Version: 1.0
Date: 05.25.17

BD Protocol #: MPS-17IPVAW10 BSL Protocol #: 1704167-103

Protocol Title: A Randomized, Single-Center, Blinded, Clinical Evaluation of the Antimicrobial Effectiveness of an Antimicrobial Cloth Compared to Vehicle and 0.9% Saline

Current Version	Version 1.0/05.25.17
Version History	Version 1.0/05.25.17 (Original)
Sponsor	BD 75 N Fairway Drive Vernon Hills, IL 60061
Sponsor Medical Monitor	[redacted]
Central (or Analytical) Laboratory	BioScience Laboratories Inc. 1765 South 19th Ave. Bozeman Montana, 59718
Sponsor Risk Assessment	☐ Significant Risk (SR) ☐ Non-significant Risk (NSR) ☒ Minimal Risk

This study will be performed in accordance with all stipulations of the protocol and in compliance with all applicable BD Policies and Procedures. This study will be conducted in accordance with the ethical principles that originate from the Declaration of Helsinki and the Belmont Report. Study conduct will comply with US FDA Regulations, applicable state and local regulations, and the Good Clinical Practice guidelines set forth by the International Conference on Harmonization (ICH-E6) and ISO14155.

1.0 SPONSOR PROTOCOL APPROVAL

Signature below indicates approval of the protocol as written.

Individual or function	Name	Signature	Date
Scientific Affairs Team Representative	[redacted]	*This document is signed electronically in the eTMF system*	
Medical Monitor / Business Unit Medical Director	[redacted]	*This document is signed electronically in the eTMF system*	
Study Statistician	[redacted]	*This document is signed electronically in the eTMF system*	
Study Manager	[redacted]	*This document is signed electronically in the eTMF system*	

GIRB Approved
Date: 6-5-17
Initial:

2.0 INVESTIGATOR SIGNATURE PAGE

Principal Investigator	Christopher M. Beausoleil, CCRP
Investigational Site	BioScience Laboratories Inc. 1765 South 19th Ave. Bozeman Montana, 59718

I have read this protocol and agree to conduct this study in accordance with all stipulations of the protocol and in compliance with all applicable Good Clinical Practices and regulations.

Signature of Principal Investigator

06/01/2017
Date

GIRB Approved
Date: 6-5-17
Initial:

3.0 SUMMARY

Protocol Number:	MPS-17IPVAW10
Protocol Title:	A Randomized, Single-Center, Blinded, Clinical Evaluation of the Antimicrobial Effectiveness of an Antimicrobial Cloth Compared to Vehicle and 0.9% Saline
Development Phase:	Phase 2
Product Intended Use:	Preoperative Skin Preparation
Planned Study Period:	May 2017 – July 24, 2017
Planned Sample Size:	24 evaluable treatments per each anatomical site per investigational product treatment arm
Objectives:	**Primary Objective :** To compare the immediate antimicrobial activity of a thermally treated cloth impregnated with 0.4% w/v Octenidine Dihydrocloride in aqueous solution to thermally treated polyester cloths impregnated with vehicle formulation and polyester cloths containing 0.9% normal saline. **Secondary Objectives:** To evaluate safety using skin irritation scores and the incidence of adverse events reported during the study.
Study Design:	This single site study is a randomized, blinded design employing a minimum of 36 healthy volunteers, where each subject receives two of the planned treatments on the abdomen and/or groin.
Study Population:	Healthy male and female volunteers, 18 years of age or older, with no dermatological conditions or known history of sensitivity to natural rubber latex, adhesive skin products (e.g., Band-Aids, medical tapes), polyester, octenidine dihydrochloride, or common personal care or beauty products will be enrolled into this study.
Study Product(s):	**Test Product:** Thermally Treated Polyester Cloths Impregnated with 0.4% w/v Octenidine Dihydrochloride **Vehicle Control:** Thermally Treated Polyester Cloths Impregnated with vehicle formulation. **Saline Control:** 0.9% saline applied with Polyester cloths
Study Methodology:	ASTM Standard Test Method E1173-15, *Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations*
Primary Study Endpoints and Acceptance Criteria:	For assessment of immediate activity at 10 min post application, a > 1.2 $\log_{10}$ superiority criterion is implemented for the mean treatment effect of the Investigational Product to the Vehicle Control and the Saline Control.
Secondary Study Endpoints and Acceptance Criteria:	Safety will be evaluated using skin irritation scores and the incidence of adverse events.

Protocol Number:	MPS-17IPVAW10
Statistical Methods:	A linear regression model will be used for primary analysis. In the model, the response is the post-treatment $\log_{10}$ bacterial counts and predictors are the treatment effect and the pre-treatment $\log_{10}$ bacterial counts as a covariate.

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4.0 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
BD	Becton Dickinson and Company
cc	cubic centimeter
CFR	Code of Federal Regulations
CFU	Colony forming units
CI	Confidence Interval
CRF	Case Report/Record Form
CRO	Contract Research Organization
FDA	Food and Drug Administration
FDAAA	FDA Amendments Act of 2007
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonization
IRB/EC	Institutional or Independent Review Board/Ethics Committee
mL	Milliliter
mm	Millimeter
OCT	Octenidine Dihydrochloride
SAE	Serious Adverse Event
PHI	Personal Health Information
SD	Standard deviation
SOP	Standard Operating Procedure
TFM	Tentative Final Monograph
WHO	World Health Organization

5.0 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 pp 31448 to 31450) describes *in-vivo* procedures for evaluating this type of product. Consistent with the 1994 TFM and The 2015 FDA proposed changes to the TFM, 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record;* Proposed Rule (Vol. 80: No. 84, 1 May 2015. pp 25166 to 25205), recent letters published by the FDA on January 19, 2017 describe revised statistical analyses and expected performance criteria for antiseptic health care products tested in microbial log reduction studies.

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] The current study will evaluate the immediate antimicrobial efficacy of thermally treated polyester cloths impregnated with 0.4% w/v octenidine dihydrochloride in aqueous formulation to two negative controls, the thermally treated polyester cloth impregnated with vehicle formulation and a polyester cloth containing 0.9% normal saline, according to the revised statistical analyses and expected performance criteria published by the FDA on January 19, 2017.

6.0 OBJECTIVES

6.1 Primary Objectives

The primary objective of this study is to compare the immediate antimicrobial activities of a thermally treated polyester cloth impregnated with 0.4% w/v octenidine dihydrochloride in aqueous solution to thermally treated polyester cloths impregnated with vehicle formulation and a polyester cloth containing 0.9% saline. In agreement with the Food and Drug Administration Tentative Final Monograph (TFM) for *Effectiveness Testing of a Patient Preoperative Skin Preparation,* a log reduction study will be used to determine antimicrobial efficacy based on the statistical analyses outlined in published letters from the FDA on January 19, 2017. 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, *Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations* and the test criteria from the deferal letters published by the FDA on January 19, 2017.

At 10 minutes post-prep, the test article should achieve a mean treatment effect >1.2 $\log_{10}$ greater than both negative controls on both the abdomen and the groin with 95% confidence. In this protocol two negative controls will be used, the Vehicle Control and the Saline Control.

6.2 Secondary Objectives

Safety will be evaluated using skin irritation scores and the incidence of adverse events.

7.0 STUDY DESIGN

7.1 Overall Study Design

This single site study is a randomized, blinded design employing a minimum of 36 healthy volunteers, where each subject receives two of the planned treatments on the abdomen and groin.

7.2 Specification of Study Endpoints

7.2.1 Primary Endpoints

The primary endpoints are the microbial counts for each product at the 10 minutes post-application time point for both the abdomen and groin.

7.2.2 Secondary Endpoints

The secondary endpoints are safety data including evaluations for skin reactions using the modified Berger Bowman irritation assessment scale and the incidence of adverse events reported during the study for all randomized subjects (the full Intent-to-Treat data set). Post treatment skin irritation scores of 3 on those scales are also considered adverse events.

7.3 Acceptance Criteria

The following table summarizes the minimum baseline criteria for each of the test sites and the expected minimum efficacy standards.

Table 3.3: Minimum Treatment Baseline and Expected Mean Log_{10} Reduction per Anatomical Site		
Anatomical Site	**Treatment Day Baseline Criteria**	**Expected Efficacy Standards***
Abdomen	3.00 to 5.50 log_{10}/cm^2	At 10 minutes the mean treatment effect of the IP should be > 1.2 log_{10} the negative controls with 95% confidence
Groin	5.00 to 7.50 log_{10}/cm^2	At 10 minutes the mean treatment effect of the IP should be > 1.2 log_{10} the negative controls with 95% confidence

7.4 Treatment Allocation and Methods to Reduce Bias

7.4.1 Randomization

The randomization scheme for the study will be provided by the Study Sponsor. Subjects will be randomized to treatment using the following block design:

7.4.1.1 Treatment Balance

Each subject will receive two of the three possible treatments, one on the right side of the body and one on the left. A minimum of 36 evaluable subjects will be required to achieve a total of at least 24 test sites for groin and abdominal regions for each treatment.

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

7.4.1.2 Left/Right Balance

The application will be randomized so that each treatment is applied on an equal number of left and right sides of the body. Due to baseline requirements the final data set may be imbalanced with regard to right and left sides upon achieving the minimum number of qualifying body sites.

7.4.1.3 Site and Sample Time Balance

Each groin and abdomen sample site is divided into four areas and two of these areas are sampled once – one at baseline and one at 10 minutes ± 30 seconds, and two areas 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 (study products, left/right, and sampling order) is a multiple of 144 (3*2*24). The exact subject numbers 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 ratio of 1:1:1 for all three study products.
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-duplicative 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 above at once, with priority using the order listed above.

The Investigator is responsible for ensuring that the randomization is followed. A basic outline of a randomization schedule for the abdominal and groin sites is provided in Appendix 22.4. The final randomization schedule will be prepared by the study statistician before the initial treatment.

Subjects will be identified by their initials, and a subject number.

- Randomized subjects will be assigned numbers ranging from 001 to a three digit number equal to the total number of test subjects needed (36 for the estimated numbers).

7.5 Masking/Blinding

Treatment materials for the following treatment groups will be blinded with treatment codes A or B:

- Thermally treated cloth impregnated with 0.4% w/v octenidine dihydrochloride aqueous solution (test article)
- Thermally treated cloth impregnated with vehicle formulation (Vehicle control)

The 0.9% Saline Control cannot be blinded due to product application requirements and will be assigned to treatment code C.

The staff member(s) performing product applications, sample collections, and irritation assessments will not be blinded from the identification of treatment assignments. Staff member(s) performing bacterial enumeration will be blinded from the identification of all treatment assignments. The study staff performing the bacterial enumeration will not be involved in the study material application, or the collection of samples or skin irritation assessments. The Raw Data Sheet sections of the case report

form will be maintained separately (from the pages within the case report form which include study treatment identifications) during the conduct phase of the study. The study staff performing the bacterial enumeration will record counts directly onto the Raw Data Sheet pages of the case report form without accessing the subject study documentation folder containing the other case report form pages. The Raw Data Sheets will be compiled with the entire case report form after all data recording has been completed.

7.5.1 Emergency Procedure for Unmasking

Response of medical personnel to an adverse event (AE) potentially associated with the test materials will not necessitate translation of the blinding code. The Sponsor Quality Assurance will provide the code translation to BSL Quality Assurance in the individual sealed envelopes (one for each blinded treatment code), which will be secured by BSL Quality Assurance and will remain unopened in the study file. If an emergency requires unblinding, the envelope corresponding to the treatment code (A-B) that is associated with the AE will be opened by the BSL Quality Assurance Manager 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 the 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. The BSL Quality Assurance Manager 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.

7.6 Stopping Rules

No stopping rules for the study have been developed by the Sponsor. The Principal Investigator is responsible for suspending study enrollment for reasons of subject/clinician safety and well-being.

8.0 STUDY POPULATION

Healthy male and female volunteers, 18 years of age or older, with no dermatological conditions or known history of sensitivity to natural rubber latex, adhesive skin products (e.g., Band-Aids, medical tapes), polyester, Octenidine Dihydrochloride, or common personal care or beauty products will be enrolled into this study. A sufficient number of volunteers will be enrolled, such that a minimum total of 24 readings for the groin (sebaceous rich) region and 24 readings for the abdominal (sebaceous poor) region are evaluable for the Investigation Product (test article) and Negative Control arms; a minimum total of 24 readings for the groin region and 24 readings for the abdominal region are evaluable for the Vehicle Control arm and a minimum total of 24 readings for the groin region and 24 readings for the abdominal region are evaluable for the Saline Control arm

Subjects must satisfy all Treatment Day Inclusion/Exclusion Criteria prior to Treatment Day procedures. Subjects may qualify on the abdominal and/or inguinal sites and be admitted into testing for one or both anatomical sites, 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. To be included in the primary analysis (mITT data set), a treatment site must have a Treatment Day microbial baseline equal to or greater than the stated minimum requirement. Enrollment will continue until minimum number of subjects meeting baseline criteria on each body region per treatment arm is identified.

A minimum of 36 human subjects will be treated utilizing bilateral applications to ensure that the treatments will be evaluated on the sites as described in the following table. Each subject has four independent sampling sites: left and right sides for the abdomen and left and right sides for the groin.

Table 4.0: Estimated No. of readings per anatomical site (abdomen and groin), per treatment, per sampling interval (10-minutes)

Arm	Treatment	Number of Abdomen Evaluations	Number of Groin Evaluations
Test article	Thermally treated polyester cloth impregnated with 0.4% w/v octenidine dihydrochloride	24	24
Vehicle Control	Thermally treated polyester cloth impregnated with vehicle formulation	24	24
Saline control	0.9% normal saline applied with polyester cloth	24	24

Unless specified otherwise, these criteria apply at screening and throughout the study.

8.1 Inclusion Criteria

Individuals that satisfy all of the following conditions will be considered for participation:

a. Males and/or females, at least 18 years or older and of any race.
b. Are in good general health.
c. Unable to become pregnant, or willing to use an acceptable method of contraception (i.e. oral contraception, intra-uterine device [IUD], diaphragm, condom, abstinence, bilateral Tubal ligation, or are in a monogamous relationship with a partner who has had a Vasectomy) to prevent pregnancy for at least 14 days immediately preceding Treatment Day and throughout the duration of the study, if female of child-bearing potential.
d. All female subjects must have a negative urine pregnancy test on Treatment Day prior to any applications of the study products.
e. Have skin within 6 inches of the test sites that is free of dermatoses, abrasions, cuts, lesions or other skin disorders.
f. Cooperative and willing to follow Subject Instructions (Appendix 22.6).
g. Cooperative and willing to sign Consent Form and HIPAA Authorization Form.
h. Able to read, write and follow instructions in English.

8.2 Exclusion Criteria

Subjects with any one of the following characteristics will be excluded from participation in this study:

a. Exposure to topical or systemic antimicrobials or any other product known to affect the normal microbial flora of the skin, antibiotics or steroids (other than hormones for contraception or post-menopausal reasons) exposure within 14 days prior to Treatment Day and for the remainder of the study. Restrictions include, but are not limited to antimicrobial-containing soaps, antiperspirants/deodorants, shampoos, lotions, perfumes, after shaves, and colognes.
b. Swimming in chemically treated pools or bathing in hot tubs, spas and whirlpools within 14 days prior to Treatment Day and for the remainder of the study.
c. Use of tanning beds, hot waxes, or depilatories, including shaving (in the applicable test areas) within 14 days prior to Treatment Day and for the remainder of the study.
d. Contact with strong detergents, solvents, acids, bases, bug repellant, fabric softener-treated clothing, UV treated clothing or other household chemicals in the applicable test areas within 14 days of the Treatment Day and for the remainder of the study.
e. Subjects who have a history of sensitivity to vinyl, natural rubber latex, adhesive skin products (e.g., Band-Aids, medical tapes), polyester, metals, inks, common antibacterial agents found in common personal beauty or personal care soaps, lotions, or ointments particularly octenidine dihydrochloride.
f. Subjects who have asthma requiring medication, diabetes, hepatitis B or C, an organ transplant, mitral valve prolapse with a heart murmur, congenital heart disease, lupus, Crohn's disease, medicated multiple sclerosis, internal prosthesis or any immunocompromised conditions (such as AIDS or HIV positive).
g. Subjects who have a history of skin allergies.
h. Subjects who have a history of skin cancer within 6 inches of the applicable test areas or have received treatment for any type of internal cancer within the 5 years prior to enrollment.
i. Any tattoos or scars on the test sites or 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.
j. Dermatoses, cuts, lesions, active skin rashes, scabs, breaks in the skin or other skin disorders within 6 inches on or around the test sites.
k. A currently active skin disease or inflammatory skin condition (for example contact dermatitis; psoriasis, and eczema) anywhere on the body.
l. Subjects who are pregnant, attempting pregnancy, or nursing.
m. Subjects who have showered or bathed within at least 72 hours of the Treatment Day (sponge baths may be taken, however, the lower abdomen and upper thigh region must be avoided).
n. 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 Treatment Day baseline sample collection.
o. Participation in another clinical trial in the 30 days prior to signing the informed consent for this study, current enrollment in another clinical trial, or have already participated in this study.

8.3 Subject Selection

Number of Subjects

A sufficient number of male and female, overtly healthy volunteer subjects at least 18 years of age, will be enrolled to ensure that the total number of evaluable samples collected from groin and abdominal regions are not less than 24 evaluable tests per anatomical site from each treatment arm (minimum of 24 each for groin and abdominal regions). A minimum of 36 evaluable subjects is required to achieve a total of at least 24 test sites for groin and abdominal regions. Subjects must satisfy all Treatment Day Inclusion/Exclusion Criteria prior to Treatment Day procedures. If the required numbers of subjects do not qualify from the initial screening group, additional volunteers will be recruited. At least 4 male or female, overtly healthy volunteer subjects at least 18 years of age will be utilized for the neutralization study.

Based upon historical microbial baseline counts, approximately 76 subjects will be screened such that sufficient subjects are treated to meet the requirement for 24 evaluable treatment sites for each treatment arm on abdomen and groin. The three study products will be assigned for bilateral applications blocks of 24 subjects to assure that each product is evaluated on the skin of each anatomical site – the abdomen and the inguen – at each post-treatment sample time from at least 36 subjects meeting treatment day baseline requirements.

Subject Recruitment

Following approval of the Study Protocol, Informed Consent Form, and other study specific documents by the IRB 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 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 15-SR-008). A List of Restricted Products will be provided to each subject prior to beginning the study (Form SR-002-113016). 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 may 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 IRB-approved study descriptions online or in person. 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 IRB prior to their use for recruiting subjects.

9.0 DESCRIPTION OF STUDY PRODUCTS

9.1 Test Product(s)

The materials identified in Table 5.1 will be used in the study. Specific product lot numbers will be included on separate Investigational Product (study materials) release/receipt form provided by the sponsor with the shipment of the materials.

Table 5.1: Study Materials

Study Arm	Treatment Code	Name	Lot No.	Exp.
Test Article	A or B	Thermally treated polyester cloth impregnated with 0.4% w/v octenidine dihydrochloride	TBD	TBD
Vehicle Control	A or B	Thermally treated polyester cloth impregnated with vehicle formulation	TBD	TBD
Saline Control	C	0.9 % normal saline applied with polyester cloths	TBD	TBD

*BD will supply the polyester cloth for the saline control. BSL will purchase 0.9% normal saline.

9.2 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.

9.3 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.

9.4 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 BSL

9.5 Product Labeling

BD will label, package and ship the study materials to the research facility. Test article and vehicle control materials will be labeled randomly assigned blinded codes A or B prior to shipment to BSL.

The polyester cloth for saline application will be labeled with code C. BSL personnel will document receipt and storage of study materials using the assigned code.

Investigational products (or the immediate packaging) shall be labeled in accordance with regulatory requirements, including the following statement: "CAUTION-Investigational product. Limited by Federal (or United States) law to investigational use."

Commercial products will be supplied as labeled by the manufacturer.

9.6 Maintenance and Storage of Study Products

The test materials will be received and stored by BSL in accordance with instructions from the Sponsor and BSL procedures and retained in secure quarantine when not being used in testing. BSL will maintain an inventory of the test materials in secure quarantine and a log of use. Unused, sealed test materials will be stored by BSL 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 Products and Active Control will be provided to BSL by the Sponsor at the completion of the study when unblinding has occurred.

10.0 STUDY PROCEDURES

Procedure	14 days or more prior to Treatment	3 or more days prior to Treatment	Treatment Day
Informed Consent Obtained	X		
Product-Restriction Period	X	X	
Inclusion/Exclusion Criteria including Medical History Reviewed	X	X	X
Clipping Hair From Test sites		X	
Pregnancy Test			X

Test-Day Baseline Sample		X
Product Application		X
10-Minute Post-Product Application Sample		X
Adverse Events	X	X

Note: Visual evaluations of the skin on each test area will be performed at each laboratory visit prior to treatment, and prior the10-minute post-treatment microbial sampling.

Note: All sampling times will be calculated from the completion of the dry time of each product following application.

10.1 Enrollment

The Inclusion/Exclusion Criteria will be reviewed with each subject to ensure eligibility for the study. Prior to the scheduled Treatment Day, subjects will undergo a minimum 14-day washout period. The subjects will be instructed to avoid contact with any topical or systemic antimicrobial products for the duration of their involvement in the study as written in the Subject Instructions. If it becomes necessary to take systemic antibiotics or to apply topical medications to the test areas within this pretreatment period, the subject must contact the Investigator as soon as reasonably possible so that another volunteer may be recruited.

Restrictions include, but are not limited to:

- Use of antimicrobial containing soaps, shampoos, lotions, perfumes, after shaves or powders, colognes, antiperspirants, deodorants
- Contact with materials such as acids, bases, solvents, bug repellant, fabric softener-treated clothing, UV treated clothing or other household chemicals ….
- Swimming in chemically treated pools and bathing in hot tubs, spas and/or whirlpools
- Use of tanning beds, hot waxes or depilatories (including shaving)

Subjects will be provided a kit with non-antimicrobial personal care products for exclusive use during the study. Subjects will also be provided with written instructions regarding the use of these products.

Subjects will be asked about the condition of the skin at the test areas and their response will be documented. All subjects will be scheduled to return to the test facility approximately 96 hours before the Treatment Day for an initial visual assessment of the test areas and hair removal from the sites to facilitate sample collection, if required.

Subjects will be required to refrain from bathing or showering for at least 72 hours prior to Treatment Day.

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

10.2 Pre-Treatment Day

At least 4 days (96 hours) prior to Treatment Day, the Investigator or a designee will complete the Pre-Treatment Day Inclusion/Exclusion Criteria page in each subject's source document CRF. If these criteria are satisfied, a visual skin assessment will be performed to evaluate the condition of each test area and hair will be clipped, if required.

Subjects will be required to refrain from bathing or showering at least 72 hours prior to Treatment Day.

10.3 Treatment Day

The Investigator or a designee will complete the Treatment Day Inclusion/Exclusion Criteria source/CRF. If these criteria are satisfied, subjects will don a disposable undergarment and a visual skin assessment will be performed to evaluate the condition of each test area.

The randomization schedule will designate the treatment to each side of the abdomen and groin.

10.3.1 Preparation of Abdominal Test Area on Treatment Day

The test site within the abdominal region (abdominal test area) is defined as the area to the right and left sides of the abdomen, adjacent to the umbilicus that appear to be similar in condition and above the groin. Using a 5" x 5" sterile template, the corners of each abdominal test area will be marked directly on the skin using a non-toxic skin marker. Four sampling sites will be numbered within each abdominal test area, on each side of the abdominal region. The positioning and numbering of the abdominal sampling sites are standard for all subjects. Sampling sites on the contra-lateral side of the abdomen will be numbered in a mirror-image orientation. The four sampling sites within each abdominal test area represent one baseline (pre-prep) site, one post-prep sample sites (10-minutes) and two sites will be designated as not sampled.

Prior to performing the Treatment Day baseline sample collection, a skin irritation assessment will be performed by the Investigator or designee trained by the Investigator.

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

10.3.2 Preparation of Groin Test Area on Treatment Day

The test site within the groin region (groin test area) is defined as the inner aspect of the upper thigh within and parallel to the inguinal crease below the groin. Using a 2" x 5" sterile template, the corners of each groin test area will be marked directly on the skin using a non-toxic skin marker. Four sampling sites will be numbered within each groin test area, on each side of the groin region. The positioning and numbering of the groin sampling sites are standard for all subjects. Sampling sites on the contra-lateral side of the groin will be numbered in a mirror-image orientation. The four sampling sites within

each groin test area represent one baseline (pre-prep) site, one post-prep sample sites (10-minutes), and two sites will be designated as not sampled.

Prior to performing the Treatment Day baseline sample collection, a skin irritation assessment will be performed by the Investigator or designee trained by the Investigator.

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

After groin test areas are marked and sample sites numbered, baseline samples will be collected from the appropriate site per the randomization schedule in each test area using the Williamson-Kligman scrub cup technique.

10.3.3 Treatment Materials Application

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

The treatment materials will be applied and the sampling configurations will be performed per the Randomization Schedule and the Study Material Treatment Application Instructions (Appendix 22.7). The duration of each application procedure will be recorded on the appropriate CRF.

All test materials, including packaging, will be weighed before and immediately following application (Appendix 22.7) and the weights will be recorded.

10.3.4 Timing of Post Application Sample Collection

Microbial samples will be collected at 10-minutes (± 30 sec.) post treatment application for both the abdomen and the groin regions. Post application timing begins upon completion of the treatment material application, including drying time. Microbial samples will be collected using the Williamson-Kligman scrub cup technique.

A skin irritation assessment will be performed by the Investigator or designee trained by the Investigator prior to collection of each post treatment microbial sample collection (10- minutes) and a corresponding rating score for each individual skin condition will be recorded in the subject's CRF.

If a subject receives a score of 1 for erythema, edema, rash, and/or dryness on Treatment Day prior to product application, they will be dismissed from testing that day, and can move to another group and participate once the irritation subsides.

If an irritation score of 3 is assigned for any individual skin condition at any post treatment observation, the subject will be discontinued from the study and an adverse event will be recorded.

Following final sample collection, 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.

10.3.5 Microbial Sample Collection / Scrub Cup Technique

Quantitative cultures (treatment baselines and post treatment application) will be obtained by a modification of the cylinder sampling technique of Williamson-Kligman scrub cup technique. To collect the samples, a sterile scrub cup (inner skin surface area of 3.46 cm^2) will be placed on the site and held firmly to the skin. Sampling solution (SS) (3.0 mL) will be pipetted into the cup and the skin will be massaged in a sweeping manner for one minute using a sterile rubber policeman. Using a sterile transfer pipette, the SS will be removed and placed in a sterile test tube. An additional 3.0 mL of fresh SS will be pipetted into the cup and the scrub procedure will be repeated. This solution will be pooled with the first solution collected.

Sampling Solution (SS)

[redacted]

Bacterial Enumeration Methods

Following sample collection, 10-fold serial dilutions (1 mL sample + 9 mL PBW) will be prepared using Butterfield's phosphate buffered water (PBW). One-mL aliquots of appropriate dilutions will be pour-plated in duplicate using trypticase soy agar containing neutralizers (TSA+). Duplicate plating of sample dilutions, according to the method described in ASTM E1173-15, is considered adequate for the objectives of this phase 2 clinical evaluation. Samples must be plated within 30 minutes of collection. After 72 ± 4 hours of aerobic incubation at 30 ± 2C, colonies will be counted and viable cells in the original sample will be calculated according to Standard Operating Procedures. After incubation, plates may be refrigerated up to 48 hours prior to counting.

Raw colony counts from each dilution will be recorded on the appropriate CRFs for each subject. The average CFU/cm^2 of skin will be calculated using a validated Excel spreadsheet using this formula: CFU/cm^2 = Average CFU/mL x 6 mL/3.46 cm^2. The average number of microorganisms recovered (CFU/cm^2) of skin for the treatment day baseline samples will be calculated using the formula to convert the volume of sample collected into CFU/cm^2 of skin:

$$R = \log_{10}\left[\frac{F\left(\frac{\sum_{i=1}^{2} c_i}{n}\right)D}{A}\right]$$

Where: R = the average CFU count in $\log_{10}$ scale per cm^2 of skin.

F = total mL of sampling solution (SS) added to the sampling cylinder (6 mL);

$\frac{\sum_{i=1}^{2} c_i}{n}$ = average of the duplicate colony counts used for each sample collected

D = Dilution factor of the plates counted. One of 10^0, 10^1, 10^2, 10^3, 10^4, or 10^5.

A = Inside area of the sampling cylinder (3.46 cm^2)

The average CFU/mL, CFU/cm^2, and log_{10} CFU/cm^2 will be calculated for samples from each test site in the same manner.

In order to avoid potential calculation problems due to taking the logarithm of zero, counts of less than 1 CFU/cm^2 will be treated as 1 CFU/cm^2, such that the log_{10} transformation is no less than zero.

Neutralizer Validation

The effectiveness of the neutralizer system must be validated prior to the study start date to demonstrate that the antimicrobial is effectively neutralized and there is no effect on the growth of microorganisms. A procedure that will include *in-vivo* sampling will be combined with an *in-vitro* evaluation using procedures in accordance with ASTM E1054-08(2013).[12] The procedure is in Appendix 22.8. Two organisms, Methicillin-resistant *Staphylococcus epidermidis* (MRSE), ATCC 51625 and Methicillin-sensitive *Staphylococcus epidermidis* (MSSE), ATCC 12228, will be evaluated. These data will be provided in the final report.

11.0 INTERRUPTION OR DISCONTINUATION OF PARTICIPATION/TESTING

11.1 Discontinuation of study subjects

Subjects may request withdrawal from the study at any time or may be withdrawn at the discretion of the Principal Investigator for any of the following reasons:

- Adverse Event/Concurrent Illness
- Noncompliance with study requirements or restrictions
- Failure to meet ongoing inclusion criteria, or development of an excluding condition
- Protocol deviation
- Withdrawal of consent
- Subject is lost to follow up
- Administrative issues
- Any other reason which, in the opinion of the PI, makes the subject's participation in the study not in his or her best interest.

11.2 Replacement of Discontinued Subjects

Discontinued subjects requiring replacement due to any of the reasons mentioned above will require additional enrollment until a sufficient number of subjects meet Treatment day baseline counts. Retention of Data from Discontinued Subjects

No data will be collected from subjects after the point of discontinuation except as needed to follow ongoing adverse events. All study data collected from the subject up to the point of discontinuation will be recorded

on the Case Report Form, entered into the study database, and included in subsequent analyses, as appropriate.

11.3 Discontinuation Visits and Follow-up Procedures

For subjects discontinued due to adverse events, the clinical course of the event will be followed according to accepted standards of medical practice until the event resolves, stabilizes, or in the opinion of the Investigator, is no longer considered clinically significant.

12.0 RISK / BENEFIT ASSESSMENT

12.1 Potential Risks

The risks anticipated during the study are the following skin reactions:

- dryness
- redness
- chapping of the skin
- rash
- skin irritation or sensitization
- mild abrasion due to sampling
- folliculitis from clipping

There may be risks from participating in this study that are unknown, including allergic reactions.

12.2 Potential Benefits

There are no direct benefits to the subject for participation in this study. The findings may reveal information that will allow for a better understanding of the antimicrobial effectiveness of thermally treated polyester cloth impregnated with 0.4% octenidine dihydrochloride in aqueous solution, and thus may improve medical care for persons that require invasive procedures, including surgery.

13.0 SAFETY

13.1 Adverse Event Definitions

Adverse Event (AE): Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease in a subject that is temporally associated with the use of an investigational product or procedures, even if the event is not considered to be related to the study product or procedures.

This includes events not seen at initial assessment (Pre-Treatment Day) and events that have worsened if present at initial assessment. The term AE will refer to all adverse events (serious and non-serious) occurring during participation in a study of either investigational devices and/or drugs.

Serious Adverse Event (SAE): An SAE is any AE occurring during study participation that results in any of the following outcomes:

- Death

- Life Threatening (refers to any event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Hospitalization or prolongation of a hospital stay
- Persistent or significant disability or incapacitation (refers to any event which results in a substantial and/or permanent disruption of the subject's ability to carry out normal life functions)
- Required intervention to prevent permanent impairment/damage
- Congenital anomaly/birth defect
- Important medical event that may require intervention to prevent one of the preceding conditions.

13.2 Adverse Event (AE) Management

At each study contact, subjects will be questioned in an open-ended manner regarding any new or worsening undesirable signs or symptoms they may have experienced since the previous contact. All clearly related signs, symptoms, and abnormal diagnostic procedure results should be comprehensively recorded on the appropriate source document, though should be grouped under one diagnosis.

Each sign, symptom, disease or illness reported must be evaluated by the Investigator or designee to determine if it meets the definition of an Adverse Event.

The clinical course of the event will be followed according to accepted standards of medical practice until the event resolves, stabilizes, or in the opinion of the Investigator, is no longer considered clinically significant. The Investigator must supply the Sponsor with information concerning the follow up and/or resolution of the AE.

13.3 Assessment of Adverse Events (AEs)

All AEs must be assessed for Seriousness, Severity, and Relationship. All AEs, regardless of classification, must be comprehensively documented in the CRF and on the SAE form, if applicable, and reported to BD. This includes AEs related to marketed study products.

The study period during which adverse events must be reported is normally defined as the period from initiation of study procedures and/or exposure to study product, to the end of the study treatment follow-up. For this study, the study treatment follow-up for ongoing AEs is defined as 30 days following the last application of study treatment; or in the opinion of the Investigator, needs to be followed longer or is no longer considered clinically significant. The Investigator must supply the Sponsor with information concerning the follow up and/or resolution of the AE.

All AEs must be assessed for Seriousness, Severity, and Relationship. All AEs, regardless of classification, must be comprehensively documented in the CRF and on the SAE form, if applicable, and reported to BD. The following information about the event is to be reported on the AE CRF:

- Seriousness, classified as: Non-Serious or Serious
- Severity, classified as:
  - Mild: Transient symptoms, easily tolerated, no interference with daily activities
  - Moderate: Marked symptoms, moderate interference with daily activities, tolerable
  - Severe: Considerable interference with daily activities, intolerable
- Relationship, to the study product or study procedures:
  - Not Related: Evidence suggests absolutely no possible causal relationship between the event and the investigational study device (or procedures).

- Unlikely Related: Evidence suggests that other possible causes or contributing etiological factors may have caused the event other than the investigational study device (or procedures).
- Possibly Related: Evidence suggests a causal relationship between the event and the investigational study device (or procedures) cannot be ruled out
- Related: Evidence suggests a reasonable causal relationship between the event and the device (or procedures) is likely

In addition, the following should be recorded for each AE:

- Action(s) taken to remedy the AE, including change in study treatment or participation, or medical/surgical treatments
- Duration of the AE from onset through resolution, as applicable
- Cause (including suspected product/procedure and/or other cause)
- Outcome of the event, including resolution and sequelae, as applicable

13.4 Additional procedures for Assessing & Reporting Serious Adverse Events (SAE)

SAE criteria are specified in Section 9.1. All SAEs must also be assessed by the Investigator and Sponsor Medical Monitor to determine whether an SAE is expected or unexpected. An adverse event will be considered unexpected or unanticipated if the nature, severity or frequency of the event is not consistent with the risk information previously described in the protocol, Informed Consent, or Investigator's Brochure (if applicable).

Any adverse event meeting the criteria for 'Serious', regardless of the Investigator's opinion of expectedness or relationship to the study product, must be reported to BD within 24 hours. The Investigator or designee must report the event by telephone or email to the Study Monitor. In addition to reporting the SAE to the Study Monitor, the Investigator must also submit a completed SAE form to the BD Trial Safety Dept. via fax or email listed below within 24 hours of receipt of the information.

Sponsor Notification of Serious Adverse Event:

[redacted]

Medical questions about study safety issues and serious adverse events can be directed to the Sponsor Medical Monitor.

The Investigator must report any adverse events which are serious, unanticipated/unexpected and probably or possibly related to the study product or procedures to the reviewing IRB/EC. This report must be submitted as soon as possible, but in no event later than 10 working days after the Investigator first learns of the event.

The Investigator may also have additional responsibilities for AE reporting to their governing Health Authority which they are responsible for identifying and fulfilling.

The Sponsor will provide results of any evaluation of an unanticipated/unexpected adverse device effect to appropriate Health Authorities, to all Investigators, and to all reviewing IRB/ECs within 10 working days after the Sponsor is notified of the event. If the Investigator wishes to assume responsibility for filing reports of evaluation results to their own IRB/EC in lieu of the Sponsor, they must notify the Sponsor in writing of this preference and must retain evidence of their compliance with this requirement.

BD will comply with all other Sponsor safety reporting requirements and timelines for other entities (e.g., Data Safety Monitoring Boards) and local health authorities in other countries where this study or other studies with the same product are being conducted, in compliance with study procedures and applicable local regulatory requirements and BD Standard Operating Procedures.

14.0 INCIDENTS

A Clinical Study Incident is defined as any problem or issue involving the investigational product(s), reference methods, associated procedures or equipment, or represents a product-related injury (or potential for injury) to study subjects or personnel as a result of execution of this protocol. Clinical Study Incidents may adversely (or potentially adversely) affect human safety, the integrity of the evaluation data, or the operation of devices or systems, and warrant prompt attention.

Incidents involving injury to study subjects will also be reported as Adverse Events (refer to Section 9). Examples of Clinical Study Incidents that are not Adverse Events might be **mislabeling or adulteration of the investigational product, equipment malfunctions, errors in the application instructions, damage to Investigational Product caused by shipping or handling or improper storage, or injury to study personnel due to execution of the protocol**. If appropriate, an Incident may also be documented and reported as a protocol deviation.

Study-specific procedures for reporting Incidents, as well as adverse events and protocol deviations, will be provided to the study site prior to study execution. The Monitor should be contacted immediately when site becomes aware of or suspects any defective or malfunctioning product. This includes:

- Products that are involved in Study Incidents,
- Products that are found to be expired, damaged or defective,
- Products that are possibly the cause of an adverse effect, regardless of whether the product was believed to be damaged, defective or malfunctioning.

Such products (whether investigational or marketed) should be segregated and returned with appropriate documentation to the BD address below, unless instructed otherwise by BD. The Study Monitor should be contacted with any questions regarding return of study products. BD will supply mailing kits specifically intended for product contaminated with potentially bio-hazardous material.

15.0 RETURN OR DESTRUCTION OF STUDY PRODUCT

All disposable, used products not failed, damaged or otherwise involved in an Incident or Adverse Event are to be discarded into appropriate waste containers at the investigational site.

Unless instructed otherwise by BD, the Investigator will return all remaining unused or unopened or damaged test, reference, and ancillary study products to BD. At the conclusion of the study, and as appropriate during the course of the study, any products, supplies or BD equipment that are required to be returned will be shipped to BD at the address below, unless instructed otherwise:

BD, ATTN: R&D Clinical Supply Shipments Coordinator
75 N. Fairway Drive
Vernon Hills IL 60061
630-606-9392

16.0 DATA COLLECTION AND MANAGEMENT

16.1 Source Documents

Source data includes all information in original records (and certified copies of original records) of clinical findings, observations, or other activities (in a clinical study) used for the reconstruction and evaluation of the study. Source data are contained in source documents (original records or certified copies) and are used to verify the authenticity of information recorded on the Source/CRF. Typical source documents include the hospital chart, medical office file, laboratory report, clinician notes, patient record, recorded data from automated instruments or other documentation prepared and maintained by the investigator/staff or ancillary services which contains a record of all observations and other data pertinent to the investigation on a study subject.

16.2 Case Report Forms (CRF)

The Source/CRFs will be provided by the Site. The Investigator may delegate Source/CRF completion to study personnel. However, the Sponsor must be apprised in writing of the name of such persons. The Principal Investigator or designee is obligated to review each Source/CRF page and sign or initial the indicated pages using ink. An individual record will be kept for each subject that provided informed consent.

All entries to a paper CRF should be made clearly in black or dark blue indelible ballpoint pen to ensure the legibility of self-copying or photocopied pages. Corrections are made by placing a single horizontal line through the incorrect entry, so that the original entry can still be seen, and placing the revised entry beside it. The revised entry must be initialed and dated by a member of the Investigator's research team authorized to make CRF entries. Correction fluid must not be used.

Source documents/CRFs will be 100% reviewed by the study Monitor during the course of the study.

17.0 STATISTICAL METHODS

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

17.1 Sample Size Determination

This is a phase 2 study to assess the immediate antimicrobial effect of the Investigational Products relative to the two negative controls using a log reduction study 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) and the test criteria

outlined in the deferal letters from the FDA published on January 19, 2017.The purpose is to inform design of future 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.

17.2 Data Evaluability

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 3.00 to 5.50 $\log_{10}/cm^2$, inclusive, on the abdomen and 5.00 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. Data collected will be excluded from Per Protocol data if the Treatment-Day baseline counts are outside the acceptable range. Analyses conducted on the mITT data set will also be conducted on the Per Protocol data set as supportive analyses when Per Protocol data are different from mITT data.

- For $\log_{10}$ CFU/cm^2 determinations, missing data will not be imputed for either mITT data or Per Protocol data and will be excluded from analysis.

17.3 Statistical Methods

17.3.1 Primary Analysis

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

The primary purpose of the study is to compare the immediate antimicrobial activity of the Investigational Products to two negative controls according to the methods described in the FDA TFM using the statistical analyses outlined in the deferral letters published by the FDA on January 19, 2017.

Statistical hypotheses are:

Ho: Treatment effect of the Investigational Product vs. Vehicle Control / Saline Control ≤ 1.2

Ha: Treatment effect of the Investigational Product vs. Vehicle Control / Saline Control > 1.2

Ho: is the null hypothesis and Ha is the alternative hypothesis.

A linear regression model will be used for primary analysis. In the model, the response is the post-treatment $\log_{10}$ bacterial counts and predictors are the treatment effect and the pre-treatment $\log_{10}$ bacterial counts as a covariate. The interaction between the treatment and pre-treatment $\log_{10}$ bacterial counts will be also explored. If there is no significant interaction detected between treatment and pre-treatment $\log_{10}$ bacterial counts, the interaction term will be removed from the model and the average difference between treatments will be estimated from the linear regression model after correcting for pre-treatment $\log_{10}$ bacterial counts. If there is a significant interaction detected between treatment and pre-treatment $\log_{10}$ bacterial counts, the average difference between treatments will be estimated from the linear regression model after averaging over the interaction term. The objective is to achieve a difference

in treatment effect (log_{10} reduction) for investigational products vs. negative controls greater than 1.2 log10 on both the abdomen and groin with 95% confidence. The 95% confidence intervals will be summarized for each product tested, grouped by anatomical site and the 10 minute post-application time point and compared to the superiority margin of 1.2 log_{10} for investigational products vs. each Negative Control.

17.3.2 Secondary Analysis

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 treatment day or the 10 minute post-application timepoint) 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 Products and Negative Controls will be evaluated using Fisher's exact test on skin irritation data summarized as follows: any reaction above zero (no reaction) on the skin irritation rating scale for any category (erythema, edema, rash, and dryness) will be considered a positive signal for that substance. If Fisher's exact test shows statistically significant skin irritation between the three study products, a secondary analysis will be conducted to determine how the reactions differ.

17.3.3 Exploratory Analysis:

Log Reductions:

The following descriptive statistics for log_{10} CFU/cm^2 reductions will be computed for the mITT and Per Protocol populations for each product tested, grouped by anatomical site at the 10 minute application sampling time point: mean, median, standard deviation, minimum, maximum, and count.

Antimicrobial activity will be compared using log_{10} reductions between the Investigational Product, and both negative controls for each body area at the 10-minute sampling time. Differences in log_{10} CFU/cm^2 reductions between treatments and confidence intervals will be estimated by an analysis of variance (ANOVA). In the ANOVA model, the response is the log_{10} reduction and the predictors are treatment and time point as well as the interaction between treatment and time point. The average difference in log reduction between treatments per time point will be estimated. However, all conclusions for the treatment effect (difference between treatments in log_{10} reduction) will be based on the primary analysis only.

17.3.4 Informational Analysis:

Product Expression Volumes:

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.

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 separately for each body site 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.

17.3.5 Microbial Baseline Effects

Additional analyses may be conducted using the same methods as for the primary and secondary analysis using the Per Protocol data set, but with modified baseline requirements to determine the effect of baselines on the outcomes. This is for informational purposes only.

17.4 Demographics/Other descriptive information

Demographics of the study population will be collected by the clinical sites.

17.5 Interim analysis

No interim analysis will be performed.

18.0 QUALITY CONTROL AND ASSURANCE

18.1 Accountability of Study Products

Investigational study products will be released only for use by Investigators who have obtained written IRB/EC approval (as required) for participation in this study, who have completed all required study documentation, and who have been qualified by the Sponsor. Investigators must maintain control over all study products, and ensure they are used in accordance with this protocol. Failure to do so may result in the Sponsor suspending or terminating the study at the Investigator's site.

The Investigator will ensure that study products are only applied to subjects (or used for specimens) properly enrolled in the study. The Investigator must maintain records of receipt, disposition, return and/or destruction of all study products. All investigational study products released to the site must be accounted for at the unit level prior to study close out, regardless of disposition. The Study Monitor will regularly review all records regarding study product accountability.

The Sponsor will maintain records that document the shipment, receipt, disposition, return and/or destruction of study products.

18.2 Monitoring

BD, the study sponsor, will designate trained and qualified personnel to monitor the progress of this clinical study in accordance with BD Monitoring SOPs and the study-specific Monitoring Plan. A pre-study site qualification visit will be conducted to assess the adequacy of the site facilities and staff with respect to study requirements, if required

Prior to study start, a study initiation visit will be conducted to provide training to site staff with regard to the protocol, the completion of study documentation and Case Report Forms (CRFs), the monitoring schedule, and all regulatory requirements. During the study, routine monitoring visits will be conducted to assure the site continues to adhere to the protocol, the investigator agreement, and regulations regarding conduct of clinical studies. Assessments will be made regarding the subjects' protection and safety, when relevant, as well as the quality, completeness, and integrity of the data. The Study Monitor will assist the investigative site with query resolution and will perform site close-out activities once all queries have been resolved.

Additional visits may be carried out depending upon site activity and performance. The Investigator must agree to the inspection of all study related records and give direct access to source documents for verification of data on CRFs.

The Investigator is responsible for ensuring that any site-owned equipment required for use in the study is properly installed and maintained (e.g., inspected, calibrated, alarmed). Documentation of equipment maintenance procedures must be available for review by the Monitor.

18.3 Audits and Inspections

If the study is selected for audit by the Sponsor or if there is an inspection by the appropriate Health Authorities, then the Investigator and his team will make themselves available during the visit. The Investigator must agree to the inspection of all study related records and give the auditor/inspector direct access to source documents. The subject's anonymity must be safeguarded and data checked during the audit remain confidential.

As soon as the Investigator is aware of an upcoming inspection/audit by the Health Authorities, he/she will promptly inform BD. As agreed with the Investigator, BD personnel may be present at the site during the inspection.

18.4 Protocol Deviations

Protocol deviations are not permitted and should be implemented prospectively as a protocol amendment whenever practical or appropriate, unless required to protect the safety and well-being of the subject. The Investigator must notify the Sponsor immediately of any such deviation resulting from the need to protect a subject.

Protocol deviations (other than those required to protect the safety and well-being of a subject) may impact the evaluability of study data, and may place subjects at risk. If the Investigator or their staff inadvertently deviates from the study plan, the Investigator should implement appropriate corrective and preventive procedures, and should notify the Sponsor at their earliest convenience. Significant deviations may also need to be reported to the IRB/EC and local health authority.

The Study Monitor will evaluate records of study conduct at the site to identify any deviations, and will also report them to the Sponsor. Upon evaluation by the Sponsor, actions may be required to prevent additional deviations, such as retraining of the site, implementation of additional site procedures, and more frequent monitoring. If these steps fail, more serious measures, up to and including termination of the site and withdrawal of study product may be necessary.

19.0 ETHICAL AND REGULATORY STANDARDS

19.1 IRB/EC

An appropriate IRB/EC must review this protocol, the Informed Consent Form (if applicable), and any other supporting study documents which affect subject or study personnel safety, prior to study initiation at an investigational site. No investigational site may begin the study until the IRB/EC has given its written approval, signed by the IRB/EC chairperson or authorized personnel, and a copy of the approval letter and the approved Informed Consent Form (if applicable) has been provided to the Sponsor.

19.2 Informed Consent

Prior to giving informed consent, each candidate will have the opportunity to review the study procedures, risks and benefits and ask any questions he or she may have regarding the study. Before enrollment, each subject must give informed consent, documented by signing a written form, created and approved in compliance with 21 CFR Part 50.25 and 21 CFR Part 56. Each subject should be given a copy of the signed informed consent document.

19.3 Confidentiality of Data

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and BD and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating subjects. Subject confidentiality and anonymity will be maintained at all times by removal of all identifiers from any data, clinical samples or documentation submitted for this study.

Any data collected meeting the definition of PHI will be collected and maintained using the designated authorizations and following all privacy procedures as specified in the applicable health authority regulations.

BD will maintain the security and confidentiality of all clinical study data sent to BD. BD clinical study databases will not be shared with any third party without the express written consent of the Principal Investigator and/or Site.

The Study Monitor or other authorized representatives of BD may inspect all documents and records required to be maintained by the Investigator. The Site will permit access to such records. BD and the Site may be required to provide regulatory agencies access to clinical study data and records, as well as source documents.

All other agreements as to confidentiality by BD, the Principal Investigator, and the Site may be found in the Confidential Disclosure Agreement and the Clinical Trial Agreement.

19.4 Protocol Modifications

Amendments to the protocol will not be implemented without agreement from the Sponsor and prior submission to and written approval from the governing IRB/EC, except when necessary to eliminate an immediate hazard to the subject. Notice of an emergency modification shall be given to the Sponsor and the reviewing IRB/EC as soon as possible, but in no event later than 5 working days after the emergency occurred. Protocol amendments may affect Informed Consent Forms for current and future subjects.

Minor changes to the protocol, such as correction of typographical errors or changes in personnel names (other than the PI) or contact information will be processed as administrative changes. Administrative

changes will be submitted to the governing IRB/EC but implementation of the administrative change may proceed without prior IRB/EC approval, unless so required by the IRB/EC or site SOPs.

19.5 Study Discontinuation

BD reserves the right to temporarily suspend or prematurely discontinue the study at a single site or at all sites at any time and for any reason. If such action is taken, BD will discuss the reasons with all Investigators (the Investigator). If the study is terminated or suspended due to safety reasons, the sponsor will inform the health authorities as required, and provide the reason(s) for the action. Investigator(s) must inform their IRB/EC promptly and provide the reason(s) for the suspension or termination.

19.6 Clinical Study Registration

In compliance with Title VIII of Public Law 110-85, known as FDA Amendments Act of 2007 (FDAAA), BD will register all applicable studies and disclose study results in a publicly accessible database, e.g. the ClinicalTrials.gov web site. Applicable studies will be registered no later than 21 days after commencing enrollment. Study results for applicable studies will be posted to the website within 12 months of the last subject visit for collection of primary outcome data, or after health authority approval for previously unapproved devices. BD has responsibility for determining whether this study qualifies as an "applicable" study under the law, and if so, will take responsibility for registration and disclosure as required by law.

19.7 Publication of Results

No presentation or publication of data from this study can be initiated without the explicit written consent of the Sponsor and in direct collaboration with the Sponsor. If required, publication of the protocol and publication of study results will be posted to Clinical Trials.gov.

BD must receive copies of any intended communication in advance of publication as specified in the Clinical Trial Agreement. In a timely manner, BD will review the communications for accuracy (thus avoiding potential discrepancies with submissions to health authorities), verify that confidential information is not being inadvertently divulged and to provide any relevant supplementary information to the investigators.

19.8 Record Retention

If the Principal Investigator or Clinical Center withdraws from the responsibility of keeping the study records, custody must be transferred to a person or entity who will accept the responsibility. BD must be notified in writing of the name and address of the new custodian.

Federal regulations require that a copy of all essential study documents (e.g., IRB/EC approvals, signed informed consent forms, source documents, CRF copies, safety reports, test article dispensing records, etc.), must be retained in the files of the responsible Investigator for a minimum of 2 years following notification by BD that all investigations are completed, terminated, or discontinued, or that the FDA has approved the application (21 CFR 812.140).

20.0 BIBLIOGRAPHY/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.*

21.0 PROTOCOL REVISION HISTORY

Version #	Rationale for Change	Section or Page affected	Description of change
1.0	New Protocol		

22.0 APPENDICES

The following appendices are included:

22.1 Key Study Personnel, Titles, Responsibilities

22.2 Study Summary

22.3 Abdomen and Groin Diagram

22.4 Randomization Scheme

22.5 Required Elements of Informed Consent

22.6 Subject Instructions

22.7 Treatment Application Instructions

22.8 Procedure for Neutralizer Validation

22.9 Skin Irritation Rating Scale

22.1 Key Study Personnel, Titles, Responsibilities

[redacted]

BSL Personnel:

Christopher M. Beausoleil, CCRP	Principal Investigator
Patricia A. Mays Suko	Subinvestigator
Samantha Comstock	Subinvestigator

[redacted]

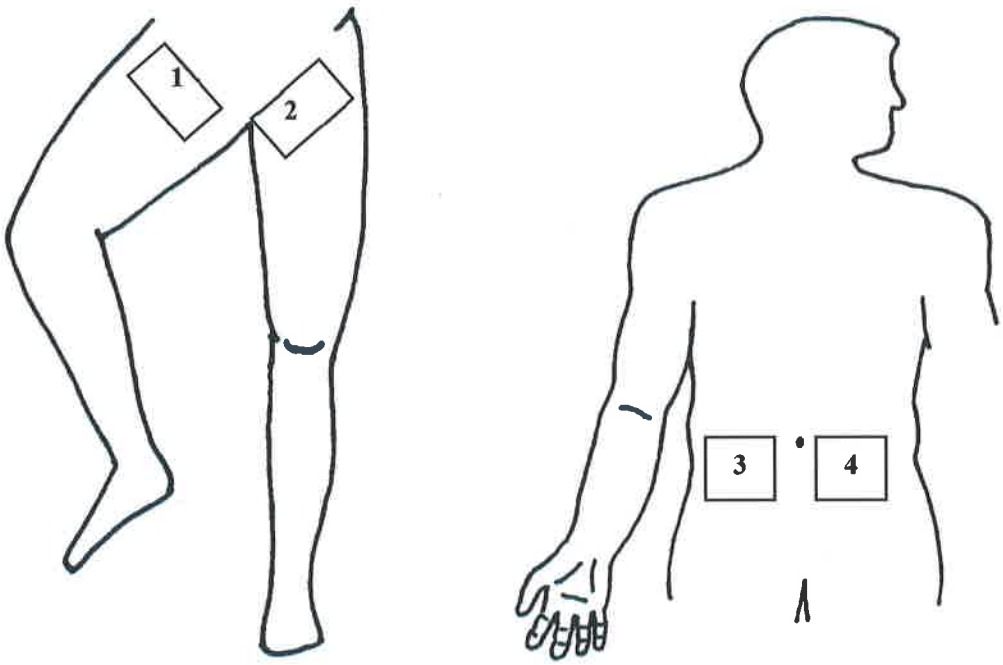
Note: additional subinvestigators will be identified prior to study initiation.

22.2 Study Summary

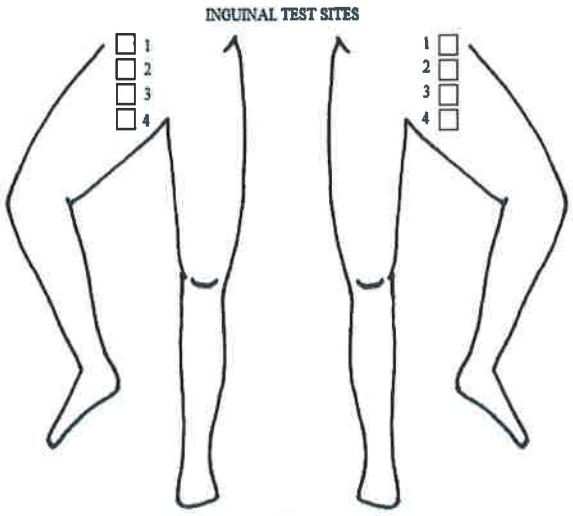
Pre-Study Preparation	Screening Phase 14 Day Washout Period	Treatment Phase	
		Abdominal Region	Groin Region
Staff reviews study protocol	Initiation of consenting process	Complete Treatment Inclusion/ Exclusion Criteria form	
Prepare consent form	Review study Consent Form and Inclusion/ Exclusion Criteria	Visual skin assessment pre-baseline sample and after 10 minute sampling	
Obtain IRB approval	Review subject instructions	Mark test areas, Collect baseline samples	
Recruit volunteers for Treatment phase	Subject signs consent form	Apply test articles	
Prepare subject kits	Product Restriction Period begins	Visual skin assessment, 10-minute (± 30 sec.) post-prep sample	Visual skin assessment, 10-minute (± 30 sec.) post-prep sample
	Visual skin assessment (abdominal and groin regions)	Count Treatment Day Baseline plates, determine qualification and enroll replacement subjects as required	Count Treatment Day Baseline plates, determine qualification and enroll replacement subjects as required
	Dispense subject kits		
	Schedule for clipping, if needed, 96 hrs. prior to treatment day		
	No bathing / showering 72 hrs. prior to treatment day		

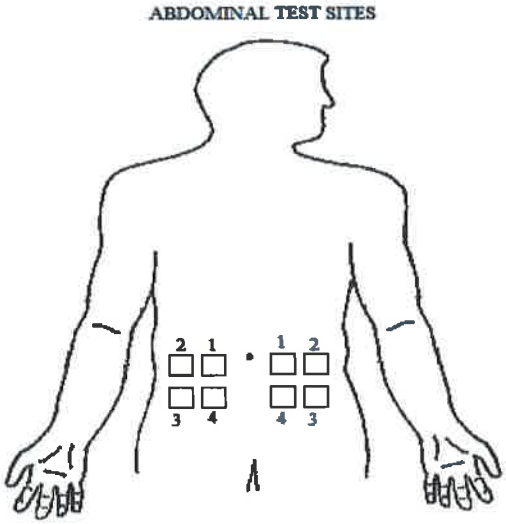
22.3 Abdomen and Groin Diagram

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



1-2: Groin (anterior) – 2' x 5" area; 3-4: Abdomen (anterior) – 5" x 5" area


INGUINAL TEST SITES
1
2
3
4
1
2
3
4


ABDOMINAL TEST SITES
2
1
1
2
3
4
4
3

22.4 Randomization Scheme

Example Randomization Scheme – 30 second and 6 hour sites will not be used in this protocol.

Random-ization Number	Subject Number	Treatment Area	Treatment Code Left Side	Treatment Code Right Side	Site 1	Site 2	Site 3	Site 4
R001		Ab / Groin / Both	A	C	Baseline	10 Minutes	30 Seconds	6 Hours
R002		Ab / Groin / Both	D	B	10 Minutes	Baseline	30 Seconds	6 Hours
R003		Ab / Groin / Both	A	B	30 Seconds	Baseline	10 Minutes	6 Hours
R004		Ab / Groin / Both	B	D	10 Minutes	Baseline	6 Hours	30 Seconds
R005		Ab / Groin / Both	C	B	10 Minutes	30 Seconds	6 Hours	Baseline
R006		Ab / Groin / Both	A	D	Baseline	6 Hours	10 Minutes	30 Seconds
R007		Ab / Groin / Both	B	C	30 Seconds	10 Minutes	Baseline	6 Hours
R008		Ab / Groin / Both	D	C	30 Seconds	Baseline	6 Hours	10 Minutes
R009		Ab / Groin / Both	D	A	30 Seconds	10 Minutes	6 Hours	Baseline
R010		Ab / Groin / Both	B	A	6 Hours	30 Seconds	10 Minutes	Baseline
R011		Ab / Groin / Both	C	A	Baseline	30 Seconds	6 Hours	10 Minutes
R012		Ab / Groin / Both	C	D	6 Hours	10 Minutes	Baseline	30 Seconds
R013		Ab / Groin / Both	D	A	Baseline	30 Seconds	10 Minutes	6 Hours
R014		Ab / Groin / Both	C	A	30 Seconds	6 Hours	10 Minutes	Baseline
R015		Ab / Groin / Both	C	B	6 Hours	Baseline	10 Minutes	30 Seconds
R016		Ab / Groin / Both	D	B	6 Hours	10 Minutes	30 Seconds	Baseline
R017		Ab / Groin / Both	A	C	10 Minutes	6 Hours	30 Seconds	Baseline
R018		Ab / Groin / Both	B	A	10 Minutes	30 Seconds	Baseline	6 Hours
R019		Ab / Groin / Both	B	C	6 Hours	30 Seconds	Baseline	10 Minutes
R020		Ab / Groin / Both	A	D	Baseline	10 Minutes	6 Hours	30 Seconds
R021		Ab / Groin / Both	A	B	30 Seconds	6 Hours	Baseline	10 Minutes

22.5 Required Elements of Informed Consent

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

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

ADDITIONAL ELEMENTS OF CONSENT

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

13. A statement that the particular treatment or procedure may involve risks to the subject (or embryo or fetus, if subject became pregnant) which are currently unforeseeable.
14. Anticipated circumstances under which subject's participation may be terminated by the Investigator without regard to subject's consent.

15. Any additional costs to the subject that may result from participation in the research.
16. A statement explaining the consequences of subject's decision to withdraw during the course of the research which may relate to subject's willingness to continue participation will be provided to the subject.
17. A statement that significant new findings developed during the course of the research which may relate to subject's willingness to continue participation will be provided to the subject.
18. Approximate number of subjects involved in the study.

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

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

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

22.6 Subject Instructions – Washout, Treatment Visit

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

- Use <u>only</u> the soap provided for all bathing, sponge bathing and hand washing.
- Use <u>only</u> the shampoo provided when washing your hair.
- <u>Do not use</u> antiperspirants or deodorants (other than those provided to you in the kit), lotions, colognes, perfumes, after shaves or powders.
- <u>Do not</u> come in contact with solvents, acids, bases, fabric softener-treated clothing or other household chemicals in the abdominal and upper thigh body regions.
- <u>Do not swim</u> in chemically treated pools or bathe in hot tubs, whirlpools or spas.
- <u>Do not</u> use tanning beds.
- <u>Do not</u> shave, use depilatories or hot waxes on the abdomen or upper thigh areas. If hair is present, allow study staff to clip hair at a designated time.
- <u>Do not apply</u> any medicated creams or ointments to any area of your skin, nor should you take any antibiotics. If antibiotics are necessary due to illness, please report this to either
  Christopher M. Beausoleil at [redacted] (Principal Investigator)
  OR TO
  Patricia A. Mays Suko at [redacted] or [redacted]
  (Subinvestigator)
  OR TO
  Samantha Comstock [redacted] or [redacted]
  (Subinvestigator)

<u>Additional Instructions for Treatment Visit:</u>

- Do not bathe or shower in the MINIMUM 72-hour period before your scheduled appointment. A sponge bath may be taken, but avoid the areas of the lower abdomen and/or upper thigh.
- You will be required to return to the lab approximately 96 hours before your Treatment Visit for clipping.
- On the Treatment Visit, you will return to the laboratory for treatment and the initial sampling.

If you have questions about this study or in case of emergency, contact, Principal Investigator at (406)580-8556 any time during or after business hours.

GIRB Approved
Date: 6-5-17
Initial: BC

22.7 Treatment Application Instructions

Investigational Product and Vehicle Control

Procedure:

1. Weigh the unopened cloth package and record weight.
2. Remove a single cloth from the package.
3. Apply product as described below (Cloth Treatment Site Application Instructions) .
4. Following product application, return the used cloth to the open package. Weigh and record weight.
5. Retain the labeled package and discard both the used and unused cloths.
6. Place the label on the designated source label page.

Saline Control

Procedure: (Prior to treatment application)

1. Using a sterile pipette, transfer 25 mL of 0.9% normal saline into the bag containing the single wipe, distributing the formulation over the maximum amount of surface area possible.
2. Remove the excess air from the bag and then seal the bag tightly.
3. Place the sealed bag on a flat surface and use a roller, or suitable device (e.g. bottles, test tubes, etc.) to evenly distribute the 0.9% normal saline throughout the wipe by rolling the rolling device across the wipe.
4. Weigh the sealed bag containing the wipe material and formulation and record weight.
5. Apply product as described below (Cloth Treatment Site Application Instructions)
6. Following product application, return the used wipe to the bag. Weigh and record weight.

Cloth Treatment Site Application Instructions

Procedure (Abdomen and Groin):

1. Using a single cloth for each anatomical region, vigorously scrub skin back and forth for three (3) minutes completely wetting the treatment area (5" x 5" for the abdomen or 2" x 5" for the groin).
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 contact times.

22.8 Procedure for Neutralization Validation

The sampling solution, (SS), [redacted] is a buffered detergent solution that is commonly used in studies where microbial sampling of skin is conducted. Neutralizers have been added to inactivate the antimicrobials (Octenidine Dihydrochloride and chlorhexidine gluconate) present in test article. The effectiveness and toxicity of this neutralizer system must be assessed to demonstrate that there is no effect on the growth of microorganisms and that the active ingredient is inactivated.

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

This is a test where the test article is applied to the right and left sides of the abdomen and the treated areas will be sampled. Each sample will then be processed using procedures in accordance with ASTM E1054-08(2013). One sample will be taken from each treatment area and will be processed using methicillin-resistant *Staphylococcus epidermidis* (MRSE) or methicillin-sensitive *Staphylococcus epidermidis* (MSSE).

1. Objective

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

2. Subject Entry Criteria

Four subjects will be used for the neutralization validation required for this study, four per each challenge microorganism. Each subject must meet the inclusion and exclusion criteria described in the protocol except for the baseline bacterial count, the 72-hour exclusion from showering/bathing, and the length of the washout period. No minimum bacterial count is required and the washout period is only necessary for 7 days (not 14 days). An additional exclusion criteria for this procedure is sensitivity to isopropyl alcohol. The subjects will be asked to provide information on demographics and inclusion/exclusion criteria and sign the Consent and Authorization Forms before beginning the 7-day washout period. When the subjects return to begin their participation in the study they will again be asked to provide information relative to inclusion/exclusion criteria. If they meet all inclusion/exclusion criteria, they may be enrolled. The subjects will be identified by the letter "N" for neutralization and a subject number of 01 to 04.

The test article will be applied to the abdomen regions so that four applications (n = 4) are performed for each treatment and each test organism using bilateral application (a total of four subjects per test organism). Each Treatment site will have one sampling site.

The test organisms for this study is:

a. Methicillin-resistant *S. epidermidis* (MRSE), ATCC 51625, marker organism
b. Methicillin-sensitive *S. epidermidis* (MSSE), ATCC 12228, marker organism

3. Treatment Materials

a. Test article: Thermally treated polyester cloth impregnated with 0.4% w/v octenidine dihyrdochloride.
b. Vehicle Control: Thermally treated cloth impregnated with vehicle formulation (not evaluated)
c. Saline Control: 0.9% normal saline applied with polyester cloth (not evaluated)

4. *In-vivo* Test Procedures (collection of samples)

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

- On one side of the body, mark the abdominal test area using a sterile 2" x 5" template.
- After the test area is marked, the area will be processed using three 70% isopropyl alcohol swabs for a total of one minute to prepare the site; the area will be allowed to dry. This step is to prepare the skin for the neutralization test.
- Prep the test area with the Test Article according to the instructions provided in Appendix 22.7.
- Using the scrub cup technique at approximately 10-minutes post-prep, begin collecting samples from the site using SS. This technique is described in the Protocol.
- The procedure is repeated on the other side of the body with the Test Article.

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

Inoculum Preparation:

Two days prior to beginning the neutralization assay, *S. epidermidis* MRSE (ATCC #51625) or *S. 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 1.5×10^8 to 5.0×10^8 CFU/mL. The suspension will then be serially diluted in PBS to achieve an inoculum titer of approximately 1.5×10^3 to 5.0×10^3 CFU/mL, and used as test inoculum. .

(note – the reference to test article applies to all treatment materials; all procedures outlined, where applicable, will employ all treatment materials.).

Neutralizer effectiveness (Test 1):

a. Out of the 6.0 mL aliquot of SS taken from the volunteer, 4.90 mL will be transferred to a new sterile tube and inoculated with 0.1 mL aliquot of the challenge microorganism so that the resulting suspension contains approximately 30 – 100 colony-forming units (CFU) /mL of the challenge microorganism).
b. Within one minute after the addition of the challenge microorganism, the microorganisms will be enumerated by standard microbiological methods extant in the laboratory.
c. Duplicate one mL aliquots will be removed and plated using TSA pour plates.
d. After 30 minutes, the microorganisms will be enumerated a second time using the same procedures.
e. Duplicate one mL aliquots will be removed and plated using TSA pour plates.
f. This procedure will be repeated and additional three times for a total of four replicates for each test organism.

Neutralizer toxicity (Test 2):

a. A 4.90 mL aliquot of SS will be inoculated with 0.1 mL aliquot of the challenge microorganism so that the resulting suspension contains approximately 30 – 100 colony-forming units (CFU) /mL of the challenge microorganism in the same manner as Test 1.
b. Within one minute after the addition of the challenge microorganism, the microorganisms will be enumerated by standard microbiological methods extant in the laboratory.
c. Duplicate one mL aliquots will be removed and plated using TSA pour plates.
d. After 30 minutes, the microorganisms will be enumerated a second time using the same procedures.
e. Duplicate one mL aliquots will be removed and plated using TSA pour plates.
f. This procedure will be repeated an additional three times for a total of four replicates for each test organism.

Test microorganism viability control (Test 3):

a. A 4.90 mL aliquot of PBW will be inoculated with 0.1 mL aliquot of the challenge microorganism so that the resulting suspension contains approximately 30 – 100 CFU/mL in the same manner as Test 1.
b. Within one minute the microorganisms will be enumerated by standard microbiological methods extant in the laboratory.
c. Duplicate one mL aliquots will be removed and plated using TSA pour plates.
d. After 30 minutes, the microorganisms will be enumerated a second time using the same procedures.
e. Duplicate one mL aliquots will be removed and plated using TSA pour plates.
f. This procedure will be repeated an additional three times for a total of four replicates for each test organism.

Test article control (Test 4):

a. A 4.90 mL aliquot of the test or control article will be inoculated with 0.1 mL aliquot of the challenge microorganism so that the resulting suspension contains approximately 30 – 100 CFU/mL in the same manner as Test 1.
b. Within one minute the microorganisms will be enumerated by standard microbiological methods extant in the laboratory.
c. Duplicate one mL aliquots will be removed and plated using TSA pour plates.
d. After 30 minutes, the microorganisms will be enumerated a second time using the same procedures.
e. Duplicate one mL aliquots will be removed and plated using TSA pour plates.
f. This procedure will be repeated an additional three times for a total of four replicates for each test organism.

Incubation:

All plates for Tests 1, 2, 3 and 4 will be incubated for 48 ± 4 hours at 35° ± 2°C.

Interpretation of data:

a. The number of surviving challenge microorganisms for each replicate from each test will be average count of the duplicate plates.
b. The number of survivor values will be transformed to $\log_{10}$.
c. The number of survivors ($\log_{10}$) from each Test (1, 2, and 4) will be compared to the test microorganism viability population (Test 3).
d. Neutralization aspects of the SS will be considered adequate if the mean $\log_{10}$ CFU/mL of Test 1 is not more than 0.20 $\log_{10}$ less than the mean $\log_{10}$ CFU/mL of Test 3 (Mean $\log_{10}$ CFU/mL from Test 3 – Mean $\log_{10}$ CFU/mL from Test 1 using corresponding time points).
e. The SS will be considered non-toxic if the mean $\log_{10}$ CFU/mL of Test 2 is not more than 0.20 $\log_{10}$ less than the mean $\log_{10}$ CFU/mL of Test 3 (Mean $\log_{10}$ CFU/mL from Test 3 – Mean $\log_{10}$ CFU/mL from Test 2 using corresponding time points).
f. All sterility controls must be negative for growth.
g. The amount of CFU added for each aspect must be confirmed to yield a final suspension containing approximately 30-100 CFU/mL (validated in Test 3).

Controls:

Sterility Control

Duplicate plates of TSA and TSA+ used will be incubated with the test. In addition, duplicate 1.0-mL aliquots of SS and PBW will be plated using TSA pour plates. All plates will be incubated with the test plates.

Challenge microorganism confirmation:

Visually confirm the morphology characteristics of both the test organisms following all Tests with all products.

Challenge microorganism Assays:

An initial assay confirming the amount of microorganism present will be performed prior to testing and a final assay will be performed to confirm the amount of microorganism present at the completion of the assay, pour plated in duplicate using TSA.

22.9 Skin Irritation Rating Scale

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

[a] = Represents significant irritation in any category and may require subject's removal from the study.

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

[C] = If a subject receives a score of 1 for erythema, edema, rash, and/or dryness prior to baseline sampling on Treatment Day, they will be dismissed from testing that day, and can move to another group and participate once the irritation subsides.

BD PROTOCOL # MPS-17IPVAW10
BIOSCIENCE LABORATORIES, INC., PROTOCOL #1704167-103

Protocol Title: A Randomized, Single-Center, Blinded, Clinical Evaluation of the Antimicrobial Effectiveness of an Antimicrobial Cloth Compared to Vehicle and 0.9% Saline

Protocol Version: Version 1.0/05.25.17

ACCEPTED BY: BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1765 South 19th Avenue
Bozeman, Montana 59718

Principal Investigator: _____ 06/01/2017
Christopher M. Beausoleil, CCRP — Date of Study Initiation

GIRB Approved
Date: 6-5-17
Initial: BL

Signature Page for MPS-17IPVAW10 Protocol 30May2017

Signature Page for MPS-17IPVAW10 Protocol 30May2017 - System Version No. 1.0

GIRB Approved
Date: 6-5-17
Initial: