**Study Number and Title:** **Zurex Pharma, Inc. Project No. ZX-ZP-0092**
**Microbac Project No. 865-107**

**Pivotal Clinical Evaluation of the Antimicrobial Effectiveness of Topically Applied ZuraPrep™** [redacted]

**NCT:** **03782103**

**Date:** **December 4, 2018**

MICROBAC®

# Microbac Clinical Protocol

| | |
|---|---|
| Study Number and Title: | Zurex Pharma, Inc. Project No. ZX-ZP-0092<br>Microbac Project No. 865-107<br><br>Pivotal Clinical Evaluation of the Antimicrobial Effectiveness of Topically Applied ZuraPrep™ [redacted] |
| Investigational Test Article: | ZuraPrep™ [redacted] |
| Reference Control Article: | ChloraPrep® [redacted] |
| Negative Control Article: | ZuraPrep™ [redacted] |
| Principal Investigator: | M. Hamid Bashir, MD, CCRP<br>[redacted] |
| Sub-Investigator: | Angela L. Hollingsworth<br>[redacted] |
| Qualified Physician / Sub-Investigator: | Rajeev Khanna, MD<br>[redacted] |
| Research Facility: | Microbac Laboratories, Inc.<br>105 Carpenter Drive<br>Sterling, Virginia 20164<br>Telephone: 703-925-0100<br>[redacted] |
| IRB: | MicroBioTest Internal IRB<br>[redacted] |
| Sponsor: | Zurex Pharma, Inc.<br>2113 Eagle Drive<br>Middleton, WI 53562 |
| Date: | December 4, 2018 |

**Confidentiality Statement**

This document contains the confidential information of Zurex Pharma, Inc., and Microbac Laboratories, Inc. (Microbac). It is intended solely for the guidance of the clinical investigation. This protocol may not be disclosed to parties not associated with the clinical investigation or used for any purpose without the prior written consent of Zurex Pharma, Inc., and/or Microbac.

## TITLE PAGE (continued)

Zurex Pharma, Inc.
Sponsor Representative:

[redacted]

Study Monitor

[redacted]

Statistical Consultant:

[redacted]

## SIGNATURE OF APPROVAL

**Investigator Agreement: I have read the attached protocol and I agree to conduct the study in accordance with the ethical principles that have their origin in the Declaration of Helsinki, Good Clinical Practice (GCP) and applicable state and federal regulations.**

Principal Investigator: _____ Date: 12/04/2018

M. Hamid Bashir, MD, CCRP
Principal Investigator

Sponsor(s): [redacted]

## Table of Contents

# 1. Background Information

## 1.1 Name, Description and Intended Use of the Test and Control Articles (Treatments)

The single investigational test article, ZuraPrep™ [redacted] is being evaluated for efficacy as a preoperative skin preparation solution by demonstrating its immediate and persistent antimicrobial properties. Testing will be performed based upon procedures outlined in the FDA Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use, Final Rule (Federal Register Volume 82, Number 243, Wednesday, December 20, 2017) and ASTM E1173 – 15 Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations. [redacted]

ZuraPrep™ [redacted] Solution Vehicle, [redacted] will be evaluated as a negative control (ZuraPrep™ [redacted]

ChloraPrep® [redacted] will be evaluated as an active control (reference control).

## 1.2 Risk/Benefit Summary

There are minimal anticipated adverse health risks for the participants of this study. Considerable safety and efficacy data are available for the active ingredient in the test and control formulations as described in the investigator brochure (IB).

## 1.3 Treatment Application Configurations

This study treatment will be conducted using ZuraPrep™ [redacted], ZuraPrep™ [redacted] Vehicle, [redacted] and ChloraPrep®, [redacted]. The product (including packaging) will be weighed pre-application and post-application.

The treatments will be applied topically to intact skin of the abdomen and groin regions of each subject. The treatments will be applied per the Treatment Application Instructions (Appendix 14.7).

## 1.4 Good Clinical Practice and Regulatory Requirements

This study will be conducted in compliance with this protocol, Good Clinical Practice (GCP) including 45 CFR 160 and 164 (Authorization for Use/Disclosure of Protected Health Information (PHI), 21 CFR 50 (Protection of Human Subjects), 56 (Institutional Review Boards), 330 (Over-The-Counter Human Drugs which are generally recognized as safe and effective and not misbranded) and Tentative Final Monograph for Health Care Antiseptic Drug Products (TFM), International Conference on

Harmonisation ICH Harmonised Tripartite Guideline E6: Good Clinical Practice, the Standard Operating Procedures of Microbac, the study protocol and any protocol amendments.

### 1.5 Study Population

Healthy male and female volunteers, 18 years of age or older, with no dermatological conditions or known history of sensitivity to natural rubber latex, adhesive skin products (e.g., Band-Aids, medical tapes), isopropyl alcohol or chlorhexidine gluconate will be enrolled into this study. For this trial, a sufficient number of volunteers will be recruited in the screening phase such that a total of at least 72 abdominal (sebaceous poor) regions and 72 groin (sebaceous rich) regions are evaluable at the completion of the study for each treatment. Subjects must satisfy all Screening Day and Treatment Day Inclusion/Exclusion Criteria prior to screening sample collection and Treatment Day procedures.

The right and left sides of both the abdomen and the groin must meet the minimum Screening Day baseline values stated in the Inclusion Criteria (Section 4.1) to qualify for the study.

At least 108 human subjects will be employed utilizing bilateral applications assuring that the treatments will be evaluated on the sites as described in the following table:

**Table 1.5:** Minimum number of readings per anatomical site (abdomen and groin), per treatment, per sampling interval (30 seconds and 6 hours post-application)

| Treatment | Number of Abdomen Evaluations | Number of Groin Evaluations |
|---|---|---|
| ZuraPrep™ [redacted] | 72 | 72 |
| ChloraPrep® [redacted] (active control) | 72 | 72 |
| ZuraPrep™ [redacted] Vehicle, [redacted] (negative control) | 72 | 72 |

## 2. Study Objectives and Purpose

The primary objective of this study is to measure the antimicrobial effectiveness of a single investigational test article, ZuraPrep™ [redacted] as specified by the Healthcare Antiseptics, Topical Antimicrobial Drug Products Final Rule of December 20, 2017. The treatments will be ZuraPrep™ [redacted] as the investigational test article, ChloraPrep® [redacted] as an active control, and ZuraPrep™ [redacted] Vehicle [redacted] as a negative control. For efficacy at 30 seconds post-prep, the test article should be superior to the negative control using a margin of 1.2 $\log_{10}$ CFU/cm$^2$ and non-inferior to the active control using a margin of 0.5 $\log_{10}$ CFU/cm$^2$.

The secondary objective of this study is to show persistent effect as defined in the Health Care Antiseptics, Topical Antimicrobial Drug Products Final Rule of December 20, 2017,

(Final Rule). Due to the normal variability associated with this biological system, the test article is considered to have persistent effect if the percentage of body sites with $\log_{10}$ CFU/cm$^2$ values less than or equal to baseline for the 6-hour sample is between 95 to 100%.

## 3. Study Design

### 3.1 Study Type

This is a randomized, paired-comparisons design where each subject receives two of the planned treatments.

**Table 3.1**: Treatments, Anatomical Sites of Evaluation, Application and Dry Times and Coverage Areas (See Appendix 14.7 for detailed application instructions)

| Treatment (Quantity/Volume) | Body Site | Application Time | Dry Time | Area of Coverage |
|---|---|---|---|---|
| ZuraPrep™ [redacted] | Abdomen (sebaceous poor) | 30 seconds | 3 minutes | 5” x 5” |
| | Groin (sebaceous rich) | 2 minutes | 3 minutes | 1.5” x 5” |
| ChloraPrep® [redacted] (active control) | Abdomen (sebaceous poor) | 30 seconds | 3 minutes | 5” x 5” |
| | Groin (sebaceous rich) | 2 minutes | 3 minutes | 1.5” x 5” |
| ZuraPrep™ [redacted] Vehicle, [redacted] (negative control) | Abdomen (sebaceous poor) | 30seconds | 3 minutes | 5” x 5” |
| | Groin (sebaceous rich) | 2 minutes | 3 minutes | 1.5” x 5” |

### 3.2 Primary Endpoint/Analysis

The primary analysis for the study utilizes the standards of the Health Care Antiseptics, Topical Antimicrobial Drug Products Final Rule of December 20, 2017. The primary efficacy criteria will be assessed based on $\log_{10}$ CFU/cm$^2$ values at 30 seconds for immediate effect and at 6 hours for persistent effect.

Product effectiveness will be measured using superiority and non-inferiority estimates for the 30-second sample time calculated using average treatment effects (ATEs). ATEs will be estimated from a linear regression of the 30 second post-treatment bacterial counts ($\log_{10}$ scale) on the additive effect of a treatment indicator and the baseline or pre-treatment measurements ($\log_{10}$ scale). To show effectiveness the test product will be (1) non-inferior to ChloraPrep® [redacted] with a 0.5 margin (upper bound of 95% confidence interval of the ATE of the test product versus active control value < 0.5) and (2) superior to the ZuraPrep [redacted] Vehicle (negative control) by a margin of 1.2 (lower bound of the 95% confidence interval of the ATE of the test product versus

the vehicle value > 1.2). Superiority and non-inferiority will be calculated separately for each body area (abdomen and groin).

A site is considered evaluable for efficacy (part of the per-protocol data set) if the criteria in the following table are met:

**Table 3.2**: Minimum and Maximum Treatment Day Baseline Values per Anatomical Site

| Anatomical Site | Minimum and Maximum Treatment Day Baseline* |
|---|---|
| Abdomen | $1.0 \times 10^3 – 3.2 \times 10^5$ CFU/cm$^2$ ($3.0$ Log$_{10}$ – $5.5$ Log$_{10}$) |
| Groin | $1.0 \times 10^5 – 3.2 \times 10^7$ CFU/cm$^2$ ($5.0$ Log$_{10}$ – $7.5$ Log$_{10}$) |

*Note: For the Screening Day baseline, the maximum upper limit does not apply. Only the minimum criteria must be met.

### 3.2.1 Secondary Endpoints/Analysis

Persistence or long-term efficacy is defined in the Final Rule as having the 6 hour post-treatment log$_{10}$ CFU/cm$^2$ values lower than or equal to the baseline log$_{10}$ CFU/cm$^2$ values for 100 percent of the subjects in each indication and body area. Due to the normal variability associated with this biological system, the test article is considered to have persistent effect if the percentage of body sites with log$_{10}$ CFU/cm$^2$ values less than or equal to baseline for the 6-hour sample is between 95 to 100%. All subjects that do not achieve the 6-hour efficacy goal will be documented and discussed. This only applies to ZuraPrep™ [redacted] for the purpose of determining efficacy but the same calculation will be performed for ChloraPrep® [redacted] for information purposes only.

### 3.2.2 Study Validity

There are no study validity criteria. All calculations performed for ZuraPrep™ [redacted] will be performed for ChloraPrep® [redacted], but for informational purposes only.

## 3.3 Randomization and Blinding

Subjects will be randomized before treatment, after screening eligibility is determined. Subjects will be randomized to treatment using the following block design:

Treatment Balance
Each subject will receive two different treatments, one on the right side of the body and one on the left.

The treatment assignments will be balanced such that the number of readings per anatomical site matches the calculated requirements.

Left/Right Balance
The application will be randomized so that each treatment is used on an equal number of left and right sides of the body.

Site and Sample Time Balance
Within each anatomical site, sample order with respect to sampling area number will be balanced with respect to treatments.

The randomization plan is based on the estimated population size [redacted] Subjects will be randomized to treatments in blocks of 12, randomly ordered within the block. Sample order will be randomized separately in blocks of 24 based on the 24 possible sample orders; 12 of the sample orders will be matched to the 12 treatment assignments in such a way to balance treatment with respect to sample order. Additional details on the randomization will be found in the randomization document.

The Investigator is responsible for ensuring that the randomization is followed. A basic outline of a randomization schedule for the abdominal and groin sites is provided in Appendix 14.4. The final randomization schedule will be prepared before the initial treatment. The test and control articles will be labeled with the appropriate codes as designated by the study randomization.

Subjects will be identified by their initials, a Screening ID Number, and a Subject Number. Subject Numbers will not be assigned until a subject has passed the screening criteria, including baseline bacterial counts (at least $1.0 \times 10^5$ CFU/cm$^2$ in the groin and at least $1.0 \times 10^3$ CFU/cm$^2$ on the abdomen). Subjects whose abdominal and groin regions qualify will be assigned the Subject Number on Treatment Day. Therefore, each of the participating treated subjects will be assigned two identification numbers: Screening Identification Number and a Subject Number.

- Screening subjects will be assigned numbers ranging from 9001 to 9999.
- Subjects to be treated (including treatment day baseline collection) will be assigned sequential numbers ranging from 1001 up to the total number of test subjects treated.

The study materials will not be blinded from the Investigator or other study staff performing the study material application or bacterial sample collections. The staff member(s) performing bacterial enumeration will be blinded from the identification of treatment assignment. The study staff performing the bacterial enumeration will not be involved in the study material application or the collection of samples. The Raw Data Capture form sections will be maintained separately (from the pages which include study treatment identifications) during the conduct phase of the study. The study staff performing the bacterial enumeration will record counts directly onto the Raw Data Capture forms without accessing the subject study documentation folder containing the other source document pages. The Raw Data Capture forms will be compiled with

the entire subject source record after all data recording has been completed. The data capture forms will serve as the source document.

Data will first be recorded onto the data capture forms, followed by entry into the electronic Case Report Form (eCRF). Wherever the data are entered first is considered the source document.

### 3.4 Study Materials

The materials identified in Table 3.4 will be used in the study. Specific product identification codes and lot numbers will also be included on the form titled "Confirmation of Release and Receipt of Study Materials" (Appendix 14.8) at the time the clinical supplies are shipped to the study site.

**Table 3.4**: Study Materials

| Study Arm | Name | Description | Lot No. | Exp. |
|---|---|---|---|---|
| Test Article | ZuraPrep™ | | | |
| Reference Control Article (Active Control) | ChloraPrep® | 2% Chlorhexidine Gluconate (w/v) and 70% isopropyl alcohol (v/v) | 8218679 | 07/31/21 |
| Negative Control Article | ZuraPrep™ Vehicle, | ZuraPrep™ Vehicle | | |
| | | Zurex | | |

### 3.4.1 Study Materials Labeling

Zurex Pharma, Inc. will label, package and ship the study materials required for ZuraPrep™ [redacted] and the negative control (ZuraPrep™ [redacted] Vehicle [redacted]) to the research facility. [redacted]

[redacted]

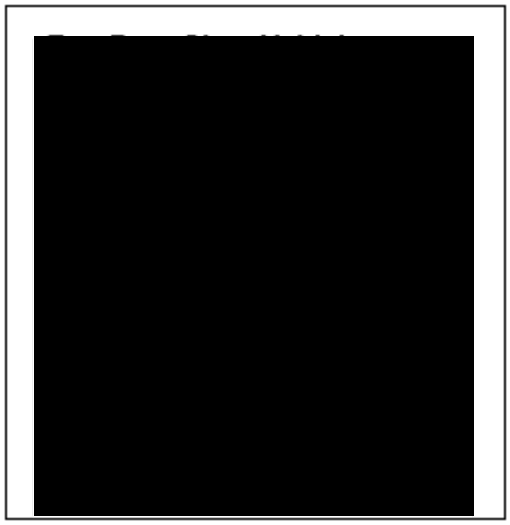
[redacted]

[redacted]

[redacted]

[redacted]

[redacted]



[redacted]

### 3.4.2 Study Supplies Provided by Sponsor

- Test article treatment materials and negative control treatment materials.

### 3.4.3 Study Supplies Provided by Study Site

- Reference Control Article
- Treatment Material Disposition forms
- Product Washout Kits (toiletry items to be used by subjects during study)
- Consent / Authorization forms, IRB-approved
- Case Report Forms
- Sampling Solution, sterile [redacted]
- Butterfield's sterile Phosphate Buffered Water containing neutralizers, [redacted]
- High-purity deionized water, sterile
- Trypticase Soy Agar (TSA)
- Trypticase Soy Agar containing 0.5% Tween 80 and 0.07% lecithin (TSA+N)
- Transfer pipettes, polyethylene, sterile
- Serological pipettes [redacted], sterile, [redacted]
- Pipetting device, calibrated to accurately dispense 5.0 mL
- Tubes with sealable caps, polypropylene or glass, sterile
- Petri dishes, 100 mm, sterile

- Gloves, sterile
- Gauze, sterile (2" x 2")
- Marking templates, 5" x 5" & 1.5" x 5", sterile (for marking test sites)
- Non-toxic marking pen (Sharpie or equivalent)
- Rubber policemen, sterile
- Scrub cups [redacted]
- Timers or stopwatches
- Pipette Aid or similar apparatus
- Vortex mixer
- Surgical Clipper & clipper blades
- Water bath (45° ± 2°C)
- Incubator (30° ± 2°C)
- Disposable underwear for subjects
- Tryptic Soy Broth (for the Neutralization Validation Procedure only – See Appendix 14.10) (TSB)
- [redacted] Urine Strip Test (for Pregnancy) or equivalent.

## 3.5 Study Duration

The expected duration of this study for each subject is up to 3-4 weeks. Subjects will undergo at least a 14-day washout period followed by a qualification screening baseline visit. Subjects whose screening baseline samples meet the minimum values described in the Inclusion Criteria (Section 4.1) will be notified and invited to participate in the treatment phase of the study. The treatment phase will be scheduled no sooner than 72 hours from the screening baseline collection.

## 3.6 Study Termination/Subject Discontinuation or Withdrawal/Subject Revocation of Authorization

### 3.6.1 Study Termination

Zurex Pharma, Inc. or the Investigator has the right to discontinue the study at any time for medical and/or administrative reasons. As far as possible, this should occur after mutual consultation.

### 3.6.2 Subject Discontinuation and Withdrawal

The Investigator may discontinue individual subjects from the study at any time. Subjects may voluntarily withdraw from the study at any time without reason or consequence. The subject will be asked to report the reason for withdrawal, when possible. The Investigator will record the reason for early discontinuation on the data capture form and transcribe on the appropriate eCRF including the date and reason for discontinuance. Subjects who qualify on Screening Day and begin the treatment phase may not be re-entered into the study, regardless of whether or not they completed the study.

Any enrolled subject will be discontinued for the following reasons:

1. A skin irritation rating of 3 for any individual skin condition at any evaluation following the application of study treatment. (A skin irritation rating of 2 for any individual skin condition at any evaluation following the application of study material may also be the cause for subject discontinuation at the discretion of the Investigator.)
2. Experiencing a serious protocol deviation that compromises the data results, for example, using a topical antibiotic at a test site during the study.

See Section 8.5 for handling of withdrawn subject data.

### 3.6.3 Subject Revocation of Authorization to Use and Disclose Protected Health Information

In order to implement a valid revocation of authorization, the subject or their representative must make the request in writing to Microbac, [redacted]. The revocation cannot stop the use or disclosure of information that has been collected prior to the revocation, or is needed to ensure complete and accurate study results, and/or is required by law or government regulation (e.g., reporting adverse events, etc.). Revocation of an authorization may not be used to withhold normal medical care from the subject, but may [or will] make the subject ineligible to receive the study treatment or care.

## 3.7 Treatment Material Accountability

Zurex Pharma, Inc. requires Investigators to maintain accountability and adequate inventory security of the study material at all times. The Investigator or designee will:

- complete the Confirmation of Release and Receipt of Study Materials form (Appendix 14.8) upon receipt of the shipment and maintain and account for inventory on the Study Material Disposition form (Appendix 14.9).
- keep study materials in a secure storage area, accessible only to authorized individuals.
- dispense study material only to subjects properly enrolled into the study.
- return all unused study materials to Zurex Pharma, Inc. at the end of the study or dispose of unused study materials as agreed upon.

## 3.8 Source Data

Data capture forms will serve as the source document. Data will be recorded directly onto the data capture form and then transcribed into the eCRF. The electronic data will be consistent with the source documents or the discrepancies will be explained. Wherever the data are entered first is considered the source document.

## 3.9 Protocol Modifications

### 3.9.1 Protocol Amendments

The party initiating an amendment must confirm it clearly in writing using the Project Sheet that will contain the Amendment. It must be signed and dated by Zurex Pharma, Inc. and, in the case of a significant amendment, the Investigator. A significant amendment means one that affects the safety, rights or welfare of subjects, the scope of the investigation or the scientific quality of the study.

Zurex Pharma, Inc. will submit significant protocol amendments to the Investigator for submission to the IRB. Zurex Pharma, Inc. will also notify the Investigator when a protocol amendment may be implemented.

### 3.9.2 Protocol Deviations

A deviation is a departure from the protocol that will likely affect the safety, rights or welfare of subjects, the scope of the investigation or the scientific quality of the study. Protocol deviations are documented on a Protocol Deviation Form and transcribed on the appropriate eCRF, if applicable.

Sponsor Notification

Deviations that potentially affect 1) subject safety, rights or welfare, 2) data integrity or 3) compromise the statistical analysis of the study require immediate communication to Zurex Pharma, Inc. The Investigator must contact the Zurex Pharma, Inc. study monitor within 24 hours of occurrence at the following phone number: [redacted] A Protocol Deviation Form must be completed by the Investigator and include a description of the circumstances surrounding and the reason for the deviation, any actions taken, and whether or not the subject was allowed to continue in the study. A copy of the deviation form or completion in the eCRF must be sent to the Zurex Pharma, Inc. study monitor within 24 hours of identifying the occurrence.

IRB Notification

Deviations which are made to protect the life or physical well-being of a subject in an emergency must be reported by the Investigator to the IRB as soon as possible, or no later than 5 working days after the investigative site learns of the occurrence.

## 3.10 Computerized System

- [redacted]

## 4. Subject Selection

Healthy volunteers with no dermatological conditions or known history of sensitivity to natural rubber latex, adhesive skin products (e.g., Band-Aids, medical tapes), isopropyl alcohol, citric acid, methylparaben, propylparaben, or chlorhexidine gluconate will be enrolled into this study. A sufficient number of volunteers will be enrolled in the screening phase such that the total number of abdominal regions and the total number of groin regions meets or exceeds the number determined for the study (72 abdominal regions and 72 groin regions for each treatment). Subjects must satisfy all Screening Day and Treatment Day Inclusion/Exclusion Criteria prior to screening sample collection and Treatment Day procedures. Volunteers will be recruited and treated until the count of results in the per protocol data set meets or exceeds the required test material/region counts.

A subject must qualify for both the abdominal portion and the groin portion of the study and the right and left sides of the abdomen and groin must meet the minimum baseline values stated in the Inclusion Criteria in order to be eligible for Treatment Day procedures.

Sites which are treated but discovered to have Treatment Day baseline counts below the minimum values or above the maximum values stated in Table 3.2 will not be included in the primary efficacy analysis (per-protocol data set). Additional subjects will be recruited and treated identically to the treatments for the subjects who had non-qualifying sites until a sufficient number of qualifying sites have been treated.

Subjects will be identified at screening by their initials and a screening ID number. Subject numbers will not be assigned until a subject has passed the screening criteria, including baseline bacterial counts.

All volunteers will be given verbal and written information about the study procedures, and Subject Instructions (Appendix 14.6) will be provided to each subject for the pre-treatment phase of the study. The following Inclusion/Exclusion Criteria will be reviewed on Screening Day and on Treatment Day to establish eligibility for participation:

### 4.1 Subject Inclusion Criteria

Subjects to whom all of these conditions apply will be eligible for enrollment in this study:

- Healthy male and female volunteers, 18 years of age or older.
- Are in good general health.
- Be able to present a valid identification card that includes name and date of birth.
- Have skin within 6 inches of the test sites that is free of tattoos, dermatoses, abrasions, cuts, lesions or other skin disorders.
- Willing to avoid antihistamines, immunosuppressants, or oral steroids for the duration of the study. (other than hormones for contraception or post-menopausal reasons).
- Wiling to avoid topical or systemic antimicrobial exposure for the duration of the study. Restrictions include, but are not limited to antimicrobial soaps,

antiperspirants/deodorants, shampoos, lotions, perfumes, after shaves, colognes, and topical or systemic antibiotics.

- Cooperative and willing to follow Subject Instructions (Appendix 14.6).
- Cooperative and willing to sign Consent Form / HIPAA Authorization Form.
- Have Screening Day baseline counts of at least 1.0 x $10^3$ CFU/$cm^2$ per abdominal site (left and right) and at least 1.0 x $10^5$ CFU/$cm^2$ per groin site (left and right).

## 4.2 Subject Exclusion Criteria

Subjects to whom *any* of these conditions apply will be excluded from this study:

- Topical or systemic antimicrobial exposure within 14 days prior to Screening Day and up to and including Treatment Day. Restrictions include, but are not limited to antimicrobial soaps, antiperspirants/deodorants, shampoos, lotions, perfumes, after shaves, colognes, and topical or systemic antibiotics.
- Taking antihistamines, immunosuppressants, or oral steroids within 14 days prior to Screening Day and up to and including Treatment Day (other than hormones for contraception or post-menopausal reasons).
- Swimming in chemically treated pools or bathing in hot tubs, spas and whirlpools within 14 days prior to Screening Day and up to and including Treatment Day.
- Use of tanning beds, hot waxes, or depilatories, including shaving (in the applicable test areas) within 14 days prior to Screening Day and up to and including Treatment Day.
- Contact with solvents, acids, bases, fabric softener-treated clothing or other household chemicals in the applicable test areas within 14 days of the Screening Day and up to and including Treatment Day.
- Subjects who have a history of sensitivity to natural rubber latex, adhesive skin products (e.g., Band-Aids, medical tapes), isopropyl alcohol, citric acid, methylparaben, propylparaben, or chlorhexidine gluconate products.
- Subjects who have a history of skin allergies.
- Subjects who have a history of skin cancer within 6 inches of the applicable test areas.
- Subjects who are pregnant, attempting pregnancy or nursing. For all females of child-bearing potential (<60 years of age), a pregnancy test will be performed before treatment on treatment day.
- Subjects who have showered or bathed within 72 hours of the Screening Day or Treatment Day (sponge baths may be taken, however, the lower abdomen and upper thigh region must be avoided).
- Subjects who receive an irritation score of 1 for any individual skin condition prior to the Screening Day baseline or Treatment Day baseline sample collection.
- Participation in another clinical trial in the 30 days prior to Test Day of this study (treatment with test materials in this study), currently enrolled in another clinical trial, or has previously participated in this study.

### 4.3 Subject Consent

The Investigator, or designated sub-investigators trained by the Investigator, must ensure that written informed consent to participate in the investigation is obtained before including any individual as a subject in the investigation. The Investigator, or designated sub-investigators trained by the Investigator, must provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate, and minimize the possibility of coercion or undue influence. The process is designed to 1) give the subject all the information needed, 2) ensure that the subject understands the information, and 3) give the subject a chance to consider study participation. The process should permit the subject to ask questions and exchange information freely.

Specifically, the Investigator, or designated sub-investigators trained by the Investigator, is to explain to each subject all elements of informed consent as specified in 21 CFR 50.25 (Appendix 14.5). After the explanation, subjects or their representative will voluntarily sign and date the consent form if they wish to participate in the study. A copy of the consent form must be provided to the subject. A signed and dated consent form must be maintained in the Investigator study documentation file at all times.

### 4.4 Subject Authorization for Use and Disclosure of Protected Health Information

The Investigator, or designated sub-investigators trained by the Investigator, must ensure that written authorization for use and disclosure of Protected Health Information (PHI) is obtained before including any individual as a subject in the investigation.

Specifically, the Investigator, or designated sub-investigators trained by the Investigator, is to explain to each subject all elements of authorization as specified in 45 CFR 164.508. After the explanation, subjects or their representative **must** voluntarily sign and date the authorization form if they wish to participate in the study. A copy of the authorization form must be provided to the subject. A signed and dated authorization form must be maintained in the Investigator study documentation file at all times.

An authorization form may be combined with a consent form (i.e., compound authorization) if required by the IRB. All required elements for both informed consent and authorization must be included in a compound authorization.

## 5. Study Treatment

These study procedures are based on the American Society for Testing and Materials (ASTM) “Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations” (E 1173-15[1]).

## 5.1 Study Procedures

Procedures will be performed by the Investigator or designated personnel trained by the Investigator.

### 5.1.1 Screening Phase Study Procedures

#### 5.1.1.1 Washout Period

The Inclusion/Exclusion Criteria will be reviewed with each subject to ensure eligibility for the study. If these criteria are satisfied, subjects will sign the consent form / HIPAA Authorization Form before screening phase study procedures begin. Prior to the scheduled Screening Day, subjects will undergo a minimum 14-day washout period. The subjects will be instructed to avoid contact with any topical or systemic antimicrobial products for the duration of their involvement in the study as written in the Subject Instructions (Appendix 14.6). If it becomes necessary to take systemic antibiotics or to apply topical medications to the test areas within this pre-treatment period, the subject must contact the Investigator as soon as reasonably possible so that another volunteer may be recruited.

Restrictions include, but are not limited to:

- Use of antimicrobial soaps, shampoos, lotions, perfumes, after shaves, colognes, antiperspirants, deodorants
- Contact with materials such as acids, bases, solvents
- Swimming in chemically treated pools and bathing in hot tubs, spas and/or whirlpools
- Use of tanning beds, hot waxes or depilatories (including shaving)

Subjects will be provided a Product Washout Kit with non-antimicrobial personal care products for exclusive use during the study. Subjects will also be provided with written instructions regarding the use of these products (Appendix 14.6).

A visual skin assessment of the test areas will be performed. If subjects require hair removal to facilitate sample collection, the subject will be asked to return to the test facility at least 48 hours before the Screening Day.

Subjects will be required to refrain from bathing or showering for 72 hours prior to both the Screening Day and Treatment Day.

Sponge bathing will be allowed, however, the subject must avoid the lower abdomen and upper thigh region.

#### 5.1.1.2 Screening Day (Qualification Baseline Sampling)

Subjects will be required to refrain from bathing or showering 72 hours prior to Treatment Day and hair will be clipped at least 48 hours prior to Screening Day.

After the washout period and at least 3-days before Treatment Day, the Investigator or a designated assistant will complete the Screening Day Inclusion/Exclusion Criteria data capture form.

Prior to performing the Screening Day baseline sample collection, a skin irritation assessment will be performed

If an irritation score of 1 for any individual skin condition at the Screening Day baseline is assigned, the subject will be excluded from the study.

A baseline screening sample will be collected from each test area using the Williamson-Kligman scrub cup technique[3]. Baseline samples will be taken from the center of each contra lateral test area within each anatomical region. Samples from both the left and right sides of a body region must meet the minimum value indicated in the Inclusion Criteria for the subject to be enrolled into the treatment phase of the study for that region. Subjects must qualify for both the abdominal portion and the groin portion of the study.

Subjects who qualify for the study will be notified and will continue to follow the subject instructions until completion of the scheduled Treatment Day.

Subjects will again be required to refrain from bathing or showering 72 hours prior to Treatment Day and hair will be clipped at least 48 hours prior to Treatment Day.

### 5.1.2 Treatment Phase Study Procedures

A sufficient number of subjects who meet the entrance criteria will be enrolled into the treatment phase of the study for each region, such that the total number of abdominal regions and the total number of groin regions meets or exceeds the number required (72 abdominal regions and 72 groin regions for each treatment). The Randomization Scheme, given in the randomization document, will designate the treatment to each side of the abdomen and groin. A basic outline of a Randomization Scheme for the abdominal and groin sites is provided in Appendix 14.4. Additional details on the randomization will be found in the randomization document.

The Treatment Day Inclusion/Exclusion Criteria will be completed on the source document and transcribed in the eCRF. If these criteria are satisfied,

a visual skin assessment will be performed to evaluate the condition of each test area.

#### 5.1.2.1 Preparation of Test Areas on Treatment Day

A Test Site Diagram for the abdominal and groin test areas is shown in Appendix 14.3. Note: skin irritation will be assessed for all assigned test areas prior to treatment of any test area.

##### 5.1.2.1.1 Preparation of Abdominal Test Area

The test site within the abdominal region (abdominal test area) is defined as the area below the umbilicus and above the groin. Using a 5" x 5" sterile template, the corners of each abdominal test area will be marked directly on the skin using a non-toxic skin marker. Four sampling sites will be numbered within each abdominal test area, on each side of the abdominal region. The positioning and numbering of the abdominal sampling sites are standard for all subjects. Sampling sites on the contra-lateral side of the abdomen will be numbered in a mirror-image orientation. The four sampling sites within each abdominal test area represent one baseline (pre-prep) site, one post-prep sampling site for each of two sampling times (30 seconds and 6 hours), and one unused sampling site.

Prior to performing the Treatment Day baseline sample collection, a skin irritation assessment will be performed.

If an irritation score of 1 for any individual skin condition at the Treatment Day baseline is assigned, the subject will be excluded from the treatment phase of the study. After abdominal test areas are marked and sample sites numbered, baseline samples will be collected from the appropriate site per the Randomization Scheme in each test area using the scrub cup technique.

##### 5.1.2.1.2 Preparation of Groin Test Area

The test site within the groin region (groin test area) is defined as the inner aspect of the upper thigh within and parallel to the inguinal crease below the groin. Using a 1.5" x 5" sterile template, the corners of each groin test area will be marked directly on the skin using a non-toxic skin marker. Four sampling sites will be numbered within each groin test area, on each side of the groin region. The positioning and numbering of the groin sampling

sites are standard for all subjects. Sampling sites on the contra-lateral side of the groin will be numbered in a mirror-image orientation. The four sampling sites within each groin test area represent one baseline (pre-prep) site, one post-prep sampling site for each of two sampling times (30 seconds and 6 hours), and one unused sampling site.

Prior to performing the Treatment Day baseline sample collection, a skin irritation assessment will be performed.

If an irritation score of 1 for any individual skin condition at the Treatment Day baseline is assigned, the subject will be excluded from the treatment phase of the study. After groin test areas are marked and sample sites numbered, baseline samples will be collected from the appropriate site per the Randomization Scheme in each test area using the scrub cup technique.

#### 5.1.2.2 Treatment Materials Application

Following baseline sample collection, randomly assigned contra-lateral test areas will be treated with the applicable treatment materials. The post-application sampling times will be randomized among the sampling sites within a test area.

The treatment materials will be applied and the sampling configurations will be performed per the Randomization Scheme and the Study Material Treatment Application Instructions (Appendix 14.7). The duration of each application procedure will be recorded on the appropriate data capture form and transcribed in the eCRF.

The applicator weight (ZuraPrep™ [redacted] or the pre-filled applicator for the reference control article or vehicle and empty applicator for the negative control) will be measured and documented before and after application to the treatment site. See Appendix 14.7 for details.

#### 5.1.2.3 Timing of Post-Application Sample Collection

Microbial samples will be collected at 30 seconds (± 5 seconds) and 6 hours (± 30 minutes) post-treatment application for both the abdomen and the groin regions. Post-application timing begins upon completion of the treatment material application, including drying time. Microbial samples will be collected using the scrub cup technique (see Section 5.2.1).

After the 30 seconds (± 5 seconds) samples have been collected, sampling sites for 6 hours (± 30 minutes) will be covered indvidually with a piece of sterile gauze and a non-occlusive dressing to allow subjects restricted mobility and to protect the sites from contamination between sampling times. The subjects will be allowed to leave the clinical test facility, but must return at 6 hours (± 30 minutes) for the final post application sample collection

A skin irritation assessment will be performed prior to collection of the post-treatment microbial sample collection (30 seconds and 6 hours) and a corresponding rating score using the Skin Irritation Rating Scale for each individual skin condition will be recorded on the subject's data capture form and transcribed in the eCRF. See Appendix 14.11.

If an irritation score of 3 for any individual skin condition at any post-treatment observation is assigned, the subject will be discontinued from the study and an adverse event will be recorded. See Section 7.3 (Adverse Events). Following final sample collection, residual study materials will be wiped/cleansed from the subject's skin using mild soap and/or tap water with a paper towel.

## 5.2 Microbiological Methods

### 5.2.1 Microbial Sample Collection / Scrub Cup Technique

Quantitative cultures (screening baselines, treatment baselines and post-treatment application) will be obtained by a modification of the cylinder sampling technique of Williamson-Kligman scrub cup technique. To collect the samples, a sterile scrub cup [redacted] will be placed on the site and held firmly to the skin. Sampling solution (SS) [redacted] will be pipetted into the cup and the skin will be scrubbed in a circular motion with moderate pressure for 1 minute using a sterile rubber policeman. Using a sterile transfer pipette, the SS will be removed and placed in a sterile test tube. An additional [redacted] of fresh sampling solution will be pipetted into the cup and the scrub procedure will be repeated. This solution will be pooled with the first solution collected.

### 5.2.2 Sampling Solution (SS)

The SS consists of sterile [75mM phosphate buffer [redacted] containing [redacted] SS).

### 5.2.3 Bacterial Enumeration Methods

Following sample collection, 10-fold serial dilutions [redacted] will be prepared using Butterfield's Phosphate Buffered Water with

neutralizers (PBW). One mL aliquots of appropriate dilutions will be pour-plated in triplicate using Trypticase Soy Agar containing neutralizers (TSA+N). Samples must be plated within 30 minutes of collection. After 72 ± 4 hours of aerobic incubation at 30° ± 2°C, colonies will be counted and viable cells in the original sample will be calculated according to Standard Operating Procedures. After incubation, plates may be refrigerated up to 48 hours prior to counting.

Raw colony counts from each dilution will be recorded on the appropriate data capture forms for each subject. The average number of microorganisms recovered (CFU/cm$^2$) of skin for the screening and treatment day baseline samples will be calculated using the following formula to convert the plate CFU values into $\log_{10}$ CFU/cm$^2$ of skin:

$$R = \log_{10}\left[\frac{F\left(\frac{\Sigma c_i}{n}\right)D}{A}\right]$$

Where:

R = the average CFU count in $\log_{10}$ scale per cm$^2$ of skin.
*F* = total mL of stripping fluid added to the sampling cylinder [redacted]
$\Sigma c_i/n$ = average of the triplicate colony counts for each sample collected
*D* = Dilution of the plates counted ($10^0$, $10^1$, $10^2$, $10^3$, $10^4$, or $10^5$)
*A* = Inside area of the sampling cylinder ([redacted]

Dilution may be reported as dilution factor, which is the base 10 logarithm of the dilution. In that case, the factor of 'D' in the equation above will be replaced with $10^D$.

### 5.2.4 Growth Promotion Control

For each batch of plating medium, TSA+N, fewer than 100 CFU of [redacted] will be inoculated in a single plate pour plate. A 20-26 hours aged culture of [redacted] will be serially diluted in dilution fluid. The CFU added will be confirmed using triplicate trypticase soy agar (TSA) spread plates. The plates will be incubated for 72 ± 4 hours of aerobic incubation at 30° ± 2°C.

### 5.2.5 Neutralizer Validation

The effectiveness of the neutralizer system must be validated prior to the study start date to demonstrate that the antimicrobial is effectively neutralized and there is no effect on the growth of microorganisms. A procedure that will include in-vivo sampling will be combined with an in-vitro evaluation using procedures in accordance with ASTM E1054-08[4] (2013). The procedure is

attached as Appendix 14.10. These data must be provided in the final report.

### 5.3 Medication(s)/Treatment(s) Not Permitted

Topical or systemic antimicrobial exposure is not permitted within 14 days prior to the day of Screening Day, and during the study. Restrictions include, but are not limited to antimicrobial soaps, antiperspirants/deodorants, shampoos, lotions, perfumes, after shaves, colognes, and topical or systemic antibiotics. Exposure to antihistamines, immunosuppressants, or oral steroids within 14 days prior to Screening Day and up to and including Treatment Day (other than hormones for contraception or post-menopausal reasons) is not permitted.

### 5.4 Subject Compliance

Answers to the inclusion/exclusion questions (captured in the source documents and entered in the subject's eCRF) asked at the beginning of the screening and treatment phases will determine compliance to the Subject Instructions (Appendix 14.6) provided to each subject upon study participation. Documentation of the Inclusion/Exclusion criteria shall serve as confirmation of subject compliance with the Protocol.

## 6. Assessment of Efficacy

### 6.1 Efficacy Parameters

The primary measure of antimicrobial efficacy is the reduction of skin flora on the abdominal and groin sites 30 seconds following application of the study treatments relative to the Treatment Day baseline counts.

Product effectiveness will be measured using superiority and non-inferiority estimates for the 30-second sample time calculated using Average Treatment Effects (ATEs). The ATE will be estimated from a linear regression of the 30-second post-treatment bacterial count ($log_{10}$ scale) on the additive effect of a treatment indicator and the baseline or pre-treatment measurements ($log_{10}$ scale). To show effectiveness the test product will be 1) non-inferior to ChloraPrep® [redacted] with a 0.5 margin (upper bound of 95% confidence interval of the ATE of the test product versus active control value < 0.5) and (2) superior to the ZuraPrep [redacted] Vehicle (negative control) by a margin of 1.2 (lower bound of 95% confidence interval of the ATE of the test product versus the vehicle value > 1.2). Superiority and non-inferiority will be calculated separately for each body area (abdomen and groin).

#### 6.1.1 Secondary Endpoints/Analysis

Persistence or long-term efficacy is defined in the Final Rule as having the 6 hours post-treatment $log_{10}$ CFU/$cm^2$ values lower than or equal to the baseline $log_{10}$ CFU/$cm^2$ values for 100 percent of the subjects in each indication and body area. Due to the normal variability associated with this biological system, the test article is considered to have persistent effect if the percentage of body sites with $log_{10}$ CFU/$cm^2$ values less than or equal to

baseline for the 6-hour sample is between 95 to 100%. All subjects that do not achieve the 6-hour efficacy goal will be documented and discussed. This only applies to ZuraPrep™ [redacted] for the purpose of determining efficacy, but the same calculation will be performed for ChloraPrep® [redacted] for information purposes only.

#### 6.1.2 Study Validity

There are no study validity criteria. All calculations performed for ZuraPrep™ [redacted] will be performed for ChloraPrep® [redacted], but for informational purposes only.

### 6.2 Assessment Methods

Efficacy will be assessed by sampling the skin using the cup scrub method described in Section 5.2. See Section 8 for analysis methods.

## 7. Assessment of Safety

### 7.1 Safety Parameters

The principal measures of safety will be the recording of skin irritation scores and the incidence of adverse events reported during the study.

### 7.2 Assessment Methods for Skin Irritation

After the washout period, the Investigator or designated sub-investigators trained by the Investigator will assess the subject's skin condition and assign a skin irritation rating score using the Skin Irritation Rating Scale (see Appendix 14.11). A skin irritation score will be recorded at both the screening and treatment phases prior to baseline sample collections and prior to each post-treatment application sample collection (30 seconds, and 6 hours).

A corresponding rating score for each individual skin condition, for each site will be recorded on the appropriate data capture form and entered in the subject's eCRF. (See Appendix 14.11, which includes the following four independent evaluation categories: Erythema, Edema, Rash, and Dryness).

If an irritation score of 1 or greater for any individual skin condition prior to the baseline sample collection (at either the screening or treatment day phases) is assigned, the subject will be excluded from the study (no study treatment will be applied).

If an irritation score of 3 for any individual skin condition at any observation period is assigned, the subject will be discontinued from the study and an adverse event will be recorded. See Section 7.3 (Adverse Events).

### 7.3 Adverse Events

The Investigator is responsible for identifying adverse events that occur to each

subject throughout the study and follow-up period. An adverse event can occur at any time during the conduct of the study. Adverse events will be captured for all subjects from the time of screening baselines are taken until the time of discharge from the study. An adverse event can be identified by the Investigator or reported by the subject.

*Note: The Federal Privacy Rule (HIPAA) specifically permits the use and disclosure of protected health information "without written authorization of the individual" when used for public health activities such as reporting adverse events, tracking FDA-related products, enabling recalls, repairs, replacements, lookbacks, or conducting post-market surveillance [45 CFR 164.512]. This use and disclosure is subject to the minimum necessary standard, i.e. "the minimum necessary to accomplish the intended use, disclosure, or request" [45 CFR 164.502(b)(1)].*

Definitions:

Adverse Event/Experience

An Adverse Event/Experience is any unexpected or undesirable experience occurring to a subject during a study, which may or may not be related to the test article. All adverse event/experiences will be recorded and reported according to the Standard Operating Procedures of the laboratory.

All adverse events, regardless of severity or the causal/effect relationship, will be recorded. The severity of the effect will be noted as "*Mild,*" "*Moderat*e," or "*Severe*" according the following definitions:

| | |
|---|---|
| Mild | Awareness of signs or symptom, but easily tolerated. |
| Moderate | Discomfort to a degree as to cause interference with normal daily life activities and /or requiring medication. |
| Severe | Incapacity with inability to work or do usual daily life activities and requiring medical attention/intervention. |

Causal Relations of Adverse Event/Experience

When determining the causal/effect relationship to the test article, the relationship will be described as "*None," "Possible," "Probable,"* or *"Definite*." The following definitions will be utilized:

| | |
|---|---|
| None | No association to the test article. Related to other etiologies such as concomitant medications or conditions or subject's known clinical state. |
| Possible | Uncertain association. Other etiologies are also possible. |
| Probable | Clear-cut association with improvement upon withdrawal of the test article. Not reasonably explained by the subject's known clinical state |

Definite — An adverse event with a clear-cut temporal association with exposure to study materials and cannot reasonably be explained by the subject's known clinical state. Association with study material is confirmed by laboratory testing if possible.

Serious Adverse Event/Experience

A Serious Adverse Event/Experience is any adverse experience occurring at any dose that results in any of the following outcomes:

- Death;
- A life-threatening adverse drug experience;
- Inpatient hospitalization or prolongation of existing hospitalization;
- A persistent or significant disability/incapacity;
- Congenital anomaly/birth defect;
- An important medical event that may require medical or surgical intervention to prevent one of the previously listed outcomes.

Unexpected Adverse Event/Experience

An Unexpected Adverse Event/Experience is any adverse drug event/experience not listed in the current labeling for the test article or the current Investigator's brochure. Where test article labeling or Investigator's brochure is not available, anticipated experiences will be based on the known pharmacological/toxicological properties of the test article or ingredients.

Recording and Reporting

The Investigator or designee records all adverse events on an Adverse Event Record that will be transcribed in the subject's eCRF. Documentation includes the AE description, term, severity, seriousness, date of onset and resolution, relationship to the test article, action taken and outcome.

The Investigator must promptly report all treatment related adverse events to the Sponsor within two business days. All serious adverse events must be reported to the Sponsor and to the IRB within 24 hours of the Investigator awareness/notification of the event.

If a subject has no adverse event during the study, the absence of such must be recorded on the eCRF.

## 7.4 Follow-up

If an adverse event/experience occurs, the subject under the direction of the Investigator (or designee) may be referred to the nearest acute care facility for treatment. Serious or Unexpected Drug Event/Experience will be followed to resolution or until the AE has stabilized. Any adverse event will be documented on an Adverse Event Report and entered on the eCRF.

# 8. Statistics

## 8.1 Data Sets Analyzed

The Intent-to-Treat (ITT) population will consist of all subjects who pass the pre-test period prior to baseline screening and are assigned a subject number for treatment. The ITT data set will include data for all of the ITT population. The ITT data set will be used for the safety analysis.

Since each body site (left or right for abdomen or groin) can pass or fail Treatment Day baseline requirements separately, the per-protocol data set will be defined as those sites passing treatment day baselines on a site-by-site basis. Subjects are therefore included in the per-protocol population if and only if they have at least one qualifying body site. The per-protocol data set will be evaluated for efficacy.

## 8.2 Statistical Methods

### 8.2.1 Efficacy Analyses

CFU values will be calculated as per the Bacterial Enumeration methods above. Individual plate CFU counts that are zero will be treated as 0.5 for further calculations.

Product effectiveness will be measured using superiority and non-inferiority estimates for the 30-second sample time calculated using average treatment effects (ATEs). ATEs will be estimated from a linear regression of the 30 second post-treatment bacterial counts ($log_{10}$ scale) on the additive effect of a treatment indicator and the baseline or pre-treatment measurements ($log_{10}$ scale). Superiority and non-inferiority will be estimated as follows:

**a.** ZuraPrep™ [redacted] will be compared to the ChloraPrep® [redacted]. To show effectiveness, ZuraPrep™ [redacted] should be non-inferior to ChloraPrep® [redacted] with a 0.5 margin at 30 seconds. Specifically, the upper bound of the 95% confidence interval of the ATE of ZuraPrep™ [redacted] minus the ATE of ChloraPrep® [redacted] should be less than 0.5.

**b.** ZuraPrep™ [redacted] will be compared to ZuraPrep™ [redacted] Vehicle. To show effectiveness, ZuraPrep™ [redacted] should be superior to ZuraPrep™ [redacted] Vehicle by a 1.2 margin at 30 seconds. Specifically, the lower bound of the 95% confidence interval of the ATE of ZuraPrep™ [redacted] Vehicle minus the ATE of ZuraPrep™ [redacted] should be greater than 1.2.

Superiority and non-inferiority will be calculated separately for each body area.

Persistence or long-term efficacy is defined as having the 6 hours post-

treatment $log_{10}$ CFU/$cm^2$ values lower than or equal to the baseline $log_{10}$ CFU/$cm^2$ values for 100% of the subjects in each body area. Due to the normal variability associated with this biological system, the test article is considered to have persistent effect if the percentage of body sites with $log_{10}$ CFU/$cm^2$ values less than or equal to baseline for the 6-hour sample is between 95 to 100%. All subjects that do not achieve the 6-hour efficacy goal will be documented and discussed.

The efficacy calculations (superiority and persistence) only apply to ZuraPrep™ [redacted] for the purpose of determining efficacy, but the same calculations will be performed for ChloraPrep® [redacted] for information purposes only.

Summary statistics for the $log_{10}$ CFU/$cm^2$ values at each time point and the respective changes from baseline will be calculated, grouped by time point, test product, and body area. These statistics are for information purposes only and are not part of efficacy calculations.

### 8.2.2 Safety Analysis

The ITT population will be analyzed for safety. Skin irritation scores assessed in accordance with Appendix 14.11 will be reported for any subject who is scored with a 1 or more at any observation [baseline (screening day and treatment day), post-application/prior to 30 second and 6 hour sampling procedures], in any category for any site.

Adverse events (including post-treatment skin irritation scores of 3 in accordance with Appendix 14.11, will be summarized. Summary tables will present incidence rates of adverse events by treatment group for all subjects who enter the treatment period. Listings of adverse events will be provided.

The statistical significance of differences in skin irritation between the three treatments will be evaluated using Fisher's exact test on skin irritation data summarized as follows: any reaction above zero (no reaction) on the skin irritation rating scale for any category (erythema, edema, rash, and dryness) for any post-treatment sampling time will be considered a positive signal for that substance. If Fisher's exact test shows statistically significant skin irritation between the three treatments, a secondary analysis will be conducted to determine how the reactions differ.

## 8.3 Sample Size Justification

The number of subjects for each treatment (ZuraPrep™ [redacted], ChloraPrep® [redacted] and ZuraPrep™ [redacted] Vehicle) is based off of the following assumptions: the primary efficacy parameters are as defined above; the power is 80%; and the ATEs (as approximated by mean reductions from baseline) and standard deviations of the ATEs are equivalent to the results [redacted]

[redacted] all other components/ingredients remain consistent [redacted]. Using the standard normal approximation, this requires 65 subjects per treatment. The number 108 was used to make randomization blocking easier and to be conservative. See Appendix 14.12 for the full Estimation of Sample Size report.

### 8.4 Subject Discontinuation Criteria

A summary and/or listing of reasons for discontinuation will be provided.

### 8.5 Procedures for Accounting for Missing Data and Protocol Deviations

Missing data will be reported as missing. No imputation will be performed. Calculations that use missing data points will still be performed if the calculation is still logically possible.

Other irregularities or deviations which could affect the results will be handled on a case-by-case basis. Handling will be documented in the statistical report.

### 8.6 Deviations to Statistical Plan

Any deviation(s) from the original statistical analysis plan will be described and justified in the final report.

## 9. Monitoring

Zurex Pharma, Inc., as sponsor of this study along with the Investigator, is responsible for ensuring the proper conduct of the study with regard to protocol adherence and validity of the data recorded on the source documents and eCRFs. Zurex Pharma, Inc. has, therefore, assigned a study monitor to this study. The progress of the study will be monitored by:

- Periodic on-site review
- Telephone communications and e-mail
- Review of eCRFs and source documents

The Investigator will give the Zurex Pharma, Inc. study monitor direct access to source documents that support data on the eCRFs and make available such records to authorized Zurex Pharma, Inc., quality assurance, IRB, and regulatory personnel for inspection and/or copying.

*Note: The Federal Privacy rule (HIPAA) specifically permits the use and disclosure of protected health information "to a person subject to the jurisdiction of the Food and Drug Administration (FDA) [e.g., study sponsor] with respect to an FDA-related product or activity for which that person has responsibility, for the purpose of activities related to the quality, safety, or effectiveness of such FDA-regulated product or activity" [45 CFR 164.512(b)(1)(iii)].*

## 10. Quality Control and Quality Assurance

Zurex Pharma, Inc. and the Investigator are responsible for implementing and maintaining quality assurance and quality control systems through written standard operating procedures (SOPs) to ensure that this study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and regulations cited in Section 1.4 of this protocol. Study monitoring may be carried out to accomplish this.

## 11. Ethics

This study will be conducted in accordance with the principles that have their origin in the Declaration of Helsinki, 21 CFR 50 (Informed Consent) and 56 (IRBs). Laboratory operations will be conducted in accordance with 21 CFR 58 (Good Laboratory Practice for Nonclinical Laboratory Studies).

The study will start only after approval of the protocol and consent form by the IRB. The approval letter or notice must contain the IRB name and identification number, meeting date, and sufficient information to identify the protocol and informed consent by name and number that were reviewed. Zurex Pharma, Inc., prior to study initiation, must receive a copy of the IRB approval letter.

## 12. Data Handling and Record Keeping

### 12.1 Study Personnel

Prior to study initiation, the Investigator must provide Zurex Pharma, Inc. with a signed Investigator agreement as documentation of the Investigator's commitment to conduct the study according to the protocol and all applicable state and federal regulations.

### 12.2 Pre-Study Documentation Requirements

Prior to study initiation, the Investigator must provide Zurex Pharma, Inc. with the following documents:

- Signed protocol including any amendments in place prior to study initiation
- Curriculum vitae for the Investigator and any co-Investigators
- IRB-approved informed consent form containing subject authorization for use and disclosure of Protected Health Information statement.
- IRB study approval letter (including approval of the protocol, consent form, Investigator and study facility)
- IRB name, location and board membership listing
- Signed Investigator agreement
- Signed Study Agreement and Confidentiality agreement

### 12.3 Completion of Electronic Case Report Forms

The primary source document for this study will be the subject's data capture form. The source documents will be retained at the site.

For this study, an electronic data management system (EDMS) will be used for the collection of the study data in an electronic format. The EDMS will be designed based on the protocol requirements, the approved eCRF layouts and specifications, and in accordance with 21 CFR Part 11. The eCRF layouts and specifications define and identify the applicable source data that will be collected and captured into the EDMS. The applicable source data will be electronically transcribed by the site designee onto the eCRF (data entry screens) in the EDMS. The investigator is ultimately responsible for the accuracy of the data entered into eCRFs. Data monitoring and management will be performed in the EDMS by the study clinical monitor.

### 12.4 Final Report

The Investigator will prepare a final study report. The final report will be a record of the total study conduct and will be subject to review by Zurex Pharma, Inc. The final report will assess and summarize all data collected and include: test material identification, type of assay, randomization schedule, dates of study initiation and completion, and data demonstrating neutralization effectiveness. A review of the final report by Quality Assurance, including compliance statements will also be included with the final report. A copy of the final report will be sent to the study sponsor.

### 12.5 Records, Reports and Retention Requirements

The Investigator will maintain study records for a minimum of 2 years following the date a marketing application is approved for the indication for which is being investigated, or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified. Records that must be maintained by the Investigator include, but are not restricted to:

- Signed study protocol, amendments, deviations
- IRB approval of protocol, consent form, authorization form, waiver of consent and/or authorization and amendments to any of these documents
- Applications to the IRB
- Signed consent and authorization forms
- eCRFs
- Adverse event reports
- Records of receipt, use or disposition of the study material
- Correspondence relating to the study
- Investigator Final Report
- Sponsor Final Report (if provided)

## 13. References

1. Annual Book of ASTM Standards, Vol 11.08. E 1173-15 (Reapproved 2009) Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations.
2. Butterfield, C.T. The Selection of a Dilution Water for Bacteriological Examinations. J. Bacteriol. 23: 355-368, 1931.
3. Williamson, P., Kligman, A.M. A New Method for the Quantitative Investigation of Cutaneous Bacteria. J. Invest. Dermatol. 45:498-503, 1965.
4. ASTM International. ASTM E1054-08 (2013), standard test methods for evaluation of inactivators of antimicrobial agents. West Conshohocken [PA]: ASTM Int'l; 2013.

# 14. Appendices

## 14.1 Key Study Personnel, Titles, Responsibilities

**Zurex Pharma, Inc. Personnel:**

[redacted]

**Microbac Personnel:**

M. Hamid Bashir, MD, CCRP, Laboratory Manager — Principal Investigator

[redacted]

**Statistical Consultant:**

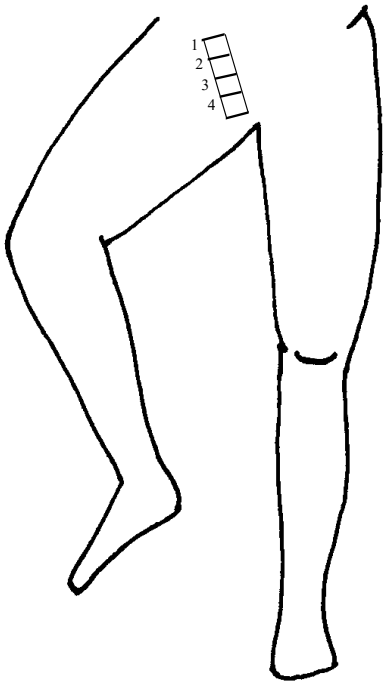
[redacted]

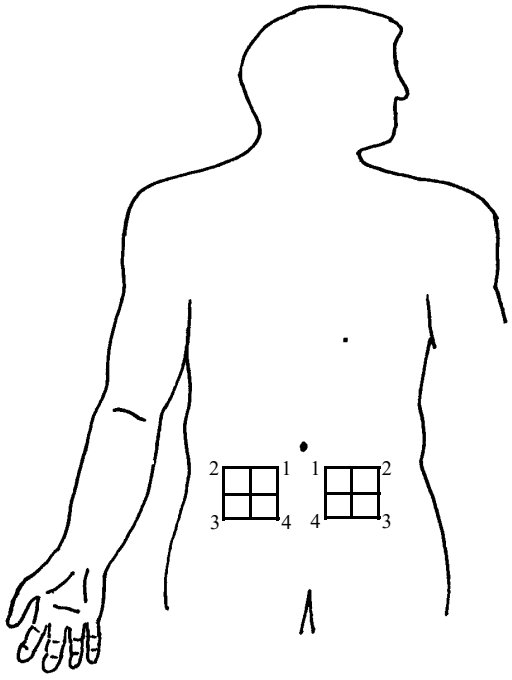
### 14.2 Study Summary

| Pre-Study Preparation | Screening Phase | | Treatment Phase | |
|---|---|---|---|---|
| | 14 Day Washout Period | Screening Day | Abdominal Region | Groin Region |
| Staff reviews study protocol | Initiation of consenting process | Complete Screening Inclusion/ Exclusion Criteria form | Complete Treatment Inclusion/ Exclusion Criteria form | |
| Prepare consent form | Review study Consent Form and Inclusion/ Exclusion Criteria | Visual skin assessment | Visual skin assessment | |
| Obtain IRB approval | Review subject instructions | Collect screening baseline samples from abdominal and groin regions | Mark test areas, Collect baseline samples | |
| Recruit volunteers for screening phase (14 day washout / screening day) | Subject signs consent form | Count screening plates, determine which volunteers qualify for study | Apply test articles | |
| Prepare subject kits | Visual skin assessment (abdominal and groin regions) | Contact and enroll eligible subjects, schedule Treatment Day | Visual skin assessment, 30 seconds (± 5 sec.) and 6 hours (± 30 min.) post-prep sample | Visual skin assessment, 30 seconds (± 5 sec.) and 6 hours (± 30 min.) post-prep sample |
| | Dispense subject kits | Schedule for clipping, if needed, 48 hrs. prior to Treatment Day visit | | |
| | Schedule for clipping, if needed, 48 hrs. prior to screening visit | No bathing / showering 72 hrs. prior to Treatment Day visit | Count Treatment Day Baseline plates, determine qualification and enroll additional subjects as required | Count Treatment Day Baseline plates, determine qualification and enroll additional subjects as required |
| | No bathing / showering 72 hrs. prior to screening visit | | | |

### 14.3 Abdomen and Groin Diagram

Follow the randomization scheme for each subject for the exact placement of study materials.




| | |
|---|---|
| Groin (anterior) – 1.5”’ x 5” area | Abdomen (anterior) – 5” x 5” area |

### 14.4 Randomization Scheme Example

**Abdominal and Groin Sites Bilateral Sampling Procedure Configurations**

**GROUP 1**

Key: A = ZuraPrep™ [redacted]

B = ChloraPrep® [redacted] (active control)

C = ZuraPrep™ [redacted] Vehicle (negative control)

| Subject | Left Treatment | Right Treatment | Site 1 | Site 2 | Site 3 | Site 4 |
|---|---|---|---|---|---|---|
| 1001 | Treatment block 1 | | Subjects will be numbered sequentially from 1001 to the number of subjects treated during the study. Randomization will be performed by group issued prior to study initiation. | | | |
| 1002 | | | | | | |
| 1003 | | | | | | |
| 1004 | | | | | | |
| 1005 | | | | | | |
| 1006 | | | | | | |
| 1007 | | | | | | |
| 1008 | | | | | | |
| 1009 | | | | | | |
| 1010 | | | | | | |
| 1011 | | | | | | |
| 1012 | | | | | | |
| 1013 | Treatment block 2 | | Sample order group 1 | | | |
| 1014 | | | | | | |
| 1015 | | | | | | |
| 1016 | | | | | | |
| 1017 | | | | | | |
| 1018 | | | | | | |
| 1019 | | | | | | |
| 1020 | | | | | | |
| 1021 | | | | | | |
| 1022 | | | | | | |
| 1023 | | | | | | |
| 1024 | | | | | | |
| … | | | | | | |

### 14.5 Required Elements of Informed Consent

These elements of consent should be included as applicable to the study being conducted.

1. Statement that the study involves research.
2. Purpose(s) of the research.
3. Expected duration of subject's participation.
4. Procedures to be followed and identification of any procedures that are experimental.
5. A description of any reasonable foreseeable risks or discomforts to the subject.
   a) Risks/discomforts from study procedures.
   b) Foreseeable risks, which include adverse experiences listed in the Investigator's Brochure or package insert.
6. A description of any benefits to the subject or to others which may reasonably be expected from the research.
7. A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject.
8. Extent to which confidentiality of records identifying subject will be maintained.
   a) Possibility that representatives of Zurex Pharma, Inc. and the FDA may inspect and make copies of the records.
   b) Suggested text: "Information on the Confidential Follow-up form (where used) will be held and treated with strict confidentiality and will be used only in the event that long-term follow-up is needed.
   c) Suggested text: "I understand that, at any time, an agent of Zurex Pharma, Inc. may also review any hospital, physician, or insurance billing or any other costs which relate to therapy incurred as a direct result of my participating in this study.
9. An explanation as to whether any compensation or medical treatments are available if injury occurs for research involving more than minimal risk. The explanation should involve a description of the compensation or treatment available or a statement describing where further information may be obtained.
10. Whom to contact for answers to pertinent questions about research and research subject's rights.
11. Whom to contact in the event of research-related injury to the subject.
12. Participation is voluntary:
    a) Refusal to participate will involve no penalty or loss of benefits to which subject is otherwise entitled.
    b) Subject may discontinue participation at any time without penalty or loss of benefit to which subject is otherwise entitled.

ADDITIONAL ELEMENTS OF CONSENT

When appropriate, one or more of the following elements of information shall also be provided to each subject.

13. A statement that the particular treatment or procedure may involve risks to the subject (or embryo or fetus, if subject became pregnant) which are currently unforeseeable.
14. Anticipated circumstances under which subject's participation may be terminated by the Investigator without regard to subject's consent.
15. Any additional costs to the subject that may result from participation in the research.
16. A statement explaining the consequences of subject's decision to withdraw during the course of the research which may relate to subject's willingness to continue participation will be provided to the subject.
17. A statement that significant new findings developed during the course of the research which may relate to subject's willingness to continue participation will be provided to the subject.
18. Approximate number of subjects involved in the study.

Nothing in these regulations is intended to limit the authority of a physician to provide emergency medical care to the extent the physician is permitted to do so under applicable federal, state, or local laws.

Informed consent allows the subject to fully understand his/her participation and serves to protect the Investigator and Sponsor from potential negligence claims. A fully informed subject is the best protection against such claims.

The informed consent requirements in these regulations are not intended to preempt any applicable federal, state, or local laws that require additional information be disclosed for informed consent to be legally effective. Some states, such as California and Oregon, require further action on the Investigator's part concerning subject consent.

## 14.6 Subject Instructions – Washout, Screening Visit, Treatment Visit

**The following instructions are to be followed <u>until the completion of the study.</u>**

- [redacted]

---

**<u>Additional Instructions for Screening Visit:</u>**

- [redacted]

You will be contacted by telephone as to whether you have met the established criteria and therefore will be returning for a Treatment visit (if selected). Remember to continue to use your kit products until you have been eliminated or have completed the study.

---

**<u>Additional Instructions for Treatment Visit (if you are selected):</u>**

- [redacted]

[redacted]

## 14.7 Treatment Application Instructions

**ZuraPrep™ [redacted]**

[redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

**Treatment Site Application Instructions**

Abdominal Test Site

1. Using repeated back-and-forth strokes of the sponge for thirty (30) seconds, completely wet the treatment area with test material.
2. Allow the area to air-dry for three (3) minutes. Do not blot or wipe away.

Inguinal Test Site

1. Using repeated back-and-forth strokes of the sponge for two (2) minutes, completely wet the treatment area with test material.
2. Allow the area to air-dry for three (3) minutes. Do not blot or wipe away.

**ChloraPrep® [redacted] (active control)**

[redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

**Treatment Site Application Instructions**

Abdominal Test Site

1. Using repeated back-and-forth strokes of the sponge for thirty (30) seconds, completely wet the treatment area with test material.
2. Allow the area to air-dry for three (3) minutes. Do not blot or wipe away.

Inguinal Test Site

1. Using repeated back-and-forth strokes of the sponge for two (2) minutes, completely wet the treatment area with test material.
2. Allow the area to air-dry for three (3) minutes. Do not blot or wipe away.

**ZuraPrep™ [redacted] Vehicle (negative control)**

[redacted]

**Treatment Site Application Instructions**

Abdominal Test Site

1. Using repeated back-and-forth strokes of the sponge for thirty (30) seconds, completely wet the treatment area with test material.
2. Allow the area to air-dry for three (3) minutes. Do not blot or wipe away.

Inguinal Test Site

1. Using repeated back-and-forth strokes of the sponge for two (2) minutes. Completely wet the treatment area with test material.
2. Allow the area to air-dry for three (3) minutes. Do not blot or wipe away.

## 14.8 Confirmation of Release and Receipt of Study Materials

**CONFIRMATION OF RELEASE and RECEIPT OF STUDY MATERIALS**

**Investigator:** M. Hamid Bashir, MD, CCRP
**Study No:** Microbac 865-107
**Test Site:** Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164

.

| Quantity (Units) | Description | ID/Lot Number | Exp. Date |
|---|---|---|---|
| | | | |
| | | | |
| | | | |

Supplies Released to Site by: _____
Sponsor Signature

Supplies Sent to Site (Date): _____

Supplies Checked and Verified by: _____
Signature Date

_____
Print Name, Title

Once the supplies have been verified and this form is signed / dated, a signature copy will be sent to the sponsor representative: [redacted]

### 14.9 Study Material Disposition Form

Use one form for each study material.

| | |
|---|---|
| **Study Number:** Microbac 865-107 | |
| **Investigator:** M. Hamid Bashir, MD, CCRP | **Investigator Site:** Microbac Laboratories Inc., [redacted] |

| **Study Material ID:** | **Date Received:** | **Quantity Received:** | **Lot Number/ Serial Number:** | **Date Returned to Sponsor:** |
|---|---|---|---|---|
| | | | | |

| **Date Dispensed/Distributed** | **Subject Number** | **Quantity Dispensed** | **Quantity Remaining** |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

### 14.10 Procedure for Neutralization Validation

1. Background

The sampling solution, SS, sterile [75mM phosphate buffer [redacted] containing [redacted] lecithin, [redacted] Tween® 80, and [redacted] Tamol, [redacted] is a buffered detergent solution that is commonly used in studies where microbial sampling of skin is conducted. Neutralizers have been added to inactivate the antimicrobial, isopropyl alcohol and chlorhexidine gluconate, present in test treatments. The effectiveness and toxicity of this neutralizer system must be assessed to demonstrate that there is no effect on the growth of microorganisms and that the active ingredient is inactivated.

The density of normal human skin flora generally ranges from $10^2$ to $10^5$ CFU/$cm^2$ depending on the body site. However, since significant neutralizer or toxic effects are more easily detected at a lower cell density, the efficacy and toxicity of this neutralizer system will be assessed against a lower bacterial concentration.

This is a test where the study materials are applied to the abdomen and the treated areas will be sampled. After sampling, [redacted] a selected representative of normal skin flora will be added into a portion of each sample. Each sample will then be processed using procedures in accordance with ASTM E1054-08 (2013).

2. Objective

This control assay will determine the ability of the SS to completely neutralize the active ingredients in the test treatments when applied to the abdomen by recovering and quantifying microorganism populations on agar media and is appropriate for antimicrobial agents that can be chemically inactivated or diluted to sub-inhibitory levels.

Neutralization validation will be conducted prior to the conduct of efficacy testing.

3. Subject Entry Criteria

[redacted] subjects will be used for the neutralization validation required for this study. Each subject must meet the inclusion and exclusion criteria described in Sections 4.1 and 4.2 except for the baseline bacterial count, the 72-hour exclusion from showering/bathing and the length of the washout period. No minimum bacterial count is required and the washout period is only necessary for 7 days (not 14 days). The subjects will be asked to provide information on demographics and inclusion/exclusion criteria and sign the Consent and Authorization Forms before beginning the 7-day washout period. When the subjects return to begin their participation in the study they will again be asked to provide information relative to inclusion/exclusion criteria. If they meet all inclusion/exclusion criteria, they may be enrolled. The subjects will be identified by the letter “N” for neutralization and a subject number of [redacted]

The test, vehicle and reference control articles will be applied to the abdomen regions using bilateral applications so that [redacted] applications are performed for each treatment using bilateral

application [redacted] One area will be located on one side of the body and the remaining area on the other side.

The treatments per subject will be randomized. The randomization scheme will be generated before the test.

4. Test Organisms

The test organisms for this study are:

a. [redacted]

[redacted]

5. Treatment Materials

a. ZuraPrep™ [redacted] (containing 70% v/v Isopropyl Alcohol)

b. ChloraPrep® [redacted] (reference control article containing 2% chlorhexidine gluconate)

c. ZuraPrep™ [redacted] Vehicle (negative control article)

6. Materials, Supplies and Equipment

See section 3.4.3 of the Microbac 865-107 Protocol. In addition, 70% v/v isopropyl alcohol swabs.

7. In-vivo Test Procedures (collection of samples)

Preparation of Test Area and Post-Prep Sampling: Neutralization samples will be taken from the abdomen. The subject number, location of the prep application, location of the sites sampled within the prep area, and the time of sample collection will be documented on the appropriate data capture form. The subject will be treated with the study materials based on the following.

- For each side of the body, mark the abdominal test areas using a sterile 1.5" x 5" template. The 1.5" x 5" areas will be delineated with each containing <u>two</u> 1" x 1" sampling site.
- After the test areas are marked, each area will be processed using three 70% isopropyl alcohol swabs for a total of one minute to prepare the site; the areas will be allowed to dry. This step is to prepare the skin for the neutralization test.
- Prep the test areas with the appropriate treatment according to the instructions provided in Appendix 14.7, Microbac 865-107 Protocol for groin.
- Using the scrub cup technique at approximately 30 seconds post-prep, begin collecting samples from each of two sampling sites within each test area using

SS by two technicians simultaneously. This technique is described in Section 5.2.1 of the Microbac 865-107 Protocol.

8. In-vitro Test Procedures (performed using the collected samples in accordance with ASTM E1054-08 (2013))

8.1 Inoculum Preparation:

For each test organism, the organism will be prepared from and overnight broth culture (24 ± 4 hours) grown in TSB at 35 ± 2C to yield a concentration of approximately [redacted]. The culture will be diluted using PBW in a manner such that [redacted] will be delivered into the neutralizer tube (a [redacted] aliquot of the collected SS sample will be used).

8.2 Test:

(note – the reference to test article applies to test article, the vehicle and the reference control article; all procedures outlined, where applicable will employ all articles.)

8.2.1 Neutralizer effectiveness (Test 1):

a Out of the [redacted] aliquot of sampling solution taken from the volunteer, [redacted] will be transferred to a new sterile tube and inoculated with the challenge microorganism so that the final concentration will equal [redacted] colony-forming units (CFU) / mL of the challenge microorganism (the prepared inoculum will be diluted using PBW to achieve the desired concentration, [redacted] aliquot from the [redacted] dilution will be used).

b Within one min after the addition of the challenge microorganism, the microorganisms will be enumerated by standard microbiological methods extant in the laboratory.

c Triplicate [redacted] aliquots will be removed and plated using TSA+N pour plates.

d After 30 minutes, the microorganisms will be enumerated a second time using the same procedures.

e Triplicate [redacted] aliquots will be removed and plated using TSA+N pour plates.

8.2.2 Neutralizer toxicity (Test 2):

a A [redacted] aliquot of sampling solution will be inoculated with the challenge microorganism so that the final concentration will equal [redacted] colony-forming units (CFU) / mL of the challenge microorganism in the same manner as Test 1.

b Within one min after the addition of the challenge microorganism, the microorganisms will be enumerated by standard microbiological methods extant in the laboratory.

c Triplicate [redacted] aliquots will be removed and plated using TSA+N pour plates.

d After 30 minutes, the microorganisms will be enumerated a second time using the same procedures.

e Triplicate [redacted] aliquots will be removed and plated using TSA+N pour plates.

f This procedure will be repeated two times for a total of three replicates.

8.2.3 Test microorganism viability control (Test 3):

a A [redacted] aliquot of PBW will be inoculated with a volume of the challenge microorganism so that the resulting suspension contains [redacted] CFU/mL in the same manner as Test 1.

b Within one min the microorganisms will be enumerated (in triplicate) by standard microbiological methods extant in the laboratory.

c Triplicate [redacted] mL aliquots will be removed and plated using TSA pour plates.

d After 30 minutes, the microorganisms will be enumerated a second time using the same procedures.

e Triplicate [redacted] aliquots will be removed and plated using TSA pour plates.

f This procedure will be repeated two times for a total of three replicates.

8.2.4 Test article control (Test 4):

a A [redacted] aliquot of the test or control article will be inoculated with a volume of the challenge microorganism so that the resulting suspension contains [redacted] CFU/mL in the same manner as Test 1.

b Within one min the microorganisms will be enumerated (in triplicate) by standard microbiological methods extant in the laboratory.

c Triplicate [redacted]mL aliquots will be removed and plated using TSA pour plates.

d After 30 minutes, the microorganisms will be enumerated a second time using the same procedures.

e Triplicate [redacted] mL aliquots will be removed and plated using TSA pour plates.

f This procedure will be repeated two times for a total of three replicates.

8.3 Incubation:

All plates for Tests 1, 2, 3 and 4 will be incubated for 72±4 hours at 30°±2°C.

8.4 Interpretation of data:

a. The number of surviving challenge microorganisms for each replicate from each test will be average count of the three plates.

b. The number of survivor values will be transformed to $\log_{10}$.

c. The number of survivors ($\log_{10}$) from each test (1, 2, and 4) will be compared to the test microorganism viability population (test 3).

d. Neutralization aspects of the sampling solution will be considered adequate if the mean $\log_{10}$ CFU/mL of Test 1 is not more than 0.20 $\log_{10}$ less than the mean $\log_{10}$ CFU/mL of Test 3 (Mean $\log_{10}$ CFU/mL from Test 3 – Mean $\log_{10}$ CFU/mL from Test 1 using corresponding time points).

e. The sampling solution will be considered non-toxic if the mean $\log_{10}$ CFU/mL of Test 2 is not more than 0.20 $\log_{10}$ less than the mean $\log_{10}$ CFU/mL of Test 3 (Mean $\log_{10}$ CFU/mL from Test 3 − Mean $\log_{10}$ CFU/mL from Test 2 using corresponding time points).

f. The mean $\log_{10}$ CFU/mL from Test 4 must be at least 0.20 $\log_{10}$ less than the mean $\log_{10}$ CFU/mL of Test 3.

g. The amount of CFU added for each aspect must be confirmed to yield a final suspension containing [redacted] CFU/mL (validated in test 3).

h. The sterility controls must be negative for growth.

8.5 Controls:

8.5.1 Sterility control:

Triplicate plates of TSA and TSA+N used will be incubated with the test. In addition, triplicate [redacted] aliquots of sampling solution and PBW will be plated using TSA pour plates used for a particular test date. All plates will be incubated with the test.

8.5.2 Challenge microorganism confirmation:

In order to confirm growth consistent with the challenge microorganism, Gram stains will be performed from a representative colony from a test plate. The colony morphology will be noted as well.

### 14.11 Skin Irritation Rating Scale

| Skin Irritation Rating Scale<br>Reactive area(s) within the treatment site only | | |
|---|---|---|
| **Condition** | **Rating** | **Description** |
| Erythema | 0 | No reaction |
| | 1 | Mild and/or transient redness |
| | 2 | Moderate redness |
| | 3[a] | Severe redness |
| Edema | 0 | No reaction |
| | 1 | Mild and/or transient swelling |
| | 2 | Moderate swelling |
| | 3[a] | Severe swelling |
| Rash | 0 | No reaction |
| | 1 | Mild and/or transient rash |
| | 2 | Moderate rash |
| | 3[a] | Severe rash |
| Dryness | 0 | No reaction |
| | 1 | Mild and/or transient dryness |
| | 2 | Moderate dryness |
| | 3[a] | Severe dryness |

[a] = A rating of 3 on the skin irritation scale in any category will be recorded as Adverse Event and will require subject's removal from the study.

## 14.12 Estimation of Sample Size Report

See attached report.

## 14.13 Informed Consent Forms

See attached forms.