens as the predicate CHG product. The duration of the study is for six hours in each case. The differences in each study are indicated in Table 20 and include the protocol number, principal investigators, number of subjects and their sex, age, location of the final report, and differences in the results.

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**Table 20. Differences in Design and Results of the Controlled Safety and Efficacy Studies.**

Protocol Number	Principal Investigators	Number of Subjects	Age	Sex	Location of Final Report	Result
01-109381-11		69 enrolled 51 qualified	18 - 69	M (19) F (32)	Appendix 6	Test product met required log reduction on abdominal sites but <i>did not</i> meet required log reduction on the inguinal sites
020125-103		88 enrolled 35 qualified	18-70	M (25) F (10)	Appendix 7	Test product met required log reduction on both anatomical sites
500-102		43 enrolled 32 qualified	18-61	M (14) F (18)	Appendix 8	The test product met the required log reduction on the inguinal site

While the objective of these studies was similar, the results varied. In each study, the objective was to evaluate the efficacy of the drug product as a preoperative skin preparation. Studies to evaluate the efficacy of the drug product on both the abdominal and inguinal sites were conducted at the [redacted] and [redacted] laboratories. Studies at [redacted] evaluated the inguinal site only. The differences lie in the results of the studies as indicated by Table 20.

The preoperative skin preparation studies were modeled after 21 CFR 333.470(b)(3); Tentative Final Monograph for Topical Antimicrobial Drug Products for Over-the-Counter Use; Federal Register; Volume 59, No. 116; June 17, 1994. They also utilized ASTM method E 1054, "Standard Test Method for Evaluation of Inactivators of Antimicrobial Agents". The pivotal studies were conducted at three sites using Sage's [redacted] product to establish that the product met the standard for preoperative skin prep as described in the TFM.

In the first two controlled pivotal trials performed at [redacted] and [redacted], 2% CHG met the TFM requirement of a two-log reduction in baseline microbial counts for the abdominal site within 10 minutes with counts not to exceed baseline in six hours. Therefore, the Applicant met the TFM requirements for the abdominal site.

However, the study conducted at [redacted] laboratory failed to meet the monograph criteria for the marketed control product at both the abdominal and inguinal sites. In addition, the study at [redacted] laboratory site failed the three-log reduction requirement both for the comparator (Hibiclens) and the study product at the inguinal site. [redacted] also conducted two pilot studies and neither of these studies met the three-log reduction at the inguinal site.

Because the marketed product (Hibiclens) failed to meet the monograph criteria at [redacted] Research, the Applicant questioned the reliability of the results from this study site. Therefore, the Applicant paid additional attention to the excluded subjects at this site. Data from all three laboratories conducting the three pivotal

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studies for this NDA were evaluated. The number of subjects treated and the numbers that passed or failed the baseline criteria in the inguinal sites at all laboratories were assessed. Comparing the disposition of the treated subjects for all three laboratories, the proportion failing baseline criteria was statistically significant  $P=0.0003$ . However, taking the [REDACTED] data alone, the proportion failing baseline criteria was not statistically significant  $P=0.1144$ . Therefore, the Applicant concluded that [REDACTED] had a disproportionate number of subjects who failed the inclusion criteria at baseline for the inguinal site data.

Comparing the disposition of treated subjects with regard to the abdominal site, the proportion failing was not statistically significant  $P=0.1998$ . Therefore, the number of subjects failing the inclusion criteria at baseline at [REDACTED] for the abdominal site was not different from those failing at [REDACTED]. The Applicant believes the statistical analyses confirm the abdominal site data but not the inguinal site data from the [REDACTED] study are valid.

After deliberation with the Agency during a teleconference on dated October, 25, 2002 regarding the failures of the studies at [REDACTED], the Applicant identified a third independent site to conduct the second pivotal trial for the inguinal site. Therefore, a third controlled trial was conducted at [REDACTED] to confirm the three-log reduction at the inguinal site.

Sage product 2% CHG met the two-log reduction for the abdominal site and the three-log reduction for the inguinal site at two independent laboratories. This fulfilled the requirements set by FDA for the preoperative skin preparation as described in the TFM.

**Reviewer's comments:** Clinical studies performed for the Applicant by [REDACTED] failed to meet the TFM requirements for the application of a preoperative skin preparation product to the inguinal site. In addition, the data for the positive control product, Hibiclens, also failed to meet the TFM criteria for both the abdominal and inguinal sites. Since data from studies performed at both [REDACTED] met the TFM criteria for the proposed product at both the abdominal and inguinal sites, the Applicant concludes that the data from the studies at [REDACTED] are suspect. This Reviewer believes the conclusion made by the Applicant is premature.

While a number of discrepancies in procedure and administration were noted in a recent [REDACTED] Visit Report, a proper assessment of the data is not possible. To properly assess the data, an extensive comparison of the procedures and administration of this type of study at the other two sites is necessary.

The failure of subjects at [REDACTED] to meet the inclusion criteria at baseline is certainly one variable that may help to explain the variation in the results for the log reductions at the inguinal site.

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### **Controlled Trial Study Description: Preoperative Skin Preparation**

The studies are designed to evaluate the antimicrobial effectiveness of the Applicant's product. The studies contain a positive control formulation (Hibiclens by Astrazeneca) previously approved by the FDA. The studies were run initially at two facilities: \_\_\_\_\_

The sample size is a minimum of 30 qualified sites per time point at each study location. The sample size is determined by the following statistical analysis:

$$n \geq \frac{2S^2(Z_{\alpha/2} + Z_b)^2}{D^2}$$

$$S^2 = 0.69$$

$$Z_{\alpha/2} = 1.96 \text{ with } \alpha=0.05$$

$$Z_b = 0.842 \text{ when } \beta = 20$$

$$D = 0.5 \log_{10}$$

$$n \geq \frac{2(0.69)^2[1.96 + 0.842]^2}{0.5^2} = 29.904$$

$$n \geq 30$$

Since each subject is treated with both products, the total minimum sample size is 30 qualified sites (see Table 20). The subjects are treated in one or both of two anatomical locations: the abdomen and the inguinal. A baseline measurement of microbial cell count is used to qualify the sites prior to the start of the study ( $>2.5 \log_{10}$  CFU/cm<sup>2</sup> for abdominal sites and  $>5.0 \log_{10}$  CFU/cm<sup>2</sup> for inguinal sites). Only qualified anatomical sites are used for the study.

The test product is applied by rubbing the article onto the test site for three minutes using the saturated cloth to simulate use conditions. The application time period is based on the results of the pilot studies. The time chosen is the time needed to achieve a  $3\text{-log}_{10}$  reduction in microorganism count from baseline in the inguinal region.

The studies are randomized and blinded. Each subject has both the test article and control applied contralaterally; test product and control application to the left side and the right side are randomized. Each subject has four test sites per side; baseline, 10 minutes, 30 minutes, and 6 hour sampling intervals; these sites are

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randomized. The laboratory personnel evaluating the samples are blinded to the identity of the sample.

After the baseline sample is taken, the designated product ( [REDACTED] or Hibiclens) are applied to the skin according the directions for use. For the [REDACTED] the direction is to scrub the skin for three minutes and allow to air dry. For Hibiclens, the product is applied liberally and swabbed for two minutes, dried with a sterile towel, and the procedure is repeated for an additional two minutes.

Once the product has dried and adjacent samples have been taken, the site designated for the 6 hour sampling time is covered with sterile gauze held in place with sterile non-occlusive dressing ( [REDACTED] ) at [REDACTED] and fenestration bandage ( [REDACTED] ) at [REDACTED]. This allows the subject to be mobile during the time interval between the 30-minute and 6-hour sampling times without compromising the test site.

Using the cylinder sampling (scrub cup) technique, a sample is taken 10 minutes after the product has dried on the skin. A sterile scrubbing cylinder is held firmly to the skin over the site to be sampled. Scrub solution with validated antimicrobial neutralizers specific to the product formulation is added to the cylinder.

The area inside the cylinder is scrubbed for one minute using a sterile rubber policeman. The scrub solution is aspirated into a sterile test tube using a sterile pipette. The method is repeated using a second aliquot of scrub solution with validated antimicrobial neutralizers. The two aliquots are pooled. This technique is repeated for the 30-minute and 6-hour sample times. Table 21 shows a comparison between areas of the scrub cup and the volume of solution added used by the two pivotal studies. The Applicant states that the difference in the size of the cylinders used does not impact the results of the study because any differences are accounted for in the microbial count calculations.

**Table 21: Scrubbing Cup Comparison**

Clinical Trial Location	Internal Area of Scrubbing Cup	Volume of Scrub Solution Added
[REDACTED]	3.8 cm <sup>2</sup>	3.0 mL
[REDACTED]	3.46 cm <sup>2</sup>	2.5 mL

The pooled aliquots are diluted in 10-fold steps using Butterfield's Phosphate Buffer and appropriate validated antimicrobial neutralizers. The dilutions are plated on agar containing validated product neutralizers. The plates are incubated aerobically for 72±4 hours at 30±2°C.

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After incubation of the media, the colonies are counted. The average number of microorganisms recovered per square centimeter of skin is determined and reported. This number is used to calculate colony-forming units per milliliter (CFU/mL); \_\_\_\_\_ utilized the formula  $CFU/cm^2 = \frac{CFU/mL \times 6mL}{3.8cm^2}$ , \_\_\_\_\_, utilized the formula  $CFU/cm^2 = \frac{CFU/mL \times 5mL}{3.46cm^2}$ . The difference in formula is due to difference in cylinder size and scrub solution volumes used.

The CFU/mL data are converted to  $\log_{10}$  and evaluated using statistics. The  $\log_{10}$  calculated from the post-application sample is subtracted from the  $\log_{10}$  calculated from the baseline sample, giving the result of the  $\log_{10}$  reduction from the baseline count. An average count reduction from baseline counts is calculated using data from all qualified subjects. The data gathered are evaluated by using descriptive statistics to determine the mean, standard deviation, 95% confidence interval and  $\log_{10}$  reduction from baseline.

**Reviewer's comments:** A number of inconsistencies in the procedure are noted and require mention. These inconsistencies pertain to variation in cup scrub diameter, neutralization composition, processing of bacterial samples, and variation in formulas for the determination of CFU/cm<sup>2</sup>.

First, the Applicant notes that the internal diameters of the scrubbing cups used by the different contract laboratories i.e. \_\_\_\_\_ for the sampling are different; the Applicant states that this variation in size does not affect the microbial count data obtained. However, the Applicant has not referenced or supplied such data.

Next, it is unclear if the neutralizers used by all three contract laboratories are identical in composition or concentration. Both \_\_\_\_\_ state the neutralizer consists of 1% Polysorbate 80 and 0.3% lecithin (vol. 14, p 5928 and vol. 12, p 4641, respectively); the composition and concentration of the neutralizer used by \_\_\_\_\_ could not be located. While these are appropriate neutralizers for use against chlorhexidine according to the ASTM document E 1054-02 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents", consistency in the protocol is required to reduce variability in the protocol.

Finally, the Applicant states that after incubation of the media, the colonies are counted. Due to the variation in the cup scrub diameter, different formulas are used by \_\_\_\_\_ to determine the CFU/cm<sup>2</sup> of skin. It is unclear as to which formula is used by \_\_\_\_\_ to calculate the number of bacteria on the skin.

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**Results of the Preoperative Skin Preparation Studies**

Tables 22 and 23 summarize the results of the preoperative skin preparation pivotal studies for the *abdominal* site.

**Table 22. Abdominal Site Results for Test Product [REDACTED] at [REDACTED]**

Sample	Number of samples	Mean Log <sub>10</sub> Value	Standard Deviation	Log <sub>10</sub> Reduction from Baseline
Average Baseline	30	3.36	0.45	N/A
10 Min. post-prep	30	0.86	0.85	2.50
30 Min. post-prep	30	1.03	1.06	2.33
6 Hours post-prep	30	0.82	1.09	2.54
Average Baseline	31	3.34	0.44	N/A
10 Min post-prep	31	0.97	0.90	2.37
30 Min post-prep	31	0.83	0.75	2.51
6 Hours post-prep	31	0.92	0.76	2.42

**Table 23. Abdominal Site Results for Control Product (Hibiclens) at [REDACTED]**

Sample	Number of samples	Mean Log <sub>10</sub> Value	Standard Deviation	Log <sub>10</sub> Reduction from Baseline
Average Baseline	30	3.51	0.57	N/A
10 Min post-prep	30	1.33	1.09	2.18
30 Min post-prep	30	1.32	1.29	2.19
6 Hours post-prep	30	0.74	1.01	2.77
Average Baseline	31	3.32	0.43	N/A
10 Min post-prep	31	1.37	0.88	1.95
30 Min post-prep	31	1.01	0.83	2.32
6 Hours post-prep	31	1.03	0.86	2.29

Tables 24 and 25 summarize the results of the preoperative skin preparation pivotal studies for the *inguinal* site.

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**Table 24. Inguinal Site Results for Test Product** at

Sample	Number of samples	Mean Log <sub>10</sub> Value	Standard Deviation	Log <sub>10</sub> Reduction from Baseline
Average Baseline	30	6.15	0.34	N/A
10 Min post-prep	30	2.70	0.82	3.45
30 Min post-prep	30	2.65	0.89	3.50
6 Hours post-prep	30	2.51	1.04	3.64
Average Baseline	31	6.42	0.47	N/A
10 Min post-prep	31	3.79	1.04	2.63
30 Min post-prep	31	3.61	0.95	2.82
6 Hours post-prep	31	3.25	0.85	3.18
Baseline	32	5.31	0.40	N/A
10 Min post-prep	32	1.12	0.74	4.19
30 Min post-prep	32	1.29	0.65	4.02
6 Hours post-prep	32	2.18	0.73	3.12

**Table 25. Inguinal Site Results for Reference Product (Hibiclens) at**

Sample	Number of samples	Mean Log <sub>10</sub> Value	Standard Deviation	Log <sub>10</sub> Reduction from Baseline
Average Baseline	30	6.16	0.43	N/A
10 Min post-prep	30	3.38	0.94	2.78
30 Min post-prep	30	3.53	0.77	2.63
6 Hours post-prep	30	3.01	1.10	3.15
Average Baseline	31	6.44	0.44*	N/A
10 Min post-prep	31	4.48	0.86	1.96
30 Min post-prep	31	4.46	0.75	1.99
6 Hours post-prep	27	3.97	1.00	2.46
Baseline	32	5.30	0.42	N/A
10 Min post-prep	32	1.47	1.01	3.83
30 Min post-prep	32	1.44	0.84	3.86
6 Hours post-prep	32	2.70	0.66	3.03

\*Baseline standard deviation for the 27 samples at the 6-hour sampling time was 0.46

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The data gathered at [redacted] meets the log<sub>10</sub> reduction requirement defined in the TFM for both the abdominal and inguinal sites. The data gathered at [redacted] meets the required log<sub>10</sub> reduction from baseline for the abdominal site, but not for the inguinal site. After discussion with the FDA during a teleconference dated October 25, 2002, the study was repeated at [redacted] in order to provide two independent studies that show reproducible results for the inguinal area.

All the parameters of the study performed at [redacted] are identical or nearly identical to those parameters followed at [redacted] with one exception. This exception is that the subjects are treated in the inguinal site only, based upon the Agency's comments dated March 5, 2003.

A baseline measurement of microbial count of >5.0 log<sub>10</sub> CFU/cm<sup>2</sup> is used to qualify the sites prior to the start of the study. Only qualified inguinal sites are used for the study.

The results meet the three-log reduction requirement and shows the results obtained at [redacted] are reproducible. The final report for this study is located in volume 14, appendix 8 of the NDA briefing package.

**Table 26. Results Summary.**

Anatomical site	Requirement per Tentative Final Monograph	Study location	Result at 10-minutes post-prep for test product	Result at 6 hours post-prep for test product
Abdomen	<ul style="list-style-type: none"> <li>&gt; 2.0 log<sub>10</sub> reduction from baseline within 10 minutes</li> <li>Log<sub>10</sub> does not exceed baseline at 6 hours</li> </ul>		> 2.0 log <sub>10</sub> reduction from baseline	Log <sub>10</sub> does not exceed baseline
			> 2.0 log <sub>10</sub> reduction from baseline	Log <sub>10</sub> does not exceed baseline
Inguinal	<ul style="list-style-type: none"> <li>&gt; 3.0 log<sub>10</sub> reduction from baseline within 10 minutes</li> <li>Log<sub>10</sub> does not exceed baseline at 6 hours</li> </ul>		> 3.0 log <sub>10</sub> reduction from baseline	Log <sub>10</sub> does not exceed baseline
			> 3.0 log <sub>10</sub> reduction from baseline	Log <sub>10</sub> does not exceed baseline

Studies at two independent laboratories demonstrate that Sage's [redacted] meet the requirements set in the TFM for preoperative skin preparations.

**Reviewer's comments:** The procedures for the application of the product are very similar for two of the contract laboratories, [redacted] but this procedure was omitted in the report from [redacted]

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The procedure for the application of the Sage product at [REDACTED] reads as follows (volume 12, appendix 6, p 4620):

**“Test Formulation—Antiseptic Cleanser Cloth chlorhexidine gluconate.**

1. Vigorously scrub skin using a back and forth motion for 3 minutes, turning the washcloth over halfway through application. If necessary, hold skin taut to ensure that the maximum amount of the washcloth contacts the area to be cleansed or prepped throughout application.
2. Use 1 washcloth per each area to be cleansed or prepped.
3. Allow to dry; rinsing is not required.”

The procedure for the application of the Sage product at [REDACTED] (volume 13, appendix 7, pp 5155-6) is similar but contains additional information. The additional information includes the following: the subject donned disposable undergarments, the technician donned sterile gloves, and for the reference product, Hibiclens, the reference product was applied twice and dried with a sterile towel.

The third contract laboratory, [REDACTED] references the procedure (volume 14, appendix 8, p 5928), but the page is blank. The procedure is found in volume 22, p 9997. The procedure is nearly identical to the [REDACTED] protocol with the following additions. The [REDACTED] protocol states that “the product will be allowed to air dry for one minute. Timing for each contact time will begin after drying is completed.”

A second area of concern is the failure of the positive control, Hibiclens, to meet the TFM requirements for a 2- $\log_{10}$  reduction after 10 minutes in the abdominal site, performed at [REDACTED]. As Hibiclens is often a positive control for most clinical simulations for patient preoperative preparation products, these data do not support the validity of the test product results.

*However, the most flagrant deficiency in the design of the clinical simulation trials is the lack of negative controls. Since the test product is a CHG solution applied with a washcloth, there is a device component to the product. Thus, there are two possible mechanisms for the removal of bacteria from the skin: the chemical action of the CHG and the physical action of the washcloth. Since it is known that the mechanical action of washing with soap and water removes microbes from the skin, the responsibility for the bacterial reductions of both the chemical and mechanical action must be determined separately. Therefore, two negative controls are necessary to determine the cause of the bacterial reductions on the skin. One negative control is the application of the test product without mechanical action of the applicator (washcloth). This control may be achieved by layering the test product on the skin. A second negative control is to omit the active ingredient from the applicator (washcloth) during the application of the test product. The washcloth here may only include vehicle. Without both negative controls it is impossible to determine the contribution of the chemical component or the device component of the product to the antimicrobial activity. Therefore, the Applicant has not demonstrated the efficacy of the product in the clinical simulations.*

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**Validation of the Neutralization Studies: Studies at \_\_\_\_\_**

A neutralization study was performed to assure the validity of the neutralizers used in the recovery medium. The neutralization follows the guidelines set forth in ASTM E 1054-02 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents". The test microorganism used for the neutralization study is *Staphylococcus epidermidis* (ATCC #12228).

The following tables provide summaries of the data generated during the study. Raw data and statistical analysis can be found in Volume 21, pp. 9739-9757 of the NDA submission.

Table 27 [p 8 of the NDA amendment 21-669 N000(B2)] shows the percent recovery for the test bacterium *Staphylococcus epidermidis* (ATCC #12228) used for the neutralization study. Table 28 [p 9 of the NDA amendment 21-669 N000(B2)] presents the mean log<sub>10</sub> values and differences from the numbers control.

**Table 27: % Recovery**

Article	Plating Time	Plate Counts						Ave. CFU/mL	% Recovery
Numbers Control 9.9 mL Saline	< 1 min.	33	37	30	32	31	35	33	100
	> 30 min.	26	32	28	22	29	26	27.17	82.33
Toxicity Control 9.9 mL Scrub Solution with Neutralizers	> 30 min.	27	28	24	25	26	34	27.33	82.82
	> 30 min.	36	31	39	31	23	40	33.33	101
1.0 mL Test Product 8.9 ml Scrub Solution with Neutralizers	< 1 min.	27	31	28	26	22	28	27	81.82
	> 30 min.	30	20	23	22	29	23	24.5	74.24
1.0 mL Control Product 8.9 ml Scrub Solution with Neutralizers	< 1 min.	26	21	26	25	28	21	24.5	74.24
	> 30 min.	25	21	22	25	20	23	22.67	68.7

$$\begin{aligned}
 < 1 \text{ Minute \% Recovery} &= \frac{\text{Count at 0 min.}}{\text{Numbers Control Count at 0 minutes}} \times 100 \\
 30 \text{ Minute \% Recovery} &= \frac{\text{Count at 30 min.}}{\text{Numbers Control Count at 30 minutes}} \times 100
 \end{aligned}$$

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**Table 28: Mean log<sub>10</sub> values and differences**

Test Procedure	Mean Log <sub>10</sub> Value	Difference from Numbers Control	Within 0.2 of Numbers Control*	P value
Numbers Control: <1 minute	1.52	NA	NA	NA
Numbers Control: >30 minutes	1.43	0.09	Yes	0.036
Product Control: Test product	0	1.52	No	ND
Product Control: Reference product	0	1.52	No	ND
Neutralizing Fluid Toxicity Control: >30 minutes	1.44	0.08	Yes	0.075
Diluting Fluid Toxicity Control: >30 minutes	1.52	0	Yes	0.747
Neutralizer Efficacy, Test product <1 minute	1.43	0.09	Yes	0.031
Neutralizer Efficacy, Test product <30 minutes	1.39	0.13	Yes	0.012
Neutralizer Efficacy, Reference product <1 minute	1.39	0.13	Yes	0.006
Neutralizer Efficacy, Reference product <30 minutes	1.35	0.17	Yes	0.003

\*If "Yes", then no difference from Numbers Control;  
If "No", then difference from Numbers Control.

The data are evaluated according to ASTM Standard E 1054-02 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents". The average number of challenge microorganisms are determined. The number of survivors are converted to log<sub>10</sub> values. A Student's t-test is performed to statistically compare the results of the test to the numbers control.

The results of the statistical analyses indicate:

- No significant difference is found between the mean log<sub>10</sub> values of the numbers control and the mean log<sub>10</sub> values for the neutralizer efficacy using the *test* product.
- No significant difference is found between the mean log<sub>10</sub> values of the numbers control and the mean log<sub>10</sub> values for the neutralizer efficacy using the *control* product.
- Since there is no recovery in the product controls, the results are considered statistically less than the numbers control.
- No significant difference is found between the recovery population of the toxicity control and the test organism population of the numbers control.

These results indicate that the neutralizer is effective and non-toxic.

#### **Validation of the Neutralization Studies: Studies at \_\_\_\_\_**

A neutralization study was performed to determine the effectiveness of the neutralizer system for inactivating the microbicidal properties of the antimicrobial agents used in the study and to ensure that no components of the neutralizing procedures and agents exert an inhibitory effect on microorganisms targeted for recovery. The neutralization is performed as defined in Appendix VII of the Final

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Report (Volume 20, pp 8712-8715 of the NDA submission). The test microorganism used for the neutralization study is *Staphylococcus aureus* (ATCC #27217).

The following table provides a summary of the data generated during the study. Table 29 [p 13 of the NDA amendment 21-669 N000(B2)] shows the percent recovery for the test bacterium) used for the neutralization study.

**Table 29: % Recovery**

Article	Plating Time	Plate Counts*						Ave. CFU/mL	% Recovery
Numbers Control 5.0 mL Saline	0 min.	33	33	38	38	38	55	$1.2 \times 10^2$	NA**
	30 min.	23	38	46	43	40	46	$1.2 \times 10^2$	NA
Toxicity Control 5.0 mL Scrub Solution with Neutralizers	0 min.	42	54	52	35	66	37	$1.4 \times 10^2$	117
	30 min.	50	53	63	48	64	37	$1.6 \times 10^2$	133
Toxicity Control 5.0 mL Scrub Solution with Neutralizers	0 min.	41	32	41	89	56	35	$1.5 \times 10^2$	125
	30 min.	38	31	53	22	56	40	$1.2 \times 10^2$	100
Test Product 5.0 mL Scrub Solution with Neutralizers	0 min.	39	41	46	39	56	41	$1.3 \times 10^2$	108
	30 min.	39	59	43	54	47	50	$1.5 \times 10^2$	125
Test Product 5.0 mL Scrub Solution with Neutralizers into 4.5 mL Dilution Fluid with Neutralizers	0 min.	46	47	45	39	60	50	$1.4 \times 10^2$	117
	30 min.	39	28	61	52	54	38	$1.4 \times 10^2$	117
Control Product 5.0 mL Scrub Solution with Neutralizers	0 min.	41	38	43	36	40	61	$1.3 \times 10^2$	108
	30 min.	36	39	40	46	33	32	$1.1 \times 10^2$	92
Control Product 5.0 mL Scrub Solution with Neutralizers into 4.5 mL Dilution Fluid with Neutralizers	0 min.	31	30	48	40	36	52	$1.2 \times 10^2$	100
	30 min.	56	51	35	45	45	29	$1.3 \times 10^2$	108

\*CFU/ml is the sum of 1.0 mL spread across three plates in duplicate

\*\*NA = Not Applicable

$$0 \text{ Minute \% Recovery} = \frac{\text{Average Count at 0 minutes}}{\text{Average Numbers Control Count at 0 minutes}} \times 100$$

$$30 \text{ Minute \% Recovery} = \frac{\text{Average Count at 30 minutes}}{\text{Average Numbers Control Count at 30 minutes}} \times 100$$

The data are evaluated by determining the average number of microorganisms for each test. The percent recovery is calculated to compare the results of the test product to the numbers control. The neutralizer system is considered effective if recovery is  $\geq 50\%$  of the corresponding numbers control. In this study, the neutralizer effectively neutralizes the antimicrobials and is non-toxic.

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**Validation of the Neutralization Studies: Studies at \_\_\_\_\_** A neutralization study was performed to assure the validity of the neutralizer(s) used in the recovery medium. The neutralization followed the guidelines set forth in ASTM E 1054-02 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents". The test microorganism used for the neutralization study is *Staphylococcus epidermidis* (ATCC #12228).

The following tables provide summaries of the data generated during the study. Raw data and statistical analysis can be found in Volume 19, pp. 8369-8373 of the NDA submission. Table 30 [pp 17-18 of the NDA amendment 21-669 N000(B2)] shows the percent recovery for the test bacterium used for the neutralization study. Table 31 [p 19 of the NDA amendment 21-669 N000(B2)] shows the average log<sub>10</sub> values and differences from the numbers control.

**Table 30: % Recovery**

Article	Plating Time	Plate Counts						Ave. CFU/mL*	% Recovery
Numbers Control	< 1 min.	72	69	63	60	57	65	64.3	NA
	30 min.	67	68	62	59	54	59	61.5	NA
Test Article Control	< 1 min.	0	0	0	0	0	0	0	NA
	30 min.	0	0	0	0	0	0	0	NA
Toxicity Control 12 mL Scrub Solution with Neutralizers (Tube#1)-TSA	< 1 min.	59	63	57	56	72	61	61.3	95.3
	30 min.	47	56	51	53	49	53	51.7	80.4
Toxicity Control 12 mL Scrub Solution with Neutralizers (Tube#1)-TSA+	< 1 min.	61	63	59	56	60	57	59.5	92.5
	30 min.	56	55	51	50	42	44	49.7	77.3
Toxicity Control 45 mL Dilution Fluid with Neutralizers (Tube#2)-TSA	< 1 min.	30	12	19	22	17	21	60.9	94.7
		17	15	26	21	19	20		
		27	19	23	17	15	26		
	30 min.	18	14	16	9	17	28	47.5	73.9
		12	10	19	17	18	11		
		9	12	27	23	19	6		
Toxicity Control 45 mL Dilution Fluid with Neutralizers (Tube#2)-TSA+	< 1 min.	28	17	10	12	16	31	55.7	86.6
		21	13	13	17	16	22		
		24	14	20	11	20	23		
	30 min.	25	12	16	13	17	15	45.5	70.8
		20	16	12	14	15	17		
		10	17	14	9	11	20		

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**Table 30: % Recovery (continued)**

Article	Plating Time	Plate Counts						Ave. CFU/mL*	% Recovery
Test Product 12 mL Scrub Solution with Neutralizers	< 1 min.	39	42	37	44	42	41	40.8	63.5
	30 min.	36	32	41	33	39	37	36.3	56.5
Test Product 0.5 mL Scrub Solution into 45 mL Dilution Fluid with Neutralizers	< 1 min.	10	15	17	11	13	19	41.2	64.1
		9	12	15	17	16	12		
		16	10	14	14	12	15		
	30 min.	9	17	13	15	9	12	39.2	61
		8	12	19	13	12	15		
		20	11	10	17	16	7		
Control Product 12 mL Scrub Solution with Neutralizers	< 1 min.	71	57	62	51	67	47	59.2	92.1
	30 min.	69	52	55	54	51	49	55	85.5
Control Product 0.5 mL Scrub Solution into 45 mL Dilution Fluid with Neutralizers	< 1 min.	30	19	11	27	20	21	59.7	92.9
		7	22	26	23	25	11		
		18	19	22	16	24	17		
	30 min.	12	24	28	23	15	16	53.2	82.7
		21	17	18	20	12	10		
		15	16	23	12	19	18		

\*The average cfu/ml for the scrub solution + dilution fluid is reported in CFU/10mL. To calculate the % recovery, the number was converted to CFU/mL by dividing by 10, then multiplied by the dilution factor (1:10).

$$\begin{aligned}
 <1 \text{ Minute \% Recovery} &= \frac{\text{Average Count at } <1 \text{ minute}}{\text{Average Numbers Control Count at } <1 \text{ minute}} \times 100 \\
 30 \text{ Minute \% Recovery} &= \frac{\text{Average Count at } 30 \text{ minutes}}{\text{Average Numbers Control Count at } <1 \text{ minute}} \times 100
 \end{aligned}$$

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**Table 31: Mean log<sub>10</sub> values and differences**

Test Procedure	Time	Average Log <sub>10</sub> Value	Difference from Numbers Control	Within 0.2 of Numbers Control*
Numbers Control:	<1 min.	1.81	NA	NA
	>30 min.	1.79	0.02	Yes
Product Control: Test product	<1 min.	0	1.81	No
	>30 min.	0	1.81	No
Neutralizing Fluid Toxicity Control - TSA	<1 min.	1.79	0.02	Yes
	>30 min.	1.71	0.1	Yes
Neutralizing Fluid Toxicity Control - TSA+	<1 min.	1.77	0.04	Yes
	>30 min.	1.69	0.12	Yes
Diluting Fluid Toxicity Control - TSA	<1 min.	1.78	0.03	Yes
	>30 min.	1.68	0.13	Yes
Diluting Fluid Toxicity Control - TSA+	<1 min.	1.75	0.06	Yes
	>30 min.	1.66	0.15	Yes
Neutralizer Efficacy, Test product – Scrub Solution	<1 min.	1.61	0.2	Yes
	>30 min.	1.56	0.25	No
Neutralizer Efficacy, Test product – Scrub Solution + Dilution Fluid	<1 min.	1.61	0.2	Yes
	>30 min.	1.59	0.22	No
Neutralizer Efficacy, Reference product - Scrub Solution	<1 min.	1.77	0.04	Yes
	>30 min.	1.74	0.07	Yes
Neutralizer Efficacy, Reference product - Scrub Solution + Dilution Fluid	<1 min.	1.78	0.03	Yes
	>30 min.	1.73	0.08	Yes

\*If "Yes", then no difference from Numbers Control; If "No", then difference from Numbers Control.

The data are evaluated according to ASTM Standard E 1054-02 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents". The average number of challenge microorganisms is determined. The number of survivors is converted to log<sub>10</sub> values. A Student's t-test is performed to statistically compare the results of the test to the numbers control.

The results of the statistical analyses indicate:

- No significant differences are found between the mean log<sub>10</sub> values of the numbers control and the mean log<sub>10</sub> values for the neutralizer efficacy using the test product.
- No significant differences are found between the mean log<sub>10</sub> values of the numbers control and the mean log<sub>10</sub> values for the neutralizer efficacy using the control product.
- Since there is no recovery in the product controls, the results are considered statistically less than the numbers control.
- No significant differences are found between the recovery population of the toxicity control and the test organism population of the numbers control.

These results indicate the neutralizer is effective and non-toxic.

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**Reviewer's comments:** Neutralization validation assays for the clinical simulation studies were performed by all three contract laboratories: [REDACTED] Neutralization validation assays performed by [REDACTED] utilized *Staphylococcus aureus* (ATCC# 27217) as the test organism while neutralization validation assays performed by [REDACTED] utilized *Staphylococcus epidermidis* (ATCC# 12228). Neutralization validation assays performed by all three laboratories demonstrated that the neutralizer was efficacious and non-toxic. However, to be consistent and reduce the potential for variability, the test organisms should be the same for the neutralization validation assays for all three laboratories.

Although two of the efficacy controls for the test products are statistically different from the numbers control i.e. greater than 0.2 log difference, the recoveries were more than 50% of the numbers control. Thus, the neutralizer is considered effective.

#### **Conclusions/Recommendations:**

This NDA submission is for a product containing a 2% CHG solution impregnated into a non-woven polyester cloth intended for use as a patient preoperative skin preparation. The Applicant has demonstrated the preclinical (*in vitro*) efficacy of the product; **however, there is no clear evidence that the chemical properties of the CHG component of the product are responsible for efficacy *in vivo*.** What follows are comments and deficiencies noted by this Reviewer.

9. In general, the application is poorly organized and presented. In some cases, data is not easily available and required time-consuming searches. In some cases, data is presented in an incorrect format. For example, data from the time-kill studies required conversion from percent reduction to log<sub>10</sub> reduction.
10. Clearly, the product demonstrates efficacy against a broad spectrum of microorganisms *in vitro*. MICs were obtained from 25 fresh clinical isolates and 25 laboratory strains of the organisms listed in the TFM. In some cases, less than 25 laboratory strains or less than 25 clinical strains were tested; however, 50 total isolates were tested and thus the low number of clinical isolates or laboratory isolates is allowed.
11. The product exhibits rapid bactericidal action as demonstrated by time-kill kinetics. However, some organisms show a more rapid reduction in microbial numbers. All Gram-negative organisms exhibit at least a 5-log reduction within 15 sec. However, some organisms, particularly Gram-positive and yeast are slower to exhibit a 3-log reduction. Thus, this Reviewer is concerned about the fast-acting claims and efficacy of the product since these three organisms, *E. faecalis*, *E. faecium*, and *S. aureus*, are often indicated in post surgical infections.
12. A comparison of the MIC results with the time-kill kinetics results should demonstrate that the product kills organisms at higher dilutions, and therefore more sensitive to CHG, *more* rapidly. Conversely, one should observe that the product kills organisms with lower dilutions and therefore more resistant to CHG, *less* rapidly. Organisms killed at high dilutions but slower time-kill kinetics include:

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*Enterococcus faecalis*, *Enterococcus faecium*, and *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*. These observations suggest that neither spectrum of activity data nor time-kill kinetic data be used separately but in conjunction with one another in the determination of the efficacy of active ingredients and product performance.

13. From the neutralization validation studies for the time-kill kinetics, this Reviewer concludes that the neutralizer is both effective and not toxic. Although there are statistical differences between the numbers controls versus the toxicity and efficacy controls, the toxicity and efficacy controls are *higher*, not lower than the numbers controls. In addition, the toxicity and efficacy controls demonstrate greater than 50% recovery compared to the numbers control.

14. The Applicant presents MIC data for a number of antibiotic resistant organisms. Most but not all of these organisms have high dilution values suggesting sensitivity to CHG. The Applicant also presents time-kill kinetic data for several antibiotic resistant organisms, specifically, [REDACTED]

[REDACTED] The time-kill kinetics for these organisms show slow killing indicated by less than 3-log reductions at 15 sec. However, from the data given, it is impossible to determine if the MIC values and the time-kill kinetics data are for the same organisms. Therefore, a correlation between MIC dilution values and the time-kill kinetics data are not possible.

[REDACTED]

15. Since several antibiotic resistant organisms show lower MIC dilution values but slower time-kill kinetics for CHG, it would be prudent to be aware of any future changes in antibiotic resistance or CHG resistance patterns.

16. A number of procedural inconsistencies for the application of the product during the clinical simulations are noted and require mention. These inconsistencies include variation in cup scrub diameter, neutralization composition, processing of bacterial samples, and variation in formulas for the determination of CFU/cm<sup>2</sup>.

d. First, the Applicant notes that the internal diameters of the scrubbing cups used by the different contract laboratories i.e. [REDACTED]

[REDACTED], for the sampling are different; the Applicant states that this variation in size does not affect the microbial count data obtained. However, the Applicant does not reference or supply data to support this statement.

e. It is unclear if the neutralizers used by all the contract laboratories are identical in composition or concentration. Both [REDACTED] state the neutralizer consists of 1% Polysorbate 80 and 0.3% lecithin; the composition and concentration of the neutralizer used by [REDACTED] could not be located. While these are appropriate neutralizers for use against chlorhexidine according to the ASTM document E 1054-02 "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents", consistency in the protocol is required to eliminate variability.

f. Finally, the Applicant states that after incubation of the media, the colonies are counted. Due to the variation in the cup scrub diameter, different formulas are

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- used by [REDACTED] to determine the CFU/cm<sup>2</sup> of skin. It is unclear as to which formula is used by [REDACTED]
14. The preoperative preparation application procedures are very similar for the three contract laboratories, but some differences in the protocol are present. The significance of these differences is not immediately apparent.
  15. 10.A second area of concern with the clinical simulations is the failure of the positive control, Hibiclens, to meet the TFM requirements. Hibiclens failed to meet the 2- $\log_{10}$  reduction after 10 minutes in the abdominal site, performed at [REDACTED] and the 3- $\log_{10}$  reduction in the inguinal site at both [REDACTED]. As Hibiclens is often a positive control for most clinical simulations for patient preoperative preparation products, these data may be reason for concern regarding the validity of the positive control.
  16. *However, the most flagrant deficiency in the design of the clinical simulation trials is the lack of negative controls.* Since the test product is a 2% CHG solution applied with a washcloth, there is a device component to the product. Thus, there are two possible mechanisms for the removal of bacteria from the skin: the chemical action of the CHG and the physical action of the washcloth. Since the mechanical action of washing with soap and water removes microbes from the skin, the responsibility for the bacterial reductions of both the chemical and mechanical action must be determined separately. Therefore, two negative controls are necessary to determine the cause of the bacterial reductions on the skin. One negative control is the application of the test product without mechanical action of the washcloth. This control may be achieved by layering the test product on the skin. A second negative control is to omit the active ingredient from the washcloth during the application. The washcloth here may only include vehicle. Without both negative controls, it is impossible to determine the contribution of the chemical component or the device component of the product. ***Therefore, the Applicant has not demonstrated the efficacy of the product in the clinical simulations.***
  17. Neutralization validation assays for the clinical simulations performed by all three laboratories demonstrate the neutralizer is effective and non-toxic. However, to be consistent and reduce the potential for variability, the test organisms should be the same for the neutralization validation assays for all three laboratories.
  18. **A package insert was not submitted by the Applicant.**

The Applicant presents data that demonstrate *in vitro* efficacy of the product by determining the antimicrobial spectrum of activity of the active ingredient, CHG. However, these MIC studies do have limitations since the concentration and duration of exposure are fixed and may not mimic actual preoperative conditions. This limitation is important because antimicrobial activity for antiseptics is time and concentration dependent for antiseptics. Therefore, time-kill kinetics and clinical simulations are necessary to determine the antimicrobial activity of the antiseptic in actual use conditions.

The purpose of the time-kill kinetic studies is to attempt to establish a relationship between the *in vitro* kill rates caused by the product and the *in vivo* kill rates during clinical simulation studies. These studies measure bacterial  $\log_{10}$  reductions at reference

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time points. The TFM requires a 1:10 dilution of the product to perform the studies in order to simulate the preoperative situation in which the product may become diluted by blood and other body fluids during surgery. The product was not diluted since the product is a leave-on product, that is, it is not intended to be used with water.

The time-kill kinetics studies indicate the product is fast-acting against most organisms tested. As an arbitrary time point, a 3-log reduction at 15 sec. is considered the criterion for a designation of fast-acting. Most organisms meet this criterion with the exception of: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus faecalis* and *Enterococcus faecium*. In addition, the following antibiotic resistant organisms failed the 3-log reduction including: \_\_\_\_\_

\_\_\_\_\_. In a comparison between time-kill kinetics and MICs, all of these organisms demonstrate slow time-kill kinetics but high MICs. The apparent disconnect between the MICs and time-kill kinetics suggest that while the product may be effective against these organisms *in vitro*, the microbicidal action is slow and thus may not be as effective *in vivo* in a surgical environment for which the product is intended. These observations are additionally disconcerting since many of these organisms are responsible for most infections seen post-surgery.

Judgement of the efficacy of a patient preoperative preparation is based upon *in vitro* evidence that includes MIC studies and time-kill kinetics, and *in vivo* evidence that includes clinical simulations. While the spectrum of activity evidence is strong, the time-kill kinetics evidence wavers. But most notably, the *in vivo* clinical simulation evidence is weak.

It is important to recognize that the *in vivo* evidence relies upon clinical simulations in which healthy volunteers act as surrogates for surgical personnel. Thus, it is imperative that the evidence from the clinical simulations is strong in order to establish the connection between the *in vitro* evidence and the *in vivo* evidence.

The *in vivo* evidence from the clinical simulations is weak. There is no clear evidence that the efficacy of the product is due to the chemical properties of CHG and not to the mechanical application of the product. The Applicant's product is a mechanical device impregnated with a chemical that is purported to be antimicrobial. Proper controls were incorporated with the MIC data; the MIC data was derived for not just the bulk drug product, but also a negative control (vehicle) as well as a positive control (active ingredient). No such negative control was utilized in the clinical simulations, thus, it is not possible to determine whether the *chemical* action of the CHG contained in the product or the *mechanical* action of the cloth on the treated skin was responsible for the log<sub>10</sub> reductions.

**This Reviewer recommends that this application not be approved** until the clinical simulations are completed with the required negative controls to the satisfaction of the Agency.

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2 Page(s) Withheld

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           § 552(b)(5) Deliberative Process

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Peter Coderre, Ph.D.

Microbiology Reviewer

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