Appendix A

August 20, 2003

FINAL REPORT #030408-150

EVALUATION OF THE IMMEDIATE, PERSISTENT, AND RESIDUAL ANTIMICROBIAL PROPERTIES OF FOUR TEST PRODUCTS AND A REFERENCE PRODUCT USED AS HEALTHCARE PERSONNEL HANDWASHES

Prepared for:

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EXECUTIVE SUMMARY

Overview

This surgical scrub study evaluated the immediate, persistent, and residual antimicrobial properties of four (4) test products used in several configurations: two (2) waterless and three (3) water-aided, and of one (1) reference product. The Triseptin Water-Optional formulation was used in both waterless and water-aided configurations. Evaluation methods were based on a Surgical Scrub procedure. A reference product was used to assure the internal validity of the study. The test period of the study was five (5) consecutive days. Nine (9) subjects were randomly assigned to each of the five (5) test product configurations, and four (4) subjects were to the reference product, for a total of forty-nine (49) subjects. Three (3) test time point samples -- immediate, four (4) hour, and eight (8) hour -- were collected for the test product configurations. Two (2) time points -- immediate and eight (8) hour -- were collected for the reference product. The study required a baseline week with hand-sampling on Days 1 and 5. During test week, hand-sampling was performed on Test Days 1, 3 and 5, with product applications performed over the entire five (5) day test period. All results must be interpreted on the basis of the specific product-use instructions provided by the Study Protocol.

Results: Waterless Applications

The Triseptin Waterless Surgical Scrub (Test Product Configuration #1) demonstrated reductions from baseline population of more than three (3) $\log_{10}$. Additionally, the product demonstrated significant persistent antimicrobial properties (no regrowth of microbial populations to baseline level). It is difficult from the results to determine the residual properties of this product, for it demonstrated greater than a 3 $\log_{10}$ reduction in microorganisms on all three (3) sample days. This product demonstrated significantly greater antimicrobial activity than any of the other products tested. Avagard™ D (Test Product Configuration #3) demonstrated statistically significant $\log_{10}$ microbial reductions from baseline, but did not achieve a 3 $\log_{10}$ reduction on Test Day 5. Additionally, microbial regrowth eight (8) hours post-product-application averaged about 1.9 $\log_{10}$ greater than the population in the immediate time sample. The reference product, Hibiclens® with 4% chlorhexidine gluconate (Reference Product Configuration #6), demonstrated $\log_{10}$ reductions similar to those observed under comparable conditions.

Results: Water-aided Applications

The results from the test product, Triseptin (Test Product Configuration #2), when used as a water-aided surgical scrub formulation, were compared with those for the other three (3) water-aided products. Triseptin demonstrated highly significant antimicrobial properties when used as a water-aided product but did not achieve a 3 $\log_{10}$ reduction on Day 5. It did, however, demonstrate greater antimicrobial properties than any of the other products tested. It also demonstrated a high degree of antimicrobial persistence over the eight (8) hour post-product-application period and a significant residual effect, increasing in immediate microbial kill an average of 0.25 $\log_{10}$ per day of use. Bactoshield® Antimicrobial Skin Cleanser (Test Product Configuration #5) and Hibiclens® with 4% chlorhexidine gluconate (Reference Product Configuration #6) were statistically equivalent in $\log_{10}$ microbial reductions over the five (5) day test period. Both products produced adequate antimicrobial persistence and demonstrated significant residual antimicrobial activity, increasing in immediate microbial kill at a rate of about one-third $\log_{10}$ per day. The CV® Medicated Lotion Soap (Test Product Configuration #4) did not demonstrate significant antimicrobial properties over the five (5) day test period.

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1.0 TITLE: **EVALUATION OF THE IMMEDIATE, PERSISTENT, AND RESIDUAL ANTIMICROBIAL PROPERTIES OF FOUR TEST PRODUCTS AND A REFERENCE PRODUCT USED AS HEALTHCARE PERSONNEL HANDWASHES**

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4.0 STUDY DIRECTORS:

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Daryl S. Paulson, Ph.D. - Statistical Analysis and Interpretation

5.0 PURPOSE:

This study evaluated the immediate, persistent, and residual antimicrobial properties of four (4) test products used in several configurations, two (2) waterless and three (3) water-aided, and of one (1) internal reference product. Triseptin Water-Optional was used in both waterless and water-aided configurations. Evaluation methods were based on a Surgical Scrub procedure. The test period of the study was five (5) consecutive days.

6.0 SCOPE:

This study evaluated and compared the immediate, persistent, and residual antimicrobial effectiveness of four (4) test products. A reference product was used to assure the internal validity of the study. Nine (9) subjects were utilized for each of the five (5) test product application configurations, and four (4) subjects were used for the reference product configuration, for a total of forty-nine (49) subjects. Three (3) test time point samples -- immediate, four (4) hour, and eight (8) hour (see Protocol and/or SOP Deviation Recording Form in Addendum I of this Final Report) -- were collected for the test product configurations. Two (2) time points -- immediate and eight (8) hour (see Protocol and/or SOP Deviation Recording Form in Addendum I of this Final Report) -- were collected for the reference product configuration. The study required a baseline week with hand-sampling on Days 1 and 5. During test week, hand-sampling was performed on Test Days 1, 3 and 5, with product applications performed over the entire five (5) day test period.

7.0 TEST MATERIALS:

The Test and Control Products were supplied by Sponsor and identified by lot number. Records of lot numbers utilized were maintained by Sponsor. Responsibility for the identity, strength, purity, composition, and stability of the Triseptin test product lay with Sponsor. Other test products and the reference product were obtained OTC.

Test Product Configuration #1:	Triseptin Water-Optional (used in a Waterless application)
Lot Number:	TBDI
Expiration Date:	07/04

Test Product Configuration #2:	Triseptin Water-Optional (used in a Water-aided application)
Lot Number:	TBDI
Expiration Date:	07/04
Test Product Configuration #3:	Avagard™ D (Waterless Test Product)
Lot Number:	JAN 03 002
Expiration Date:	12/04
Test Product Configuration #4:	CV® Medicated Lotion Soap (Water-aided Test Product)
Lot Number:	22924924A3
Expiration Date:	12/04
Test Product Configuration #5:	Bactoshield® Antimicrobial Skin Cleanser (Water-aided Test Product)
Lot Number:	210-880
Expiration Date:	09/04
Reference Product Configuration #6:	Hibiclens® 4% CHG (Water-aided Reference Product)
Lot Number:	300700
Expiration Date:	02/05

8.0 EQUIPMENT:

8.1 Steam Autoclaves: BSLI 91113, BSLI 91127, and BSLI 010501
8.2 Laminar Biological Flowhood (certified): BSLI 91119
8.3 Wash Sinks: BSLI 91114 and BSLI 960101
8.4 Wash Sink Thermometers: BSLI 000701, BSLI 000902, and BSLI 020110
8.5 Continuously Adjustable Pipetter, 20 µL - 200 µL Capacity: BSLI 991205
8.6 Continuously Adjustable Pipetters, 100 µL - 1000 µL Capacity: BSLI 991001, BSLI 991204, and BSLI 011002
8.7 Portable Pipetters: BSLI 971206, BSLI 980601, BSLI 980602, and BSLI 001002
8.8 Positive Displacement Pipetter: BSLI 011203
8.9 Autoplate 4000 Spiral Plater: BSLI 980409
8.10 Q-Count Plate Counting System: BSLI 000906
8.11 Incubators, 30° ± 2°C: BSLI 930712 and BSLI 011011
8.12 Incubator Thermometers: BSLI TI-011109, BSLI TI-000814, and BSLI TI-000815
8.13 Environmental Chamber, 30° ± 2°C: BSLI 930214
8.14 Environmental Chamber Thermometers: BSLI TI-000813 and BSLI TI-960611
8.15 Refrigerators, 2° - 8°C: BSLI 000401, BSLI 011204, and BSLI 021201
8.16 Refrigerator Thermometers: BSLI TI-000804, BSLI TI-000805, BSLI TI-011103, BSLI TI-011102, BSLI TI-021013, and BSLI TI-021012
8.17 Environmental Chamber, 2° - 8°C: BSLI 930212
8.18 Environmental Chamber Thermometers: BSLI TI-960106 and BSLI TI-021007
8.19 Vortex Mixers: BSLI 931201 and BSLI 980103
8.20 Calibrated Minute/Second Timers: BSLI 961010, BSLI 980405, BSLI 020201, BSLI 020202, BSLI 020203, BSLI 020204, BSLI 020205, BSLI 020206, BSLI 021016, BSLI 021017, BSLI 021018, and BSLI 021019
8.21 Calibrated NIST Traceable Clocks: BSLI 020107, BSLI 020108, BSLI 020109, and BSLI 030303
8.22 Orion pH Meter Model 720: BSLI 931104
8.23 A & D Balance Model EK-2000G: BSLI 960801
8.24 Scientech 404D Balance: BSLI 011009
8.25 Troemner Weights: BSLI 930408
8.26 Ohaus Weights: BSLI 961011
8.27 Hewlett-Packard HP-15C Hand Calculator
8.28 Texas Instruments TI-35X and TI-36X Hand Calculators
8.29 MiniTab® Statistical Software (PC Version, 10xtra and 13)

9.0 SUPPLIES:

9.1 Sterile 1 cc Capacity Syringes: Becton Dickinson Lot Number 1004514
9.2 Sterile 10 cc Capacity Syringes: Becton Dickinson Lot Numbers 2336892 and 3020709
9.3 Sterile 5.0 mL Capacity, Serological Pipettes: Sterilin Lot Numbers 306408, expires 11/07 and 318408, expires 01/08
9.4 Sterile Dilution Bottles and Tubes
9.5 Sterile Disposable Petri Plates, 100mm x 15mm: American Precision Plastics Lot Numbers 03726821 and 03765721
9.6 Sterile 400 mL Capacity Beakers
9.7 Disposable Aprons: Ansell Edmont, no lot numbers available
9.8 Sterile Powder-Free, Latex Gloves: Ansell Edmont Lot Numbers 0209520604, 0210535904, and 0301404404
9.9 Non-Sterile Exam Gloves: American Health and Safety, no lot numbers available, High Five Lot Number DY98036, and Ansell Edmont Lot Number 0210535904
9.10 Sterile Scrub Brushes/Nail Cleaners: Becton Dickinson, no lot numbers available
9.11 Nonmedicated Soap (Baby San®): Ecolab Lot Number L1216911, container expires 12/14/06
9.12 Universal 1.0 mL Pipette Tips, Sterilized
9.13 Sterile 1.0 mL Capacity Tips: IBP Lot Numbers 70,202, 70,262, and 81,765
9.14 Sterile 0.1 mL Capacity Tips: IBP Lot Number 66,495
9.15 Positive Displacement Tips: Gilson Batch Number B0172223S

10.0 TEST SOLUTIONS AND MEDIA:

Sampling Solution

10.1 Sterile Stripping Fluid (SSF): SSF030829A, SF030829B, SSF030830C, SSF030830D, and SSF030919D

Neutralizing/Diluting Fluid

10.2 Butterfield's Phosphate Buffer Solution with Product Neutralizers (BBP++): BBP++030710D, BBP++030904D, and BBP++030923B

10.3 Phosphate Buffered Saline Solution (PBS) for Neutralization Assay: PBS030809A

Media

10.4 Tryptic Soy Agar with product neutralizers (TSA+): TSA+030828B, TSA+030904A, TSA+030904B, TSA+030911F, TSA+030911G, TSA+030911M, TSA+030912A, TSA+030913C, TSA+030916A, TSA+030916B, TSA+030918A, TSA+030918B, TSA+030919A, TSA+030919B, TSA+030924C, TSA+030927A, and TSA+031008A

10.5 Tryptic Soy Agar (TSA) for Neutralization Assay: TSA030815B and TSA030924A

10.6 Tryptic Soy Broth (TSB) for Neutralization Assay: TSB030704C

11.0 NEUTRALIZATION:

A neutralization study was performed to assure the validity of the neutralizer(s) used in the recovery medium. The neutralization followed guidelines set forth in ASTM E 1054-02, *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*, except that the microorganisms were added to the neutralizer prior to the addition of the test or internal reference antiseptic. Simulated-use neutralization study was performed with the Triseptin Waterless application. *Staphylococcus epidermidis* (ATCC #12228) was used as the challenge species in the neutralizer validation study. Effective neutralization of the antimicrobial activity of the test and reference products was demonstrated. Results are presented in Addendum VI.

12.0 TEST METHODS:

Institutional Review Board

12.1 Informed Consent Forms and any other supportive material relevant to the safety of the subjects were supplied by principal investigators to the Gallatin Institutional Review Board (GIRB) for their review and approval. The primary purpose of the GIRB is the protection of the rights and welfare of the subjects involved (reference CFR 21 Parts 50, 56, 312, and 314). This study began only after GIRB approval was obtained. This study was conducted in compliance with Good Clinical Practices requirements; the standard operating procedures of BioScience Laboratories, Inc., and the study protocol (see Protocol and/or SOP Deviation Recording Forms in Addendum I of this Final Report).

Subjects

12.2 One-hundred and three (103) overtly healthy subjects over the age of eighteen (18), but under the age of seventy (70) years were admitted into the study. Forty-nine (49) subjects completed the study. Of the forty-nine (49) subjects who completed the study, sixteen (16) were male, thirty-three (33) were female, forty-six (46) were Caucasian, one (1) was African American, one (1) was Hispanic/American, and two (2) were Caucasian/Native American. The median age of the forty-nine (49) subjects who completed the study was twenty-seven (27) years, with nineteen (19) being the youngest and sixty-five (65) the oldest. All subjects were free from clinically-evident dermatoses or injuries to the hands or forearms. All subjects signed Informed-Consent Forms and Study Descriptions (Addendum I, Appendix I) prior to participating in the study.

12.3 A Study Description and the Informed Consent statement were provided to each subject prior to beginning the study. Trained laboratory personnel explained the study to each participant and were available to answer any questions that arose. Only subjects meeting the inclusion criteria (Addendum I, Sections 12.6 - 12.10) and none of the exclusion criteria (Addendum I, Sections 12.11 - 12.21) were admitted into the study (see Protocol and/or SOP Deviation Recording Form in Addendum I of this Final Report). After admission into the study, subjects could withdraw at any time for any reason. Details of subject withdrawals are explained in the Subject Disposition Table in Addendum VII of this Final Report.

Pre-Test Period

12.4 The seven (7) days prior to the baseline portion of the study constituted the pre-test period. The pre-test product restriction period allows for stabilization of the normal microbial populations residing on the hands. During this time, subjects avoided the use of medicated soaps, lotions, and shampoos, as well as skin contact with solvents, detergents, acids and bases, or any other products known to effect the normal microbial populations of the skin (Addendum I, Appendix II). Non-antimicrobial personal hygiene products were supplied to the subjects, and these products were used exclusively throughout the period of study. Subjects also avoided using UV tanning beds and swimming or bathing in biocide-treated pools or hot tubs.

Randomization

12.5 Subjects were assigned randomly to one (1) of the five (5) test configurations or to the reference product. Thus, each person was assigned to any one (1), and only one (1), of the six (6) product-use configurations.

12.6 Test product subjects were randomly assigned two (2) of three (3) sample times, one (1) for each hand. Reference product subjects only had two (2) sample times, one (1) for each hand.

Baseline Period

12.7 Baseline-sampling was conducted on Days 1 and 5 during the week (baseline week) following the seven (7) day pre-test period. The baseline populations measured constituted the baseline values for each subject.

12.8 Subjects clipped their fingernails to = 1 mm free-edge and removed all jewelry from their hands and forearms. For each baseline determination, the hands were washed under laboratory supervision using a liquid, nonmedicated baseline soap (BabySan®), as follows:

12.7.1 All washes/rinses were performed in tap water regulated at 40° ± 2°C (see Protocol and/or SOP Deviation Recording Form in Addendum I of this Final Report).

12.7.2 Subjects rinsed their hands, including the lower two-thirds (2/3) of their forearms, under running tap water for thirty (30) seconds. During this rinse, the subjects cleaned their fingernails using a nail cleaner.

12.7.3 A 5 mL aliquot of nonmedicated, baseline soap (BabySan®) was dispensed into the subjects' cupped hands. Subjects then washed their hands and forearms for thirty (30) seconds, using water as required to lather the soap. During this procedure, subjects maintained their hands positioned higher than their elbows.

12.7.4 Subjects rinsed their hands and forearms thoroughly for thirty (30) seconds under running tap water to remove all lather.

12.7.5 This procedure was followed by performance of the Glove Juice Procedure (Section 12.12).

Experimental Period

12.9 The forty-nine (49) subjects accepted into the study were assigned randomly to one (1) of the six (6) product configurations. Test product sample times were randomized between hands for two (2) of the three (3) sample times – immediate, four (4) hours, or eight (8) hours (see Protocol and/or SOP Deviation Recording Form in Addendum I of this Final Report). This entailed using a blocked design, where each set of three (3) subjects was assigned randomly one (1) of nine (9) sample time configurations (Table I). Reference product sample times were randomized to hands for the two (2) sample times – immediate and eight (8) hours.

TABLE I: TEST PRODUCT SAMPLE TIMES

Sample Time Block Configuration					
Block			Sample Times		
1	2	3	Immediate	4 Hours	8 Hours
1	4	7	X	X	
2	5	8	X		X
3	6	9		X	X

Test Period

12.10 Each subject was utilized for approximately one and one-half (1.5) hours each day for five (5) consecutive days. Prior to being admitted into testing, subjects were questioned regarding their adherence to the Protocol requirements.

Product Application

12.11 The products were used by subjects according to the following directions four (4) times each day, with a minimum of fifteen (15) minutes between each product application (see Protocol and/or SOP Deviation Recording Form in Addendum I of this Final Report). The water temperature used for the hand washes was regulated at 40° ± 2°C (see Protocol and/or SOP Deviation Recording Form in Addendum I of this Final Report).

Test Product Configuration #1 and Test Product Configuration #3 (Waterless Test Products)

12.11.1 Subjects clipped their fingernails to = 1 mm free-edge and removed all jewelry from their hands and forearms.

12.11.2 Subjects were supplied a sufficient amount of test product (five [5] to nine [9] mL, depending upon their hand-size) to wet all surfaces thoroughly. The product was applied to clean, dry hands and nails, paying particular attention to inter-digital spaces, fingernails, and cuticles.

12.11.3 The subjects' hands were allowed to dry without wiping.

12.11.4 When the hands were to be sampled, subjects' hands and forearms were allowed to air-dry for five (5) minutes prior to donning gloves for the Glove Juice Sampling Procedure (Section 12.12).

Test Product Configuration #2 (Water-aided Test Product)

12.11.5 Subjects clipped their fingernails to = 1mm free-edge and removed all jewelry from their hands and forearms.

12.11.6 Subjects were supplied a sufficient amount of test product (five [5] to nine [9] mL, depending upon their hand-size) to wet all surfaces thoroughly. The product was applied to clean, dry hands and nails, paying particular attention to inter-digital spaces, fingernails, and cuticles. Subjects rubbed their hands together for fifteen (15) seconds.

12.11.7 Subjects rinsed their hands with tap water.

12.11.8 For samples immediately following washes, the hands were gloved wet. For washes not followed immediately by a sample, the hands were patted dry, using a disposable paper towel. The hands were sampled using the Glove Juice Sampling Procedure (Section 12.12).

Test Product Configuration #4, Test Configuration #5, and Reference Product Configuration #6 (Water-aided Test Products)

12.11.9 Subjects clipped their fingernails to = 1mm free-edge and removed all jewelry from their hands and forearms.

12.11.10 Subjects wet their hands thoroughly.

12.11.11 A 5 mL aliquot of the reference product was dispensed into the subjects' cupped hands.

12.11.12 Subjects rubbed their hands together for thirty (30) seconds, covering all surfaces of hands and fingers.

12.11.13 Subjects rinsed their hands for thirty (30) seconds.

12.11.14 For samples immediately following washes, the hands were gloved wet. For washes not followed immediately by a sample, the hands were patted dry, using a disposable paper towel. The hands were sampled using the Glove Juice Sampling Procedure (Section 12.12).

Glove Juice Procedure

12.12 Following the fourth wash on Test Days 1, 3, and 5, powder-free, loose-fitting, sterile latex gloves were placed on subjects' hands at Time Zero (0), immediately after product usage. At the assigned sample time (immediately, four [4] hours, or eight [8] hours post-wash; see Protocol and/or SOP Deviation Recording Form in Addendum I of this Final Report), or for baseline samples, 75.0 mL of Sterile Stripping Fluid without product neutralizers were instilled into the appropriate glove (according to the randomization). The wrist was secured, and an attendant massaged the hand through the glove in a standardized manner for sixty (60) seconds. A 5 mL aliquot of the glove juice was removed from the glove and diluted in 5 mL of Butterfield's Phosphate Buffer Solution with product neutralizers (dilution 10^0). The 10^0 dilution was then serially diluted in Butterfield's Phosphate Buffer Solution with product neutralizers.

Plating

12.13 Duplicate spread and/or spiral plates were prepared from the appropriate glove-juice dilutions using Tryptic Soy Agar with 0.07% Lecithin (w/v) and 0.5% Tween 80 (w/v). The plates were incubated at 30° ± 2°C for approximately seventy-two (72) hours, or until sufficient growth was observed.

Data Collection

12.14 Following incubation, the colonies on the plates were counted and the data recorded using the computerized Q-count® plate-counting system. If 10^0 plates gave an average count of zero (0), the average plate count was expressed as 1.00.

Data-Handling

12.15 The estimated $\log_{10}$ number of viable microorganisms recovered from each hand was designated the "R-value," the adjusted average $\log_{10}$ colony count measurement for each subject at each sampling time. Each R-value was determined using the following formula:

$$R = \log_{10}[F \times C_i \times 10^{-D}] \times 2$$

Where:

F	=	The amount of sterile sampling solution instilled into glove (In this Study, F = 75 mL);
C_i	=	The arithmetic average colony-count from the two (2) plates for each hand at a particular dilution level;
D	=	The dilution factor; and
2	=	The neutralization dilution.

NOTE: The reason that a $\log_{10}$ transformation was performed on the collected data was to convert them to linear scale. A linear scale, more appropriately a $\log_{10}$ linear scale, is a basic requirement of the statistical models used in this study.

13.0 STATISTICAL ANALYSIS:

13.1 The MiniTab® statistical computer package was used for all statistical calculations.

13.2 The six (6) test product configurations were analyzed statistically on the basis of application procedure. Test product configuration #1 was compared to test product configuration #3. Test product configuration #2 was compared to test product configurations #4 and #5. The reference product configuration was used for internal validation only.

14.0 ADVERSE EVENTS:

Subject #95 experienced an adverse event possibly related to use of Test Product Configuration #5 (Bactoshield® Antimicrobial Skin Cleanser), which resolved following treatment.

15.0 RESULTS:

Table II presents the descriptive statistics obtained following application according to Test Product Configuration #1 (Triseptin [used in a Waterless application]). Table III presents the descriptive statistics obtained following application according to Test Product Configuration #2 (Triseptin [used in a Water-aided application]). Table IV presents the descriptive statistics obtained following application according to Test Product Configuration #3 (Avagard™ D [Waterless Test Product]). Table V presents the descriptive statistics obtained following application according to Test Product Configuration #4 (CV® Medicated Lotion Soap [Water-aided Test Product]). Table VI presents the descriptive statistics obtained following application according to Test Product Configuration #5 (Bactoshield® Antimicrobial Skin Cleanser [Water-aided Test Product]). Table VII presents the descriptive statistics obtained following application according to Reference Product Configuration #6 (Hibiclens® 4% CHG [Water-aided Reference Product]). Figure 1 is the graphical presentation of the $\log_{10}$ reductions from baseline values for Test Product Configuration #1, Test Product Configuration #3, and Reference Product Configuration #6. Figure 2 is the graphical presentation of the $\log_{10}$ reductions from baseline values for Test Product Configuration #2, Test Product Configuration #4, Test Product Configuration #5, and Reference Product Configuration #6. All results should be interpreted on the basis of the specific product-use instructions provided by the Study Protocol.

Table II: Statistical Summary of the $\log_{10}$ Recovery Values for Test Product Configuration #1 Triseptin Water-Optional (used in a Waterless application)

Sample	Sample Size	Mean	Standard Deviation	95% Confidence Interval	$\log_{10}$ Reduction
Baseline	18	6.06	0.29	5.92 to 6.21	N/A
Day 1, Immediate	6	2.56	1.24	1.26 to 3.87	3.50
Day 1, 4 Hour	6	2.57	0.27	2.29 to 2.85	3.49
Day 1, 8 Hour	6	2.76	0.51	2.22 to 3.29	3.30
Day 3, Immediate	6	2.23	0.37	1.84 to 2.61	3.83
Day 3, 4 Hour	6	2.76	1.47	1.22 to 4.30	3.30
Day 3, 8 Hour	6	2.45	0.73	1.68 to 3.21	3.61
Day 5, Immediate	6	2.31	0.24	2.06 to 2.56	3.75
Day 5, 4 Hour	6	2.10	0.28	1.81 to 2.38	3.96
Day 5, 8 Hour	6	2.29	0.58	1.68 to 2.90	3.77

Table III: Statistical Summary of the log_{10} Recovery Values for Test Product Configuration #2 Triseptin Water-Optional (used in a Water-aided application)

Sample	Sample Size	Mean	Standard Deviation	95% Confidence Interval	log_{10} Reduction
Baseline	18	6.02	0.56	5.74 to 6.29	N/A
Day 1, Immediate	6	4.40	0.53	3.85 to 4.95	1.62
Day 1, 4 Hour	6	4.80	0.61	4.16 to 5.44	1.22
Day 1, 8 Hour	6	4.61	0.70	3.87 to 5.34	1.41
Day 3, Immediate	6	3.30	0.76	2.50 to 4.09	2.72
Day 3, 4 Hour	6	3.63	0.99	2.59 to 4.66	2.39
Day 3, 8 Hour	6	3.91	0.88	2.99 to 4.83	2.11
Day 5, Immediate	6	3.39	1.01	2.33 to 4.45	2.63
Day 5, 4 Hour	6	4.17	0.74	3.39 to 4.95	1.85
Day 5, 8 Hour	6	3.91	0.64	3.24 to 4.59	2.11

Table IV: Statistical Summary of the log_{10} Recovery Values for Test Product Configuration #3 Avagard™ D (Waterless Test Product)

Sample	Sample Size	Mean	Standard Deviation	95% Confidence Interval	log_{10} Reduction
Baseline	18	6.00	0.25	5.88 to 6.12	N/A
Day 1, Immediate	6	3.78	0.84	2.89 to 4.66	2.22
Day 1, 4 Hour	6	4.45	1.40	2.99 to 5.92	1.55
Day 1, 8 Hour	6	6.03	0.52	5.48 to 6.58	0.00
Day 3, Immediate	6	3.58	1.19	2.33 to 4.84	2.42
Day 3, 4 Hour	6	4.02	1.42	2.52 to 5.51	1.98
Day 3, 8 Hour	6	5.18	1.25	3.87 to 6.49	0.82
Day 5, Immediate	6	3.81	0.99	2.78 to 4.85	2.19
Day 5, 4 Hour	6	4.23	0.56	3.64 to 4.82	1.77
Day 5, 8 Hour	6	5.58	1.21	4.31 to 6.85	0.42

Table V: Statistical Summary of the log_{10} Recovery Values for Test Product Configuration #4 CV® Medicated Lotion Soap (Water-aided Test Product)

Sample	Sample Size	Mean	Standard Deviation	95% Confidence Interval	log_{10} Reduction
Baseline	18	6.25	0.36	6.07 to 6.43	N/A
Day 1, Immediate	6	6.34	0.27	6.06 to 6.63	0.00
Day 1, 4 Hour	6	6.45	0.23	6.20 to 6.69	0.00
Day 1, 8 Hour	6	6.65	0.32	6.31 to 6.98	0.00
Day 3, Immediate	6	6.04	0.37	5.65 to 6.42	0.21
Day 3, 4 Hour	6	6.28	0.67	5.57 to 6.98	0.00
Day 3, 8 Hour	6	6.83	0.35	6.46 to 7.19	0.00
Day 5, Immediate	6	6.04	0.42	5.60 to 6.48	0.21
Day 5, 4 Hour	6	6.64	0.45	6.18 to 7.11	0.00
Day 5, 8 Hour	5	6.73	0.38	6.26 to 7.20	0.00

Table VI: Statistical Summary of the log_{10} Recovery Values for Test Product Configuration #5 Bactoshield® Antimicrobial Skin Cleanser (Water-aided Test Product)

Sample	Sample Size	Mean	Standard Deviation	95% Confidence Interval	log_{10} Reduction
Baseline	18	5.95	0.33	5.79 to 6.11	N/A
Day 1, Immediate	6	4.51	0.54	3.94 to 5.07	1.44
Day 1, 4 Hour	6	5.23	0.71	4.48 to 5.97	0.72
Day 1, 8 Hour	6	5.34	0.23	5.10 to 5.57	0.61
Day 3, Immediate	6	3.81	0.75	3.03 to 4.59	2.14
Day 3, 4 Hour	6	4.21	0.69	3.49 to 4.93	1.74
Day 3, 8 Hour	6	4.69	0.66	4.00 to 5.38	1.26
Day 5, Immediate	6	3.17	0.59	2.55 to 3.79	2.78
Day 5, 4 Hour	6	4.04	1.11	2.88 to 5.21	1.91
Day 5, 8 Hour	6	5.09	0.99	4.06 to 6.12	0.86

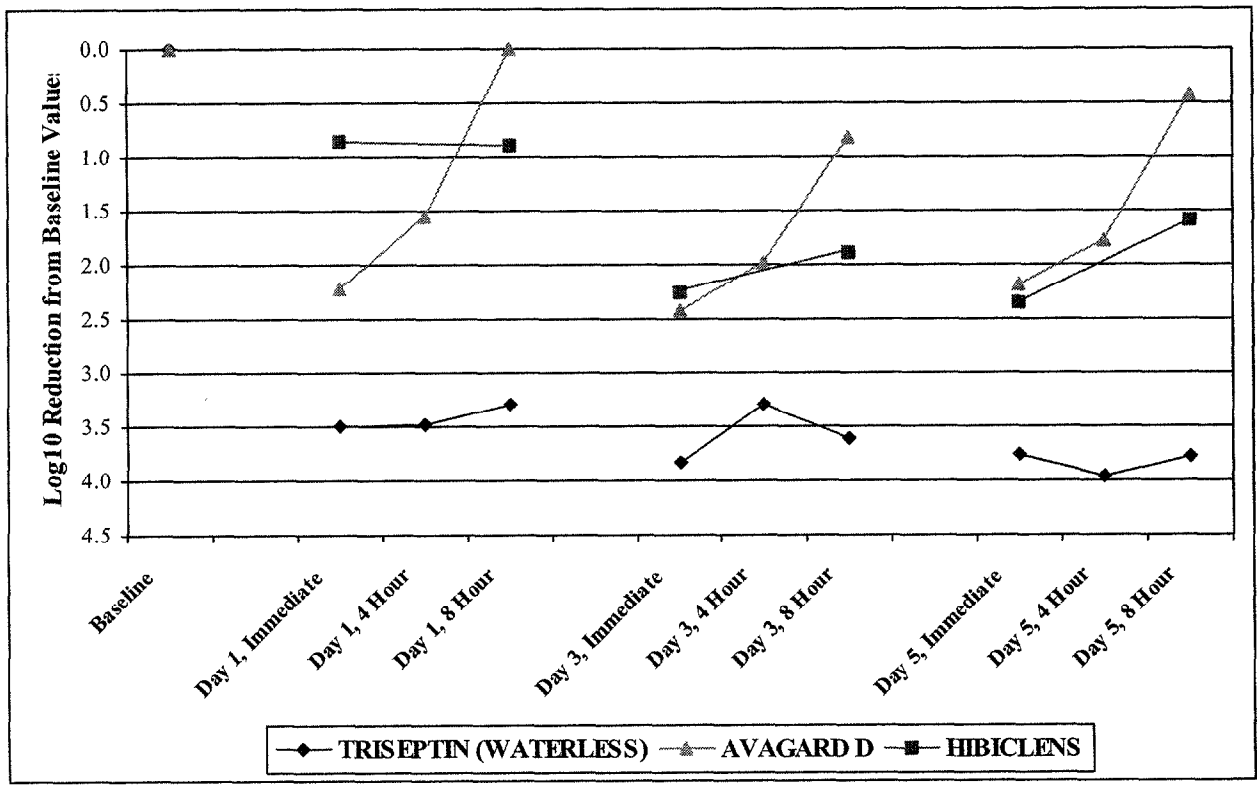
Table VII: Statistical Summary of the log_{10} Recovery Values for Reference Product Configuration #6 Hibiclens® 4% CHG (Water-aided Reference Product)

Sample	Sample Size	Mean	Standard Deviation	95% Confidence Interval	log_{10} Reduction
Baseline	8	6.02	0.44	5.65 to 6.38	N/A
Day 1, Immediate	4	5.16	0.40	4.52 to 5.79	0.86
Day 1, 8 Hour	4	5.13	1.44	2.84 to 7.42	0.89
Day 3, Immediate	4	3.76	0.78	2.51 to 5.00	2.26
Day 3, 8 Hour	4	4.14	1.75	1.35 to 6.93	1.88
Day 5, Immediate	4	3.66	0.83	2.33 to 4.99	2.36
Day 5, 8 Hour	4	4.42	1.72	1.69 to 7.15	1.60

16.0 CONCLUSIONS:

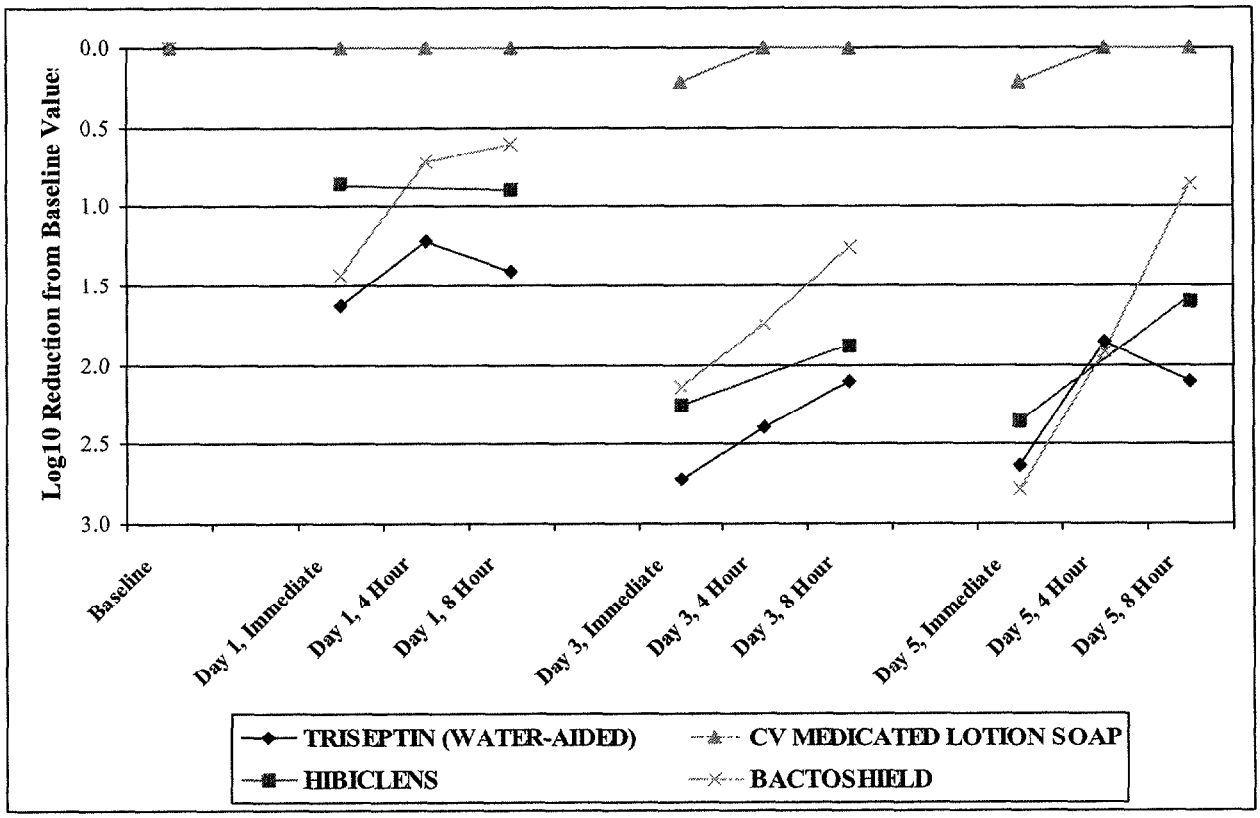
The Triseptin Waterless Surgical Scrub (Test Product Configuration #1) demonstrated reductions from baseline population of more than three (3) $\log_{10}$ reductions. Additionally, the product demonstrated significant persistent antimicrobial properties (no regrowth of microbial populations to baseline level). It is difficult from the results to determine the residual properties of this product, for it demonstrated greater than a 3 $\log_{10}$ reduction in microorganisms on all three (3) sample days. This product demonstrated significantly greater antimicrobial activity than any of the other products tested. Avagard™ D (Test Product Configuration #3) demonstrated statistically significant $\log_{10}$ microbial reductions from baseline, but did not achieve a 3 $\log_{10}$ reduction on Test Day 5. Additionally, microbial regrowth eight (8) hours post-product-application averaged about 1.9 $\log_{10}$ greater than the population in the immediate time sample. The reference product, Hibiclens® with 4% chlorhexidine gluconate (Reference Product Configuration #6), demonstrated $\log_{10}$ reductions similar to those observed under comparable conditions.

Figure 1: Graphical Presentation of the Log_{10} Reduction from Baseline Values for the Two Waterless Configurations and the Reference Product Configuration



The results from the test product, Triseptin (Test Product Configuration #2), when used as a water-aided surgical scrub formulation, were compared with those for the other three (3) water-aided products. Triseptin demonstrated highly significant antimicrobial properties when used as a water-aided product but did not achieve a 3 $\log_{10}$ reduction on Day 5. It did, however, demonstrate greater antimicrobial properties than any of the other products tested. It also demonstrated a high degree of antimicrobial persistence over the eight (8) hour post-product-application period and a significant residual effect, increasing in immediate microbial kill an average of 0.25 $\log_{10}$ per day of use. Bactoshield® Antimicrobial Skin Cleanser (Test Product Configuration #5) and Hibiclens® with 4% chlorhexidine gluconate (Reference Product Configuration #6) were statistically equivalent in $\log_{10}$ microbial reductions over the five (5) day test period. Both products produced adequate antimicrobial persistence and demonstrated significant residual antimicrobial activity, increasing in immediate microbial kill at a rate of about one-third $\log_{10}$ per day. The CV® Medicated Lotion Soap (Test Product Configuration #4) did not demonstrate significant antimicrobial properties over the five (5) day test period.

Figure 2: Graphical Presentation of the Log_{10} Reduction from Baseline Values for the Three Water-Aided Configurations and the Reference Product Configuration



17.0 ACCEPTANCE:

BIOSCIENCE LABORATORIES, INC.
300 N. Willson Avenue
Bozeman, Montana 59715

Executive Sign-Off

President
and CEO: _____ _____
Daryl S. Paulson, Ph.D. Date

Laboratory Sign-Off

Principal
Study Director: _____ _____
Robert R. McCormack, CCRP Study Completion Date

Associate
Study Director: _____ _____
Christopher M. Beausoleil, CCRP Date

Statistical Analysis Sign-Off

President
and CEO: _____ _____
Daryl S. Paulson, Ph.D. Date

QUALITY ASSURANCE STATEMENT:

This study was inspected by the Quality Assurance Unit, and reports were submitted to the Study Director and Management in accordance with Standard Operating Procedures, as follows:

	Date
Neutralization Assay	06/19/03
Product Testing	06/16/03, 06/23/03, & 07/14/03
Data Audit	08/01/03
Final Report Review	08/15/03 & 8/18/03
Reports to Study Director and Management	06/17/03, 06/19/03, 06/23/03, 07/14/03, 08/15/03, & 08/18/03

This study was conducted in compliance with Good Laboratory Practices standards, as described by the FDA (reference CFR 21 Parts 50, 56, 312, and 314), with the following exception: test article preparations were not analyzed at BioScience Laboratories, Inc., to confirm concentration, stability, or homogeneity.

Director of
Quality
Assurance: _____ _____
John A. Mitchell, Ph.D. Date

INDEX OF ADDENDA

Figure 1b: Bar Graph Presentation of the Log_{10} Reduction from Baseline Values for the Two Waterless Configurations and the Reference Product Configuration

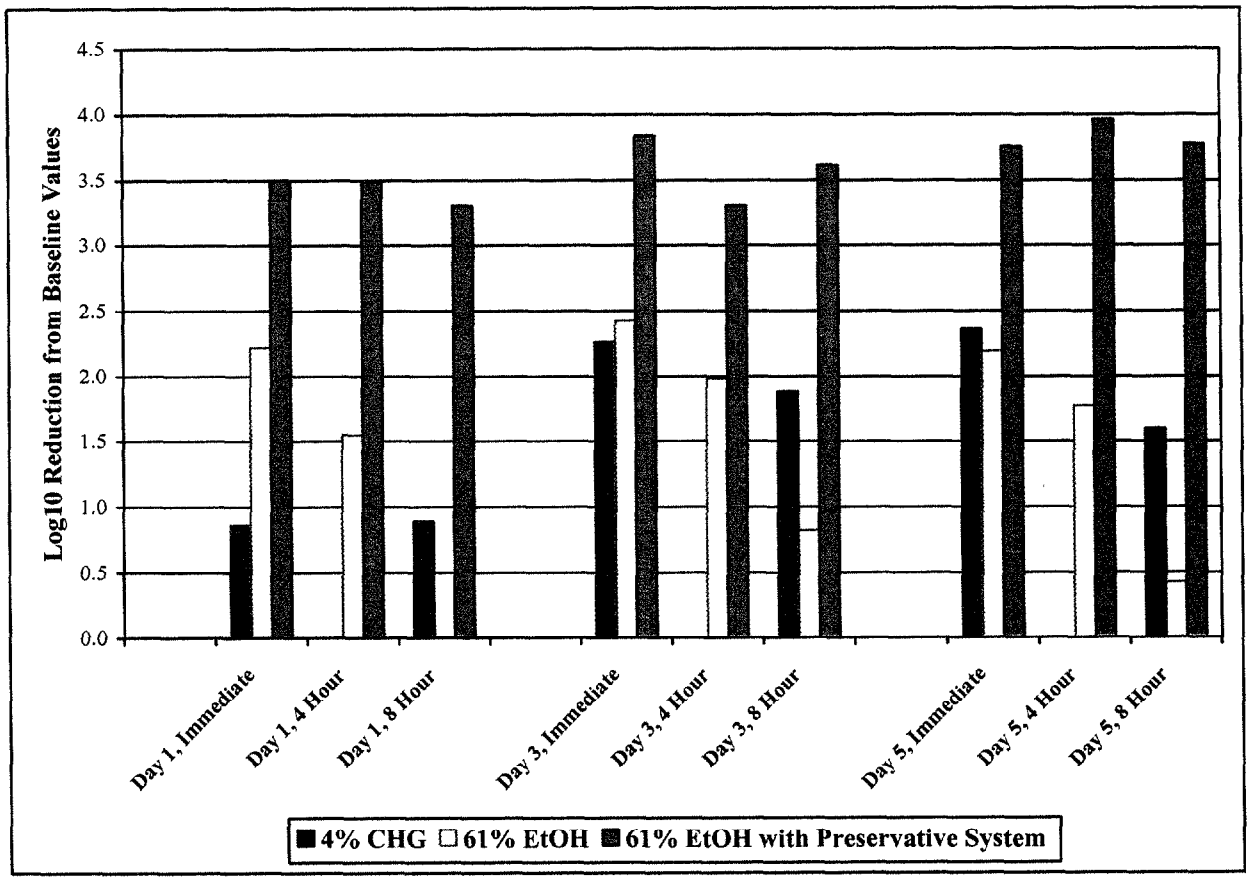
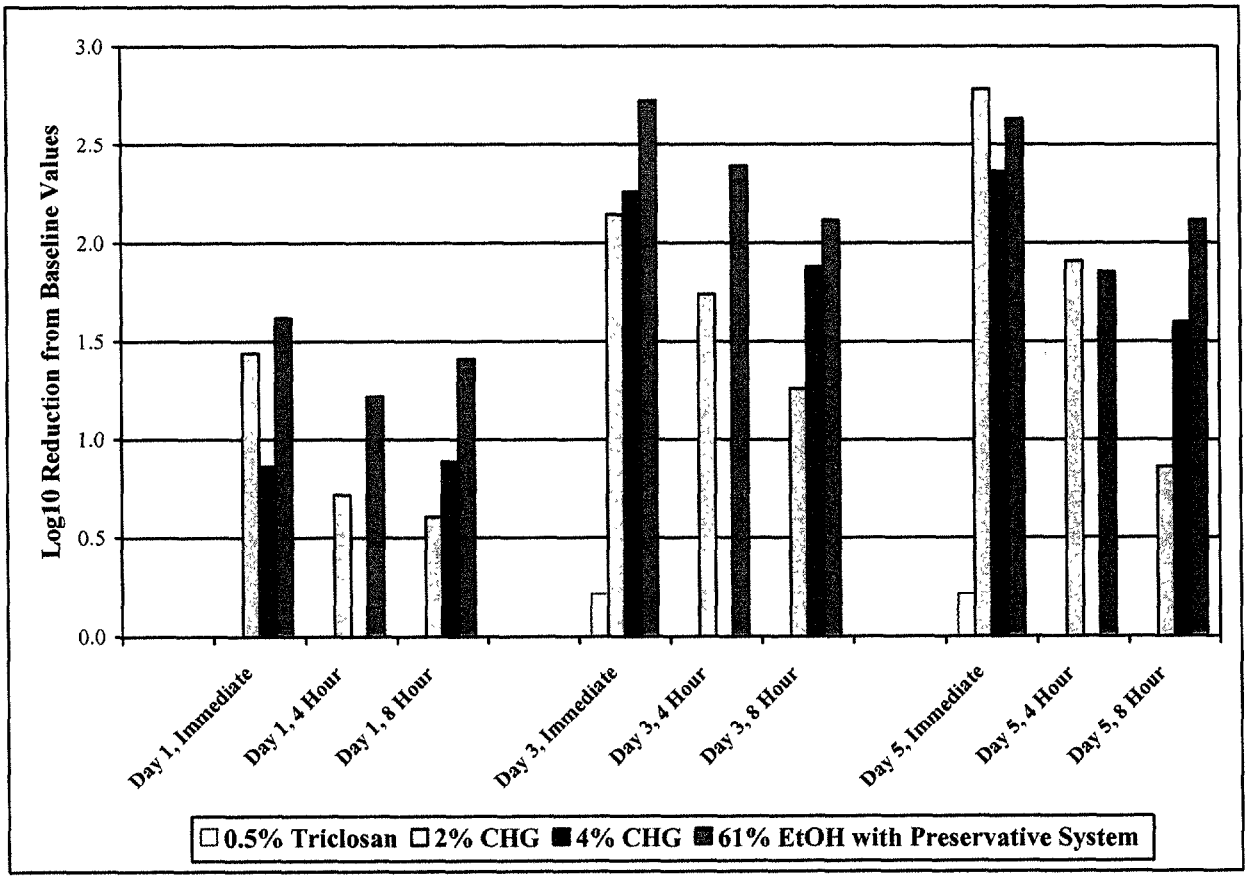


Figure 2b: Bar Graph Presentation of the Log_{10} Reduction from Baseline Values for the Three Water-Aided Configurations and the Reference Product Configuration



TRISEPTIN®
WATER-OPTIONAL
Healthcare Personnel Handwash and Rub

TECHNICAL BULLETIN

HEALTHPOINT®

TRISEPTIN® *WATER-OPTIONAL* Healthcare Personnel Handwash and Rub

Table of Contents

TRISEPTIN® *WATER-OPTIONAL* Healthcare Personnel Handwash and Rub

Healthcare Personnel Handwash *in vivo* Efficacy Study, Waterless Application

Objective:

The test for a Healthcare Personnel Handwash was designed to evaluate the degree of rapid reduction of a marker organism.

Test criteria are established in the FDA's Tentative Final Monograph (TFM) for Healthcare Antiseptic Drug Products[1], which relies upon the ASTM[2] "Standard Test Method for Evaluation of the Effectiveness of Healthcare Personnel or Consumer Handwash Formulations."

10 Washes, 1-Day Period	
Baseline	Determine by inoculation with 5 x 10^8 cfu of *S. marcescens* (ATCC[3] 14756) followed by a single wash with a non-antimicrobial soap
All Washes (post baseline through #9)	Re-inoculate with not less than 5 x 10^8 cfu of *S. marcescens*
Wash 1	Minimum reduction of 2.0 $\log_{10}$
Wash 10	Minimum reduction of 3.0 $\log_{10}$

Test Method:

- A statistically relevant number of subjects underwent a one-week period of "wash out" (no use/exposure to topical or systemic antimicrobials).
- Baseline obtained and hands re-inoculated with *S. marcescens* (ATCC 14756).
- The product was applied according to label directions as a waterless product and sampling was performed.
- Above step was repeated for a total of 10 cycles with samples obtained at wash 1, 3, 7 and 10.
- Sampling was performed using the "glove juice method." Soon after product application, sterile gloves were donned, and 75 ml of sampling fluid was inserted into the gloves. The hands were vigorously massaged for 1 minute. A 5 ml sample of fluid was collected and neutralized 5-minutes post application.
- Samples were plated and enumerated, and the $\log_{10}$ difference between baseline and the selected sample was determined.

Test Results:

Subjects applied TRISEPTIN *WATER-OPTIONAL* as a waterless healthcare personnel handwash per label directions. The product demonstrated superior results compared to the FDA's TFM testing criteria for a healthcare personnel handwash (Figure 1).

TRISEPTIN® *WATER-OPTIONAL* Healthcare Personnel Handwash and Rub

FIGURE 1: Testing Results for the FDA's Tentative Final Monograph for Healthcare Antiseptic Drug Products *in vivo* Testing Criteria for TRISEPTIN *WATER-OPTIONAL* as a Waterless Healthcare Personnel Handwash

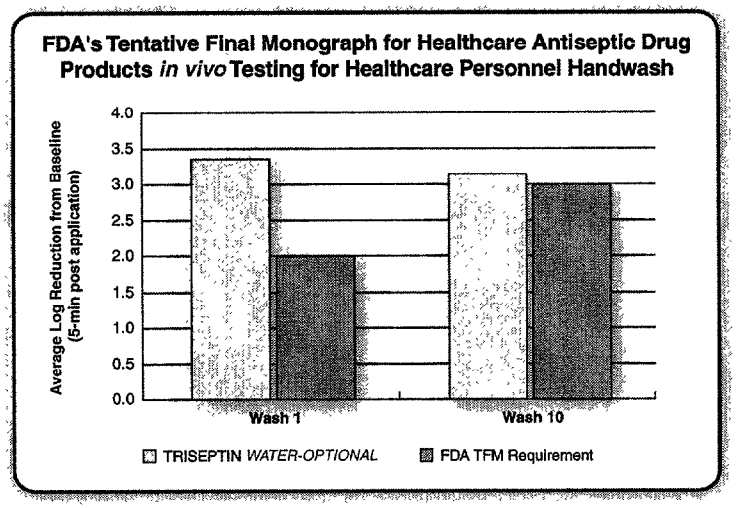
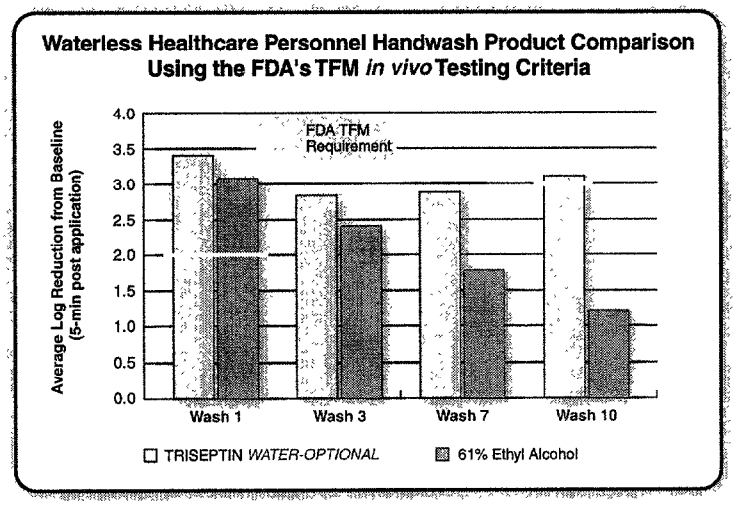


FIGURE 2: Comparison of TRISEPTIN *WATER-OPTIONAL* (Waterless Application) to a 61% Ethyl Alcohol Formulation[4] Using the FDA's Tentative Final Monograph for Healthcare Antiseptic Drug Products *in vivo* Testing Criteria for Healthcare Personnel Handwash



Conclusions:

- In the waterless application, TRISEPTIN *WATER-OPTIONAL* met and exceeded the FDA's TFM testing for healthcare personnel handwash classification.
- In the waterless application, TRISEPTIN *WATER-OPTIONAL* demonstrated superior results at all wash points (1, 3, 7 and 10) compared to a 61% ethyl alcohol formulation.
- The 61% ethyl alcohol formulation did not demonstrate the level of efficacy at the Wash 10 test point required by the FDA's TFM for Healthcare Personnel Handwash classification.

TRISEPTIN® *WATER-OPTIONAL* Healthcare Personnel Handwash and Rub

Healthcare Personnel Handwash *in vivo* Efficacy Study, Water-Aided Application

Objective:

The test for a Healthcare Personnel Handwash was designed to evaluate the degree of rapid reduction of a marker organism.

Test criteria are established in the FDA's Tentative Final Monograph (TFM) for Healthcare Antiseptic Drug Products, which relies upon the ASTM "Standard Test Method for Evaluation of the Effectiveness of Healthcare Personnel or Consumer Handwash Formulations."

10 Washes, 1-Day Period	
Baseline	Determine by inoculation with 5 x 10^8 cfu of *S. marcescens* (ATCC 14756) followed by a single wash with a non-antimicrobial soap
All Washes (post baseline through #9)	Re-inoculate with not less than 5 x 10^8 cfu of *S. marcescens*
Wash 1	Minimum reduction of 2.0 $\log_{10}$
Wash 10	Minimum reduction of 3.0 $\log_{10}$

Test Method:

- A statistically relevant number of subjects underwent a one-week period of "wash out" (no use/exposure to topical or systemic antimicrobials).
- Baseline obtained and hands re-inoculated with *S. marcescens* (ATCC 14756).
- The product was applied according to label directions as a water-aided product and sampling was performed.
- Above step was repeated for a total of 10 cycles with samples obtained at wash 1, 3, 7 and 10.
- Sampling was performed using the "glove juice method." Soon after product application, sterile gloves were donned, and 75 ml of sampling fluid was inserted into the gloves. The hands were vigorously massaged for 1 minute. A 5 ml sample of fluid was collected and neutralized 5-minutes post application.
- Samples were plated and enumerated, and the $\log_{10}$ difference between baseline and the selected sample was determined.

Test Results:

Subjects applied TRISEPTIN *WATER-OPTIONAL* as a water-aided healthcare personnel handwash per label directions. The product demonstrated superior results compared to the FDA's TFM testing criteria for a healthcare personnel handwash (Figure 3).

TRISEPTIN® *WATER-OPTIONAL* Healthcare Personnel Handwash and Rub

FIGURE 3: Testing Results for the FDA's Tentative Final Monograph for Healthcare Antiseptic Drug Products *in vivo* Testing Criteria for TRISEPTIN *WATER-OPTIONAL* as a Water-Aided Healthcare Personnel Handwash

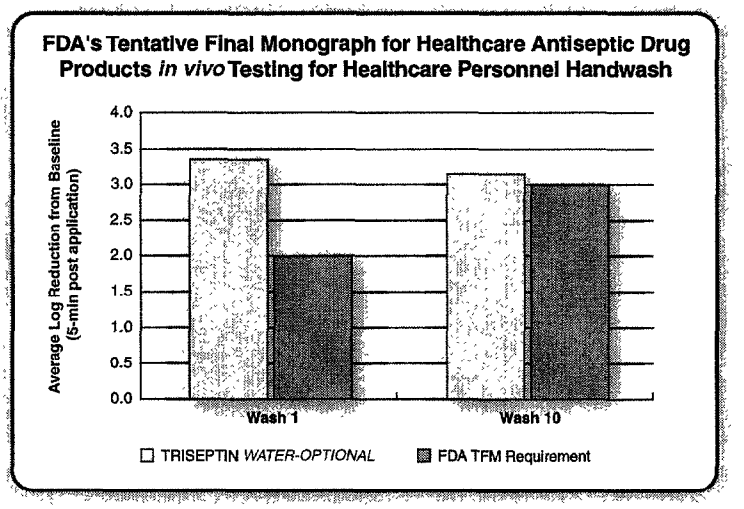
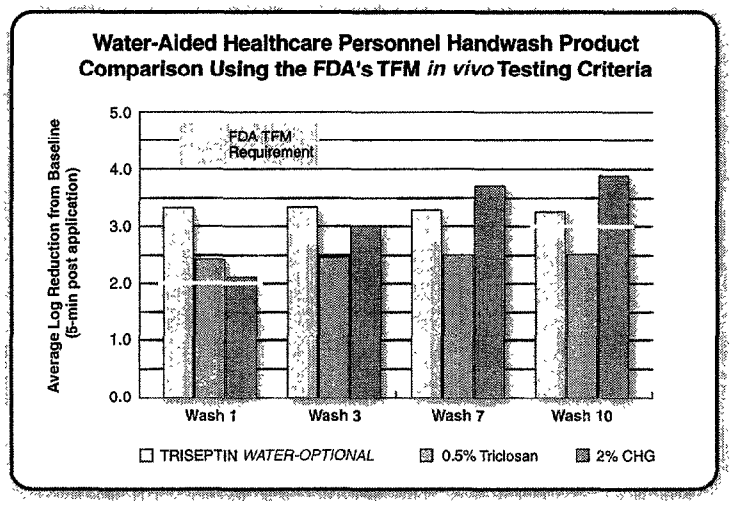


FIGURE 4: Comparison of TRISEPTIN *WATER-OPTIONAL* (Water-Aided Application) to a 2% Chlorhexidine Gluconate (CHG Formulation) and a 0.5% Triclosan Formulation Using the FDA's Tentative Final Monograph for Healthcare Antiseptic Drug Products *in vivo* Testing Criteria for Healthcare Personnel Handwash



Conclusions:

- In the water-aided application, TRISEPTIN *WATER-OPTIONAL* met and exceeded the FDA's TFM testing for healthcare personnel handwash classification.
- In the water-aided application, TRISEPTIN *WATER-OPTIONAL* demonstrated superior results at Wash 1 compared to 0.5% Triclosan and 2% CHG.
- In the water-aided application, TRISEPTIN *WATER-OPTIONAL*, 2% CHG, and 0.5% Triclosan met the FDA's TFM *in vivo* testing criteria at Wash 1; however, only TRISEPTIN and 2% CHG met the Wash 10 test point for healthcare personnel handwash classification.

TRISEPTIN® *WATER-OPTIONAL* Healthcare Personnel Handwash and Rub

In Vitro Time-Kill Study

Objective:

To determine how rapidly TRISEPTIN *WATER-OPTIONAL* achieves its antimicrobial effect.

Test Method:

TRISEPTIN *WATER-OPTIONAL* was tested against the following species of ATCC microorganisms and corresponding clinical isolates.

Test Results:

- TRISEPTIN *WATER-OPTIONAL* killed the ATCC microorganisms and corresponding clinical isolates.
- For the ATCC strains listed below, greater than 4-$\log_{10}$ reduction (>99.99%) at 15 seconds was observed for all organisms.

TRISEPTIN *WATER-OPTIONAL* Time-Kill Data, $\log_{10}$ Reduction			
Organism	**15 seconds**	**Organism**	**15 seconds**
Acinetobacter baumannii, ATCC 19606	5.4564	*Micrococcus luteus*, CI	5.4624
Acinetobacter baumannii, CI	5.4099	*Proteus mirabilis*, ATCC 7002	4.9934
Bacteroides fragilis, ATCC 29762	6.1303	*Proteus mirabilis*, CI	5.0374
Bacteroides fragilis, CI	5.7782	*Pseudomonas aeruginosa*, ATCC 15442	4.9058
Candida albicans, ATCC 10231	5.1804	*Pseudomonas aeruginosa*, CI 0701	4.8692
Candida albicans, CI	5.0354	*Pseudomonas aeruginosa*, ATCC 27853	4.9445
Candida tropicalis, ATCC 750	4.1399	*Pseudomonas aeruginosa*, CI 0404	4.6580
Candida tropicalis, CI	5.2529	*Serratia marcescens*, ATCC 14756	5.1319
Enterobacter cloacae, ATCC 39979	5.1383	*Serratia marcescens*, CI	4.6445
Enterobacter cloacae, CI	4.8543	*Staphylococcus aureus*, ATCC 6538	5.0607
Enterococcus faecalis, ATCC 29212	6.0663	*Staphylococcus aureus*, CI (MRSA)	4.6628
Enterococcus faecalis, CI	5.3243	*Staphylococcus aureus*, ATCC 29213	4.9217
Enterococcus faecium, ATCC 51559	4.8976	*Staphylococcus aureus* (vancomycin-intermediate), CI	4.5315
Enterococcus faecium, CI	5.3589	*Staphylococcus epidermidis*, ATCC 12228	4.9890
Escherichia coli, ATCC 11229	5.0107	*Staphylococcus epidermidis*, CI (VISE)	4.9823
Escherichia coli, CI 0601	4.6128	*Staphylococcus haemolyticus*, ATCC 19970	5.0755
Escherichia coli, ATCC 25922	4.9085	*Staphylococcus haemolyticus*, CI	5.0107
Escherichia coli, CI 0703	4.6721	*Staphylococcus hominis*, ATCC 51634	4.3636
Haemophilus influenzae, ATCC 33929	5.2380	*Staphylococcus hominis*, CI	5.3560
Haemophilus influenzae, CI	4.2355	*Staphylococcus saprophyticus*, ATCC 15305	5.1072
Klebsiella oxytoca, ATCC 15764	4.6021	*Staphylococcus saprophyticus*, CI	5.0149
Klebsiella oxytoca, CI	4.7924	*Streptococcus pneumoniae*, ATCC 33400	4.9638
Klebsiella pneumoniae, ATCC 51504	4.4346	*Streptococcus pneumoniae*, CI	4.5250
Klebsiella pneumoniae, CI	4.6180	*Streptococcus pyogenes*, ATCC 19615	5.7818
Micrococcus luteus, ATCC 7468	4.1847	*Streptococcus pyogenes*, CI	5.4533

CI = clinical isolate

Conclusions:

- TRISEPTIN *WATER-OPTIONAL* rapidly kills ATCC reference microorganisms and clinical isolates.
- TRISEPTIN *WATER-OPTIONAL* is a fast-acting and broad-spectrum antimicrobial agent.

Virucidal Efficacy Test Results

Objective:

To determine the virucidal capacity of TRISEPTIN *WATER-OPTIONAL*.

Test Method:

TRISEPTIN *WATER-OPTIONAL* was tested against several clinically important viruses via a standard ASTM suspension test method with 15-second to 3-minute exposure times.

Test Results:

TRISEPTIN *WATER-OPTIONAL* was proven effective at killing the following viruses.

Challenge Virus	Initial $CCID_{50}$/ml	Log_{10} Virus Reduction
Herpes Simplex Virus	$\geq 10^{6.67}$	>3.0
Human Immunodeficiency Virus	$\geq 10^{7.50}$	≥4.0
Hepatitis A Virus	$\geq 10^{6.50}$	≥3.0
Human Rotavirus	$\geq 10^{6.50}$	≥3.0
Human Coronavirus (SARS virus family)	$>10^{5.77}$	>4.0*

* 30-second exposure time

Conclusion:

TRISEPTIN *WATER-OPTIONAL* inactivates RNA and DNA viruses and meets or exceeds the ASTM requirements to be classified as a virucide.

TRISEPTIN® *WATER-OPTIONAL* Healthcare Personnel Handwash and Rub

Antimicrobial Spectrum Study

Objective:

To test the efficacy of TRISEPTIN *WATER-OPTIONAL* against a broad spectrum of microorganisms as listed in the Tentative Final Monograph for Healthcare Antiseptic Drug Products.

Test Method:

The active ingredient, the vehicle and TRISEPTIN *WATER-OPTIONAL* were tested against the following species of ATCC microorganisms and corresponding clinical isolates.

Test Results:

TRISEPTIN *WATER-OPTIONAL* provided greater than a 4-$\log_{10}$ reduction (>99.99%) after 15 seconds of exposure.

Organisms	
Acinetobacter baumannii, ATCC 19606	*Micrococcus luteus*, CI
Acinetobacter baumannii, CI	*Proteus mirabilis*, ATCC 7002
Bacteroides fragilis, ATCC 29762	*Proteus mirabilis*, CI
Bacteroides fragilis, CI	*Pseudomonas aeruginosa*, ATCC 15442
Candida albicans, ATCC 10231	*Pseudomonas aeruginosa*, CI 070199Pa
Candida albicans, CI	*Pseudomonas aeruginosa*, ATCC 27853
Candida tropicalis, ATCC 750	*Pseudomonas aeruginosa*, CI 040400Pa5
Candida tropicalis, CI	*Serratia marcescens*, ATCC 14756
Enterobacter cloacae, ATCC 39979	*Serratia marcescens*, CI
Enterobacter cloacae, CI	*Staphylococcus aureus*, ATCC 6538
Enterococcus faecalis, ATCC 29212	*Staphylococcus aureus*, CI 032301MMRSA13*
Enterococcus faecalis, CI	*Staphylococcus aureus*, ATCC 29213
Enterococcus faecium, ATCC 51559	*Staphylococcus aureus*, CI 042800VISA*
Enterococcus faecium, CI	*Staphylococcus epidermidis*, ATCC 12228
Escherichia coli, ATCC 11229	*Staphylococcus epidermidis*, CI 102599VISE*
Escherichia coli, CI 060199Ec	*Staphylococcus haemolyticus*, ATCC 29970
Escherichia coli, ATCC 25922	*Staphylococcus haemolyticus*, CI
Escherichia coli, CI 070399Ec	*Staphylococcus hominis*, ATCC 51624
Haemophilus influenzae, ATCC 33929	*Staphylococcus hominis*, CI
Haemophilus influenzae, CI	*Staphylococcus saprophyticus*, ATCC 15305
Klebsiella oxytoca, ATCC 15764	*Staphylococcus saprophyticus*, CI
Klebsiella oxytoca, CI	*Streptococcus pneumoniae*, ATCC 33400
Klebsiella pneumoniae, ATCC 51504	*Streptococcus pneumoniae*, CI
Klebsiella pneumoniae, CI	*Streptococcus pyogenes*, ATCC 19615
Micrococcus luteus, ATCC 7468	*Streptococcus pyogenes*, CI

Conclusions:

- TRISEPTIN *WATER-OPTIONAL* kills ATCC microorganisms and corresponding clinical isolates, some of which are antibiotic-resistant strains. These are identified by an asterisk (*).
- TRISEPTIN *WATER-OPTIONAL* is an effective, fast-acting and broad-spectrum antimicrobial.

Resistant Organism Time-Kill Study

Objective:

To determine how rapidly TRISEPTIN *WATER-OPTIONAL* achieves its antimicrobial effect when tested against known drug-resistant bacteria.

Test Method:

Several clinically important antibiotic-resistant organisms were exposed to TRISEPTIN *WATER-OPTIONAL* to measure $\log_{10}$ reduction after 15 seconds of exposure.

Test Results:

TRISEPTIN *WATER-OPTIONAL* showed strong bactericidal activity against the following antibiotic-resistant organisms.

Time-Kill Data for Antibiotic-Resistant Organisms Exposed to TRISEPTIN *WATER-OPTIONAL*

No.	Microorganism Species	ATCC or Isolate #	Log_{10} Reduction at 15 Seconds
1	*Acinetobacter baumannii*	061700Ab16	5.41
2	*Bacteroides fragilis*	29762	6.13
3	*Bacteroides fragilis*	090800Bf	5.78
4	*Enterobacter cloacae*	39979	5.13
5	*Enterobacter cloacae*	070700Ec11	4.85
6	*Enterococcus faecalis*	061700Efs12	5.32
7	*Enterococcus faecium*	51559	4.90
8	*Enterococcus faecium* (VRE)	062999VRE	5.36
9	*Escherichia coli*	060199Ec	4.61
10	*Escherichia coli*	070399Ec	4.67
11	*Haemophilus influenzae*	33929	5.24
12	*Haemophilus influenzae*	121699Hi3	4.23
13	*Klebsiella oxytoca*	15764	4.60
14	*Klebsiella oxytoca*	060199Ko	4.79
15	*Klebsiella pneumoniae*	51504	4.43
16	*Klebsiella pneumoniae*	040400Kpm2	4.62
17	*Proteus mirabilis*	121699Pm2	5.03
18	*Pseudomonas aeruginosa*	070199Pa	4.87
19	*Pseudomonas aeruginosa*	040400Pa5	4.66
20	*Serratia marcescens*	081499Sm	4.94
21	*Staphylococcus aureus* (MRSA)	032301MMRSA13	4.66
22	*Staphylococcus aureus* (VISA)	042800VISA	4.53
23	*Staphylococcus epidermidis* (VISE)	102599VISE	4.98
24	*Staphylococcus hominis*	51624	4.37
25	*Staphylococcus hominis*	060700Sho4	5.35
26	*Staphylococcus saprophyticus*	062900Ss	5.01

Conclusion:

TRISEPTIN *WATER-OPTIONAL* demonstrated exceptional bactericidal action against known antibiotic-resistant organisms.

Minimum Inhibitory Concentration (MIC) Study

Objective:

To determine the minimum concentration level at which TRISEPTIN *WATER-OPTIONAL* inhibits bacterial growth of the following ATCC microorganisms and corresponding clinical isolates.

Test Method:

The following ATCC microorganisms and corresponding clinical isolates were exposed to various dilutions of TRISEPTIN *WATER-OPTIONAL* and tested to determine the highest dilution factor (lowest product concentration) which inhibits growth of the microorganisms.

Test Results:

TRISEPTIN *WATER-OPTIONAL* showed strong activity against ATCC organisms and clinical isolates, including antibiotic-resistant organisms. The data table below is representative of the 1,100 isolates tested:

Organism	Percentage Concentration of TRISEPTIN	Organism	Percentage Concentration of TRISEPTIN
Acinetobacter sp., ATCC 9957 (–) (DR)	0.0977%	*Klebsiella pneumoniae*, CI 081599Kp (–) (DR)	0.1953%
Acinetobacter baumannii, CI 121799Asp3 (–) (DR)	0.1953%	*Micrococcus luteus*, ATCC 15957 (+) (DR)	0.0977%
Bacteroides caccae, CI 090800Bc1 (DR)	0.0244%	*Micrococcus sp.*, CI 070700Ms1 (+) (DR)	0.7813%
Bacteroides fragilis, ATCC 29762 (-9) (DR)	0.0488%	*Proteus mirabilis*, ATCC 29855 (–)	0.3906%
Bacteroides fragilis, CI 090800Bf (-9) (DR)	0.0244%	*Proteus mirabilis*, CI 121699Pm1 (–) (DR)	0.7813%
Candida albicans, ATCC 11651	<0.7813%	*Pseudomonas aeruginosa*, ATCC 9027 (–)	0.7813%
Candida albicans, CI 081599Ca	<0.7813%	*Pseudomonas aeruginosa*, CI 052299Pa (–) (DR)	0.7813%
Candida tropicalis, ATCC 750	<0.7813%	*Pseudomonas aeruginosa*, ATCC 15442	<0.7813%
Candida parapsilosis, CI 040400Cp5	0.7813%	*Serratia marcescens*, ATCC 43297 (–) (DR)	0.3906%
E. faecalis, ATCC 29212 (+)	0.1953%	*Serratia marcescens*, CI 081499Sm (–) (DR)	0.3906%
Enterobacter aerogenes, CI 013100Ea (–) (DR)	0.1953%	*Staphylococcus aureus*, CI 051599MRSA (+) (DR)	0.0977%
Enterobacter aerogenes, ATCC 29940 (–)	0.1953%	*Staphylococcus aureus*, ATCC 33591 (+) (DR)	0.0488%
Enterobacter cloacae, CI 39979 (–) (DR)	0.1953%	*Staphylococcus aureus*, ATCC 27660 (+) (DR)	0.0977%
Enterococcus faecalis, CI 010500Efs (–) (DR)	0.1953%	*Staphylococcus epidermidis*, CI 060700Sel (+)	0.0488%
Enterococcus faecalis, ATCC 49533 (+) (DR)	0.1953%	*Staphylococcus epidermidis*, CI 061700Sel4 (+)	0.0244%
Enterococcus faecium, ATCC 700221 (+) (DR)	0.0244%	*Staphylococcus epidermidis*, ATCC 51625 (+) (DR)	0.0244%
Enterococcus faecium, CI 071499VRE (+) (DR)	0.0977%	*Staphylococcus haemolyticus*, ATCC 15796 (+)	0.0012%
Escherichia coli, CI 051599Ec (–) (DR)	0.1953%	*Staphylococcus hominis*, ATCC 25615 (+)	0.0488%
Escherichia coli, ATCC 25253 (–) (DR)	0.1953%	*Staphylococcus saprophyticus*, ATCC 15305 (+)	0.0977%
Escherichia coli, ATCC 29214 (–) (DR)	0.1953%	*Staphylococcus aureus*, ATCC 6538 (+)	0.3906%
Escherichia coli, CI 070399Ec (–) (DR)	0.1953%	*Streptococcus pneumoniae*, ATCC 700674 (+)	0.0977%
Klebsiella oxytoca, ATCC 15764 (–) (DR)	0.1953%	*Streptococcus pyogenes*, ATCC 25663 (+)	0.0244%
Klebsiella pneumoniae, ATCC 51503 (–) (DR)	0.1953%	*Streptococcus pyogenes*, ATCC 12384 (+)	0.0488%

CI = clinical isolate
(+) gram positive
(–) gram negative
(DR) drug-resistant organisms

Conclusion:

TRISEPTIN *WATER-OPTIONAL* is an effective antimicrobial agent that inhibits growth of a broad spectrum of microorganisms.

Primary Skin Irritation Study (Rabbits)

Objective:

The objective of the study was to compare TRISEPTIN *WATER-OPTIONAL* to a marketed waterless, alcohol-based healthcare personnel hand antiseptic to determine the irritation potential under occlusion.

Test Method:

- Both products were administered once daily for 4 consecutive days. A volume of 0.2 ml was applied to a four-site, 40-cm^2 area on the back of rabbits.
- Observations were recorded for 8 days. Dosed sites were covered with an impervious material for 1 hour.
- Observations were scored for irritation and other effects at the site of application, and an irritation potential was evaluated.

Test Results:

TRISEPTIN *WATER-OPTIONAL* proved to be comparable to the marketed waterless, brushless, alcohol-based surgical scrub.

Conclusion:

The irritation study was conducted on a species that is bred to be more sensitive than human subjects. These results were encouraging and served as a basis for further testing in humans.

Exaggerated Hand Exposure Tests

Objective:

The study design evaluates the potential of the test product, TRISEPTIN *WATER-OPTIONAL*, to produce irritation under exaggerated exposure tests.

Methodology:

A total of 21 subjects completed the study using the test materials identified above per the manufacturers label claim for directions for use. At baseline, subjects' hands were examined for inappropriate skin conditions and clinically graded for erythema and burning. Qualified subjects participated in the following clinical grading and instrumental procedures:

- Expert Dermatological Grader: Subjects were clinically graded by an expert grader on the back of the right and left hands for erythema and burning.
- Subject Self-Assessment: Subjects rated the backs of their right and left hands for overall moisture and conditioning.
- NOVA™ Dermal Phase Meter (DPM) Measurements: Triplicate NOVA DPM measurements were taken on the back of each subject's right and left hand to measure the moisture content of the stratum corneum.

Test Method:

Washout	Day 1	Day 2	Day 3	Day 4	Day 5
1 week	Baseline				Endpoint
	Clinical Grading	Clinical Grading	Clinical Grading	Clinical Grading	Clinical Grading
	5 washes	5 washes	5 washes	5 washes	5 washes
	DPM		DPM		DPM
			Subject Grading		Subject Grading

After completion of baseline clinical grading and instrumental measurements, subjects washed both hands using Dove® liquid soap. After completion of the wash, subjects participated in five cycles of product applications (one test material per hand).

Subjects returned to the clinic for Day 2, Day 3, Day 4 and Day 5. Prior to beginning the wash cycle each day, subjects were clinically examined for inappropriate skin conditions and graded for erythema, dryness and burning on the back of each hand. Subjects were disqualified from further study participation if they presented with an inappropriate skin condition or a score of 3 or greater for any of the graded parameters. Disqualified subjects participated in endpoint procedures (as described below). After initial (pre-wash) clinical grading at each visit, subjects participated in a hand wash and five product application procedures as described for baseline. At Day 3, subjects received clinical grading and instrumentation (NOVA DPM) approximately 2 hours after completion of the last test material application.

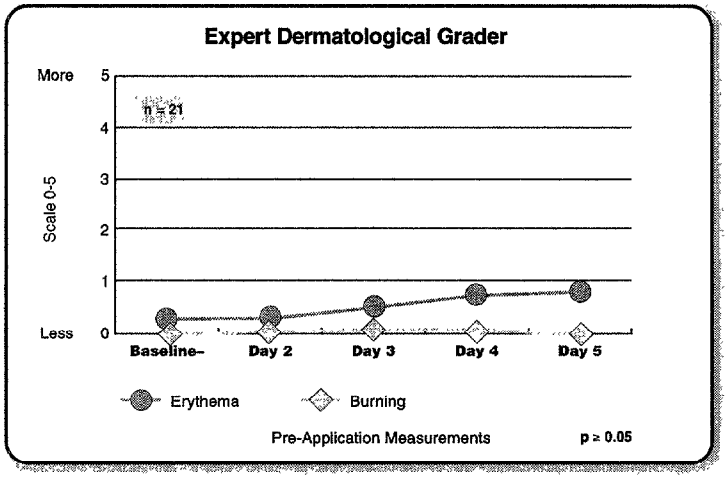
The endpoint procedures for all subjects took place 2 hours after final product application visit Day 5 or when a subject was disqualified according to specifications at an earlier time. At the endpoint, all subjects participated in the following procedures as described for baseline:

- Clinical grading of erythema and burning symptoms
- Self-assessment of hand skin condition by subjects
- NOVA DPM measurements on the back of each hand

Conclusion:

Overall the results of the 5-day study demonstrate that after 25 repeated applications, TRISEPTIN *WATER-OPTIONAL* maintains approximately the same skin condition as existed prior to repeated product exposure.

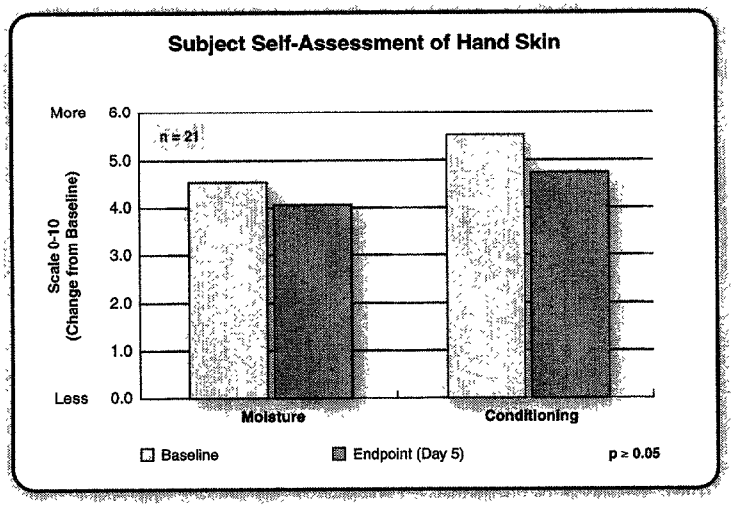
Expert Dermatological Grader: Erythema and Burning



Conclusions:

- After 25 applications, hands treated with TRISEPTIN *WATER-OPTIONAL* demonstrated no statistical difference between the baseline and the endpoint (Day 5) in the area of erythema and burning.
- Based on the data presented, the user should not experience a significant difference in skin condition after repeated applications of TRISEPTIN *WATER-OPTIONAL*.

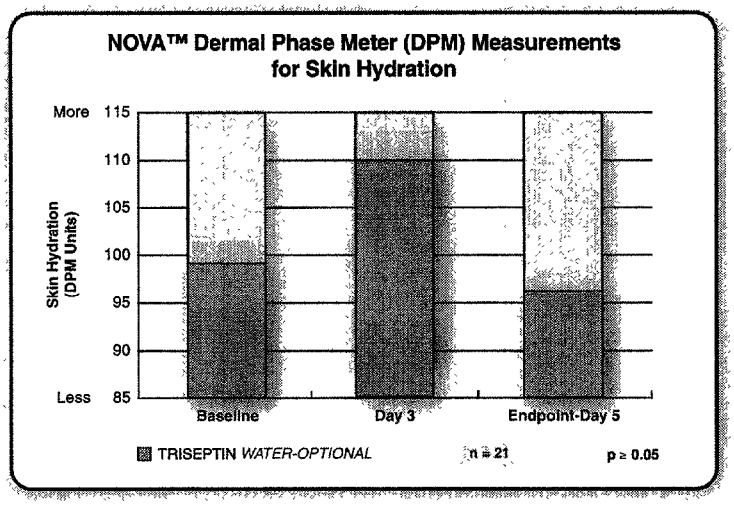
Subject Self-Assessment of Hand Skin: Moisture and Conditioning



Conclusions:

- After 25 applications, hands treated with TRISEPTIN *WATER-OPTIONAL* demonstrated no statistical difference between the baseline and the endpoint (Day 5) in the area of moisture and conditioning based on the user's self-assessment.
- Based on the data presented, the user should not experience a significant difference in skin condition after repeated applications of TRISEPTIN *WATER-OPTIONAL*.

Skin Hydration Analysis



Conclusions:

- TRISEPTIN *WATER-OPTIONAL* increased the skin hydration level at the Day 3 test point after 15 washes. This would suggest that TRISEPTIN's emollient-rich formulation improves the amount of water retained in the skin, avoiding skin dehydration and preserving normal moisture and conditioning.
- At Day 5, after 25 repeated applications, TRISEPTIN *WATER-OPTIONAL* demonstrated results very similar to the baseline results, which would indicate that there was virtually no change in skin's hydration after five days of repeated use, suggesting preservation of normal skin moisture and conditioning.

References

1. Tentative Final Monograph (TFM) for Healthcare Antiseptic Drug Products, Proposed Rule Federal Register Part III, Vol. 59, No. 116 (1994).
2. ASTM: American Society for Testing and Materials.
3. ATCC: American Type Culture Collection.
4. The product tested was Avagard D®.

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