ACTIPREP™ One-Step Prep Solution

TECHNICAL BULLETIN

HEALTHPOINT®

ACTIPREP™ One-Step Prep Solution

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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 transient and resident human flora below the baseline population level.

Test criteria were established in the FDA Tentative Final Monograph for Healthcare Antiseptic Drug Products (TFM), which described the ASTM "Standard Test Method for Evaluation of a Patient Preoperative Skin Preparation," Designation 1173.

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 meet or exceed bacterial population 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 wand 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 (subject sites randomized, used once) 10 minutes, 6 hours, and 24 hours after application.
5. Samples were plated and enumerated, and the $\log_{10}$ difference between baseline and sample time was determined.

Test Results:

After the two sites were prepped with ACTIPREP per the label directions, the data demonstrated superior results exceeding the FDA TFM testing criteria for the Patient Preoperative Skin Preparation classification, as shown in Figure 1 and Figure 2.

FIGURE 1: FDA Tentative Final Monograph for Healthcare Antiseptic Drug Products *in Vivo* Testing Criteria for Patient Preoperative Skin Preparation Classification

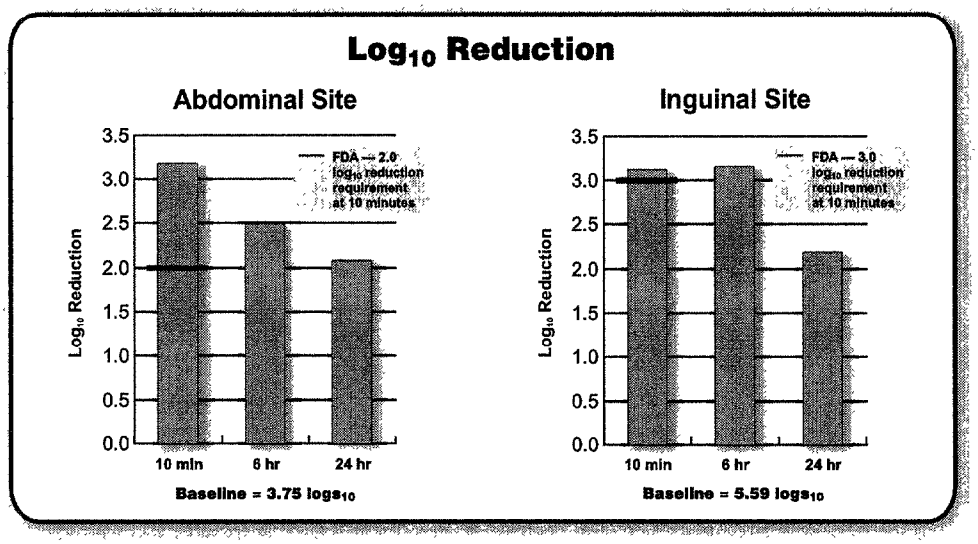
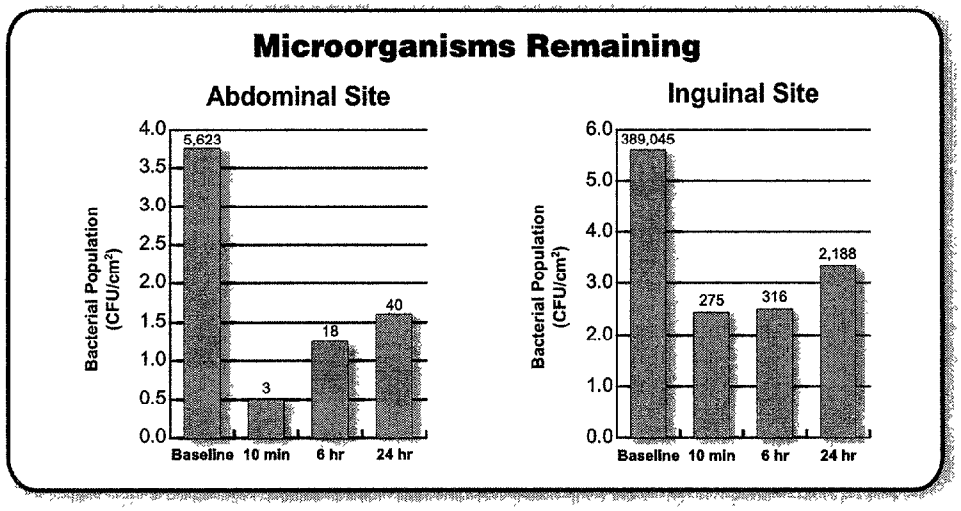


FIGURE 2: Baseline Cultures and Microorganisms Remaining Following One Application of ACTIPREP Antiseptic Skin Preparation and Subsequent *in Vivo* Testing Per the FDA Tentative Final Monograph for Healthcare Antiseptic Drug Products



Conclusions:

- ACTIPREP demonstrated rapid reduction of resident and transient flora, exceeding efficacy requirements of a patient preoperative skin preparation as outlined by the FDA TFM testing criteria.
- ACTIPREP demonstrated persistence, maintaining microbial reduction at 6 hours, exceeding efficacy requirements of a patient preoperative skin preparation as outlined by the FDA TFM testing criteria.
- ACTIPREP reduced microorganisms in the inguinal site from 389,045 CFU/cm² to only 275 CFU/cm² 10-minute post prep.
- The reduction of flora count remained below baseline for the extended 24-hour test period.

In Vitro Time-Kill Study

Objective:

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

Test Method:

1. Each of the following ATCC reference strains was grown under optimal conditions for the organism (aerobic, anaerobic, gram-positive, gram-negative or yeast).
2. A 1:10 dilution of the product was challenged with $\geq 10^6$ CFU/ml-test organism.
3. Samples were withdrawn at 9 minutes, antimicrobial activity neutralized, then samples were plated for enumeration.

Test Results:

- The 1:10 dilution of ACTIPREP reduced the ATCC strain populations.
- For the ATCC strains listed below, greater than 3-$\log_{10}$ reduction at 9 minutes was observed for most organisms.

Organism	% Reduction at 9 Minutes
Acinetobacter baumanii, ATCC 15308 (–)	99.9997
Bacteroides fragilis, ATCC 25285 (–)	99.9999
Candida albicans, ATCC 10231 (yeast)	99.9998
Candida tropicalis, ATCC 750 (yeast)	100
Enterobacter aerogenes, ATCC 13048 (–)	99.9961
Enterococcus faecalis, ATCC 29212 (+)	99.9999
Enterococcus faecium, ATCC 6569 (+)	99.9998
Escherichia coli, ATCC 11229 (–)	99.8919
Hemophilus influenzae, ATCC 19418 (–)	99.9999
Klebsiella oxytoca, ATCC 43165 (–)	100
Klebsiella pneumoniae, ATCC 29995 (–)	99.9333
Micrococcus luteus, ATCC 7468 (+)	100
Proteus mirabilis, ATCC 7002 (–)	99.9000
Pseudomonas aeruginosa, ATCC 15442 (–)	99.9999
Pseudomonas aeruginosa, ATCC 27853 (–)	100
Serratia marcescens, ATCC 14756 (–)	99.7250
Staphylococcus aureus, ATCC 29213 (+)	99.9894
Staphylococcus aureus, ATCC 6538 (+)	99.9998
Staphylococcus epidermidis, ATCC 12228 (+)	99.9999
Staphylococcus haemolyticus, ATCC 29970 (+)	99.9999
Staphylococcus hominis, ATCC 29885 (+)	99.9945
Staphylococcus saprophyticus, ATCC 15305 (+)	99 9999
Streptococcus pneumoniae, ATCC 6303 (+)	100
Streptococcus pyogenes, ATCC 19615 (+)	100

(+) gram positive
(–) gram negative

Conclusions:

- ACTIPREP rapidly kills ATCC microorganisms at a 1:10 dilution.
- ACTIPREP is an effective, fast-acting and broad-spectrum antimicrobial.

Minimum Inhibitory Concentration (MIC) Study

Objective:

To determine the minimum concentration level at which ACTIPREP inhibits bacterial growth of the following ATCC microorganisms and corresponding clinical isolates.

Test Method:

The ATCC microorganisms and corresponding clinical isolates were exposed to various dilutions of ACTIPREP to determine the highest dilution factor (lowest concentration) which inhibits growth of the microorganisms.

Test Results:

ACTIPREP showed strong inhibition activity against ATCC organisms and corresponding clinical isolates, including the antibiotic-resistant organisms.

Summary of Minimum Inhibitory Concentration Test Results for ACTIPREP

Organism	Percentage Concentration of ACTIPREP	Organism	Percentage Concentration of ACTIPREP
Acinetobacter sp, CI 99220 (–)	0.0391	*Micrococcus luteus*, ATCC 7468 (+)	0.0098
Acinetobacter baumanii, ATCC 15308 (–)	0.6250	*Micrococcus luteus*, CI 99462 (+)	0.0781
Bacteroides fragilis, ATCC 25285 (–)	0.0780	*Proteus mirabilis*, ATCC 7002 (–)	0.1563
Bacteroides fragilis, CI 99463 (–)	0.0391	*Proteus mirabilis*, CI 99113 (–)	0.3125
Candida albicans, ATCC 10231 (yeast)	0.3125	*Pseudomonas aeruginosa*, ATCC 15442 (–)	0.6250
Candida albicans, CI 99244 (yeast)	0.0195	*Pseudomonas aeruginosa*, ATCC 27853 (–)	0.6250
Candida tropicalis, CI 99464 (yeast)	0.0195	*Pseudomonas aeruginosa*, CI 9952 (–)	0.1563
Candida tropicalis, ATCC 750 (yeast)	0.3125	*Pseudomonas aeruginosa*, CI 9975 (–)	0.6250
Enterobacter aerogenes, ATCC 13048 (–)	0.6250	*Serratia marcescens*, ATCC 14756 (–)	0.0391
Enterobacter sp., CI 9902 (–)	0.0391	*Serratia marcescens*, CI 99413 (–)	0.0781
Enterococcus faecalis, ATCC 29212 (+)	0.0391	*Staphylococcus aureus*, ATCC 29213 (+)	0.0049
Enterococcus faecium, ATCC 6569 (+)	0.0391	*Staphylococcus aureus*, CI 99161 (+)	0.0049
Enterococcus faecalis, CI 99137 (+)	0.0391	*Staphylococcus aureus*, CI 9976 (+)	0.0391
Enterococcus faecium, CI 99168 (+)	0.0391	*Staphylococcus aureus*, ATCC 6538 (+)	0.0781
Escherichia coli, CI 99166 (–)	0.0391	*Staphylococcus epidermidis*, CI 9998 (+)	0.0049
Escherichia coli, ATCC 11229 (–)	0.0195	*Staphylococcus haemolyticus*, CI 99418 (+)	0.0098
Escherichia coli, ATCC 25922 (–)	0.0391	*Staphylococcus epidermidis*, ATCC 12228 (+)	0.0098
Escherichia coli, CI 99421 (–)	0.0098	*Staphylococcus haemolyticus*, ATCC 29970 (+)	0.0195
Haemophilus influenzae, ATCC 19418 (–)	0.0098	*Staphylococcus saprophyticus*, ATCC 15305 (+)	0.0195
Haemophilus influenzae, CI 99414 (–)	0.0098	*Staphylococcus hominis*, ATCC 29885 (+)	0.0049
Klebsiella pneumoniae, ATCC 29995 (–)	0.0391	*Streptococcus saprophyticus*, CI 99415 (+)	0.0098
Klebsiella pneumoniae, CI 9928 (–)	0.0781	*Streptococcus pyogenes*, CI 99417 (+)	0.0098
Klebsiella oxytoca, CI 99416 (–)	0.0391	*Streptococcus pneumoniae*, ATCC 6303 (+)	0.0195
Klebsiella oxytoca, ATCC 43165 (–)	0.0098	*Streptococcus pneumoniae*, CI 99369 (+)	0.0098
Staphylococcus hominis, CI 99408 (+)	0.0195	*Streptococcus pyogenes*, ATCC 19615 (+)	0.0391

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

Conclusion:

ACTIPREP is an effective antimicrobial agent at inhibiting growth of a broad spectrum of microorganisms.

Resistant Organism MIC Testing

Objective:

To determine the minimum-concentration level at which ACTIPREP inhibits bacterial growth of antibiotic-resistant microorganisms.

Test Method:

Several clinically important antibiotic-resistant organisms were exposed to various dilutions of ACTIPREP to determine the highest dilution factor (lowest concentration) which inhibits growth of the microorganisms.

Test Results:

Organism	Percentage Concentration of ACTIPREP
Enterococcus faecalis, ATCC 51575 (VRE)	0.0488
Enterococcus faecium, ATCC 51559 (VRE)	0.0244
Enterococcus faecium, Clinical Isolate (VRE)	0.0244
Staphylococcus aureus, ATCC 33592 (MRSA)	0.0061
Staphylococcus epidermidis, ATCC 5124 (MRSE)	0.0122

Conclusion:

ACTIPREP is effective against known multiple drug-resistant bacteria.

Development of Antimicrobial Resistance Study

Objective:

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

Test Method:

ACTIPREP was tested against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 11229, and *Pseudomonas aeruginosa* ATCC 15442 in a series of ascending two-fold dilutions, with repeated subculturing of the last dilution demonstrating growth.

Test Results:

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

Organism	Outgrowth Demonstrated	Evidence of Resistance
Staphylococcus aureus, ATCC 6538	No	No
Escherichia coli, ATCC 11229	No	No
Pseudomonas aeruginosa, ATCC 15442	No	No

Conclusion:

ACTIPREP did not induce point mutations resulting in development of antimicrobial resistance.

Primary Skin Irritation (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:

1. ACTIPREP was applied to intact skin and abraded skin sites.
2. Sites were covered with a 2 x 2 cm non-occlusive dressing and then wrapped with a semi-occlusive bandage.
3. Dressings were removed and the application sites graded.
4. 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:

ACTIPREP meets the guidelines as a non-irritating preparation.

Conclusion:

ACTIPREP is a non-irritating preoperative skin preparation.

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