Document Cover Page

Official Title of Study:

Pilot Evaluation of Two Test Materials With a Positive Control When Used as a Patient Preoperative Skin Preparation

NCT Number: NCT03861780

Date of Document: March 5, 2019

BIOSCIENCE LABORATORIES, INC. PROTOCOL 1810480-103.01

PILOT EVALUATION OF TWO TEST MATERIALS WITH A POSITIVE CONTROL WHEN USED AS A PATIENT PREOPERATIVE SKIN PREPARATION

1.0 TITLE PAGE

Investigational Products:	26 mL Project X Chlorhexidine Gluconate 3.15% (w/v) solution and Isopropyl Alcohol (70% v/v) 5.1 mL Project X Chlorhexidine Gluconate 3.15% (w/v) solution and Isopropyl Alcohol (70% v/v)
Sponsor:	Professional Disposables International, Inc. (PDI) 400 Chestnut Ridge Road Woodcliff Lake, New Jersey 07677
Study Number:	1810480-103
Sponsor Representative:	James Clayton
Principal Investigator:	Collette Duley
Subinvestigators:	Chelsey Allison, CCRC, Christopher Beausoleil, CCRP, Brett Griggs, Eric Olson
Clinical Site:	BioScience Laboratories, Inc. (BSLI) 1755 South 19th Avenue Bozeman, Montana 59718 Telephone: (406) 587-5735
Date:	March 5, 2019

This Protocol has been approved by the GIRB on March 6-19
RL

Confidentiality Statement

This document contains the confidential information of Professional Disposables International, Inc. (PDI) and BioScience Laboratories, Inc. (BSLI). 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 PDI and BSLI. 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.

This Protocol has been approved by the GIRB on 03/06/19

2.0 PROTOCOL SYNOPSIS

Name of Sponsor: Professional Disposables International, Inc. (PDI)	
Name and Concentration of Test Product Active Ingredients: 26 mL Chlorhexidine Gluconate 3.15% (w/v) and Isopropyl Alcohol 70% (v/v) solution 5.1 mL Chlorhexidine Gluconate 3.15% (w/v) and Isopropyl Alcohol 70% (v/v) solution	**Protocol Number** **1810480-103.01**
Title of Study:	**Pilot Evaluation of Two Test Materials with a Positive Control When Used as a Patient Preoperative Skin Preparation**
Principal Investigator:	Collette Duley
Sub-Investigators:	Chelsey Allison, CCRC, Christopher Beausoleil, CCRP, Brett Griggs, Eric Olson
Study Center:	BioScience Laboratories, Inc.
Publications (References)	ASTM Standard Test Method E1173-15, *Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations* ASTM E1054-08(2013), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents.* 2017 FDA, 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use,* Final Rule. (FR82: No. 243, December 20, 2017. pp 60474 to 60503)
Study Duration:	A pre-test period of 14 days and a test period of 1 day.
Objectives:	The purpose of this pilot study is to evaluate the antimicrobial efficacy of two test products containing a chlorhexidine gluconate and isopropyl alcohol solution (26 mL and 5.1 mL), when used as a patient preoperative skin preparation compared to a positive control (Prevantics® Maxi Swabstick 5.1 mL). The evaluation will determine the correct application procedures and the sample size to be used for the pivotal study. The $\log_{10}$ recoveries of the normal flora will be used to determine the antimicrobial effectiveness of the two test materials using a multiple linear regression model, consistent with the requirements of the 2017 FDA 21 CFR

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Name of Sponsor: Professional Disposables International, Inc. (PDI)	**Protocol Number 1810480-103.01**
Name and Concentration of Test Product Active Ingredients: 26 mL Chlorhexidine Gluconate 3.15% (w/v) and Isopropyl Alcohol 70% (v/v) solution 5.1 mL Chlorhexidine Gluconate 3.15% (w/v) and Isopropyl Alcohol 70% (v/v) solution	
	Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use*, Final Rule (December 20, 2017); the difference between the two regression lines is the Average Treatment Effect (ATE).
Methodology:	The testing methods are based on the standardized test method ASTM E1173-15, *Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations*. Following a 14-day restriction period, subjects will be sampled for baseline, 30-seconds, 10-minutes, and 6-hours microbial reduction evaluations post-product application. Subjects will be sequestered for the 6-hour evaluation. Test day baseline criteria will be set at: abdomen: 3.0 - 5.5 $\log_{10}$ CFU/cm^2, and inguen: 5.0 - 7.5 $\log_{10}$ CFU/cm^2.
Number of Subjects:	A sufficient number of subjects will be entered into testing to complete 50 subjects per each of the 2 test and 1 control materials. A minimum of 75 subjects meeting the test day baseline criteria will test bi-laterally (150 abdomen and inguinal sites; 50 abdomen and inguinal sites per test and control materials).
Main Criteria for Inclusion:	A sufficient number of overtly healthy subjects at least 18 years of age will be admitted into the study so that a minimum of 75 subjects complete the study. Insofar as possible, the groups of subjects selected will be of mixed sex, age, and race. The subject recruiters will verbally and/or visually 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.
Duration of treatment:	For each subject, two of the three test materials will be applied bi-laterally to abdomen and inguinal sites, a single time.

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Name of Sponsor: Professional Disposables International, Inc. (PDI)	
Name and Concentration of Test Product Active Ingredients: 26 mL Chlorhexidine Gluconate 3.15% (w/v) and Isopropyl Alcohol 70% (v/v) solution 5.1 mL Chlorhexidine Gluconate 3.15% (w/v) and Isopropyl Alcohol 70% (v/v) solution	**Protocol Number** **1810480-103.01**

Criteria for Evaluation:	**Efficacy:** The primary efficacy variables for this study are the immediate antimicrobial effects at 30-seconds and 10-minutes post-application and persistent antimicrobial effects at 6-hours post-application. The effectiveness criteria are that the $\log_{10}$ recoveries of the two test products (26 mL and 5.1 mL) are non-inferior to the Positive Control (Prevantics® Maxi Swabstick 5.1 mL) with a 0.5 margin ($\log_{10}$ scale) per square centimeter on the abdominal and inguinal sites within 30 seconds after drying and within 10 minutes after drying. The determination of non-inferiority will be based on a $\log_{10}$ multiple linear regression model. The Average Treatment Effect (ATE) is the mean difference of the test and the positive control products used in performing a non-inferiority statistic. To demonstrate persistence for the test materials, the 6-hour post-treatment measurement should be lower than or equal to the baseline measurement for 100 percent of the subjects on the abdominal and inguinal sites. The efficacy criteria will be consistent with the requirements of the 2017 FDA 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use*, Final Rule (December 20, 2017).
	Safety: Evaluation for safety of use of the test materials will consist of Adverse Event-reporting and assessment for skin reactions during testing.

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Name of Sponsor: Professional Disposables International, Inc. (PDI)	
Name and Concentration of Test Product Active Ingredients: 26 mL Chlorhexidine Gluconate 3.15% (w/v) and Isopropyl Alcohol 70% (v/v) solution 5.1 mL Chlorhexidine Gluconate 3.15% (w/v) and Isopropyl Alcohol 70% (v/v) solution	**Protocol Number** **1810480-103.01**
Statistical Methods:	A statistical analysis of the data will be performed to determine the non-inferiority of the two test products to the Positive Control. Separate analyses will be performed for data sets for the abdomen and the inguinal test sites and for the 30-seconds and 10-minutes post-application sample times. The statistic to be used is a multiple linear regression with one quantitative and one qualitative variable. The average treatment effect (ATE) is the mean difference of the two products with the 95% confidence intervals in place that will determine if the test products will pass the non-inferiority evaluation. To pass, the upper bounds of the 95% confidence interval must be equal to or less than 0.5 $\log_{10}$. The number of subjects necessary to pass this requirement will be determined. Descriptive statistics will be generated, including sample sizes, means, standard deviations, and ranges.

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4.0 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse Event
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
ATE	Average Treatment Effect
BBP++	Butterfield's Phosphate Buffer Solution with product neutralizers
BSLI	BioScience Laboratories, Inc.
CFR	Code of Federal Regulations
CFU	Colony Forming Units
CHG	Chlorhexidine gluconate
cm	Centimeter
CRF	Case Report/Record Form
CRO	Contract Research Organization
FDA	Food and Drug Administration
FR	Federal Register
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GIRB	Gallatin Institutional Review Board
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonization
in	Inch
mL	Milliliter
PBS	Phosphate Buffered Saline
SAE	Serious Adverse Event
SD	Standard Deviation
SSF++	Stripping Suspending Fluid with product neutralizers
SOP	Standard Operating Procedure
TSA	Tryptic Soy Agar
TSA+	Tryptic Soy Agar with product neutralizers
TSB	Tryptic Soy Broth

TFM	Tentative Final Monograph
UV	Ultraviolet
Cylinder Sampling Procedure	A procedure used to sample bacteria from the skin using a sterile cylinder, sterile rubber policemen and a specified volume of sampling fluid.
Mean $\log_{10}$ reductions	Average of the differences between the baseline microbial populations expressed as $\log_{10}$ CFU/cm^2 and the populations in $\log_{10}$ CFU/cm^2 recovered from the post-application samples.
Positive Control	A product that is to be tested according to procedures in this protocol in order to validate the testing procedures and to be used as a control.
Source Documents	Recorded results of original observations and activities of a clinical investigation.
Subjects	Healthy human paid participant that has consented to test in the study.
Test Material	The test products or positive control that are to be tested according to procedures in this protocol.

5.0 ETHICS

5.1 Institutional Review Board

Informed Consent Forms and any other supportive material relevant to the safety of the subjects will be supplied to the Gallatin Institutional Review Board (GIRB) for their review and approval. The primary purpose of the GIRB is the protection of the rights and welfare of the subjects involved (21 CFR Parts 50, 56, 312, and 314). This study will begin only after GIRB approval has been obtained.

5.2 Ethical Conduct of Study

The study will be conducted in compliance with Good Clinical Practice standards (21 CFR Parts 50, 56, 312, and 314, and ICH E6), the United States Food and Drug Administration regulations, Standard Operating Procedures of BioScience Laboratories, Inc., the study protocol, and any protocol amendments.

5.3 Subject Information and Consent

The Informed Consent Form and List of Restricted Products will be provided to each subject prior to beginning the study. Subjects must also have a current Authorization to Use and Disclose Protected Health Information Form on file at the testing facility. Trained personnel will explain the study to each subject and will be available to answer

any questions that may arise.

6.0 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

BioScience Laboratories, Inc.
1755 South 19th Avenue.
Bozeman, Montana 59718

Contact: Collette Duley
Phone: (406) 587-5735 Ext. 110
Fax: (406) 586-7930
Email: cduley@biosciencelabs.com

Principal Investigator: Collette Duley

Subinvestigators: Chelsey Allison, CCRC, Christopher Beausoleil, CCRP, Brett Griggs, Eric Olson

Quality Assurance Monitor: Amy L. Juhnke, RQAP-GLP

Statistician: Daryl S. Paulson, Ph.D.

Consulting Medical Experts: David McLaughlin, M.D., Gabor Benda, M.D.

Sponsor Study Monitor: Clinical Research Works

Gallatin Institutional Review Board (GIRB)
168 Timberview Circle
Bozeman, Montana 59718
Phone: (406) 581-8559
DHHS Number: IRB00005939

6.1 Monitoring

Professional Disposables International, Inc. (PDI), as Sponsor of this study, is responsible for ensuring the proper conduct of the study with regard to Protocol adherence and validity of the data recorded on the study documents. PDI has therefore assigned a study monitor to this study. The progress of the study may be monitored by:

- Periodic on-site review
- Telephone communications
- E-mail communications
- Review of sample data sheets and source documents

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

Note: The Federal Privacy rule (HIPAA) specifically permits the use and disclosure of protected health information "to a person subject to the jurisdiction of the Food and Drug Administration (FDA) [e.g. study sponsor] with respect to an FDA-

related product or activity for which that person has responsibility, for the purpose of activities related to the quality, safety, or effectiveness of such FDA-regulated product or activity" [45 CFR 164.512(b)(1)(iii)].

7.0 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 2017 FDA 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use*, Final Rule requires that the immediate and 10-minute $\log_{10}$ recoveries of the test product is non-inferior to an positive control by a margin of 0.5 $\log_{10}$, superior to that of a negative control by a margin of 1.2 $\log_{10}$, and that 100% of subjects' microbial populations recovered in the 6-hour samples are less than baseline populations. The testing methods for performing such evaluations are also described in the standardized test method ASTM E1173-15, *Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations.*

8.0 STUDY OBJECTIVES

The purpose of this pilot study is to evaluate the antimicrobial efficacy of two test products containing a chlorhexidine gluconate and isopropyl alcohol solution (26 mL and 5.1 mL), when used as a patient preoperative skin preparation. The evaluation will determine the correct application procedures and the number of subjects needed to pass the pivotal study. The $\log_{10}$ recoveries of the normal flora will be used to determine the antimicrobial effectiveness of the two test products consistent with the requirements of the 2017 FDA 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use*, Final Rule (December 20, 2017).

9.0 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This pilot study is designed to determine the antimicrobial effectiveness of two test products (Test Products #1 and #2), intended for use as Patient Preoperative Skin Preparations. The testing methods are based on the standardized test method ASTM E1173-15, *Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations,* and using the requirements specified by the 2017 FDA 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use*, Final Rule (December 20, 2017). The immediate and persistent antimicrobial effects will be measured to evaluate the efficacy.

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At least 75 subjects will be treated bilaterally with two of the three test materials (Test Product #1, Test Product #2, and Positive Control), one per each side of the abdomen and inguinal test sites. Subjects will be required to complete a 14-day pre-test conditioning period. Subjects will complete a 1-day test period, during which time subjects' sites will be treated with the test materials and samples taken following treatment. The Cylinder Sampling Technique will be performed for baseline and for sampling 30-seconds, 10-minutes, and 6-hours post-test material-application on Test Day.

The effectiveness criteria are that the $\log_{10}$ recoveries of the two test products (26 mL and 5.1 mL) are non-inferior to the Positive Control (Prevantics® Maxi Swabstick 5.1 mL) with a 0.5 margin ($\log_{10}$ scale) per square centimeter on the abdominal and inguinal sites within 30 seconds after drying and within 10 minutes after drying. The determination of non-inferiority will be based on the upper bounds of the 95% confidence interval being equal to or less than 0.5. Superiority will not be evaluated in this study. To show persistence for the test materials, the 6 hours post-treatment measurement should be lower than or equal to the baseline measurement for microbial reductions for 100 percent of the subjects on the abdominal and inguinal sites. This evaluation will determine the correct application procedures and the power for the pivotal study.

9.2 Discussion of Study Design, Including Choice of Sample Size

The sample size of a minimum of 100 subjects treated per test material is required for performing this type of evaluation based on the 2017 FDA 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use*, Final Rule (December 20, 2017). For this pilot study, only a minimum of 50 subjects treated per test material will be evaluated to determine if a study utilizing 100 subjects will meet the requirements in a pivotal study.

9.3 Selection of Study Population

A sufficient number of overtly healthy subjects at least 18 years of age will be admitted into the study so that 50 abdomen and 50 inguinal site evaluations are completed per each test material at each post-treatment sample time from sites passing Treatment Day baseline criteria (3.0 -5.5 $\log_{10}$ CFU/cm^2 from the skin of the abdomen and 5.0 -7.5 $\log_{10}$ CFU/cm^2 from the skin of the inguen). Insofar as possible, the group of subjects selected will be of mixed gender, age, and race. All subjects will be free from clinically evident dermatoses, injuries, wounds, and/or any other disorders that may compromise the subject and the study. All subjects will sign the Informed Consent Form and List of Restricted Products prior to participating in the study. The above forms are provided as separate Informed Consent documents. Subjects will receive a copy of these documents once consented to the study. Subjects must also have a current Authorization to Use and Disclose Protected Health Information Form on file at the testing facility. The Informed Consent Form will include a list of the active ingredients and post study product-removal

materials to which subjects may be exposed. The Informed Consent Form provides the subject with contact information for the Principal Investigator, and Subinvestigator(s), as appropriate.

9.3.1 Inclusion Criteria

- 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 have read and signed an Informed Consent Form and List of Restricted Products prior to participating in the study; located in the separate Informed Consent documents, as well as have a signed Authorization to Use and Disclose Protected Health Information Form on file at the test facility.
- Subjects must be in good general health and have no medical diagnosis of a physical condition, such as a current or recent severe illness, medicated or uncontrolled diabetes, hepatitis B, hepatitis C, an organ transplant, mitral valve prolapse with heart murmur, fibromyalgia, ulcerative colitis, Crohn's disease, an immunocompromised condition such as AIDS (or HIV positive), lupus, or medicated multiple sclerosis.
- Subjects will have test sites on the skin of the abdomen and/or inguinal free of injury and in good condition (no active skin rashes, excessive freckling, moles, scratches, breaks in the skin, etc.) and have no currently active skin diseases or skin conditions (for example, contact dermatitis, psoriasis or eczema) that may compromise subject safety or study integrity.
- Subjects must 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, scars, active skin rashes, or breaks in the skin of test sites, skin blemishes, such as dry scabs or warts, may be admitted at the discretion of the Principal Investigator, Subinvestigators, or Consulting Physicians.

9.3.2 Exclusion Criteria

Potential subjects will be excluded from this study if any of the following criteria apply to them:

- Known allergies to vinyl, latex (rubber), alcohols, metals, tapes or adhesives, inks, sunscreens, deodorants, laundry detergents, topically applied fragrances, cleansers, or to common antibacterial agents found in soaps or lotions, particularly chlorhexidine gluconate or ethanol.
- Have experienced hives (raised welts) as a reaction to anything that contacted the skin with the exception of items that cause hives as a reaction to the general population (e.g. poison oak and poison ivy).
- Use of systemic or topical antibiotic medications during the 14-day pre-test period

through completion of testing on the single test day.

- Use of systemic or topical steroids, other than for contraception, hormone therapy, post-menopausal indications, during the 14-day pre-test period through completion of testing on the single test day. This includes steroid medications used to treat asthma. Note: topically applied hormonal steroids used for post-menopausal reasons must not get on the test sites.
- Any type of port (or portacath) or Peripherally Inserted Central Catheter (PICC).
- Pregnancy, plans to become pregnant or impregnate a sexual partner within the pre-test and test period of the study, or nursing a child. Female subjects must have a negative urine pregnancy test documented before treatment with test materials.
- Current participation or participation in a clinical study in the past 14 days.
- Any medical condition or use of any medications that, in the opinion of the Principal Investigator or Consulting Physicians, would preclude participation.

9.3.3 Subject Responsibilities

During the 14-day pre-test period or through completion of testing on the single test day, subjects:

- Must not expose test sites to strong detergents, solvents, other irritants, antimicrobial agents, medicated soaps, medicated shampoos, medicated lotions, hot waxes or depilatories, including shaving (in the applicable test areas).
- Must not wear fabric softener-treated or antimicrobial-treated clothing (including bug-repellent and UV-treated clothing).
- Must not use biocide-treated pools or hot tubs, tanning beds, or sunbathe.
- Must not shower, bath, or swim within the 72-hour period prior to sampling (sponge baths may be taken, for example to wash the feet, however, the test areas must be avoided).
- May clip hair from the test sites prior to the day product restriction begins if they have hair density on the abdomen or inguen as pictured in the bottom two images in Appendix 3.
- Must be willing to fulfill the performance requirements of the study.

9.3.4 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 and withdrawn from the study.

9.4 Test Methods

9.4.1 Equipment, Supplies, Test Solutions and Media

The equipment and supplies used during this study will be detailed on tracking forms.

The Test Solutions and Media used for this study are listed below:

Sampling Solution

Stripping Suspending Fluid with product neutralizers (SSF++). Neutralizers in this solution are Lecithin, Polysorbate 80, Sodium thiosulfate, and Tamol™ SN.

Diluting Fluid

Butterfield's Phosphate Buffer Solution with product neutralizers (BBP++). Neutralizers in this solution are Lecithin, Polysorbate 80, Sodium thiosulfate, and Tamol™ SN.

Media

Tryptic Soy Agar with product neutralizers (TSA+). Neutralizers in this agar are Lecithin and Polysorbate 80

Tryptic Soy Agar (TSA)

Tryptic Soy Broth (TSB)

Phosphate Buffered Saline (PBS)

9.4.2 Identity of Test Materials

The test materials will be provided to the Testing Facility by the Study Sponsor, complete with appropriate documentation. 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 and a log of use. Responsibility for determination of the identity, strength, purity, composition, stability, and solubility of the test materials, as well as responsibility for retention of the test materials rests with the Sponsor. Unused, sealed test materials will be stored by BSLI until the Sponsor specifies their disposition. In the absence of a disposition request from the Sponsor within 1 year of their planned usage, the test materials will be returned to the Sponsor. No test materials will be destroyed unless so requested by the Sponsor.

Test Product #1: 26 mL Project X Chlorhexidine Gluconate 3.15% (w/v) solution and Isopropyl Alcohol (70% v/v)

Active Ingredients: Chlorhexidine Gluconate 3.15% (w/v) solution and Isopropyl Alcohol (70% v/v)

Lot Number:	FA81
Expiration Date:	7/24/2019
Manufacture Date:	1/24/2019

Test Product #2:	5.1 mL Project X Chlorhexidine Gluconate 3.15% (w/v) solution and Isopropyl Alcohol (70% v/v)
Active Ingredients:	Chlorhexidine Gluconate 3.15% (w/v) solution and Isopropyl Alcohol (70% v/v)
Lot Number:	FA80
Expiration Date:	7/21/2019
Manufacture Date:	1/21/2019

Positive Control:	Prevantics® Maxi Swabstick 5.1mL
Active Ingredients:	Chlorhexidine Gluconate 3.15% (w/v) solution and Isopropyl Alcohol (70% v/v)
Lot Number:	11801602
Expiration Date:	11/2020
Manufacture Date:	11/2018

9.4.3 Method of Assigning Subjects to Treatment Groups

The three test materials will be assigned randomly to the test sites per a computer-generated randomization schedule, such that two of the three test materials are used on each subject at the abdomen and/or inguinal sites, one test material on one side, and another test material on the opposite side of the subject. The randomization schedule will be continued until it is determined that the required sample sizes are fulfilled.

The specific sites for post-application sampling (i.e., 30 seconds, 10 minutes, and 6 hours after skin preparation on the abdominal and inguinal test sites) will be assigned according to a computer-generated randomization scheme.

9.4.4 Pre-Test Conditioning Period

The 14 days prior to the test portion of the study will constitute the pre-test period. During this time, subjects will avoid the use of medicated soaps, lotions, deodorants and shampoos, as well as skin contact with solvents, detergents, acids and bases, or any other products known to affect the normal microbial populations of the skin. Subjects will be supplied a personal hygiene kit containing non-medicated soap, shampoo, antiperspirant, lotion, and rubber gloves to be worn when contact with antimicrobials, solvents, detergents, acids, or bases cannot be avoided. Subjects will be instructed to use the contents of this kit exclusively during their participation in the study. Subjects must also avoid using UV tanning beds or sunbathing and swimming or bathing in biocide-treated pools or hot tubs.

At least 3 days (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 on the test areas. Hair present on test areas may be clipped if needed, to ensure that bandages used in testing will remain secure to the test sites.

9.4.5 Test Period

Prior to sampling, the subjects will be questioned regarding adherence to the protocol. Females must have a negative urine pregnancy test documented before treatment with test materials. Subjects will don a disposable undergarment prior to sampling and will be examined physically to ensure no evidence of injury, dermatosis, or dermatitis is present at the sampling sites. Subjects will also don disposable exam gloves prior to product application to guard against contamination of the test sites during testing.

On the abdomen (dry-skin sites), a sterile surgical marker will be used to demarcate bilaterally two 5" × 5" areas of skin (Appendix 1) that appear to be similar in condition, and not including the waistband area of clothing the subject was wearing, to the right and left side of the navel. In the inguinal region (moist-skin sites), a sterile surgical marker will be used to demarcate bilaterally two 1.5" × 5" areas of skin (Appendix 1) that appear to be similar in condition.

Subjects will be sampled for baseline microbial population at the abdominal and inguinal sites on both sides using the Cylinder Sampling Technique (Section 9.4.7).

The randomly assigned test materials will be applied to the abdominal and inguinal test sites per directions presented in Appendix 2. All test materials, including packaging, will be weighed before and immediately following application (Appendix 2) and the weights will be recorded.

The abdominal and inguinal test sites will be sampled 30 seconds + 5 seconds (30 seconds to 35 seconds) and 10 minutes ± 30 seconds post-product application using the Cylinder Sampling Technique (Section 9.4.7). At the completion of the 10-minute sample, the assigned 6-hour sample site will be covered with a dressing consisting of sterile gauze and semi-occlusive bandages to protect the site until the 6-hour sample window. All sampling times will be calculated from the completion of the dry time of each test material following application. Samples will be taken from the assigned sites at 6 hours ± 30 minutes post-product application using the Cylinder Sampling Technique (Section 9.4.7). If the semi-occlusive bandage 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.

Each subject who volunteers to participate in this study will be required to remain/be sequestered at the test facility through the 6-hours post-treatment sampling. Following collection of the last 6-hour ± 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 towels.

9.4.6 Blinding

Technicians who participate in test material application or bacterial sample collections, will not be blinded to the test material identity due to the physical differences of the test materials.

Technicians who participate in plating samples and/or counting plates from samples will not participate in the test product application or sample collection procedures.

9.4.7 Cylinder Sampling Technique

A sterile cylinder with an inside area of 3.46 cm^2 will be held firmly onto the test site to be sampled. 3.0-mL of sterile Stripping Suspending Fluid with product neutralizers (SSF++) will be placed into the cylinder, and the skin area inside the cylinder will be massaged in a circumferential manner for 1 minute with a sterile rubber policeman. The SSF++ will be removed with a sterile pipette and transferred to a sterile test tube. A second 3.0-mL aliquot of SSF++ will be placed into the cylinder, and the skin area again massaged for 1 minute with a sterile rubber policeman. Both aliquots will be pooled together for enumeration of microorganisms.

9.4.8 Plating

Aliquots of the microorganism suspension (10^0 dilution) will be serially diluted in Butterfield's Phosphate Buffer Solution with product neutralizers (BBP++), as appropriate. Triplicate pour-plates will be prepared from each of these dilutions in Tryptic Soy Agar with product neutralizers (TSA+) and incubated at 30 °C ± 2 °C for approximately 72 hours, or until sufficient growth is observed.

9.5 Efficacy and Safety Variables

Subject safety will be monitored by irritation evaluations and Adverse Event reporting (Section 11.0).

9.5.1 Efficacy and Safety Measurements and Flow Chart

Mean log_{10} reductions in normal flora will be used to determine the antimicrobial effectiveness of the test materials.

Flow Chart

Procedure	Day		
	-14 or more	-14 to 0	0
Informed Consent Signed	X		
Inclusion/Exclusion Criteria Reviewed	X		X
Pre-Test Conditioning Period		X	
Evaluation of Skin Condition			X
Test Material Application Locations and Sampling Sites Assigned			X
Baseline Sample			X
Test Material Application			X
30-second Post-Application Sample			X
10-minute Post-Application Sample			X
6-hour Post-Application Sample			X

9.5.2 Appropriateness of Measurements

The testing methods are based on the standardized test method ASTM E1173-15, *Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations.* The effectiveness criteria are that the $\log_{10}$ recoveries of the two test products (26 mL and 5.1 mL) are non-inferior to the Positive Control (Prevantics® Maxi Swabstick 5.1 mL) by no more than the upper margin of the 95% confidence interval of a 0.5 margin ($\log_{10}$ scale) per square centimeter on the abdominal and inguinal sites within 30 seconds after drying and within 10 minutes after drying. To show persistence of effect for the test materials, the 6 hours post-treatment measurement should be lower than or equal to the baseline measurement for 100 percent of the subjects on the abdominal and inguinal sites. The efficacy criteria will be consistent with the requirements of the 2017 FDA 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use*, Final Rule (December 20, 2017).

9.5.2.1 Neutralization

A neutralization study will be performed to assure that the neutralizers used in the recovery medium quench the antimicrobial activity of each test material and are not toxic to the challenge species. 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. Escherichia coli* (ATCC 11229), *Staphylococcus aureus* (ATCC 6538) and methicillin-

resistant *Staphylococcus aureus* MRSA (ATCC 33591) will be used as the challenge species in both components of the neutralizer validation study. Reference Appendix 4 for the procedure for validation of neutralization effectiveness.

9.5.3 Primary Efficacy Variables

The primary efficacy variables for this study are the immediate antimicrobial effects at 30-seconds and 10-minutes post-application and persistent antimicrobial effects at 6-hours post-application. The effectiveness criteria are that the $\log_{10}$ recoveries of the two test products (26 mL and 5.1 mL) are non-inferior to the Positive Control (Prevantics® Maxi Swabstick 5.1 mL) with a 0.5 margin ($\log_{10}$ scale) per square centimeter on the abdominal and inguinal sites within 30 seconds after drying and within 10 minutes after drying. The determination of non-inferiority will be based on the regression model used with a 95% confidence interval around the Average Treatment Effect (ATE). The upper bound of the 95% confidence interval cannot exceed 0.5. To show persistence of effect for the test materials, the 6-hours post-treatment measurement should be lower than or equal to the baseline measurement for 100 percent of the subjects on the abdominal and inguinal sites. The efficacy criteria will be consistent with the requirements of the 2017 FDA 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use*, Final Rule (December 20, 2017).

9.5.4 Data Collection and Microbial Recoveries

Colonies will be manually counted and data recorded on appropriate data collection forms. If one of the plates is uncountable, the count on the remaining plate will be used for calculations and noted in the source record.

The estimated $\log_{10}$ number of viable microorganisms 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

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$\frac{\sum c_i}{n}$ = average of the triplicate 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, $A = 3.46\ cm^2$

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.

Average colony counts ($\frac{\sum c_i}{n}$) of 0 will be set to 0.5 prior to calculating R-values. Any calculated CFU/cm^2 values ($\frac{F\left(\frac{\sum c_i}{n}\right)10^{-D}}{A}$) of less than 1 will be set to 1 CFU/cm^2, such that the $\log_{10}$ transformation will be zero for the calculated R-Value.

Data collected from subjects with low or high test-day baseline counts on an anatomical test site (< 3.0 $\log_{10}$ CFU/cm^2/ > 5.5 $\log_{10}$ CFU/cm^2 from the skin of the abdomen, or < 5.0 $\log_{10}$ CFU/cm^2/> 7.5 $\log_{10}$ CFU/cm^2 on the skin on the inguen) will not be used in the Efficacy Analyses (Section 9.7.1). This is a common occurrence in clinical-simulation studies. Hence, more subjects will be enrolled and tested to account for test-day baseline failures.

9.6 Data Quality Assurance

The Sponsor's designated Quality Assurance Representative may conduct audits at the study site. Audits will include, but are not limited to test materials, presence of required documents, the informed consent process, and review of source documents. The Investigator agrees to participate with audits conducted at a reasonable time in a reasonable manner.

The study will be inspected by Quality Assurance at BioScience Laboratories, Inc., and reports will be submitted to the Principal Investigator and Management in accordance with BioScience Laboratories, Inc., Standard Operating Procedures.

9.7 Statistical Methods and Determination of Sample Size

The statistical analysis will be performed by BSLI statistician, Daryl S. Paulson, PhD, to determine the correct sample size to use for the abdominal and inguinal sites with a Beta error of 0.20 (Power of 1 – 0.20 = 0.80), an alpha error of 0.05, and a detectable difference of 0.5. The PASS Version 16 statistical package will be used to determine the number of subjects needed in the pivotal study in order to pass the evaluation.

9.7.1 Statistical Methods

The Minitab® Version 18 statistical computer package will be used for all statistical calculations.

Non-Inferiority Analysis

An analysis of the data will be performed to determine the non-inferiority of the two test products to the Positive Control. Separate analyses will be performed for data sets for both the abdomen and the inguinal test sites and for the 30-seconds and 10-minutes post-application sample time. Only those data from sites meeting the baseline criteria (3.0 - 5.5 $\log_{10}$ CFU/cm^2 from the skin of the abdomen and 5.0 - 7.5 $\log_{10}$ CFU/cm^2 from the skin of the inguen) will be used in the analysis.

The statistic to be used is a multiple linear regression with one quantitative and one qualitative predictor. It has the form:

$$\hat{y} = B_0 + B_1 x_1 + B_2 x_2 + e$$

where:

$\hat{y}$ = Log_{10} Recoveries

B_0 = y intercept

B_1 = Regression slope

B_2 = Difference between the two products

x_1 = Log_{10} Baseline

x_2 = Products
1, if Test Product
2, if Positive Control

e = Error term

The average treatment effect (ATE) is the mean of the 95% confidence interval. For the non-inferiority test, the upper bound of the 95% confidence interval must be equal to or less than 0.5 $\log_{10}$.

For the non-inferiority test:

$$\bar{x}_C - \bar{x}_T \leq 0.5$$

where:

$\bar{x}_T$ = mean of the test product

$\bar{x}_C$ = mean of the positive control product

0.5 = non-inferiority margin

Descriptive statistics will be generated, including sample sizes, means, standard deviations, and ranges of the recovery data.

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Sample Size Determination Analysis

When the study is complete, the PASS 16 statistical package will determine the number of subjects used in a pivotal study for the abdominal and inguinal regions.

The formula that will be used is:

$$n \geq \frac{2\, s^2 \left(z\alpha_{/2} + z_\beta\right)^2}{0.5^2}$$

where:

$n \geq$ minimum number of subjects employed in the study

2 = number of products tested; 3 test materials (Test Product #1, Test Product #2 and Positive Control)

s^2 = the largest variance of the two products tested at the abdominal or inguinal sites

$z_{\alpha/2}$ = 1.96 (z-scale) for 1 – 0.05 = 95% confidence of Type I error

z_β = 0.842 (z-scale) for 1 – 0.20 = 0.80% confidence of Type II error

0.5 = Detection level requirement

9.7.2 Determination of Sample Size

The sample size of a minimum of 100 subjects treated per test material is required for performing this type of evaluation based on the 2017 FDA 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use*, Final Rule (December 20, 2017). For this pilot study, only a minimum of 50 subjects treated per test material will be evaluated to determine if a study utilizing 100 subjects will be likely to meet the requirements in a pivotal study.

9.8 Changes in the Conduct of the Study or Planned Analyses

Neither the Investigators nor the Sponsor will modify or alter this protocol without first obtaining agreement from the other parties with one exception: when necessary to eliminate an apparent immediate hazard to the subject. 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.

10.0 STUDY SUBJECTS

10.1 Disposition of Subjects

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 Parts 50 and 56.

The Investigator may discontinue individual subjects from the study at any time. Subjects may voluntarily withdraw from the study at any time. The Investigator or designee will provide a written report on the appropriate study document describing the reason for discontinuance and the date of discontinuance. The discontinued or withdrawn subject who participated in treatment may not re-enter the study. The Investigator will provide a disposition table that specifies the number of subject's enrolled, randomized, treated, and discontinued as well as reasons for discontinuations.

In order to implement a valid revocation of authorization to use and disclose private health information, the subject or their representative **must** make the request in writing to BioScience Laboratories, Inc., 1755 South 19th Avenue, Bozeman, Montana 59718. The revocation cannot stop the use or disclosure of information that has been collected prior to the revocation, is needed to ensure complete and accurate study results, or is required by law or government regulation (e.g. reporting adverse events, etc.). Revocation of an authorization may not be used to withhold normal medical care from the subject, but will make the subject ineligible to receive the study treatment or care.

10.2 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 Principal 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 BSLI. 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 or the Investigator has the right to discontinue the study at any time for medical and/or administrative reasons. This should occur after mutual consultation, where possible.

11.0 SAFETY EVALUATION

11.1 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.

11.2 Evaluation of Test Sites on Completion

Skin reactions will be evaluated prior to and post-product application (baseline, 30-seconds, 10-minutes, and 6-hours) using the following Skin Irritation Scoring System (Draize).

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

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

11.3 Adverse Events

Adverse events will be captured for all subjects from the time of informed consent to the time of subject discharge from the study. Adverse events will be categorized in relationship to the test materials that were applied. 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.

11.3.1 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 the test materials. All adverse event/experiences will be recorded and reported using appropriate Adverse Event documentation according to the Standard Operating Procedures of the laboratory.

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 to the following definitions:

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

11.3.2 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 the test materials. Related to other etiologies such as concomitant medications or conditions or subject's known clinical state.
Possible	Uncertain association. Other etiologies are also possible.
Probable	Clear-cut association with improvement upon withdrawal of the test materials. Not reasonably explained by the subject's known clinical state but not an anticipated event.
Definite	An adverse event with a clear-cut temporal association and laboratory confirmation if possible.

11.3.3 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.

11.3.4 Unexpected Adverse Event/Experience

An Unexpected Adverse Event/Experience is any adverse event/experience not listed in the current labeling for the test materials, the current investigator's brochure, or the Anticipated Reactions Section 11.3.7 of this Protocol. Where test material labeling or investigator's brochure is not available, anticipated experiences will be based on the known pharmacological/toxicological properties of the test materials or ingredients.

11.3.5 Follow-up

If an adverse event/experience 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. Serious or Unexpected Event/Experiences will be followed to resolution. Any adverse event will be documented on an Adverse Event Report Form.

11.3.6 Notification

The Sponsor and the reviewing IRB will be notified of all adverse events/experiences that are related to product usage or require medical treatment within 2 business days. Any Serious or Unexpected (in nature or severity) Adverse Event/Experience that occurs during the study must be reported immediately by the Principal Investigator to the Sponsor and the reviewing IRB, followed by written notification within one business day of the information being reported to the investigative study team. Adverse events/experiences not linked to test product exposure or not requiring medical treatment will be reported to the Sponsor and reviewing IRB approximately weekly or monthly, as determined by the Principal Investigator (or more frequently at the discretion of the Principal Investigator).

The Principal Investigator is 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 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.

11.3.7 Anticipated Reactions

Minimal risks to the study subjects are anticipated during the study. The risks associated with this study are primarily related to application of the test materials. Reactions to adhesives are also possible. Mild skin irritation or mild local irritant reactions such as burning and itchiness are anticipated. Although not anticipated, more severe reactions might occur, including moderate to severe erythema, swelling, cracking, peeling, or, in rare cases, blistering and/or an allergic reaction. Other anticipated reactions that may occur may result from testing procedures. Irritation and/or folliculitis may occur after clipping. A mild or moderate abrasion may occur from the Cylinder Sampling Technique.

A Chlorhexidine gluconate (CHG) solution will be utilized during testing. CHG can cause a rare, but serious allergic reaction that may be life-threatening. Subjects will be instructed to seek immediate emergency medical help if they experience any of the

following symptoms: hives, severe skin rash, wheezing, difficulty breathing, cold sweats, feeling light-headed, or swelling of the face/lips/tongue/throat.

12.0 EXCEPTIONAL CONDITIONS

The Sponsor will be notified within 24 hours by telephone, email, and/or letter of any exceptions encountered in this study that may affect subject safety or the results of the study. The exceptional conditions or occurrences will be detailed in full and formally recorded. Exceptional conditions that occur and are not addressed in this Protocol will be subject to Out-of-Scope charges (see Proposal/Contract).

13.0 REFERENCES

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.*

Code of Federal Regulations Title 21 Parts 50, 54, 56, 58, 310, and 312.

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).

ICH E6 Good Clinical Practice Guidelines.

14.0 FINAL REPORT

A Final Report will be prepared in ICH format describing the methodology and results of the study in a clear and concise manner.

15.0 DOCUMENTATION AND RECORD KEEPING

All documentation and records will be compiled and retained by BioScience Laboratories, Inc., at its facility in Bozeman, Montana. All raw data for this study, as well as the Final Report, will be retained in safe storage by the Testing Facility for a period of at least 2 years following the date a marketing application is approved for the test products for the indication for which they are being investigated. BioScience Laboratories, Inc. will notify the Study Sponsor before any documents or records are destroyed.

15.1 Study Center File Management

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

- Investigational Brochure (if applicable) or other appropriate test material safety information
- GIRB-Approved Signed Protocol
- Revised Protocol (if applicable)
- GIRB-Approved Informed Consent Form (blank)
- Financial Disclosure for the Principal Investigator and Subinvestigators (if applicable)
- Curriculum Vitae of Principal Investigator and Subinvestigators
- DHHS Number for GIRB, or other documentation of IRB compliance with FDA regulation (Included in this Protocol)
- Documentation of GIRB approval of protocol, consent form, any protocol amendments and any consent form revisions
- All correspondence between the Principal Investigator, IRB, and Sponsor relating to study conduct
- Copies of information related to SAE and the information on Immediately Reported Adverse Events
- Copy of the Approval Letter from the GIRB
- Copy of Notification of Completion of Clinical Testing (Completion Letter to the GIRB)
- Research Site Signature Log/Delegation of Duties

To protect privacy and maintain the confidentiality of data, each subject will be assigned a unique study number, with all study samples and research records identified using the subject's study number. Research records will be kept in a room with access limited to study personnel, and electronic databases will be maintained on password-protected computers.

16.0 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 test material for use as defined in the Study Protocol.

This Protocol has been approved by the GIRB on 03/06/19

17.0 ACCEPTANCE

PILOT EVALUATION OF TWO TEST MATERIALS WITH A POSITIVE CONTROL WHEN USED AS A PATIENT PREOPERATIVE SKIN PREPARATION

ACCEPTED BY:

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718

President & CEO: Daryl S. Paulson, Ph.D. — 03-05-19 Date

Principal Investigator: Collette Duley — 03/06/19 Date of Study Initiation

REVIEWED BY:

Director of Quality Assurance: for Amy L. Juhnke, RQAP-GLP — 03/05/19 Date

ACCEPTED BY:

PROFESSIONAL DISPOSABLES INTERNATIONAL, INC. (PDI) (SPONSOR)
400 Chestnut Ridge Road
Woodcliff Lake, New Jersey
07677

Representative — 3/6/19 Date

R+D Director Laboratory Sciences
Title

This Protocol has been approved by the GIRB on March 6-19 BL

17.0 ACCEPTANCE

PILOT EVALUATION OF TWO TEST MATERIALS WITH A POSITIVE CONTROL WHEN USED AS A PATIENT PREOPERATIVE SKIN PREPARATION

ACCEPTED BY:

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718

President & CEO: Daryl S Paulson _____ 03-05-19
Daryl S. Paulson, Ph.D. — Date

Principal Investigator: _____ _____
Collette Duley — Date of Study Initiation

REVIEWED BY:

Director of Quality Assurance: Renee Dahl for Amy L Juhnke _____ 03/05/19
Amy L. Juhnke, RQAP-GLP — Date

ACCEPTED BY:

PROFESSIONAL DISPOSABLES INTERNATIONAL, INC. (PDI) (SPONSOR)
400 Chestnut Ridge Road
Woodcliff Lake, New Jersey
07677

_____ _____
Representative — Date

_____
Title

This Protocol has been approved by the GIRB on March 6-19
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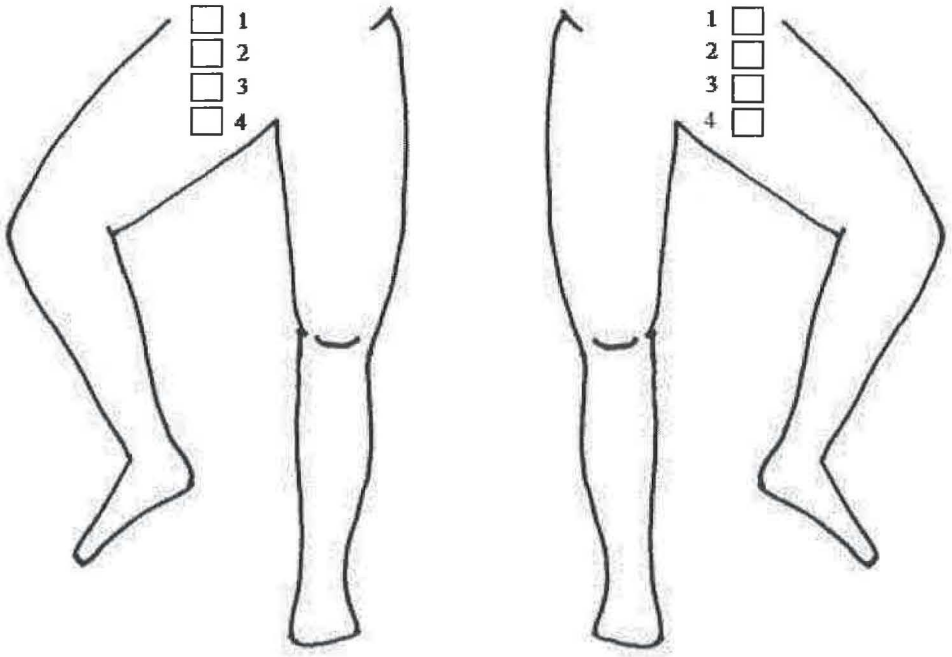
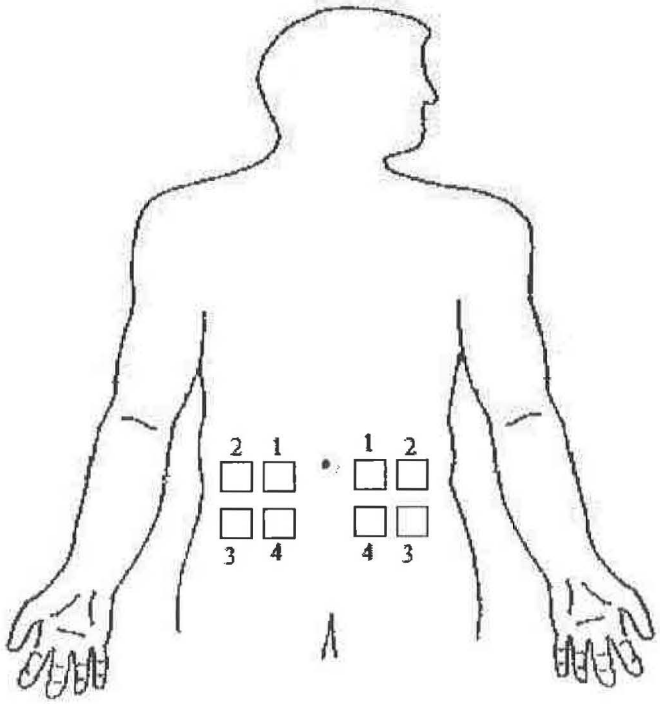
APPENDIX 1

ANATOMICAL DIAGRAM OF SAMPLING SITES

INGUINAL TEST SITES



ABDOMINAL TEST SITES



This Protocol has been approved by the GIRB on 03/06/19

APPENDIX 2

PRODUCT APPLICATION INSTRUCTIONS

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

1) Test Product #1

- Peel open package and remove applicator – do not touch swab.
- Hold the applicator with swab down.
- Press down the antiseptic reservoir to release the antiseptic to the swab.
- Wet the swab by repeated blotting against the treatment area until antiseptic is visible on the skin.
- Thoroughly wet the treatment area with antiseptic using back-and-forth, left-and-right strokes repeated for 30 seconds.
- Do not apply swab beyond borders of marked skin areas by more than one swab width
- Allow antiseptic to completely dry (minimum of 3 minutes).
- Do not blot or wipe dry.

2) Test Product #2

- Peel open package and remove applicator – do not touch swab.
- Hold the applicator with swab down.
- Press down the antiseptic reservoir to release the antiseptic to the swab.
- Wet the swab by repeated blotting against the treatment area until antiseptic is visible on the skin.
- Thoroughly wet the treatment area with antiseptic using back-and-forth, left-and-right strokes repeated for 30 seconds.
- Do not apply swab beyond borders of marked skin areas by more than one swab width.
- Allow antiseptic to completely dry (minimum of 30 seconds).
- Do not blot or wipe dry.

3) Positive Control

- Peel open package and remove swabstick – do not touch swab.
- Place one flat side of the foam tip within the pre-measured treatment area, and prep with back-and-forth repeated strokes for 15 seconds.

- Turn the Maxi-swabstick over and repeat the procedure for 15 seconds.
- Do not apply swab beyond borders of marked skin areas by more than one swab width.
- Allow the area to completely air-dry; approximately 30 seconds.

Inguinal Test Site (1.5in. x 5in.)

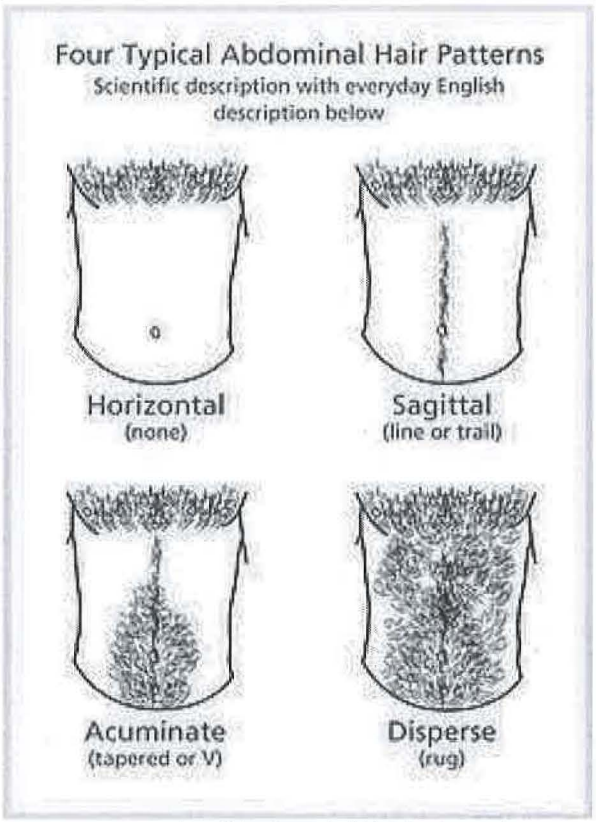
1) Test Product #1
   - Peel open package and remove applicator – do not touch swab.
   - Hold the applicator with swab down.
   - Press down the antiseptic reservoir to release the antiseptic to the swab.
   - Wet the swab by repeated blotting against the treatment area until antiseptic is visible on the skin.
   - Thoroughly wet the treatment area with antiseptic using back-and-forth, left-and-right strokes repeated for 2 minutes.
   - Do not apply swab beyond borders of marked skin areas by more than one swab width.
   - Allow antiseptic to completely dry (minimum of 3 minutes).
   - Do not blot or wipe dry.
2) Test Product #2
   - Peel open package and remove applicator – do not touch swab.
   - Hold the applicator with swab down.
   - Press down the antiseptic reservoir to release the antiseptic to the swab.
   - Wet the swab by repeated blotting against the treatment area until antiseptic is visible on the skin.
   - Thoroughly wet the treatment area with antiseptic using back-and-forth, left-and-right strokes repeated for 2 minutes.
   - Do not apply swab beyond borders of marked skin areas by more than one swab width.
   - Allow antiseptic to completely dry (minimum of 90 seconds).
   - Do not blot or wipe dry.
3) Positive Control
   - Peel open package and remove swabstick – do not touch swab.
   - Place one flat side of the foam tip within the pre-measured treatment area, and

prep with back-and-forth repeated strokes for 60 seconds.

- Turn the Maxi-swabstick over and repeat the procedure for 60 seconds
- Do not apply swab beyond borders of marked skin areas by more than one swab width.
- Allow the area to completely air-dry; approximately 90 seconds.
- Do not blot or wipe dry.

APPENDIX 3

HAIR DENSITY IMAGE



https://en.wikipedia.org/wiki/Abdominal_hair

This Protocol has been approved by the GIRB on 03/06/19

APPENDIX 4

VALIDATION OF NEUTRALIZER EFFECTIVENESS

1.0 PURPOSE OF THE 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. *Escherichia coli* (ATCC 11229), *Staphylococcus aureus* (ATCC 6538) and methicillin-resistant *Staphylococcus aureus* MRSA (ATCC 33591) 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 evaluate the three test materials. At least 18 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 *Escherichia coli* (ATCC 11229), *Staphylococcus aureus* (ATCC 6538) and methicillin-resistant *Staphylococcus aureus* MRSA (ATCC 33591).

3.0 TEST MATERIALS

Test Materials are as described in the Study Protocol, Section 9.4.2.

4.0 EQUIPMENT AND SUPPLIES

The equipment and supplies used during this neutralization will be detailed on tracking form(s).

5.0 TEST SOLUTIONS AND MEDIA

Sampling Solution

Stripping Suspending Fluid with product neutralizers (SSF++). Neutralizers in this solution are Lecithin, Polysorbate 80, Sodium thiosulfate, and Tamol™ SN.

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Diluting Fluid

Butterfield's Phosphate Buffer Solution with product neutralizers (BBP++). Neutralizers in this solution are Lecithin, Polysorbate 80, Sodium thiosulfate, and Tamol™ SN.

Media

Tryptic Soy Agar with product neutralizers (TSA+). Neutralizers in this agar are Lecithin and Polysorbate 80

Tryptic Soy Agar (TSA)

Tryptic Soy Broth (TSB)

Phosphate Buffered Saline (PBS)

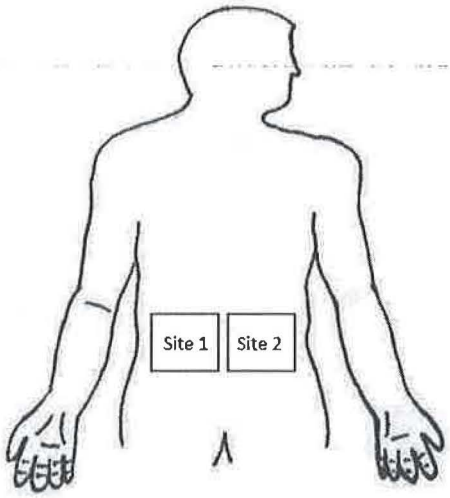
6.0 SUBJECT SELECTION

6.1 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 three test materials (18 subjects).

6.2 Subjects must meet the inclusion and exclusion criteria in Sections 9.3.1 and 9.3.2 of the Protocol to which this neutralizer validation is attached, except for the 72-hour exclusion from showering/bathing criteria, and the length of the washout period. Neutralization subjects only need to avoid topical and systemic antimicrobials for 7 days (not 14 days) prior to Test Day. One additional criterion for subjects is that they must not have hair on the test sites of the abdomen; this to minimize normal flora in the samples. Subjects will be asked to provide information on demographics and inclusion/exclusion criteria and sign the Informed Consent 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.

6.3 Each subject will receive two of the three test materials, 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 Figure 1 below.

Figure 1. Neutralization Test Sites Diagram



7.0 PROCEDURES

Compliance with Good Clinical Practices and Regulatory Requirement

7.1 The study will be conducted in compliance with the Good Clinical Practice regulations in Section 5.0 of the Protocol to which this neutralizer validation is attached.

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

Product Restriction Period

7.3 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.

7.4 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.

Randomization

7.5 The test materials 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.

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Test Inoculum Preparation

7.6 Prior to beginning the neutralization assay, *Escherichia coli* (ATCC 11229), *Staphylococcus aureus* (ATCC 6538) and methicillin-resistant *Staphylococcus aureus* MRSA (ATCC 33591) from a stock culture slant, lyophilized vial, or cryogenic stock culture will be transferred into separate tubes containing Tryptic Soy Broth (TSB). The tube will be incubated for 24 hours ± 4 hours at 35 °C ± 2 °C.

7.7 One day prior to beginning the neutralization assay, loopfuls of each broth culture will be streaked onto Tryptic Soy Agar (TSA) plates, and the plates will be incubated for 24 hours ± 4 hours at 35 °C ± 2 °C.

7.8 Immediately prior to initiating the neutralization assay, an inoculum suspension will be prepared in Phosphate Buffer Saline (PBS) solution from each culture on an agar plate, and the concentrations adjusted to approximately 3.0×10^8 to 1.0×10^9 CFU/mL. The suspensions 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 for each species.

Inoculum Assay (Initial Population) – Test C

7.9 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 triplicate, 1 mL aliquots of the test material with TSA. This assay will be performed three additional times for a total of four replicates.

7.10 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 triplicate, with TSA.

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

7.11 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.

7.12 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 triplicate, with TSA.

7.13 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 triplicate, with TSA.

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

7.14 This phase of the neutralization assay assures that the sterile Stripping Suspending Fluid with product neutralizers (SSF++) the sampling solution employed in the

evaluation, is not inherently toxic to the microorganism. This assay will be performed in four replicates.

7.15 To each of four test tubes containing 5.0 mL of one of the sampling solutions, 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 triplicate, with Tryptic Soy Agar with product neutralizers (TSA+).

7.16 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 triplicate, with TSA+.

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

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

7.18 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 triplicate, with TSA+.

7.19 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 triplicate, with TSA+.

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

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

7.21 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.

7.22 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.

7.23 A test material (reference Section 9.4.2 of the Study Protocol) will be applied to a 1.5" x 5" site on the abdomen of the subjects following the instructions for inguinal application in Appendix 2, with the randomly assigned test material.

7.24 The site will then be sampled using the Cylinder Sampling Technique 30 seconds + 5 seconds (30 seconds to 35 seconds) post product-application completion.

7.25 The Cylinder Sampling Technique will be performed as described in Section 9.4.7 of the Study Protocol (using SSF++).

7.26 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 triplicate, with TSA+.

7.27 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 triplicate, with TSA+.

7.28 The process will be repeated on the remaining abdominal site with the randomly assigned test material. A total of at least 18 subjects will be treated with two of the three test materials with samples from at least 6 subjects being used for each challenge microorganism.

7.29 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.

Incubation

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

8.0 CALCULATIONS

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

$$Log10\left\{\frac{A+B+C}{3}\right\}$$

Where: A, B, and C = Individual Plate Counts

8.2 If colonies on one of the plates are uncountable, the count(s) from the remaining plate(s) will be used.

9.0 STATISTICS

9.1 After calculating the $\log_{10}$ populations recovered from Tests D, B, and A, these will be statistically compared to the Initial Population Test C (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.

9.2 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.

9.3 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 significantly different.

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

9.5 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.

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

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

10.0 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.

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