

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
21-074

MICROBIOLOGY REVIEW

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

IND/NDA # 21-074 **Date Completed:** May 30, 2000
(New Correspondence)

Applicant (NDA): 3M Health Care
3M Center, Building 275-3E-08
St. Paul, MN 55122-1000

Chem/Ther. Type: Topical Antimicrobial

Submission Reviewed: New Drug Application

Providing for: Use as a surgical hand scrub
Use as a Healthcare Personnel hand wash

Product Name(s):
Proprietary: Currently known as Avagard –CHG Hand Prep (HPD-5a)

Non-proprietary/USAN: Chlorhexidine gluconate

Compendia: Chlorhexidine gluconate and ethanol

Code name/number: HPD-5a

Chemical name: 1,1'-Hexamethylenebis[5-(*p*-chlorophenyl) biguanide] di-D-gluconate

Structural formula: See USAN Dictionary

Molecular formula: C₂₂H₃₀Cl₂N₁₀2C₆H₁₂O₇

Dosage form(s): Solution

Route(s) of administration: Topical

Pharmacological Category: Antibacterial, antiseptic

Dispensed: Rx X OTC

Initial Submission Dates

Received by CDER: June 25, 1999
Received by Reviewer: June 30, 1999
Review Completed:

Supplements/Amendments:

Received by CDER: September 3, 1999
Received by Reviewer: September 5, 1999
Review Completed:

Related Documents: [REDACTED]

Remarks:

The subject of this NDA is HPD-5a-CHG Hand Prep, which contains 1-% chlorhexidine gluconate and 61-% ethyl alcohol. The scientific rationale for the development of a combination products such as this is two fold. First, the alcohol is a fast acting, wide spectrum antimicrobial, and provides the product with rapid antimicrobial effect as it evaporates from the hands. Second, the chlorhexidine gluconate, a wide spectrum antimicrobial, functions to augment suppression of the resident flora and is also thought to function as a protective barrier against the transient flora that may be acquired during health care activities. Since this product contains two active ingredients, it must meet the drug combination policy. This policy requires that the contribution of each active ingredient be demonstrated in adequate and well-controlled clinical studies. Thus, this NDA will be evaluated with this regulatory perspective in mind.

Also, based on an evaluation of the proposed product label provided in Volume 1.2 of 50, Section 2-Labeling, pages 2-1 to 1-10, the applicant seeks approval for use as a healthcare personnel handwash, surgical hand scrub [REDACTED] Unlike traditional products of this type, which require use with water, HPD-5a is designed as a "leave on" product. That is, it is painted or rubbed on and allowed to air dry.

The requirements for establishment of microbiological and clinical efficacy for products this type have been published in a Federal Register (FR) Notice.¹ This notice requires that products intended for such use be evaluated by assessing the *in vitro* spectrum of activity of the product, the rate of kill by time-kill kinetic studies, and by performance of two adequate and well-controlled clinical simulation trials for each indication proposed in the product label. The FR Notice also characterizes alcohol as an active ingredient that is safe and effective for the indications of [REDACTED] health care personnel hand was, and as a surgical hand scrub when formulated to contain 60% to 95 % alcohol. This will be taken into consideration during the evaluation of the product.

Conclusions/Recommendations:

Key words: chlorhexidine gluconate, topical antiseptic, topical antimicrobial, surgical hand scrub, health care personnel hand wash, Federal Register Notice.

Summary:

1. The NDA review by the Microbiology Reviewing Officer revealed major problems with the *in vitro* susceptibility studies. The problems included not following the standardized procedures described in the FR Notice published by the FDA and the lack of rigor in the analysis of the data. Due to the modifications of the methods and lack of appropriate analysis of the *in vitro* data, this information can not be used to support the approval of the NDA.

¹ Topical Antimicrobial Drug Products for Over-the-Counter Human Use, Tentative Final Monograph for Healthcare Antiseptic Drug Products. (1994) Volume 59, No. 116, pages 31402-31451

2. In addition, the neutralization validation systems used for the time-kill kinetic studies require clarification. Of particular concern is the time that the active ingredient was in contact with the neutralizer before the marker organism was introduced. This step must mimic as closely as possible what happens during the time-kill kinetic study.
3. The neutralization validation studies submitted for the surgical hand rub indication are not satisfactory because there appear to be errors in the technical conduct of the protocol and the sample used to validate the study does not represent the worst case scenario expected in the clinical simulation studies. It appears, based on the protocol provided that the product was only used once and that sample used to validate the study



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Microbiological Review

Introduction

Hand washing is considered one of the most important and effective procedures performed by healthcare professionals because it helps reduce the incidence of nosocomial infections. Hand washing is considered an important intervention strategy that incorporates both physical/mechanical action and presumed chemical barrier strategies to remove transient and resident microorganisms, thus prevent the transmission of nosocomial pathogens. The Food and Drug Administration recognizes this important strategy and supports the evaluation of products intended for such uses. In this regard, the agency published a FR Notice¹, which describes two indications that support the use of these chemical/physical intervention strategies when used as surgical hand scrubs, or health care personnel hand wash products. A [redacted] indication provides for the labeling of a product as a [redacted]

[redacted] All [redacted] possible indications are sought for the HPD-5a-CHG Hand Prep product.

The characteristics desired and of interest for products of this type are exemplified by the products intended use but in general we ask that they be wide spectrum, fast acting, and persistent antimicrobials that are safe and effective for the intended uses as described within the notice.

Preclinical Studies

The hand washing [redacted] studies proposed in the FR Notice¹ have limitations in that they only allow assessment of product efficacy against the resident flora of test panelists or measurement of efficacy versus a surrogate marker. In reality, these test panelists are surrogates for healthcare personnel. As such, they may not carry, transiently, nor are the panelists exposed to the same pathogens encounter by healthcare professionals during daily activities. Since the clinical simulation tests have this inherent limitation, the agency must gather information on product efficacy from *in vitro* studies. Thus, the FR Notice¹ requires that the *in vitro* spectrum of activity and time-kill kinetic studies also be performed to gather additional information on product efficacy. The purpose of these preclinical studies is to demonstrate that products have a satisfactory spectrum of activity against pathogens that are likely to be encountered in these setting. The desired method for this assessment are the *in vitro* spectrum of activity and time-kill kinetic studies, which are performed with organisms that represent nosocomial pathogens¹. The requirements for clinical simulation studies and *in vitro* studies could be reduced if clinical studies were performed in settings, such as hospitals, where the intended use of the product is germane.

In vitro Spectrum of activity

The FR Notice requires that the *in vitro* spectrum of activity be assessed using standardized minimal inhibitory concentration (MIC) methods² against a selected panel of bacteria that are described within that notice. The requirement states that 50 strains of each species must be tested. Twenty-five of the strains must be fresh clinical isolates and the remaining 25 can be stocks strains obtained from the American Type Culture Collection (ATCC). The *in vitro* spectrum of each battery of 50 strains for each listed species must be evaluated against the product, the product vehicle to assess the contribution of the vehicle to the spectrum of activity, and to the active ingredient alone. For this review, the active ingredient is chlorhexidine gluconate. The vehicle and the active ingredient results are also compared to the product formulation results to determine whether the vehicle has an effect on the intrinsic activity of the active ingredient or whether it augments it.

² Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically-Fourth Edition, Approved Standard. NCCLS Document M7-A4. NCCLS, 940 West Valley Road, Suite 1400 Wayne, Pennsylvania.

Some applicants have complained that the *in vitro* spectrum of activity requirement is excessive. In order to address this complaint and not compromise the scientific information required, I have agreed to let applicants test only 10 strains for the active ingredient (CHG) and the vehicle. The active ingredient is represented by Hibiclens. However, the 10 strains tested must be selected from the 50 strains tested with the finished product, which remains the standard requirement as described in the FR Notice. The ten strains must include 5 of the 25 ATCC strains tested versus the test product and 5 of the 25 fresh clinical isolates for a total number of 10.

3M Health Care provided a summary of the *in vitro* spectrum of activity (study LIMS 7801) in volume 1.19 (Section 7.5.1), and the actual study protocol, results and conclusions in volumes 1.20 -1.24.

The *in vitro* spectrum of activity studies were evaluated to determine whether the methodology recommended in the FR Notice, the National Committee for Clinical Laboratory Standards method, was used. The testing laboratory conveys that the study was performed with the procedure described in the Federal Register, June 17, 1994, which is the National Committee for Clinical Laboratory Standards² for broth microdilution testing (Volume 3.0, section 3.1, page 3-3). [REDACTED] performed the MIC studies.

MIC testing was performed using the Mueller-Hinton broth microdilution procedure. The NCCLS method suggests the addition of additives to the Mueller-Hinton broth depending on the organisms being tested. Therefore, the media was evaluated to assure that the appropriate Mueller-Hinton media was used. For *Enterobacteriaceae*, *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Staphylococcus* spp., and *Enterococcus* spp., the requirement is cation-adjusted Mueller-Hinton broth. Inoculum should be prepared in water or saline to achieve a final concentration of 5×10^5 CFU/well and trays incubated at 35°C in ambient air for 18-20 hours.

Evaluation of the summary of the protocol suggests that the Mueller-Hinton broth was not adjusted with cations, the inoculum was prepared with [REDACTED] buffered water, and diluted to a turbidity of 0.5% [REDACTED] standard. This approximation should yield a cell density of 1×10^5 CFU/mL in each well. Incubation temperature and duration of incubation were $35^\circ\text{C} \pm 2^\circ\text{C}$ for duration's that ranged from 16.0 to 24.0 hours. The 96-well plates were read with a spectrophotometer.

Reviewer's comments: Regulatory agencies require the use of standardized methods to assure a level playing field in terms of the requirements that pharmaceutical companies are required to meet. In addition, standardization of methods is a scientifically valid approach used by regulatory agencies to assure comparability of product performance and performance of test methods in the hands of different investigators.

In summary, the *in vitro* susceptibility study provided by the applicant for *Enterobacteriaceae*, *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Staphylococcus* spp., and *Enterococcus* spp., were not performed as directed in the FR Notice and the NCCLS reference in that cation-adjusted Mueller-Hinton broth was not used in the study. Mueller-Hinton broth was used but it was not supplemented with cations as required. Further, inocula were not prepared in water or saline as required, and the incubation temperatures were inconsistently applied and ranged outside of recommended time duration for incubation. The effect of these modifications on the outcome of susceptibility testing is unclear.

Evaluation of the methods used for the fastidious microorganisms was also performed. The requirement for susceptibility testing of *Haemophilus influenzae* is the use of Haemophilus Test Medium (HTM) and for *Streptococcus pneumoniae* and *Streptococcus* spp., the use of cation-adjusted Mueller-Hinton broth with 2-5% lysed horse blood is recommended. Review of the inoculum used and incubation temperatures was also performed.

According to the information provided (volume 1.20, section 7.14.1.5), *Haemophilus influenzae* was tested in Mueller-Hinton broth with horse lysed blood or in [REDACTED] Broth with lysed blood (horse?). This is

not consistent with the media recommendations of the NCCLS for standardized susceptibility testing of this organism which requires the use of Haemophilus Test Medium (HTM). Inoculum preparation requires that the organism be grown on a chocolate agar plate, and a suspension made in Mueller-Hinton or 0.9% saline and adjusted to the turbidity of a 0.5% [REDACTED] standard. The cell density should be $1-4 \times 10^8$ CFU/mL, which is used to produce a final inoculum of 5×10^5 CFU/well. For this study, the inoculum was prepared in [REDACTED] buffered water to obtain a cell density of 6×10^5 to 4×10^6 CFU/mL. The cell density is somewhat lighter than recommended by the NCCLS and is not prepared as suggested. Incubation should be performed at 35°C at ambient air for 20-24 hours. In this study, incubation was at $35^\circ\text{C} \pm 2^\circ\text{C}$ and $5 \pm 1\%$ CO₂ for 19-71 hours. The disparate incubation temperatures are also not acceptable.

A similar type of review and analysis was performed for Streptococcus spp. including *S. pneumoniae* (Volume 1.23, Section 7.14.1.20). The susceptibility testing requirement described in the FR Notice for *S. pneumoniae* is the use of cation-adjusted Mueller-Hinton broth containing lysed horse blood (2-5%) and trays are to be incubated at 35°C in ambient air for 20-24 hours. The inoculum is prepared in M-H broth or 0.9% saline using colonies taken directly from an overnight (18-20-hour) sheep blood agar culture.

The study (vol. 1.23, section 7.14.1.20) was performed with Mueller-Hinton broth with no lysed horse blood. The inoculum was prepared from a blood agar plate and colonies scrapped and diluted in [REDACTED] buffered water, and adjusted to a turbidity equivalent to the 0.5% [REDACTED] standard. The plates were incubated at $35^\circ\text{C} \pm 2^\circ\text{C}$ and $5 \pm 1\%$ CO₂ for 17-48 hours. The disparate incubation temperatures are also not acceptable.

Reviewer's comments: Susceptibility testing for the fastidious microorganisms was not performed according to established standardized methods. The summary provided by the applicant states that *Haemophilus influenzae* was tested in Mueller-Hinton broth or [REDACTED] Broth with lysed horse blood. As stated previously, Haemophilus Test Medium is the correct broth. In addition, the inoculum was not prepared according to NCCLS recommendations and the incubation temperature and duration of incubation was not appropriate. Thus, the *in vitro* spectrum data for *H. influenzae* can not be used to support efficacy of this organism against this product. Similar deviations from established protocols were noted for *Streptococcus pneumoniae*. The effect of these modifications on the outcome of susceptibility testing is unclear and the data will not be accepted.

Although the applicant did not perform the susceptibility studies as required, the data may still provide some insight into the spectrum of HPD-5a by relating the results of the test product to the control product, Hibiclens. The products tested are HPD-5a (1% CHG and 61% ethyl alcohol, lot # Jan98 004), HPD-5a vehicle formula HPD-5b [REDACTED] and Hibiclens (4% CHG, lot # 4882-Y). The battery of isolates used for the MIC evaluation included 21 bacterial species (10 gram-positive, 9 gram-negative and two yeast species). The number tested is approximately 100 (50 clinical isolates and 50 ATCC/lab isolates) for HPD-5a (HPD-5a) and approximately 10 for the vehicle and Hibiclens as agreed to and previously discussed. The results are presented in Table 1 for all isolates.

The evaluation of the data performed by the applicant is at best minimal. Basically, the MIC values were provided as raw data and the data compiled as a range of MICs by pathogen tested. The data was presented in tabular format. The discussion provided in the summary focused only on the comparison of ranges observed between the two products.

Reviewer's comments: The analysis performed by the applicant on this data was exclusively a comparison of ranges obtained with the test and control product. This analysis is minimal at best and is not sufficient rigorous to draw conclusions that the test and control products produce analogous results. The following types of data presentation would be substantially more informative and it is highly recommended that they be provided for strains that have been tested using acceptable standardized methodology.

1. The applicant should perform frequency distributions of the data and present them as histograms to allow comparison of results between test and control product. A

discussion of the results should be provided for each organism and a summary of this data as a whole.

2. A comparison should be performed between the histograms of laboratory and clinical isolates to determine where similar patterns are noted. Although the sample size is small, the discussion should describe population distributions between the laboratory and clinical isolates.
3. A discussion should be provided on the susceptibility profile of antibiotic susceptible and resistant laboratory and clinical isolates. Does the fact that the organism has a resistance phenotype to an antibiotic increase the likelihood that the CHG MIC will be elevated relative to antibiotic susceptible population?

Since the data is presented as MIC ranges, very basic types of comparisons can be performed with this data. For example, a comparison of laboratory and clinical isolate MIC ranges for HPD-5a suggest that the MIC ranges are within one tube dilution of each other, which is within the error of the assay. The data in presented in Table 1 and does suggest that the highest MIC values occur with the clinical isolates such as *P. mirabilis*, *S. aureus*, *S. pyogenes*, *E. faecalis*, *E. faecium*, and *S. pneumoniae* and histograms would help to clarify this MIC distribution. The isolates with the highest MIC's should be evaluated to determine if they are resistant to any particular antibiotic(s). It would also be interesting to determine whether the antibiotic resistant strains had higher CHG MICs than the antibiotic susceptible strains. As expected, a similar pattern is seen with Hibiclens but the number of isolates tested is generally less than 8. The exception is *S. aureus* (including MRSA) which included at least 22 strains. An attempt was made to evaluate these strains to determine whether the resistant stains had the higher CHG MIC values but an analysis could not be performed because the MRSA strains could not be segregated from the existing data set. Susceptibility testing was also performed with the HPD-5a vehicle and as expected, no inhibitory effect was seen with the concentrations tested, which were 0.25 to 256 µg/mL.

Table 1. Minimum inhibitory concentration (µg/mL) ranges of laboratory and clinical isolates for HPD-5a and Hibiclens (Study LIMS 7801)

Microorganism	MIC range for laboratory isolates		MIC range for clinical isolates	
	HPD-5a	Hibiclens	HPD-5a	Hibiclens
<i>Acinetobacter sp.</i>				
<i>B. fragilis</i>				
<i>H. influenzae</i>				
<i>Enterobacter sp.</i>				
<i>E. coli</i>				
<i>Klebsiella sp.</i>				
<i>P. aeruginosa</i>				
<i>P. mirabilis</i>				
<i>S. marcescens</i>				
<i>S. aureus</i> (including MRSA)				
<i>S. epidermidis</i> (including MRSE)				
<i>S. hominis</i>				
<i>S. haemolyticus</i>				
<i>S. saprophyticus</i>				
<i>M. luteus</i>				
<i>S. pyogenes</i>				
<i>E. faecalis</i> (including MDR)				
<i>E. faecium</i> (including VRE)				
<i>S. pneumoniae</i>				
<i>Candida sp.</i>				
<i>C. albicans</i>				

also performed, at the request of the FDA, quality control (QC) testing to assess the reproducibility of the method in the hands of these investigators. The lab used *S. aureus* (ATCC 29213)

and *E. coli* (ATCC 25922) for the evaluation and a target of 1-4 and 2-8 µg/mL with HDP5a and Hibiclens. It is stated that QC testing was performed for the 77 days that susceptibility testing was conducted and outliers occurred only during 5 of the 77 days because lysed blood was used as a component of the media. Data generated during this interval was not included in the analysis. Unfortunately, the investigator apparently did not understand that an antibiotic should have been used to conduct QC testing. However, since the QC ranges used by the investigator are no greater than three tube dilutions, the data suggests that the method is reproducibility in the hands of the investigator.

Global summary of the *in vitro* spectrum studies

The *in vitro* spectrum of activity studies are important because they help characterize the “microbiological potential” of the product in a manner that is not feasible through clinical simulation studies. The *in vitro* spectrums of activity studies are also more important for products that are formulated for use with water because of the dilution factors associated with such use.

When evaluating HPD-5a, the MIC study measures the effective concentration of the chlorhexidine gluconate exclusively because dilution factors eliminate the effect of the alcohol by reducing it to a non-effective concentration. Since this product is formulated for use as a leave on product, there is no dilution factor associated with the formulation during use. Since the alcohol contributes the immediate effect and the chlorhexidine gluconate the immediate and persistent effect, the importance of the MIC studies is not as pivotal. I would recommend that the existent data be evaluated as proposed by this reviewer and resubmitted for a review and analysis. This could be performed as a Phase 4 commitment if no other deficiencies are noted.

Time-kill kinetic studies

The FR Notice requires that the applicant perform time-kill kinetic studies with the ATCC strains described in that document. It is realized that standardized methods are not currently available but the methodological conditions that need to be controlled have been described by others.^{3,4} Generally, the end-point that is measured and considered significant is the time required to produce a 3 log₁₀ reduction (99.9%) from the initial baseline. The FR Notice does state that a 1:10 dilution of the product should be evaluated especially if the product is used with water. This becomes problematic for products, such as HPD-5a, which are leave-on products and not intended to be used with water.

The time-kill kinetic study is performed to assess how quickly a 1:10 dilution of the test product and appropriate comparative controls kill bacteria. A 1:10 concentration is selected as an example of the concentration that is likely to reside on the hands during hand washing with water. It is assumed that the test product will be diluted to a concentration of 1:10 with water during product use. The recommended time-kill time measurements described in the FR Notice are 0, 3, 6, 9, 12, 15, 20, and 30 minutes. The purpose of this study is to attempt to establish a relationship between the rates of kill in a test tube by the test product versus the rate of kill during the clinical simulation studies where bacterial reductions at reference time points are also assessed. There is no standardized method for this study but it is evaluated to assure that it follows accepted scientific principles. [REDACTED]

[REDACTED] performed the study.

In this instance and as stated previously, the product is a leave-on product, and the proposed product label states that the product should be used in three washes cycles with 2 milliliters (mLs) of product per cycle for surgical hand scrub use resulting in a total exposure of 6 mLs. The volume of HPD-5a used for the health care personnel hand wash is 2.0 mL total per hand wash. Basically, the 1:10 dilution required of products used with water is not applicable to this product but the applicant performed one of the studies in this manner anyway (LIMS 8071). The second study (LIMS 8257) was performed undiluted to reflect actual product use conditions.

3M Health Care provided a summary of the time-kill kinetic studies (Study LIMS 8071 and LIMS 8257) in volume 1.19 (Section 7.5.1), and the actual study protocols, results and conclusions in volume 1.25. Five time points (0.25, 0.5, 1.0, 5.0 and 10 minute) were sampled and enumerated. The inoculum was approximately 5×10^5 (± 0.5 log) CFU/mL. Since these studies are 1:10 dilutions of the test and control

HPD-5a Antiseptic Hand Prep
3M Health Care

products, the concentrations that were tested are 0.1% (1000 µg/mL) of HPD-5a and 0.4% (4000 µg/mL) of Hibiclens.

The analysis performed by the applicant was to rank-order the results of each product by using the time required to produce a $\geq 5\text{-log}_{10}$ reduction and 5 minutes was considered acceptable in reducing the bacterial population (Table 2). Based on this algorithm, the organisms most resistant to kill by HPD-5a were *Enterococcus faecium* (ATCC 19434), *Micrococcus luteus* (ATCC 7468), *Enterococcus faecalis* (ATCC 29212), *Proteus mirabilis* (ATCC 7002), and *Haemophilus influenzae* (ATCC19418). That is, the 5-log_{10} reduction was not achieved at the end of 5 minutes. However, within 10 minutes all had $\geq 5\text{-log}_{10}$ reduction except *Micrococcus luteus* (3.50 \log_{10}), *Enterococcus faecium* (2.93 \log_{10}), and *Haemophilus influenzae* (4.88 \log_{10}). We must take into consideration how long the product is likely to remain on the hands when used as directed when attempting to interpret this time-kill kinetic data. It is unlikely that the alcohol will remain on the hands for a 5-minute interval but this product is formulated with moisturizers. It is possible that the evaporative rate may be reduced sufficiently to produce a microbiologically significant contact time. The organisms most resistant to kill with Hibiclens as measured by the algorithm were *Enterococcus faecium* (ATCC 19434), *S. aureus* (ATCC 6538), and *Haemophilus influenzae* (ATCC19418). These results suggest that both products produce similar results as measured by the time to product a $\geq 5\text{-log}_{10}$ reduction in bacterial population numbers. When measured at 10 minutes (data not shown) HPD-5a produced a $\geq 5\text{-log}_{10}$ reduction in 21 of 24 species and Hibiclens in 23 of 24 species evaluated. However, it should be noted that Hibiclens is a 4% CHG product and contains 4X more CHG than HPD-5a so the results are not surprising. This observation suggests that kill is time dependent and not antimicrobial drug concentration dependent at the concentrations evaluated.

Table 2. Bacterial reductions (\log_{10}) achieved at specified time intervals. Data derived from time-kill kinetic studies of a 1:10 dilution of test and control products measured from a pre-established baseline.

Microorganism Name	HPD-5a		Hibiclens	
	60 S ^a	300S	60S	300S
<i>Enterococcus faecalis</i> ATCC 29212	0.60	3.97	5.50	5.34
<i>E. faecalis</i> (MDR) ATCC 51299	1.56	5.10	5.46	5.75
<i>Escherichia coli</i> ATCC 11229	5.66	5.37	5.66	5.87
<i>E. coli</i> ATCC 25922	5.86	6.04	5.57	5.41
<i>Micrococcus luteus</i> ATCC 7468	0.48	2.80	5.26	5.23
<i>Pseudomonas aeruginosa</i> ATCC 15442	5.50	5.38	5.18	5.32
<i>P. aeruginosa</i> ATCC 27853	5.70	5.71	5.41	5.53
<i>Serratia marcescens</i> ATCC 14756	5.56	5.32	5.89	5.78
<i>Staphylococcus aureus</i> ATCC 29213	2.60	5.45	4.38	5.62
<i>S. aureus</i> ATCC 6538	4.24	5.66	2.23	4.27
<i>S. aureus</i> ATCC 335923	3.30	5.75	3.41	5.75
<i>S. epidermidis</i> ATCC 12228	5.56	5.67	5.56	5.75
<i>Acinetobacter lwoffii</i> ATCC 15309	5.38	5.38	5.38	5.38
<i>Candida albicans</i> ATCC 10231	5.53	5.20	5.03	5.20
<i>Enterobacter aerogenes</i> ATCC 13048	5.61	5.58	5.61	5.58
<i>Enterococcus faecium</i> ATCC 19434	0.11	1.67	1.45	3.66
<i>Haemophilus influenzae</i> ATCC 19418	5.00	4.81	5.00	4.81
<i>Klebsiella pneumoniae</i> ATCC 11296	5.26	5.41	5.26	5.41
<i>Proteus mirabilis</i> ATCC 7002	3.50	4.58	5.66	5.34
<i>Staphylococcus haemolyticus</i> 29970ATCC	5.63	5.48	5.63	5.48
<i>S. hominis</i> ATCC 27844	5.20	5.34	5.20	5.34
<i>S. saprophyticus</i> ATCC 15305	5.30	5.28	5.30	5.28
<i>S. pneumoniae</i> ATCC 6303	5.46	5.34	5.46	5.34
<i>S. pyogenes</i> ATCC 19615	2.74	5.63	1.18	5.18

a. Time interval present in seconds where 60S = one minute and 300S = 5 minutes.

A time-kill kinetic study was also performed as previously described with undiluted HPD-5a and Hibiclens (LISM 8257, section 7.14.3). The time intervals enumerated were 0.25 (15S), 0.5 (30S) and 1.0 (60S) minutes. The results of this study are more realistic for a “leave on” product such as HPD-5a because they mimic actual product use. The results of this time-kill study are based on the algorithm of $\geq 3 \log_{10}$ in 0.5 minutes and are presented in Table 3. It should be noted that this study is measuring primarily the intrinsic activity of the alcoholic component of HPD-5a and not that of CHG as was performed in the 1:10 study. So it is not surprising that HPD-5a produced the $\geq 3 \log_{10}$ in 0.5 minutes for all 15 and Hibiclens for 12 of the 15 test organisms evaluated. The results are presented in Table 3. At 1 minute, practically the same results were noted. These results clearly demonstrate the fast-acting alcoholic component of the HPD-5a ingredient. Comparison of the Table 3 data with the Table 2 data suggests that the contribution of both active ingredients is important and that both contribute to product efficacy in a different manner.

Table 3. Bacterial reductions (\log_{10}) achieved at specified time intervals. Data derived from time-kill kinetic studies of undiluted test and control products measured from a pre-established baseline.

Microorganism Name	HPD-5a ^b		Hibiclens	
	30 S ^a	60S	30S	60S
<i>Enterococcus faecalis</i> ATCC 29212	4.08	6.00	1.66	3.03
<i>E. faecalis</i> (MDR) ATCC 51299	3.40	6.03	3.75	5.32
<i>Escherichia coli</i> ATCC 11229	5.11	6.51	5.96	4.76
<i>E. coli</i> ATCC 25922	6.34	6.45	3.02	3.20
<i>Micrococcus luteus</i> ATCC 7468	3.94	3.78	3.05	3.58
<i>Pseudomonas aeruginosa</i> ATCC 15442	5.51	5.57	3.83	3.79
<i>P. aeruginosa</i> ATCC 27853	3.14	4.85	3.05	2.89
<i>Serratia marcescens</i> ATCC 14756	5.81	7.00	2.41	3.54
<i>Staphylococcus aureus</i> ATCC 29213	5.06	6.71	4.80	4.56
<i>S. aureus</i> ATCC 6538	4.69	4.84	2.87	4.27
<i>S. aureus</i> ATCC 335923	6.24	6.68	3.07	3.82
<i>S. epidermidis</i> ATCC 12228	4.15	5.57	5.62	5.62
<i>Candida albicans</i> ATCC 10231	3.72	4.40	3.30	3.79
<i>Staphylococcus epidermidis</i> (MRSE) ATCC 51625	6.26	6.23	4.86	4.29
<i>Enterococcus faecium</i> (VRE) ATCC 51559	4.08	4.44	0.08	0.48

^a Time interval present in seconds where 30S = 0.5 minute and 60S = 1 minutes.

^b HPD-5a is manufactured to contain 1% CHG in a vehicle [REDACTED]. Dilution of 1:10 results in 0.1% CHG and 6.1% etho. The Hibiclens product is formulated to contain 4% CHG so a 1:10 dilution results in 0.4% CHG.

Time-Kill Kinetic Study Summary

Comparison of the Table 2 and Table 3 results provide insight into the potential utility of HPD-5a. The Table 2 results provide the reductions noted at the specified time intervals for both drugs. Since this study was actually performed with a 1:10 dilution of the test drug, the results obtained from this study do not provide us with the potential utility of the alcoholic component of the leave on product. In fact, the potential activity of the chlorhexidine gluconate component can be gleaned from the Hibiclens test results because the concentration more closely approximates the final concentration to be applied onto the site by the leave on product. Based on the Hibiclens 30 and 60-second results, we can expect the HPD-5a product to produce $\geq 3 \log_{10}$ reductions that are consistent with the clinical simulation study results. This result is further supported by the results in Table 3, which demonstrate the fast acting antimicrobial activity of the alcoholic component. These results also suggest that kill is time dependent and not antimicrobial concentration dependent since more rapid kill is not seen with increasing concentration of CHG (Table 2). In summary, the *in vitro* time-kill kinetic studies results suggest that the HPD-5a should perform equally well in the *in vivo* clinical simulation studies.

Neutralizer validation for the time-kill kinetic study LIMS 8071

Since the LIMS 8071 time-kill kinetic study was conducted with a 1:10 dilutions of the test and control products, the concentrations tested in the time-kill kinetic studies were 0.1% (1000 µg/mL) of HPD-5a and 0.4% (4000 µg/mL) of Hibiclens. Thus, the target concentrations that must be assessed for the neutralization validation are the concentrations that are carried over in aliquots for dilution. Since this information was not provided in the time-kill kinetic study, but a volume of 1.0 mL appears to have been used, this observation provides us with an estimation of the carry-over concentration of 1000 µg/mL for HPD-5a and 4000 µg/mL for Hibiclens. When this volume is added to the first dilution tube (10^{-1}), the concentrations will be reduced 10 fold by dilution.

Neutralization effectiveness: The neutralization validation protocol states that to 9.0 mL of neutralizer is added 1.0 mL of a 1:10 dilution of HPD-5a or Hibiclens, which produces the desired concentrations of 100 µg/mL for HPD-5a and 400 µg/mL of Hibiclens for evaluation. A 1.0-mL sample was taken and serially diluted 10^{-1} , 10^{-2} and 10^{-3} . To each tube was added 1.0 mL of marker organism, mixed and plated to monitor survival. The duration of time that elapsed before the inoculum was introduced was not provided. This is a pivotal step in the validation of the neutralization system because it must mimic what occurs during the *in vitro* time-kill kinetic study. The neutralizer used for these time-kill kinetic studies is Trypticase Soy Broth containing 10% Tween 80, 3% lecithin, 0.5% sodium thiosulfate, and 0.1% histidine. The pH is adjusted to 7.2 ± 0.1 and the neutralizer tested for toxicity and neutralization using *E. coli* ATCC 11229, *M. luteus* ATCC 7468, and *S. aureus* ATCC 6538 as the marker organisms.

The results presented in volume 1.19, section 7.10, and page 7-45, are reproduced in Tables 4 and 5. The data demonstrates that at dilutions of 10^{-1} survival of *E. coli* was 83% and 61%, for *M. luteus* 103% and 96%, and for *S. aureus* 88% and 54%, respectively for HPD-5a and Hibiclens as measured from an established baseline. Survival of 50% has been considered acceptable for a study of this type. Since the first sample enumerated in the time-kill study is placed directly into neutralizer, results obtained with 1:10 dilutions actually would underestimate the neutralizer potential. Since neutralization is seen at this level, we can only assume that undiluted neutralizer will be a more effective neutralizer system.

Reviewer’s comments: The results of the neutralizer effectiveness study are based on time of exposure of the CHG to neutralizer. This duration of exposure must mimic what was used in the time-kill study. During the conduct of the time-kill kinetic study, it is not clear whether the duration of time that a sample was held prior to serial dilution is the same as the duration of time used to validate neutralization. This information must be provided. What were the time frames that samples were stored prior to dilution in the time-kill kinetic studies and how do these values compare to the duration of time used in these neutralization studies?

Toxicity

The method employed to perform the toxicity study required the addition of 1 ml of water to 9.0 ml of neutralizer and then serially diluting in phosphate buffered water to achieve dilutions of 10^{-1} , 10^{-2} and 10^{-3} of the neutralizer. To these tubes was added 1.0 mL of one of the three microorganisms to achieve 75-125 cfu/mL. From each dilution, 1.0 ml was plated to measure survival. The applicant concludes that 50% survival was achieved suggesting that the neutralizer is not toxic. The results are reproduced in Tables 4 (HPD-5a) and 5 (Hibiclens) exactly as provided by the applicant.

Table 4. Summary of Toxicity and Neutralization Effectiveness when evaluated with HPD-5a.

Neutralizer Test	Neutralizer Dilution	% Recovery*		
		<i>E. coli</i>	<i>M. luteus</i>	<i>S. aureus</i>
Toxicity	NA**	95	99	88

Effectiveness	10 ⁻¹	83	103	80
	10 ⁻²	96	96	93
	10 ⁻³	100	104	102

Table 5. Summary of Toxicity and Neutralization Effectiveness when evaluated with Hibiclens.

Neutralizer Test	Neutralizer Dilution	% Recovery*		
		<i>E. coli</i>	<i>M. luteus</i>	<i>S. aureus</i>
Toxicity	NA**	95	99	88
Effectiveness	10 ⁻¹	61	966	54
	10 ⁻²	86	118	80
	10 ⁻³	88	105	89

* Compared to blank baseline, which is assumed to be 100% recovery.

** Not applicable

Reviewer's comments for study LIMS 8071: Based on the information that was provided in volume 1.19, Section 7.4, and reproduced in Tables 4 and 5 above, it appears that the toxicity studies were performed with neutralizer that has been dilute 10⁻¹, 10⁻² and 10⁻³ as stated in the protocol. But, it states in the presentation of the data in the aforementioned Tables that neutralizer dilution is not applicable (NA). The study should be performed with undiluted neutralizer since that is how it is used in the time-kill kinetic study. The method used is not clear and currently is interpreted as not confirming that the neutralizer is non-toxic. In addition, the duration of exposure of the marker organisms to the neutralizer prior to enumeration was not provided. The duration should be a time interval greater than the time interval from the time the time-kill kinetic study sample was obtained and stored prior to dilution.

Neutralization validation for the in vitro time-kill kinetic study (LIMS 8257)

The neutralizer system and the marker organisms used in this study were they same as previously described except that *M. luteus* was excluded.

Neutralization effectiveness

To 45 mL of neutralizer was added approximately 4.4 grams of HPD-5a or Hibiclens resulting in a 1:10 dilution of these products which results in concentrations of 1000 µg/mL for HPD-5a and 4000 µg/mL for Hibiclens. This dilution step mimics the time-kill experiment. One (1.0) mL aliquots are then serially diluted to 10⁻¹, 10⁻², 10⁻³ and 10⁻⁴ and to each dilution tube was added 1.0 mL of marker organism mixed, and plated to measure survival. The duration of time that elapsed before the inoculum was introduced was not provided and is a pivotal step because it must mimic the procedure that was used during the study. As before, the neutralization system was considered non-toxic and effective if >50% of the original inoculum was recovered.

The results of the study are presented in Tables 6 and 7 below. The data presented in Table 7 for the two marker organisms at the 10⁻¹ dilution of Hibiclens indicates that both *E. coli* and *S. aureus* could not be cultivated from this tube. The data is interpreted to suggest that at this dilution of Hibiclens (400µg/mL), the neutralizer is not effective. Survival was >80% for subsequent dilutions of Hibiclens (<40 µg/mL). The results obtained with HPD-5a suggest that at the 10⁻¹ dilution, organisms could be recovered in 3 of the 4 tubes in numbers >65% of the baseline.

Toxicity

To 45 mL of neutralizer was added approximately 5.0 of sterile water, mixed and 1.0 mL aliquots serially diluted 10⁻¹, 10⁻² and 10⁻³. To each dilution tube was added 1.0 mL of

marker organism mixed, and plated to measure survival. The tests were produced in duplicate on two separate days. Survival of *E. coli* was 65 and 81% for HPD-5a and 81 and 87% for Hibiclens. With *S. aureus* survival was 76% for both compounds (Tables 7-21 and 7-22, volume 1.19, section 7.11). No tests were performed on the second day due to laboratory error.

Table 6 Summary of Toxicity and Neutralization Effectiveness for HPD-5a (HPD-5a) performed on two separate days.

Neutralizer Test	Neutralizer Dilution	% Recovery*					
		Feb. 12	<i>E. coli</i>	Feb. 26	Feb. 12	<i>S. aureus</i>	Feb. 26
Toxicity	NA**	65		81	76		NT
Effectiveness	10 ⁻¹	87		74	48		91
	10 ⁻²	65		97	94		87
	10 ⁻³	60		83	65		92
	10 ⁻⁴	73		77	74		89

*Compared to blank baseline which is assumed to be 100% recovery.

** Not applicable

Table 7. Summary of Toxicity and Neutralization Effectiveness for Hibiclens performed on two separate days.

Neutralizer Test	Neutralizer Dilution	% Recovery*					
		Feb. 12	<i>E. coli</i>	Feb. 26	Feb. 12	<i>S. aureus</i>	
Toxicity	NA**	87		81	76		NT
Effectiveness	10 ⁻¹	0		0	0		NT
	10 ⁻²	92		93	81		NT
	10 ⁻³	64		109	85		NT
	10 ⁻⁴	87		98	78		NT

* Compared to blank baseline which is assumed to be 100% recovery.

** Not applicable

Reviewer's comments for study LIMS 8257: The duration of exposure of the test and control products to neutralizer prior to inoculation is pivotal information that was not provided in the protocol. The active ingredient, CHG and the inoculum should be added almost at the same time because that is how the sample is handled in the experiment. The inoculum should closely follow the active ingredient and it is difficult to assess from the protocol that this was done. The duration of exposure used to assess neutralization should be the duration expected during the conduct of the time-kill kinetic study prior to the enumeration step. In addition, the toxicity study was not performed with the same concentration that is used in the study. The neutralizer was serially diluted before it was tested for toxicity. Also there is a contradiction in the presentation of the tabulated data and how the study description states that it was performed.

Clinical Simulation Studies

Surgical Hand scrub studies

Staphylococcal mediated prosthetic valve endocarditis and postoperative wound infections in patients undergoing cardiac surgery has been documented.^{3,4} However, few reports actually address common source epidemics of staphylococci infections in such patients.⁵ Several papers have been published which conclusively establish surgeons as the source of outbreaks^{6,7} in some cases. Control measures instituted at one facility included hand washing with a chlorhexidine-based product. This resulted in the elimination of *Staphylococcus epidermidis* from the hands of a surgeon identified as the carrier whose patients had a higher incidence of postoperative infections than other surgeons.

Surgical hand scrubbing is performed to remove transient bacteria and reduce the resident flora of the hands of surgeons and health care personnel involved in surgical suites. The product is also designed to have a persistent effect in case the surgeon's glove is breached. It is assumed that the persistence effect will prevent the multiplication of resident flora in the surgical glove occluded hand thus preventing contamination of the surgical field. The typical duration of surgical scrubbing in the United States is usually 5 minutes.⁸

The Food and Drug Administration supports the practices of hand washing and encourages the development of these products for the intended use by establishing efficacy requirements. The surgical hand scrub protocol described in the FR Notice is designed to mimic, microbiologically, the use of the product in the clinical setting. Thus, the applicant is required to perform a surgical hand scrub simulation study as described in the FR Notice.¹

The randomized, parallel design study is performed by establishing a test panel of individuals, which function as a surrogate for surgeons or health care professionals. Inclusion criteria require that the hands of panelists contain numerable baselines $\geq 1.5 \times 10^5$ cfu/hand. Panelist are randomized into groups of 6 as described in Table 8 to maximize the information obtained from this study design. On days 2, 3 and 4, surgical scrubs are performed at hourly intervals as described in Table 9.

Table 8. Randomization of Subjects for the Surgical hand scrub effectiveness study described in the FR Notice.

Subjects	Emuneration times (Hours)		
	1/60	3	6
A	R	L	-
B	L	-	R
C	-	L	R
D	L	R	-
E	R	-	L
F	-	R	L
Total observations	4	4	4

³ Archer GL, Armstrong BC. (1983) Alterations of staphylococcal florain cardiac surgerypatients receiving antibiotic prophylaxix. J. Inf. Dis. 147:642-649.

⁴ Karchmer, AW, Archer GL, Dismukes WE. (1983) Prosthetic valve endocarditis: microbiologic and clinical observations as guides to therapy. Ann. Inter. Med, 98:447-455.

⁵ Archer GL, Vishniavsky N, Stiver HG. (1982) Plasmid pattern analysis of *Staphylococcus epidermidis* isolates from patients with prostitic valve endocarditis. Infect Immun 35:627-632.

⁶ van Den Broek PJ, Lampe AS, et. al. (1985) Epidemic of prosthetic valve endocarditis caused by *Staphylococcus epidermidis*. Br. Med. J {Clin Res} 291:949-950.

⁷ Boyce JM, Potter-Byone G, et. al. (1990) A Common Source Outbreak of *Staphylococcus epidermidis* Infections among patients undergoing cardiac surgery. JID. 161:493-499.

⁸ Larson, EL. (1995) APIC Guideline for Infection Control Practices: APIC guideline for handwashing and hand antiseptis in health care settings. AJIC Am Infect Control. 23:251-269.

Table 9. Surgical hand scrub and enumerations scheme described in the FR Notice.

Scrub Interval	Days of test				
	1	2	3	4	5
1	X*	X*	X	X	X*
2		X	X	X	
3		X	X	X	

*After the surgical scrub is performed, enumerations of the hands are performed on this day. Enumerations are performed five minutes after product use and after 3 and 6 hours as described in the scheme presented in Table 3.

Panelists are required to use the volume of drug and surgical scrub time recommended in the product label in performing the simulated surgical hand scrub. Then they occlude the hands by donning sterile surgical gloves or plastic bags and at the time intervals specified in Table 9, sampling solution is instilled into the glove, the hands are massaged for three minutes and an aliquot removed for enumeration. The time from aliquot removal to placement of this sample into neutralizer containing dilution blanks is critical for accurate enumeration.

The efficacy requirements for surgical hand scrub are a 1- \log_{10} reduction per hand from the established baseline approximately five minutes after product use on day 1 (1st wash). Regrowth of the resident flora must not supercede the established baseline by the 6-hour enumeration time frame. On day two (2nd wash), the product should produce a 2- \log_{10} reduction of the microbial flora per hand and on the 5th day (11th wash), the product should produce a 3- \log_{10} per hand. In no day should the regrowth of the microbial flora supercede the established baseline.

The product labels are provided in volume 1.2 of this NDA and evaluation of the label suggests that the applicant requests [redacted] indications. They are the surgical hand scrub, the health care personnel hand wash [redacted] indications. A quick evaluation of the clinical simulation studies reveals that studies for the surgical hand scrub and health care personnel hand wash indications are provided. [redacted]

[redacted] will not be approved. It should be noted that this product is not used as a surgical hand scrub or health care personnel hand wash [redacted]

[redacted] surgical hand scrub studies were submitted in the NDA. They are studies LIMS 7838, LIMS 7957, and LIMS 7588. The first two are pivotal studies and the third is a pilot. I will focus primarily on the pivotal studies and will use the pilot as supportive.

1st Pivotal study to assess the Antimicrobial Effectiveness of Surgical Rub Formulations (LIMS 7838)

This study is submitted in Volume 1.26, section 7.14.4 pages 7-2138 to 7-2258 and was evaluated for compliance with the FR Notice requirements regarding protocol design and performance for surgical hand scrub studies. The study is a prospectively designed, randomized (Table 8), partially blinded (HPD-5a, HPD5b), parallel group, three arm study that is designed to evaluate the efficacy of HPD-5a, the vehicle (HPD-5b), and Hibiclens. The HPD-5a is the test product and must demonstrate the reductions at the time frames described in the introductory paragraph of this segment of the review. Sampling intervals were performed as described in Table 9. The vehicle is included in the study to allow comparison of its active ingredient, 61% ethanol, with the finished product, which also contains 1.0 % chlorhexidine gluconate. This three-arm comparison will allow differentiation of the contributions of the individual active ingredients. Hibiclens is included in the study as a positive control and is present only to validate the conduct of the study in the hands of the investigator. Due to its unique formulation characteristics and use directions of use, Hibiclens is not blinded to the investigator or user but is blinded to the microbiologist performing the sequential steps of enumeration. Approximately 119 subjects were screened and those

having bacterial population counts $\geq 1 \times 10^5$ cfu/hand at the end of the first and second baseline were enrolled in the trial. A panel of 85 subjects was included in the study and allocated as presented in Table 10. With the exception of the scrubbing directions specific for each product, all test assessments appear to be performed in an identical manner. For study LOMS 7838, the rubbing directions of the test and vehicle are to clean under the finger nails with a pick, dispense two-(2) mL of product in the palm of one hand, dip the finger tips of the opposing hand into the product and work under the nails. Spread the remainder over the top of the hand and up to just above the elbow. Repeat the same procedure with the opposite hand using a second 2 mLs of product. Dispense a third volume of 2 mLs into the hand and reapply to all aspects of both hands up to the wrists. Allow to air dry. The Hibiclens control was used according to approved labeling directions. HPD-5a, lot Jan94 004, was used for this study. Validation of the neutralization system was also performed and will be evaluated later in this review. This study was performed at the [REDACTED]

Study LIMS 7838 summary

The results of the study are presented in Table 10 as \log_{10} transformation of raw data. Log reductions are calculated by subtracting post treatment log values from the pre-established baseline counts for each hand. Descriptive statistics are calculated to assess significance of product performances versus baseline, and between HPD-5a and vehicle (HPD-5b) to determine the contribution of the chlorhexidine gluconate to the alcoholic vehicle. The latter analysis is of particular interest because it addresses the combination drug policy as discussed later.

The results of the study indicate that the control product Hibiclens performed as expected (Table 10). On the first day of surgical hand wash, a 1.6 ± 1.47 (0.7, 2.5) \log_{10} reduction was achieved 1 minute after product use and suppression of the microbial flora was maintained to a level below that established for the baseline at the 6th hour (1.4 ± 0.90 (0.8, 1.9)). On the second day, 2nd surgical wash (Table 10, first scrub, day 2) a 2.4 ± 0.54 (2.1, 2.8) \log_{10} reduction was achieved at minute 1 and suppression was maintained for the duration of the 6 hours. On the 11th surgical wash (Table 9, day 5), a 3.6 ± 0.82 (3.1, 4.1) \log_{10} reduction was demonstrated and this suppression was maintained for 6 hours. The Hibiclens results and the descriptive statistics suggest that after the second day, the resident flora of the hands of the test subjects is suppressed $\geq 2 \log_{10}$ for the duration of the experiment at the 3 and 6 hour time frames.

The conduct of the study and the results obtained with Hibiclens by these investigators validate the study. The results of the test product and vehicle control can now be accepted as being an accurate representation of performance. The data presented in Table 10 indicates that HPD-5a (Avagard) produced reductions that meet the TFM requirements for surgical hand scrubs at all time points as previously described for Hibiclens. HPD-5a has been shown to be effective when used as a surgical rub as demonstrated by this clinical simulation study. The results suggest that HPD-5a is an excellent antimicrobial at day 1 and that the antimicrobial affect persists for 6 hours. When compared to Hibiclens, it certainly appears to be superior. By day 5, wash 11, both products produce similar results. Thus it would appear that the HPD-5a product results are consistent through out the study and do not change as the results for Hibiclens do (Table 10). The change in Hibiclens performance is due to the cumulative effect of the CHG. With the HPD-5a product, there does not appear to be as dramatic a cumulative effect but the effect is higher than that of Hibiclens.

The superiority of HPD-5a is shown in Table 11. However, it should be noted that this type of comparison is not required to gain approval of the HPD-5a product. The product is only required to meet the efficacy requirements described in the TFM.

Of interest to this reviewer is the regulatory issue, which requires that the contribution of all active ingredients be demonstrated to justify the need of each. Since HPD-5a contains a [REDACTED] vehicle and 1-% CHG, the applicant is required to demonstrate the contribution of both of these active ingredients. Evaluation of the results of the test (HPD-5a) and vehicle (HPD-5b) control was performed by comparative statistical analysis of these arms of the study. The analysis indicates that HPD-5a produced results, which are statistically significantly better when compared to the vehicle (Table 11). We conclude that the statistically significant difference between the test product and vehicle control is due to the contribution of the chlorhexidine gluconate component thus justifying its use to augment the activity of the alcoholic

vehicle product. The alcoholic vehicle does contribute at least a 1-log₁₀ reduction at all time points, which is equivalent to a 90% reduction of the transient and resident flora of the hands. A comparison of HPD-5a and HPD-5b results in Table 10 clearly demonstrate the added effect of the CHG.

Reviewer's comments: Study LIMS 7838 demonstrated that HPD-5a is capable of meeting the efficacy requirements for surgical hand scrub as required by the Tentative Final Monograph (TFM) as published in the FR Notice. In addition study LIMS 7838 also provided evidence which demonstrates the contribution of the chlorhexidine gluconate to the alcoholic vehicle thus justifying the requirements of the drug combination policy.

Table 10. Post-treatment log reductions achieved and descriptive statistics calculated for the test and control products Hibiclens, HPD-5a vehicle (HPD-5b) and HPD-5a (Avagard) for as-treated subjects.*

Day/Time point for enumeration	Treatment groups		
	HDP-5a (N=34)	HDP-5b (N=31)	Hibiclens (N=20)
Day 1 Log Reductions			
N	21	21	13
1 minute±SD (95% CI)***	2.6±1.53 (1.9, 3.3)	1.1±1.61 (0.4, 1.8)	1.6±1.47 (0.7, 2.5)
N	23	21	14
3 Hours±SD (95% CI)	3.1±0.94 (2.7, 3.6)	1.4±0.83 (1.0, 1.8)	1.8±0.98 (1.2, 2.4)
N	24	20	13
6 hours±SD (95% CI)	2.8±1.06 (2.3, 3.2)	0.5±0.69 (0.2, 0.8)	1.4±0.90 (0.8, 1.9)
Day 2 Log Reductions			
N	21	21	21
1 minute±SD (95% CI)	3.2±0.82 (2.9, 3.2)	2.0±0.70 (1.6, 2.3)	2.4±0.54 (2.1, 2.8)
N	21	21	14
3 hours±SD (95% CI)	3.8±0.72 (3.2, 4.0)	1.3±1.01 (0.9, 1.8)	2.3±1.05 (1.7, 2.7)
N	22	20	13
6 hours±SD (95% CI)	3.6±0.76 (3.2, 3.9)	0.5±1.06 (0.1, 1.0)	2.3±0.60 (1.9, 2.7)
Day 5 Log Reductions			
N	20	20	13
1 minute±SD (95% CI)	3.5±0.86 (3.1, 3.9)	1.5±0.87 (1.1, 1.9)	3.6±0.82 (3.1, 4.1)
N	21	20	13
3 hours±SD (95% CI)	3.9±0.50 (3.7, 4.2)	1.4±0.83 (1.0, 1.8)	3.6±0.64 (3.2, 4.0)
N	21	18	12
6 hours±SD (95% CI)	3.5±0.71 (3.2, 3.8)	0.5±0.87 (0.1, 0.9)	3.0±1.18 (2.3, 3.7)
Baseline mean**	6.1	6.0	6.0

*The "as treated" and intent to treat (ITT) populations differ by one subject that was randomized to the PHD-5b arm but received Hibiclens. The as treated population was used in the evaluation.

** Statistical mean value calculated for the three baseline counts.

*** The log reduction is statistically significant if the confidence interval does not contain zero.

Table 11. Group Differences in Log₁₀ Reductions from Baseline Bacterial Counts (CFU/Hand) for As-Treated Populations (LIMS 7838). George Rochester, Ph.D., mathematical statistician, performed the descriptive statistics for this NDA.

Day/Time Point	HPD-5a vs. Hibiclens®			HPD-5a vs. HPD-5b		
	Log ₁₀ Reduction Difference	95% Confidence Interval	p-value	Log ₁₀ Reduction Difference	95% Confidence Interval	p-value
Day 1 Log Reduction						
1 minute	1.04	(-0.05, 2.12)	0.0602	1.52	(0.54, 2.50)	0.0032
3 hours	1.36	(0.07, 2.01)	0.0002	1.72	(1.19, 2.26)	<0.0001
6 hours	1.40	(0.69, 2.10)	0.0003	2.29	(1.73, 2.85)	<0.0001
Day 2 Log Reduction						
1 minute	0.81	(0.29, 1.33)	0.0034	1.28	(0.80, 1.75)	<0.0001
3 hours	1.37	(0.76, 1.97)	0.0001	2.34	(1.79, 2.88)	<0.0001
6 hours	1.29	(0.79, 1.79)	<0.0001	3.03	(2.46, 3.60)	<0.0001
Day 5 Log Reduction						
1 minute	-0.06	(-0.68, 0.55)	0.8380	2.00	(1.44, 2.55)	<0.0001
3 hours	0.34	(-0.06, 0.74)	0.0920	2.50	(2.07, 2.93)	<0.0001
6 hours	0.51	(-0.16, 1.17)	0.1295	3.00	(2.48, 3.51)	<0.0001

Neutralizer validation for Study LIMS 7838

Since accurate enumeration of the transient and resident bacterial flora must be achieved during the study, it is incumbent on the applicant to demonstrate that neutralization of any residual antimicrobial transferred with the microbiological sample is achieved. This validation is required because we are attempting to capture a snapshot in time where we are attempting to enumerate the effect of the antimicrobial. Any residual carry over that is not neutralized can continue to produce kill, which affects the outcome of the study in favor of the products being tested.

[redacted] using a protocol that was provided to them by the 3M Corporation, performed validation of the neutralization system. The validation study is required to demonstrate neutralization of the HPD-5a (test substance, F), HPD-5b (the test substance vehicle, H) and Hibiclens (test substance G). It must also show that the neutralization system is not toxic to the marker organism used in the validation study (*Staphylococcus aureus* ATCC 14154). The results are presented in Table 12

The protocol used is described in volume 1.26, section 7.14.4, and pages 7-2215 to 7-2218 as follows:

1. The marker organism is grown overnight, diluted in sterile [redacted] phosphate buffer to a concentration of 1×10^3 CFU/mL, which will be used in the neutralizer validation procedures.
2. A subject is selected and directed to use one of the test products in a surgical hand scrub. The hands are sampled using a procedure that was not described. The sampling time is one minute after the scrub.
3. Two (2) mLs of the sampling solution are added to 18 mLs of [redacted] phosphate buffer neutralization solution (0.3% Lecithin, 3% Polysorbate 80) resulting in a 1:10 dilution of the microbial/neutralizer sample.
4. To the 1:10 microbial/neutralizer sample is added 200 μ L of the previously prepared test organism suspension which has a bacterial density of 1×10^3 CFU/mL. This results in a bacterial density of 10 CFU/mL in the 1:10 microbial/neutralizer sample and is considered the 10^0 dilution (neat sample).

5. This last sample is vortexed, serially diluted and plated on [redacted]. This is considered the time zero sample. Thirty (30) minutes later another sample is taken, serially diluted and plated as before.
6. Plates are incubated at 35-37°C for an unspecified time and examined for the presence of test organisms.

Table 12. Validation of Neutralization by measuring survival of the marker organism *S. aureus*.

Neutralization Validation Plate Count Results (CFU/mL)						
Test Substance	Organism	Exposure Time	10 ⁰ dilution	10 ⁻¹ dilution	10 ⁻² dilution	10 ⁻³ dilution
HPD-5a	<i>S. aureus</i>	0 minutes	TMTC*	TMTC	104, 127	20, 11
		30 minutes	TMTC	TMTC	82, 69	10, 9
Hibiclens	<i>S. aureus</i>	0 minutes	TMTC	TMTC	201, 160	25, 31
		30 minutes	TMTC	TMTC	154, 115	6, 11
HPD-5b	<i>S. aureus</i>	0 minutes	TMTC	TMTC	161, 107	23, 16
		30 minutes	TMTC	TMTC	145, 163	12, 9
Numbers control	<i>S. aureus</i>	0 minutes	TMTC	TMTC	167, 179	14, 24
		30 minutes	TMTC	TMTC	77, 76	3, 10
Toxicity control	<i>S. aureus</i>	0 minutes	TMTC	TMTC	142, 139	8, 6
		30 minutes	TMTC	TMTC	110, 136	8, 10

Reviewer's comments regarding the LIMS 7838 neutralizer validation study: The neutralization validation study performed for surgical hand scrub LIMS 7838 and the results derived from that studies are not acceptable for the following reasons. First, based on the protocol that was provided, the marker organism concentration at time zero should be 10 CFU/mL. The applicant had to perform serial dilutions up to 10⁻³ to achieve this value. The applicant is required to explain this noted discrepancy. Second, the more appropriate plating medium should have been the plating medium used in the clinical simulation studies thus eliminating a potential bias introduced by the [redacted]. Third, it is not clear what duration of time elapsed between the addition of the microbial sample to the neutralizer and the addition of the inoculum to the aforementioned combination. Fourth, the subjects, who provide the samples to be used in the neutralization validation experiments, should be exposed to 11 surgical hand scrubs and the microbial sample obtained after the 11th wash. This mimics the worst case scenario regarding carry over of CHG.

2nd pivotal study to assess the Antimicrobial Effectiveness of Surgical Rub Formulations (LIMS 7957)
 This study is submitted in Volume 1.27, section 7.14.5, and pages 7-2533 to 7-2695. The reviewer evaluated the study for compliance with the FR Notice requirements regarding protocol design and performance of surgical hand scrub studies as previously described above. They appear to have complied with the TFM requirements. The study is a prospectively designed, randomized (Table 8), partially blinded (HPD-5a), parallel group, two arm study that is designed to evaluate the efficacy of HPD-5a and Hibiclens. A total of 52 subjects were enrolled and randomized to the HPD-5a arm (27 subjects) and Hibiclens arm (25 subjects). This study did not include a vehicle arm nor did it include the 3-hour time period for enumeration, which is a time frame that is optional but informative [redacted] performed this surgical hand scrub study.

Study LIMS 7957 summary

The results of study LIMS 7957 are presented in Table 13 and demonstrate that Hibiclens met all requirements for surgical hand scrubs described in the TFM. That is, it produced the 1.0 log₁₀ reduction on day 1 (wash 1), 2.0 log₁₀ reduction at day 2 (wash 2), and a 3.0 log₁₀ reduction at day 5 (wash 11). The initial reductions did not rise above the pre-established baselines at any time period. The performance of

HPD-5a Antiseptic Hand Prep
3M Health Care

the study and the results obtained for Hibiclens by these investigators validate the study and we can now accept the results of the test product (HPD-5a) as being an accurate representation of their performance.

The results of this study (Table 13) also demonstrate that HPD-5a achieved the efficacy requirements for surgical hand scrubs as defined in the TFM. A 2- \log_{10} reduction was achieved on the 1st hand wash and a 3- \log_{10} reduction on the 2nd and 11th wash of the study as required. In no case did the initial bacterial reductions observed grow back to a level higher than the initial baseline. The Table 13 results also demonstrate the consistent level of antimicrobial performance of HPD-5a when compared is made of the 1st, 2nd, and 11th wash cycles. The results are reasonable similar to each other and to those results presented in study LIMS 7838 (Table 10). The results are also similar to the cumulative effect of Hibiclens at day 5, wash 11 as previously noted. That is, HPD-5a results look more like Hibiclens results at day 5, wash 11. In addition, Table 14 descriptive statistics demonstrate that Hibiclens was not superior to HPD-5a or vice versa. The results are contrary to the observation made in study LIMS 7838, Table 11. The analysis presented in Table 14 is not required for approval of the product under investigation. The applicant is only required to achieve the efficacy requirements described in the TFM FR Notice¹ and the comparative statistics provided are not required.

Reviewer's comments: Study LIMS 7957 confirms the results of study LIMS 7838 and we conclude that the HPD-5a product is an effective surgical hand scrub. The statistician should confirm that the number of subjects used to evaluate the efficacy of each product at each time point is satisfactory. A total of 27 subjects were evaluated for the HPD-5a arm but it is not clear what the allocation of subjects was to each time point enumerated.

Table 13. Post-treatment log reductions achieved and descriptive statistics calculated for HPD-5a and Hibiclens, for intent-to-treated subjects (LIMS 7957).

Day/Time point for enumeration	Treatment groups	
	HDP-5a (N=27)	Hibiclens (N=25)
Day 1 Log Reductions		
N	18	17
1 minute \pm SD (95% CI)***	2.5 \pm 0.82(2.1, 2.9)	1.8 \pm 0.55(1.5, 2.1)
N	19	16
3 Hours \pm SD (95% CI)	2.6 \pm 0.92(2.0, 3.0)	1.8 \pm 0.8(1.3, 2.1)
N	17	17
6 hours \pm SD (95% CI)	2.2 \pm 1.04(1.6, 2.7)	1.9 \pm 0.66(1.6, 2.3)
Day 2 Log Reductions		
N	16	17
1 minute \pm SD (95% CI)	3.0 \pm 0.98(2.5, 3.5)	2.6 \pm 0.73(2.2, 2.9)
N	16	16
3 hours \pm SD (95% CI)	3.1 \pm 0.67 (2.8, 3.5)	2.7 \pm 0.70(2.3, 3.1)
N	16	17
6 hours \pm SD (95% CI)	3.3 \pm 0.53(3.0, 3.6)	2.3 \pm 0.78(1.9, 2.7)
Day 5 Log Reductions		
N	16	16
1 minute \pm SD (95% CI)	3.7 \pm 0.73(3.3, 4.1)	3.7 \pm 0.76(3.3, 4.1)
N	16	16
3 hours \pm SD (95% CI)	3.6 \pm 0.74(3.2, 4.0)	3.7 \pm 0.89(3.2, 4.1)
N	16	16
6 hours \pm SD (95% CI)	3.8 \pm 0.59(3.5, 4.1)	3.5 \pm 0.84(3.1, 4.0)
Baseline mean**	6.3	6.4

** This is the mean of three baseline counts.

Table 14. Log Reductions in Bacterial Counts (CFU/Hand) and Log Reduction Differences Between HPD-5a and Hibiclens® in LIMS 7957 – Surgical Hand Scrub.

Day/Time Point	HPD-5a (N=27)	Hibiclens® (N=25)	Log Reduction Difference	P-value** (95% CI)
Baseline Period Mean*	6.3	6.4	N/A	N/A
Day 1 Log Reduction 1 min (95% CI)	2.5 (2.1, 2.9)	1.8 (1.5, 2.1)	0.65	0.0095 (0.17, 1.13)
6 hr (95% CI)	2.2 (1.6, 2.7)	1.9 (1.6, 2.3)	0.25	0.3989 (-0.35, 0.86)
Day 2 Log Reduction 1 min (95% CI)	3.0 (2.5, 3.5)	2.6 (2.2, 2.9)	0.44	0.1546 (-0.17, 1.05)
Day 5 Log Reduction 1 min (95% CI)	3.7 (3.3, 4.1)	3.7 (3.3, 4.1)	0.00	0.9974 (-0.54, 0.54)
6 hr (95% CI)	3.8 (3.5, 4.1)	3.5 (3.1, 4.0)	0.32	0.236 2 (-0.21, 0.84)

* This is the mean of all three-baseline counts.

** HPD-5a vs. Hibiclens®

Neutralizer validation for Study LIMS 7957

Since accurate enumeration of the transient and resident bacterial flora must be achieved during the study, it is incumbent on the applicant to demonstrate that neutralization of any residual antimicrobial that is carried over in the microbiological sample is achieved. This step is required because we are attempting to capture a microbiological picture of effect in time when enumerating the effect of the antimicrobial. Any residual carry over that is not neutralized can continue to produce kill, which affects the outcome of the study in favor of the products being tested.

_____ performed validation of the neutralization system and this information is found in Volume 1.27, section 7.14.5, and pages 7-2677 to 2679. The validation study must demonstrate neutralization of the HPD-5a (test substance, F), HPD-5b (the test substance vehicle, H) and Hibiclens (test substance G). The study is also required to demonstrate that the neutralization system is not toxic to the marker organism used in the study (*Staphylococcus aureus*).

The protocol used is described in volume 1.26, section 7.14.4, and pages 7-2215 to 7-2218 as follows:

- Two subjects use the products according to labeling directions and the hands sampled according to the clinical simulation protocol. A 1.0-mL aliquot is placed into a 9.0-mL dilution tube containing the appropriate neutralizers. The neutralizers used in this study are the same qualitatively and quantitatively as used in the previous neutralization study. That is _____ phosphate buffer containing 0.3% lecithin and 3.0% Polysorbate 80 (Tween 80) at a pH of 7.2.
- A 24-hour overnight culture of *Staphylococcus aureus* ATCC 27217 was serially diluted to achieve approximately 10³ to 10⁴ CFU/mL.
- A 0.1 mL volume of the marker organism was added to the 1:10 tube containing the surgical hand scrub sample in neutralizer to achieve a final bacterial concentration of 10 to 100 CFU/mL (dilution volumes are 0.1:10.0 = 100 fold dilution). The tubes are mixed.

4. At time zero minutes, a 1.0-mL sample is taken and plated on Trypticase Soy agar containing 3.0 µg/mL tetracycline. The sampling and dilution step is repeated at 30 minutes.
5. A toxicity and numbers control was also prepared by inoculating as before, dilution tubes with neutralizer to assess toxicity and into 0.9% saline tubes to monitor the true population inoculum.

The results of the study are presented in Table 15. These results clearly demonstrate that the neutralizer is capable of neutralizing any carry over CHG under the conditions of this test. The neutralizer does not appear to be toxic to the indicator organism.

Table 15. Validation of Neutralization using survival of the marker organism *S. aureus* ATCC 27217 as a measure of efficacy.

Test substance	Exposure Time	Plate counts		Average CFU/MI	% recovery
HPD-5a	0 minutes	66	58	6.2 x 10 ¹	103
	30 minutes	65	57	6.1 x 10 ¹	105
Hibiclens	0 minutes	58	48	5.3 x 10 ¹	88
	30 minutes	82	68	7.5 x 10 ¹	129
Numbers control	0 minutes	56	65	6.0 x 10 ¹	NA*
	30 minutes	62	54	5.8 x 10 ¹	NA*
Toxicity control	0 minutes	58	50	5.4 x 10 ¹	90
	30 minutes	65	53	5.9 x 10 ¹	102

Reviewer’s comments regarding the LIMS 7957 neutralizer validation study: The results of the study presented in Table 14 indicate that neutralization was demonstrated since the number of organisms recovered in the HPD-5a and Hibiclens studies are similar to those recovered in the numbers control. Also the toxicity and numbers control values are similar and suggest that the neutralizer is not toxic. However, the study is not acceptable because the subjects appear to have used the test and control products only once. The worst case scenario must be evaluated to assure that the neutralizer works efficiently. Thus samples should be taken from subjects that have used the products a total of 11 surgical scrubs. This is especially true for Hibiclens because its microbiological effect appears to be exposure dependent. In addition, the time that has elapsed between the addition of the subject’s samples to the neutralizer before the inoculum is added, needs to be provided. Ideally, the inoculum should be added to the neutralizer before the subjects sample is added to the neutralizer.

Pilot study to assess the Antimicrobial Effectiveness of Surgical Rub Formulations (LIMS 7588)

A randomized, prospective, parallel design surgical hand scrub pilot study was performed with HPD-5a and Hibiclens. The study followed TFM requirements for entry, conduct of the study, and performance requirements. Eight subjects were used in each of the Hibiclens, HPD-5a, and HPD-5b arms. The purpose of this experiment was to characterize the efficacy of the antiseptics, the regrowth potential of the resident flora after product use, and variability of the test method to aid in the estimation of subject numbers. In addition, enumeration methods and neutralization procedures were evaluated. For example, serial dilutions were performed of samples and then specified volumes plated or samples filtered and placed on agar plates, incubated, and enumerated. Neutralization was performed using six different methods to assess the effects of different procedures on neutralization. [redacted] performed the study, the protocol, raw data and results are found in Volume 1.28, section 7.14.7 and summarized in Table 16 of this review. Hibiclens performed as expected thus validating the study. The studies provided insight as to the potential efficacy of the test and vehicle while the control provided insight as to the validity of the study and of the performance of the control in the hands of these investigators. Descriptive statistics for samples sizes of n=3 are not performed nor are they required for this study.

Table 16. Post-treatment log reductions achieved for Hibiclens, HPD-5a vehicle (HPD-5b) and HPD-5a for as-treated subjects.*

Day/Time point for enumeration	Treatment groups		
	HDP-5a (N=8)	HDP-5b (N=8)	Hibiclens (N=8)
Day 1 Log Reductions			
N			
1 minute±SD	3.0±.52	0.7±0.56	1.5±0.78
N			
6 hours±SD	3.1±0.37	0.0±0.61	1.6±0.98
Day 2 Log Reductions			
N			
1 minute±SD	3.4±0.45	1.0±0.68	2.1±0.63
N			
6 hours±SD	3.6±0.41	0.4±0.86	2.1±0.81
Day 5 Log Reductions			
N			
1 minute±SD	3.4±0.41	1.0±0.90	2.9±1.24
N			
6 hours±SD	3.2±0.73	0.2±0.92	2.8±1.04
Baseline mean**	5.67	5.67	5.67

*The "as treated" and intent to treat (ITT) populations differ by one subject that was randomized to the PHD-5b arm but received Hibiclens. The as treated population was used in the evaluation.

** Statistical mean value calculated for the three baseline counts.

Neutralization validation for Pilot Surgical Scrub study (LIMS 7588)

In order to produce accurate enumeration values of the microbial flora of the hands post product use, any carry over antimicrobials must be neutralized. The purpose of this study is to provide proof of concept that the neutralizer system used in the pilot study is effective. In this pilot study, six different methods were evaluated.

Neutralizer effectiveness method #1: The neutralization validation protocol states that to 9 bottles containing 75 mLs of neutralizer solution (0.3% Lecithin, 3.0% Polysorbate 80) are added 0.75 mLs of a stock *E. coli* (ATCC 11229) or *Staphylococcus aureus* (ATCC 11228) containing approximately 1×10^5 CFU/mL. (The placement of 0.75ml of the organism into 75ml of neutralizer should produce a dilution of 100 fold. Thus the 10^0 -dilution tube should produce counts of 1000 cfu/mL). This is followed by either 0.2 mL Hibiclens (4% CHG) to produce a concentration of 105 µg/mL of chlorhexidine gluconate or 2 pumps (4.0 mLs of 1% CHG) of HPD-5a, which results in a CHG concentration of 526 µg/mL of chlorhexidine gluconate. It is not clear why different concentrations were evaluated. The scheme produces 3 bottles per organism/drug combination. The tubes were held at room temperature (RT) for approximately 30 minutes prior to plating.

The results (not presented here) suggest that the neutralizer is capable of neutralizing the concentrations of antiseptics evaluated when neutralization exposure is at least 30 minutes and when compared to the water controls. The counts were >1000 CFU/mL for all samples as expected.

Reviewer's comments: We can interpret these results as suggesting that the placement of the sample directly into neutralizer is critical to appropriate neutralization. It is not clear how the concentrations of CHG found in these products and used in this study relate to the concentrations encountered during the clinical simulations studies.

Neutralizer effectiveness method #2: The same procedure described in method #1 was used except that the 75-mL bottles did not contain neutralizers. In addition, the bottles were incubated at RT for 10 minutes, 1.0 ml portions transferred to dilution tubes containing neutralizers, the tubes allowed to sit at RT for 30 minutes, serially diluted and plated onto Trypticase Soy Agar (TYA) plates.

The results (not shown here) indicate that organisms exposed to antiseptics as described did not survive relative to the water control.

Reviewer's comment: We can interpret these results and those of method #1, as suggesting that placement of the antiseptic-containing sample immediately into the neutralizer is essential to proper survival and accurate enumeration of the microorganism.

Neutralizer effectiveness method #3: The same procedure described in method #2 was used except that the dilution tubes were plated immediately after dilution onto TYA plates containing neutralizers. The results (not shown) suggest that kill occurred in the first 10 minutes since neutralization at the plating level did not yield viable colonies.

Reviewer's comment: Again the data suggest that placing of the antiseptic-containing sample into neutralizer immediately after it is taken is essential to proper enumeration.

Neutralizer effectiveness method #4: The same procedure described in method #1 was used except that the 75-mL sampling solution bottles did not contain neutralizers. The inoculated bottles were allowed to sit for 60 seconds and then 4ml samples placed into 36 mL of [redacted] phosphate buffer with neutralizers. A 1.0-mL sample was serially diluted and plated. 30mL were passed through a filter, which was washed with [redacted] phosphate buffer with neutralizers. The filter and the serially diluted samples were plated onto TSA plates. The results (not shown) demonstrate that no viable microorganism was seen growing on the TSA plates irrespective of method used to quantitate the samples. The results suggest that 1-minute exposure of the organisms to the antiseptics is a sufficient time interval to produce lethality since the neutralization after this time interval did not produce survival.

Reviewers comment: This data clearly demonstrates that if any time delay occurs when taking the sample to be enumerated from the glove and placing it directly into neutralizers, we will get results that are biased. This requires that we ask whether time delays occurred in placing the sample to be enumerated into the neutralizer during the clinical simulation studies. The experiment that needs to be performed is one that determines whether neutralization after 1 minute will enhance survival. It is possible that the time taken to dilute the product prior to plating on neutralization containing agar was sufficient to provide the kill even though the product was diluted.

Neutralizer effectiveness method #5: In this experiment, antiseptic was added to nine bottles containing 75 mL sampling solution as previously described in method #1. The bottles were allowed to sit for 1 minute. A 4-mL of sample was taken and added to 36-mL bottles containing [redacted] phosphate buffer with neutralizers. After 1 minute samples were enumerated by serial dilution and plating on to TSA plates or by filtration and plating of the filter on to TSA plates as described in method #4. The results (not shown) suggest that neutralization occurred in one minute for all samples and viability of the microorganism was equivalent to that seen with the water control.

Reviewer's comments: Since neutralizer is contained in the 75-mL bottles, I expect the results to be the same as those described in method #1 above. The addition of neutralizers to the plating medium may not have any additional effect but it is not possible to assess because the appropriate experiments have not been provided.

Global Summary for the Surgical Hand scrub indication

The effect of HPD-5a is reasonably reproducible if we look at the data presented in Tables 10 and 13. The day 1 reduction range from 2.5-2.6 log₁₀ and the effect of the product is of sufficient duration after one wash as to suppress the microbial flora for at least 2 log₁₀ below the baseline out to 6 hours. The day 2 results also demonstrate further reduction of the resident flora to a level >3 log₁₀ (range 3.0-3.2) below the baseline in both studies. The effect of the antiseptic is evident post-use since the day 2 bacterial reduction is maintained below this initial value out to 6 hours. Day 5 results are practically identical between the two labs (range 3.5-3.7) and are below the efficacy requirement of >3 log₁₀ below the baseline with no rebound of the resident flora back to baseline. In addition, the inter-laboratory consistency of the Hibiclens results also demonstrates the highly reproducible nature of this surgical hand scrub protocol in the hands of qualified investigators. We conclude that HPD-5a is a satisfactory surgical hand scrub when assessed by the surgical hand scrub method.

Health care Personnel hand wash studies LIMS 9739 and LIMS 7938

One objective that health care professionals are urged to accomplish in the conduct of daily activities is to wash their hands to prevent the dissemination of nosocomial pathogens within the health care setting. It is a well-established fact that hand washing prevents the dissemination of pathogens by health care professionals between patients thus reducing the incidence of nosocomial infections.

The FR Notice describes a clinical simulation protocol that is designed to mimic, microbiologically, the daily activities of a health care professional as they move from one patient to the next during rounds. It is assumed that the health care practitioner's hands will become contaminated when they interact with a patient. Thus, before they move on to the next patient, they should wash their hands to prevent the dissemination of potential pathogens to the next patient. The purpose of this clinical simulation health care personnel hand wash study is to mimic the acquisition of a transient microorganism load, which the test product is, required to remove or reduce to specified levels.

The randomized, parallel design study is accomplished by establishing a test panel of individuals to represent the health care professional. The test panelist wash their hands with bland soap to remove transient organisms, the hands are then contaminated with a marker organism and allowed to air dry. The marker organism used in this particular study is the pigmented organism *Serratia marcescens* ATCC 14756. The hands are occluded with latex gloves or vinyl Baggies, a sampling fluid containing no neutralizers instilled into the glove/baggie, the hand massaged and a sample obtained and quantitated. This first step establishes the baseline by which to measure product-induced change. This contamination/wash procedure is repeated 10 times and quantitation performed at the 1st, 3rd, 7th, and 10th wash intervals. The product is required to show a 2-log₁₀ reduction of the marker organism from baseline per hand no more than 5 minutes after the 1st handwash. It is also required to show a 3-log₁₀ reduction of the marker organism from baseline per hand no more than 5 minutes after the 10th handwash as a demonstration of efficacy.

One pivotal and one pilot health care personnel hand wash study for HPD-5a were submitted in fulfillment of the efficacy requirements described in the FR Notice. [REDACTED]

[REDACTED] performed the pivotal study (LIMS 7939, Volume 1.28, section 7.14.6, and pages 7-2968 to 7-3043). The applicant 3M Health Care, Medical Surgical Division, performed the pilot study (LIMS 7938, Volume 1.28, section 7.14.8, and pages 7-3332 to 7-3378).

Both of these studies were evaluated for compliance with the protocol described in the FR Notice¹ for health care personnel hand wash use. The protocol for the pivotal study is a prospective, randomized, partially blinded, parallel arm study designed to evaluate the effectiveness of HPD-5a (lot #Jan98 004). Approximately 48 panelists were recruited and randomly assigned to test or control product (Hibiclens, lot # 4269-A). Evaluation of the protocol suggests that principle investigator followed the recommendations described in the FR Notice. A washout period of 7 days was followed by hand washing with a bland soap prior to inoculation with two (2) 1.5-mL volumes of an overnight culture of the marker organism, *Serratia*

HPD-5a Antiseptic Hand Prep
3M Health Care

marcescens ATCC 14756. Sampling was performed by the hand occlusion method by instilling 75 mLs of sampling solution, serially diluting in neutralizer containing diluent and plating. Panelists used 2-mL (1 pump for the device) of HPD-5a per hand wash and 5 mL of Hibiclens per hand wash. Hibiclens was used according to labeling directions (15-second hand wash). The inoculation/hand wash cycle was repeated 10 consecutive times and emuneration were performed after the 1st, 3rd, 7th, and 10th wash intervals using the hand occlusion method.

The results of the pivotal healthcare personnel hand wash study are present in Table 17. The control product, Hibiclens, performed as expected. A 2.6- \log_{10} reduction was achieved at the 1st wash and a 3.7- \log_{10} reduction was achieved at the 10th wash thus validating the study. The results of the test product can now be considered an accurate representation of the efficacy of the product as assessed by this protocol and the efficacy requirements. The test product produced a 2.14- \log_{10} reduction at the 1st wash and a 3.7- \log_{10} reduction at the 10th wash. Both of these reductions meet the minimum efficacy requirements for the health care personnel hand wash indication. The Table 17 results also demonstrate that the reduction noted with Hibiclens is statistically superior to that produced HPD-5a at the 1st ad 3rd wash. The CI at both of these testing intervals also suggests an outcome in favor of Hibiclens. However, HPD-5a meets the efficacy requirements and continued use of the test product results in indistinguishable outcomes when compared to Hibiclens. Since this is a health care personnel indication, it is likely that HPD-5a will be used multiple times per day and the efficacy of the product is more likely to reflect that expected with Hibiclens. It should be noted that assessment of efficacy only requires that the test product meet the TFM efficacy requirements. The test product is not required to meet an equivalence or superiority claim compared to the control, Hibiclens.

Table 17. Pivotal Health care personnel hand was post-treatment log reductions achieved and descriptive statistics for HPD-5a and Hibiclens for intent to treat subjects.

Day/Time point	HPD-5a N=24	Hibiclens N=24	Log reduction differences	P-value* (95 CI)
\bar{x} Baseline	7.0	7.0	NA	NA
1 st Wash \pm SD (95% CI)	2.1 \pm 0.055 (1.9, 2.4)	2.6 \pm 0.45 (2.4, 2.8)	-0.43	0.0051 (-0.72, -0.14)
3 rd Wash \pm SD (95% CI)	2.4 \pm 0.87 (2.1, 2.8)	2.9 \pm 0.47 (2.7, 3.1)	-0.43	0.0405 (-0.83, -0.02)
7 th Wash \pm SD (95% CI)	3.5 \pm 0.94 (3.1, 3.9)	3.7 \pm 0.70 (3.2, 3.6)	0.04	0.8409 (-0.04, 0.49)
10 th Wash \pm SD (95% CI)	3.7 \pm 0.98 (3.3, 4.2)	3.7 \pm 0.70 (3.4, 4.0)	0.04	0.857 (-0.45, 0.54)

* Based on the average log reduction of right and left hands for each subject.

The objective of the pilot study (Volume 1.28, section 7.14.8, and pages 7-3331 to 7-3378) was to help characterize the efficacy of HPD-5a, HPD-5b (the vehicle) and control products, which would aid in the design of future studies. Especially an estimation of the number of panelist that would be needed to demonstrate efficacy of the products tested. This pilot study was a parallel, randomized design, in which 12 panelists were enrolled to assure that 3 panelists per arm completed the study. The results of the pilot health care personnel hand study are presented in Table 18. Due to the small number of subjects used in this study, descriptive statistics were not performed. The results presented in this study are atypical. The Hibiclens results are not consistent with results obtained from other laboratories. The \log_{10} reductions obtained at each wash interval measured are of much greater magnitude than normally seen. For example the results presented in Tables 10 and 13 are typical of results seen with Hibiclens and should be compared with the results found in Table 18. This comparison clearly demonstrates that this study produced reductions that would be statistically different than those found in Tables 10 and 13. If the results had been submitted as a pivotal trial, the validity of the study would be questioned. Since the study was used only to

HPD-5a Antiseptic Hand Prep
3M Health Care

characterize the number of subjects needed in the pivotal study, the results will not be included in the analysis.

Table 18. Pilot Health care personnel hand was post-treatment log reductions achieved for HPD-5a, HPD-5b, and Hibiclens.

Day/Time point	HPD-5a N=3	HPD-5b N=3	Hibiclens N=3
× Baseline±SD	8.50±0.25	8.84±0.37	8.57±0.42
1 st Wash±SD	2.67±1.22	2.47±1.53	4.25±0.42
3 rd Wash±SD	4.79±1.42	1.68±0.55	5.53±0.37
7 th Wash±SD	7.02±0.18	0.68±0.69	6.52±0.36
10 th Wash±SD	7.10±0.25	0.62±0.48	6.69±0.26

Neutralized validation for Study LIMS 7939

Since accurate enumeration of the marker organism, *Serratia marcescens*, must be achieved during the study, it is incumbent on the applicant to demonstrate that neutralization of any residual antimicrobial carried over in the sample is achieved. This step is required because we are attempting to capture a snapshot in time when enumerating the effect of the antimicrobial. Any residual carry over that is not neutralized can continue to produce kill, which affects the outcome of the study in favor of the products being tested.

[redacted] performed validation of the neutralization system and this information is found in Volume 1.28, section 7.14.6, and pages 7-3034 to 7-3036. The validation study must demonstrate neutralization of the HPD-5a (test substance, F), and Hibiclens (test substance G) and that the neutralization system is not toxic to the marker organism.

The protocol used to make this assessment is as follows:

1. The hands of two subjects are washed 10 times with the test or control product according to labeling directions. This is ideal because it provided the worst case scenario.
2. Hand sampling is performed after the 1st, 3rd, 7th, and 10th hand wash using sampling solution without neutralizers.
3. One (1.0) mL of the sampling solution from the 10th wash is added to four 9.0 mL tubes containing [redacted] phosphate with 0.3% lecithin and 3.0% Tween 80 (Polysorbate).
4. Each of the four tubes is inoculated with a *Serratia marcescens* ATCC 14756 culture to achieve a concentration of 100-1000 cfu/mL. The duration of time that elapses between the addition of the sample to the neutralizer and the addition of the marker organism to the neutralized solution is a critical step.
5. Numbers control and toxicity of neutralizer tubes are set up. The numbers control is performed in 0.9% saline and the toxicity control in 9.0 mL of neutralizer.
6. After mixing, a sample is taken, serially diluted and plated to obtain viability. The same procedure is repeated after 30 minutes.

The results are presented in Table 19 and include the numbers control, toxicity control and the neutralization efficacy data. The results suggest that the exposure of the marker to the neutralizer for up to 30 minutes yield no less than 83% of the initial population quantitated from the saline control. This suggests that the neutralizer is not toxic to the marker organism. Comparison of the HPD-5a with the saline control suggests that at 0 minutes, survival of the marker is >83.8% of the saline control. However, at 30 minutes, there is substantial reduction of the original inoculum yielding >69.7% survival. The Hibiclens data suggests that neutralization of the CHG is reasonable complete given that there is >80% survival of the marker organism. We conclude that the neutralizer used by [redacted] for the health care personnel hand wash study is effective in neutralizing any carry CHG with the enumeration sample.

Table 19. Summary of toxicity and neutralizer effectiveness for HPD-5a and Hibiclens as measured by survival of the marker organism.

Article	Time	Plate counts		× cfu/mL	% recovery
Numbers control	0 min.	84	101	9.2×10^1	NA*
	30 min.	103	75	8.9×10^1	NA*
Toxicity control	0 min.	79	98	8.8×10^1	95.7
	30 min.	83	66	7.4×10^1	83.7
HPD-5a	0 min.	77	78	7.8×10^1	83.8
	30 min.	71	53	6.2×10^1	69.7
Hibiclens	0 min.	81	68	7.4×10^1	80.5
	30 min.	85	81	8.3×10^1	93.3

* Not Applicable

% Recovery = counts at time 0 or 30 minutes/numbers controls at time 0 or 30 minutes x 100

Review's comments

The validation of the neutralization system used for the health care personnel hand wash study can not be used to justify the use of the same neutralization system in the surgical hand scrub study.

Although the neutralizer validation for health care personnel is satisfactory as performed by [redacted] [redacted] the neutralizer validation study they performed for the surgical hand rub is not.

The directions for use for each of these indications result in radically different volumes and duration of exposure of the hands to HPD-5a between the two indications.

Global summary of the health care personnel hand wash indication

The results of the pivotal health care personnel hand wash study (LIMS 7939) presented in Table 17 demonstrate that HPD-5a is capable of achieving the efficacy requirements described in the TFM for this indication. However, since the two pivotal surgical hand scrub studies are not acceptable, the indication of health care personnel wash is not supported by that data. Therefore, it can not be approved since an adequate and well-controlled study and a supporting study are not available to justify approval.

Albert T. Sheldon, Jr. Ph.D.
Team Leader, Microbiology Reviewer

Cc: Original NDA No. 21-074
Microbiologist, HFD-520
File name: 21-074

DepDir/LGavrilovich

Cc: Original NDA 21-074
HFD-473
HFD-520/DepDir/LGavrilovich
HFD-520/Smicro/ATSheldon
HFD-520/MO/DBostwick
HFD-520/Pharm/AEllis
HFD-520/Chem/MSloan
HFD-520/CSO/MDillon-Parker
HFD-520
HFD-502
HFD-635

Division of Anti-Infective Drug Products
Clinical Microbiological Review # 2

IND/NDA # 21-074 **Date Completed:** June 6, 2000
(BI)

Applicant (NDA): 3M Health Care
3M Center, Building 275-3E-08
St. Paul, MN 55122-1000

Chem/Ther. Type: Topical Antimicrobial

Submission Reviewed: New Drug Application

Providing for: Use as a surgical hand scrub
Use as a Healthcare Personnel hand wash

Product Name(s):
Proprietary: Currently known as Avagard –CHG Hand Prep (HPD-5a)

Non-proprietary/USAN: Chlorhexidine gluconate

Compendia: Chlorhexidine gluconate and ethanol

Code name/number: HPD-5a

Chemical name: 1,1'-Hexamethylenebis[5-(*p*-chlorophenyl) biguanide] di-D-gluconate

Structural formula: See USAN Dictionary

Molecular formula: C₂₂H₃₀Cl₂N₁₀2C₆H₁₂O₇

Dosage form(s): Solution

Route(s) of administration: Topical

Pharmacological Category: Antibacterial, antiseptic

Dispensed: Rx OTC

Initial Submission Dates

Received by CDER: June 25, 1999
Received by Reviewer: June 30, 1999
Review Completed:

Supplements/Amendments:

Received by CDER: May 5, 2000, May 18, 2000, May 30, 2000
Received by Reviewer: May 12, 2000, June 1, 2000
Review Completed: June 6, 2000

Related Documents: 

Remarks:

The May 4, 2000 submission is a response to the Agency's letter of April 10, 2000, which listed numerous outstanding issues to the NDA as defined from the perspective of the Microbiology Review Officer. The letter was sent to the applicant in advance of the due date of the submission with the thought that if responses could be obtained in a timely manner, then the official regulatory letter that is to be issued may more appropriately reflect a positive outcome.

In this review of the information submitted, the deficiency and the response are discussed in the summary section of this review. The question is provided first followed by the applicant's response to the question, followed by the conclusion reached by this reviewer. Where needed, specific details of the deficiencies will be summarized in this review to aid the reader understand the issues.

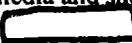
Conclusions/Recommendations:

The applicant provided clarification to all of the questions that were listed in Review #1 by providing discussion, new information, analysis, reanalysis, and supporting documentation to address the issues. I find the information acceptable and capable of supporting the efficacy of HPD-5a when used as surgical hand scrub and health care personnel hand wash.

Key words: chlorhexidine gluconate, topical antiseptic, topical antimicrobial, surgical hand scrub, health care personnel hand wash, Federal Register Notice.

Summary:

A). The *in vitro* susceptibility study provided by the applicant for *Enterobacteriaceae*, *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Staphylococcus* spp., and *Enterococcus* spp., were not performed as directed in the FR Notice and the NCCLS reference in that cation-adjusted Mueller-Hinton broth was not used in the study. Mueller-Hinton broth was used but it was not supplemented with cations as required. Further, inocula were not prepared in water or saline as required, and the incubation temperatures were inconsistently applied and ranged outside of recommended time duration for incubation. The effect of these modifications on the outcome of susceptibility testing is unclear.

Applicant's response: The studies were conducted as described in the TFM using NCCLS methods but minor modifications were made to reflect standard microbiological methods and practices. The NCCLS document (M7-A2, Vol. 10 No. 8, 1990) provides for inclusion of cations to Mueller Hinton Broth (MHB) under specified situations (i.e., testing of tetracycline for all bacteria and aminoglycosides for *P. aeruginosa*). Since the test and control materials tested were not antibiotics, cation adjusted MHB was not necessary. In addition the cultures tested had been passed in artificial media and shown to grow in cultures with out the supplements. The inocula were prepared with  phosphate buffer to overcome the potential toxic effect of water and the variability of pH noted in water supplies. This buffer is recommended by the FDA in its document "Bacteriological Analytical Methods", by the Standard Methods for the Examination of Water and Wastewater, and that of Dairy products, and by the American Society for Testing and

Materials. The incubation temperatures used are those referenced by the NCCLS and American Type Culture Collection (ATCC). The duration of incubation is based on NCCLS requirements or discretionary measures described there in.

Reviewer's response and Reviewer's comments: The *in vitro* susceptibility studies provided by the applicant included susceptibility testing of *Enterobacteriaceae*, *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Staphylococcus* spp., and *Enterococcus* spp. The NCCLS method used by the applicant is based on the document provided by the applicant as Reference 1 of this submission and is, in fact, that reference described in the TFM. The reference provided in the TFM is 10 years old and does not reflect current accepted changes in the conduct of *in vitro* susceptibility methods but does justify some of the flexibility described by the applicant. The most recent NCCLS standards were used by this reviewer as the basis for Review #1 and it requires that cations adjusted MHB (CAMHB) be used for the organisms previously described above. The applicant has responded by performing susceptibility studies as described in the TFM and the agency will have to accept that data as provided even though the document referenced in that document is now outdated.

[Editorial Note: That is a perfect example why this reviewer objected to the use of references with fixed dates in the TFM when it was written. It makes the regulatory requirements for efficacy outdated and provides no flexibility to the review and scientific process as new methods are developed or existing methods evolve. The NDA regulatory process should develop its own independent standards for topical antiseptics.]

The incubation temperatures used for this study (LIMS 7801) for the genera and species listed in Table 1 are described as 35°C±2°C or appropriate temperature for individual isolates and duration's of incubation ranged from 16.0 to 72.0 hours (Volume 1.20, Tab 7.14.1.1, section 10.2.13 and 11.9). Evaluations of each of the reports for each genera/species suggest wide variability in the duration of incubation. The requirements described by the NCCLS as referenced by the applicant are 35°C for 16 to 20 hours in a forced air incubator. When testing *Haemophilus*, trays should be incubated for a total of 20 to 24 hours prior to determining results. What the applicant presented is in clear violation of the standard they referenced.

During a May 18, 2000 teleconference where the issue of incubation temperature was addressed, the applicant was asked to justify the use of time frames that ranged outside the time frames recommended by the reference method. The representatives responded that they were aware that some of the strains had been tested outside the recommended time frames but not all and they were willing to separate the data for us so that we could assess whether sufficient strains were available to justify inclusion in the package insert. The data was to be present for those strains that complied with the standard method of incubation for 16 to 20 hours and those for the time frames that supercede the recommended time frames. This data was provided in a May 30, 2000 fax transmission.

The data provided by the applicant is divided into two separate categories as requested. The first are supposed to be the strains that represent the genera and species that were incubated for 16 to 20 hours. Instead, the applicant provided time frames that ranged from 16 to 24 hours. The other time frames ranged from 24 hours to 71 hours. Clearly, the applicant did not analyze the data as requested so I will have to perform an independent analysis of the data to assess how many strains are actually over the 20-hour time frame for all but *Haemophilus* spp.

Evaluation of the information suggests that increased incubation time did not produce results that differed from those that were performed according to the reference standard. The results are indistinguishable and the modification is accepted.

Conclusions: The applicant provided arguments, which are reasonable and are supported by the requirements of the TFM even though, outdated. All of the organisms studied and currently listed

in the PI will be accepted with the exception of *H. influenzae* and *Streptococcus pneumoniae* as discussed below.

Questions for the *In vitro* spectrum of activity studies. (LIMS 7801):

a). Why were the *in vitro* susceptibility studies performed with a method that differs from that recommended by the tentative final monograph (TFM)? The TFM requires the use of very specific standardized methods as described by the National Committee for Clinical Laboratory Standards.

Applicant's response: The LIMS 7801 MIC study "was performed under the methodology referenced by the NCCLS document M7-A2. Since the NCCLS documents are prepared for antibiotic susceptibility testing and not for MIC's of topical antiseptics, it necessitated modification to be made for use with topical antiseptic products and the organisms specified by the TFM. The study was performed under Good Laboratory Practices guidelines. Every test parameter contained a positive, negative and reference control."

Reviewer's response: The question asked of the applicant is based on observations of MIC methodologies used in studies performed with *H. influenzae* and *Streptococcus pneumoniae*. The 1990 standard referenced by the applicant provides part of the answer to this question since the question was actually based on the use of the most recent standard. However, Haemophilus should have been tested in Haemophilus Test Medium (HTM) and incubated at 35°C/20-24 hours. HTM is CAMHB with the addition of hematin. Review of the Protocol presented in Volume 1.20, Tab 7.14.1.5 states that the antiseptics were prepared in MHB in horse defibrinated lysed blood, or in [redacted] Broth in lysed horse blood and incubated for 19-71 hours at 35°C ± 2°C in 5% ± 1% CO₂. Thus the methodology used by the applicant differs from that of the NCCLS in that different media is used, the duration of incubation is much longer than allowed and there is no requirement for CO₂.

Similar deviations from accepted methodology were noted for *Streptococcus pneumoniae* in that CAMHB with laked sheep blood was not used to perform the broth MIC study and incubation was extended from 18-24 hours to 17-48 hours.

In conclusion, *H. influenzae* and *Streptococcus pneumoniae* will not be allowed in the PI until studies are performed with the methods described in their standard.

b). The analysis performed by the applicant on preclinical data was exclusively a comparison of ranges obtained with the test and control product and is not sufficient to draw conclusions that the test and control products produce analogous results. The following types of data presentation would be substantially more informative and it is highly recommended that they be provided for strains that have been tested using acceptable standardized methodology.

1). The applicant should perform frequency distributions of the data and present them as histograms to allow comparison of results between test and control product. A discussion of the results should be provided for each organism and a summary of this data as a whole.

Applicant's Response: Appendix A of the May 4, 2000 submission provided the histograms and a summary table, which compared the frequency distributions of MIC's for HPD-5a and Hibiclens versus 272 isolates. The frequency distributions are very similar but "there is a slight shift to the right of about 1 dilution stop" for the test versus the control.

Reviewer's response: The population distribution presented in Appendix A.1 shows that there is overlap of the susceptibility patterns of the test and control drugs as expected but the Hibiclens does appear to have a slight advantage in that MIC's are shifted towards the more susceptible side. The MIC range for both antiseptics is 0.5 to 128 µg/mL.

Organisms with the highest MIC's ($\geq 16 \mu\text{g/mL}$) were *Acinetobacter* spp., *Enterobacter* spp., *P. aeruginosa*, *P. mirabilis*, *S. marcescens*, *S. aureus*, and *S. pneumoniae* but in all cases the MIC value is substantially below the maximal possible exposure of 20000 $\mu\text{g/mL}$. The analysis presented by the applicant is substantially more informative than that previously submitted and is satisfactory. However, the applicant is correct in that the number of strains required for each genera/species is well below the number suggested by the TFM and the data is not acceptable.

***In vitro* summary of the preclinical studies:** The applicant did not use the most recent standardized methods required to demonstrate susceptibility of nosocomial pathogens to their product. They did use the method described in the TFM, which is now 10 years old. We have no recourse but to accept the data since clear direction was not provided to the applicant and they did not have the initiative to request clarity of the use of a standard which is 10 years old. The standard is update every 3 years and the applicant should have used the most current references to perform their studies.

With the exception of *Streptococcus pneumoniae* and *S. pyogenes*, and *Haemophilus influenzae*, all genera and species studied are supported by acceptable data and can be included in the package insert.

2). A comparison should be performed between the histograms of laboratory and clinical isolates to determine where similar patterns are noted. Although the sample size is small, the discussion should describe population distributions between the laboratory and clinical isolates.

Applicant's response: The applicant performed the analysis as requested and concluded that frequency distributions of the ATCC and clinical isolates are similar.

Reviewer's response: Table B.1 of the May 4, 2000 amendment (BI) provides the frequency distributions, which allow me to compare the clinical and nonclinical isolates (ATCC). These histograms show clearly that they are no differences between the two collections. The analysis is satisfactory and acceptable.

3). A discussion should be provided on the susceptibility profile of antibiotic susceptible and resistant laboratory and clinical isolates. Does the fact that the organism has a resistance phenotype to an antibiotic increase the likelihood that the CHG MIC will be elevated relative to antibiotic susceptible population?

Applicant's response: The applicant states that the TFM reference does not require MIC testing with resistant strains and few were included in the study. The applicant is correct but they did provide a discussion in the original submission and clarity was sought as to the number and type of strains that were evaluated.

Reviewer's response: The labeling of this product will not allow any statements that suggest that HPD-5a is active against *Staphylococcus aureus* methicillin resistant (MRSA), *S epidermidis* methicillin resistant (MRSE), nor *Enterococcus faecalis* multi-drug resistant based on MIC studies.

B. The results of the neutralizer effectiveness study are based on time of exposure of the CHG to neutralizer. This duration of exposure must mimic what was used in the time-kill study. During the conduct of the time-kill kinetic study, it is not clear from reading the protocol whether the duration of time that a sample was held prior to serial dilution is the same as the duration of time used to validate neutralization. This information must be provided.

Question for the *in vitro* time-kill kinetic study (LIMS 8071):

1) What is the maximum time interval that time-kill kinetic samples were stored in neutralizer prior to dilution? How does this value compare to the duration of time used in the neutralization validation studies?

Applicant's response: In the time kill kinetic studies, the samples were not stored for any amount of time after neutralization and were serially diluted immediately. The neutralizer validation test was conducted using the same procedure.

Reviewer's response: The response is satisfactory since the time between sampling and neutralization was immediately after acquisition of the sample.

Questions for the *in vitro* time-kill kinetic neutralizer validation study (LIMS 8257):

2) How long were neutralizer and the 1:10 dilution of test/control product allowed to sit after mixing before the indicator organism was added to the neutralizer? The indicator organism should be added prior to the test/control product. Why was a 1:10 dilution of the test/control product selected to validate the study?

Applicant's response: In the neutralizer validation study, the organisms were added immediately after the product had been neutralized in order to assure the test product inactivation. The ASTM E 1054-91 method "Standard Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant Sanitizers, Antiseptics or Preserved Products" was followed. Based on this protocol, validation requires that the neutralizer be added to the product prior to inoculation with the indicator organism.

Reviewer's response: I disagree with this concept because the study is biased towards success of neutralization if the antiseptic and neutralizer are allowed to sit for any length of time before the indicator organism is added to the mixture. Any time delay in the addition of the organism to the mixture of neutralizer and product results in a potential bias that needs to be addressed. The organism and neutralizer should be mixed first followed by the addition of the antiseptic because this more closely follows the conditions of the clinical simulation study. This issue was conveyed to the applicant in a teleconference on May 18, 2000. The representatives responded by stating that the addition of the antiseptic was followed immediately by the indicator organism. There was no time delay between the two steps. Therefore, the validation study and data are accepted, as are the time-kill kinetic studies.

3) According to the information provided in volume 1.19, Section 7.4, it appears that the toxicity studies were performed with neutralizer that has been dilute 10^{-1} , 10^{-2} and 10^{-3} as stated in the protocol. Why was the toxicity study performed with diluted neutralizer?

Applicant's response: The applicant states that the toxicity studies were not performed with diluted neutralizer and provides wording that describes the process more clearly than provided originally in original submission.

Reviewer's response: The explanation is accepted.

C) There appear to be errors in the technical conduct of the neutralization validation studies submitted for the surgical hand rub indication, and the sample used to validate the study does not represent the worst case scenario expected in the clinical simulation studies. It appears, based on the protocol provided that the product was only used once and that sample used to validate the study.

Questions for the surgical rub neutralization validation study (LIMS 7838):

1) Based on the protocol that was provided, the marker organism concentration at time zero should be 10 CFU/mL. According to the results presented serial dilutions up to 10^{-3} were necessary to achieve this value. Please explain this noted discrepancy.

Applicant's response: Again clarity was provided regarding the description of the process. Basically, the applicant made an incorrect estimation of the cell density.

Reviewer's response: The response is acceptable.

2) The conduct of the neutralization study should mimic the conduct of the clinical simulation study in every aspect possible to reduce bias and the introduction of variability. Why was agar containing 0.02% tellurite and 3.0-g/mL tetracycline HCL used as the plating media to measure survival? Why couldn't the plating media used in the clinical simulation studies have been used in this step?

Applicant's response: The use of agar with tetracycline was required to assure the selection of the *Staphylococcus aureus* marker organism used in the study. The media and antibiotic were used to assured that selective conditions were created to provide differential characteristics that selected for the marker organism only.

Reviewer's response: I understand why this approach was used but it is unclear what effect this process may have on the validation of the neutralization process since it introduces another variable to the study. Although informative, it is an indirect method of performing validation of the method. In order to assure consistency and reproducibility in future validation of methods, all future studies must be performed with the media and reagents used in the clinical simulation studies. The response is accepted.

3) It is not clear from the information provided what the duration of time was between the addition of the CHG containing microbial sample to the neutralizer and the addition of the inoculum to the aforementioned combination. Please provide an explanation of the time intervals between the addition of the CHG to the neutralizer and the addition of the marker organism to the aforementioned mixture. What was the duration of "incubation"?

Applicant's response: The sample was removed from the glove, placed into the neutralizer and immediately inoculated, diluted and plated for the time $T_{=0}$ time point.

Reviewer's response: The issue was discussed at length with the applicant and it was discovered that all neutralization validation studies were performed by adding the sample to the neutralizer and immediately adding the marker organism to the mixture. In addition, a study was performed and submitted on May 18, 2000, which supports the neutralization validation results. The response is satisfactory.

Questions for both the surgical rub neutralization validation studies (LIMS 7838 and LIMS 7957):

4). The subjects, who provide the samples to be used in the neutralization validation experiments, appear to have used the product only once. The sample used to validate the neutralizer should be obtained from individuals that represent the maximal number of exposures as described in the surgical hand scrub protocol. This mimics the worst case scenario regarding carry over of CHG. Thus, it seems that the subjects should have used the product at least 11 times and neutralization validation samples obtained from them. Why was this not done?

Applicant's response: The applicant responded by stating that the test facility has considerable experience in the conduct of these studies and has always performed the neutralization study after the first exposure. Panelists also used products other than the test and control products during the 5 days of study and therefore it would be highly unlikely that differences would be seen. The applicant did provide a study dated May 17, 2000 in which panelists performed 11 wash exposures and then were sampled for neutralization validation. This provides the worst case scenario.

It was also stated that the neutralization validation was performed by adding the antiseptic to the neutralizer, mixing, and followed by the indicator organism. No time elapsed between the addition of any of the components to the neutralizer.

Reviewer's response: The issue was discussed at length with the applicant and it was discovered that all neutralization validation studies were performed by adding the sample to the neutralizer and immediately adding the marker organism to the mixture. In addition, a study was performed and submitted on May 18, 2000, which supported the neutralization validation results. The response is satisfactory.

Question for the surgical rub neutralization validation study (LIMS 7957):

5) It is not clear from the information provided what the duration of time was between the addition of the CHG containing microbial sample to the neutralizer and the addition of the inoculum to the aforementioned combination. Please provide an explanation of the time intervals between the addition of the CHG to the neutralizer and the addition of the marker organism to the aforementioned mixture. What was the duration of "incubation"?

Applicant's response: The sample was removed immediately from the glove and placed into neutralizer and immediately inoculated, plated and diluted.

Reviewer's response: The issue was discussed at length with the applicant and it was discovered that all neutralization validation studies were performed by adding the sample to the neutralizer and immediately adding the marker organism to the mixture. In addition, a study was performed and submitted on May 18, 2000, which supported the neutralization validation results. The response is satisfactory.

/S/

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Team Leader, Microbiology Reviewer

Cc: Original NDA No. 21-074
Microbiologist, HFD-520
File name: 21-074_#2

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/S/ 6/7/00

Cc: Original NDA 21-074
HFD-473
HFD-520/DepDir/LGavrilovich
HFD-520/Smicro/ATSheldon
HFD-520/MO/DBostwick
HFD-520/Pharm/AEllis
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20 page(s) have been removed because it contains trade secret and/or confidential information that is not disclosable.