TRISEPTIN®

- **Brush-Free Surgical Scrub**
- **Hand and Body Antiseptic**

TECHNICAL BULLETIN

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Surgical Scrub *In Vivo* Efficacy Study

Objective:

The test for a surgical scrub was designed to evaluate the degree of rapid and persistent reduction of normal resident and transient human flora.

Test criteria were established in the FDA Tentative Final Monograph (TFM) for Healthcare Antiseptic Drug Products, which described the ASTM "Standard Test Method for Evaluation of a Surgical Scrub."

11 Washes over a 5-Day Period		
Day 1	1 wash	1-$\log_{10}$ reduction of microbial flora within 1 minute after application
Day 2	3 washes	2-$\log_{10}$ reduction of microbial flora within 1 minute after application
Day 3	3 washes	No test
Day 4	3 washes	No test
Day 5	1 wash	3-$\log_{10}$ reduction of microbial flora within 1 minute after application

Test Method:

1. A statistically relevant number of subjects underwent a two-week period of "wash-out" (no use of antimicrobial products, internally or externally, for two weeks).
2. Baseline populations must meet or exceed 1 x 10^5 CFU/hand at a minimum.
3. The product was applied according to label directions and sampling was performed (subject sites randomized, used once) 10 minutes, 3 hours and 6 hours after application. Additional longer time points may be added.
4. Sampling was performed using the "glove juice method." After product application per label directions, sterile gloves were donned. A 75 ml neutral medium was inserted into the gloves and the hands were massaged for 1 minute. The antimicrobial action in the sample was neutralized. The fluid was collected, the wash repeated and the samples pooled.
5. Samples were plated and enumerated, and the $\log_{10}$ difference between baseline and sample time was determined.

Test Results:

Subjects scrubbed with TRISEPTIN Brush-Free Alcohol Surgical Scrub per the label directions. The test demonstrated superior results exceeding the FDA TFM testing criteria for the surgical scrub classification and surpassed performance of 4% Chlorhexidine Gluconate (CHG) and 7.5% PVP-I scrub brush solutions, as shown in Figure 1.

FIGURE 1: FDA Tentative Final Monograph for Healthcare Antiseptic Drug Products *in Vivo* Testing Criteria for Surgical Scrub Classification

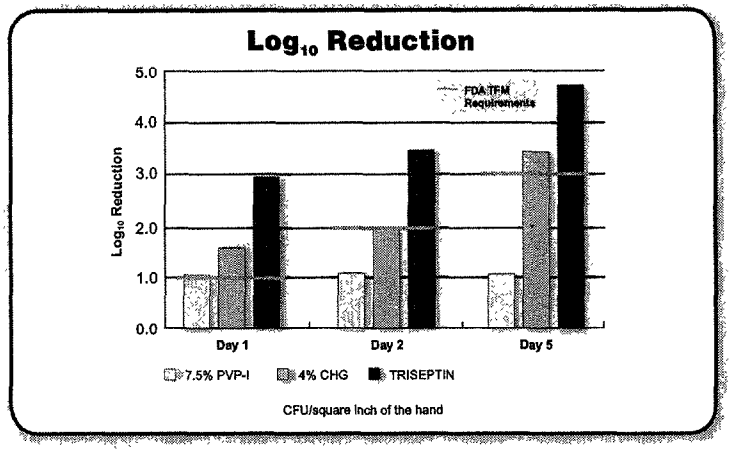
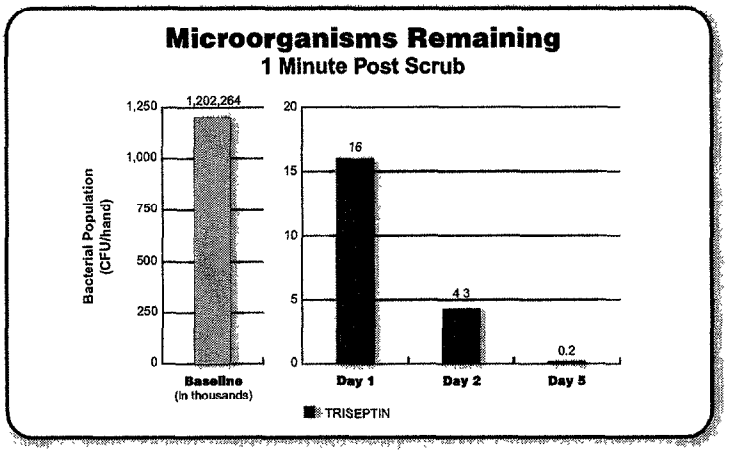


FIGURE 2: Microorganisms Remaining 1 Minute Post Application



Conclusions:

- TRISEPTIN Brush-Free Alcohol Surgical Scrub demonstrated rapid reduction of resident and transient flora.
- TRISEPTIN demonstrated superior persistence, maintaining microbial reduction at all three test points, with efficacy that exceeds that required of a surgical scrub as outlined by the FDA TFM testing criteria.
- TRISEPTIN reduced microorganisms from 1,202,264 CFU/hand to only 0.2 CFU/hand on the Day 5 test point.
- TRISEPTIN demonstrated superior efficacy compared to 4% Chlorhexidine Gluconate (CHG) and 7.5% PVP-I at all three test points.

Hand and Body Antiseptic *In Vivo* Efficacy Study

Patient Preoperative Skin Preparation

Objective:

The test for a patient preoperative skin preparation was designed to evaluate the degree of rapid and persistent microbial reduction of normal resident human flora.

Test criteria were established in the FDA Tentative Final Monograph for Healthcare Antiseptic Drug Products, which described the ASTM "Standard Test Method for Evaluation of a Patient Preoperative Skin Preparation."

Inguinal Test Site	3-$\log_{10}$ reduction of microbial flora per cm^2 within 10 minutes after application
Abdominal Test Site	2-$\log_{10}$ reduction of microbial flora per cm^2 within 10 minutes after application
Suppression of bacterial growth below baseline for 6 hours after application	

Test Method:

1. A statistically relevant number of subjects underwent a two-week period of "wash-out" (no use of antimicrobial products, internally or externally, for two weeks).
2. Baseline populations must be sufficient to demonstrate, at a minimum, a 3-$\log_{10}$ reduction (inguinal or axilla area as a moist site) and a 2-$\log_{10}$ reduction (abdominal area as a dry site). Subjects demonstrating the minimum baseline population of 4.5 $\log_{10}/cm^2$ (moist site) and 3.5 $\log_{10}/cm^2$ (dry site) were included in the study.
3. Sampling was performed using the "cup scrub method." An open ended cup was placed securely over the sample site, a small amount of sampling fluid was placed within the cup and the skin surface rubbed with a rubber spatula for 1 minute. The fluid was collected, the wash repeated and the samples pooled.
4. The product was applied according to label directions and sampling was performed 10 minutes and 6 hours after application.
5. Samples were collected as above, plated and enumerated, and the $\log_{10}$ difference between baseline and sample time was determined.

Test Results:

The test demonstrated superior results exceeding the FDA TFM testing criteria for the Patient Preoperative Skin Preparation classification, as shown in Figure 3 and Figure 4.

FIGURE 3: FDA Tentative Final Monograph for Healthcare Antiseptic Drug Products *in Vivo* Testing Criteria for Patient Preoperative Skin Preparation Classification

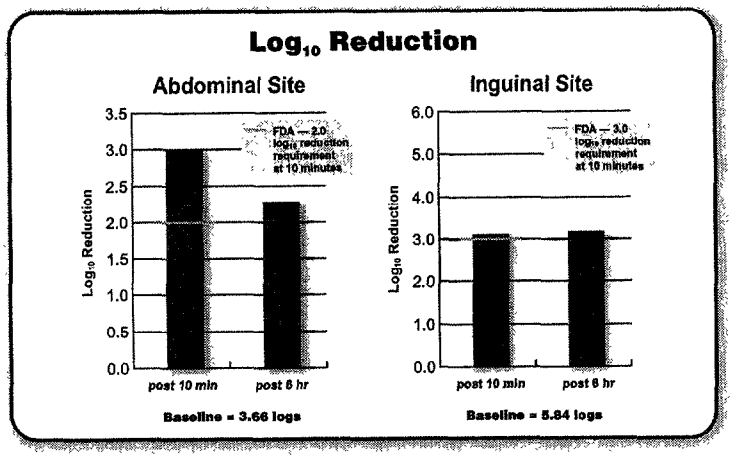
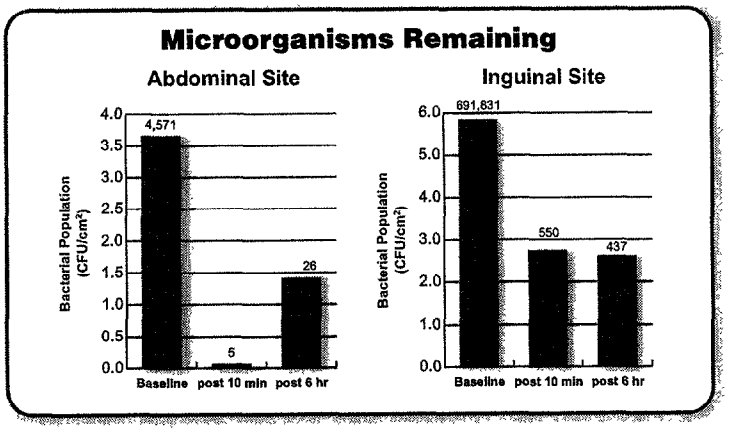


FIGURE 4: Baseline Cultures and Microorganisms Remaining Following TRISEPTIN Hand and Body Antiseptic Application as an Antiseptic Patient Skin Preparation and Subsequent *in Vivo* Testing per the FDA Tentative Final Monograph for Healthcare Antiseptic Drug Products



Conclusions:

- TRISEPTIN Hand and Body Antiseptic used as an antiseptic patient preoperative skin preparation demonstrated rapid reduction of resident and transient flora. Such flora on the surface of the skin can lead to surgical site infections.
- TRISEPTIN demonstrated persistence, maintaining microbial reduction at 6 hours, with efficacy exceeding that of a preoperative site preparation as outlined by the FDA TFM testing criteria.
- TRISEPTIN reduced microorganisms in the inguinal site from 691,831 CFU/cm² to only 9 CFU/cm² 10 minutes post prep.

In Vitro Time-Kill Study

Objective:

To determine how rapidly a 1:10 dilution of TRISEPTIN achieves its antimicrobial effect.

Test Method:

A 1:10 dilution of TRISEPTIN was tested against the following species of ATCC microorganisms and corresponding clinical isolates at three minutes.

Test Results:

- The 1:10 dilution of TRISEPTIN reduced the ATCC microorganisms and corresponding clinical isolates.
- For the ATCC strains listed below, greater than 4-$\log_{10}$ reduction at three minutes was observed for most organisms.

TRISEPTIN Time-Kill Data, $\log_{10}$ Reduction

Organism	3 min	Organism	3 min
Acinetobacter sp., CI 317 (–)	5.2304	*Micrococcus roseus*, ATTCC 186 (+)	6.5052
Acinetobacter sp., ATCC 15308 (–)	6.6902	*Moraxella catarrhalis*, CI 326 (–)	6.2304
Bacteroides fragilis, ATCC 25285 (–)	6.2788	*Proteus mirabilis*, ATCC 7002 (–)	6.8921
Candida albicans, ATCC 10231 (yeast)	6.6385	*Proteus mirabilis*, CI 312 (–)	3.2175
Candida albicans, CI 304 (yeast)	6.2553	*Pseudomonas aeruginosa*, ATCC 15442 (–)	3.0022
Candida krusei, CI 302 (yeast)	6.6232	*Pseudomonas aeruginosa*, ATCC 27853 (–)	6.7243
Candida sp., CI 327 (yeast)	6.1139	*Pseudomonas aeruginosa*, CI 309 (–)	2.9542
Candida tropicalis, ATCC 750 (yeast)	6.1271	*Serratia marcescens*, ATCC 14756 (–)	6.8692
Candida tropicalis, CI 303 (yeast)	6.5682	*Serratia sp.*, CI 320 (–)	5.8808
Citrobacter freundii, CI 306 (–)	5.1761	*Staphylococcus aureus*, CI 301 (+)	6.0792
Enterobacter aerogenes, CI 308 (–)	4.5399	*Staphylococcus aureus*, ATCC 29213 (+)	6.4914
Enterobacter aerogenes, ATCC 13048 (–)	6.8124	*Staphylococcus aureus*, ATCC 6538 (+)	5.4590
Enterobacter sp., CI 318 (–)	6.5563	*Staphylococcus Coag. Neg.*, CI 319 (+)	6.7243
Enterobacter sp., CI 325 (–)	6.8976	*Staphylococcus Coag. Neg.*, CI 323 (+)	6.7324
Enterococcus faecalis, ATCC 29212 (+)	6.2989	*Staphylococcus epidermidis*, ATCC 12228 (+)	6.2304
Enterococcus faecium, ATCC 6569 (+)	6.7243	*Staphylococcus haemolyticus*, ATCC 29970 (+)	5.2041
Enterococcus sp., CI 322 (+)	6.7243	*Staphylococcus hominis*, ATCC 29885 (+)	6.3010
Escherichia coli, CI 307 (–)	6.7482	*Staphylococcus saprophyticus*, ATCC 15305 (+)	6.7709
Escherichia coli, ATCC 11229 (–)	6.5911	*Streptococcus bovis*, CI 313 (+)	6.6021
Escherichia coli, ATCC 25922 (–)	6.2356	*Streptococcus* Group B, CI 321 (+)	6.6721
Escherichia coli, CI 310 (–)	6.2553	*Streptococcus* Group D, CI 324 (+)	6.7559
Hemophilus influenzae, ATCC 19418 (–)	5.3802	*Streptococcus pneumoniae*, ATCC 6303 (+)	5.0414
Klebsiella pneumoniae, ATCC 29995 (–)	6.1761	*Streptococcus pneumoniae*, CI 305 (+)	6.5315
Klebsiella sp., CI 316 (–)	6.6435	*Streptococcus pyogenes*, ATCC 19615 (+)	6.7993
Micrococcus luteus, ATCC 7468 (+)	6.6384	*Streptococcus pyogenes*, CI 314 (+)	6.6732

CI = clinical isolate
(+) gram positive
(–) gram negative

Conclusions:

- TRISEPTIN rapidly kills ATCC microorganisms at a 1:10 dilution.
- TRISEPTIN is a fast-acting and broad-spectrum antimicrobial agent.

Resistant Organism Time-Kill Study

Objective:

To determine how rapidly TRISEPTIN achieves its antimicrobial effect.

Test Method:

Several clinically important antibiotic resistant organisms were exposed to TRISEPTIN to measure $\log_{10}$ reduction over time.

Test Results:

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

Organism	% Kill Log_{10} Reduction at 15 Seconds
Enterococcus faecalis, ATCC 51575 (VRE)	>99.9984
Enterococcus faecium, ATCC 51559 (VRE)	>99.9956
Enterococcus faecium, Clinical Isolate (VRE)	>99.9918
Staphylococcus aureus, ATCC 33592 (MRSA)	>99.9937
Staphylococcus aureus, Clinical Isolate (VISA)	>99.9704
Staphylococcus epidermidis, ATCC 51642 (MRSE)	>99.9854

Conclusion:

TRISEPTIN demonstrated exceptional bactericidal action.

Virucidal Efficacy Test Results

Objective:

To determine how rapidly TRISEPTIN achieves its antiviral effect.

Test Method:

TRISEPTIN was tested against several clinically important viruses.

Test Results:

TRISEPTIN was proven effective at reducing the following viruses.

Virus	Initial PFU*	Level of Virucidal Reduction
Herpes Simplex 1 Virus	$\geq 10^{6.33}$	$>10^3$ (>99.9%)
Hepatitis A Virus	$10^{5.77}$	$>10^2$ (>99%)
Human Immunodeficiency Virus	$10^{6.0}$	$>10^3$ (>99.9%)

** Test limits reached at >3 $\log_{10}$ reduction. PFU is the viral counterpart to CFU. PFU represents plague-forming units.*

Conclusion:

TRISEPTIN inactivates RNA and DNA viruses.

Antimicrobial Spectrum Study

Objective:

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

Test Method:

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

Test Results:

TRISEPTIN provided greater than a 3-$\log_{10}$ reduction after 30 seconds of exposure.

Organism	
Acinetobacter sp., CI 317 (–)	*Micrococcus roseus*, ATTCC 186 (+)
Acinetobacter sp., ATCC 15308 (–)	*Moraxella catarrhalis*, CI 326 (–)
Bacteroides fragilis, ATCC 25285 (–)	*Proteus mirabilis*, ATCC 7002 (–)
Candida albicans, ATCC 10231 (yeast)	*Proteus mirabilis*, CI 312 (–)
Candida albicans, CI 304 (yeast)	*Pseudomonas aeruginosa*, ATCC 15442 (–)
Candida krusei, CI 302 (yeast)	*Pseudomonas aeruginosa*, ATCC 27853 (–)
Candida sp., CI 327 (yeast)	*Serratia marcescens*, ATCC 14756 (–)
Candida tropicalis, ATCC 750 (yeast)	*Serratia sp.*, CI 320 (–)
Candida tropicalis, CI 303 (yeast)	*Staphylococcus aureus*, CI 301 (+)
Citrobacter freundii, CI 306 (–)	*Staphylococcus aureus*, ATCC 29213 (+)
Enterobacter aerogenes, CI 308 (–)	*Staphylococcus aureus*, ATCC 6538 (+)
Enterobacter aerogenes, ATCC 13048 (–)	*Staphylococcus Coag. Neg.*, CI 319 (+)
Enterobacter sp., CI 318 (–)	*Staphylococcus Coag. Neg.*, CI 323 (+)
Enterobacter sp., CI 325 (–)	*Staphylococcus epidermidis*, ATCC 12228 (+)
Enterococcus faecalis, ATCC 29212 (+)	*Staphylococcus haemolyticus*, ATCC 29970 (+)
Enterococcus faecium, ATCC 6569 (+)	*Staphylococcus hominis*, ATCC 29885 (+)
Enterococcus sp., CI 322 (+)	*Staphylococcus saprophyticus*, ATCC 15305 (+)
Escherichia coli, CI 307 (–)	*Streptococcus bovis*, CI 313 (+)
Escherichia coli, ATCC 11229 (–)	*Streptococcus* Group B, CI 321 (+)
Escherichia coli, ATCC 25922 (–)	*Streptococcus* Group D, CI 324 (+)
Escherichia coli, CI 310 (–)	*Streptococcus pneumoniae*, ATCC 6303 (+)
Hemophilus influenzae, ATCC 19418 (–)	*Streptococcus pneumoniae*, CI 305 (+)
Klebsiella pneumoniae, ATCC 29995 (–)	*Streptococcus pyogenes*, ATCC 19615 (+)
Klebsiella sp., CI 316 (–)	*Streptococcus pyogenes*, CI 314 (+)

CI = clinical isolate
(+) gram positive
(–) gram negative

Conclusions:

- TRISEPTIN kills ATCC microorganisms and corresponding clinical isolates.
- TRISEPTIN is an effective, fast-acting and broad-spectrum antimicrobial.

Minimum Inhibitory Concentration (MIC) Study

Objective:

To determine the minimum concentration level at which TRISEPTIN solution 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 and tested to determine the highest dilution factor (lowest product concentration) which inhibits growth of the microorganisms.

Test Results:

TRISEPTIN showed strong inhibition activity against ATCC organisms and clinical isolates, including the antibiotic-resistant organisms as follows:

Organism	Percentage Concentration of TRISEPTIN	Organism	Percentage Concentration of TRISEPTIN
Acinetobacter sp., CI 317 (–)	0.0195	*Micrococcus luteus*, ATCC 7468 (+)	0.0195
Acinetobacter sp , ATCC 15308 (–)	0.0195	*Micrococcus roseus*, ATTCC 186 (+)	0.0195
Candida albicans, ATCC 1023 (yeast)	0.0195	*Moraxella catarrhalis*, CI 326 (–)	0.0195
Candida albicans, CI 304 (yeast)	0.0195	*Proteus mirabilis*, ATCC 7002 (–)	0.0195
Candida krusei, CI 302 (yeast)	0.0195	*Proteus mirabilis*, CI 312 (–)	0.0195
Candida sp , CI 327 (yeast)	0.0195	*Pseudomonas aeruginosa*, ATCC 27853 (–)	0.0781
Candida tropicalis, ATCC 750 (yeast)	0.0195	*Pseudomonas aeruginosa*, CI 309 (–)	0.1563
Candida tropicalis, CI 303 (yeast)	0.0195	*Serratia marcescens*, ATCC 14756 (–)	0.1563
Citrobacter freundii, CI 306 (–)	0.0195	*Serratia sp* , CI 320 (–)	0.0781
Enterobacter aerogenes, CI 308 (–)	0.0195	*Staphylococcus aureus*, CI 301 (+)	0.0195
Enterobacter aerogenes, ATCC 13048 (–)	0.0195	*Staphylococcus aureus*, ATCC 29213 (+)	0.0781
Enterobacter sp., CI 318 (–)	0.0195	*Staphylococcus aureus*, ATCC 6538 (+)	0.0195
Enterobacter sp., CI 325 (–)	0.0195	*Staphylococcus Coag. Neg.*, CI 319 (+)	0.0195
Enterococcus faecalis, ATCC 29212 (+)	0.0195	*Staphylococcus Coag. Neg.*, CI 323 (+)	0.0195
Enterococcus faecium, ATCC 6569 (+)	0.0195	*Staphylococcus epidermidis*, ATCC 12228 (+)	0.0195
Enterococcus sp., CI 322 (+)	0.0195	*Staphylococcus haemolyticus*, ATCC 29970 (+)	0.0195
Escherichia coli, CI 307 (–)	0.0195	*Staphylococcus hominis*, ATCC 29885 (+)	0.0195
Escherichia coli, ATCC 11229 (–)	0.0195	*Staphylococcus saprophyticus*, ATCC 15305 (+)	0.0195
Escherichia coli, ATCC 25922 (–)	0.0195	*Streptococcus* Group B, CI 321 (+)	0.0195
Escherichia coli, CI 310 (–)	0.0391	*Streptococcus* Group D, CI 324 (+)	0.0195
Hemophilus influenzae, ATCC 19418 (–)	0.0391	*Streptococcus pneumoniae*, ATCC 6303 (+)	0.6250
Klebsiella pneumoniae, ATCC 29995 (–)	0.0195	*Streptococcus pyogenes*, ATCC 19615 (+)	0.0391
Klebsiella sp., CI 316 (–)	0.0195	*Streptococcus pyogenes*, CI 314 (+)	0.0195

CI = clinical isolate
(+) gram positive
(–) gram negative

Conclusion:

TRISEPTIN is an effective antimicrobial agent that inhibits growth of a broad spectrum of microorganisms.

Development of Antimicrobial Resistance Study

Objective:

To determine the development of resistance to TRISEPTIN. For patient preoperative skin preparations, the Tentative Final Monograph for Antiseptic Drug Products proposes that the formulations be tested for development of antimicrobial resistance via point mutation.

Test Method:

TRISEPTIN was tested against *Escherichia coli* ATCC 8739, *Staphylococcus* species ATCC 6538, and *Pseudomonas aeruginosa* ATCC 15442 in a series of ascending two-fold dilutions, using repeated subculturing of the last solution demonstrating growth.

Test Results:

No growth was observed in increasingly concentrated solutions from the initial inoculation.

Organism	Outgrowth Demonstrated In Subsequent Challenge	Evidence of Resistance
Escherichia coli, ATCC 8739	No	No
Staphylococcus aureus, ATCC 6538	No	No
Pseudomonas aeruginosa, ATCC 15442	No	No

Conclusion:

Organisms, when challenged with TRISEPTIN, do not demonstrate resistance to the product.

Primary Skin Irritation Study (Human)

Objective:

To determine by repetitive epidermal contact the primary or cumulative irritation and/or allergic contact sensitization potential of a test material.

Test Method:

- TRISEPTIN was applied to intact skin and abraded skin sites.
- Sites were covered with a 2.5 x 2.5 cm non-occlusive dressing and then wrapped with a semi-occlusive bandage.
- Dressings were removed and the application sites graded.
- Sites were observed for signs of erythema and edema at 24 and 72 hours. Observations were scored according to the "Classification System for Scoring Skin Reactions."

Test Results:

TRISEPTIN meets the guidelines as a non-irritating preparation.

Conclusion:

TRISEPTIN is a non-irritating preoperative skin preparation.

Buehler Test for Sensitization (Animal)

Objective:

To determine by repetitive epidermal contact the primary or cumulative irritation and/or allergic contact sensitization potential of a test material to guinea pigs.

Test Method:

- Skin sites were treated repeatedly with occluded applications of TRISEPTIN at 50% strength for three weeks.
- After a cessation of applications for two weeks, TRISEPTIN was applied to virgin sites.
- Observations were scored for irritation and other effects at the application sites and a determination was made regarding sensitization.

Test Results:

TRISEPTIN-treated sites demonstrated no reactions.

Conclusion:

TRISEPTIN is not a topical sensitizer as demonstrated under the conditions of this test.

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TRISEPTIN® *WATERLESS* Surgical Scrub

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Surgical Scrub *In Vivo* Efficacy Study

Objective:

The test for a surgical scrub was designed to evaluate the degree of rapid and persistent reduction of normal resident and transient human flora.

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 a Surgical Scrub."

11 Washes over a 5-Day Period		
Day 1	1 wash	1-$\log_{10}$ reduction of microbial flora within 1 minute after application
Day 2	3 washes	2-$\log_{10}$ reduction of microbial flora within 1 minute after application
Day 3	3 washes	No test
Day 4	3 washes	No test
Day 5	1 wash	3-$\log_{10}$ reduction of microbial flora within 1 minute after application

Test Method:

- A statistically relevant number of subjects underwent a two-week period of "wash-out" (no use of antimicrobial products, internally or externally, for two weeks).
- Baseline populations must meet or exceed 1 x 10^5 CFU/hand at a minimum.
- The product was applied according to label directions and sampling was performed (subject sites randomized, used once) at 1 minute, 3 hours and 6 hours after application.
- Sampling was performed using the "glove juice method." Soon after product application per label directions, sterile gloves were donned, and a 75 ml sampling medium was inserted into the gloves. The hands were massaged for 1 minute. The antimicrobial action in the sample was neutralized. The fluid was collected, the wash repeated and the samples pooled.
- Samples were plated and enumerated, and the $\log_{10}$ difference between baseline and sample time was determined.

Test Results:

Subjects applied TRISEPTIN *WATERLESS* brush-free surgical scrub per the label directions. The test demonstrated superior results compared to the FDA's TFM testing criteria for the surgical scrub classification.

FIGURE 1: Testing Results for the FDA's Tentative Final Monograph for Healthcare Antiseptic Drug Products *in Vivo* Testing Criteria for Surgical Scrub Classification for TRISEPTIN *WATERLESS* Surgical Scrub

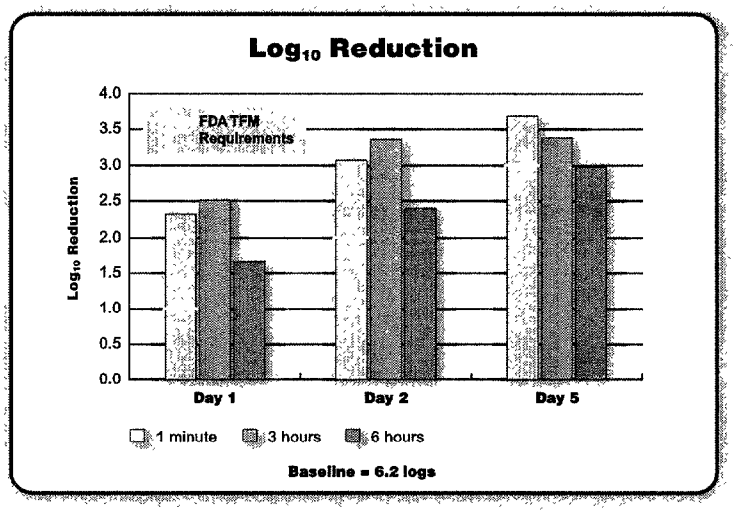
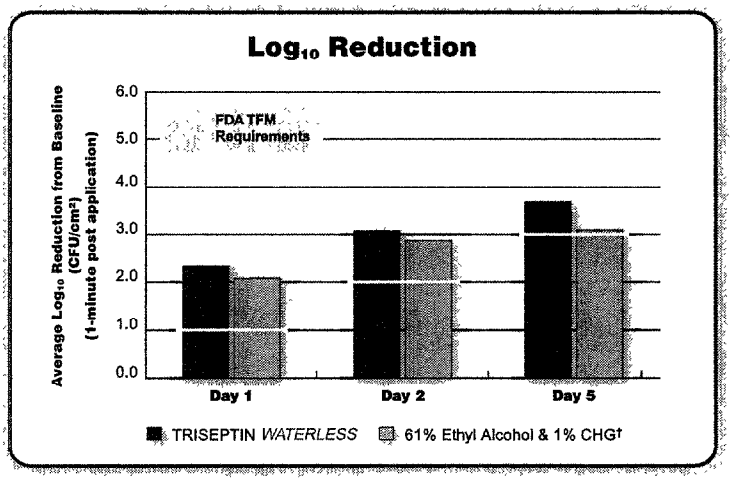


FIGURE 2: Comparison of TRISEPTIN *WATERLESS* Surgical Scrub to 61% Ethyl Alcohol with 1% CHG Waterless Scrub Agent to the FDA's Tentative Final Monograph for Healthcare Antiseptic Drug Products *in Vivo* Testing Criteria for Surgical Scrub Classification



Conclusions:

- TRISEPTIN *WATERLESS* brush-free surgical scrub demonstrated rapid reduction of resident and transient flora.
- TRISEPTIN *WATERLESS* brush-free surgical scrub demonstrated superior persistence, maintaining microbial reduction at all three test points, with efficacy that exceeds that required of a surgical scrub as outlined by the FDA's TFM testing criteria.
- TRISEPTIN *WATERLESS* brush-free surgical scrub reduced the microorganisms from a baseline of 6.2 logs (1,584,893 CFU/cm^2) to an average of 2.52 logs (331 CFU/cm^2) remaining on the Day 5 1-minute post application test point.

In Vitro Time-Kill Study

Objective:

To determine how rapidly TRISEPTIN *WATERLESS* surgical scrub achieves its antimicrobial effect.

Test Method:

TRISEPTIN *WATERLESS* surgical scrub was tested against the following species of ATCC[3] microorganisms and corresponding clinical isolates.

Test Results:

- TRISEPTIN *WATERLESS* surgical scrub 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 *WATERLESS* Surgical Scrub 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 *WATERLESS* surgical scrub rapidly kills ATCC reference microorganisms and clinical isolates.
- TRISEPTIN *WATERLESS* surgical scrub is a fast-acting and broad-spectrum antimicrobial agent.

Virucidal Efficacy Test Results

Objective:

To determine the virucidal capacity of TRISEPTIN *WATERLESS* surgical scrub.

Test Method:

TRISEPTIN *WATERLESS* surgical scrub was tested against several clinically important viruses via a standard ASTM suspension test method with a 3-minute exposure.

Test Results:

TRISEPTIN *WATERLESS* surgical scrub 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 Rota Virus	$\geq 10^{6.50}$	≥3.0

Conclusion:

TRISEPTIN *WATERLESS* surgical scrub inactivates RNA and DNA viruses and meets or exceeds the ASTM requirements to be classified as a virucide.

Antimicrobial Spectrum Study

Objective:

To test the efficacy of TRISEPTIN *WATERLESS* surgical scrub 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 *WATERLESS* surgical scrub were tested against the following species of ATCC microorganisms and corresponding clinical isolates.

Test Results:

TRISEPTIN *WATERLESS* surgical scrub provided greater than a 4.0-$\log_{10}$ 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 *WATERLESS* surgical scrub kills ATCC microorganisms and corresponding clinical isolates, some of which are antibiotic-resistant strains. These are identified by an asterisk (*).
- TRISEPTIN *WATERLESS* surgical scrub is an effective, fast-acting and broad-spectrum antimicrobial.

Resistant Organism Time-Kill Study

Objective:

To determine how rapidly TRISEPTIN *WATERLESS* surgical scrub achieves its antimicrobial effect when tested against known drug-resistant bacteria.

Test Method:

Several clinically important antibiotic-resistant organisms were exposed to TRISEPTIN *WATERLESS* surgical scrub to measure $\log_{10}$ reduction at 15 seconds.

Test Results:

TRISEPTIN *WATERLESS* surgical scrub showed strong bactericidal activity against the following antibiotic-resistant organisms.

Time-Kill Data for Antibiotic-Resistant Organisms Exposed to TRISEPTIN *WATERLESS* Surgical Scrub

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 *WATERLESS* surgical scrub demonstrated exceptional bactericidal action against known antibiotic-resistant organisms.

Minimum Inhibitory Concentration (MIC) Study

Objective:

To determine the minimum concentration level at which TRISEPTIN *WATERLESS* surgical scrub solution 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 *WATERLESS* surgical scrub and tested to determine the highest dilution factor (lowest product concentration) which inhibits growth of the microorganisms.

Test Results:

TRISEPTIN *WATERLESS* surgical scrub showed strong inhibition activity against ATCC organisms and clinical isolates, including the antibiotic-resistant organisms as follows:

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 *WATERLESS* surgical scrub 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 *WATERLESS* surgical scrub to a marketed waterless, brushless, alcohol-based surgical scrub and 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 *WATERLESS* surgical scrub 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 test products to produce irritation under exaggerated exposure. In this study, TRISEPTIN *WATERLESS* surgical scrub was compared to a 61% ethyl alcohol with 1% Chlorhexidine Gluconate (CHG) scrub agent† and a 4% Chlorhexidine Gluconate‡ scrub agent.

Methodology:

A total of 29 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 dryness. 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 dryness/scaling and erythema.
- Subject Self-Assessment: Subjects rated the backs of their right and left hands for overall conditioning and cracking.
- Transepidermal Water Loss (TEWL) Measurements: To determine the barrier function of the skin, measurements of the electrical conductance of the skin were taken on the back of each subject's right and left hand to measure transepidermal water loss (TEWL) through the skin using a DermaLab®.
- 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
	TEWL		TEWL		TEWL
	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 and dryness 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 (TEWL and 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:

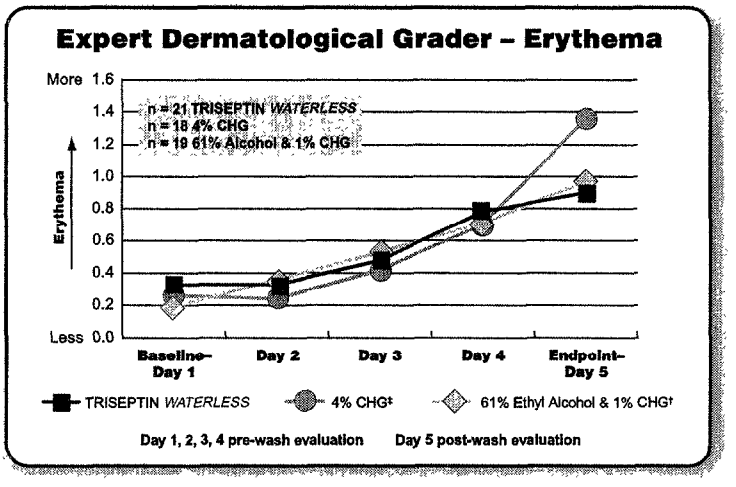
- Clinical grading of dryness and erythema symptoms
- Self-assessment of hand skin condition by subjects
- TEWL measurements on the back of each hand
- NOVA DPM measurements on the back of each hand

Conclusion:

Overall, the results of the study strongly show that TRISEPTIN *WATERLESS* surgical scrub and the surgical hand scrub with 61% alcohol with 1% Chlorhexidine Gluconate† were superior to 4% Chlorhexidine Gluconate‡ in nearly all parameters assessed.

Expert Dermatological Grader

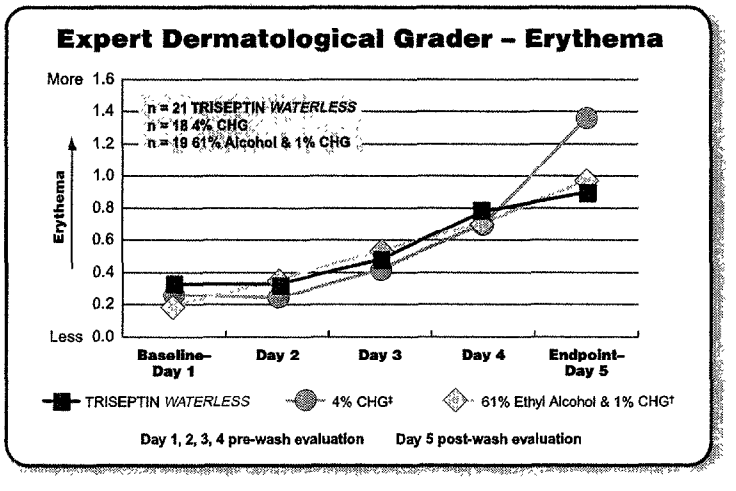
Clinical Dryness



Conclusions:

- At the endpoint (Day 5) hands treated with 4% Chlorhexidine Gluconate (CHG) were shown to be significantly drier than both TRISEPTIN *WATERLESS* surgical scrub and the surgical hand scrub with 61% alcohol with 1% Chlorhexidine Gluconate.
- TRISEPTIN *WATERLESS* surgical scrub and the surgical hand scrub with 61% alcohol with 1% Chlorhexidine Gluconate were statistically equivalent; no difference was found.

Clinical Erythema

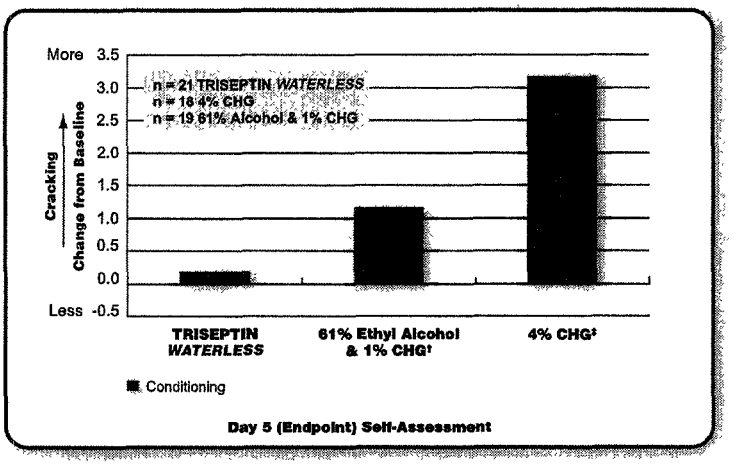


Conclusions:

- At the endpoint (Day 5) hands treated with 4% Chlorhexidine Gluconate showed significantly more erythema than both TRISEPTIN *WATERLESS* surgical scrub and the surgical hand scrub with 61% alcohol with 1% Chlorhexidine Gluconate.
- TRISEPTIN *WATERLESS* surgical scrub and the surgical hand scrub with 61% alcohol with 1% Chlorhexidine Gluconate were statistically equivalent; no difference was found.

Subject Self-Assessment of Hand Skin

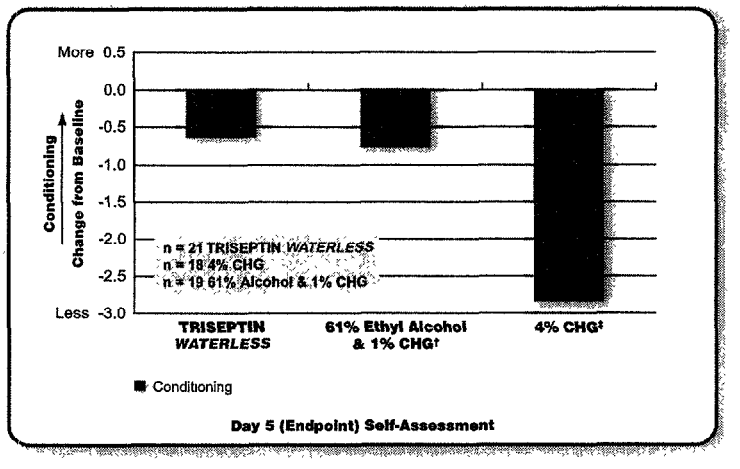
Skin Cracking



Conclusion:

At the endpoint (Day 5), hands treated with TRISEPTIN *WATERLESS* surgical scrub or 61% alcohol with 1% Chlorhexidine Gluconate demonstrated significantly less skin cracking than hands treated with 4% Chlorhexidine Gluconate.

Skin Conditioning

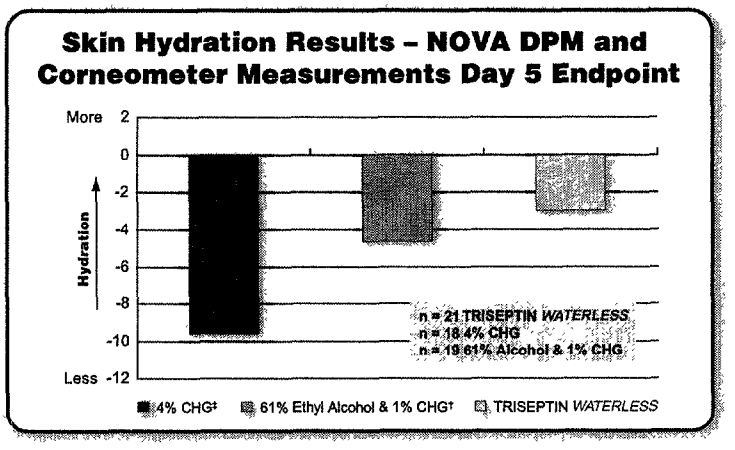


Conclusion:

At the endpoint (Day 5), hands treated with TRISEPTIN *WATERLESS* surgical scrub or 61% alcohol with 1% Chlorhexidine Gluconate demonstrated significantly less skin conditioning change than hands treated with 4% Chlorhexidine Gluconate.

NOVA™ Dermal Phase Meter (DPM) Measurements

Skin Hydration

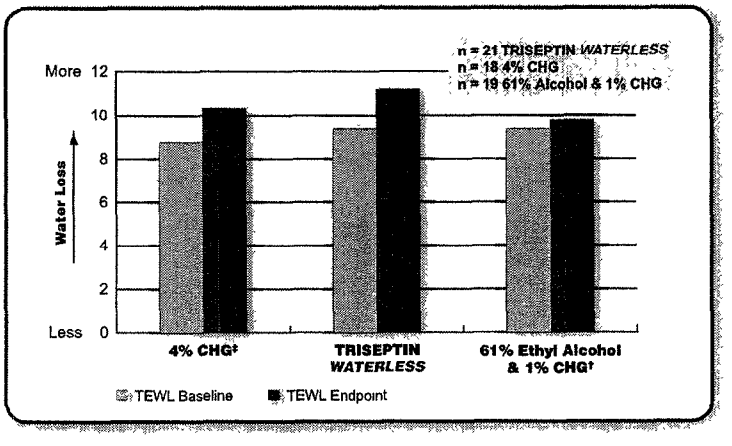


Conclusion:

While all products tested had an impact on skin hydration, both TRISEPTIN *WATERLESS* surgical scrub and 61% alcohol with 1% Chlorhexidine Gluconate retained significantly more hydration compared to hand skin treated with 4% Chlorhexidine Gluconate.

Transepidermal Water Loss (TEWL) Measurements

TEWL



Conclusions:

- No product tested caused a significant change in the transepidermal water loss measurements from baseline to the endpoint (Day 5).
- There were no statistical differences between TRISEPTIN *WATERLESS* surgical scrub, 61% alcohol with 1% Chlorhexidine Gluconate and 4% Chlorhexidine Gluconate from baseline to the endpoint (Day 5).

21-Day Cumulative Irritation Study in Humans

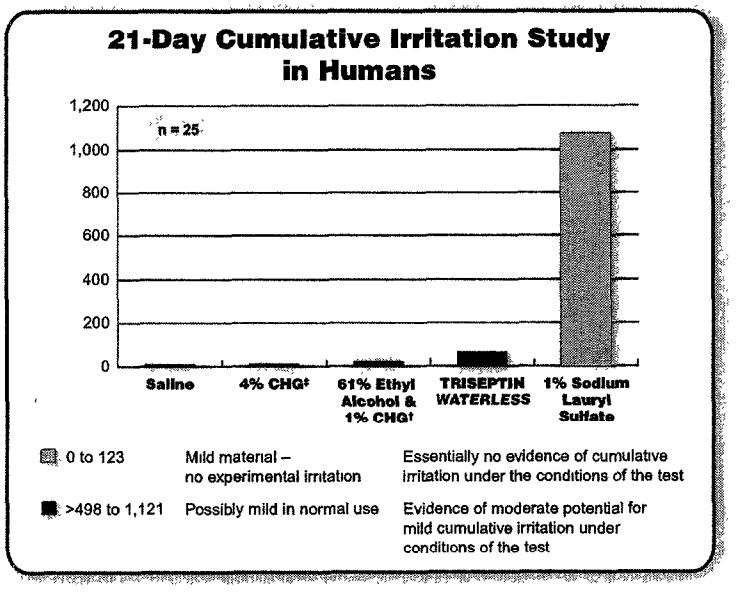
Objective:

To determine the cumulative irritation potential of a test product.

Test Method:

- Testing was done on 30 healthy subjects (male and female), between 23 and 61 years of age with Fitzpatrick Skin Type II to IV.
- Occlusive patches containing a defined amount of test product were placed on subjects' backs by laboratory professionals every day for a period of 21 days.
- Test sites were evaluated every day for irritation at least 2 hours after patch removal by the expert dermatological grader.
- After clinical evaluation, a new patch with test material was re-applied to the same area of the back.

Test Results:



Conclusion:

After 21 days of exposure, TRISEPTIN *WATERLESS* surgical scrub, 4% Chlorhexidine Gluconate and the surgical hand scrub with 61% alcohol with 1% Chlorhexidine Gluconate were statistically equivalent and demonstrated essentially no evidence of cumulative irritation under the conditions of the test.

Ingredient Description

The table below provides the list of ingredients and their function in TRISEPTIN *WATERLESS* Surgical Scrub.

TRISEPTIN *WATERLESS* Surgical Scrub	
Ingredient	**Function**
1. Alcohol (Ethanol) 61%	Active antimicrobial ingredient
2. Glyceryl Laurate	Emollient
3. Linoleic Acid	Emollient
4. Lauryl Lactate	Emollient
5. Capryl Glycol	Skin moisturizer
6. Cyclomethicone	Silicone emollient
7. Dimethicone Copolyol	Silicone emollient
8. Isopropyl Myristate	Emollient
9. Diisopropyl Sebacate	Emollient
10. Glycerin	Humectant and skin moisturizer
11. Poloxamer 124	Non-foaming surfactant
12. Citric Acid	pH adjusting agent
13. Purified Water	Vehicle
14. Fragrance	Fragrance
15. Zinc Pyrithione	Preservative
16. Ammonium Acryloyldimethyltaurate/VP Copolymer	Thickener

- TRISEPTIN *WATERLESS* surgical scrub uses a 61% ethyl alcohol active combined with a preservative to prolong persistence.
- TRISEPTIN *WATERLESS* surgical scrub was specially formulated with 9 different emollients and moisturizers and a humectant to help improve skin 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.

† Avagard® was used as a comparator in the "Exaggerated Hand Exposure Test" and the "Cumulative 21-Day Irritation Test in Humans."

‡ Hibiclens® was used as a comparator in the "Exaggerated Hand Exposure Test" and the "Cumulative 21-Day Irritation Test in Humans."

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