BD Protocol #: **MPS-17IPVSS02**

Eurofins Evic Romania Study #: **ER18/149**

Protocol Title: **A Randomized, Single-Center, Partially Blinded, Evaluation of the Time-Dependent Antimicrobial Effectiveness of Swabsticks Impregnated with 2% (w/v) Chlorhexidine Gluconate in 70% (v/v) Isopropyl Alcohol in Healthy Volunteers**

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Sponsor	BD – Becton, Dickinson & Company 75 N. Fairway Dr., Vernon Hills, IL 60061
Sponsor Medical Monitor	[redacted] VP, Medical Affairs, Interventional Segment [redacted]

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 applicable regulatory requirements and the Good Clinical Practice guidelines set forth by the International Conference on Harmonization (ICH-E6).

All Study Product(s) used in this study should be considered to be for investigational use only.

NCT Number: [NCT04035161] (Number added post-approval per CT.gov requirement)

SPONSOR PROTOCOL APPROVAL

Signature below indicates approval of the protocol as written.			
Individual or function	**Name**	**Signature**	**Date**
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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 Investigator Date

SUMMARY

Protocol Number:	MPS-17IPVSS02
Protocol Title:	A Randomized, Single-Center, Partially Blinded, Evaluation of the Time-Dependent Antimicrobial Effectivess of Swabsticks Impregnated with 2% (w/v) Chlorhexidine Gluconate in 70% (v/v) Isopropyl Alcohol in Healthy Volunteers
Planned Sample Size:	This study will include a minimum of 516 evaluable subjects to achieve 280 evaluable sites per anatomical location for the investigational product (IP), active control (AC), and negative control (NC) and 192 evaluable sites per anatomical location for the reference standard (RS).
Study Design:	This single site study is a randomized, controlled, partially-blinded design enrolling a minimum of 516 healthy volunteers, where each subject will receive two of the planned study products on the product application sites of the abdomen and/or groin.
Study Methodology:	ASTM Standard Test Method E1173-15
Study Population:	Healthy male and female volunteers, 18 years of age or older. Screening Day bacterial count requirements of at least 3.20 $\log_{10}/cm^2$ on the abdomen and 5.50 $\log_{10}/cm^2$, on the groin. Product Application Day baseline bacterial count requirements are in the range of 3.20 to 6.00 $\log_{10}/cm^2$, inclusive, on the abdomen or 5.50 to 7.50 $\log_{10}/cm^2$, inclusive, on the groin.
Objectives:	To demonstrate the antimicrobial activity of the Investigational Product (IP) administered as a single Swabstick application by comparing counts of resident skin microbes, obtained after IP application to intact skin of the abdomen and groin, to counts obtained prior to application. In this study, the antimicrobial activity of the IP will be investigated according to established testing requirements described by Health Canada (HC) and the US FDA. **<u>Efficacy Objectives according to HC testing requirements</u>** **Primary Objective:** To demonstrate the immediate and persistent antimicrobial activity of a single Swabstick impregnated with 2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol at 30 seconds and 6 hours post product application. **Exploratory Objectives:** 1) To evaluate the $\log_{10}$ reductions from baseline for the IP and RS at each anatomical site at 10 minutes post product application. 2) To determine if the 30-second, 10 minutes and 6 hour $\log_{10}$ reductions on the abdomen and groin for the IP are not statistically worse than those for 60% v/v 1-propanol post product application.

Protocol Number:	MPS-17IPVSS02
	Efficacy Objectives according to FDA testing requirements **Primary Objectives:** 1) To demonstrate superior antimicrobial activity of the IP, a single Swabstick impregnated with 2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol, to a negative control (NC) of 0.9% normal saline and the non-inferiority of the IP to an active control (AC) at the immediate time point of 10 minutes on the abdomen and groin. 2) To demonstrate persistence of the IP at 6 hours post product application on the abdomen and the groin. **Secondary Objective:** To demonstrate superior antimicrobial activity of the IP, a single Swabstick impregnated with 2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol, to a negative control (NC) of 0.9% normal saline and the non-inferiority of the IP to an active control (AC) at 30 seconds post product application on the abdomen. **Exploratory Objectives:** 1) To evaluate the $\log_{10}$ reductions from baseline for the IP, AC and NC at each anatomical site at 30 seconds, 10 minutes, and 6 hours post product application. 2) To evaluate the amount of product applied (g) to each anatomical site for each product. **Safety Objectives:** To evaluate safety using skin irritation scores and the incidence of adverse events reported during the study.
Study Endpoints and Acceptance Criteria:	**Efficacy Endpoints:** The primary endpoint will be $\log_{10}$ CFU/cm^2 of skin before and after product application at 30 seconds, 10 minutes and 6 hours on the abdomen and groin. **Acceptance Criteria according to HC analysis:** For the IP: 1. Immediate effectiveness is at least a 2-$\log_{10}$ CFU/cm^2 reduction from baseline skin flora counts for the abdomen at 30 seconds. 2. Immediate effectiveness is at least a 3-$\log_{10}$ CFU/cm^2 reduction from baseline skin flora counts for the groin at 30 seconds. 3. Persistence effectiveness is at least a 2- $\log_{10}$ CFU/cm^2 reduction from baseline skin flora counts for the abdomen at 6 hours. 4. Persistence effectiveness is at least a 3- $\log_{10}$ CFU/cm^2 reduction from baseline skin flora counts for the groin at 6 hours. The reference standard, 60% v/v 1-Propanol, should meet the same efficacy standards as the investigational product.

Protocol Number:	MPS-17IPVSS02
	Acceptance Criteria according to FDA analysis: For assessment of the primary objectives (i.e., immediate activity at 10 minutes post application on the abdomen and groin): 1. A non-inferiority criterion with a 0.5 $\log_{10}$ margin will be implemented for the average treatment effect (ATE) of the IP compared to the AC (i.e., the upper two-sided 95% confidence bound of the post-product application bacterial load corrected for pre-product application bacterial load of the IP – AC should be less than 0.5 $\log_{10}$). 2. A 1.2 $\log_{10}$ superiority criterion will be implemented for the ATE of the IP compared to the NC (i.e., the lower two-sided 95% confidence bound of the post-product application bacterial load corrected for pre-product application bacterial load of the NC – IP should be greater than 1.2 $\log_{10}$). Persistence of efficacy will be defined at 6 hours post-application as having $\geq$0 $\log_{10}$ reduction from baseline bacterial load. No inferential analysis will be performed on the 6 hour time point data. For assessment of the secondary objective (i.e., antimicrobial activity at 30 seconds post application on the abdomen): 1. A non-inferiority criterion with a 0.5 $\log_{10}$ margin will be implemented for the ATE of the IP compared to the AC (i.e., the upper two-sided 95% confidence bound of the post-product application bacterial load corrected for pre-product application bacterial load of the IP – AC should be less than 0.5 $\log_{10}$). 2. A 1.2 $\log_{10}$ superiority criterion will be implemented for the ATE of the IP compared to the NC (i.e., the lower two-sided 95% confidence bound of the post-product application bacterial load corrected for pre-product application bacterial load of the NC – IP should be greater than 1.2 $\log_{10}$). Assessment of the primary endpoints (i.e., antimicrobial activity at 10 minutes post application on both abdomen and groin) and secondary endpoints (i.e., antimicrobial activity at 30 seconds post application on the abdomen) will follow a gatekeeping procedure: the secondary endpoints (i.e., antimicrobial activity at 30 seconds post application on the abdomen) will be evaluated only when the assessment of the primary endpoints (i.e., immediate activity at 10 minutes post application on the abdomen and groin) passes non-inferiority and superiority criteria on both abdomen and groin. **Safety endpoints:** Safety will be evaluated using skin irritation scores and the incidence of AEs reported during the study.

<table>
<tr><td>Protocol Number:</td><td>MPS-17IPVSS02</td></tr>
<tr><td>Study Products:</td><td>
Investigational Products (IP):
Single Swabstick impregnated with 1.75 mL of 2% (w/v) chlorhexidine gluconate (CHG) in 70% (v/v) isopropyl alcohol (thermally treated)
Reference Standard (RS):
1.75 mL of 60% (v/v) 1-propanol applied with a single Swabstick
Active Control (AC):
ChloraPrep® SEPP® clear applicator-2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol
Negative Control (NC):
1.75 mL 0.9% normal saline applied with a single Swabstick

Study Products, Anatomical Sites Evaluated, Application and Dry Times, and Coverage Areas:
<table>
<tr><th>Study Products</th><th>Anatomical Site</th><th>Application Time</th><th>Dry Time</th><th>Area of Coverage</th></tr>
<tr><td rowspan="2">IP</td><td>Abdomen</td><td>30 seconds</td><td>30 seconds</td><td>2.5” x 2.5” (6.4 cm x 6.4 cm)</td></tr>
<tr><td>Groin</td><td>2 minutes</td><td>1 minutes</td><td>1.3” x 5” (3.3 cm x 12.7 cm)</td></tr>
<tr><td rowspan="2">AC</td><td>Abdomen</td><td>30 seconds</td><td>30 seconds</td><td>2.5” x 2.5” (6.4 cm x 6.4 cm)</td></tr>
<tr><td>Groin</td><td>2 minutes</td><td>1 minutes</td><td>1.3” x 5” (3.3 cm x 12.7 cm)</td></tr>
<tr><td rowspan="2">NC</td><td>Abdomen</td><td>30 seconds</td><td>30 seconds</td><td>2.5” x 2.5” (6.4 cm x 6.4 cm)</td></tr>
<tr><td>Groin</td><td>2 minutes</td><td>1 minutes</td><td>1.3” x 5” (3.3 cm x 12.7 cm)</td></tr>
<tr><td rowspan="2">RS</td><td>Abdomen</td><td>30 seconds</td><td>30 seconds</td><td>2.5” x 2.5” (6.4 cm x 6.4 cm)</td></tr>
<tr><td>Groin</td><td>2 minutes</td><td>1 minutes</td><td>1.3” x 5” (3.3 cm x 12.7 cm)</td></tr>
</table>
</td></tr>
</table>

Statistical Methods:	The full intent-to-treat (ITT) data set (all randomized subjects that received study product) will be used for the safety analysis. A modified intent-to-treat (mITT) data set will be used for efficacy analyses for both the FDA and HC analyses. Data included in the mITT data set is evaluated for each anatomical site (left and right for the groin and abdomen). For each anatomical site, if the Product Application Day baseline bacterial count is in the range of 3.20 to 6.00 $\log_{10}/cm^2$, inclusive, on the abdomen or 5.50 to 7.50 $\log_{10}/cm^2$, inclusive, on the groin, then the data from that anatomical site are included in the mITT data set. Supportive analysis for both the FDA and HC analyses will be performed on the per-protocol population (PP). The PP data set will include evaluable product application sites from the mITT data set that adhere to defined assessments and procedures in the protocol central to patient enrollment, safety, rights or wellbeing, as well as the completeness, accuracy and reliability of study data. Additional details on qualification for the PP data set are outlined in the Statistical Analysis Plan (SAP). A summary table will present $\log_{10}$ CFU/cm^2 for each study product on each anatomical site at each time point. 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 study product arms with similar baseline CFU/cm^2 values. **Safety Analysis:** The statistical significance of differences in skin irritation between the study products at each post application sampling time will be evaluated by Fisher's exact test on skin irritation data summarized for safety analysis as follows: any reaction above a 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 study products, follow-up analyses will be conducted to determine how the reactions differ. No statistical analysis will be performed on the incidence of AEs. **HC Efficacy Analyses** **HC Primary Analysis:** Log_{10} CFU/cm^2 changes from baseline will be calculated separately for each subject, each of the four sites (left and right for the abdomen and groin), and each post-product application sampling time by taking the baseline $\log_{10}$ CFU/cm^2 values and then subtracting the $\log_{10}$ CFU/cm^2 values for the samples taken after baseline. The mean $\log_{10}$ CFU/cm^2 changes from baseline will be calculated separately for the IP and the RS, at each anatomical site (abdomen and groin), and at each post-product application sampling time point (30 seconds and 6 hours). The following descriptive statistics for $\log_{10}$ CFU/cm^2 reductions will be computed for the IP and RS, grouped by anatomical site and each post application sampling time point (30 seconds and 6 hours): mean, median, standard deviation, minimum, maximum, and count. The 95% confidence intervals (two-sided) will be calculated for the observed log reductions for the IP and RS at each time point on each anatomical area using one-sample t-test.

	The goal for the IP is that the mean log_{10} reduction of all four primary objectives meets the following criteria: 1. The lower limit of the 95% confidence interval (two-sided) of mean log reduction at 30 seconds will be greater than or equal to 2 for the abdomen. 2. The lower limit of the 95% confidence interval (two-sided) of mean log reduction at 30 seconds will be greater than or equal to 3 for the groin. 3. The lower limit of the 95% confidence interval (two-sided) of mean log reduction at 6 hours will be greater than or equal to 2 for the abdomen. 4. The lower limit of the 95% confidence interval (two-sided) of mean log reduction at 6 hours will be greater than or equal to 3 for the groin The HC testing requirements will only be considered met when and only when all four primary objectives pass HC analysis acceptance criteria. No multiplicity adjustment is required. The RS should meet the same efficacy standards as the IP. **HC Exploratory Analysis:** 1) The following descriptive statistics for log_{10} CFU/cm^2 reductions will be computed for the IP and RS, grouped by anatomical site and each post application sampling for the 10 minute time point: mean, median, standard deviation, minimum, maximum, and count. The 95% confidence intervals (two-sided) will be calculated for the observed log reductions for the IP and RS at the 10 minute time point on each anatomical area. 2) Log_{10} CFU/cm^2 reductions at 30 seconds, 10 minutes, and 6 hours on the abdomen and the groin for the IP will be compared to the RS using two-sample t-test. **<u>FDA Efficacy Analyses</u>** **FDA Primary Analysis:** A linear regression model for each body site (abdomen and groin) will be used for primary analysis of efficacy at 10 minutes. In the model, the response is the post-product application bacterial counts at 10 minutes and predictors are the study products as a fixed effect and the pre-product application bacterial loads as a covariate. The ATE corrected for pre-product application bacterial loads will be estimated from the model and compared to both non-inferiority and superiority criteria. The FDA testing requirements for the primary analysis will only be considered met when and only when all four primary objectives (10 minutes for both abdomen and groin) pass FDA analysis acceptance criteria, no multiplicity adjustment is required. To evaluate the persistent antimicrobial properties of a single Swabstick impregnated with 2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol, log_{10} bacterial counts at 6 hours post product application for each product application site will be converted to a binary (yes or no) success measure with success defined as having skin flora counts that are less than or equal to the baseline skin flora counts. Responder rates at 6 hours post product application will be summarized descriptively for each

	product on each body site. No inferential analysis will be performed on the 6 hour time point data. **FDA Secondary Analysis:** The secondary objectives will be evaluated only when the four co-primary objectives pass both non-inferiority and superiority criteria. A linear regression model for the abdomen will be used for a secondary analysis of efficacy at 30 seconds. In the model, the response is the post-product application bacterial counts at 30 seconds and predictors are the study products as a fixed effect and the pre-product application bacterial loads as a covariate. The ATE corrected for pre-product application bacterial loads will be estimated from the model and compared to both non-inferiority and superiority criteria. **FDA Exploratory Analyses:** The following descriptive statistics for $\log_{10}$ CFU/cm^2 reductions will be computed for the IP, AC and NC at each anatomical site at 30 seconds, 10 minutes, and 6 hours post product application: mean, median, standard deviation, minimum, maximum, and count. Product weight expression will be calculated as product weight prior to product application (g) – product weight post-product application (g). The following descriptive statistics for expression weight will be computed for each product at each anatomical site: mean, median, standard deviation, minimum, maximum, and count.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AC	Active control
AE	Adverse event
ANOVA	Analysis of variance
ATE	Average treatment effect
BD	Becton, Dickinson and Company
CFR	Code of Federal Regulations
CFU	Colony Forming Units
CHG	Chlorhexidine gluconate
CI	Confidence Interval
CRF	Case Report/Record Form
DMC	Data Monitoring Committee
EDC	Electronic Data Capture
FDA	Food and Drug Administration
FDAAA	FDA Amendments Act of 2007
GCP	Good Clinical Practice
GMP	Good Manufacturing Practices
HC	Health Canada
IB	Investigator's brochure
ICH	International Conference on Harmonization
IP	Investigational product
IRB/EC	Institutional or Independent Review Board/Ethics Committee
ITT	Intent to treat
IUD	Intra-uterine device
LLOQ	Lower limit of quantification
mITT	Modified Intent to Treat
NC	Negative control
NDA	New Drug Application
PBW	Phosphate Buffered Water
PP	Per-Protocol
QA	Quality assurance
RS	Reference Standard
SAE	Serious Adverse Event
SD	Standard deviation
SOP	Standard Operating Procedure
SS	Sampling solution
TSA	Trypticase Soy Agar
TSA + N	Trypticase soy agar containing neutralizers
TFM	Tentative Final Monograph
UPT	Urine pregnancy test
v/v	Volume per volume
WHO	World Health Organization
w/v	Weight per volume

1.0 INTRODUCTION

Topical antimicrobial products are used prior to surgery in order to reduce the risk of nosocomial infection by reducing the number of microorganisms on the skin.

Chlorhexidine gluconate (CHG) was first introduced in the United States in the 1970s as a hand washing agent for healthcare workers. It is a broad-spectrum antiseptic against Gram-positive and Gram-negative bacteria and has fair fungicidal effects. CHG binds strongly to skin and has residual properties documented up to 48 hours. Isopropyl alcohol (IPA) has a long history of use as a topical antiseptic and is included in many over-the-counter products, such as hand sanitizers and other skin preparations products, due to its immediate broad-spectrum antimicrobial effects. The combination of CHG and IPA provides an antiseptic product for intact skin that produces immediate and persistent antimicrobial effects against many species of microorganisms. Numerous clinical studies support the use of the alcoholic CHG formulations, driving its wide adoption by clinicians worldwide.

In this study, the antimicrobial activity of the investigational product (IP), a single Swabstick impregnated with 1.75 mL of 2% (w/v) chlorhexidine gluconate (CHG) in 70% (v/v) isopropyl alcohol, will be investigated according to established testing requirements described by Health Canada (HC) and the US FDA. For evaluation of efficacy using HC analysis criteria, to meet the immediate and persistent effectiveness standards, the IP should achieve a mean 2-$\log_{10}$ per cm^2 reduction on the abdomen site and a mean 3-$\log_{10}$ per cm^2 reduction on the groin site. A reference standard, 60% v/v 1-Propanol, will be applied with a single Swabstick and will also be compared to the same log reduction standards the IP. Immediate antimicrobial activity will be evaluated at 30 seconds and persistent antimicrobial activity will be evaluated at 6 hours post-product application on the abdomen and the groin.

For evaluation of antimicrobial activity using the FDA analysis criteria, the immediate and persistent antimicrobial efficacy of the IP will be evaluated. For immediate activity, the IP will be compared to a negative control (NC) of 0.9% saline and the ChloraPrep™ SEPP® Clear Applicator active control (AC). The primary objective is to demonstrate superiority of the IP compared to the NC and non-inferiority of the IP to the AC at the immediate time point of 10 minutes post product application on the abdomen and the groin. The persistent antimicrobial primary objective is to demonstrate that microbial levels remain at or below baseline levels 6 hours post product application. If the primary immediate objective passes both the non-inferiority and superiority criteria on abdomen and groin, then the secondary objective will be evaluated for antimicrobial efficacy. The secondary objective is to demonstrate superior antimicrobial activity of the IP compared to the NC and non-inferiority of the IP to the AC at 30 seconds post product application on the abdomen only.

2.0 OBJECTIVES

2.1 Safety Objectives

To evaluate safety using skin irritation scores and the incidence of adverse events reported during the study for all study products.

2.2 Health Canada Analysis

2.2.1 Primary Efficacy Objectives

To demonstrate the immediate and persistent antimicrobial activity of a single Swabstick impregnated with 2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol at 30 seconds and 6 hours post product application.

To meet the immediate effectiveness standards, the IP should achieve a mean 2-$\log_{10}$ per cm^2 reduction on the abdomen site and a mean 3-$\log_{10}$ per cm^2 reduction on the groin site at 30 seconds. The primary efficacy objective

for persistent effect is the IP meets the same log_{10} CFU/ cm^2 reduction goals at 6 hours as for 30 seconds. The reference standard of 60% v/v 1-propanol (RS) applied with a Swabstick, should meet the same efficacy standards as the IP.

2.2.2 Exploratory Objectives

The first exploratory objective is to evaluate the log_{10} CFU/cm^2 reductions from baseline for the IP and RS at each anatomical site, abdomen and groin, at 10 minutes post-product application. The second exploratory objective is to determine if the 30-second, 10 minutes and 6 hour log_{10} CFU/cm^2 reductions on the abdomen and groin for the IP are not statistically worse than those for 60% v/v 1-propanol post product application.

2.3 FDA Efficacy Analysis

2.3.1 Primary Objectives

To demonstrate the immediate and persistent antimicrobial properties of a single Swabstick impregnated with 2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol to ChloraPrep® SEPP® Clear Applicator AC and a NC of 0.9% normal saline at 10 minutes and 6 hours post product application.

To demonstrate immediate antimicrobial efficacy at 10 minutes post-application, there are four co-primary objectives for the IP. The first two objectives are to demonstrate the non-inferiority of the average treatment effect (ATE) of the IP compared to the AC (i.e., the upper two-sided 95% confidence bound of the post-product application bacterial counts corrected for pre-product application bacterial load of the IP – AC should be less than 0.5 log_{10}) on both the abdomen and the groin. The second two objectives are to demonstrate the superiority of the ATE of the IP compared to the NC (i.e., the lower two-sided 95% confidence bound of the post-product application bacterial counts corrected for pre-product application bacterial load of the NC – IP should be greater than 1.2 log_{10}) on both the abdomen and the groin.

To evaluate persistent antimicrobial efficacy at 6 hours post-product application, log_{10} bacterial counts for each product application site will be converted to a binary (yes or no) success measure with success defined as having skin flora counts that are less than or equal to the baseline skin flora counts. Responder rates at 6 hours post product application will be summarized descriptively for each product on each body site.

2.3.2 Secondary Objectives

To demonstrate antimicrobial efficacy at 30 seconds post-application, there are two objectives for the IP. The first objective is to demonstrate the non-inferiority of the average treatment effect (ATE) of the IP compared to the AC (i.e., the upper two-sided 95% confidence bound of the post-product application bacterial counts corrected for pre-product application bacterial load of the IP – AC should be less than 0.5 log10) on the abdomen. The second objective is to demonstrate the superiority of the ATE of the IP compared to the NC (i.e., the lower two-sided 95% confidence bound of the post-product application bacterial counts corrected for pre-product application bacterial load of the NC – IP should be greater than 1.2 log10) on the abdomen. The two secondary objectives will be evaluated only when the four co-primary objectives pass both non-inferiority and superiority criteria.

2.3.3 Exploratory Objectives

The first exploratory objective is to evaluate the log_{10} CFU/cm^2 reductions from baseline for the IP, AC, and NC at each anatomical site at 30 seconds, 10 minutes and 6 hours. For the second exploratory objective, product weight expression from product application will be calculated as product weight prior to product application (g) – product weight post-product application (g).

3.0 STUDY DESIGN

3.1 Overall Study Design

This single site study is a randomized, controlled, partially blinded, design enrolling a minimum of 516 healthy volunteers, where each subject will receive two of the planned study products on the product application sites of the abdomen and/or groin. In this study, the antimicrobial activity of the investigational product (IP), a single swabstick impregnated with 1.75 mL of 2% (w/v) chlorhexidine gluconate (CHG) in 70% (v/v) isopropyl alcohol, will be investigated according to established testing requirements described by Health Canada (HC) and the US FDA. The study methods for this evaluation will be based on ASTM Standard Test Method E1173-15.

3.1.1 Efficacy Endpoints

The evaluation of antimicrobial activity will be based on $\log_{10}$ CFU/cm^2 of resident microbes on skin before and after each product application at 30 seconds, 10 minutes, and 6 hours on the abdomen and groin.

3.1.2 Safety Endpoints

The principal measures of safety will be skin reactions using the modified Berger Bowman irritation assessment scale and the incidence of AEs reported during the study.

3.2 Acceptance Criteria

To be included in the primary analysis (modified intent-to-treat (mITT) data set), a product application site must have a Product Application Day microbial baseline within the stated requirements. **Error! Reference source not found.** summarizes the baseline criteria for the mITT data set for each anatomical site and the expected minimum efficacy country specific standards.

Table 1: mITT data set for each anatomical site and expected minimum efficacy standards					
Anatomical Product Application Site	**Product Application Day Baseline Criteria**	**HC Analysis**		**FDA Analysis**	
		Acceptance Criteria at 30 sec	**Acceptance Criteria at 6 hours**	**Acceptance Criteria at 30 sec**	**Acceptance Criteria at 10 min**
Abdomen	3.20 to 6.00 log_{10}/cm^2	Immediate effectiveness for the IP is at least a 2-log_{10} CFU/cm^2 reduction from baseline skin flora counts at 30 seconds.	Persistence effectiveness for the IP is at least a 2-log_{10} CFU/cm^2 reduction from baseline skin. flora counts at 6 hours.	At 30 seconds the upper two-sided 95% confidence bound of the average treatment effect (ATE) of IP - AC should be less than 0.5 log_{10} At 30 seconds the lower two-sided 95% confidence bound of the ATE of NC - IP should be greater than 1.2 log_{10}	At 10 minutes the upper two-sided 95% confidence bound of the average treatment effect (ATE) of IP - AC should be less than 0.5 log_{10} At 10 minutes the lower two-sided 95% confidence bound of the ATE of NC - IP should be greater than 1.2 log_{10}
Groin	5.50 to 7.50 log_{10}/cm^2	Immediate effectiveness for the IP is at least a 3-log_{10} CFU/cm^2 reduction from baseline skin flora counts at 30 seconds.	Persistence effectiveness for the IP is at least a 3-log_{10} CFU/cm^2 reduction from baseline skin. flora counts at 6 hours.	None	At 10 minutes the upper two-sided 95% confidence bound of the ATE of IP - AC should be less than 0.5 log_{10} At 10 minutes the lower two-sided 95% confidence bound of the ATE of NC - IP should be greater than 1.2 log_{10}

3.3 Product Application Allocation and Methods to Reduce Bias

3.3.1 Randomization

The randomization scheme for the study will be provided by the Sponsor. A sufficient number of subjects will be recruited and newly randomized to meet the minimum number of evaluable product application sites per study product arm, preserving the randomization assignment. The number of evaluable product application sites per study product arm per anatomical area will be monitored to determine additional subject enrollment and randomization to meet the required evaluable sample size. Subjects who fail to meet Product Application Day baselines will not be replaced. Additional subjects will be randomized to account for product application day failures.

Subjects will be randomized to study product arms using the following block design:

Study Product Balance

Each subject will receive two different study products, one on the right side of the body and one on the left. Therefore, there are six possible study product combinations: A-B, A-C, A-D, B-C, B-D and C-D.

A minimum of 516 evaluable subjects will be required to achieve a total of a minimum of 192 evaluable product application sites for groin and abdominal regions for 60% (v/v) 1-propanol (RS) and 280 evaluable product application sites for groin and abdominal regions for the other three study product arms. Due to the imbalance of the sample size between RS and other products, RS cannot be blinded. Assuming the treatment code for RS is B and all the other three products are either A, C or D, within each small block [i.e., every 129 (516/4) evaluable subjects randomized], number of subjects who receive each product combination is shown below. With 4 blocks in total (i.e., 516 evaluable subjects), the minimum of 192 evaluable product application sites for groin and abdominal regions for 60% (v/v) 1-propanol (RS) and 280 evaluable product application sites for groin and abdominal regions for the other three study product arms can be achieved.

Product Combination	No. of Subjects in Each Block
A-B	16
A-C	27
A-D	27
B-C	16
B-D	16
C-D	27
Total	129

Left/Right Balance

The application will be randomized so that each study product is applied on an equal number of left and right sides of the body within each block as much as possible. Due to baseline bacterial requirements the mITT data set may be imbalanced with regard to right and left sides upon achieving the minimum number of qualifying body sites.

Site and Sample Time Balance

Each groin and abdomen product application site is divided into four sampling areas and each of these sampling areas is sampled once – one at baseline, one at 30 seconds, one at 10 minutes, and one at 6 hours post product application. Therefore, for any groin or abdomen site there are 24 possible sampling orders.

Overall the following priority order will be used for randomization schedule design:

1. Study product combinations will always be applied with assigned numbers in each block to preserve the 6 potential study product combinations of the four study products.
2. Left/right balance will be preserved – each study product will be applied an approximately 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 with respect to all three factors 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 2. The final randomization schedule will be prepared by the study statistician before the initial product application.

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

Randomized subjects will be assigned numbers ranging from 0001 to a four digit number equal to the total number of subjects needed (sufficient randomization codes will be prepared to ensure sufficient codes are available for the indicated maximum evaluable sample size plus subjects not meeting product application day baseline requirements).

3.3.2 Blinding

Study products for the following groups will be blinded with study product codes A, B, C, or D:

Table 1 Blinded Study Product Codes

Study Arm	Study Product Code	Product
Investigational Product (IP)	A, C, or D	Single Swabstick impregnated with 2% (w/v) chlorhexidine gluconate (CHG) in 70% (v/v) isopropyl alcohol
Reference Standard (RS)	B	60% 1-propanol applied with a single Swabstick
Active Control (AC)	A, C, or D	ChloraPrep SEPP- 2% chlorhexidine gluconate in 70% isopropyl alcohol
Negative Control (NC)	A, C, or D	0.9% normal saline applied with a single Swabstick

Study products cannot be blinded to staff member(s) performing product applications, sample collections, and irritation assessments due to package labeling of the marketed product (AC) or study procedures for the negative control and reference control. Additionally, the RS cannot be blinded due to the imbalanced randomization.

Staff member(s) performing bacterial enumeration will be blinded from the identification of all study product assignments. In addition, 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 product 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.

3.3.2.1 Emergency Procedure for Unblinding

The BD Quality Assurance (QA) manager will provide study unblinding information directly to site QA department manager in individual sealed envelopes (one for each blinded study product code), which will remain unopened and secured on site. Response of medical personnel to an AE potentially associated with the study products will not necessitate unblinding unless treatment is impacted. If an emergency requires unblinding, the envelope corresponding to the study product code that is associated with the AE will be opened by the site QA manager to reveal the study product identification for that single study product. If possible, the Investigator or designee will contact the Sponsor with notification of the intent to unblind the study product codes prior to the actual unblinding. If it is not possible to notify the Sponsor prior to the unblinding, the 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 Investigator or designee shall also provide written notification of the unblinding to the IRB. The site QA manager will communicate the study product identification of the code associated with the AE to only the study personnel who require the information to manage the emergency.

3.3.2.2 Measures to Control Bias

For this study, several measures are being used to control or reduce potential bias. Those measures include, but are not limited to the following:

• Subject randomization;

• Statistical Analysis Plan lists potential protocol deviations and rules for use of associated data in analysis;

• Products will be labeled with treatment codes (A, B, C, D);

• Data Management Plan outlines steps to prevent Sponsor study team members from becoming aware of treatment codes.

3.4 Stopping Rules

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

4.0 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), isopropyl alcohol or chlorhexidine gluconate will be enrolled into this study. A sufficient number of volunteers will be enrolled such that a minimum number of evaluable product application sites are available for the groin (sebaceous rich) region and the abdominal (sebaceous poor) region for each of the study products. A minimum of 280 evaluable sites per anatomical location is required for the IP, AC, and NC. A minimum number of 192 evaluable site per anatomical location is required for the RS.

The minimum number of volunteers enrolled will be 516 based on the objective to assess four study product arms on the required number of unique skin sites for the groin and abdomen. Subjects must satisfy all Screening Day and Product Application Day Inclusion/Exclusion Criteria prior to Product Application Day procedures.

Subjects must qualify on all four abdomen and inguinal sites to be admitted into the study until such time that one of the anatomical sites has completed the minimal required evaluable sites, then subjects will be screened and treated on the remaining anatomical site. To be included in the primary analysis (modified Intent to Treat (mITT) data set), a product application site must have a Product Application Day microbial baseline within the stated requirements. Enrollment will continue until a minimum number of subjects meeting baseline criteria on each anatomical site per study product arm is identified.

Subjects will be treated utilizing bilateral applications to ensure that the study products will be evaluated on the sites as described in Table . Each subject has four independent product application sites: left and right sides for the abdomen and left and right sides for the groin.

Table 3 Estimated Number of Evaluations

Arm	Study Product	Number of Abdomen Evaluations	Number of Groin Evaluations
Investigational Product	Single Swabstick impregnated with 2% (w/v) chlorhexidine gluconate (CHG) in 70% (v/v) isopropyl alcohol	280	280
Reference Standard	60% 1-propanol applied with a single Swabstick	192	192
Negative Control	0.9% normal saline applied with a single Swabstick	280	280
Active Control	ChloraPrep SEPP- 2% chlorhexidine gluconate in 70% isopropyl alcohol	280	280

4.1 Inclusion Criteria

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

1. Read, understand, and provide signed informed consent.
2. Are healthy subjects in good general health
3. Are 18 years of age or older
4. If females of childbearing potential, are using an acceptable form of birth control for at least 28 days immediately preceding Day 1 and throughout the duration of the study. Acceptable methods are established, effective hormonal contraception (oral/implant/injectable/transdermal/intra-vaginal), intra-uterine device [IUD], diaphragm with spermicide, condom with spermicide, abstinence, bilateral tubal ligation, or are in a monogamous relationship with a partner who has had a vasectomy
5. In the case of females of childbearing potential, have a negative urine pregnancy test (UPT) on Product Application Day prior to any applications of the study products..
6. Are free of any systemic or dermatologic disorder, which, in the opinion of the Investigator, will interfere with the study results or increase the risk of AEs
7. Have minimal Screening Day bacterial count requirements of 3.20 $\log_{10}/cm^2$ on the abdomen and 5.50 $\log_{10}/cm^2$ on the groin.

4.2 Exclusion Criteria

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

1. Have had 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) within the product restriction period and for the remainder of the study. Restrictions include, but are not limited to antimicrobial soaps, antiperspirants/deodorants, shampoos, lotions, perfumes, after shaves, and colognes
2. Subjects who have a history of skin allergies.
3. Have known allergies or sensitivity to vinyl, latex (rubber), alcohols, metals, inks, common consumer/beauty products, tape adhesives, or to common antibacterial agents found in soaps, lotions, or ointments, particularly chlorhexidine, or any other product components
4. Swimming in chemically treated pools or bathing in hot tubs, spas and whirlpools within the product restriction period and for the remainder of the study.
5. Use of tanning beds, hot waxes, or depilatories, including shaving (in the applicable product application areas) within the product restriction period and for the remainder of the study
6. Have had exposure of the product application sites to strong detergents, acids, bases, bug repellant, fabric softener-treated clothing, UV treated clothing, other household chemicals in the applicable product application areas or other irritants during the product restriction period or during the study period
7. Have a history of skin cancer within 6 inches of applicable product application areas, or are currently being treated for skin cancer or have received treatment for any type of internal cancer within the 5 years prior to enrollment

8. Subjects who have a history of asthma, 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)
9. A currently active skin disease or inflammatory skin condition (for example contact dermatitis) anywhere on the body
10. Any tattoos or scars (including stretch marks) on the product application sites or within 2 inches of the product application sites; skin blemishes or warts may be permissible with the specific approval of the Investigator or consulting physician
11. Dermatoses, cuts, lesions, active skin rashes, scabs, breaks in the skin or other skin disorders within 6 inches on or around the product application sites
12. Subjects who have showered or bathed within at least 72 hours of the Product Application Day. Sponge baths may be taken, however, the lower abdomen and upper thigh region must be avoided
13. Subjects who have an irritation score of 1 or greater (any redness, swelling, rash, or dryness present at any product application area) for any individual skin condition prior to the Product Application Day baseline sample collection
14. Are females who are pregnant, plan to become pregnant during the study, or are breastfeeding a child
15. Are unable to adhere to or understand the protocol
16. Have used an investigational drug or participated in an investigational study within 30 days prior to signing the informed consent for this study or are currently participating in a clinical study.
17. Are employed by or are a family member of staff of BD or the study site conducting the study.

5.0 DESCRIPTION OF STUDY PRODUCTS

Specific blinded product lot numbers will be included on separate Study Product release/receipt form provided by the Sponsor with the shipment of the materials.

5.1 Investigational Product

Single Swabstick impregnated with 1.75mL of 2% (w/v) chlorhexidine gluconate (CHG) in 70% (v/v) isopropyl alcohol (thermally treated)

a. Reference Standard

1.75 mL of 60% (v/v) 1-propanol applied with a Swabstick. The 1-propanol will be European Pharmacopeia grade material and diluted per BS EN 12791:2016[10].

5.2 Active Control

ChloraPrep® SEPP® clear applicator -2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol

5.3 Negative Control

1.75mL 0.9% normal saline applied with a Swabstick

5.4 Reference Products and Methods

The equipment and supplies used during this study will be detailed on Equipment and Supplies Tracking Form, and the form will be included in the Final Report.

5.4.1 Neutralization Validation

The effectiveness of the neutralizer system must be validated prior to the study start date to demonstrate that the antimicrobials are 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. Three organisms, *Staphylococcus epidermidis*, ATCC 12228 (MSSE), *Staphylococcus epidermidis*, ATCC 51625 (MRSE), and Methicillin-resistant *Staphylococcus aureus,* ATCC 33592 (MRSA), will be evaluated. See APPENDIX 6 for full study procedures.

5.4.2 Sampling Solution (SS)

[redacted]

5.4.3 Butterfield's Phosphate Buffered Water (PBW)

312 µM KH_2PO_4, pH 7.2 ± 0.1

5.4.4 Tryptic Soy Agar

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

5.5 Product Labeling

The study and commercial product labels will contain, at minimum, the following information:

- Product identification code
- Sponsor BD
- Protocol number MPS-17IPVSS02
- Blinded lot Numbers

Investigational product (or the immediate packaging) shall be labeled in accordance with regulatory requirements, including the following statement: " CAUTION: New Drug – Limited by United States Law to Investigational Use Only"

5.6 Maintenance and Storage of Study Products

Investigational product and active control product will be supplied and shipped to the site by BD and stored at controlled room temperature between 20-25 °C (68-77°F). Excursions permitted to 15-30°C (59-86°F). Becton Dickinson will supply the dry single Swabsticks for the reference standard and the negative control. The reference standard of 1-propanol and the negative control of 0.9% normal saline will be supplied by the site.

The storage conditions for the study product(s) will be described on the product label(s).

All products should be stored in accordance with instructions from BD and local procedures and retained in secure quarantine when not being used in the application process. A complete inventory of the study products and a log of use will be kept at the site.

Unused, sealed study products will be stored until BD specifies its disposition. In the absence of a disposition request from BD within 1 year of planned usage, the study products will be returned to BD. No unused study products will be destroyed unless confirmed by BD.

6.0 STUDY METHODS

6.1 Study Duration

The expected duration of this study for each subject is up to 3-4 weeks. Subjects will undergo at least a 14-day product restriction period followed by a qualification screening baseline visit. Subjects whose screening baseline samples meet the minimum values described in the Inclusion Criteria (Section 3.3) will be notified and invited to participate in the product application phase of the study. The product application phase will be scheduled no sooner than 72 hours from the screening baseline collection. Each subject who chooses to participate in this study will be required to remain at the study facility for scheduled study product application and assessments to include 30-seconds, 10-minutes, and 6-hours post-prep sampling.

6.1.1 Visit Schedule

Table 4 Schedule of Events

Procedure	14 days or more prior to Screening Day	2 or more days prior to Screening Day and Treatment Day	Screening Day	Product Application Day
Informed Consent Obtained	X			
Product-Restriction Period	X	X	X	X
Inclusion/Exclusion Criteria including Medical History Reviewed	X	X	X	X
Pregnancy Test				X
Skin Irritation Assessment (Product restriction after consent)	X			
Skin Irritation Assessment (pre-Clipping)		X		
Clipping Hair From Product Application sites		X		
Skin Irritation Assessment (pre-screening sample)			X	
Screening Day Baseline Sample			X	

Skin Irritation Assessment (Pre-Product Application)					X
Randomization					X
Product Application -Day Baseline Sample					X
Product Application					X
Skin Irritation Assessment (30 sec, 10 min and 6 hour post application)					X
Post-Product Application Samples (30 sec, 10 min and 6 hour)					X
Subject Self-Assessment of 6 hour sample site					X
Technician's Assessment of 6 hour sample site					X
Adverse Events			X	X	X
Concomitant Medications	X		X	X	X
Study product removal with soap and water after the 6 hr sample collection					X

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

6.1.2 Subject Recruitment, Consent and Product Restriction Period

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 events. All study-specific advertising materials will be approved by the IRB prior to their use for recruiting subjects.

Initial screening of subjects will be conducted at least 14 days prior to the Screening Day visit. The Inclusion/Exclusion Criteria will be reviewed with each subject to ensure eligibility for the study. At consent, the site will perform a skin irritation assessment and verify that the skin of the abdomen and groin are free from clinically evident dermatoses, injuries, or any other disorders that may compromise the subject or the study. Subjects will receive any necessary written and verbal information, and the informed consent of each subject will be obtained.

A list of restricted products will be provided to each subject. Prior to the scheduled Screening Day visit, subjects will undergo a minimum 14-day product restriction 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 antimicrobial products within this pre-product application period, the subject will be discontinued from the study.

Restrictions include, but are not limited to:

- Use of antimicrobial 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)

Prior to the start of the product restriction period, 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.

A visual skin assessment (Table 6) of the product application areas will be performed. If subjects require hair removal to facilitate sample collection, the subject will be asked to return to the study facility for a Pre-Screening Clipping Day Visit. Subjects will be required to refrain from bathing or showering for 72 hours prior to the Screening Day and Product Application Day visits.

6.1.3 Pre-Screening Clipping Day Visit (if required)

Approximately 48-96 hours prior to Screening Day, the Investigator or a designee will complete the Pre-Screening Day Inclusion/Exclusion Criteria Page in each subject's case report form (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 a subject's Screening Day Visit exceeds 96 hours from clipping, then the subject will be scheduled for re-clipping to ensure hair is removed approximately 48-96 hours prior to Screening Day.
Subjects will be reminded to refrain from bathing or showering for at least 72 hours prior to Screening Day.

6.1.4 Screening Day Visit

After the product restriction period and at least 3-days before Product Application Day, the Investigator or a designated sub-investigator will complete the Screening Day Inclusion/Exclusion Criteria page in each subject's CRF.

Prior to performing the Screening Day baseline sample collection, a skin irritation assessment will be performed by the Investigator or designated sub-investigators trained by the Investigator. If an irritation score of 1 or greater is assigned for any individual skin condition at the Screening Day baseline, the subject will be excluded from the study.

The product application site within the abdominal region (abdominal product application 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 (APPENDIX 1). The product application site within the groin region (groin product application area) is defined as the inner aspect of the upper thigh within and parallel to the inguinal crease below the groin (APPENDIX 1). Using a 2.5" x 2.5" (6.4 cm x 6.4 cm) sterile template for the abdomen, or a 1.3" x 5" (3.3 cm x 12.7 cm) sterile template for the groin, the corners of each product application area will be marked directly on the skin using a non-toxic skin marker. Four sampling sites will be numbered within each abdominal product application area according to APPENDIX 1 for each anatomical location. The positioning and numbering of the

sampling sites are standard for all subjects on each anatomical location. Sampling sites on the contra-lateral side of each anatomical location will be numbered in a mirror-image orientation.

A baseline screening sample will be collected from each product application area using the Williamson-Kligman scrub cup technique. The screening baselines samples will be taken from site #1 within each product application site on the abdomen (left and right sides) and site #3 within each product application site on the groin (left and right sides). Samples from both the left and right sides of a body region must meet the minimum value indicated in the Inclusion Criteria for the subject to be enrolled into the product application phase of the study for that region. Subjects must qualify on all four abdomen and inguinal sites to be admitted into the treatment phase of the study until such time that one of the anatomical sites has completed the minimal required evaluable sites, then subjects will be screened and treated only on the remaining anatomical site.
Subjects who qualify for the study will be notified and will continue to follow the subject instructions until their participation has been completed. Subjects will again be required to refrain from bathing or showering 72 hours prior to Product Application Day and hair will be clipped, if required.

6.1.5 Pre-Treatment Day Clipping Day Visit (if required)

Approximately 48-96 hours prior to Treatment Day, the Investigator or a designee will complete the Pre-Treatment Day Inclusion/Exclusion Criteria page in each subject's case report form (CRF). If these criteria are satisfied, a visual skin irritation assessment will be performed to evaluate the condition of each test area and hair will be clipped. If a subject's Treatment Day Visit exceeds 96 hours from clipping, then the subject will be scheduled for re-clipping to ensure hair is removed approximately 48-96 hours prior to Treatment Day.

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

6.1.6 Product Application Day Visit

The Investigator or a designated sub-investigator will complete the Product Application Day Inclusion/Exclusion Criteria CRF. If these criteria are satisfied, a visual skin assessment will be performed to evaluate the condition of each product application area. If all criteria are met, the subject will be assigned a randomization number.

Subjects will be provided with gowns, disposable sterile gloves and underwear for use on Product Application Day. Each subject who chooses to participate in this study will be required to change prior to baseline sampling into the gown and underwear provided as well as remain at the study facility for the scheduled assessments to include 30 seconds, 10 minutes and 6 hours post-product application sampling. Additionally, subjects who choose to participate will be required to wear disposable sterile gloves prior to the baseline sampling scheduled assessment through the 30-second and 10- minute post-product application sampling.

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

6.1.6.1 Preparation of abdominal product application area

A skin irritation assessment will be performed by the Investigator or designee trained by the Investigator, using the Skin Irritation Rating Scale (Table 6). If an irritation score of 1 or greater is assigned for any individual skin condition at the Product Application Day baseline, the subject will be excluded from the product application phase of the study for the abdomen.

The product application site within the abdominal region (abdominal product application 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 (APPENDIX 1). Using a 2.5" x 2.5" (6.4 cm x 6.4 cm) sterile template, the corners of each abdominal product application area will be marked directly on the skin using a non-toxic skin marker. Four sampling sites will

be numbered within each abdominal product application 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 product application area represent one baseline (pre-application) site and three post-application sample sites (30seconds, 10-minutes, and 6-hours).

After abdominal product application areas are marked and sample sites numbered, baseline samples will be collected from the appropriate site per the randomization schedule in each product application area using the scrub cup technique (see Section 6.3.1.1 Microbial Sample Collection/ Scrub Cup Technique).

6.1.6.2 Preparation of Groin product application Area

A skin irritation assessment will be performed by the Investigator or designated sub-investigators trained by the Investigator, using the Skin Irritation Rating Scale (Table 6). If an irritation score of 1 or greater is assigned for any individual skin condition at the Product Application Day baseline, the subject will be excluded from the product application phase of the study for the groin.

The product application site within the groin region (groin product application area) is defined as the inner aspect of the upper thigh within and parallel to the inguinal crease below the groin (APPENDIX 1). Using a 1.3" x 5" (3.3 cm x 12.7 cm) sterile template, the corners of each groin product application area will be marked directly on the skin using a non-toxic skin marker. Four sampling sites will be numbered within each groin product application 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 product application area represent one baseline (pre-application) site and three post-application sample sites (30-seconds, 10-minutes, and 6-hours).

After groin product application areas are marked and sample sites numbered, baseline samples will be collected from the appropriate site per the randomization schedule in each product application area using the scrub cup technique (see Section 6.3.1.1 Microbial Sample Collection/ Scrub Cup Technique).

6.1.6.3 Study Product Materials Application

After baseline sample collection is completed, the randomly assigned contra-lateral product application areas will be treated with the assigned study product materials.

The study products will be applied and the sampling configurations will be performed per the randomization schedule and the Study Product Application Instructions (Appendix 5). The duration of application time for each location will be recorded.

The study product material weight will be measured before and after application to the product application site and recorded as described in the Study Product Application Instructions (Appendix 5).

Table 5 contains a summary of the study products, anatomical sites evaluated, application time, dry time and coverage area.

Table 5: Anatomical body sites, application times, dry times and coverage area for each study product.				
Study Product	**Body Site**	**Application Time**	**Dry Time**	**Area of Coverage**
IP	Abdomen	30 seconds	30 seconds	2.5" x 2.5" (6.4 cm x 6.4 cm)
	Groin	2 minutes	1 minute	1.3" x 5" (3.3 cm x 12.7 cm)
AC	Abdomen	30 seconds	30 seconds	2.5" x 2.5" (6.4 cm x 6.4 cm)
	Groin	2 minutes	1 minute	1.3" x 5" (3.3 cm x 12.7 cm)
NC	Abdomen	30 seconds	30 seconds	2.5" x 2.5" (6.4 cm x 6.4 cm)
	Groin	2 minutes	1 minute	1.3" x 5" (3.3 cm x 12.7 cm)
RS	Abdomen	30 seconds	30 seconds	2.5" x 2.5" (6.4 cm x 6.4 cm)
	Groin	2 minutes	1 minute	1.3" x 5" (3.3 cm x 12.7 cm)

6.2 Efficacy Assessments

6.3.1 Post Application Sample Collection and Assessments

The microbial samples will be collected at 30 seconds (± 5 sec.), 10 minutes (± 30 sec.) and 6 hours (± 30 min.) post product application for both the abdomen and the groin regions. Post application timing begins upon completion of the study product application, including drying time. Microbial samples will be collected using the scrub cup technique (see Section 6.3.1.1 Microbial Sample Collection/ Scrub Cup Technique).

After the 10-minute (± 30 sec.) samples have been collected, a sterile non-occlusive dressing will be secured over the remaining sample sites to protect the sites from contamination between sampling times. Subjects will be instructed to ensure the non-occlusive dressing remains intact and dry until the 6 hours sampling occurs.

Prior to collection of the 6-hour sample, the technician will perform an assessment of the 6-hour sample site to determine if the gauze appears dry, clean, and intact. Subjects will also be asked to provide a verbal assessment of the sample site regarding any adjustments to the gauze and if the gauze remained intact and dry from time of placement until the 6 hour evaluation.

A skin irritation assessment will be performed by the Investigator or designated sub-investigators trained by the Investigator prior to collection of each post product application microbial sample collection (30-seconds, 10-minutes and 6-hours) and a corresponding rating score for each individual skin condition will be recorded in the subject's CRF.

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

Following final sample collection at the 6 hour time point or early study discontinuation, the remaining study product will be removed from the subjects' skin using soap and water.

6.3.2 Microbial Sample Collection / Scrub Cup Technique

Quantitative cultures (baselines and post product 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 (2.20 cm I.D., skin surface area of 3.80 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 scrubbed with moderate pressure 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 sampling solution will be pipetted into the cup and the scrub procedure will be repeated. This solution will be pooled with the first solution collected.

6.3.3 Bacterial Enumeration Methods

Following sample collection, 10-fold serial dilutions (1 mL sample + 9 mL PBW) will be prepared using PBW. One-mL aliquots of appropriate dilutions will be pour-plated in triplicate using trypticase soy agar containing neutralizers (TSA+N). Samples must be plated within 30 minutes of collection.

Serial dilutions of collected baseline screening samples as follows:

- for groin sites: 1 serial dilution (10^{-3})
- for abdomen sites: 1 serial dilution (10^{-1})

Serial dilutions of collected treatment day (D0) samples as follows:

for groin sites

- Baseline: 3 serial dilutions (10^{-3}, 10^{-4} and 10^{-5})
- 30 seconds: 6 serial dilutions (10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5})
- 10 minutes: 6 serial dilutions (10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5})
- 6 hours: 6 serial dilutions (10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5})

for abdomen sites

- Baseline: 3 serial dilutions (10^{-1}, 10^{-2} and 10^{-3})
- 30 seconds: 4 serial dilutions (10^{0}, 10^{-1}, 10^{-2} and 10^{-3})
- 10 minutes: 4 serial dilutions (10^{0}, 10^{-1}, 10^{-2} and 10^{-3})
- 6 hours: 4 serial dilutions (10^{0}, 10^{-1}, 10^{-2} and 10^{-3})

After 72 ± 4 hours of aerobic incubation at 30 ± 2ºC, colonies will be counted and viable cells in the original sample will be calculated according to Standard Operating Procedures (SOPs). 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 number of microorganisms recovered will be calculated using the formula to convert the bacterial counts into $\log_{10}$ CFU/cm^2 of skin:

$$R = \log_{10}\left[\frac{F\left(\frac{\sum_{i=1}^{3} c_i}{n}\right)D}{A}\right]$$

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);

$\frac{\sum_{i=1}^{3} c_i}{n}$ = average of the triplicate 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 (3.80cm^2)

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

In order to avoid potential calculation problems due to taking the logarithm of zero, values of less than 1 CFU/cm^2 will be treated as 1 CFU/cm^2, such that the $\log_{10}$ transformation is not less than zero.

6.3 Safety Assessments

The principal measures of safety will be skin reactions using the modified Berger Bowman irritation assessment scale and the incidence of AEs reported during the study

6.3.1 Adverse Events

The incidence of adverse events reported during this study will be collected for assessment of safety. Details on the AE management, reporting and assessment can be found in Section 9.

6.3.2 Skin Irritation Score

Subjects that receive study products will be monitored for skin irritation using the modified Berger Bowman irritation assessment scale. Skin irritation assessment will be performed as outlines in Section 6.2 Study Visits.

If a reaction occurs on the skin test site, it may be needed to take photographs of the skin test site only. These photographs will be shared with the Principal Investigator / Sub-Investigator, the sponsor and the study staff only and will be used for the Principal Investigator / Sub-Investigator's review and assessment of the reactions.

Table 6: Modified Berger Bowman Skin Irritation Rating Scale

Skin Irritation Rating Scale Reactive area(s) within the product application 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	Mild and/or transient swelling
	2[a]	Moderate swelling
	3[b]	Severe swelling
Rash	0	No reaction
	1	Mild and/or transient rash
	2[a]	Moderate rash
	3[b]	Severe rash
Dryness	0	No reaction
	1	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 AE and will require subject's removal from the study.

7.0 INTERRUPTION OR DISCONTINUATION OF PARTICIPATION

7.1 Discontinuation of study subjects

Subjects may request withdrawal from the study at any time or may be withdrawn at the discretion of the 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 Investigator, makes the subject's participation in the study not in his or her best interest.

If a subject withdraws from the study, all efforts will be made to complete a final evaluation, if possible.

7.2 Discontinuation of specimen analysis.

Discontinued subjects will not be replaced. If needed, additional subjects will be enrolled with a sequential, unique randomization code according to the randomization schedule.

No data will be collected from subjects after the point of discontinuation except as needed to follow ongoing AEs. All study data collected from the subject up to the point of discontinuation will be recorded on the CRF, entered into the study database, and included in subsequent analyses, as appropriate.

7.3 Discontinuation Visits and Follow-up Procedures

For subjects discontinued due to AEs, the clinical course of the event will be followed according to the procedures outlined in section 9.2.1 Follow-up of Adverse Events.

8.0 RISK / BENEFIT ASSESSMENT

8.1 Potential Risks

Subjects participating in this study could experience side effects to the skin (from either the Investigational or Comparator products and/or study procedures) such as:

- skin reactions, primarily confined to the area of application
- local minimal or moderate erythema
- minimal edema
- application site pruritus
- rash
- papules
- vesicles
- burning sensation
- allergic reaction including in rare cases anaphylaxis

Subjects may also experience folliculitis from clipping.

There may be risks from participating in this study that are unknown.

8.2 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 a single Swabstick impregnated with 1.75mL of 2% (w/v) chlorhexidine gluconate (CHG) in 70% (v/v) isopropyl alcohol.

9.0 SAFETY

9.1 Adverse Event Definitions

Adverse Event (AE):

Any untoward medical occurrence in a patient or clinical investigation subject administered an investigational product (drug or device), which does not necessarily have to have a causal relationship with the treatment.

An adverse Event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product.

Note 1: This definition includes events related to the investigational product or the comparator.
Note 2: This definition includes events related to the procedures involved

Serious Adverse Event (SAE):

A serious adverse event is an event that in the view of either the Investigator or Sponsor, results in any of the following outcomes:

- Death
- life-threatening illness or injury
- permanent impairment of a body structure or body function
- in-patient hospitalization or prolongation of hospitalization
- injury, significant disability Fetal distress, fetal death, or a congenital abnormality or birth defect
- Other important medical event, that requires medical or surgical intervention to prevent any of the above

Note 1: "Planned hospitalization for pre-existing condition, or a procedure required by the protocol, without serious deterioration in health, is not considered a serious adverse event".

Note 2: "Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse".

9.2 Adverse Event (AE) Management

Collection of adverse events will begin after the subject has signed the Informed Consent form through the end of study conduct.

At each study contact, the subject 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. Signs and symptoms must be comprehensively documented on the appropriate source documentation. All related signs and symptoms should be grouped under one diagnosis if possible.

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

Any irritation of the application sites that results in the withdrawal of the subject from the study or, in the opinion of the Investigator or designate, warrants medical attention or medication will be considered AEs that will be subjected to the same criteria as all other AEs.

If an AE or SAE reaction occurs on the skin test site, it may be needed to take photographs of the skin test site only. These photographs will be shared with the Principal Investigator / Sub-Investigator, the sponsor and the study staff only and will be used for the Principal Investigator / Sub-Investigator's review and assessment of the reactions.

9.2.1 Follow-up of Adverse Events (AEs)

Adverse events that are present after the completion of the subject's scheduled study visit will be followed up to 14 days by means of the investigational site contacting the subject via telephone or by any other means considered appropriate. If the subject's adverse event is still ongoing past the 14 days, the Investigator will communicate with the sponsor Medical Monitor (via telephone and/or email) as to the need for further follow-up. In the case of discrepancies, the Investigator's judgement will prevail. All communications with the subject and medical monitor will be documented appropriately.

Adverse Events whose onset occurs up to 14 days after the end of the subject's study participation, will be recorded and will require the Investigator to communicate with the sponsor Medical Monitor as to the need for further follow-up as described above. All contacts will be documented appropriately.

The clinical course of any serious adverse 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 as it relates to the study product and/or study procedures.

9.3 Assessment of Adverse Events (AEs)

All AEs (Serious and Non-Serious) are assessed according to severity or intensity of the event experienced by the subject as either mild, moderate, or severe using the definitions below:

Note: To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe," which are not synonymous, the following note of clarification per *ICH (E2A) Definitions and Terminology associated with clinical safety experience* is provided :
The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

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 following information about the event is to be collected on the AE CRF:

- Severity, classified as:

Mild:	Signs or symptoms that do not interfere with the subject's daily activity, are usually considered self-limiting, can be treated with non-prescription type medications and do not require medical intervention.
Moderate:	Event(s) may interfere or cause low level inconvenience with the subject's daily activity. Requires medical intervention and/or treatment, however, unlikely to require hospitalization or be consider potentially life-threatening in nature.
Severe:	Event(s) may cause significant discomfort to the subject and/or interferes with the subject's daily activity. Requires medical intervention and/or treatment to preclude a permanent impairment, can be life threatening and require hospitalization.

- Relationship or Causality, assessed as:

Not Related:	The AE is independent of study intervention, and/or evidence exists that the event is related to another etiology. There must be an alternative, etiology documented by the clinician.
Unlikely Related:	A clinical event, whose temporal relationship to study intervention makes a causal relationship improbable (e.g., the event did not occur within a reasonable time of the study product use) and in which underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
Likely Related:	There is evidence to suggest a causal relationship, and the influence of other factors is less likely. The clinical event occurs within a reasonable time after use of the study product, and is less likely to be attributed to concurrent disease.
Related:	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event occurs in a plausible time relationship to use of the study product and cannot be explained by concurrent disease.

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

9.4 **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.

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 the Sponsor 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 safety email address listed below within 24 hours of receipt of the information.

Sponsor Notification of Serious Adverse Event:

- Safety Email: [redacted]

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

[redacted]
VP, Medical Affairs, Interventional Segment
[redacted]

9.4.1 Reporting Obligations to IRB/EC and Health Authorities

The Investigator must report any AEs 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 product 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.

9.4.2 Pregnancies

Investigators must instruct subjects to inform them immediately if they become pregnant during the trial.

In case of a pregnancy during the participation in the trial, the subject will be immediately considered for premature discontinuation.

The pregnancy will be registered on the CRF/eCRF protocol deviation form. In addition, the Pregnancy Form (which is independent of the CRF/eCRF) will be completed and entered into the EDC with all the available information within the same time frame and following the same routing as for a SAE, described previously. The Investigator will make every effort to obtain all information related to the pregnancy and its final outcome, which may involve follow-up after the trial has ended. Pregnancies will be followed up for maximally 4 weeks after delivery or termination of the pregnancy.

All pregnancies discovered by the Investigator after the termination of the trial but which were initiated during the participation in the trial will be reported to BD (see details above) as occurring during the trial.

10.0 INCIDENTS

A 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. 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 AEs (refer to Section 9.0). Examples of Study Incidents that are not AEs might be mislabeling or adulteration of the investigational product, equipment malfunctions, errors in the investigational product 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 AEs 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.

11.0 RETURN OR DESTRUCTION OF STUDY PRODUCT

All disposable, used products are to be discarded into appropriate waste containers at the investigational site. Failed, damaged or products involved in an Incident or AE/SAE should be returned to BD for further investigation or disposal by BD.

Unless instructed otherwise by BD, the Investigator will return all remaining unused or unopened IP, 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 Supply Shipments Coordinator
75 N. Fairway Drive
Vernon Hills IL 60061
[redacted]

12.0 DATA COLLECTION AND MANAGEMENT

12.1 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 electronic Case Report Form (e-CRF). Typical source documents can include CRFs, 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.

The investigator is required to maintain original source documents at the site according to section 15.8 "Record Retention" procedure of this protocol. Should an original source document (e.g., an instrument printout, direct entry CRF) need to be forwarded to the Sponsor for data entry, the site must retain a clearly designated certified copy. The Study Monitor will confirm that procedures for copy certification have been established at the site prior to transmittal of any original source documents.

12.2 Electronic Case Report Forms (eCRF)

The electronic case report forms (eCRF) will be provided by the Sponsor. The term "eCRF" as used in this protocol refers to eCRFs, or electronic case report forms for electronic data capture (EDC), as determined by the Sponsor.

The Investigator may delegate eCRF completion to study personnel. However, the Sponsor must be apprised in writing of the name of such persons and the scope of their authority. The Investigator or designee is obligated to review each eCRF page and sign EDC, using an electronic signature. An individual record will be kept for each subject that provided informed consent.

Electronic CRF entries will be compared to source documents by the study monitor or designated personnel. Unless specified otherwise, all information on the CRFs must be traceable to original source documents.

12.3 Electronic Source Data

Specific procedures will be described in a study-specific Data Management Plan.

12.4 Data Management and Storage

Data Management will be performed by the Sponsor. Electronic records entered at the site will be entered directly into the controlled database. Data security is ensured through password protection, limited access, audit trails, and regular backups of the data. Upon completion of the study and verification of data, data will be screened for accuracy and completeness, after which the database will be locked from any additional changes.

13.0 STATISTICAL METHODS

13.1 Sample Size Determination

To evaluate the primary objective for HC analysis a sample size of at least 192 evaluable sites per study product arm (IP or RS) per body site can achieve > 95% power to pass HC analysis acceptance criteria for all four objectives using one-sample t-test (two-sided), with the following assumptions.

- Two-sided test Type I error (α): 0.05
- Standard deviation and mean log reduction:

Time Point	Study Product	Standard Deviation		Log Reduction	
		Abdomen	Groin	Abdomen	Groin
30 Seconds	IP	1.14	1.43	3.47	3.38
6 hours	IP	1.22	1.24	3.43	3.50

To evaluate the primary and secondary objectives for the US FDA analysis a sample size of 280 evaluable sites per study product arm (IP, AC or NC) per body site can achieve > 95% power to demonstrate the non-inferiority of the Investigational Product (IP) as compared to the Active Control (AC) and the superiority of the IP as compared to the negative control (NC) in antimicrobial effect at 30-second (abdomen only) and 10-minute (abdomen and groin) post-product application time point for all six objectives, with the following assumptions:

- Two-sided test Type I error (α): 0.05
- Average Treatment Effect in $\log_{10}$ CFU/cm^2:

Average Treatment Effect	Abdomen	Groin
IP – AC	0.06	0.10
NC – IP	1.58	2.31

- Standard deviation for each treatment effect for Abdomen: 1.05 $\log_{10}$
- Standard deviation for each treatment effect for Groin: 1.2 $\log_{10}$

The overall power to pass both HC analysis and US FDA analysis acceptance criteria is > 90%.

13.2 Adjustments for Multiple Objectives

An overall significance level of 0.05 (alpha = 0.05) will be used.

This study has two sets of objectives, one for Health Canada analysis and the other for US FDA analysis. These two sets of objectives are considered independently of each other and no alpha adjustments between two sets of objectives are performed.

Within the HC analysis primary objectives, since the study HC efficacy analysis will declare success when and only when all four primary endpoints pass HC analysis acceptance criteria, no multiplicity adjustment is required[9].

Within the FDA efficacy analysis, a gatekeeping procedure will be applied: the efficacy analysis for two secondary objectives [30-second (abdomen only) post-product application time point] will be performed only when all four primary objectives [10-minute (abdomen and groin) post-product application time point] pass the acceptance criteria. No alpha adjustments between primary and secondary endpoints are performed. For primary objectives [10-minute (abdomen and groin) post-product application time point], since it will declare success when and only when all four primary objectives pass FDA analysis acceptance criteria, no multiplicity adjustment for the four primary objectives is required[9]. For secondary objectives [30-second (abdomen only) post-product application time point], since it will declare success when and only when all four primary and two secondary objectives pass FDA analysis acceptance criteria, no multiplicity adjustment for the two secondary endpoints is required[9].

13.3 Study Populations

The full intent-to-treat (ITT) data set (all randomized subjects that received study product) 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 anatomical site (left and right for the groin and abdomen). For each anatomical site, if the product application day baseline bacterial count is in the range of 3.20 to 6.00 $\log_{10}/cm^2$, inclusive, on the abdomen or 5.50 to 7.50 $\log_{10}/cm^2$, inclusive, on the groin, then the data from that anatomical site are included in the mITT data set.

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.

The Per-Protocol data set will include evaluable product application sites from the mITT data set that adhere to defined assessments and procedures in the protocol central to patient enrollment, safety, rights or wellbeing, as well as the completeness, accuracy and reliability of study data. Additional details on qualification for the PP data set are outlined in the SAP.

13.4 Handling of Missing Data

Missing $\log_{10}$ CFU/cm^2 determinations at 30 seconds, 10 minutes, or 6 hours, such as due to laboratory error or subject lost to follow up, will be reported as missing and will not be imputed. Inclusion of these subjects in the per-protocol data set will be based on the criteria defined above and within the SAP. Details of any missing data and rationale for inclusion/exclusion in the per-protocol data set will be described in the study report.

For FDA analysis objectives: For $\log_{10}$ CFU/cm^2 determinations, a tipping-point sensitivity analysis will use multiple imputation to impute a series of $\log_{10}$ CFU values relative to the baseline $\log_{10}$ CFU values for all missing data points in order to find the point at which the objectives at 30 seconds and 10 minutes would not be met. The multiple imputation will be performed separately for each of the 4primary and 2 secondary comparisons.

For responder rate calculations from 6-hour data (FDA analysis), subjects with missing $\log_{10}$ CFU/cm^2 determinations at 6 hours will not be excluded from analysis. Missing data will be imputed by increasing the assumed number of failures sequentially from 0/M to M/M, where M is the number of missing data points at 6 hours. This sensitivity analysis will be performed for all products on each anatomical site and will provide information on the possible effect of missing data on study results.

Detailed description of sensitivity analysis performed will be described in the statistical analysis plan.

13.5 Statistical Methods

13.5.1 HC Primary Analysis

Log_{10} CFU/cm^2 changes from baseline will be calculated separately for each subject, each of the four sites (left and right for the abdomen and groin), and each post-product application sampling time by taking the baseline $\log_{10}$ CFU/cm^2 values and then subtracting the $\log_{10}$ CFU/cm^2 values for the samples taken after baseline. The mean $\log_{10}$ CFU/cm^2 changes from baseline will be calculated separately for the IP and the RS, at each anatomical site (abdomen and groin), and at each post-product application sampling time point (30 seconds and 6 hours). The following descriptive statistics for $\log_{10}$ CFU/cm^2 reductions will be computed for the IP and RS, grouped by anatomical site and each post application sampling time point (30 seconds and 6 hours): mean, median, standard deviation, minimum, maximum, and count. The 95% confidence intervals (two-sided) will be calculated for the observed log reductions for the IP and RS at each time point on each anatomical area using one-sample t-test (two-sided).

The goal for the IP is that the mean log_{10} reduction meets the following criteria:

- The lower limit of the 95% confidence interval (two-sided) of mean log reduction at 30 seconds will be greater than or equal to 2 for the abdomen.
- The lower limit of the 95% confidence interval (two-sided) of mean log reduction at 30 seconds will be greater than or equal to 3 for the groin.
- The lower limit of the 95% confidence interval (two-sided) of mean log reduction at 6 hours will be greater than or equal to 2 for the abdomen.
- The lower limit of the 95% confidence interval (two-sided) of mean log reduction at 6 hours will be greater than or equal to 3 for the groin

The RS should meet the same efficacy standards as the IP.

13.5.2 HC Exploratory Analyses

1. The following descriptive statistics for log_{10} CFU/cm^2 reductions will be computed for the IP and RS, grouped by anatomical site and each post application sampling for the 10 minute time point: mean, median, standard deviation, minimum, maximum, and count. The 95% confidence intervals (two-sided) will be calculated for the observed log reductions for the IP and RS at the 10 minute time point on each anatomical area using one-sample t-test (two-sided).
2. Log_{10} CFU/cm^2 reductions at 30 seconds, 10 minutes, and 6 hours on the abdomen and the groin for the IP will be compared to the RS using two-sample t-test (two sided).

13.5.3 FDA Primary Analysis

A linear regression model for each body site (abdomen and groin) will be used for primary analysis of efficacy at 10 minutes. In the model, the response is the post-product application bacterial counts at 10 minutes and predictors are the study products as a fixed effect and the pre-product application bacterial loads as a covariate. The ATE corrected for pre-product application bacterial loads will be estimated from the model and compared to both non-inferiority and superiority criteria.

For assessment of the primary objectives (i.e., immediate activity at 10 minutes post application on the abdomen and groin):

A non-inferiority criterion with a 0.5 log_{10} margin will be implemented for the average treatment effect (ATE) of the IP compared to the AC (i.e., the upper two-sided 95% confidence bound of the post-product application bacterial load corrected for pre-product application bacterial load of the IP – AC should be less than 0.5 log_{10}).

A 1.2 log_{10} superiority criterion will be implemented for the ATE of the IP compared to the NC (i.e., the lower two-sided 95% confidence bound of the post-product application bacterial load corrected for pre-product application bacterial load of the NC – IP should be greater than 1.2 log_{10}).

To evaluate the persistent antimicrobial properties of a single Swabstick impregnated with 2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol, log_{10} bacterial counts at 6 hours post product application for each product application site will be converted to a binary (yes or no) success measure with success defined as having skin flora counts that are less than or equal to the baseline skin flora counts. Responder rates at 6 hours post product application will be summarized descriptively for each product on each body site. No inferential analysis will be performed on the 6 hour time point data.

13.5.4 FDA Secondary Analysis

A linear regression model for the abdomen will be used for the analysis of efficacy at 30 seconds. In the model, the response is the post-product application bacterial counts at 30 seconds and predictors are the study products as a fixed effect and the pre-product application bacterial loads as a covariate. The ATE corrected for pre-product application bacterial loads will be estimated from the model and compared to both non-inferiority and superiority criteria. The FDA analysis secondary objectives will be evaluated only when the four co-primary objectives pass both non-inferiority and superiority criteria.

For assessment of the secondary objective (i.e., antimicrobial activity at 30 seconds post application on the abdomen):

1. A non-inferiority criterion with a 0.5 $\log_{10}$ margin will be implemented for the ATE of the IP compared to the AC (i.e., the upper two-sided 95% confidence bound of the post-product application bacterial load corrected for pre-product application bacterial load of the IP – AC should be less than 0.5 $\log_{10}$).
2. A 1.2 $\log_{10}$ superiority criterion will be implemented for the ATE of the IP compared to the NC (i.e., the lower two-sided 95% confidence bound of the post-product application bacterial load corrected for pre-product application bacterial load of the NC – IP should be greater than 1.2 $\log_{10}$).

13.5.5 FDA Exploratory Analysis

Log_{10} reductions:

The following descriptive statistics for $\log_{10}$ CFU/cm^2 reductions will be computed for the IP, AC and NC at each anatomical site at 30 seconds, 10 minutes, and 6 hours post product application: mean, median, standard deviation, minimum, maximum, and count.

Product weight expression:
The weight (grams) of product solutions applied to an anatomical area will be estimated as:

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

The following descriptive statistics for expression weights will be computed for each product at each anatomical site: mean, median, standard deviation, minimum, maximum, and count.

13.5.6 Safety Analysis

The statistical significance of differences in skin irritation between the study products at each post application sampling time will be evaluated by Fisher's exact test on skin irritation data summarized for safety analysis as follows: any reaction above a 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 study products, follow-up analyses will be conducted to determine how the reactions differ. No statistical analysis will be performed on the incidence of AEs.

13.6 Demographics/Other descriptive information

Demographics of the study population will be collected by the study sites and summary statistics for demographics will be performed.

14.0 QUALITY CONTROL AND ASSURANCE

14.1 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 dispensed to subjects 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.

14.2 Monitoring

BD, the study sponsor, will designate trained and qualified personnel to monitor the progress of this study in accordance with BD Monitoring SOPs and the study-specific Monitoring Plan.

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 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 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.

14.3 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 for verification of data on CRFs. 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.

14.4 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 and Investigator. Upon evaluation by the Sponsor and Investigator, 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.

15.0 ETHICAL AND REGULATORY STANDARDS

The study will be conducted in compliance with FDA regulations, the ethical principles of the Declaration of Helsinki (Fortaleza, Brazil; 2013), the Belmont Report, the International Conference on Harmonization (ICH) – Good Clinical Practice (GCP) Guidelines as currently amended, and all applicable SOPs at the study site.

15.1 IRB/EC

An appropriate IRB/EC must review this protocol, the Informed Consent Form, 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 has been provided to the Sponsor.

15.2 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.

15.3 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 analysis of biological samples in addition to the information relating to participating subjects. Subject confidentiality and anonymity will be maintained at all times by removal of all identifiers from any data, samples or documentation submitted for this study.

Any data collected meeting the definition of protected health information 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 study data sent to BD. BD study databases will not be shared with any third party without the express written consent of the 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 study data and records, as well as source documents.

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

15.4 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 Investigator) 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.

15.5 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.

15.6 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. 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 products. 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.

15.7 Publication of Results

BD believes that results of applicable studies of our products should be published in peer-reviewed literature in a timely, accurate, complete and balanced manner, regardless of study outcomes. BD is committed to making information public whenever it relates to the safety and efficacy of its marketed products.

Any formal presentation or publication of data collected from this study will be considered as a joint publication by the investigator(s) and the appropriate personnel of BD. Authorship will be based on generally accepted criteria of the International Committee of Medical Journal Editors and determined by mutual agreement. For multi-center studies, the first publication will be based on data from all centers, analyzed as stipulated in the protocol by BD statisticians, and not based on data from single sites or a subset of sites. Investigators participating in multi-center studies agree not to present data gathered from one center or a small group of centers before the full, initial publication, unless formally agreed to by all other investigators and BD (the sole exception being an unanticipated AE that is product-related and which might have clinically significant safety implications for a marketed product or a class of products).

15.8 Record Retention

The Investigator shall retain all essential study documents (e.g., IRB/EC approvals, signed informed consent forms, source documents, CRF copies, safety reports, study product dispensing records, etc.), for a minimum of 5 years after submission of study results, or 2 years after approval of application, or 2 years after study is completed, terminated, or discontinued, whichever is longer. BD will notify the Investigator(s)/institution(s) in writing, when the study-related records are no longer required. If the Investigator or Study 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.

16.0 BIBLIOGRAPHY/REFERENCES

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2. ASTM E 1054-08, Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents, ASTM International, West Conshohocken, PA. 2013.
3. Bretz F, Dmitrienko A. (Ed.), Tamhane A. (Ed.), Chow S. Multiple Testing Problems in Pharmaceutical Statistics. New York: Chapman and Hall/CRC. 2009s
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6. Food and Drug Administration (FDA). Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Final Rule. December 2017. Accessed at https://www.gpo.gov/fdsys/pkg/FR-2017-12-20/pdf/2017-27317.pdf
7. Williamson P., Kligman A.M. A New Method for the Quantitative Investigation of Cutaneous Bacteria. *J. Invest. Dermatol*. 1965;45: 498-503,.
8. World Health Organization. WHO guidelines on hand hygiene in health care. 2009.
9. Alex Dmitrienko, Ajit C.Tamhane, Frank Bretz Multiple Testing Problems in Pharmaceutical Statistics
10. BS EN 12791:2016, Chemical disinfectants and antiseptics. Surgical hand disinfection. Test method and requirements (phase 2, step 2).

17.0 PROTOCOL REVISION HISTORY

Version #	Rationale for Change	Section or Page affected	Description of change
1.0	New Protocol		
2.0	Updated Health Canada primary efficacy objective for clarity	*Summary Objectives, Page 4*	**Before:** **Efficacy Objectives according to HC testing requirements** **Primary Objective:** To demonstrate the immediate and persistent antimicrobial activity of a single Swabstick impregnated with 2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol compared to 60% (v/v) 1-propanol (RS) applied with a Swabstick at 30 seconds and 6 hours post product application. **After:** **Efficacy Objectives according to HC testing requirements** **Primary Objective:** To demonstrate the immediate and persistent antimicrobial activity of a single Swabstick impregnated with 2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol at 30 seconds and 6 hours post product application.
2.0	Updated Health Canada primary efficacy objective for clarity	*2.0: Health Canada Analysis, 2.2.1 Primary Efficacy Analysis*	***Before:*** To demonstrate the immediate and persistent antimicrobial activity of a single Swabstick impregnated with 2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol compared to 60% (v/v) 1-propanol (RS) applied with a Swabstick at 30 seconds and 6 hours post product application. To meet the immediate effectiveness standards, the IP should achieve a mean 2-$\log_{10}$ per cm^2 reduction on the abdomen site and a mean 3-$\log_{10}$ per cm^2 reduction on the groin site at 30 seconds. The primary efficacy objective for persistent effect is the IP meets the same $\log_{10}$ CFU/ cm^2 reduction goals at 6 hours as for 30 seconds. The reference standard of 60% v/v 1-propanol should meet the same efficacy standards as the IP. ***After:*** To demonstrate the immediate and persistent antimicrobial activity of a single Swabstick impregnated with 2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol at 30 seconds and 6 hours post product application. To meet the immediate effectiveness standards, the IP should achieve a mean 2-$\log_{10}$ per cm^2 reduction on the abdomen site and a mean 3-$\log_{10}$ per cm^2 reduction on the groin site at 30 seconds. The primary efficacy objective for persistent effect is the IP meets the same $\log_{10}$ CFU/ cm^2 reduction goals at 6 hours as for 30 seconds. The reference standard of

Version #	Rationale for Change	Section or Page affected	Description of change
			60% v/v 1-propanol,(RS) applied with a Swabstick should meet the same efficacy standards as the IP.
2.0	Updated FDA Analysis for clarity	*2.3: FDA Analysis, 2.3.1 Primary Objectives*	***Added:*** To evaluate persistent antimicrobial efficacy at 6 hours post-product application, log_{10} bacterial counts for each product application site will be converted to a binary (yes or no) success measure with success defined as having skin flora counts that are less than or equal to the baseline skin flora counts. Responder rates at 6 hours post product application will be summarized descriptively for each product on each body site.
2.0	Updated for clarity that there is no official acceptance criteria for the RS.	*3.2 Acceptance Criteria Table 1, HC Analysis – Abdomen 30 sec*	***Before:*** Immediate effectiveness for the IP is at least a 2-log_{10} CFU/cm^2 reduction from baseline skin flora counts at 30 seconds. 60% v/v 1-Propanol, should meet the same efficacy standards as the IP. ***After:*** Immediate effectiveness for the IP is at least a 2-log_{10} CFU/cm^2 reduction from baseline skin flora counts at 30 seconds.
2.0	Updated for clarity that there is no official acceptance criteria for the RS.	*3.2 Acceptance Criteria Table 1, HC Analysis – Groin 30 sec*	***Before:*** Immediate effectiveness for the IP is at least a 3-log_{10} CFU/cm^2 reduction from baseline skin flora counts at 30 seconds. 60% v/v 1-Propanol, should meet the same efficacy standards as the IP. ***After:*** Immediate effectiveness for the IP is at least a 3-log_{10} CFU/cm^2 reduction from baseline skin flora counts at 30 seconds.
2.0	Updated for clarity that there is no official acceptance criteria for the RS.	*3.2 Acceptance Criteria Table 1, HC Analysis – Abdomen 6 hours*	***Before:*** Persistence effectiveness for the IP is at least a 2-log_{10} CFU/cm^2 reduction from baseline skin. flora counts at 6 hours. 60% v/v 1-Propanol, should meet the same efficacy standards as the IP. ***After:*** Persistence effectiveness for the IP is at least a 2-log_{10} CFU/cm^2 reduction from baseline skin. flora counts at 6 hours.

Version #	Rationale for Change	Section or Page affected	Description of change
2.0	Updated for clarity that there is no official acceptance criteria for the RS.	*3.2 Acceptance Criteria Table 1, HC Analysis – Groin 6 hours*	***Before:*** Persistence effectiveness for the IP is at least a 3-$\log_{10}$ CFU/cm^2 reduction from baseline skin. flora counts at 6 hours. 60% v/v 1-Propanol, should meet the same efficacy standards as the IP. ***After:*** Persistence effectiveness for the IP is at least a 3-$\log_{10}$ CFU/cm^2 reduction from baseline skin. flora counts at 6 hours.
2.0	Updated blinding section to include measures to control bias	*3.3.2 Blinding*	***Added:*** ***3.3.2.2 Measures to Control Bias*** For this study, several measures are being used to control or reduce potential bias. Those measures include, but are not limited to the following: • Subject randomization; • Statistical Analysis Plan lists potential protocol deviations and rules for use of associated data in analysis; • Products will be labeled with treatment codes (A, B, C, D); • Data Management Plan outlines steps to prevent Sponsor study team members from becoming aware of treatment codes.
2.0	Updated Table numbering	*3.3.2 Blinding*	***Before:*** Table 1 Blinded Product Codes ***After:*** Table 2 Blinded Product Codes
2.0	Updated Table numbering	*4.0 Study Population*	***Before:*** Table 2 Estimated Number of Evaluations ***After:*** Table 3 Estimated Number of Evaluations
2.0	Updated Table numbering	*6.1.1 Visit Schedule*	***Before:*** Table 3 Schedule of Events ***After:*** Table 4 Schedule of Events

Version #	Rationale for Change	Section or Page affected	Description of change
2.0	Updated Table 4: Schedule of Events with a new procedure	*6.2.1 Visit Schedule*	***Procedure Added to Application Day Column:*** "Study product removal with soap and water after the 6 hr sample collection".
2.0	Inserted procedure to align with ICF	*6.4.2 Skin Irritation Score*	***Added:*** If a reaction occurs on the skin test site, it may be needed to take photographs of the skin test site only. These photographs will be shared with the Principal Investigator / Sub-Investigator, the sponsor and the study staff only and will be used for the Principal Investigator / Sub-Investigator's review and assessment of the reactions.
2.0	Updated paragraph to correct procedure	*7.1 Discontinuation of study subjects*	***Before:*** If a subject withdraws from the study, all efforts will be made to complete a final evaluation, if possible. Subjects discontinued due to an AE will be followed until the AE is resolved, a reasonable explanation is provided for the event, or the subject is referred to his/her own primary medical doctor. The specific AE in question will be recorded on the appropriate CRF. ***After:*** If a subject withdraws from the study, all efforts will be made to complete a final evaluation, if possible.
2.0	Updated paragraph to correct procedure	*7.3 Discontinuation Visits and Follow-up Procedures*	***Before:*** For subjects discontinued due to AEs, 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. ***After:*** For subjects discontinued due to AEs, the clinical course of the event will be followed according to the procedures outlined in section 9.2.1 Follow-up of Adverse Events.
2.0	Updated bullet points to remove duplicate statements	*9.1 Adverse Event Definition; Serious Adverse Events*	***Before:*** • Death • life-threatening illness or injury • permanent impairment of a body structure or body function • in-patient hospitalization or prolongation of hospitalization • injury, significant disability, permanent impairment of a body structure or function • Fetal distress, fetal death, or a congenital abnormality or birth defect • Other important medical event, that requires medical or surgical intervention to prevent any of the above ***After:***

Version #	Rationale for Change	Section or Page affected	Description of change
			• Death • life-threatening illness or injury • permanent impairment of a body structure or body function • in-patient hospitalization or prolongation of hospitalization • injury, significant disability • Fetal distress, fetal death, or a congenital abnormality or birth defect • Other important medical event, that requires medical or surgical intervention to prevent any of the above
2.0	Updated section for clarity on how to manage Adverse Event	*9.2 Adverse Event (AE) Management*	***Before:*** At each study contact, the subject 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. Signs and symptoms must be comprehensively documented on the appropriate source documentation. All related signs and symptoms should be grouped under one diagnosis if possible. Each sign, symptom, disease or illness reported must be evaluated by the Investigator or designee to determine if it meets the definition of an AE. 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. Any irritation of the application sites that results in the withdrawal of the subject from the study or, in the opinion of the Investigator or designate, warrants medical attention or medication will be considered AEs that will be subjected to the same criteria as all other AEs. Adverse events will be collected up to 14 calendar days after the subject completes all study procedures. The Investigator or designee, will phone the subject once a week during the 14 day period to confirm if the AE has resolved. Should the AE not resolved within the 14 days, the Investigator or designee shall consider the follow up period complete and instruct the subject to contact their primary care physician for further care. ***After:*** Collection of adverse events will begin after the subject has signed the Informed Consent form through the end of study conduct. At each study contact, the subject 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. Signs and symptoms must be comprehensively documented on the appropriate source

Version #	Rationale for Change	Section or Page affected	Description of change
			documentation. All related signs and symptoms should be grouped under one diagnosis if possible. Each sign, symptom, disease or illness reported must be evaluated by the Investigator or designee to determine if it meets the definition of an AE. Any irritation of the application sites that results in the withdrawal of the subject from the study or, in the opinion of the Investigator or designate, warrants medical attention or medication will be considered AEs that will be subjected to the same criteria as all other AEs. If an AE or SAE reaction occurs on the skin test site, it may be needed to take photographs of the skin test site only. These photographs will be shared with the Principal Investigator / Sub-Investigator, the sponsor and the study staff only and will be used for the Principal Investigator / Sub-Investigator's review and assessment of the reactions.
2.0	Added new section to clarify Adverse Event Follow ups	*9.2.1 Follow-up of Adverse Events (AEs)*	***Added:*** Adverse events that are present after the completion of the subject's scheduled study visit will be followed up to 14 days by means of the investigational site contacting the subject via telephone or by any other means considered appropriate. If the subject's adverse event is still ongoing past the 14 days, the Investigator will communicate with the sponsor Medical Monitor (via telephone and/or email) as to the need for further follow-up. In the case of discrepancies, the Investigator's judgement will prevail. All communications with the subject and medical monitor will be documented appropriately. Adverse Events whose onset occurs up to 14 days after the end of the subject's study participation, will be recorded and will require the Investigator to communicate with the sponsor Medical Monitor as to the need for further follow-up as described above. All contacts will be documented appropriately. The clinical course of any serious adverse 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 as it relates to the study product and/or study procedures.

<table>
<tr><th>Version #</th><th>Rationale for Change</th><th>Section or Page affected</th><th>Description of change</th></tr>
<tr><td>2.0</td><td>Updated introduction sentence for clarity</td><td>9.3 Assessment of Adverse Events (AEs)</td><td>Before: All AEs (Serious and Non-Serious) are assessed according to severity or intensity of the event experienced by the subject as either mild, moderate, severe, life threatening or death using the following definitions:

After: All AEs (Serious and Non-Serious) are assessed according to severity or intensity of the event experienced by the subject as either mild, moderate, or severe using the definitions below:</td></tr>
<tr><td>2.0</td><td>Updated AE Severity Description</td><td>9.3 Assessment of Adverse Events (AEs)</td><td>Before:
<table>
<tr><td>Mild:</td><td>Minor injury, symptoms or signs, without a significant discomfort or any degree of disability. Symptoms are transient, easily tolerated with no interference with subject's daily activities. Minor injury is self-limited and may require non-medical intervention. Reasonably managed with OTC products and/or palliative measures. Inconsequential prolongation of a diagnostic or therapeutic procedure.</td></tr>
<tr><td>Moderate:</td><td>Injury which may include significant discomfort, minor non-permanent injury and/or temporary disability. Symptoms are temporary and /or reversible. These injuries usually require medical intervention to treat however, are not considered life-threatening.</td></tr>
<tr><td>Severe:</td><td>Injury which results in severe symptoms, significant injury, potential long-term risk to health, or permanent impairment as a direct result of product use and/or failure, or due to a delay in treatment as a result of product use and/or failure. The injury/symptoms may be irreversible despite medical intervention and may be life threatening.</td></tr>
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After:
<table>
<tr><td>Mild:</td><td>Signs or symptoms that do not interfere with the subject's daily activity, are usually considered self-limiting, can be treated with non-prescription type medications and do not require medical intervention.</td></tr>
<tr><td>Moderate:</td><td>Event(s) may interfere or cause low level inconvenience with the subject's daily activity. Requires medical intervention and/or treatment, however, unlikely to require hospitalization or be consider potentially life-threatening in nature..</td></tr>
<tr><td>Severe:</td><td>Event(s) may cause significant discomfort to the subject and/or interferes with the subject's daily activity. Requires medical intervention and/or treatment to preclude a permanent impairment, can be life threatening and require hospitalization.</td></tr>
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<tr>
<td>2.0</td>
<td>Updated AE Relationship and Causality Description</td>
<td>9.3 Assessment of Adverse Events (AEs)</td>
<td>
Before:
<table>
<tr><td>Not Related:</td><td>The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.</td></tr>
<tr><td>Unlikely Related:</td><td>A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant’s clinical condition, other concomitant treatments).</td></tr>
<tr><td>Likely Related:</td><td>There is reasonable evidence to suggest a causal relationship, and the influence of other factors is less likely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is less likely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (if applicable).</td></tr>
<tr><td>Related:</td><td>There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention should be clinically plausible.</td></tr>
</table>
After:
<table>
<tr><td>Not Related:</td><td>The AE is independent of study intervention, and/or evidence exists that the event is related to another etiology. There must be an alternative, etiology documented by the clinician.</td></tr>
<tr><td>Unlikely Related:</td><td>A clinical event, whose temporal relationship to study intervention makes a causal relationship improbable (e.g., the event did not occur within a reasonable time of the study product use) and in which underlying disease provides plausible explanations (e.g., the participant’s clinical condition, other concomitant treatments).</td></tr>
<tr><td>Likely Related:</td><td>There is evidence to suggest a causal relationship, and the influence of other factors is less likely. The clinical event occurs within a reasonable time after use of the study product, and is less likely to be attributed to concurrent disease.</td></tr>
<tr><td>Related:</td><td>There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The</td></tr>
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			clinical event occurs in a plausible time relationship to use of the study product and cannot be explained by concurrent disease.
2.0	Updated subtitle to be consistent with product description	*Appendix 5 Study Product Application Instructions*	***Before:*** Investigational Product: 60% v/v 1-propanol and 0.9% normal saline ***After:*** Reference Standard and Negative Control: 60% v/v 1-propanol and 0.9% normal saline
2.0	Updated subtitle numbering	*Appendix 6 Neutralization Study*	***Before:*** 6.2.1 Neutralizer effectiveness (Test1) and 6.2.2 Neutralizer toxicity (Test 2) ***After:*** 7.2.1 Neutralizer effectiveness (Test1) and 7.2.2 Neutralizer toxicity (Test 2)

18.0 APPENDICES

The following appendices are included:

APPENDIX 1: Diagram of Anatomical Sites

APPENDIX 2: Example Randomization Scheme

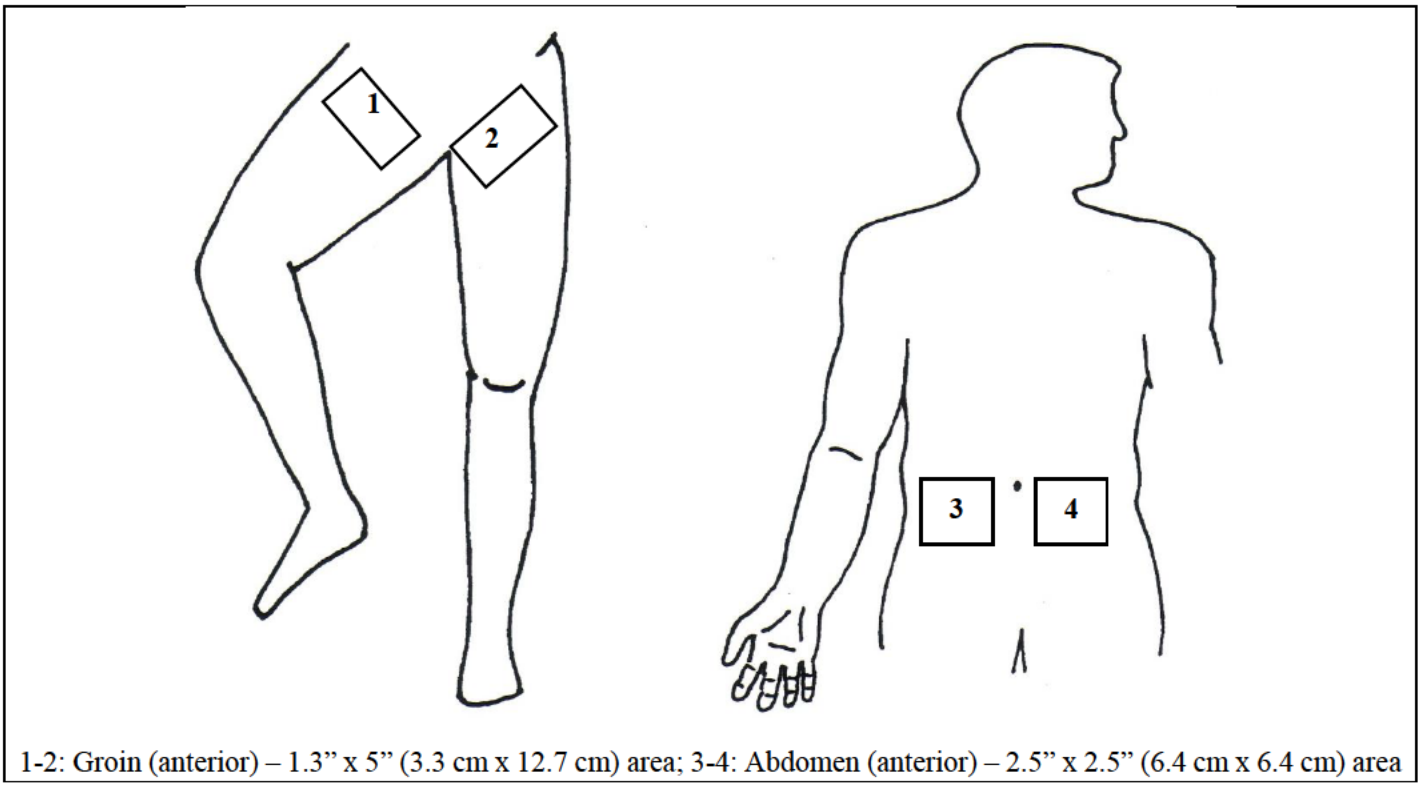
APPENDIX 3: Subject Instructions for Neutralization Study

APPENDIX 4: Subject Instructions for Main Study

APPENDIX 5: Product Application Instructions

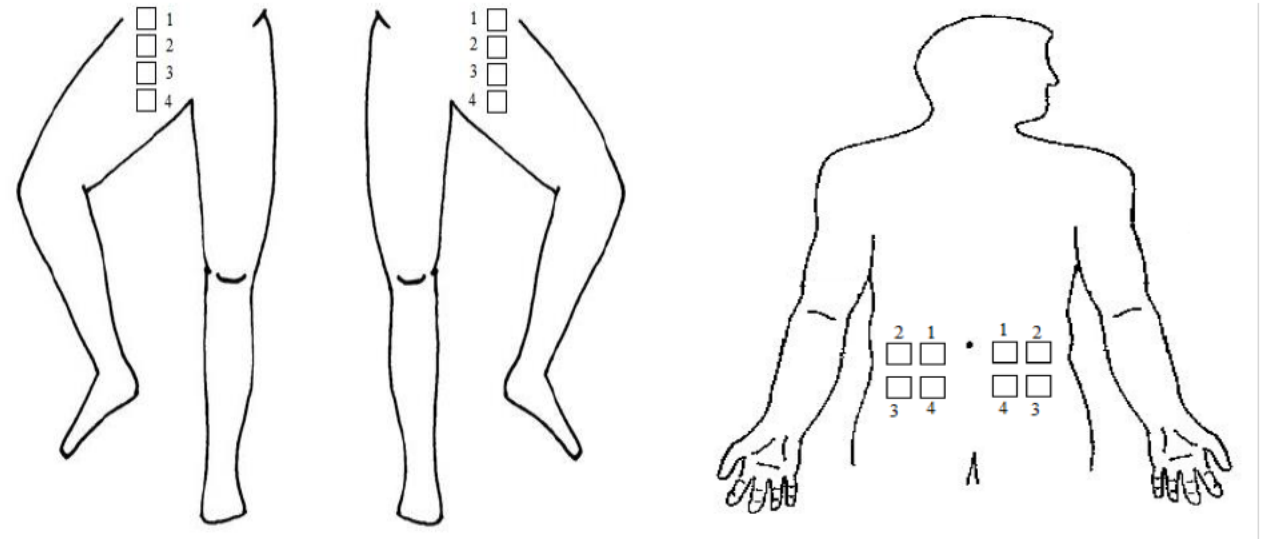
APPENDIX 6: Neutralization Study

APPENDIX 1 DIAGRAM OF ANATOMICAL SITES



1-2: Groin (anterior) – 1.3" x 5" (3.3 cm x 12.7 cm) area; 3-4: Abdomen (anterior) – 2.5" x 2.5" (6.4 cm x 6.4 cm) area

Diagram of sampling sites within the anatomical sites



APPENDIX 2 EXAMPLE RANDOMIZATION SCHEME

Randomization Number	Subject Number	Anatomical Area	Product Code Left Side	Product Code Right Side	Site 1	Site 2	Site 3	Site 4
R0001		Ab / Groin / Both	B	C	10 Minutes	30 seconds	Baseline	6 Hours
R0002		Ab / Groin / Both	A	C	10 Minutes	Baseline	30 seconds	6 Hours
R0003		Ab / Groin / Both	A	B	10 Minutes	6 Hours	Baseline	30 seconds
R0004		Ab / Groin / Both	D	A	30 Seconds	10 Minutes	Baseline	6 Hours
R0005		Ab / Groin / Both	B	D	Baseline	10 Minutes	6 Hours	30 seconds
R0006		Ab / Groin / Both	C	D	Baseline	6 Hours	10 Minutes	30 seconds

APPENDIX 3 SUBJECT INSTRUCTIONS FOR NEUTRALIZATION STUDY

SUBJECT INSTRUCTIONS FOR NEUTRALIZATION STUDY

The following instructions are to be followed until the completion of the study

- Use only the soap provided for all bathing, sponge bathing and hand washing.
- Use only the shampoo provided when washing your hair.
- Do not use antiperspirants or deodorants (other than those provided to you in the kit), lotions, colognes, perfumes, after shaves or powders.
- Do not come in contact with strong detergents, acids, bases, solvents, bug repellant, fabric softener-treated clothing, UV treated clothing or other household chemicals in the in the abdominal body regions.
- Do not swim in chemically treated pools or bathe in hot tubs, whirlpools or spas.
- Do not use tanning beds.
- Do not shave, use depilatories or hot waxes on the abdomen area. If hair is present, allow study staff to clip hair at a designated time.
- Do not apply any medicated creams or ointments to any area of your skin, nor should you take any antibiotics, systemic antimicrobials or steroids (other than hormones for contraception or post-menopausal reasons), antihistamine medication. If these medications are necessary due to illness, please report this to [redacted] Resident Dermatologist at [redacted] immediately.

Additional Instructions for Neutralization study dd/mm/yyyy _ _ / _ _ / _ _ _ _

- You may be required to return to the Research facility approximatively 48 – 96 hours before Neutralization study for clipping / reclipping.
- On the Neutralization study, you will return to the investigational site for products application and sampling.

If you have questions about this study or in case of emergency, contact any time during business hours or/and after business hours:

[redacted] **RESIDENT DERMATOLOGIST**

[redacted] [redacted]

APPENDIX 4 SUBJECT INSTRUCTIONS FOR MAIN STUDY

SUBJECT INSTRUCTIONS FOR MAIN STUDY

The following instructions are to be followed until the completion of the study

- Use only the soap provided for all bathing, sponge bathing and hand washing.
- Use only the shampoo provided when washing your hair.
- Do not use antiperspirants or deodorants (other than those provided to you in the kit), lotions, colognes, perfumes, after shaves or powders.
- Do not come in contact with strong detergents, acids, bases, solvents, bug repellant, fabric softener-treated clothing, UV treated clothing or other household chemicals in the abdominal and upper thigh body regions.
- Do not swim in chemically treated pools or bathe in hot tubs, whirlpools or spas.
- Do not use tanning beds.
- Do not 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.
- Do not apply any medicated creams or ointments to any area of your skin, nor should you take any antibiotics, systemic antimicrobials or steroids (other than hormones for contraception or post-menopausal reasons), antihistamine medication. If these medications are necessary due to illness, please report this to [redacted] Resident Dermatologist at [redacted] immediately.

Additional Instructions for Screening Day visit dd/mm/yyyy _ _ / _ _ / _ _ _ _

- Do not bathe or shower in the 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 may be required to return to the investigational site approximatively 48 – 96 hours before your Screening visit for clipping / reclipping.
- On the day of the Screening visit, you will be required to return to the investigational site for the screening baseline sampling procedures.
- You will be contacted by telephone as to whether you have met the established criteria and therefore will be returning for a Product Application visit (if selected). Remember to continue to use your kit products until you have been eliminated or have completed the study.

Additional Instructions for Product Application Day visit dd/mm/yyyy _ _ / _ _ / _ _ _ _

(if you are selected)

- Do not bathe or shower in the 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 may be required to return to the investigational site approximatively 48 – 96 hours before your Product Application Day visit for clipping / reclipping.
- On the Product Application Day visit, you will return to the investigational site for products application and the initial sampling.
- You will be required to remain at the study facility through the 6-hours ± 30 minutes post-application skin sampling (as scheduled). You must limit physical activity and getting the product application sites wet during this time period.

If you have questions about this study or in case of emergency, contact any time during business hours or/and after business hours: [redacted] RESIDENT DERMATOLOGIST

Phone: [redacted] Mobile: [redacted]

APPENDIX 5 STUDY PRODUCT APPLICATION INSTRUCTIONS

Investigational Product: Single Swabstick

Preparation

1. Weigh the Swabstick package and record the weight.
2. Remove the Swabstick from the package without touching the sponge tip.

Product Application Site Application Instructions – Abdomen

1. Place foam flat side down on the product application area. Using repeated back-and-forth strokes of the sponge for thirty (30) seconds, completely wet the product application area with the study product.
2. Allow the area to air-dry for thirty (30) seconds. Do not blot or wipe away.

Product Application Site Application Instructions – Groin

1. Place foam flat side down on the product application area. Using repeated back-and-forth strokes of the sponge for two (2) minutes, completely wet the product application area with the study product.
2. Allow the area to air-dry for one (1) minute. Do not blot or wipe away.

Post-Product Application Instructions

1. Weigh the Swabstick including package and record the weight.

Active Control: ChloraPrep® SEPP®

Preparation

1. Weigh the ChloraPrep® SEPP® package and record the weight.

2. Remove the ChloraPrep® SEPP® from the package without touching the applicator tip.

Product Application Site Application Instructions – Abdomen

1. Place sponge tip down on the product application area and pinch the barrel of the applicator only once to activate the ampule and release the antiseptic. Visualize that the foam is wet prior to starting application timer. Using repeated back-and-forth strokes of the sponge for thirty (30) seconds, completely wet the product application area with the study product.
2. Allow the area to air-dry for thirty (30) seconds. Do not blot or wipe away.

Product Application Site Application Instructions – Groin

1. Place sponge tip down on the product application area and pinch the barrel of the applicator only once to activate the ampule and release the antiseptic. Using repeated back-and-forth strokes of the sponge for two (2) minutes, completely wet the product application area with the study product.
2. Allow the area to air-dry for one (1) minute. Do not blot or wipe away.

Post-Product Application Instructions

1. Weigh the SEPP applicator including package and record the weight.

Reference Standard and Negative Control: 60% v/v 1-propanol and 0.9% normal saline

Preparation

1. Using a sterile pipette, transfer 1.75mL of 60% v/v 1-propanol or 0.9% normal saline into a suitable sized sterile Petri dish
2. Weigh the Swabstick and the Petri dish containing 1-propranol or 0.9% normal saline and record the weight.
3. Immediately prior to application, without touching the sponge tip, dip the Swabstick in the 60% v/v 1-propanol or the 0.9% normal saline absorbing as much liquid as possible.

Product Application Site Application Instructions – Abdomen

1. Place foam flat side down on the product application area. Using repeated back-and-forth strokes of the sponge for thirty (30) seconds, completely wet the product application area with the study product.
2. Allow the area to air-dry for thirty (30) seconds. Do not blot or wipe away.

Product Application Site Application Instructions – Groin

1. Place foam flat side down on the product application area. Using repeated back-and-forth strokes of the sponge for two (2) minutes, completely wet the product application area with the study product.
2. Allow the area to air-dry for one (1) minute. Do not blot or wipe away.

Post-Product Application Instructions

1. Weigh the Petri dish and Swabstick and record the weight.

APPENDIX 6 NEUTRALIZATION STUDY

1. **Background**

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 (chlorhexidine gluconate) present in the investigational product and active control. 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 study where the study material is applied to the abdomen and the anatomical areas will be sampled. Each sample will then be processed using procedures in accordance with ASTM E1054-08(2013). Three samples will be taken from each anatomical area and one sample will be processed using *Staphylococcus epidermidis* (SE), ATCC 12228, a second sample using *Staphylococcus epidermidis* (MRSE) ATCC 51625, and a third sample using Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33592.

2. **Objective**

This control assay will determine the ability of the SS to completely neutralize the active ingredients in the study products 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 in triplicate, with the exception of Test 1 which will performed with N=6.

3. **Subject Entry Criteria**

Twelve subjects will be used for the neutralization validation required for this study. Each subject must meet the inclusion criteria (1-6) listed in Section 4.1 and exclusion criteria (1-17) described in Section 4.2 of the protocol with the exception of the 72-hour exclusion from showering/bathing and the length of the product restriction period. The product restriction period for neutralization is only necessary for 7 days (not 14 days). The subjects will be asked to provide information on demographics and inclusion/exclusion criteria and sign the Consent and Authorization Forms before beginning the 7-day product restriction 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 12.

The study material will be applied to bilateral areas of the abdomen so that six applications (n = 6) are performed for each of the 4 study products (a total of twelve subjects and 24 tests). Each anatomical site will have three sampling sites.

The test organisms for this study are:

a. *STAPHYLOCOCCUS EPIDERMIDIS* (MSSE), ATCC 12228, MARKER ORGANISM
b. *STAPHYLOCOCCUS EPIDERMIDIS* (MRSE), ATCC 51625, MARKER ORGANISM
c. METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) ATCC 33592, MARKER ORGANISM

4. Study Products

a. Investigational Product: Single swabstick impregnated with 1.75mL of 2% (w/v) chlorhexidine gluconate (CHG) in 70% (v/v) isopropyl alcohol (thermally treated)
b. Reference Standard: 1.75 mL of 60% (v/v) 1-propanol applied with a swabstick
c. Active control: ChloraPrep® SEPP® clear applicator-2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol
d. Negative control: 1.75mL 0.9% normal saline applied with a swabstick

5. Randomization

Subjects participating in the neutralization portion of the study will be randomized using a separate randomization schedule. An example of the randomization schedule for the neutralization portion of the protocol is provided below.

Randomization Number	Subject Number	Anatomical Area	Left	Right	Site 1	Site 2	Site 3
N001	01	Abdomen	A	B	MSSE	MRSA	MRSE
N002	02	Abdomen	C	D	MRSE	MSSE	MRSA

6. *In-vivo* Procedures (collection of samples)

Anatomical area and post-product application sampling: Neutralization samples will be taken from the abdomen. Treatment sites and product application will utilize those for the groin area in the main study to simulate the maximum case scenario for product deposition to the treatment site. The subject number, location of the product application (left or right), location of the sites sampled within the anatomical area, and the time of sample collection will be documented on the CRF. The subject will be treated with the study materials based on the following:

- On one side of the body, mark the abdominal area using a sterile 1.3" x 5" (3.3 cm x 12.7 cm) template. The 1.3" x 5" (3.3 cm x 12.7 cm) will be delineated with three 1" x 1" sampling sites (one sampling site will be used for each test organism).
- After the anatomical area is marked, the area will be processed using three 70% isopropyl alcohol swabs for a total of one minute; the area will be allowed to dry. This step is to cleanse the skin for the neutralization test.
- Apply study product to the appropriate abdominal area for 2 minutes with a 1-minute dry time according to the instructions provided in Appendix 5 for the groin.
- Using the scrub cup technique at approximately 30 seconds post-product application (including dry time), begin collecting samples from each site using SS. This technique is described in Section 6.3.1.1. For the three sites within each 1.3" x 5" (3.3 cm x 12.7 cm) area; the samples will be collected simultaneously by three technicians.

- Repeat on the bilateral application site with the second study product according to the randomization scheme.

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

7.1 Inoculum Preparation: For each test organism, the organism will be prepared from an overnight broth culture (24 ± 4 hours) grown in TSB at 35° ± 2°C to yield a concentration of approximately 10^8 – 10^9 CFU/mL. The culture will be diluted using PBW in a manner such that 30 – 100 CFU/mL will be delivered into the neutralizer tube (a 5 mL aliquot of the collected SS sample will be used).

7.2 Test: (note – the reference to investigational product applies to all study products; all procedures outlined, where applicable, will employ all study products. In addition, using the three samples taken within each 1.3" x 5" area; all procedures, as applicable, will be performed using one sample each for each of the three test organisms).

7.2.1 Neutralizer effectiveness (Test 1):

a. Out of the 6.0 mL aliquot of sampling solution taken from the volunteer, 5.0 mL will be transferred to a new sterile tube and inoculated with the challenge microorganism so that the final concentration will equal 30 – 100 colony-forming units (CFU) /mL of the challenge microorganism (the prepared inoculum will be diluted using PBW to achieve the desired concentration, a 0.1-mL aliquot from the 10^{-4} dilution will be used).
b. Within one min after the addition of the challenge microorganism, the microorganisms will be enumerated by standard microbiological methods extant in the laboratory.
c. Triplicate 0.75 mL aliquots will be removed and plated using TSA+N pour plates.
d. After 30 minutes, the microorganisms will be enumerated a second time using the same procedures.
e. Triplicate 0.75 mL aliquots will be removed and plated using TSA+N pour plates.

7.2.2 Neutralizer toxicity (Test 2):

a. A 5.0 mL aliquot of sampling solution will be inoculated with the challenge microorganism so that the final concentration will equal 30 – 100 colony-forming units (CFU) /mL of the challenge microorganism in the same manner as Test 1.
b. Within one min after the addition of the challenge microorganism, the microorganisms will be enumerated by standard microbiological methods extant in the laboratory.
c. Triplicate 0.75 mL aliquots will be removed and plated using TSA+N pour plates.
d. After 30 minutes, the microorganisms will be enumerated a second time using the same procedures.
e. Triplicate 0.75 mL aliquots will be removed and plated using TSA+N pour plates.
f. This procedure will be repeated two times for a total of three replicates.

7.2.3 Test microorganism viability control (Test 3):

a. A 5.0mL aliquot of PBW will be inoculated with a volume of the challenge microorganism so that the resulting suspension contains 30 – 100 CFU/mL in the same manner as Test 1.
b. Within one min the microorganisms will be enumerated by standard microbiological

methods extant in the laboratory.
c. Triplicate 0.75 mL aliquots will be removed and plated using TSA pour plates.
d. After 30 minutes, the microorganisms will be enumerated a second time using the same procedures.
e. Triplicate 0.75 mL aliquots will be removed and plated using TSA pour plates.
f. This procedure will be repeated two times for a total of three replicates.

7.2.4 Investigational Product control (Test 4):

a. A 5.0mL aliquot of the Investigational Product will be inoculated with a volume of the challenge microorganism so that the resulting suspension contains 30 – 100 CFU/mL in the same manner as Test 1.
b. Within one min the microorganisms will be enumerated by standard microbiological methods extant in the laboratory.
c. Triplicate 0.75 mL aliquots will be removed and plated using TSA pour plates.
d. After 30 minutes, the microorganisms will be enumerated a second time using the same procedures.
e. Triplicate 0.75 mL aliquots will be removed and plated using TSA pour plates.
f. This procedure will be repeated two times for a total of three replicates.

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

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

7.5 Controls:
7.5.1 Sterility Control
Triplicate plates of TSA and TSA+N used will be incubated with the test. In addition, triplicate 1.0-mL aliquots of sampling solution and PBW will be plated using TSA pour plates used for a particular test date. All plates will be incubated with the test.

Signature Page for MPS-17IPVSS02 Swabsticks Protocol v2

Approved	[redacted] Senior Clinical Project Manager 07-May-2019 20:06:27 GMT+0000
Approved	[redacted] Medical Director nagement 07-May-2019 20:59:19 GMT+0000
Approved	[redacted] Director, R&D 07-May-2019 21:38:21 GMT+0000
Approved	[redacted] Principal Statistician n 08-May-2019 12:39:57 GMT+0000
Approved	[redacted] Medical Safety, VP 08-May-2019 14:20:20 GMT+0000