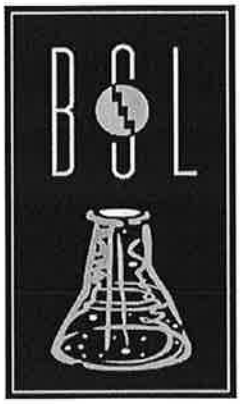

BIOSCIENCE LABORATORIES, INC., PROTOCOL #1608374-103
BD PROTOCOL NUMBER: MPS-16IPVAW01

A RANDOMIZED, SINGLE-CENTER, BLINDED, PILOT CLINICAL EVALUATION OF THE ANTIMICROBIAL EFFECTIVENESS OF THERMALLY TREATED CLOTHS IMPREGNATED WITH 0.4% OCTENIDINE DIHYDROCHLORIDE AQUEOUS SOLUTION COMPARED TO THERMALLY TREATED CLOTHS IMPREGNATED WITH VEHICLE FORMULATION AND TO SAGE 2% CHLORHEXIDINE GLUCONATE CLOTH FOR PREOPERATIVE SKIN PREPARATION

Test Products:	Thermally treated Polyester Cloths Impregnated with 0.4% w/v Octenidine Dihydrochloride Aqueous Solution Thermally treated Polyester Cloths Impregnated with Vehicle Formulation Sage 2% Chlorhexidine Gluconate Cloth
Principal Investigator:	Alicia Bogert
Subinvestigators:	J. Jill Lawrence, Judi Schutte, and Christopher Beausoleil, CCRP
Institution:	BioScience Laboratories, Inc. (BSLI) 600 South Excelsior Avenue Butte, Montana 59701 Telephone: (406) 782-5498
Sponsor:	Becton, Dickinson and Company (BD) 75 North Fairway Drive Vernon Hills, Illinois 60061 Telephone: (847)362-9062
IND Number:	128827
Phase:	2
Date:	09/12/16

GIRB Approved
Date: 9/20/16
Initial:

This Protocol has been approved by the GIRB on_____

CONFIDENTIALITY STATEMENT

This Protocol has been approved by the GIRB on 09/20/16

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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 Food and Drug Administration (FDA) 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 $\log_{10}$ reduction from baseline bacterial counts on abdominal sites for samples taken 10 minutes post product treatment, ≥ 3 $\log_{10}$ reduction from baseline bacterial counts on inguinal sites for samples taken 10 minutes post product treatment, and >0 $\log_{10}$ reduction from baseline bacterial counts on abdominal and groin sites for samples taken 6 hours post product treatment

Octenidine dihydrochloride (OCT) is an antiseptic agent that has a broad spectrum of activity against bacteria, yeast, and fungi. Its activity is persistent for hours to days after application, rendering it a suitable antimicrobial product to develop for use as a skin antiseptic. [redacted]

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2.0 OBJECTIVE

The objective of this study is to compare the immediate and persistent antimicrobial properties of thermally treated polyester cloth impregnated with 0.4% w/v octenidine dihydrochloride in clear aqueous solution, thermally treated polyester cloth impregnated with vehicle formulation, and the FDA-approved Sage 2% Chlorhexidine Gluconate Cloth to the log reduction and response rate standards set forth in the Food and Drug Administration Tentative Final Monograph (TFM) for *Effectiveness Testing of a Patient Preoperative Skin Preparation*. Testing will be performed according to the procedures outlined in the Food and Drug Administration Tentative Final Monograph (TFM) for *Effectiveness Testing of a Patient Preoperative Skin Preparation* (FR 59:116, 17 June 94, pp. 31450-31452). The test methods for this evaluation will be based on ASTM Standard Test Method E1173-15,E*valuation of Preoperative, Precatheterization, or Preinjection Skin Preparations* and the test criteria from the 1994 FDA TFM.

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3.0 SPONSOR

BD
75 North Fairway Drive
Vernon Hills, Illinois 60061
Telephone: (847) 362-9062

4.0 INVESTIGATIVE ORGANIZATION AND PERSONNEL

BioScience Laboratories, Inc.
600 South Excelsior Avenue
Butte, Montana 59701

Contact: Alicia Bogert
[redacted]

Principal Investigator: Alicia Bogert

Subinvestigators: J. Jill Lawrence, Judi Schutte, and Christopher Beausoleil, CCRP

Study Statistician: [redacted]

Director of Quality Assurance: [redacted]

Consulting Physician/Medical Expert: [redacted]

Subject Recruitment and Consenting, IRB Coordination: [redacted] the Subject Recruitment Department or designee

Sponsor Contact(s): [redacted]

Sponsor Medical Monitor: [redacted]

Study Monitor: [redacted]

4.1 Name of the IRB

Gallatin Institutional Review Board (GIRB)
3006 Secor Avenue
Bozeman, Montana 59715
DHHS Number: IRB00005939

5.0 ROLES AND RESPONSIBILITIES

5.1 Principal Investigator

Alicia Bogert is responsible for conducting the study.

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5.2 Subinvestigators

J. Jill Lawrence, Judi Schutte, and Christopher Beausoleil are responsible for assisting the Investigator in conducting the study.

5.3 Investigational Site

BioScience Laboratories, Inc.
600 South Excelsior Avenue
Butte, Montana 59701
Telephone: (406) 782-5498

5.4 Subject Recruiter(s)

[redacted] the Subject Recruitment Department, and trained designees are responsible for recruiting all subjects for the study, including screening subjects per the inclusion/exclusion criteria, the consenting process, scheduling, and answering questions from subjects, all of whom will be voluntary participants not open to coercion or any undue influence by the recruiter.

5.5 Study Contact

[redacted] shall act as the person authorized to sign the protocol and protocol amendments on behalf of the Sponsor.

5.6 Medical Expert

[redacted], shall act as the medical expert and the qualified consulting physician responsible for all trial-site related medical decisions.

5.7 Sponsor Medical Monitor

[redacted] shall act as the Sponsor's medical monitor.

5.8 Study Monitor

[redacted] shall act as the Sponsor's study monitor.

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 Board 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

This Protocol has been approved by the GIRB on 09/20/16

requirements of the United States Food and Drug Administration (FDA) and ICH.

7.0 SCOPE

The objective of this study is to compare the immediate and persistent antimicrobial properties of thermally treated polyester cloth impregnated with 0.4% w/v octenidine dihydrochloride in clear aqueous solution, thermally treated polyester cloth impregnated with Vehicle formulation, and the FDA-approved Sage 2% Chlorhexidine Gluconate Cloth against the log reduction and response rate standards set forth in the Food and Drug Administration Tentative Final Monograph (TFM) for *Effectiveness Testing of a Patient Preoperative Skin Preparation*. Testing will be performed according to the procedures outlined in the Food and Drug Administration Tentative Final Monograph (TFM) for *Effectiveness Testing of a Patient Preoperative Skin Preparation* (FR 59:116, 17 June 94, pp. 31450-31452). The test methods for this evaluation will be based on ASTM E1173-15, , *Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations* and the test criteria from the 1994 FDA TFM.

A sufficient number of male and female, overtly healthy, volunteer subjects at least 18 years of age, will be enrolled in the screening phase to ensure that the total numbers of evaluable samples collected from groin and abdominal regions meet or exceed 48 evaluable test sites from Investigational Product and Active Control arms and 16 evaluable test sites from Vehicle Control arm (minimum of 112 each for groin and abdominal regions). Subjects must satisfy all Screening Day and Treatment Day Inclusion/Exclusion Criteria prior to screening sample collection and Treatment Day procedures. If the required numbers of subjects do not qualify from the initial screening group, additional volunteers will be recruited.

Following a 14- day restriction period, subjects with resident bacterial flora that meet the specified criteria will be eligible to proceed to the treatment phase of testing. Specific baseline criteria for subjects will be ≥ 3.11 $\log_{10}$ CFU/cm^2 from the skin of the abdomen and ≥ 5.5 $\log_{10}$ CFU/cm^2 from the skin of the inguen. Evaluations will consist of samples collected at baseline, 10 minutes ± 30 seconds post-treatment, and at 6 hours ± 30 minutes post-treatment. All plating for this study will be conducted in duplicate with the pour-plating technique and incubated at 30 ± 2 °C for 72 hours ± 4 hours. The left and right abdominal test sites will be 5" × 5" areas of skin. The abdominal sampling sites will not include skin that shows evidence of the subjects clothing waistband. The test sites on the left and right inguina will be 2" × 5" areas of skin that appear to be similar in condition. Subjects passing into the treatment phase of testing will be sampled for baseline microbial populations on randomly assigned sites of each abdominal and inguinal test area.

7.1 Primary Analysis

A modified intent to treat (mITT) data set will be used for efficacy analyses. Inclusion for the mITT data set is evaluated for each body area (left and right for the groin and abdomen). For each body area, if the treatment day baseline bacterial count requirements are in the range of 3.11 to 5.50 $\log_{10}/cm^2$, inclusive, on the abdomen and 5.50 to 7.50 $\log_{10}/cm^2$, inclusive, on the groin, then the data from that body area are included in the mITT data. Efficacy analyses will also be conducted on the Per Protocol data set as supportive analyses when Per Protocol data are different from mITT data.

- For responder rate, missing data will be imputed as non-responders in the mITT data, while in the Per Protocol data, missing data will not be imputed and will be excluded from the responder rate Per Protocol analyses.
- For log reduction, missing data will not be imputed for either mITT data or Per Protocol data and will be excluded from log reduction analyses.

Log_{10} reductions for the analysis of the efficacy will be calculated by subtracting the post-test material application $\log_{10}$ recovery from the Treatment Day baseline $\log_{10}$ recovery. Log_{10} reductions will be calculated for all study materials for both the groin and the abdomen at both the 10 minute and 6 hour sample times.

The primary measure of antimicrobial efficacy is the responder rate. Responder status is calculated separately for each treatment, body area (abdomen and groin), and each post-application sample time. At the 10-minute sample time a subject is defined as a responder for the groin if they show at least a 3 $\log_{10}$ reduction from baseline skin flora counts; a subject is defined as a responder for the abdomen if they show at least a 2 $\log_{10}$ reduction from baseline skin flora counts. At the 6-hour sample time a subject is defined as a responder if they have a microbial mean $\log_{10}$ reduction from baseline that is greater than 0. The primary efficacy target is to show a 70% responder rate for each body area and post-application sample time for the Investigational Product.

7.2 Secondary Analysis

Based on the mITT data / Per Protocol data, at the 10-minute sample time, the efficacy targets for the Investigational Product are mean $\log_{10}$ reductions from baseline skin flora counts ≥ 3 for the groin and ≥ 2 for the abdomen. At the 6-hour sample time, the efficacy targets are mean $\log_{10}$ reductions from baseline that are greater than 0 for both groin and abdomen.

Log_{10} reductions and responder rates will be compared between study materials. The Investigational Product and Active Control should demonstrate greater mean $\log_{10}$ reductions from baseline at both 10 minutes and 6 hours and greater response rates at 10 minutes relative to the Vehicle Control.

7.3 Exploratory Analysis

Based on the mITT data / Per Protocol data, the weight (grams) of drug product solution applied to a treatment area will be compared between study materials for each body area from the product weight measured pre and post application.

7.4 Sample Size Justification

A sufficient number of volunteers will be enrolled in the screening phase such that the total numbers of evaluable samples collected from groin and abdominal regions are not less than 48 evaluable test sites from Investigational Product and Active Control and 16 evaluable test sites from the Vehicle Control arm arms (minimum of 112 each for groin and abdominal regions). Subjects must satisfy all Screening Day and Treatment Day Inclusion/Exclusion Criteria prior to screening sample collection and Treatment Day procedures. If the required numbers of subjects do not qualify from the initial screening group, additional volunteers will be recruited.

This is a Phase 2 study to assess the immediate and persistent antimicrobial effect of the Investigational Product relative to the $\log_{10}$ reduction and response rate standards set forth in the Food and Drug Administration Tentative Final Monograph (TFM) for *Effectiveness Testing of a Patient Preoperative Skin Preparation* (FR 59:116, 17 June 94, pp. 31450-31452) and inform design of future Phase 3 pivotal efficacy studies. Therefore, no sample size calculation was performed to provide statistically-powered evidence of efficacy as part of this study. The sample size is deemed adequate for these purposes.

7.5 Safety Analysis

The full Intent to Treat data set (all randomized subjects) will be used for the safety analysis. The principal measures of safety will be skin irritation scores and the incidence of adverse events reported during the study.

Differences between the Investigational Product, Vehicle Control, and Active Control will be evaluated on skin irritation data.

7.6 Study Flow Chart

Procedure	Timeline (Days)			
	14 or more prior to Baseline	3 or more prior to Baseline	0 Baseline	Treatment Day 3 or more post Baseline
Informed Consent Obtained	X			
Product-Restriction Period	X	X	X	
Inclusion/Exclusion Criteria including Medical History Reviewed	X	X	X	X
Clipping Hair From Test sites		X		
Baseline Screening			X	
Test-Day Baseline Sample				X
Product Application				X
10-Minute Post-Product Application Sample				X
6-Hour Post-Product Application Sample				X
Adverse Events		X	X	X

Note: Visual evaluations of the skin on each test area will be performed at each laboratory visit prior to treatment, and prior to 10-minute and 6-hour post-treatment microbial sampling.

8.0 TEST MATERIALS

The test materials (Investigational Product, Vehicle Control and Active Control) to be used in this evaluation will be supplied by the study Sponsor. Treatment materials will be supplied in individually packaged pre-filled packs. The Investigational Product and Vehicle Control packs will be thermally treated to simulate a heat sterilization process to address any physical impact of heating on the cloth. Thermally treated cloths will not be tested for confirmation of sterility for use in this study.

The Investigational Product and Vehicle Control will be blinded. The Active Control cannot be blinded, due to package labeling of the marketed product. 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 requested by the Sponsor. Lot Numbers and

Expiration dates will be provided by the Sponsor with each product shipment. The unblinded lot numbers of the Investigational Product and Vehicle Control will be provided to BSLI by the Sponsor at the completion of the study when unblinding has occurred.

Investigational Product (IP):	Thermally treated polyester A&W5 Cloths Impregnated with 0.4% w/v Octenidine Dihydrochloride (OCT) Aqueous Solution
Active Ingredient:	0.4% w/v Octenidine Dihydrochloride (OCT) Aqueous Solution
Lot Number:	_____
Expiration Date:	_____
Vehicle Control (VC):	Thermally treated polyester A&W5 Cloths Impregnated with Vehicle Formulation
Active Ingredient:	Not applicable
Lot Number:	_____
Expiration Date:	_____
Active Control (AC):	Sage 2% Chlorhexidine Gluconate Cloth
Active Ingredient:	2% Chlorhexidine Gluconate (CHG)
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.

9.3 Media

Sterile Sampling Solution (SS):

[redacted]

Butterfield's Phosphate Buffered Water (PBW):

312 µM KH_2PO_4, pH 7.2 ± 0.1

Tryptic Soy Agar with product neutralizers (TSA+) – may be purchased or made by BSLI

10.0 NEUTRALIZATION

A neutralization study will be performed to ensure that the neutralizers used in the recovery medium quench the antimicrobial activity of the three test materials, and that the neutralizers are not toxic to the bacteria. *Staphylococcus epidermidis* (ATCC #12228) and *Staphylococcus epidermidis* MRSE (ATCC #51625) will be used as the challenge species in the neutralizer study. The neutralization study will follow guidelines based on ASTM E1054-08(2013), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*. Reference Appendix 3 of this Protocol for specific guidelines for the Neutralization Assay. The neutralization validation will be completed prior to initiation of the clinical investigation and results will be documented in the Final Report.

11.0 SUBJECT SELECTION

11.1 Number of Subjects

A sufficient number of male and female, overtly healthy volunteer subjects at least 18 years of age, will be enrolled in the screening phase to ensure that the total numbers of evaluable samples collected from groin and abdominal regions are not less than 48 evaluable test sites from Investigational Product and Active Control arms and 16 evaluable test sites from Vehicle Control arm (minimum of 112 each for groin and abdominal regions). A minimum of 56 evaluable subjects is required to achieve a total of at least 112 test sites for groin and abdominal regions. Subjects must satisfy all Screening Day and Treatment Day Inclusion/Exclusion Criteria prior to screening sample collection and Treatment Day procedures. If the required numbers of subjects do not qualify from the initial screening group, additional volunteers will be recruited.

11.2 Subject Recruitment

Following approval of the Study Protocol, Informed Consent Form, and other study specific documents by the GIRB and Sponsor, potential subjects will be recruited. Personal information will be collected for each potential subject, using the Subject Confidential Information and Acceptance Criteria Form (SCIAC form, Form No. 15-SR-004). Each consenting subject will fill out an Informed Consent Form (ICF) and the Authorization to Use and Disclose Protected Health Information Form (HIPAA form, Form No. 15-SR-008). A List of Restricted Products will be provided to each subject prior to beginning the study. The above forms are provided as separate Informed Consent documents. During the consenting process, emergency contact information of individuals who can be contacted, should any problem arise, will be collected for each

participant. Trained personnel or subject recruiters will explain the study to each subject, review the elements of Informed Consent as specified in 21 CFR 50.25, and determine subject eligibility through direct questioning, and will be available to answer any questions that may arise. It will be made clear to subjects that their participation in this study will accrue to them no personal benefits, other than financial compensation, as stated. The Informed Consent Form will be signed and dated by the subject and the person obtaining consent prior to the start of any study procedures. The subject will receive a copy of the signed Informed Consent Form. Subjects will be notified that additional information about this study can be found at www.clinicaltrials.gov.

The subject recruiters will verbally verify with subjects that the skin of the abdomen and inguen are free from clinically evident dermatoses, injuries, or any other disorders that may compromise the subject or the study.

Potential subjects will be informed of volunteer opportunities available at the investigative site by means of general, nonspecific newspaper and radio advertisements instructing potential subjects to either read GIRB-approved study descriptions online at www.biosciencelabs.com or in person at BioScience Laboratories, Inc., Subject Recruitment Office. Additionally, subjects may be recruited from existing subject database, referrals, through response to advertising and from community outreach and events. All study-specific advertising materials will be approved by the GIRB prior to their use for recruiting subjects.

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 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.
- Female subjects must have a negative urine pregnancy test documented before treatment with test materials.
- Screening day microbial baseline requirements for subjects of ≥ 3.11 $\log_{10}$ CFU/cm^2 bilaterally from the skin of the abdomen and ≥ 5.50 $\log_{10}$ CFU/cm^2 bilaterally from the skin of the groin.

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, fragrances, vinyl, latex (rubber), alcohols, metals, inks, or to common antibacterial agents found in soaps, lotions, or ointments, particularly chlorhexidine gluconate (CHG), or octenidine dihydrochloride (OCT).
- 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 tanning beds, or sunbathing during the 14-day product-restriction period or during the test period.
- Wear fabric softener-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, steroid medications (other than for hormonal contraception or post-menopausal reasons), 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.
- A medical diagnosis of a physical condition, such as a current or recent severe illness, mitral valve prolapse with a heart murmur, congenital heart disease, hepatitis B, hepatitis C, an organ transplant, or an immunocompromised condition such as AIDS (or HIV positive), lupus, diabetes, Crohn's disease, asthma or medicated multiple sclerosis.
- Any tattoos, or scars within 2 inches of the test sites; skin blemishes or warts, may be permissible with the specific approval of the Principal Investigator or Consulting Physician.
- Dermatoses, cuts, lesions, active skin rashes, scabs, breaks in the skin or other skin disorders within 6 inches on or around the test sites.
- A currently active skin disease or inflammatory skin condition (for example, contact dermatitis, psoriasis, eczema) anywhere on the body that, in the opinion of the Principal Investigator, would compromise subject safety or study integrity.
- Subjects who receive an irritation score of 1 (any redness, swelling, rash, or dryness present at any treatment area) for any individual skin condition prior to the Screening Day baseline or Treatment Day baseline sample collection.
- Participation in another clinical study in the past 30 days or current participation in another clinical study.
- Showering, bathing, or swimming within the 72 hour period prior to sampling for baseline screening, the test day, or throughout the test period.

- Pregnancy, plans to become pregnant or impregnate a sexual partner within the pre-test and test periods of the study, or nursing a child. All female subjects will be required to complete a urine pregnancy test on the day of test material application, prior to treatment. Both gender of subjects must be willing to use an acceptable method of contraception to prevent pregnancy for at least 14 days immediately preceding Treatment Day and throughout the duration of the study.
- 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.

12.0 SUBJECT WITHDRAWAL

After admission to the study, the subject may withdraw at any time for any reason. If possible, the reason for withdrawal will be recorded. Any subject not adhering to Protocol requirements will be disqualified. At the discretion of the Principal Investigator, subjects will be allowed to make a maximum of two attempts at achieving baseline bacterial counts to qualify for treatment day procedures. Subjects who have received product are not eligible to re-enter the study.

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 standard operating procedures of BSLI, 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 during the minimum 72-hour period prior their baseline screening procedures and completion of treatment day procedures. 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 screening baseline procedures), subjects will be examined physically to ensure that there is no evidence of injury, dermatosis, or dermatitis present at the sampling sites. Any hair present on test areas will be clipped to ensure the comfort of the subject during sampling procedures, to ensure that bandages used in testing will remain secure to the test sites, and remove any variables in the test system.

13.3 Randomization

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. This means there are three possible combinations of treatments (assuming A and B are either Investigational Product or Active Control; C is Vehicle Control):

- A and B
- A and C
- B and C

The treatment assignments will be balanced such that the number of readings per anatomical site matches the calculated requirements. The Investigational Product and Active Control will be applied to an equal number of anatomical sites. The Vehicle Control will be compared against each active substance an equal number of times. The Vehicle Control will be applied to the number of anatomical sites necessary to generate a baseline for comparison to the active substances.

A minimum of 56 evaluable subjects will be required to achieve a total of at least 112 test sites for groin and abdominal regions. Out of the minimum 56 evaluable subjects, 40 evaluable subjects will receive treatments from both A and B, 8 evaluable subjects will receive treatments from both A and C and 8 evaluable subjects will receive treatments from both B and C to achieve 48 evaluable test sites from Investigational Product and Active Control arms (A and B) and 16 evaluable test sites from Vehicle Control arm (C) for groin and abdominal regions.

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

Each groin and abdomen sample site is divided into four areas and three of the areas will be sampled – one at baseline, one at 10 minutes, and one at 6 hours. The remaining area will not be used. Therefore, for any groin or abdomen site there are 24 possible sampling orders.

The number of subjects required for a completely balanced block design for all factors at once is dependent on the ratio of the number of anatomical sites used in the active treatments compared to the number of anatomical sites used in the Vehicle Control treatment but is in any case a multiple of 48 (a factor of two for left/right balance times a factor of 24 for sample order balance). The exact subject numbers and Active/Vehicle Control ratio may make it infeasible to provide a completely balanced block design, so the following priority order will be used for design:

1. Treatment combinations will always be applied in balanced blocks, with the block size being sufficient to preserve the active to active ratio at 1:1 and to preserve the active to Vehicle Control treatment ratio as determined by the Investigator and statistical consultant.
2. Left/right balance will be preserved – each treatment will be applied an equal number of times to each side of the body.
3. Sampling orders will be assigned in blocks of 24 as much as possible. If the final number of subjects is not a multiple of 24 the remaining subjects will be assigned random non-duplicate sample orders from the 24 possible sample orders.
4. The blocking will be adjusted based on the final subject numbers to be as balanced as possible with respect to all three factors at once, with priority using the order listed above.

13.4 Clipping Procedures

At least 3 days (72 hours) prior to baseline screening procedures, the subjects will be examined physically to ensure that there is no evidence of injury, dermatosis, or dermatitis present on the test areas. Any hair present on test areas will be clipped to remove any test variables between subjects, to ensure that bandages used in testing will remain secure to the test sites and ensure the comfort of the subject during sampling procedures. Subjects will be encouraged to shower after clipping to decrease the incidence of folliculitis.

13.5 Baseline Screening Procedures

Baseline-screening samples will be taken on the day following the-14 day pre-test period. Subjects will not shower at least 72 hours prior to being sampled. Subjects will don a disposable undergarment prior to baseline screening procedures and will be examined physically to ensure that there is no evidence of injury, dermatosis, folliculitis, or dermatitis present at the sampling sites (Section 14.2). A subject will be dismissed from the study if skin irritation score of 1 is observed on the test sites. If the subject continues to meet the qualification criteria, they will be sampled using the Cylinder Sampling (Scrub Cup) Technique (Section 13.7) at the center of the sampling areas on the skin of the inguina and abdomen (Appendix 2). 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 into the treatment phase of the study. Subjects may qualify on one or two anatomical site(s) and be admitted into testing for those anatomical site(s) only, although 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.

Baseline criteria for qualification for the test period are ≥ 3.11 $\log_{10}$ CFU/cm^2 from the skin of the abdomen, and/or ≥ 5.50 $\log_{10}$ CFU/cm^2 from the skin of the groin.

There will be a minimum of 72 hours between baseline screening procedures and treatment day procedures. Based upon achieving adequate baseline screening microbial counts, subjects will be eligible to continue to treatment day procedures.

13.6 Treatment Day Procedures

Prior to sampling, the subjects will be questioned regarding adherence to the qualification criteria.

Subjects will don a disposable undergarment and be examined physically to ensure no evidence of injury, dermatosis, or dermatitis is present in the test areas. One sterile surgical marker will be used per subject. The marker will be used to demarcate a 5" × 5" areas of skin on the right and left sides of the abdomen, adjacent to the navel that appear to be similar in condition. The marker will again be used to demarcate 2" x 5" areas of skin in the inguinal areas that appear to be similar in condition. During treatment day procedures, subjects will wear disposable gloves to guard against contamination of the test sites.

Subjects will be sampled for treatment day baseline microbial populations on randomly assigned sites of the abdominal and inguinal areas on both sides (Appendix 2). The Cylinder Sampling (Cup Scrub) Technique (Section 13.7) will be used for baseline and post-treatment samples.

All product treatments for a subject will be completed on a single day (Treatment Day). The test materials will be applied to the test sites in accordance with the randomization schedule using repeated back-and-forth strokes for 3 minutes on both abdominal and inguinal test sites with a dry time of 1 minute. Detailed instructions on product application are presented in Appendix 1. All test materials will be weighed before and after application and the weights will be recorded. All subjects will have samples collected 10 minutes ± 30 seconds and 6 hours ± 30 minutes post product application on both abdomen and inguinal sites. All sampling times will be calculated from the completion of the dry time of each product following application.

Following collection of the last 6 hours ± 30 minutes post-product application sample of a test site, the remaining test material will be wiped/cleansed from the test site with a mild soap and/or tap water; test sites will be dried with paper towel.

13.7 Cylinder Sampling (Scrub Cup) Technique

Abdomen and Inguinal Samples

A sterile cylinder with inner diameter of 3.46 cm^2 will be held firmly onto the test site to be sampled. A 3 mL aliquot of sterile Sampling Solution (SS) will be instilled into the cylinder, and the skin inside the cylinder will be massaged in a sweeping manner for 1 minute with a sterile rubber policeman. The SS will be removed with a sterile pipette and transferred to a sterile test tube. These procedures will be repeated for a second time. The second recovered aliquot will then be pooled in the test tube with the first aliquot, and the samples plated in duplicate using the appropriate media.

13.8 Diluting, Plating, and Counting

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

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

13.9 Blinding

The Investigational Product and Vehicle Control will be blinded. The Active Control (SAGE) cannot be blinded, due to package labeling of the marketed product. In order to guard against any bias of the study outcome, technicians who participate in product application or sample collection from subjects during treatment day procedures will not participate in the processing of samples. The technicians processing samples and/or counting the resultant plates will be blinded to the study randomization during the data

gathering processes. In the event that data and calculations are handled by BSLI staff, the plate counts and calculations will be reviewed and/or approved by separate individuals prior to being entered into a data spreadsheet where product information (un-blinding) would occur.

The Sponsor Quality Assurance Manager will provide the code translation to BSLI Director of Quality Assurance in individual sealed envelopes (one for each treatment code), which will be secured by BSLI Director of Quality Assurance and will remain unopened in the study file. If an emergency requires unblinding, the envelope corresponding to the treatment code that is associated with the Adverse Event will be opened by BSLI Director of Quality Assurance, or designee to reveal the treatment identification for that single treatment. If possible, the Principal Investigator or designee will contact the Sponsor with notification of the intent to unblind the treatment codes prior to actual unblinding. If it is not possible to notify the Sponsor prior to the unblinding, the Principal Investigator or designee will contact the Sponsor immediately following the unblinding procedure and follow with a written notification to document the exact manner in which the code was unblinded and the justification for the unblinding. The Principal Investigator or designee shall also provide written notification of the unblinding to the IRB. BSLI Director of Quality Assurance will communicate the treatment identification of the code associated with the AE to only the study personnel who require the information to manage the emergency.

13.10 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, $F = 6$ mL for all samples

$\frac{\sum c_i}{n}$ = 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, $A = 3.46\ cm^2$ (2.1 cm i.d.)

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 are uncountable, the count from the remaining plate will be used. In order to avoid potential calculation problems due to taking the logarithm of zero, counts of zero will be considered 1 for further calculations.

Data collected from subjects with low test-day baseline counts on an anatomical test site ($< 3.11\ \log_{10}$ CFU/cm^2 from the skin of the abdomen, or $< 5.50\ \log_{10}$ CFU/cm^2 on the skin on the inguen) will not be used in the mITT Efficacy Analyses (Section 15.0).

14.0 ASSESSMENT OF SAFETY

14.1 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 documented per BSLI Standard Operating Procedure, reported as an Adverse Event, and followed to resolution.

14.2 Evaluation of Test Sites

Prior to product application and before any subsequent samples, test site erythema, edema, rash, and dryness will be rated on a 4-step scale, the Skin Irritation Scoring System (Draize). If a subject receives a score of 1 for erythema, edema, rash, and/or dryness prior to Screening Baseline Day or product application on Treatment Day, they will be dismissed from testing. If a subject receives a score of 3 for erythema, edema, rash, and/or dryness at any point in the study, they will be dismissed from testing and reported as an adverse event. (Note: Skin irritation scores of 1 or 2 are expected after sampling and product application procedures and are not grounds for dismissal and will not be considered adverse events.)

SKIN IRRITATION SCORING SYSTEM (Draize)

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
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 of 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.

14.3 Adverse Events

Adverse Events will be documented for all subjects from the time of the clipping appointment to the time of discharge from the study. Adverse Events will be categorized in relationship to the product that was applied to the specific skin site. In case of a medical emergency, 911 will be called from the laboratory facility and general first aid administered until Emergency Medical Service arrives. Medical facilities/personnel are in close proximity.

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.

14.3.1 Definitions

14.3.1.1 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 Form (Appendix 3) 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.

14.3.1.2 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.

14.3.1.3 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.

14.3.1.4 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.

14.3.2 Follow-up

If an adverse event/experience related to the study procedures or study product occurs, the Sponsor will be monetarily responsible for all costs associated with the follow-up for said event including, but not limited to, medical visits and medication prescribed by a medical professional directly related to the adverse event along with an administration fee that covers the Principal Investigator's time resolving the Adverse Event. If it is determined by Test Facility Management that the Adverse Event is due to negligence on the part of the Test Facility, no cost will be passed through to the Sponsor. The subject under the direction of the Principal Investigator (or designee) may be referred to the nearest acute care facility for treatment. All Adverse Events will be followed to resolution and documented on appropriate Adverse Event paperwork.

14.3.3 Notification

The Sponsor and the reviewing IRB will be notified of all adverse event/experiences that require treatment within 2 business days. Any Serious or Unexpected Adverse Event/Experience that occurs during the study must be reported immediately by the Principal Investigator (or designee) to the Sponsor and the reviewing IRB, followed by written notification within 1 business day of the information being reported to the investigative study team.

Sponsor Notification of Serious Adverse Event:

Primary Contact: [redacted]

Telephone:

Email:

Secondary Contact: [redacted]

Telephone:

Email:

The Principal Investigator, Alicia Bogert, and Medical Expert, [redacted], 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 of the event. At a minimum, the Principal Investigator and Medical Expert 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.

14.4 Anticipated Reactions

The risks associated with this test are primarily related to application of the test materials, and/or the test methodology. Mild abrasion may occur due to cylinder sampling. Folliculitis may be present from clipping. 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.

15.0 STATISTICAL METHODS

Data processing and statistical analysis for this study will be conducted by the BD statistical group based on the final monitored data provided by the Testing Facility to the BD statistical group.

Details of the statistical analysis will be provided in a separate statistical analysis plan.

Data sets analyzed

The full intent to treat (ITT) data set (all randomized subjects) will be used for the safety analysis.

A modified Intent to Treat (mITT) data set will be used for efficacy analyses. Inclusion for the mITT data set is evaluated for each body area (left and right for the groin and abdomen). For each body area, if the treatment day baseline bacterial count requirements are in the range of 3.11 to 5.50 $\log_{10}/cm^2$, inclusive, on the abdomen and 5.50 to 7.50 $\log_{10}/cm^2$, inclusive, on the groin, then the data from that body area are included in the mITT data set. Efficacy analyses will also be conducted on the Per Protocol data set as supportive analyses when Per Protocol data are different from mITT data.

- For responder rate, missing data will be treated as non-responders in the mITT data, while in the Per Protocol data, missing data will not be imputed and will be excluded from the responder rate Per Protocol analysis.
- For log reduction, missing data will not be imputed for either mITT data or Per Protocol data and will be excluded from log reduction analysis.

15.1 Primary Analysis

An analysis of variance (ANOVA) of the baseline log_{10} CFU/cm^2 values will be performed to determine whether the randomization produced treatment arms with similar baseline CFU/cm^2 values.

Log_{10} reductions for the analysis of the efficacy and comparison endpoints will be calculated by subtracting the post-test material application log_{10} recovery from the Treatment Day baseline log_{10} recovery. Log_{10} reductions will be calculated for all study materials for both the groin and the abdomen at the 10 minute and 6 hour sample times.

Responder rates will be calculated by converting each log_{10} CFU/cm^2 reduction to a binary (yes or no) success measure. Success at 10 minutes is defined as achieving a 2 log_{10} per cm^2 reduction of skin flora on the sebaceous-poor site (abdomen) and a 3 log_{10} per cm^2 reduction of skin flora on the sebaceous-rich site (groin). Success at 6 hours is defined as having skin flora counts that are less than the baseline skin flora counts. Responder rates and 95% confidence intervals will be summarized for each product tested, grouped by anatomical site and each post-application time point (10 minutes and 6 hours).

The primary purpose of the study is to compare antimicrobial activity of the Investigational Product to the responder rate standards according to the FDA TFM. Investigational Product, Vehicle Control and Active Control will be compared to the efficacy standard of a 70% response rate at 10 minutes and 6 hours. It is expected that the mean responder rates will meet that standard, but since this is a Phase 2 study to inform design of future Phase 3 pivotal efficacy studies and is not designed to provide statistically powered evidence of efficacy, the confidence intervals may not lie entirely above 70%.

15.2 Secondary Analysis

The following descriptive statistics for log_{10} CFU/cm^2 reductions will be computed for each product tested, grouped by anatomical site and each post application sampling time point (10 minutes and 6 hours): mean, median, standard deviation, minimum, maximum, and count.

The mean log_{10} reductions of the Investigational Product, Active Control, and Vehicle Control will be compared to the FDA TFM standards at 10 minutes and 6 hours. The efficacy targets for the Investigational Product are mean log_{10} reductions from baseline skin flora counts ≥ 3 for the groin and ≥ 2 for the abdomen. At the 6-hour sample time, the efficacy targets are mean log_{10} reductions from baseline that are greater than 0 for both groin and abdomen.

Antimicrobial activity will be compared using log_{10} reductions and responder rates between the Investigational Product, Active Control and Vehicle Control for each body area at both post treatment sampling times. Differences in log_{10} CFU/cm^2 reductions between treatments and confidence intervals for will be produced by a mixed model ANOVA. Two-proportion test will be used to test differences in responder rates between treatment groups. The goal is that both the Investigational Product and Active Control demonstrate greater mean log_{10} reductions from baseline at both 10 minutes and 6 hours and greater response rates at 10 minutes relative to the Vehicle Control.

15.3 Exploratory Analysis

Product Expression Volumes:

The weight (grams) of drug product solutions applied to a treatment area will be estimated as:

Product weight prior to treatment (g) – product weight post-treatment (g)

The following descriptive statistics for expression volumes will be computed for each product tested at each anatomical site: mean, median, standard deviation, minimum, maximum, and count. An analysis of variance (ANOVA) of the applied volumes will be performed to determine whether the treatment arms had similar volumes applied. If a significant difference is found, differences between groups will be examined by appropriate follow up tests. Analysis for product expression volumes will be based on the mITT data / Per Protocol data.

15.4 Safety Analyses

The ITT data set (all randomized subjects) will be considered evaluable for safety. Skin irritation scores 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 10-minute, and 6-hours sampling procedures], in any category for any site.

Adverse Events (including post treatment skin irritation scores of 3), will also 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 Investigational Product, Active Control and Vehicle Control 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 two active treatments and Vehicle Control a secondary analysis will be conducted to determine how the reactions differ.

16.0 SPECIAL NOTES

16.1 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.

16.2 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.

16.3 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 will be reported immediately to the local GIRB and Sponsor representative.

16.4 Quality Assurance Audits

The BioScience Laboratories, Inc. Quality Assurance Unit (QAU) will conduct in-phase audits of critical testing processes at least once during testing and will advise the Principal Investigator and Management of the outcomes of these audits. On completion of testing, the QAU will perform an audit of the data and of the Final Report in its entirety.

17.0 FINAL REPORT

The final report will be generated and will summarize the method, data, and conclusions relative to the test materials and the subjects. The statistical analysis used in the report will be provided by BD. Copies of the data will be incorporated into the report.

18.0 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 (if needed) with one exception: when necessary to eliminate an apparent immediate hazard to the subject.

19.0 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).

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

ASTM E1173-15, *Standard Test Methods for Evaluation of Preoperative, Precatheterization, or Preinjection Skin preparations.*

Paulson, D. S. (2015). *Topical Antimicrobial Testing and Evaluation, 2nd Edition.* New York: CRC Press (pp. 102 – 104).

20.0 DOCUMENTATION AND RECORD-KEEPING

20.1 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.

20.2 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

This Protocol has been approved by the GIRB on 09/20/16

from each participating subject will be filed in the Study File and a copy given to the subject.

20.3 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 20.4. 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.

20.4 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 (if applicable)
- Financial Disclosure for the Investigator and Subinvestigator (if applicable)
- Curriculum Vitae of Investigator and Subinvestigator (if applicable)
- 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 provided to IRB
- Research Site Signature Log
- FDA's Clinical Investigator Information Sheets (if applicable)

- CRA Monitoring Log (if applicable)
- Drug Invoices and Accountability Records
- Study specific training records for investigation site personnel, including Site Signature Delegation Logs
- Enrollment/Disposition log showing subjects screened, enrolled, disqualified, withdrawn, and completed
- Sample Submission Form, 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.

21.0 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) and to other third parties for the fitness of the product for use as defined in the Study Protocol.

This Protocol has been approved by the GIRB on 09/20/16

(1)

23.0 PROTOCOL ACCEPTANCE

ACCEPTED BY: BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
600 South Excelsior Avenue
Butte, Montana 59701

[redacted]

[redacted]

(1) Numbering incorrect. Acceptance is section 22.0.
(cc) AGB 09/14/16

This Protocol has been approved by the GIRB on ___

GIRB Approved
Date: 9/20/16
Initial: CF

APPENDIX 1

PRODUCT APPLICATION INSTRUCTIONS

For all Test Materials: Investigational Product, Vehicle Control, and Active Control

1) Weigh the unopened cloth package and record weight.
2) Open package and remove a single cloth from the package (for Sage cloth: according to the manufacturer's instructions).
3) Follow application instructions as noted below.
4) Following treatment application, weigh the used cloth test material with the package (including unused cloth) and record the weight.
5) Retain the label from the package and discard both the used and unused cloths.

Treatment Site Application Instructions

Abdominal Test Site (5in. x 5in.)

1) Using a single cloth for each anatomical region, vigorously scrub skin back-and-forth for three (3) minutes completely wetting the treatment area.

2) At the completion of the three (3) minute application, allow the area to air dry for one (1) minute prior to the initiation of the sampling times.

Inguinal Test Site (2in. x 5in.)

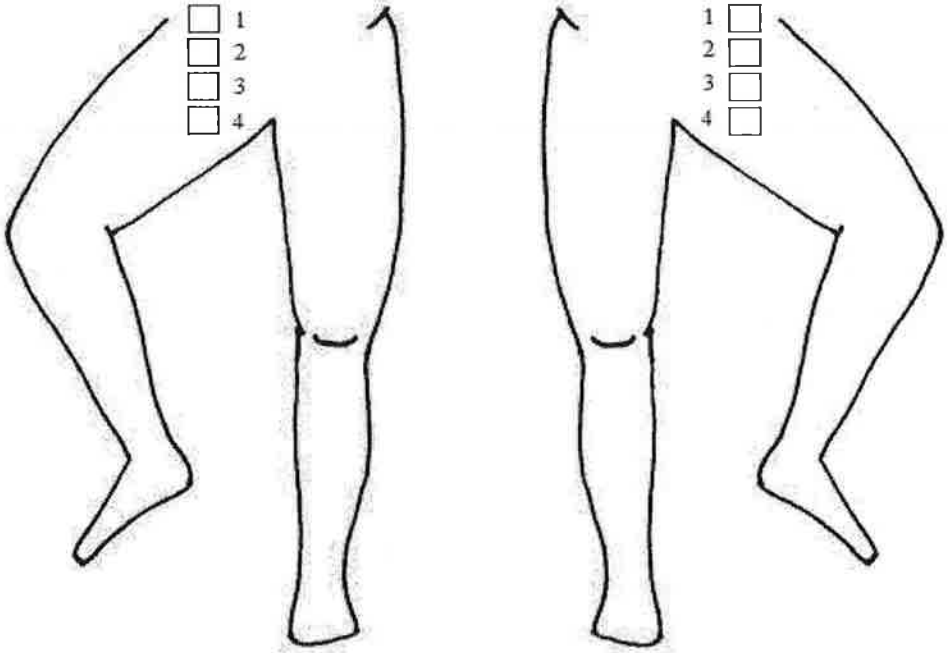
1) Using a single cloth for each anatomical region, vigorously scrub skin back-and-forth for three (3) minutes completely wetting the treatment area.

2) At the completion of the three (3) minute application, allow the area to air dry for one (1) minute prior to the initiation of the contact times.

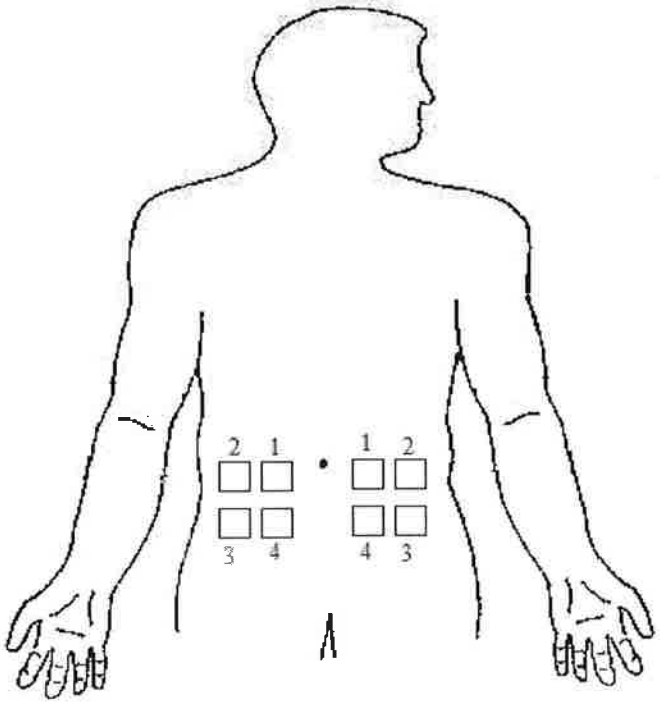
APPENDIX 2

ANATOMICAL DIAGRAM OF THE SAMPLING SITES

INGUINAL TEST SITES



ABDOMINAL TEST SITES



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APPENDIX 3

VALIDATION OF NEUTRALIZATION EFFECTIVENESS: CLINICAL TRIAL EVALUATION

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*. *Staphylococcus epidermidis* MRSE (ATCC #51625) and *Staphylococcus epidermidis* (ATCC #12228) will be used as the challenge species in both (*In-vitro and In-vivo*) 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 Investigational Product and Active Control. At least eight human subjects will be tested using product applications on the skin of the abdomen, to obtain a minimum of four samples per test material challenged with each challenge species.

3.0 TEST MATERIALS

The Investigational Product and Active Control to be used in this open label evaluation will be supplied by the Sponsor (BD). Responsibility for the identity, strength, purity, composition, and stability of the Sponsor-provided test materials used in testing rests with the Sponsor. 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 presented in the Final Report, if provided by the Sponsor.

Investigational Product:	Thermally treated polyester A&W5 Cloths Impregnated with 0.4% w/v Octenidine Dihydrochloride (OCT) Aqueous Solution
Active Ingredients:	0.4% w/ v Octenidine Dihydrochloride (OCT) Aqueous Solution
Lot Number:	_____
Expiration Date:	_____
Active Control:	Sage 2% Chlorhexidine Gluconate Cloth
Active Ingredients:	2% Chlorhexidine Gluconate (CHG)
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

Sterile Sampling Solution (SS)

Diluting Fluid

Butterfield's Phosphate Buffer Solution (PBW)

Media

Tryptic Soy Agar with product neutralizers (TSA+)

> Tryptic Soy Agar with 5 g Polysorbate 80 and 0.7 g Lecithin added to 1.0 L deionized water.

Tryptic Soy Agar (TSA)

Tryptic Soy Broth (TSB)

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 four samples for each of the two test materials and each challenge species (eight subjects).

5.2 Subject Recruitment

Subjects must meet the inclusion and exclusion criteria in Sections 11.3 and 11.4 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 both of the two test materials, one assigned to one side of the abdomen and the other assigned to the other side.

6.0 PROCEDURES

6.1 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.

6.2 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, 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 (abdomen), and hair on sampling sites will be clipped, if needed.

6.3 Randomization

The two test materials (Investigational Product and Active Control) will be assigned randomly to test sites per a computer-generated randomization schedule, such that each subject receives both of the test materials at the abdominal sites.

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

6.4 Test Inoculum Preparation

Two days prior to beginning the neutralization assay, *Staphylococcus epidermidis* MRSE (ATCC #51625) or *Staphylococcus epidermidis* (ATCC #12228) 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.

6.5 Inoculum Assay (Initial population) – Test C

Test Inoculum will be assayed by adding a 0.1 mL aliquot of the inoculum to 5.0 mL of PBS solution (IP), vortexing for at least 3 seconds, and immediately (within 1 minute) pour-plating, in duplicate, 1 mL aliquots of the IP with TSA. This assay will be performed three additional times for a total of four replicates.

The diluted test inoculum suspensions will be allowed to stand for at least 30 minutes, following which, 1 mL aliquots will be pour-plated, in duplicate, with TSA.

6.6 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 four replicates.

To each of four test tubes containing 5.0 mL of each Test Material, a 0.1 mL aliquot of the test inoculum will be added. The suspensions will be vortexed for at least 3 seconds and, immediately (within 1 minute), 1 mL 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 1 mL aliquots of each replicate will be pour-plated, in duplicate, with TSA.

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

This phase of the neutralization assay assures that the sterile Sampling Solution (SS), the sampling solution employed in the evaluation, are not inherently toxic to the microorganisms. This assay will be performed in four replicates.

To each of four test tubes containing 5.0 mL of the sampling solution, a 0.1 mL aliquot of the test inoculum will be added. The suspensions will be vortexed for at least 3 seconds and, immediately (within 1 minute), 1 mL 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 1 mL aliquots of each replicate will be pour-plated, in duplicate, with TSA+.

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

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

Four test tubes containing 5.0 mL of diluent broth to be used in the test will be prepared, and a 0.1 mL 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), 1 mL 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, 1 mL aliquots of each replicate will be pour-plated, in duplicate, with TSA+.

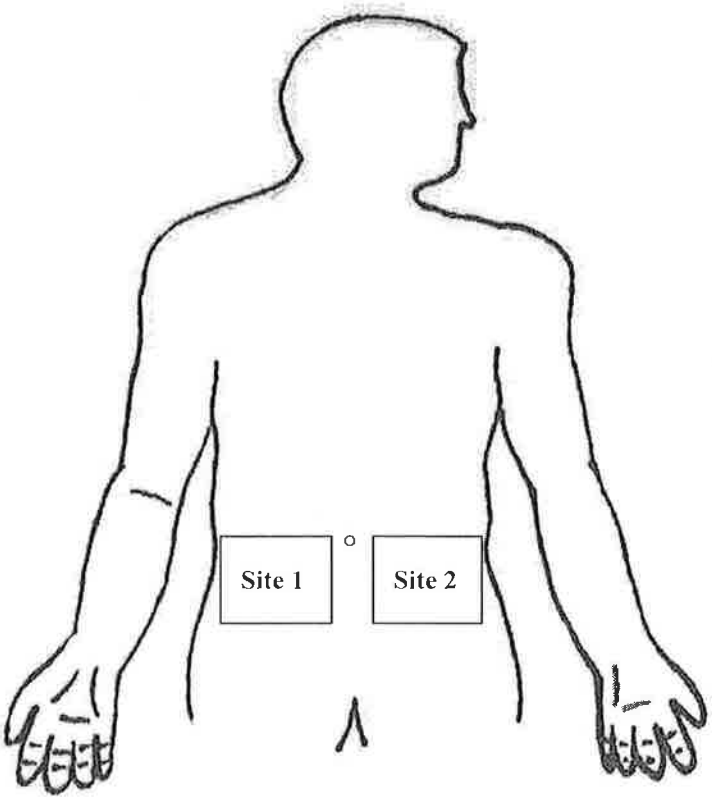
6.9 Neutralizer Efficacy Evaluation (*In Vivo*) – Test A

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 2" 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.

Neutralization Test Sites Diagram



A test material (reference Section 3.0 of the Validation of Neutralizer Effectiveness) will be applied to a 2" 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 10 minutes ± 30 seconds post product-application completion.

The Cylinder Sampling (Scrub Cup) Technique will be performed as described in Section 13.7 of the Study Protocol (using SS).

The volume of sample will be adjusted to 5.0 mL, inoculated with 0.1 mL of the test inoculum, and vortexed for at least 3 seconds. Immediately (within approximately 1 minute), 1 mL 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, 1 mL 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 8 subjects will be treated with each of the two test materials (samples from four subjects used for one microorganism, samples from the remaining four subjects used for the remaining microorganism).

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

6.10 Initial Population/Final Population

An Initial Population confirming the amount of microorganism present will be performed prior to testing, and plated in duplicate. A Final Population will be performed to confirm the amount of microorganism present at the completion of the assay.

6.11 Incubation

The inoculated plates will be incubated at 30 °C ± 2 °C for approximately 72 hours ± 4 hours.

7.0 CALCULATIONS AND DATA HANDLING

Count colonies on plates that have between 25 and 250 colonies per plate. If no plates provide counts in that range, counts from those plates closest to that range will be used for analysis.

The microbial population recovered from each replicate of Test A, Test B, Test C, and Test D will be calculated as follows:

$$\text{Log}_{10}\text{ Average Population} = \log_{10}\left(C_i \times 10^{-D}\right)$$

Where:

C_i = Average of the plates counted

D = Dilution Factor of the plates counted (for example, the dilution factor is 0 for plating 1.0 mL aliquots and is -1 when plating 0.1 mL aliquots.)

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 are uncountable, the count from the remaining plate will be used.

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

8.0 STATISTICS

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.

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 $\neq$ 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.

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

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.

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

Neutralization is considered adequate if all recovery populations are not statistically different from the Initial Population (with the exception of Test D). See table below for summary.

Neutralization Test/Phase	**Required Results for Acceptance**
Test Organism Viability/Initial Population – **Test C** (IP)	30 minute sample cannot be statistically different from 1 minute, or the evaluation fails.
Test Material Control/Product Efficacy – **Test D** (Phase I)	Must be statistically different than Test C at the within 1 minute and 30 minutes samples.
Neutralizer Toxicity Evaluation – **Test B** (Phase II)	Cannot be statistically different at the within 1 minute or 30 minute samples.
Diluent/Recovery Broth Toxicity Evaluation – **Test B** (Phase III)	
Neutralization Effectiveness Evaluation – **Test A** (Phase IV)	

All values will be compared against the IP. The 30 minute evaluations of any test are used as a time representative to the longest possible wait time of a sample prior to being plated. A comparison will be performed against the FP to ensure the challenge suspension populations were consistent through testing.

The results and all source documents of the neutralization evaluation will be presented as an addenda in the Final Report.

9.0 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 CT-1006. *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.

This Protocol has been approved by the GIRB on 09/20/16