[redacted]

[redacted] **PROTOCOL #150316-103**

(Sponsor Project Number: ZX-ZP-0074)

PIVOTAL CLINICAL EVALUATION OF [redacted], A PATIENT PREOPERATIVE SKIN PREPARATION

Test Product: ZuraPrep™

Principal Investigator: [redacted]

Subinvestigators: [redacted]

Institution: BioScience Laboratories, Inc.
[redacted]
Bozeman, Montana [redacted]
[redacted]
[redacted]

Sponsor: Zurex Pharma, Inc.
[redacted]
Middleton, Wisconsin [redacted]
[redacted]

Sponsor Project Number: ZX-ZP-0074

Date: July 15, 2016

[redacted]

Confidentiality Statement

This document contains the confidential information of Zurex Pharma, Inc. and BioScience Laboratories, Inc. 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 BioScience Laboratories, Inc. Permission to release the Protocol and Study Results to the United States Food and Drug Administration (FDA) or other regulatory agency to which this study will be submitted is explicitly granted.

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1.0 INTRODUCTION

Prior to surgery or other invasive procedures, skin must be treated with a topical antimicrobial product to minimize the risk of nosocomial infection by reducing the number of microorganisms on the skin. The proposed Tentative Final Monograph (TFM) for *Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Tentative Final Monograph for Healthcare Antiseptic Drug Products* (Vol. 59, No. 116, June 17, 1994) describes *in-vivo* procedures for evaluating this type of product, as well as expected performance criteria.

2.0 OBJECTIVE

The purpose of this study is to evaluate the antimicrobial properties of one finished test product (ZuraPrep™ [redacted]) with a positive control (ChloraPrep® [redacted]) and a negative control (ZuraPrep™ Vehicle) when used as a patient preoperative skin preparation. Testing will be performed based upon procedures outlined in the Food and Drug Administration Tentative Final Monograph (TFM) for *Effectiveness Testing of a Patient Preoperative Skin Preparation* (Vol. 59, No. 116, June 17, 1994, pp. 31450-31452) and ASTM E1173-15 *Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations.*

3.0 SPONSOR

Zurex Pharma, Inc.
[redacted]
Middleton, Wisconsin [redacted]

4.0 INVESTIGATIVE ORGANIZATION AND PERSONNEL

BioScience Laboratories, Inc.
[redacted]
Bozeman, Montana [redacted]

[redacted]

Principal Investigator: Collette Duley

[redacted]

[redacted]

Quality Assurance Monitors: [redacted]

Statistical Consultant: [redacted]

Subject Recruitment and Consenting, IRB Coordination: [redacted]

Study Sponsor Representatives(s): [redacted]

Study Monitor:

[redacted]

4.1 Name of the IRB

Gallatin Institutional Review Board (GIRB)

[redacted]

5.0 ROLES AND RESPONSIBILITIES

5.1 Principal Investigator

Collette Duley is responsible for conducting the study.

5.2 Subinvestigators

[redacted]

5.3 Investigational Sites

BioScience Laboratories, Inc.

[redacted]

Bozeman, Montana [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

5.4 Subject Recruiters

[redacted]

5.5 Study Monitor(s)

[redacted]

5.6 Medical Experts

[redacted]

6.0 CLINICAL RESEARCH STANDARDS

The clinical investigation, including the informed consent, will be approved by the Gallatin Institutional Review Board (GIRB) in accordance with Title 21 of the Code of Federal Regulations, Parts 50, 56, 58, 312, and 314, and in accordance with the International Conference on Harmonisation (ICH) guidelines. The written approval of the GIRB will be obtained prior to the initiation of the study.

The study will be conducted in accordance with Good Clinical Practice regulations, Good Laboratory Practice regulations, the Standard Operating Procedures of BioScience Laboratories, Inc., the study protocol, any protocol amendments, and the regulatory requirements of the United States Food and Drug Administration (FDA) and ICH.

7.0 SCOPE

The purpose of this study is to evaluate the antimicrobial properties of one finished test product (ZuraPrep™ [redacted], a positive control (ChloraPrep® [redacted] and a negative control (ZuraPrep™ Vehicle) when used as a patient

[redacted]

preoperative preparation. Testing will be performed according to a modification of the procedures outlined in the Food and Drug Administration Tentative Final Monograph (TFM) for *Effectiveness Testing of a Patient Preoperative Skin Preparation* (Vol. 59, No. 116, June 17, 1994, pp. 31450-31452) and ASTM E1173-15 *Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations.*

A sufficient number of subjects will be screened, meet baseline criteria, and be entered into testing in order to complete a total of 704 abdominal site evaluations and 704 groin site evaluations (minimum of 352 completed subjects). ZuraPrep™ and the positive control, ChloraPrep®, will be paired and evaluated with a sample size of at least 288 subjects, that is a minimum of 288 abdominal regions and 288 inguinal regions are treated with ZuraPrep™ on one side of the body and ChloraPrep® on the other. The negative control (ZuraPrep™ Vehicle) will be paired and evaluated with ZuraPrep™ and with the positive control, ChloraPrep®, with a sample size of at least 32 subjects for each pairing, that is a minimum of 64 abdominal regions and 64 inguinal regions are treated with the negative control (ZuraPrep™ Vehicle) on one side of the body and ZuraPrep™ or ChloraPrep® on the other for each pairing.

Following a 14-day restriction period, subjects with sufficient resident bacterial flora (abdomen: minimum of 3.0 $\log_{10}$ or 1.0×10^3 CFU/cm^2; groin: minimum of 5.0 $\log_{10}$ or 1.0×10^5 CFU/cm^2) will be tested. A total of three post treatment sample collections will be performed at each test site for all test materials (the test product, positive control and negative control). Specific sites of sampling and treatment groups will be randomized. All subjects will have samples collected at baseline. The test materials will be applied bilaterally to the skin of the abdomen and/or the groin. Samples will be collected at 30 seconds, 10 minutes, and 6 hours following completion of the product application procedure (including a 3-minute dry time) from both anatomical treatment sites. Visual evaluations of skin reactions will be conducted prior to baseline and prior to each sample interval. All plating for this study will be conducted in duplicate using the pour plating technique.

7.1 Primary Analysis

Antimicrobial efficacy will be evaluated based upon calculations of mean $\log_{10}$ reductions from baseline populations by subtracting the $\log_{10}$ number of viable microorganisms recovered at each post-product application sample from the $\log_{10}$ number of viable microorganisms recovered in the baseline samples. The study will be analyzed per the standards of the 1994 Tentative Final Monograph (TFM) for *Effectiveness Testing of a Patient Preoperative Skin Preparation* (FR 59:116, 17 June 94, pp. 31450-31452), as updated by recent recommendations by the FDA on responder rates. The primary efficacy criteria will be assessed via $\log_{10}$ CFU/cm^2 reduction at the 10-minute sampling time. The TFM indicates $\log_{10}$ CFU/cm^2 reductions as the primary

[redacted]

efficacy measure while the Proposed Amendment of the Tentative Final Monograph (FR 80:84, 01 May 2015) indicates responder rates as the primary efficacy requirement. This study will use responder rate at 10 minutes post-product application as the primary measure of efficacy but will also calculate and show $\log_{10}$ CFU/cm^2 reductions from baseline.

To be used in data analysis, day-of-test baseline populations must satisfy the inclusion criteria: 3.0 - 5.5 $\log_{10}$ ($1.0 \times 10^3 - 3.2 \times 10^5$ CFU/cm^2) from the skin of the abdomen, and 5.0 - 7.5 $\log_{10}$ ($1.0 \times 10^5 - 3.2 \times 10^7$ CFU/cm^2) from the skin of the groin.

The reduction will be calculated by a percent successes approach (i.e. percent of subjects meeting the required $\log_{10}$ reductions – responder rate). A subject is considered a responder for the treatment if the following performance criteria are met: ≥ 2.0 $\log_{10}$ CFU/cm^2 reduction from baseline on skin of the abdomen and ≥ 3.0 $\log_{10}$ CFU/cm^2 reduction from baseline on the skin of the groin at 10 minutes following product application.

Minimum and Maximum Treatment Day Baseline and Efficacy Log_{10} Reduction		
Anatomical Site	**Minimum and Maximum Treatment Day Baseline***	**Minimum Efficacy Log_{10} Reduction**
Abdomen	$1.0 \times 10^3 - 3.2 \times 10^5$ CFU/cm (3.0 - 5.5 $\log_{10}$)	2.0 $\log_{10}$ @ 10 minutes
Groin	$1.0 \times 10^5 - 3.2 \times 10^7$ CFU/cm^2 (5.0 - 7.5 $\log_{10}$)	3.0 $\log_{10}$ @ 10 minutes

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

Responder rates will be calculated separately for the abdomen and groin and separately for each test material and time point for each subject. The individual responses will then be grouped to generate an overall responder rate for each anatomical area for each test material. The efficacy goal for active test materials (test product and positive control) is to have the lower bound of the 95% confidence interval for the responder rate to be greater than or equal to 70%.

7.2 Secondary Analysis

Responder rates at 30 seconds will be calculated identically to the 10-minute rates. At 6 hours a site is considered a responder if bacterial populations are below baseline; otherwise, 6-hour responder rates will be calculated identically to the 10-minute responder rates. The secondary efficacy goal is to have the 95% confidence intervals for

[redacted]

the responder rate to be greater than or equal to 70%. Log_{10} reductions from baseline will also be calculated for all post-product application samples.

7.3 Study Validity

For a valid study, the positive control must make the same objectives that the test formulation does and both the test formulation and the positive control should be superior to the negative control for the primary endpoint. The responder rates for both active and inactive treatments at 6 hours are near 100%. Therefore, comparison of responder rates at 6 hours is unlikely to show any difference. Differences in log_{10} CFU/cm2 reductions from baseline will be used at 6 hours in order to show active treatments are more effective than the inactive control.

7.4 Sample Size Justification

[redacted]

7.5 Safety Analysis

Safety will be monitored by evaluation of reactions observed on the skin of the test sites (reference Section 15.2) and any adverse reactions. Adverse reactions will be fully documented, reported as Adverse Events, and followed to resolution.

[redacted]

7.6 Study Flow Chart

Procedure	Day			
	-14 or more	-3 or more	0	3 or more (Treatment Day)
Informed Consent Obtained	X			
Product-Restriction Period	X	X	X	X
Inclusion/Exclusion Criteria including Medical History Reviewed	X	X	X	X
Visual Skin Assessment		X		
Clipping Hair From Test sites		X		
Visual Evaluation of Skin Reaction			X	
Baseline Screening			X	
Pregnancy Test Administered (Females Only)				X
Visual Evaluation of Skin Reaction				X
Test-Day Baseline Sample				X
Product Application				X
30-Second Post-Product Application Visual Evaluation of Skin Reaction				X
30-Second Post-Product Application Sample				X
10-Minute Post-Product Application Visual Evaluation of Skin Reaction				X
10-Minute Post-Product Application Sample				X
Sample Sites Bandaged				X
6-Hour Post-Product Application Visual Evaluation of Skin Reaction				X
6-Hour Post-Product Application Sample				X
Test Materials cleaned from test sites				X

8.0 TEST MATERIALS

[redacted]

[redacted]

The test materials will be received and stored by BioScience Laboratories, Inc. (BSLI) in accordance with instructions from the Sponsor and retained in secure quarantine when not being used in testing. BSLI will maintain an inventory of the test materials in secure quarantine and a log of use. Unused, sealed test materials will be stored by BSLI until the Sponsor specifies its disposition. In the absence of a disposition request from the Sponsor within 1 year of planned usage, the test materials will be returned to the Sponsor. No test materials will be destroyed unless so requested by the Sponsor. Complete product information will be provided in the Final Report, if it is not provided below.

[redacted]

Test Product:	ZuraPrep™ [redacted]
Active Ingredient:	Isopropyl Alcohol (~70%)
Other Ingredients:	[redacted]
Manufacturer:	[redacted]
Lot Number:	[redacted]
Manufacture Date:	[redacted]
Expiration Date:	
Positive Control:	ChloraPrep® [redacted] (Scrub Teal® Tint)
Active Ingredients:	2% Chlorhexidine gluconate (w/v) and 70% isopropyl alcohol (v/v)
Lot Number:	_____
Expiration Date:	_____

[redacted]

Negative Control: ZuraPrep™ Vehicle [redacted]

Other Ingredients: [redacted]

Manufacturer:

Lot Number:

Manufacture Date:

Expiration Date:

Applicators: [redacted]

Lot Number:

Expiration Date:

9.0 LABORATORY SUPPLIES AND EQUIPMENT (provided by BioScience Laboratories, Inc.)

9.1 Equipment

The equipment used during this study will be detailed on Clinical Trials Equipment Tracking Forms, and the forms will be included in the Final Report.

9.2 Supplies

The supplies used during this study will be detailed on Clinical Trials Supplies Tracking Forms, and the forms will be included in the Final Report. [redacted]

9.3 Media

Stripping Suspending Fluid (SSF) (for baseline samples)

[redacted]

Sterile Stripping Suspending Fluid and appropriate product neutralizers (SS+) (for post-application samples)

[redacted]

[redacted]

Butterfield's Phosphate Buffer Solution with product neutralizers (PBW+) (diluting fluid)

[redacted]

Tryptic Soy Agar with product neutralizers (TSA+)

[redacted]

10.0 NEUTRALIZATION

In-vivo neutralization evaluation will be performed prior to the study start date to ensure that the neutralizers used in the recovery medium quench the antimicrobial activity of the two active test materials and the negative control. An *In-vitro* evaluation will be performed to ensure that the neutralizers and other materials used for performing and assaying samples are not toxic to the bacteria. [redacted] will be used as the challenge species in the neutralizer validation studies. The test product, positive control, and the negative control (vehicle) will be evaluated [redacted] using bilateral product applications with samples from [redacted] subjects being evaluated for each test material with [redacted] and samples from the remaining [redacted] subjects being evaluated with [redacted] *In vitro* and *in-vivo* neutralization studies will follow guidelines based on ASTM E1054-08(2013), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents* Reference Appendix 3, *Validation of Neutralizer Effectiveness*, for details of the neutralization procedures.

11.0 SUBJECT SELECTION

11.1 Number of Subjects

A sufficient number of subjects at least 18 years of age will be screened, meet baseline criteria, and be entered into testing in order to complete a total of 704 abdominal site evaluations and 704 groin site evaluations (minimum of 352 completed subjects). ZuraPrep™ and ChloraPrep® will each be paired and evaluated with a sample size of at least 288 subjects. The negative control (ZuraPrep™ Vehicle) will be paired and evaluated with ZuraPrep™ and with ChloraPrep®, with a sample size of at least 32 subjects for each pairing. A total of at least 320 inguinal and abdomen sites will be treated with ZuraPrep™ and ChloraPrep®, and at least 64 inguinal and abdomen sites will be treated with the negative control.

[redacted]

11.2 Subject Recruitment

Following approval of the study protocol and informed consent by the GIRB and sponsor authorization to start, potential subjects will be recruited. [redacted]

[redacted]

Insofar as possible, the group of subjects selected will be of mixed sex, age, and race.

[redacted]

[redacted]

11.3 Criteria for Inclusion

Potential subjects may be included in this study if they meet the following requirements:

- Subjects may be of either sex, at least 18 years of age and of any race.
- Subjects must be able to read and understand English.
- Subjects must be in good general health.
- Subjects must read and sign an Informed Consent Form, Authorization to Use and Disclose Protected Health Information Form, and List of Restricted Products prior to participating in the study.

[redacted]

- Have skin within 6 inches of the test sites that is free of tattoos, dermatoses, abrasions, cuts, lesions or other skin disorders. Subjects with tattoos may be admitted at the discretion of the Principal Investigator, Subinvestigators, or Medical Experts.
- Have Screening Day baseline counts of at least 1.0×10^3 CFU/cm^{2}per abdominal site (left and right) **and** at least 1.0×10^5 CFU/cm^{2}per groin site (left and right).
- Female subjects must have a negative urine pregnancy test documented before treatment with test materials.

11.4 Criteria for Exclusion: Medical History/Status Ascertained from Direct Questioning of a Prospective Subject

Potential subjects will be excluded from participation if any of the following criteria apply to them.

- Known allergies or sensitivities to sunscreens, deodorants, laundry detergents, topical application of fragrances, vinyl, latex (rubber), alcohols, metals, inks, or tape adhesives, or to common antibacterial agents found in soaps, lotions, or ointments, particularly chlorhexidine gluconate (CHG), citric acid, methylene blue, methylparaben, propylparaben, or isopropyl alcohol.
- Exposure of test sites to strong detergents, solvents, or other irritants within the 14-day product-restriction period or during the test period.
- Exposure of test sites to antimicrobial agents, medicated soaps, medicated shampoos, or medicated lotions, use of biocide-treated pools or hot tubs, use of hot waxes or depilatories, including shaving (in the applicable test areas), use of tanning beds, or sunbathing during the 14-day product-restriction period or during the test period.
- Wear fabric softener-treated or antimicrobial treated clothing (including bug-repellent and UV-treated clothing) during the 14-day product-restriction period or during the test period.
- Use of systemic or topical antibiotic medications, any inhaled or injection steroid medications, steroid medications (other than for hormonal contraception, for post-menopausal reasons, nasal spray, and eye drops), or any other product known to affect the normal microbial flora of the skin during the 14-day product-restriction period or during the test period. Note: topically applied hormonal steroids used for post-menopausal reasons must not get on the test sites.
- Subjects who have a history of skin allergies.
- Subjects who have a history of skin cancer within 6 inches of the applicable test areas.

[redacted]

- 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.
- Current participation in another clinical study.
- Participation in a clinical study in the past 30 days, at the time of consent.
- Showering, bathing, or swimming within the 72 hour period prior to Screening Baseline Day or Treatment Day sampling (sponge baths may be taken, however, the lower abdomen and upper thigh region must be avoided).
- Any medical condition or use of any medications that, in the opinion of the Principal Investigator or Consulting Physician, would preclude participation.
- Unwillingness to fulfill the performance requirements of the study.
- Nursing a child, pregnant, plans to become pregnant.

12.0 SUBJECT WITHDRAWAL AND DISCONTINUATION

The Investigator (or Subinvestigators) 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. The Investigator will provide a written report on the appropriate source document or Case Report Form (CRF) including the date and reason for discontinuance. Subjects who qualify on Screening Baseline 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.

Reference Sections 13.3, 13.9 and 17.3 for handling of withdrawn subject data.

13.0 PROCEDURES

13.1 Compliance with Good Clinical Practices and Regulatory Requirements

The study will be conducted in compliance with the Good Clinical Practice regulations as required by the FDA, Good Laboratory Practice regulations as required by the FDA, the

[redacted]

standard operating procedures of BioScience Laboratories, Inc., the study protocol, and any protocol amendments, and the regulatory requirements of the FDA and ICH.

Subjects will be questioned prior to and during the study to ensure compliance with study requirements.

13.2 Product Restriction Period

The 14-day period prior to the baseline-screening portion of the study will be designated the "product restriction" period. During this time, subjects will avoid the use of medicated soaps, lotions, shampoos, deodorants, etc., as well as skin contact with solvents, acids, and bases. Subjects will also avoid sunbathing, using UV tanning beds or bathing in biocide-treated (e.g., chlorinated) pools and/or hot tubs. Subjects will be provided a kit containing products that they will be instructed to use exclusively for personal hygiene during the course of the study.

Subjects must not remove hair from the anatomical sites to be treated from the beginning of the product restriction period until after the testing has been completed. The subjects will be instructed not to bathe or shower for at least 72 hours prior to the baseline-screening sampling or the day of skin-treatment. This regimen will allow for the stabilization of the normal microbial flora of the skin.

During the latter portion of the product-restriction period (at least 72 hours prior to the test period), the subjects will be examined physically to ensure that there is no evidence of injury, dermatosis, or dermatitis present at the sampling sites, and hair on sampling sites will be clipped, if needed, to ensure that bandages used in testing will remain secure to the test sites (reference Appendix 2).

13.3 Randomization

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 with the exception of replacement subjects.

[redacted]

Site and Sample Time Balance

Within each anatomical site, sampling order will be randomized for baseline, 30-second, 10-minute, and 6-hour samples.

[redacted]

The Investigator is responsible for ensuring the randomization is followed. The final randomization schedule will be prepared before the initial treatment in accordance standard operating procedures. The test materials will be labeled with the appropriate codes as designated by the study randomization plan (i.e. A = ZuraPrep™, B = ChloraPrep®, C = ZuraPrep™ Vehicle).

13.4 Baseline-Screening Sampling

Baseline-screening samples will be taken on the day following the product restriction period. Subjects will not shower within a minimum of 72 hours prior to being sampled. Subjects will don a disposable undergarment prior to sampling, will be examined physically to ensure that there is no evidence of injury, dermatosis, or dermatitis present at the sampling sites (Section 15.2). A subject will be dismissed from the study if skin irritation scores of 1 or greater are observed on the test sites. If the subject continues to meet the study criteria, they will be sampled at the center of the test areas on the skin of the inguen and abdomen (Appendix 2) according to the Cylinder Sampling (Scrub Cup) Technique (Section 13.6).

There will be a minimum of 72 hours between the time the screening period ends and the experimental period begins. Based upon adequate screening microbial counts, subjects will be eligible to continue in the study.

Baseline criteria for qualification for the test period are 3.0 $\log_{10}$ CFU/cm^2 from the skin of the abdomen, and 5.0 $\log_{10}$ CFU/cm^2 on the skin on the groin. Subjects may qualify and be admitted into testing for both anatomical sites or one if the sample size minimums have been met for an anatomical region. If more subjects qualify (pass screening baseline) than can be tested in a group, or if they have a schedule conflict (or cannot test for another reason that does not affect their eligibility to test in the study), those subjects that qualify may be moved to a future group for testing the following week without having to re-qualify (clip or go through screening baseline again). If subjects that qualify need to move to a future group later than the following week, the subjects will be required to re-qualify (clip and go through screening baseline). Subjects who do not

[redacted]

qualify may be allowed to be screened again at the discretion of the Principal Investigator.

13.5 Experimental Period

Prior to sampling, the subjects will be questioned regarding adherence to the protocol. Female subjects must have a negative urine pregnancy test documented before treatment with test materials.

Subjects will don a disposable undergarment and be examined physically to ensure no evidence of injury, dermatosis, or dermatitis is present at the test sites (Section 15.2). On the abdomen, a sterile surgical marker will be used to demarcate bilaterally 5" × 5" areas of skin to the right and left side of the navel that appear to be similar in condition. On the groin, a sterile surgical marker will be used to demarcate bilaterally 1.5" × 5" areas of skin that appear to be similar in condition.

Prior to collection of the baseline, 30-second, 10-minute, and 6-hour post-prep samples, the skin in each test area will be evaluated for indications of skin irritation, based on the Skin Irritation Scoring System (Section 15.2).

Subjects will be sampled for baseline microbial population on sites randomly assigned on the skin of the abdomen and groin on both sides (Appendix 2). The Cylinder Sampling (Scrub Cup) Technique (Section 13.6) will be used for this and for the post-treatment samples.

The randomly assigned test product, positive control, and negative control will be applied to the test sites per directions presented in Appendix 1. A total of three post treatment sample collections will be performed at each test site for all test materials. All subjects will have samples collected within 30 seconds ± 5 seconds, 10 minutes ± 30 seconds and 6 hours ± 30 minutes post application from both anatomical treatment sites. Following the 10-minute sample being obtained the remaining sample sites will be covered with sterile synthetic-blend gauze sponges and semi-occlusive catheter dressings. Subjects will return 6 hours following product application to be sampled. During product applications, subjects will wear disposable gloves to guard against contamination of the test sites.

If the semi-occlusive catheter dressing has lifted from a test site such that the gauze sponge is no longer secured, the test site will be sampled and the occurrence noted.

Following completion of the final post-product application sampling of a test site, the remaining test material will be wiped/cleansed from the test site with a mild soap and/or tap water.

[redacted]

13.6 Cylinder Sampling (Scrub Cup) Technique

Baseline samples will be performed using sterile Stripping Suspending Fluid (SSF), and post-test material application samples will be performed using sterile Stripping Suspending Fluid with product neutralizers (SS+).

A sterile cylinder [redacted] will be held firmly onto the test site to be sampled. [redacted] of the appropriate sterile Stripping Suspending Fluid will be instilled into the cylinder, and the skin area inside the cylinder will be scrubbed in a circular motion with pressure for [redacted] with a sterile rubber policeman. The sampling fluid will be removed with a sterile pipette and transferred to a sterile test tube. A second [redacted] aliquot of the appropriate sterile Stripping Suspending Fluid will be instilled into the cylinder, and the skin area again scrubbed for [redacted] with a sterile rubber policeman. The second aliquot will then be pooled in the test tube with the first aliquot.

13.7 Diluting, Plating, and Counting

Aliquots of the microorganism suspension (10^0 dilution) will be serially diluted in Butterfield's Phosphate Buffer Solution with product neutralizers (PBW+) ([redacted]). Serial dilution and plating will be completed within 30 minutes. Duplicate pour plates will be prepared from each of these dilutions with Tryptic Soy Agar with product neutralizers (TSA+) and incubated at 30 °C ± 2 °C for approximately 72 hours (± 4 hours). Following incubation, plates may be refrigerated up to ~48 hours prior to counting

Colonies will be manually counted and data recorded on appropriate data collection forms for each subject.

13.8 Blinding

The study materials will not be blinded from the Investigator or other study staff performing the study material application or bacterial sample collections. In order to ensure "blinded" microbiologists, technicians who participate in plating samples and/or counting colonies on plates resulting from testing will not participate in the test product application or sample collection procedures. The blinding does not apply to baseline-screening samples.

13.9 Data Handling

The estimated $\log_{10}$ number of viable microorganisms per cm^2 recovered from each sample site will be designated the "R-value."

To convert the volumetric measure of the sample into the number of colony-forming units per square centimeter (cm^2), the following formula will be employed:

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

Where:

R = the average colony-forming unit count in $\log_{10}$ scale per cm^2 of sampling surface

F = total number of mL of stripping fluid added to the sampling cylinder; in this study, $F =$ [redacted]

$\frac{\sum c_i}{n}$ = average of the duplicate colony counts used for each sample collected

D = dilution factor of the plate counts

A = inside area of the cylinder in cm^2; in this study, $A =$ [redacted]

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

If colonies on one of the plates is uncountable, the count from the remaining plate will be used.

Colony counts of 0 will be set to 0.5 for further calculation purposes. The average for duplicate plates each containing 0 CFU's would result in an average of 0.5 CFU's further used for calculating the R Value.

Data collected from subjects with low or high test-day baseline counts on an anatomical test site (< 3.0/> 5.5 $\log_{10}$ CFU/cm^2 from the skin of the abdomen, or < 5.0/> 7.5 $\log_{10}$ CFU/cm^2 on the skin on the groin) will not be used in the Efficacy Analyses (Section 16.2). This is a common occurrence in clinical-simulation studies. Hence, more subjects will be enrolled and tested to account for test-day baseline failures.

14.0 DATA RECORDING

The raw data from sampling of all sites evaluated on a subject will be recorded on data collection forms.

15.0 ASSESSMENT OF SAFETY

15.1 Safety Assessments

The subject's safety will be monitored by evaluations of reactions observed on the skin of the test sites and any adverse reactions. Adverse reactions will be fully documented, reported as an Adverse Event, and followed to resolution.

[redacted]

15.2 Evaluation of Skin Reactions

The skin at each site will be evaluated visually for irritation per a scoring matrix (below) immediately before screening-baseline, baseline, and post-treatment samples are taken from each site. A subject will be dismissed from the study if skin irritation scores of 1 are observed on the test sites prior to screening-baseline or treatment day baseline sampling.

SKIN IRRITATION SCORING SYSTEM

Condition	Rating	Description
Erythema	0	No reaction
	1	Mild and/or transient redness limited to sensitive area
	2	Moderate redness persisting over much of the product-exposed area
	3 *	Severe redness extending over most or all of the product-exposed area
Edema	0	No reaction
	1	Mild and/or transient swelling limited to sensitive area
	2	Moderate swelling persisting over much of the product-exposed area
	3 *	Severe swelling extending over most or all of the product-exposed area
Rash	0	No reaction
	1	Mild and/or transient rash limited to sensitive area
	2	Moderate rash persisting over much of the product-exposed area
	3 *	Severe rash extending over most or all of the product-exposed area
Dryness	0	No reaction
	1	Mild and/or transient dryness limited to sensitive area
	2	Moderate dryness persisting over much of the product-exposed area
	3 *	Severe dryness extending over most or all of the product-exposed area

* = A score of 3 in one or more of the conditions evaluated represents significant irritation and qualifies as an Adverse Event.

15.3 Adverse Events

Adverse Events will be captured for all subjects from the time screening baseline samples are taken to the time of discharge from the study. Adverse Events will be categorized in relationship to a test material that was applied to the specific skin site. Emergency treatment is available onsite in the laboratory facility, and medical facilities/personnel are in close proximity.

[redacted]

In the event that either the Principal Investigator or the Sponsor determines that continuation of the study poses a hazardous risk of serious injury or death to the subjects, the study will be stopped.

A Photograph and Video Release Form ([redacted] may be used (if subject agrees) in the even that an Adverse Event occurs for which the Principal Investigator is not onsite to observe [redacted]. This form will only be used with subject approval, and is not a requirement for participation in the study.

15.3.1 Definitions

15.3.1.1 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 a test material. All adverse event/experiences will be recorded and reported using Adverse Event documents according to the Standard Operating Procedures of the testing facility.

All Adverse Events, regardless of severity or the cause/effect relationship, are to be recorded. The severity of the effect will be noted as "Mild," "Moderate," or "Severe" according the following definitions:

Mild	Awareness of sign(s) or symptom(s), 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.

15.3.1.2 Causal Relations of Adverse Event/Experience

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

None	No association to a test material. 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 a test material. 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 if possible.

15.3.1.3 Serious Adverse Event/Experience – During this Study

A Serious Adverse Event/Experience is any adverse experience occurring 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.

15.3.1.4 Unexpected Adverse Event/Experience

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

15.3.2 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. All Adverse Events will be followed to resolution. Any Adverse Event will be documented on an Adverse Event Report Form.

15.3.3 Notification

The Sponsor and the reviewing IRB will be notified of all adverse event/experiences. Any Serious or Unexpected Adverse Drug Event/Experience that occurs during the study must be reported immediately by the Investigator to the Sponsor and the reviewing IRB, followed by written notification within 24 hours of the information being reported to the investigative study team.

The Principal Investigator, Collette Duley, and Medical Expert(s) are required to review all unanticipated problems involving risk to volunteers or others, serious adverse events, and all subject deaths associated with the protocol and provide an unbiased written report

[redacted]

of the event. At a minimum, the Principal Investigator and Medical Expert(s) must comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study.

15.4 Anticipated Reactions

The risks associated with this test are primarily related to application of a test material. Mild abrasion may occur due to cylinder sampling. Adhesive reactions are also possible. Mild skin irritation is anticipated and in some cases mild to heavy erythema, swelling, itching, cracking, peeling, or in rare cases, blistering and/or an allergic reaction might occur.

16.0 STATISTICAL METHODS

16.1 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 population (all randomized subjects) will be used for the safety analysis.

The modified Intent-to-Treat (mITT) Population will consist of all subjects who have at least one site (left or right for abdominal or inguinal) that passed the treatment day baseline (baseline values between 3.0 $\log_{10}$ -5.5 $\log_{10}$ CFU/cm^2 on the skin on the abdomen, or 5.0 $\log_{10}$ -7.5 $\log_{10}$ CFU/cm^2 on the skin on the inguen) and has CFU results for any other sample time for that site. The mITT data set will include all sites that passed the treatment day baseline bacterial counts and have CFU results for any other sample time. The mITT data set will be evaluated for efficacy.

16.2 Efficacy (Primary) Analyses

CFU values will be calculated as per Section 13.9. Individual plate CFU counts that are zero will be treated as 0.5 for further calculations. Changes from baseline will be calculated separately for each subject and for each of the three non-baseline sites by taking the baseline $\log_{10}$ CFU/cm^2 values and then subtracting the $\log_{10}$ CFU/cm^2 values for the samples taken after the baseline. Therefore, a subject who completes all treatments and passes all baseline requirements will have 4 (sites) * 3 (post-application samples per site) = 12 changes from baseline reported. Responder status will be calculated for each reported $\log_{10}$ CFU/cm2 reduction. The sites will be considered responders based on the sample time and body area:

For the groin at 30 seconds or 10 minutes, a $\log_{10}$ CFU/cm^2 reduction $\geq$ 3.0 is considered a responder.

For the abdomen at 30 seconds or 10 minutes, a $\log_{10}$ CFU/cm^2 reduction $\geq$ 2.0 is considered a responder.

[redacted]

For either the groin or the abdomen at 6 hours, a $\log_{10}$ CFU/cm^2 value less than baseline (i.e., a $\log_{10}$ CFU/cm^2 reduction > 0) is considered a responder.

Responder statuses will be grouped by body area, sample time, and test substance. Exact confidence intervals will be calculated for responder rates. The primary efficacy goal is to have the 95% confidence intervals for the responder rate at 10 minutes to be ≥ 70%. The secondary efficacy goals are to have the 30-second and 6-hour 95% confidence intervals for the responder rates to be ≥ 70%.

Differences in responder rates between treatments and their confidence intervals will be calculated using asymptotic (approximate) methods. If differences in responder rates are not reliable based on approximate methods, which happens when rates are near either 100% or 0%, $\log_{10}$ CFU/cm^2 confidence intervals for the differences will be used for comparison instead.

Two-sided confidence intervals for $\log_{10}$ CFU/cm^2 changes from baseline will also be calculated. These calculations will use an ANOVA model with subject as a random variable and test substance as a fixed variable. Body area (abdomen or groin) will be a fixed variable. Sample times (30 seconds, 10 minutes, or 6 hours) will be calculated separately using identical models. Differences in $\log_{10}$ CFU/cm^2 reductions will be calculated based on the same model.

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

16.3 Safety Analyses

All treated subjects will be considered evaluable for safety. Safety assessments will be based on Adverse Events. A listing of all Adverse Events and related variables will be presented. If a sufficiently large number of Adverse Events are observed, then summary tables will present incidence rates in terms of treatment configuration for all subjects who enter the treatment period.

The skin reactions (erythema, edema, rash, and dryness; see Section 15.2, Skin Irritation Scoring System) of the test product, positive control, and negative control will be evaluated at baseline sampling and post-product application, and will be tabulated in the final report.

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) 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.

[redacted]

17.0 SPECIAL NOTES

17.1 Informed Consent

A written consent form will be obtained from each subject and filed by the Investigator with the subject's records, in accordance with 21 CFR Part 50.

17.2 Alteration of the Study

Neither the Investigator, nor the Sponsor, will modify or alter this protocol without first obtaining the concurrence of the other parties. All protocol modifications including, but not limited to changes in the Principal Investigator, inclusion/exclusion criteria, number of subjects to be enrolled, study sites, or procedures must be submitted to the GIRB as a written amendment for review and approval prior to implementation, with one exception: when necessary to eliminate an apparent immediate hazard to the subject.

17.3 Procedures for Accounting for Missing Data

Missing microbiological data at 30 seconds, 10 minutes, or 6 hours will be reported as missing; if the entire sample is missing the site will be treated as a nonresponder for that sample time. These subjects will be included in the mITT data set based on the criteria defined above. Details of any other missing data handling will be specified in the statistical analysis.

17.4 Protocol Deviations

This study will be conducted as described in this protocol, except for an emergency situation in which the protection, safety, and well-being of the subject requires immediate interventions, based on the judgment of the Investigator. In the event of a significant deviation from the protocol due to an emergency, accident, or mistake, the Investigator or designee will document the details of the situation and any subsequent decisions. All deviations from the protocol or approved amendments shall be documented by BioScience Laboratories, Inc. Any deviation to the protocol that may have an effect on the safety or rights of the subjects must be reported immediately (within 24 hours) to the local GIRB and sponsor representative. Any deviation that may compromise the statistical analysis of the study must be reported immediately (within 24 hours) to the sponsor representative. The sponsor shall be contacted via telephone at [redacted]

18.0 FINAL REPORT

The final report will be generated using ICH format and will summarize the method, data, and conclusions relative to the test materials and the subjects. Copies of the data will be incorporated into the report.

[redacted]

19.0 APPROVAL OF PROTOCOL AMENDMENTS

No changes may be implemented to any aspects of this protocol until written approval has been obtained from the Sponsor and the GIRB with one exception: when necessary to eliminate an apparent immediate hazard to the subject.

20.0 REFERENCES

Code of Federal Regulations Title 21 Parts 50, 56, 58, 312, and 314.

ICH E6 Good Clinical Practice Guidelines.

Food and Drug Administration Tentative Final Monograph (TFM) for *Effectiveness Testing of a Patient Preoperative Skin* (Vol. 59, No. 116, June 17, 1994, pp. 31450-31452), Proposed Amendment of the Tentative Final Monograph (FR 80:84, 01 May 2015).

ASTM E1054-08(2013), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents.*

21.0 DOCUMENTATION AND RECORD-KEEPING

21.1 Data Collection

Any contact with subject via telephone or other means that provides significant clinical information will also be documented in the progress notes and/or BSLI forms as appropriate.

Any changes to information in the study progress notes and other source documents will be initialed and dated on the day the change is made by study personnel authorized to make the change. If the reason for the change is not apparent, a brief explanation will be written adjacent to the change.

If a subject needs to make a correction, the subject will initial and/or date the correction they make on the source document.

21.2 Source Document Maintenance

Source documents are defined as the results of original observations and activities of a clinical investigation. Source documents will include, but are not limited to study progress notes, computer printouts, screening logs, laboratory notebooks and recorded data from automated instruments. All source documents produced in this study will be maintained by the Investigator and made available for inspection by authorized persons. The Case Report Forms (CRFs) will be the primary source documents for the study. All data will be directly recorded on the CRFs. The original signed informed consent form from each participating subject will be filed in the Study File and a copy given to the subject.

[redacted]

21.3 File Management at the Study Site

The Investigator will not dispose of any records relevant to this study without written permission from the Sponsor and providing an opportunity for the Sponsor to collect such records. The Investigator shall take responsibility for maintaining adequate and accurate hard-copy source documents of all observations and data generated during this study including but not limited to the essential documents noted below in Section 21.4. Such documentation is subject to inspection by the Sponsor, the FDA, or any applicable regulatory agency. If the Investigator is not able to store the records, he or she will contact the Sponsor and make arrangements for the Sponsor to assume the responsibility for the continued storage.

21.4 Study File Management

It will be the responsibility of the Investigator to assure that the Study File is maintained. The Study File for this protocol will contain, but will not be limited to, the information listed below:

- Investigational Brochure or other appropriate product safety information
- Signed Protocol
- Revised Protocol (if applicable)
- IRB-Approved Informed Consent Form (blank)
- Copy of Signed Form(s) FDA-1572
- Financial Disclosure for the Principal Investigator and Subinvestigators
- Curriculum Vitae of Principal Investigator and Subinvestigators
- DHHS Number for IRB, or other documentation of IRB compliance with FDA regulation
- Documentation of IRB approval of protocol, consent form, any protocol amendments and any consent form revisions
- All correspondence between the Investigator, IRB, and Sponsor relating to study conduct
- Copies of information related to SAE and the information on Immediately Reported Adverse Events
- Copy of completed Initiation Report
- Testing Facility Site Signature Log
- FDA's Clinical Investigator Information Sheets (if applicable)
- CRA Monitoring Log (if applicable)

[redacted]

- Drug Invoices
- Study specific training records for Testing Facility site personnel
- Enrollment/Disposition log showing subjects screened, enrolled, disqualified, withdrawn, and completed
- Sample Submission Form (if provided by Sponsor), Product Receipt Logs and Product Tracking Forms

To protect privacy and maintain the confidentiality of data, subjects will be assigned a unique study number, all study samples and research records will be identified using the subject's study number, and electronic databases will be kept on password-protected computers.

22.0 LIABILITY AND INDEMNIFICATION

Test Facility's liability to Sponsor under this Protocol shall be limited to the price of this evaluation. Sponsor shall be responsible to Study Participants (when applicable) (including study related Adverse Events that occur as a part of testing in this study) and to other third parties for the fitness of the test materials for use as defined in the Study Protocol.

[redacted]

23.0 PROTOCOL ACCEPTANCE

ACCEPTED BY: BioScience Laboratories, Inc. (Testing Facility)

[redacted]

Bozeman, Montana [redacted]

[redacted]

Principal Investigator: _____ 6/15/16

Collette Duley — Date of Study Initiation

REVIEWED BY:

[redacted]

ACCEPTED BY: Zurex Pharma, Inc. (Sponsor)

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

APPENDIX 1

PRODUCT APPLICATION INSTRUCTIONS

[redacted]

APPENDIX 1

PRODUCT APPLICATION INSTRUCTIONS (Page 1 of 2)

[redacted]

Treatment Site Application Instructions

Abdominal Test Site

1) [redacted]

2)

Inguinal Test Site

1) [redacted]

2)

[redacted]

APPENDIX 1

PRODUCT APPLICATION INSTRUCTIONS (Page 2 of 2)

[redacted]

Treatment Site Application Instructions

Abdominal Test Site

[redacted]

Inguinal Test Site

[redacted]

[redacted]

APPENDIX 2

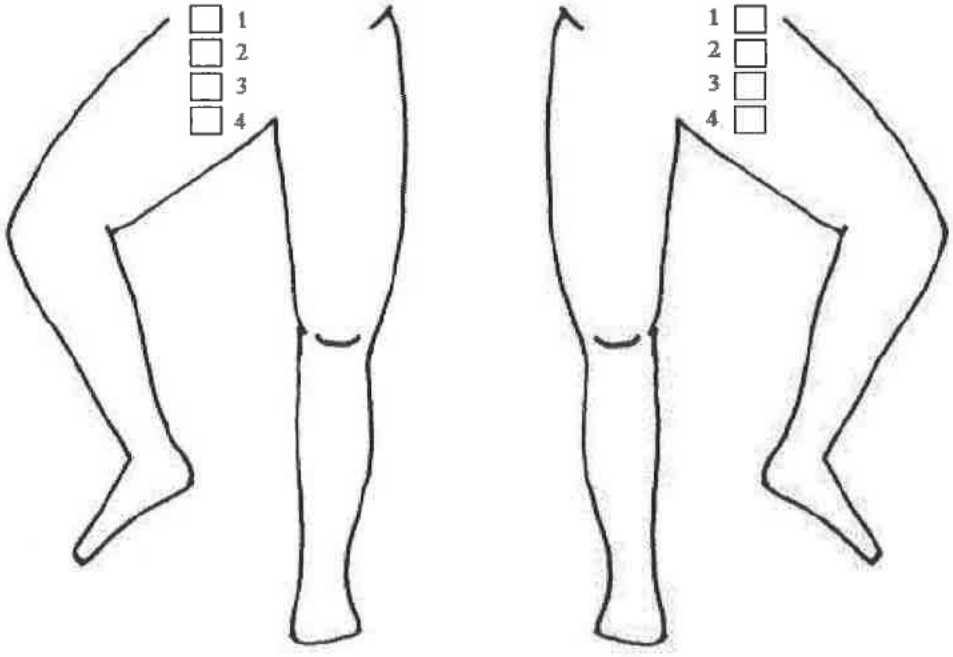
ANATOMICAL DIAGRAM OF THE SAMPLING SITES

[redacted]

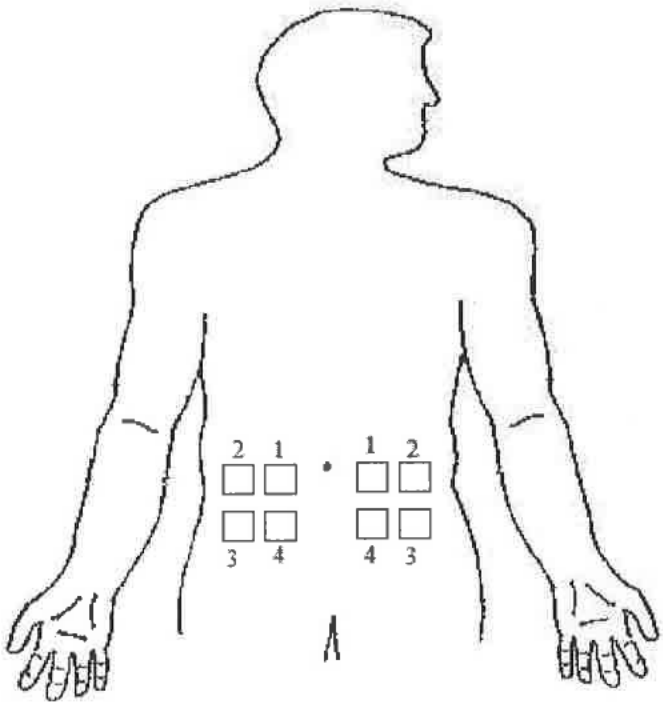
APPENDIX 2

ANATOMICAL DIAGRAM OF THE SAMPLING SITES

INGUINAL **TEST SITES**



ABDOMINAL **TEST SITES**



[redacted]

APPENDIX 3

VALIDATION OF NEUTRALIZER EFFECTIVENESS

[redacted]

APPENDIX 3

VALIDATION OF NEUTRALIZER EFFECTIVENESS

1.0 PURPOSE OF NEUTRALIZER EFFECTIVENESS STUDY

The purpose of this neutralization study is to assure that the neutralizers used in the recovery medium quench the antimicrobial activity of the test materials, and are not toxic to the bacteria. The study will comprise both an *In-Vivo* component performed using human subjects, and an *In-Vitro* component performed based on ASTM E1054-08(2013), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*.

[redacted] will be used as the challenge species in both components of the neutralizer validation study.

2.0 SCOPE

An effective nontoxic method of neutralization must be employed to eliminate the antimicrobial activity of a test material quickly. Sufficient supporting data are required to show that the neutralizing method employed is effective. A known population of microorganism must be exposed to the antimicrobial test materials, diluent/recovery media, the neutralizing solution, and the neutralizing solution plus antimicrobial test material in order to determine whether microbial inhibition is present.

Neutralizing methods include chemical inactivation, dilution of antimicrobial test material to a sub-inhibitory concentration, and membrane filtration. The procedures detailed here deal with chemical inactivation and dilution of antimicrobial test material, as well as recovery from human subjects.

The *In-Vivo* component of the neutralization study will use the two active test materials (ZuraPrep™ and ChloraPrep®) and the negative control (ZuraPrep™ Vehicle). At least [redacted] subjects will be tested using product applications on the skin of the abdomen, to obtain a minimum of [redacted]mples per test material challenged with each challenge species.

3.0 TEST MATERIALS

[redacted]

The test materials will be received and stored by BioScience Laboratories, Inc. (BSLI) in accordance with instructions from the Sponsor and retained in secure quarantine when not being used in testing. BSLI will maintain an inventory of the test materials in secure

[redacted]

quarantine and a log of use. Unused, sealed test materials will be stored by BSLI until the Sponsor specifies its disposition. In the absence of a disposition request from the Sponsor within 1 year of planned usage, the test materials will be returned to the Sponsor. No test materials will be destroyed unless so requested by the Sponsor. Complete product information will be provided in the Final Report, if it is not provided below, if provided by the Sponsor.

Test Product:	ZuraPrep™ [redacted]
Active Ingredient:	Isopropyl Alcohol (~70%)
Other Ingredients:	[redacted]
Manufacturer:	[redacted]
Lot Number:	
Manufacture Date:	
Expiration Date:	
Positive Control:	ChloraPrep® [redacted] (Scrub Teal® Tint)
Active Ingredients:	2% Chlorhexidine gluconate (w/v) and 70% isopropyl alcohol (v/v)
Lot Number:	_____
Expiration Date:	_____
Negative Control:	ZuraPrep™ Vehicle [redacted]
Other Ingredients:	[redacted]
Manufacturer:	[redacted]
Lot Number:	
Manufacture Date:	
Expiration Date:	

[redacted]

Applicators: [redacted]

Lot Number:

Expiration Date:

4.0 LABORATORY EQUIPMENT, SUPPLIES AND MEDIA

4.1 EQUIPMENT

The equipment used during this neutralization will be detailed on Clinical Trials Equipment Tracking Form(s), and the form(s) will be included in the Final Report.

4.2 SUPPLIES

The supplies used during this neutralization will be detailed on Supplies Tracking Form(s) and the form(s) will be included in the Final Report.

4.3 TEST MEDIA

Sampling Solution

Stripping Suspending Fluid (SSF)

[redacted]

Stripping Suspending Fluid with product neutralizers / 10% Tween (SS+)

[redacted]

Diluting Fluid

Butterfield's Phosphate Buffer Solution with product neutralizers (PBW+)

[redacted]

Media

Tryptic Soy Agar with product neutralizers (TSA+)

[redacted]

Tryptic Soy Agar (TSA)

Tryptic Soy Broth (TSB)

[redacted]

Phosphate Buffered Saline (PBS)

5.0 SUBJECT SELECTION

5.1 Number of Subjects

A sufficient number of overtly healthy subjects at least 18 years of age will be admitted into the study to ensure collection of at least [redacted] samples for each of the three test materials and each challenge species ([redacted]).

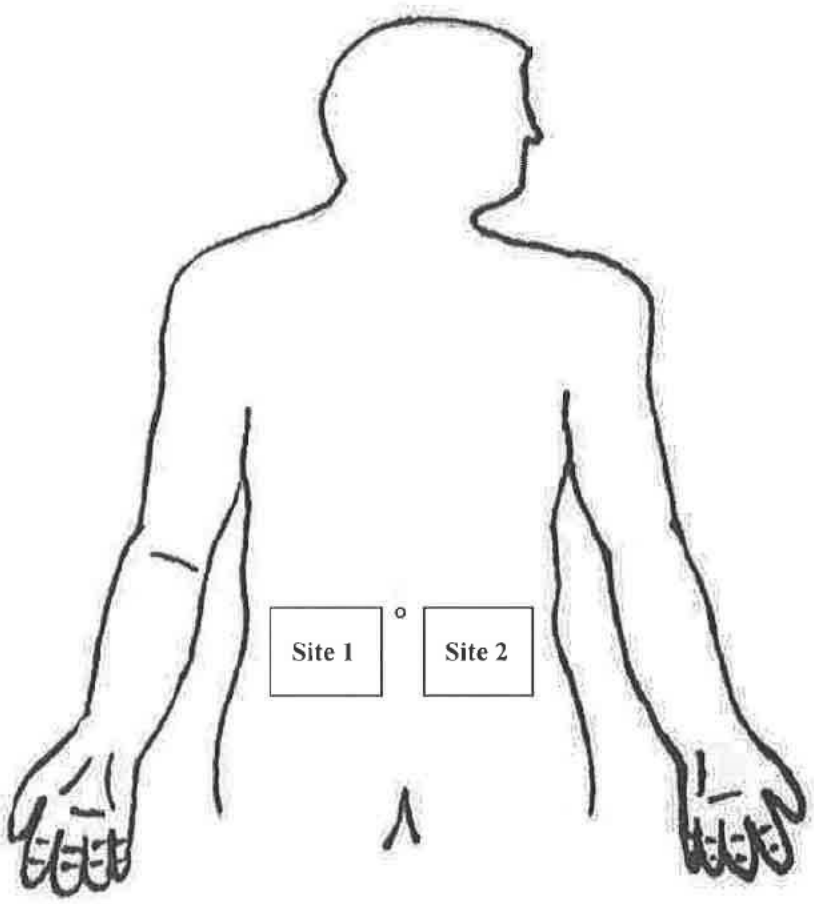
5.2 Subject Recruitment

Subjects must meet the inclusion and exclusion criteria in Section 11.0 of the protocol to which this neutralizer validation is attached, except for the baseline bacterial count, the 72-hour exclusion from showering/bathing criteria, and the length of the washout period. The neutralization subjects do not require a minimum baseline count and they only need to avoid topical and systemic antimicrobials for 7 days (not 14 days) prior to Test Day. Subjects will be asked to provide information on demographics and inclusion/exclusion criteria and sign the Informed Consent and Authorization Forms including Authorization to Use and Disclose Protected Health Information Form, and List of Restricted Products before beginning the 7-day washout period (subjects will be provided with a product restriction kit). When 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. These subjects will be identified by the letter "N" for neutralization and a subject number starting with 001.

Each subject will receive the three test materials (ZuraPrep™, ZuraPrep™ Vehicle [ZuraPrep™ without IPA], and ChloraPrep®), randomly assigned to a side of the abdomen, which will be applied to two separate 1.5" x 5" test sites on the abdomen. See diagram below.

[redacted]

Neutralization Test Sites Diagram



8.0 PROCEDURES

8.1 Compliance with Good Clinical Practices and Regulatory Requirements

The study will be conducted in compliance with the Good Clinical Practice regulations as required by the FDA, Good Laboratory Practice regulations as required by the FDA, the standard operating procedures of BioScience Laboratories, Inc., the study protocol, and any protocol amendments, and the regulatory requirements of the FDA and ICH.

Subjects will be questioned prior to and during the study to ensure compliance with study requirements.

8.2 Product-Restriction Period

The 7-day period prior to the neutralization will be designated the "product-restriction" period. During this time, subjects will avoid the use of medicated soaps, lotions,

[redacted]

shampoos, deodorants (with exception of deodorant provided in the product-restriction kit), etc., as well as skin contact with solvents, acids, and bases. Subjects will also avoid sunbathing, using UV tanning beds or bathing in biocide-treated (e.g., chlorinated) pools and/or hot tubs. Subjects will be provided a kit containing products that they will be instructed to use exclusively for personal hygiene during the course of the study.

Subjects must not remove hair from the anatomical sites to be treated from the beginning of the product restriction period until after the neutralization has been completed.

During the Product-Restriction Period (at least 72 hours prior to the test period), the subjects will be examined physically to ensure that there is no evidence of injury, dermatosis, or dermatitis present at the sampling sites, and hair on sampling sites will be clipped, if needed (reference Appendix 2).

8.3 Randomization

The test product (ZuraPrep™), the positive control (ChloraPrep®) and the negative control (ZuraPrep™ Vehicle) will be assigned randomly to test sites per a computer-generated randomization schedule, such that each subject receives two of the three test materials at the abdominal sites. See diagram above.

The challenge species will not be randomly assigned to a subject. Each challenge species will be tested separately and a group of [redacted] will provide the samples for each challenge species.

8.4 Test Inoculum Preparation

Two days prior to beginning the neutralization assay, [redacted] from a stock culture slant, lyophilized vial, or cryogenic stock culture will be transferred into a tube containing Tryptic Soy Broth (TSB). The tube will be incubated for 24 hours ± 4 hours at 30 °C ± 2 °C.

One day prior to beginning the neutralization assay, loopfuls of the broth culture will be streaked onto Tryptic Soy Agar (TSA) plates, and the plates will be incubated for 24 hours ± 4 hours at 30 °C ± 2 °C.

Immediately prior to initiating the neutralization assay, an inoculum suspension will be prepared in Phosphate Buffer Saline (PBS) solution from the culture on an agar plate, and the concentration adjusted to approximately 3.0×10^8 to 1.0×10^9 CFU/mL. The suspension will then be serially diluted in PBS to achieve an inoculum titer of approximately 3.0×10^3 to 1.0×10^4 CFU/mL, and used as test inoculum. The below procedures will be performed with both microorganisms.

[redacted]

8.5 Inoculum Assay (Initial population) – Test C

Test Inoculum will be assayed by adding a [redacted] aliquot of the inoculum to [redacted] of Phosphate Buffer Saline (PBS) solution (IP), vortexing for at least 3 seconds, and immediately (within 1 minute) pour-plating, in duplicate, [redacted] aliquots of the IP with TSA. This assay will be performed five additional times for a total of six replicates.

The diluted test inoculum suspensions will be allowed to stand for at least 30 minutes, following which, [redacted] liquots will be pour-plated, in duplicate, with TSA.

8.6 Product Efficacy Evaluation (*In Vitro*) – Test D

This phase of the neutralization assay determines whether the antimicrobial test material is able to reduce the population of the challenge microorganism. This assay will be performed in six replicates.

To each of six test tubes containing [redacted] of each Test Material, a [redacted] aliquot of the test inoculum will be added. The suspensions will be vortexed for at least 3 seconds and, immediately (within 1 minute), [redacted] aliquots of each replicate will be pour-plated, in duplicate, with TSA.

The tubes will be allowed to stand for at least 30 minutes, following which [redacted] liquots of each replicate will be pour-plated, in duplicate, with TSA.

8.7 Neutralizing/Recovery Medium Inhibition Evaluations (*In Vitro*) – Test B

This phase of the neutralization assay assures that the sterile Stripping Suspending Fluid (SSF) and the sterile Stripping Suspending Fluid with product neutralizers (SS+), the sampling solutions employed in the evaluation, are not inherently toxic to the microorganisms. This assay will be performed in six replicates.

To each of six test tubes containing [redacted] of one of the sampling solutions, a [redacted] aliquot of the test inoculum will be added. The suspensions will be vortexed for at least 3 seconds and, immediately (within 1 minute), [redacted] aliquots of each replicate will be pour-plated, in duplicate, with Tryptic Soy Agar with product neutralizers (TSA+).

The tubes will be allowed to stand for at least 30 minutes, following which [redacted] aliquots of each replicate will be pour-plated, in duplicate, with TSA+.

This same procedure will be repeated for the remaining sampling solution

8.8 Diluent Broth Inhibition Evaluation (*In Vitro*) – Test B

This phase of the neutralization assay assures that the Butterfield's Phosphate Buffer Solution with product neutralizers (PBW+) employed in the evaluation is not inherently toxic to the microorganism. This assay will be performed in six replicates.

[redacted]

Six test tubes containing [redacted] of diluent broth to be used in the test will be prepared, and a [redacted] aliquot of the test inoculum will be transferred to each tube. The suspension will be vortexed for at least 3 seconds, and immediately (within 1 minute), [redacted] aliquots of each replicate will be pour-plated, in duplicate, with TSA+.

The tubes will be allowed to stand for at least 30 minutes, following which, [redacted] aliquots of each replicate will be pour-plated, in duplicate, with TSA+.

8.9 Neutralizer Efficacy Evaluation (*In Vivo*) – Test A

This phase of the evaluation determines whether the neutralizing method chosen effectively eliminates the antimicrobial activity of the test materials contained in the applicators.

Prior to sampling, the subjects will be questioned regarding adherence to the protocol. Subjects will also be examined physically to ensure no evidence of injury, dermatosis, or dermatitis is present at the sampling sites. Female subjects will be required to provide a urine sample for a pregnancy test. Only those female subjects with a negative test will be allowed to proceed into testing.

A 1.5" x 5" test area will be demarcated on each side of the abdomen. After the test areas are marked, each area will be processed using three 70% isopropyl alcohol swabs for a total of ~ 90 seconds (~ 30 seconds each), followed by an air-dry for at least 1 minute. This step will prepare the skin for the neutralization test.

A test material (reference Section 3.0 of the Validation of Neutralizer Effectiveness) will be applied to a 1.5" x 5" site on the abdomen of the subjects following the instructions for inguinal application in Appendix 1, with the randomly assigned test material.

The site will then be sampled using the Cylinder Sampling (Scrub Cup) Technique 30 ± 5 seconds post product-application completion.

The Cylinder Sampling (Scrub Cup) Technique will be performed as described in Section 13.6 of the Study Protocol (using SS+).

The volume of sample will be adjusted to [redacted] inoculated with [redacted] of the test inoculum, and vortexed for at least 3 seconds. Immediately (within approximately 1 minute), [redacted] aliquots will be pour-plated, in duplicate, with TSA+.

The tube containing the sample and inocula will be allowed to stand for at least 30 minutes. Following the exposure, [redacted] aliquots of each sample will be pour-plated, in duplicate, with TSA+.

The process will be repeated on the remaining abdominal site with the randomly assigned test material. A total of nine subjects will be treated on the both sides of the abdomen with two of the three test material and for each challenge species. A total of [redacted] will be treated with two of the three test materials (samples from [redacted] used for

[redacted]

one microorganism, samples from the remaining nine subjects used for the remaining microorganism).

Following all sampling, each test site will be cleaned using a towelette saturated with tap water and/or mild soap to remove the test material from the skin.

8.10 Incubation

The inoculated plates will be incubated at 30 °C ± 2 °C for approximately 72 hours, or until sufficient growth is visible.

9.0 CALCULATIONS

9.1 The following formula will be used to calculate the number of viable bacteria recovered:

$$Log_{10}\left\{\frac{A+B}{2}\right\}$$

Where:

A and B = Individual Plate Counts

9.2 If colonies on one of the plates are uncountable, the count from the remaining plate will be used.

10.0 STATISTICS

10.1 After calculating the $\log_{10}$ populations recovered from Tests D, B, and A, these will be statistically compared to the Initial Population (Control) using a One-Way Analysis of Variance (ANOVA) with Dunnett Multiple Comparisons with the Control. All statistical calculations will be performed using the 0.05 level of significance for Type I (α) error.

10.2 Prior to comparing Phases to the Initial Population (Control), the 30-minute evaluation of the Initial Population should be shown to be statistically equivalent to the "time-zero" Initial Population of the microorganism. Hypotheses are:

H_0 : Initial Population = Test Phase, or

H_A : Initial Population ≠ Test Phase.

If $p \leq 0.05$ for each comparison to the control, H_0 will be rejected and the Test Phase will be considered to be significantly different from the Initial Population. There is potential for low variance of the data, which would result in rejecting H_0. The difference between the Initial Population and the Test Phase will also be used to confirm significant differences. If the difference is greater than or equal to 0.20, the two tests will be determined to be significantly different. Differences less than 0.20 between the two tests will be determined to be not different.

10.3 The Product Efficacy evaluation is effective if the antimicrobial test product produces a significant reduction in the population of the microorganism.

[redacted]

10.4 The Neutralizing/Recovery Medium Inhibition Evaluations are considered non-inhibitory if all recovered populations are not statistically different from the Initial Population of the microorganism.

10.5 The Diluent Broth Inhibition Evaluation is considered non-inhibitory if all recovered populations are not statistically different from the Initial Population of the microorganism.

10.6 Neutralization is considered adequate if all recovery populations are not statistically different from the Initial Population.

11.0 REFERENCES

ASTM E1054-08(2013), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*, ASTM International, 100 Barr Harbor Drive, P.O. Box C700, West Conshohocken, PA 19428-2959, United States.

Beausoleil, Christopher M. 2003. A Guide for Validation of Neutralizer Systems Used in Topical Antimicrobial Evaluations. In: *Handbook of Topical Antimicrobials*, D.S. Paulson, Ed. Marcel Dekker, New York. 452 pp.

BSLI SOP [redacted] *Adequacy of Neutralization Assay: Clinical Trials Evaluations.*

Sutton, Scott V. W. 1996. Neutralizer Evaluations as Control Experiments for Antimicrobial Efficacy Tests. In: *Handbook of Disinfectants and Antiseptics,* J. M. Ascenzi, Ed. Marcel Dekker, New York. 300 pp.

[redacted]

PROTOCOL AMENDMENT FORM

DATE: 08/02/2016 [redacted]

PROTOCOL NUMBER: 150316-103

SPONSOR: Zurex Pharma, Inc

PROTOCOL TITLE: PIVOTAL CLINICAL EVALUATION OF [redacted], A PATIENT PREOPERATIVE SKIN PREPARATION

REASON(S) FOR CHANGE(S): In Section 13.9, page 23, the inside area of cylinder needs to be corrected, [redacted], in Section 15.3, page 25, the name and form number of the photo/video release form need to be updated, On page26, Section 15.3.2, adverse event documentation needs to be updated.

CHANGE(S):
In Section 13.9 on page 23 of the Protocol, "A = inside area of the cylinder in cm^2; in this study, A = [redacted], will be changed to, "A = inside area of the cylinder in cm^2; in this study, A = [redacted]"

[redacted]

In Section 15.3 on page 25, "A Photograph and Video Release Form ([redacted])", will be changed to "A Medical Photograph and Video Release form ([redacted])".

In Section 15.3.2 on page 26, "Any Adverse Event will be documented on an Adverse Event Report Form." Will be changed to "Adverse Events will be documented on appropriate Adverse Event forms."

APPROVALS:

[redacted]

PRINCIPAL STUDY DIRECTOR / INVESTIGATOR — DATE: 08/02/16

[redacted]

[redacted]

PROTOCOL AMENDMENT FORM

DATE: 08/09/2016 [redacted]

PROTOCOL NUMBER: 150316-103

SPONSOR: Zurex Pharma, Inc

PROTOCOL TITLE: PIVOTAL CLINICAL EVALUATION OF [redacted], A PATIENT PREOPERATIVE SKIN PREPARATION

REASON(S) FOR CHANGE(S):
In Section 13.3, Page 19 of the Protocol, the Randomization Left/Right Balance needs to be corrected due to a typographical error about randomization replacements.

[redacted]

In Section 15.4 (Anticipated Reactions), Page 27 of the Protocol, Page 8 (Risks) of the Informed Consent Form as well as Page 7 (Risks) of the Informed Consent Form - Neutralization Assay, the anticipated reactions need to be updated to include one more anticipated reaction.

CHANGE(S):
In Section 13.3 on Page 19 of the Protocol, for the Left/Right Balance, the statement "The application will be randomized so that each treatment is used on an equal number of left and right sides of the body with the exception of replacement subjects." will be changed to, "The application will be randomized so that each treatment is used on an equal number of left and right sides of the body."

On page 2 of Appendix 1, the procedure for ZuraPrep™ Vehicle (Negative Control) [redacted] This will be changed to: [redacted]

In Section 15.4 (Anticipated Reactions), Page 27 of the Protocol, and on Page 8 of the Informed Consent Form as well as Page 7 of the Informed Consent Form - Neutralization Assay, in the Risks sections, folliculitis will be added as an anticipated reaction from clipping. For the Informed Consent Form, and new version of the Form will be created (Version 02) and approved by the GIRB. Clipping has already occurred for the neutralization, therefore, a subject letter will be signed by subjects who clipped in the neutralization – the letter will be approved by the GIRB. See attached letter for neutralization subjects, and version 02 of the Informed Consent Form.

APPROVALS:

[redacted]

PRINCIPAL STUDY DIRECTOR / INVESTIGATOR

08/09/16
DATE

PROTOCOL AMENDMENT FORM

DATE: 08/09/2016 [redacted]

PROTOCOL NUMBER: 150316-103

SPONSOR: Zurex Pharma, Inc

PROTOCOL TITLE: PIVOTAL CLINICAL EVALUATION OF [redacted] A PATIENT PREOPERATIVE SKIN PREPARATION

[redacted]

PROTOCOL AMENDMENT FORM

DATE: 03/30/2017 [redacted]

PROTOCOL NUMBER: 150316-103

SPONSOR: Zurex Pharma, Inc.

PROTOCOL TITLE: PIVOTAL CLINICAL EVALUATION OF [redacted] A PATIENT PREOPERATIVE SKIN PREPARATION (Sponsor Project Number: ZX-ZP-0074)

REASON(S) FOR CHANGE(S): The inclusion criterion regarding baseline screening results have been expanded to allow subjects to proceed to Treatment Day if they have passing Screening Day baseline counts on the left and right sides of the abdomen and /or the left and right sides of the groin due to recruitment challenges around subjects meeting the minimum screening baseline requirements in the abdomen.

[redacted]

Section 11.3, Criteria for Inclusion, second bullet point (page 17) will state the following:

> Have Screening Day baseline counts of at least 1.0×10^3 CFU/cm^{2}per abdominal site (left and right) **and/or** at least 1.0×10^5 CFU/cm^{2}per groin site (left and right).

Section 13.4, Baseline-Screening Sampling: The third paragraph will state the following:

> Baseline criteria for qualification for the test period are 3.0 $\log_{10}$ CFU/cm2 from the skin of the abdomen, and 5.0 $\log_{10}$ CFU/cm^2 on the skin on the groin. Subjects may qualify on either one or both anatomical site(s) and be admitted into testing for those anatomical site(s) only, or one if the sample size minimums have been met for an anatomical region. If more subjects qualify for treatment than can be treated in a given time period, preferential admittance into the treatment phase will be given to subjects qualifying in both the abdominal and inguinal test areas, followed by subjects who pass abdomen only. Subjects that qualify may be moved to a future group for testing the following week without having to re-qualify (clip or go through screening baseline again). If subjects that qualify need to move to a future group later than the following week, the subjects will be required to re-qualify (clip if needed and go through screening baseline). Subjects who do not qualify may be allowed to be screened again at the discretion of the Principal Investigator.

APPROVALS:

[redacted]

STUDY DIRECTOR / PRINICPAL INVESTIGATOR — DATE: 03/30/17

[redacted]

[redacted]

PROTOCOL AMENDMENT FORM

DATE: 03/30/2017 [redacted]

PROTOCOL NUMBER: 150316-103

[redacted]

[redacted]

PROTOCOL AMENDMENT FORM

DATE: 04/06/2017 [redacted]

PROTOCOL NUMBER: 150316-103

SPONSOR: Zurex Pharma, Inc.

PROTOCOL TITLE: PIVOTAL CLINICAL EVALUATION OF [redacted] A PATIENT PREOPERATIVE SKIN PREPARATION (Sponsor Project Number: ZX-ZP-0074)

REASON(S) FOR CHANGE(S): To update and clarify the randomization section of the protocol as a result of the changes implemented in Protocol Amendment Form 03 (screening day baseline criteria expansion).

[redacted]

In Section 13.3, Randomization, under "Treatment Balance", the sentence:

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

Will be changed to:

"Each subject will receive two different treatments ***per anatomical site*** (abdomen or groin), one on the left and one on the right. A subject that has qualifying screening baselines on both anatomical sites may receive up to 3 different treatments."

[redacted]

STUDY DIRECTOR / PRINICPAL INVESTIGATOR

04/06/17
DATE

[redacted]

[redacted]

PROTOCOL AMENDMENT FORM

DATE: 08/29/2017 [redacted]

PROTOCOL NUMBER: 150316-103

SPONSOR: Zurex Pharma, Inc.

PROTOCOL TITLE: PIVOTAL CLINICAL EVALUATION OF [redacted], A PATIENT PREOPERATIVE SKIN PREPARATION (Sponsor Project Number: ZX-ZP-0074)

REASON FOR CHANGE(S): This amendment is issued after the completion of testing to add additional efficacy criteria in the below sections to the study protocol in accordance with [redacted] recommendations by the Division of Nonprescription Drug Products of the Food and Drug Administration (FDA)[redacted]

CHANGE(S): The following paragraph is added to the end of *Section 7.1 Primary Analysis* of the protocol:

> Additionally, as recommended by FDA, product effectiveness will be measured using the average treatment effects (ATE). The ATE will be estimated from a linear regression of post-treatment bacterial count (log_{10} scale) at 10 minutes on the additive effect of a treatment indicator and the baseline or pretreatment measurement (log_{10} scale). To show effectiveness, the test product will be 1) non-inferior to ChloraPrep® with a 0.5 margin (log_{10} scale, upper bound of 95% confidence interval of the difference in ATE values ≤ 0.5) and 2) superior to the vehicle control by a margin of 1.2 (log_{10} scale, lower bound of 95% confidence interval of the difference in ATE values ≥ 1.2).

Section 16.2 Efficacy (Primary) Analyses title is revised to remove *(Primary)* for accuracy, to *Efficacy Analyses*.

Section 7.2 Secondary Analysis is revised (additions underlined for clarity):

> Responder rates, ATE values, and differences in ATE values at 30 seconds will be calculated identically to the 10-minute responder rates and ATE values. At 6 hours a site is considered a responder if it is below baseline; otherwise, 6-hour responder rates will be calculated identically to the 10-minute responder rates. The secondary efficacy goals are to have the 95% confidence intervals for the responder rates to be greater than or equal to 70%. Log_{10} reductions from baseline will also be calculated for all post-prep samples. The non-inferiority and superiority goals for ATE values are identical to those at 10 minutes.

The following paragraphs are added prior to the last paragraph in *Section 16.2 Efficacy Analyses* of the protocol:

> Average Treatment Effect (ATE) will be estimated from a linear regression of post-treatment bacterial count (log_{10} scale) on the additive effect of a treatment indicator and the baseline or pretreatment measurement (log_{10} scale). ATE will be calculated for the 10-minute samples separately for each product and each body area. [redacted] The ATEs will be compared as follows:
>
> a. ZuraPrep™ will be compared to the ChloraPrep®. To show effectiveness, ZuraPrep™ should be non-inferior to ChloraPrep® with a 0.5 margin. Specifically, the upper bound of the 95% confidence interval of the ATE of ChloraPrep® minus the ATE of ZuraPrep™ should be less than or equal to 0.5.
> b. ZuraPrep™ will be compared to ZuraPrep™ vehicle. To show effectiveness, ZuraPrep™ should be superior to ZuraPrep™ vehicle by a 1.2 margin. Specifically, the lower bound of the 95% confidence interval of the ATE of ZuraPrep™ minus the ATE of ZuraPrep™ vehicle should be greater than or equal to 1.2.
>
> The same ATE calculations will be performed for the 30-second time point, but the results are for informational purposes only.

[redacted]

PROTOCOL AMENDMENT FORM

[redacted]

DATE: 08/29/2017

PROTOCOL NUMBER: 150316-103

APPROVALS:

[redacted]

STUDY DIRECTOR / PRINCIPAL INVESTIGATOR — DATE: 08/29/17

[redacted]

REVIEWED BY:

[redacted]

[redacted]