Clinical Study Protocol

Study Number and Protocol Title	EM-05-014624 (MBT 109-296) Assessment of the antimicrobial efficacy of 3M™ SoluPrep™ Preoperative Skin Preparation against resident human skin flora on the inguinal region
Principal Investigator	M. Hamid Bashir, MD, CCRP Laboratory Manager, Microbiology Email: muhammad.bashir@microbac.com
Sub-investigator	Angela L. Hollingsworth Director of Microbiology Email: angela.hollingsworth@microbac.com
Research Facility	Microbac Laboratories, Inc 105 Carpenter Drive Sterling, VA 20164 Telephone: 703-925-0100 FAX: 703-925-9366
IRB	Microbac Internal IRB 105 Carpenter Drive Sterling, VA 20164
Sponsor	3M Health Care 3M Center Building 270-4N-01 St. Paul, MN 55144-1000
3M Study Monitor	Linda Olson 3M Health Care 3M Center Building 270-4N-01 St. Paul, MN 55144-1000 Telephone: 651-736-3586 FAX: 651-736-7329 Email: lkolson2@mmm.com

3M Medical Monitor

Matthew Cooper, MD, MBA, FACS
Global Senior Medical Director &
Director Patient Safety Center of Excellence
3M Health Care
3M Center Building 270-4N-01
St. Paul, MN 55144-1000
Telephone: 651-736-4689

Approver List (3M Approvers Only):

Signer	Role	Date Signed
US113168:Morse Dan J	Clinical	May 26, 2020 06:50:44 AM CDT
US233115:Olson Linda K	Clinical	May 21, 2020 11:35:47 AM CDT
A4T08ZZ:Cooper Matthew M	Clinical	May 27, 2020 09:50:20 AM CDT
a10s7zz:Paulson Joan E	Clinical	May 26, 2020 12:35:01 PM CDT
US264576:Hupperts Ann M	Regulatory	May 25, 2020 10:24:34 PM CDT

cc: Clinical Study Folder
Investigator Study Documentation File (Regulatory Binder)

Table of Contents

1. BACKGROUND INFORMATION

1.1 Name, Description and Intended Use of the Investigational Product

3M™ SoluPrep™ Film-Forming Sterile Surgical Solution (SoluPrep Film-Forming Solution), an FDA-approved over-the-counter (OTC) drug product (NDA 208288), is an antimicrobial skin preparation containing the active ingredients 2% w/v chlorhexidine gluconate (CHG) and 70% v/v isopropyl alcohol (IPA) in combination with inactive ingredients: iso-octyl acrylate/methyl methacrylate/n-vinylpyrrolidone (IOA/MMA/NVP) polymer, acetyltributyl citrate (ATBC), trisodium hydroxylethyl ethylenediamine triacetic acid (HEDTA) and purified water (water). When the solution is applied to the skin, it dries to form a film. The potential benefits of this product, as compared with currently marketed CHG/IPA containing products without a polymer, include improved adhesion of incise drapes to prepped skin. In early clinical use situations, the performance of the SoluPrep film-forming polymer did not appropriately adhere to the patient's skin, resulting in flaking or pilling of the polymer film in some cases. 3M is currently evaluating modifications to the quantitative formulation of the inactive ingredients in SoluPrep Film-Forming Solution including removal of the polymer.

3M™ SoluPrep™ Preoperative Skin Preparation (SoluPrep) is an investigational antimicrobial skin preparation containing 2% w/v chlorhexidine gluconate (CHG) and 70% v/v isopropyl alcohol (IPA) without a polymer component. The product will be available in the following configurations: 10.5-mL tinted and colorless, and 26-mL tinted. The 10.5-mL tinted configuration will be evaluated in the current study.

3M™ CHG/IPA Film-Forming Preoperative Skin Preparation (SoluPrep FF) contains 2% w/v CHG and 70% v/v IPA. SoluPrep FF 10.5-mL tinted configuration will be used as the active control.

Sterile 0.9% saline will be used as the negative control.

1.2 Summary of Relevant Studies

SoluPrep FF has been evaluated at outside labs in 4 clinical safety studies, 1) cumulative irritation patch test (21 day) with challenge (EM-05-012853), 2) clinical evaluation to assess the phototoxicity potential (EM-05-012952), 3) clinical evaluation of photoallergy potential (EM-05-013062), and 4) evaluation of impurities: human pharmacokinetics maximum usage trial (EM-05-014226). Under the conditions of these trials, the product was considered to be safe and well-tolerated.

Three in vivo pivotal studies (EM-05-012759, EM-05-012760, and EM-05-013260) conducted at outside labs to demonstrate the effectiveness of SoluPrep FF against resident skin flora showed that the product was well tolerated by the study populations.

Skin condition (erythema, edema, rash, dryness, and denudation) was evaluated in 3 clinical studies (EM-05-014626, EM-05-014733, and EM-05-014753) using a modification of the

SoluPrep FF formulation, which included reduction and removal of the polymer. These 3 studies all evaluated drape adhesion to skin. In the first 2 studies, the modified formulations used in combination with a surgical drape were well tolerated by the subject population, with no AEs reported. In the third study (EM-05-014753), 3 AEs were reported. Two of the 3 were mild in severity and the third was considered moderate. All 3 were probably related to the study products.

Refer to the Investigator Brochure (CLIN-INV-BROCH-US-05-171686) for a summary of the studies conducted.

1.3 Risk/Benefit Summary

There are minimal anticipated adverse health risks for the participants of this study. Considerable safety and efficacy data are available for the active ingredients in the investigational product and preclinical safety studies, as described above, have been completed. Application of the study products may cause skin irritation, which may manifest as minor redness, swelling, itching, cracking or peeling. The incidence of irritation is expected to be low, as is the severity of irritation. Individuals with a known history of sensitivity to acrylates, CHG- or alcohol-containing products, medical tapes, metals, natural rubber latex, vinyl, or skin-marking inks, will be excluded from this study.

There are no direct health benefits to subjects who participate in this study.

1.4 Good Clinical Practices and Regulatory Requirements

3M™ SoluPrep™ Preoperative Skin Preparation is an investigational new drug. This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and applicable state and federal regulations including 21 CFR 312 (Investigational New Drug Application), 50 (Protection of Human Subjects), 56 (Institutional Review Boards), 54 (Financial Disclosure by Clinical Investigators), and 45 CFR 160 and 164 (Authorization for Use/Disclosure of Protected Health Information).

1.5 Study Population

Healthy male and female volunteers, 18 years of age or older, with no dermatological conditions on the test sites, or known history of sensitivity to acrylates, CHG- or alcohol-containing products, medical tapes, metals, natural rubber latex, vinyl, or skin-marking inks, will be enrolled into this study. A sufficient number of subjects will be enrolled in the screening phase, such that approximately 100 applications of the investigational product (10.5-mL tinted) and the active control (10.5-mL tinted), in addition to approximately 30 applications of the negative control, are evaluable for efficacy on the inguinal region (groin) at completion of the study. In total, 230 evaluable applications of investigational product, active control, and negative control would require 115 test subjects, utilizing both the right and left sides of the inguinal region. With the expectation that 15% of subjects will not meet treatment day baseline requirements, it is anticipated that 135 subjects will need to be treated. Subjects must satisfy all Screening Day and

Treatment Day Inclusion/Exclusion Criteria prior to collection of screening baselines and Treatment Day procedures. If the required numbers of subjects do not qualify from the initial screening group, additional volunteers will be recruited, as needed.

Each subject will be identified by a screening identification number, and a subject treatment number. Subject treatment numbers will not be assigned until a subject has passed the screening criteria, including screening baseline bacterial counts.

Both the right and left sides of the groin must meet the screening baseline requirements stated in the Inclusion Criteria to qualify for treatment.

2. STUDY OBJECTIVES

The primary objective of this study is to evaluate the antimicrobial efficacy of SoluPrep by demonstrating non-inferiority to SoluPrep FF, an FDA-approved active control, with a 0.5 margin (log_{10} scale), and demonstrating a superiority margin of 1.2 log_{10}/cm^2 to saline, the negative control. In addition, both active products should demonstrate persistence of effect where the 6-hour post-treatment measurement is lower than or equal to the baseline measurement for 100% of the subjects.

Safety will also be evaluated based on the incidence of adverse events reported during the study and assessment of skin irritation ratings.

3. STUDY DESIGN

3.1 Study Type and Methodology

This is a randomized, controlled, third-party blind, single-center study in healthy volunteers where each subject receives 2 of the 3 possible study products on the groin. The study staff performing the bacterial enumeration and the statistician performing the analysis will be blinded to the study products.

Table 3.1A provides a summary of the study procedures. A schematic diagram of the study is included in Appendix 14.1.

Table 3.1A Study Summary

Pre-Study Preparation	14-day Pretreatment Phase	Screening Phase	Treatment Day
Staff reviews study protocol	Discuss study procedures including Inclusion/Exclusion Criteria	Complete Inclusion/ Exclusion Criteria form	Assign sequential subject number Complete Inclusion/Exclusion Criteria form
Prepare consent form	Perform the Informed Consent process	Perform visual skin assessment	Perform visual skin assessment
Obtain IRB approval	Assign subject screening number	Collect screening baseline samples from inguinal region	Perform skin irritation rating
Recruit volunteers for Pretreatment Phase	Complete Inclusion/Exclusion Criteria form	Count screening plates, determine which subjects qualify for study	Per randomization, mark test areas, collect baseline samples from inguinal region
Prepare subject personal hygiene kits	Review subject instructions for wash out and dispense subject personal hygiene kit	Contact eligible subjects, schedule Treatment Day (schedule for clipping, if needed)	Apply study products per randomization
	If needed, perform clipping.		Perform skin irritation rating, collect 10-minute (± 30 sec) post-prep samples
			Perform skin irritation rating, collect 6-hour (± 30 min) post-prep samples

3.2 Primary and Secondary Endpoints

Primary Endpoints:

The primary endpoints include (1) an immediate efficacy endpoint which is defined as the average treatment effect (ATE) $\log_{10}$ CFU/cm^2 of skin flora on the inguinal region at 10 minutes following application of the study products relative to the treatment day baseline skin flora, and (2) a persistent efficacy endpoint which is a binary endpoint with success/failure, where "success" is defined as $\log_{10}$ CFU/cm^2 of skin flora on the inguinal region at 6 hours following application of the study products being lower than the treatment day baseline skin flora.

Secondary Endpoints:

Secondary microbiological endpoints are the log_{10}/cm^2 baseline skin flora, log_{10}/cm^2 recovery of skin flora, and log_{10}/cm^2 reduction of skin flora relative to Treatment Day baseline log_{10}/cm^2 on the inguinal region at 10 minutes and 6 hours following application of the study products.

The principal measure of safety will be the incidence of adverse events reported during the study and a summary of the skin irritation rating scores. A skin irritation rating of 3 in any category represents significant irritation and qualifies as an Adverse Event.

3.3 Randomization and Blinding

The left and right test areas on the subject's inguinal region will be assigned according to a computer-generated randomization schedule to receive 1 of the 3 study products (see Table 3.4.1A). Randomization will be balanced between left and right sides. There are 3 different combinations of treatments. These are shown below in Table 5.3.3A. To provide an ample number of subjects, the treatment groups will be randomized into 10 blocks of 12-2-2 for each of groups 1 through 3, respectively. Baseline and post-prep samplings will be randomized to sites within each test area. The same test site randomization will be used on each side of a subject's body to reduce the possibility of sampling errors. An example of one of the blocks is shown in Table 3.3.1 below.

Table 3.4.1 Example Randomization

Random-ization Number	Treatment Group	Left Groin Treatment	Right Groin Treatment	Sampling Site		
				Baseline	10 Minute	6 Hour
1	1	SoluPrep FF	SoluPrep	4	2	3
2	1	SoluPrep FF	SoluPrep	3	2	4
3	1	SoluPrep	SoluPrep FF	4	2	3
4	1	SoluPrep FF	SoluPrep	3	2	4
5	1	SoluPrep	SoluPrep FF	3	4	2
6	3	Saline	SoluPrep FF	4	3	2
7	1	SoluPrep FF	SoluPrep	3	4	2
8	2	SoluPrep	Saline	2	3	4
9	1	SoluPrep FF	SoluPrep	3	4	2
10	1	SoluPrep	SoluPrep FF	4	2	3
11	1	SoluPrep	SoluPrep FF	2	4	3
12	1	SoluPrep	SoluPrep FF	3	4	2
13	3	SoluPrep FF	Saline	4	3	2

Random-ization Number	Treatment Group	Left Groin Treatment	Right Groin Treatment	Sampling Site		
				Baseline	10 Minute	6 Hour
14	1	SoluPrep	SoluPrep FF	4	3	2
15	1	SoluPrep FF	SoluPrep	3	2	4
16	2	Saline	SoluPrep	3	4	2

The Investigator is responsible for ensuring that the study randomization is followed. Randomization envelopes will be provided to the study site.

The study products cannot be blinded from the Investigator or study staff performing the product application, sample collection and skin assessment due to the obvious differences in applicator design, color, and other physical characteristics. However, the study staff performing the bacterial enumeration will not be involved in the study product application or the collection of samples and, therefore, will be blinded to the study products. The statistician performing the analysis will also be blinded.

Randomization Plan:

A 3M statistician not assigned to the project will develop a randomization program and generate the randomization code list. The code list will include randomization number, treatment group, left side treatment, right side treatment, baseline sample site, 10-minute sample site and 6-hour sample site. The randomization assignments will be printed and inserted into security envelopes then tightly sealed.

The Clinical Data Analyst will remove the treatment assignment from the programs that are used to download the data from Clindex®. This will keep the statisticians and programmers assigned to the study blinded until data lock.

Randomization Process:

For each eligible subject to be randomized, the investigator or designee will do the following:

- Retrieve a randomization envelope with smallest available randomization envelop number.
- Record randomization number onto a Data Collection Sheet (DCS) and/or the electronic Case Report Form (eCRF).
- When the treatments are applied, record onto a DCS and/or the eCRF; left side treatment applied, right side treatment applied, baseline sample site, 10-minute sample site and 6-hour sample site.
- Subjects will continue to be randomized until a minimum of 100 observations (with a baseline that meets the Treatment Day requirement) from each of the active treatments and

30 observations from the saline control (with a baseline that meets the Treatment Day requirement) have been collected.

3.4 Study Products and Labeling

3.4.1 Study Products

The products that will be evaluated in this study are described in Table 3.4.1A.

Table 3.4.1A Study Products

Treatment Code	Study Product	Description
SP	SoluPrep	Contains 2% w/v chlorhexidine gluconate (CHG) and 70% v/v isopropyl alcohol (IPA). 10.5-mL applicator, tinted.
SPFF	SoluPrep FF	Contains 2% w/v chlorhexidine gluconate (CHG) and 70% v/v isopropyl alcohol (IPA) in combination with an acrylate copolymer. 10.5-mL applicator, tinted.
S	Saline	Negative control. Sterile 0.9% saline. 20-mL bottle.

The SoluPrep products will be applied topically to intact skin of the inguinal (groin) region of the subject. The prep will be applied with repeated back-and-forth strokes for 2 minutes and allowed to dry for 3 minutes.

The saline control will be applied with repeated back-and-forth strokes for 2 minutes on the groin using sterile polyurethane foam applicators, aseptically saturated with 20 mL of solution, and allowed to dry for 3 minutes.

Detailed instructions for application and removal of the study materials are provided in the Investigational Product Application Instructions (Appendix 14.2).

3.4.2 Investigational Product Labeling and Accountability

3M will label, package and ship the study products to the research facility. SoluPrep will be provided in individual sterile packages and labeled as below.

Sample Investigational Product Label (ie, SoluPrep)

CAUTION: New Drug-Limited by Federal Law to Investigational Use
For use in study EM 05-014624 (MBT 109-296) only. 3M Health Care, St. Paul, MN 55144-1000
Topical Patient Prep (10.5-mL) **Subject Number:** Product ID: 42-0037-0388-3 Treatment Code: **SP**
Directions: Use as directed in protocol **Warning: Contents are flammable until dry.**

3M requires Investigators to maintain accountability and adequate inventory security of the study products at all times. The Investigator or designee will:

- Document receipt of study products on the Confirmation of Release & Receipt of Clinical Supplies form (Appendix 14.3) and return the completed form to 3M,
- Store study products in a secure facility accessible only to authorized individuals,
- Dispense study products only to subjects properly enrolled into the study,
- Account for inventory and disposition using the Study Product Disposition Form (Appendix 14.4), and
- Return all used and unused study products to 3M or dispose of used and unused study products as agreed upon. NOTE: The subject number will be recorded on the package label and retained for inventory purposes. Used applicators can be discarded.

In addition, copies of all completed Study Product Disposition Forms must be retained in the Investigator Study Documentation File. The originals of all Study Product Disposition Forms must be sent to the Sponsor's Clinical Monitor.

3.4.3 Other Product Labeling

SoluPrep FF active control will be provided in original marketed packaging with an over label, as below, placed on each applicator pouch.

Sample Marketed Active Control Label (ie, SoluPrep FF)

Commercialized Product – For use only as directed below

Study No: EM 05-014264 (MBT 109-296)
3M Health Care, St. Paul, MN 55144-1000

Topical Patient Prep (10.5-mL) **Subject Number:**
Product ID: 70-2007-8768-0
Treatment Code: **SPFF**

Directions: Use as directed in protocol
Warning: Contents are flammable until dry.

The saline solution will be supplied in original marketed 20 mL bottles and box will be overlabeled as below.

Sample Negative Control Label (ie, saline 20 mL bottles)

For use only as directed below

Study No: EM 05-014264 (MBT 109-296)
3M Health Care, St. Paul, MN 55144-1000

Saline (20 mL)
Product ID: Hospira #00409488820
Treatment Code: **S**

Directions: Use as directed in protocol

The sterile polyurethane foam applicators used for saline application will be provided in individual sterile packages labeled as below.

Sample Negative Control Foam Applicator Label (ie, saline applicators)

For use only as directed below

Study No: EM 05-014264 (MBT 109-296)
3M Health Care, St. Paul, MN 55144-1000

Saline Applicator **Subject Number:**
Treatment Code: **S**

Directions: Use as directed in protocol

3.4.4 Additional Study Supplies

Sponsor will provide the following:

- Study Product Disposition Forms
- Randomization schedule
- Data Collection Sheets (DCSs)
- Study products
- Triacetin (Glyceryl triacetate)

Study site will provide the following:

- Subject Informed Consent / HIPAA Authorization forms, IRB-approved
- Product kits (toiletry items to be used by subjects during study)
- Standard Sampling Solution (SSS), 75mM phosphate buffer (0.04% monobasic potassium phosphate, and 1.01% dibasic sodium phosphate) containing 0.1% Triton® X-100, 0.3% lecithin, 3.0% polyoxyethylene sorbitan monooleate (Tween® 80), and 1.0% Tamol™ SN; pH 7.9 $\pm$ 0.1, sterile
- Butterfield's sterile phosphate buffered water[1] (PBW), 312 µM KH_2PO_4, pH 7.2 ± 0.1
- High-purity deionized water, sterile
- Trypticase soy agar containing 0.5% Tween 80 and 0.07% lecithin (TSA+N)
- Disposable undergarments
- Gloves, sterile
- Sterile, non-occlusive dressings (to cover treated test sites)
- Alcohol wipes
- Transfer pipettes, polyethylene, sterile
- Serological pipettes, 1 mL, 2 mL, and 10 mL, sterile
- Pipettor, calibrated to accurately deliver volumes of 0.5 mL, 2.5 mL and 3.0 mL, and sterile tips
- Tubes with sealable caps, polypropylene or glass, sterile
- Petri dishes, 100-mm, sterile
- Surgical clippers with blades
- Sterile marking templates, 1.5" x 5" (for marking test sites)
- Non-toxic marking pen (Sharpie or equivalent), sterile (Note: Do not use markers containing crystal violet)
- Scrub cups (2.20 cm I.D., 3.80 cm^2), sterile
- Teflon or rubber policemen, sterile
- Timers or stopwatches
- Pipette-Aid® or similar apparatus
- Vortex mixer
- Water bath (45 ± 2C°)
- Incubator (30 ± 2C°)

3.5 Study Duration

The expected duration of this study for each subject is up to 4 weeks. Each subject's participation will involve at least a 14-day Pretreatment Phase (washout), a 1-day Screening Phase, and a 1-day Treatment Phase. Following the Pretreatment Phase, each subject will be required to visit the test facility on an arranged date for collection of screening baseline samples from the inguinal region. Subjects will remain on washout until notified that they do or do not qualify for the study. Subjects whose screening baseline samples meet the requirements described in the Inclusion Criteria (Section 4.1) will be notified and invited to participate in the Treatment Phase of the study. The Treatment 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 return to the test facility for treatment and again within 6 hours of treatment for the final sampling. Table 3.1A indicates the sequence of events throughout the study and a schematic diagram of the study is included in Appendix 14.1.

3.6 Study Termination

Conditions that may warrant termination of the study by 3M include, but are not limited to the following:

- The discovery of an unexpected, serious, or unreasonable risk to study subjects,
- Insufficient adherence to protocol requirements,
- Failure of the Investigator to enroll subjects into the study at an acceptable rate,
- Failure of the Investigator to comply with either pertinent FDA regulations or federal (eg, HIPAA) and state privacy regulations,
- Submission of knowingly false information from the Investigator to 3M,
- Termination of IND or IRB approval, or
- A decision on the part of 3M to suspend or discontinue evaluation of the investigational product.

3.7 Source Data

Electronic data capture (eDC) will be used for this study. If not entered directly into eDC, data will first be recorded onto a DCS, followed by entry into the appropriate electronic Case Report Form (eCRF). Wherever the data are entered first is considered the source document.

3.8 Computerized Systems

- Clindex® 4.4
- SAS® 9.4 or later
- R 3.4.0 or later
- Minitab 17 or later
- Excel for Office 365

3.9 Protocol Modifications

3.9.1 Protocol Amendments

The party initiating an amendment must confirm it clearly in writing using the Amendment/Administrative Revision form. It must be signed and dated by 3M and, in the case of a significant amendment, the Investigator. A significant amendment means one that affects the safety, rights or welfare of subjects, the scope of the investigation, or the scientific quality of the study.

3M will submit significant protocol amendments to the FDA and Investigator for submission to the IRB. 3M will also notify the Investigator when a protocol amendment may be implemented.

3.9.2 Protocol Deviations

A deviation is a departure from the protocol that will likely affect the safety, rights or welfare of subjects, the scope of the investigation, or the scientific quality of the study.

A protocol deviation is only for an individual subject. Protocol deviations are documented on a Protocol Deviation Form.

Deviations that potentially affect 1) subject safety, rights or welfare, 2) data integrity or 3) compromise the statistical analysis of the study require immediate communication to 3M. The Investigator must contact the 3M study monitor within 24 hours of occurrence. A Protocol Deviation Form must be completed by the Investigator and include the type of deviation and a description of the circumstances surrounding the deviation. If the deviation affects subject safety, rights or welfare, a copy is sent to the 3M study monitor within 24 hours of identifying the occurrence.

Deviations, which are made to protect the life or physical well-being of a subject in an emergency, must be reported by the Investigator to the IRB within 5 working days of occurrence and by 3M to the FDA within 5 working days after 3M learns of the occurrence.

4. SUBJECT SELECTION

4.1 Subject Inclusion Criteria

Subjects to whom *all* of these conditions apply will be eligible for enrollment in this study:

- Subjects of either sex and any race who are at least 18 years of age,
- Subjects must be in good general health,
- Subjects who satisfy all Inclusion/Exclusion Criteria and will voluntarily read and sign the Informed Consent Form including authorization to use and disclose protected health information,
- Subjects who are cooperative and willing to follow Subject Instructions for the study (Appendix 14.5),

- Subjects who are willing to report to the study facility approximately 72 hours prior to Screening or Treatment Day sampling for clipping, if needed,
- Subjects who are willing to avoid showering and tub-bathing within 72 hours prior to Screening and Treatment Days (sponge-baths may be taken; however, the groin and upper thigh regions must be avoided),
- Subjects who are willing to avoid showering, tub-bathing, swimming, and vigorous physical activity that may cause sweating during the 6-hour ± 30-minute period before the final sampling,
- Subjects who are willing to return to the study facility within 6 hours of treatment for the final sampling, and
- Subjects who have Screening Day baseline counts of ≥ 5.00 $\log_{10}$ per cm^2 bilaterally on the inguinal region.

4.2 Subject Exclusion Criteria

Subjects to whom *any* of these conditions apply will be excluded from this study:

- Participation in another clinical study in the past 30 days, current participation in another clinical study, or previous participation in this study,
- Any tattoos, scars, breaks in the skin, or any form of dermatitis, or other skin disorders (including acne) on the applicable test areas,
- A history of skin allergies,
- A history of skin cancer within 6 inches of the test areas,
- Known sensitivity to acrylates, CHG- or alcohol-containing products, medical tapes, metals, natural rubber latex, vinyl, or skin-marking inks,
- A medical diagnosis with physical condition that may put the subject at risk, such as a current or recent severe illness, hepatitis, organ transplant, congestive heart disease, or any immunocompromised conditions, such as AIDS (or HIV positive),
- Any medical condition or use of any medications that, in the opinion of the Investigator, should preclude participation,
- Pregnancy, possible pregnancy, attempting pregnancy, or nursing,
- Topical antimicrobial exposure within 14 days prior to Screening and Treatment Days. Restrictions include, but are not limited to, antimicrobial soaps, medicated shampoos, medicated lotions, antiperspirants/deodorants, perfumes, after shaves, and colognes,
- Use of systemic or topical antibiotic medications, steroid medications (other than for hormonal contraception or postmenopausal reasons), or any other product known to affect the normal microbial flora of the skin within 14 days prior to Screening and Treatment Days,
- Use of topically applied or oral antihistamine medications in the last 48 hours (eg, Claritin®, Benadryl®, Zyrtec®, Singulair®, Cromolyn, Nyquil™, Tylenol® PM),
- Exposure of the test areas to solvents, acids, bases, strong detergents, fabric softener-treated clothing, or other household chemicals within 14 days prior to Screening and Treatment Days,
- Swimming in chemically treated pools or bathing in hot tubs, spas, or whirlpools within 14 days prior to Screening and Treatment Days,

- Use of tanning beds, hot waxes, or depilatories (in the applicable test areas) within 14 days prior to Screening and Treatment Days, or
- Bathing and showering within 72 hours prior to Screening and Treatment Days.

4.3 Subject Informed Consent

The Investigator or designee must ensure that written informed consent to participate in the investigation is obtained before including any individual as a subject in the investigation. The Investigator or designee must provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate and minimize the possibility of coercion or undue influence. The process is designed to 1) give the subject all the information that they need, 2) ensure that the subject understands the information and 3) give the subject a chance to consider study participation. The process should permit the subject to ask questions and exchange information freely.

Specifically, the Investigator or designee is to explain to each subject all elements of informed consent as specified in 21 CFR 50.25 (Appendix 14.6). After the explanation, the subject or representative will voluntarily sign and date the consent form if they wish to participate in the study. A copy of the consent form must be provided to the subject. A signed and dated copy of the consent form must be maintained in the Investigator study documentation file at all times. Consent and study participation, with date, must be documented in the subject's record.

4.4 Subject Authorization for Use and Disclosure of Protected Health Information

The Investigator or designee must ensure that written authorization for use and disclosure of protected health information is obtained before including any individual as a subject in the investigation.

Specifically, the Investigator or designee is to explain to each subject all elements of authorization as specified in 45 CFR 164.508. After the explanation, the subject or representative **must** voluntarily sign and date the authorization form if they wish to participate in the study. A copy of the authorization form must be provided to the subject. A signed and dated copy of the authorization form must be maintained in the Investigator study documentation file at all times and may be placed in the subject's record.

An authorization form may be combined with a consent form (ie, compound authorization) if required by the IRB. All required elements for both informed consent and authorization must be included in a compound authorization.

5. TREATMENT OF SUBJECTS

5.1 Medication(s)/Treatment(s) Not Permitted

Exposure of the test areas to antimicrobial agents is not permitted within 14 days prior to Screening Day and for the duration of the study. Restrictions include, but are not limited to

antimicrobial soaps, antiperspirants/deodorants, shampoos, lotions, perfumes, after shaves, colognes, steroid medications (other than for hormonal contraception or postmenopausal reasons), and topical or systemic antibiotics.

5.2 Subject Compliance

Answers to the Inclusion/Exclusion Criteria questions asked at the beginning of the Screening and Treatment Phases, and prior to the final sample collection will determine compliance to the Subject Instructions (Appendix 14.5) provided to each subject upon study participation.

5.3 Study Procedures

These study procedures are based on ASTM E1173-15, Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations.[2]

5.3.1 Pretreatment Phase (Washout)

The Inclusion/Exclusion Criteria will be reviewed with each subject to ensure eligibility for the study. If these criteria are satisfied, subjects will sign the consent form. Prior to the scheduled Screening Day, subjects will undergo a minimum 14-day pretreatment phase to allow for the removal of any antimicrobial agents from the subjects' skin. Subjects are to refrain from the use of products containing antibacterial agents as written in the Subject Instructions (Appendix 14.5). Subjects will be given personal hygiene kits containing nonantimicrobial products for use during this period. Subjects will be instructed to use these products through completion of the Treatment Phase or until notified by the Investigator or designee.

In addition, subjects must avoid systemic or topical antibiotics or medications and contact with chemically treated swimming pools or hot tubs, hot waxes, and depilatories on the test areas for 14-days before the scheduled Screening Day. If it becomes necessary to take systemic antibiotics or to apply topical medications to the test areas within this pretreatment period, the subject must contact the Investigator as soon as reasonably possible so that another volunteer may be recruited.

If subjects require hair removal to facilitate sample collection, the subject will be asked to return to the test facility approximately 72 hours before the Screening Day. Subjects will not be allowed to shower or bathe for 72 hours prior to their scheduled screening appointment. Sponge-baths may be taken; however, the groin and upper thigh regions must be avoided.

5.3.2 Screening Phase

Prior to collection of the screening baseline samples, the Investigator or a designee will complete the screening Inclusion/Exclusion form. A visual skin assessment of the test areas will be performed, and the screening baseline samples will be collected using the cup scrub technique described in Section 5.3.4.1. Baseline samples will be taken from the center of each contralateral test area within the inguinal region.

Samples from both the left and right sides of the inguinal region must meet the values indicated in the Inclusion Criteria to be enrolled into the Treatment Phase of the study. Those subjects who qualify will be notified and will continue to follow the instructions in Appendix 14.5 until completion of the scheduled Treatment Day. Subjects will not be allowed to shower or bathe for 72 hours prior to Treatment Day. Sponge-baths may be taken; however, the groin and upper thigh regions must be avoided.

5.3.3 Treatment Phase

A sufficient number of subjects who meet the Inclusion Criteria will be enrolled into the Treatment Phase of the study, such that approximately 230 inguinal regions are evaluable for efficacy at completion of the study. Table 5.3.3A lists the treatment groups that will be divided amongst the subjects.

Due to the film-forming component of SoluPrep FF, a modification of the sampling solution is required to dissolve the film for bacterial recovery (see Section 5.3.4.1). The agent used to accomplish this is Triacetin, a fully saturated triglyceride.

Table 5.3.3A Subject Treatments

Treatment Combination	Left and Right Treatment
1	SoluPrep 10.5-mL and SoluPrep FF 10.5-mL
2	SoluPrep 10.5-mL and saline
3	SoluPrep FF 10.5-mL and saline

The Investigator or a designee will complete the treatment Inclusion/Exclusion form. If these criteria are satisfied, a visual skin assessment will be performed to evaluate the condition of each test area.

5.3.3.1 Preparation of Test Areas on Treatment Day

A Test Site Diagram for the inguinal test area is shown in Appendix 14.7.

The test area within the inguinal region is defined as the uppermost inner aspect of the thigh (the groin), centering the long axis of the template along the inguinal crease immediately below the

groin. If, due to a subject's anatomy, the test area cannot be centered along the inguinal crease, the area should be positioned on the upper, inner thigh as close to the crease as possible. In no instance should sampling be performed on areas not having skin-to-skin contact. Using a 1.5" x 5" sterile template, the corners of each inguinal test area will be marked directly on the skin using a non-toxic skin marker. Four sampling sites will be numbered within each inguinal test area. The positioning and numbering of the inguinal sampling sites are standard for all subjects. Sampling sites on the contralateral side of the groin will be numbered in a mirror-image orientation. The 4 sampling sites within each inguinal test area represent one baseline (pre-prep) site, 2 post-prep sample sites (10-min, 6-hr), and an unused site. Sampling sites 2, 3 and 4 will be randomized as baseline and the 2 post-prep sample sites. Sampling site 1 is designated as the unused sample site.

After inguinal test areas are marked and sample sites are numbered, baseline samples will be collected from the right and left test areas per the randomization schedule using the cup scrub technique described in Section 5.3.4.1.

5.3.3.2 Study Product Application

Following baseline sample collection, the contralateral inguinal test areas will each be prepped with 1 of the 3 study products according to the randomization codes provided. Treatments will be randomized between left and right test areas and post-prep sampling times will be randomized amongst the sampling sites within a test area.

The study products will be applied per the randomization schedule and the Investigational Product Application Instructions (Appendix 14.2). The duration of each prep procedure will be recorded on the appropriate form.

5.3.3.3 Evaluation of Skin Irritation

Prior to collection of the baseline, 10-minute, and 6-hour post-prep samples, the skin in each test area will be evaluated for indications of skin irritation, based on the Skin Irritation Rating Scale (Appendix 14.8). Skin irritation ratings for each area will be recorded on the Skin Irritation Rating form. A rating of 3 in any category during the evaluations for the 10-minute, and 6-hour post-prep samples represents significant irritation and qualifies as an Adverse Event. Any area with a rating of 3 should not be sampled.

5.3.3.4 Timing of Post-Prep Sample Collection

Microbial samples will be collected at 10 minutes (± 30 sec.) and 6 hours (± 30 min.) post-prep from the groin. Post-prep timing begins upon completion of study product application, including the 3-minute drying time. Microbial samples will be collected using the cup scrub technique described in Section 5.3.4.1.

After the 10-minute post-prep samples have been collected, a sterile non-occlusive dressing will be secured over the remaining sample sites to allow subjects restricted mobility and to protect the

sites from contamination between sampling times. The subjects will be allowed to leave the clinical test facility as long as they return within 6 hours for collection of the 6-hour samples. Subjects should be reminded that they are to avoid showering, tub-bathing, swimming, vigorous physical activity that may cause sweating, and any other activities that may compromise the integrity of the test sites until their final sample collection has been completed. In the event that the dressing on the 6-hour test site has been compromised, the site will be sampled and the loss of dressing integrity will be recorded on the appropriate DCS.

Following the final sample collection, residual study products will be removed from the subject's skin with alcohol wipes.

5.3.4 Microbiological Methods

5.3.4.1 Sample Collection and Processing

Quantitative cultures will be obtained from test sites using a modification of the cup scrub method of Williamson and Kligman.[3] Modified sampling solution (MSS) will be used to collect all microbial samples.

Modified Sampling Solution (MSS):

A sterile scrub cup will be placed and held firmly on each site. **Scrub 1:** 1.0 mL of Triacetin and 2.0 mL of SSS will be aseptically pipetted into the scrub cup. The skin will be scrubbed in a circular motion with moderate pressure for 1 minute using a sterile policeman. Using a sterile transfer pipette, the scrub solution will be transferred to a sterile collection tube. **Scrub 2:** An additional 3.0 mL of fresh SSS will be pipetted into the scrub cup and the scrub procedure will be repeated**.** This solution will be pooled with the solution from Scrub 1 for a total sample volume of approximately 6 mL per site.

Following collection, each sample tube will be capped tightly and pulsed 3 times on a vortex mixer followed by a 2-minute sonication and 30-second vortex. A 1.0 mL aliquot of each sample will be diluted into sterile tubes containing 9.0 mL PBW. Serial 10-fold dilutions will be performed in PBW. One-milliliter (1.0-mL) aliquots of selected dilutions will be pour-plated in triplicate using TSA+N. **Samples must be plated within 30 minutes of collection. Plated samples must be well swirled.** Each plate should be labeled with the subject treatment number, anatomical region sampled (groin), test site sampled (right or left and time point), and dilution plated. After 72 (± 4) hours of aerobic incubation at 30 ± 2°C, colonies will be counted and viable cells in the original sample will be calculated using standard methods. Following incubation, plates may be refrigerated for up to 48 hours prior to counting.

5.3.4.2 Collection of Raw Data

Raw colony counts from each of the dilutions will be recorded on the appropriate DCS for each subject.

Based on the scrub and dilution process, in order to have a baseline ≥ 5.0 $\log_{10}$ requires that the average of the 3 plates from the 10^{-3} dilution be ≥ 63. Averaging of the Screening Day counts will be automatically performed on the eCRF upon entry of raw data and Clindex will determine the subject's eligibility for treatment.

5.3.4.3 Neutralization of Investigational Materials

The effectiveness of the neutralizers contained in the microbial sampling solution will be tested based on ASTM E1054-08 (Reapproved 2013), Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents,[4] prior to Treatment Day. The Protocol for Validation of Neutralizer Effectiveness is provided in Appendix 14.9.

5.4 Subject Discontinuation

The Investigator or designee may discontinue individual subjects from the study at any time. Subjects may voluntarily withdraw from the study at any time. The Investigator or designee will provide a written report on the appropriate form including the date and reason for discontinuation. Subjects who have been previously screened may not be re-entered into the study.

5.5 Subject Revocation of Authorization to Use and Disclose Protected Health Information

In order to implement a valid revocation of authorization, the subject or their representative must make the request in writing to Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA, 20164. The revocation cannot stop the use or disclosure of information that has been collected and disseminated prior to the revocation, is needed to ensure complete and accurate study results, and is required by law or government regulation (eg, reporting adverse events). Revocation of an authorization may not be used to withhold normal medical care from the subject but may make the subject ineligible to receive the study treatment or care.

6. ASSESSMENT OF EFFICACY

6.1 Efficacy Parameters

Non-inferiority of the investigational product to the FDA-approved active control for $\log_{10}/cm^2$ recovery of skin flora relative to Treatment Day baseline $\log_{10}/cm^2$ on the inguinal region at 10 minutes following application. Non-inferiority is based on a non-inferiority margin of 0.5 $\log_{10}/cm^2$.

Superiority of the active products to the negative control for $\log_{10}/cm^2$ recovery of skin flora relative to Treatment Day baseline $\log_{10}/cm^2$ on the inguinal region at 10 minutes following application. A superiority margin of 1.2 $\log_{10}/cm^2$ is used.

Number of observations for which the log_{10}/cm^2 recovery of skin flora on the inguinal region at 6 hours following application of the study products is higher than treatment day log_{10}/cm^2 baseline skin flora.

6.2 Assessment Methods

Efficacy will be assessed by sampling the skin using the cup scrub method described in Section 5.3.4.1.

7. ASSESSMENT OF SAFETY

7.1 Safety Parameters

The principal measure of safety will be the incidence of adverse events reported during the study and a summary of the skin irritation rating scores. A skin irritation rating of 3 post-treatment in any category represents significant irritation and qualifies as an Adverse Event.

7.2 Assessment Methods

The Investigator or designee will assess the subject's skin condition prior to collection of the baseline, 10-minute, and 6-hour post-prep samples using the Skin Irritation Rating Scale (Appendix 14.8).

7.3 Adverse Events

The Investigator is responsible for identifying adverse events that occur to each subject on Treatment Day. An adverse event can be identified by the Investigator or reported by the subject.

Note: The Federal Privacy Rule (HIPAA) specifically permits the use and disclosure of protected health information "without written authorization of the individual" when used for public health activities such as reporting adverse events, tracking FDA-related products, enabling recalls, repairs, replacements, lookbacks, or conducting post-market surveillance. This use and disclosure is subject to the *minimum necessary* standard, ie, "the minimum necessary to accomplish the intended use, disclosure, or request."

Definitions

- Adverse event (AE) means any undesirable clinical occurrence in a subject whether or not it is considered to be drug related.

- Drug-related adverse event (ie, adverse drug experience) is an adverse event that is considered by the Investigator to have a reasonable likelihood of being associated with the investigational drug.

- Life-threatening adverse drug experience is any adverse drug experience that places the subject, in the view of the Investigator, at immediate risk of death from the reaction as it

occurred (does not include a reaction that, had it occurred in a more severe form, might have caused death).

- Serious adverse drug experience is an adverse drug experience that is fatal or life-threatening, is permanently or significantly disabling/incapacitating, requires inpatient hospitalization or prolongation of existing hospitalization, or results in a congenital anomaly/birth defect.

- Unexpected adverse drug experience is one not previously identified in nature, severity or frequency of incidence in the current Investigator brochure, in the clinical plan, or elsewhere in the current IND application and amendments.

Recording and Reporting

The Investigator records each AE occurring in the study, including those not thought to be associated with the drug, on the Adverse Event Record. Documentation includes the AE description, severity at onset, seriousness, date of onset and resolution, relationship to the investigational drug, action taken, and outcome.

The Investigator must promptly report all adverse drug experiences to the 3M study monitor. If the adverse drug experience is also considered by the Investigator to be serious and/or unexpected the Investigator must report it to the IRB as soon as possible.

3M reports (by telephone or fax) a death or life-threatening adverse drug experience to the FDA within 7 days of notification and submits a follow-up report within 15 days.

A serious and unexpected adverse drug experience involving a non-3M commercialized product is reported to the 3M study monitor and IRB.

If a subject does not experience an AE during the study, the absence of such must be recorded on the form.

8. STATISTICS

8.1 Sample Size Justification

The Final Monograph (21 CFR Part 310 Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use)[5] calls for a minimum of 100 observations per treatment. A standard deviation of 1.0 $\log_{10}/cm^2$ was observed in study EM-05-012760. One-hundred subjects per treatment arm provides 94% power using a non-inferiority margin of 0.5 $\log_{10}/cm^2$ with a 1-sided alpha of 0.025.

In the same study, a superiority to the negative control was observed to be approximately 2.5 $\log_{10}/cm^2$, with a lower 95% confidence bound of 2.26 $\log_{10}/cm^2$. A sample size of 30 for the negative control will ensure that the mean for $\log_{10}/cm^2$ reduction of skin flora relative to

Treatment Day baseline will be normally distributed. Power was assessed through simulation using an assumed superiority of 2.26 log_{10}/cm^2 (the lower 95% confidence bound observed in study EM-05-012760), sample sizes of 30 for the negative control and 100 for the active treatments, and the standard deviation of 1.0 log_{10}/cm^2 mentioned above. All 1000 simulations had a lower bound for superiority greater than 1.2 log_{10}/cm^2.

8.2 Statistical Methods

8.2.1 Efficacy Analyses

Primary Efficacy Analyses:

The average treatment effect (ATE) for the investigational product will be estimated using a linear regression of the 10-minute post-treatment bacterial count (log_{10} scale) on the additive effect of a treatment indicator and the baseline measurement (log_{10} scale). The lower bound of the 95% confidence interval for the additive treatment effect must not include the investigational product being more than 0.5 log_{10}/cm^2 lower than the positive control.

The ATE of the test product compared to the negative control is estimated as the contrast of treatment effect of negative control minus the treatment effect of the test drug in the linear regression. Likewise, the ATE of the active control compared to the test product is estimated as the contrast of treatment effect of test product minus the treatment effect of the active control in the linear regression. Superiority to negative control uses a margin of 1.2 log_{10}/cm^2, which would be demonstrated by a lower bound of the 95% confidence interval for the contrast to exceed 1.2 log_{10}/cm^2.

The percent of samples for which the 6-hours post treatment recovery is lower than the baseline recovery will be calculated by active product. The Final Monograph requires that these percentages be 100.

Secondary Efficacy Analyses:

Summary tables will be produced by treatment for log_{10}/cm^2 baseline skin flora, log_{10}/cm^2 recovery of skin flora, and log_{10}/cm^2 reduction of skin flora relative to Treatment Day baseline log_{10}/cm^2 on the inguinal region at 10 minutes and 6 hours following application of the study materials. The tables will include number, mean, standard deviation, minimum and maximum.

8.2.2 Safety Analysis

Adverse events and skin irritation scores will be summarized by product.

8.3 Data Sets Analyzed

A modified intent-to-treat data set will be used for primary efficacy analysis. All subjects who meet baseline requirements on a bilateral side will be included in the analysis for that bilateral side. However, the primary analyses involve a linear regression at the 10-minute post-treatment

time point and calculation of return to baseline at 6 hours. These analyses cannot be done without both baseline and 10-minute recovery for treatment effect or baseline and 6-hour recovery data for return to baseline percentage calculation. The exclusion of subjects who do not meet baseline requirements and who are missing recovery data at one or both of the post-treatment sampling times makes this data set a modified intent-to-treat data set.

The baseline bacterial count requirements are in the range of 5.00 to 7.50 $\log_{10}/cm^2$ on the groin. The acceptability of each bilateral baseline will be assessed separately for inclusion in the study.

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

8.4 Criteria for the Termination of the Study

3M reserves the right to terminate the study at any time for business reasons.

8.5 Procedures for Accounting for Missing, Unused, and Spurious Data

Details of any other missing data handling will be specified in the statistical analysis plan.

8.6 Deviations to Statistical Plan

A statistical analysis plan (SAP) will be written and approved prior to study initiation. Any deviation from the original SAP will be described and justified in the final report.

9. MONITORING

3M, as sponsor of this study, is responsible for ensuring the proper conduct of the study with regard to protocol adherence and validity of the data recorded. 3M has therefore assigned a study monitor to this study. The progress of the study will be monitored by:

- Periodic on-site review
- Ongoing review of electronic documentation via Clindex
- Telephone and e-mail communications
- Review of source documents

The Investigator or designee will give the 3M study monitor direct access to source documents that support data on the eCRFs and make available such records to authorized 3M, quality assurance, IRB, and regulatory personnel for inspection and/or copying.

Note: The Federal Privacy rule (HIPAA) specifically permits the use and disclosure of protected health information “to a person subject to the jurisdiction of the Food and Drug Administration (FDA) with respect to an FDA-related product or activity for which that person has responsibility, for the purpose of activities related to the quality, safety, or effectiveness of such FDA-regulated product or activity.”

10. QUALITY CONTROL AND QUALITY ASSURANCE

3M is responsible for implementing and maintaining quality assurance and quality control systems through written standard operating procedures (SOPs) to ensure that this study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and regulations cited in Section 1.4 of this protocol. Study monitoring is carried out to accomplish this.

11. ETHICS

This study will be conducted in accordance with the principles that have their origin in the Declaration of Helsinki, 21 CFR 50 (Informed Consent) and 56 (IRBs).

The study will start only after approval of the protocol and consent form by the IRB. The approval letter or notice must contain the IRB name, meeting date, and sufficient information to identify the protocol and informed consent by name and number that were reviewed. 3M, prior to study initiation, must receive a copy of the IRB approval letter.

12. DATA HANDLING AND RECORD KEEPING

12.1 Study Personnel

Prior to study initiation, the Investigator must provide 3M with a signed Form FDA 1572. This form identifies all sub-investigators who will be assisting the Investigator in the conduct of the study. The Investigator will provide a log of signatures/initials prior to study initiation.

12.2 Pre-Study Documentation Requirements

Prior to study initiation, the Investigator must provide 3M with the following documents:

- Signed protocol including any amendments in place prior to study initiation
- Curriculum vitae for the Investigator and any sub-investigators
- IRB approved consent/authorization form
- IRB study approval letter
- IRB name, location and chairperson
- IRB membership list
- Signed Form FDA 1572
- Financial Disclosure documents per 21 CFR 54
- Signed research agreement

12.3 Completion and Return of Case Report Forms

Electronic data capture (eDC) will be used for this study. The site will be trained on the eDC software prior to study enrollment. The site will be provided with a manual, including instructions on how to complete the eCRFs and how to make eCRF corrections within the eDC software. Microbiological count data will be recorded onto DCSs prior to entry into the eDC

system. The Investigator must review and electronically sign eCRFs in a timely fashion following the 3M study monitor's review.

12.4 Final Report

The Investigator will submit an Investigator Final Report to 3M within one month of the completion of statistical analysis or termination of the study. 3M will be provided a draft Investigator Final Report for review. The Investigator will make reasonable changes deemed necessary by 3M and finalize. The Investigator will submit the Investigator Final Report to 3M and the IRB.

3M will prepare and submit a Sponsor Final Report to the FDA. The study medical monitor will sign the Sponsor Final Report.

12.5 Records, Reports and Retention Requirements

Study records must be kept until 3M provides notification that the documents can be destroyed. This may extend beyond the 2 years required by law. In order to comply with regulatory requirements, the Investigator must arrange for the retention of all study records for 2 years following the date a marketing application is approved for the drug and indication investigated, or if no application is filed, until 2 years after completion/termination of the study.

Records to be retained by the Investigator include, but are not restricted to:

- Signed study protocol, amendments, deviations
- IRB approval of protocol, consent form, authorization form, waiver of consent and/or authorization and amendments to any of these documents
- Applications to the IRB
- Signed consent/authorization forms
- DCSs
- eCRFs
- Records of receipt, use or disposition of the investigational drug
- Correspondence relating to the study
- Investigator Brochure
- Financial disclosure documents
- Sponsor Final Report
- Investigator Final Report

13. REFERENCES

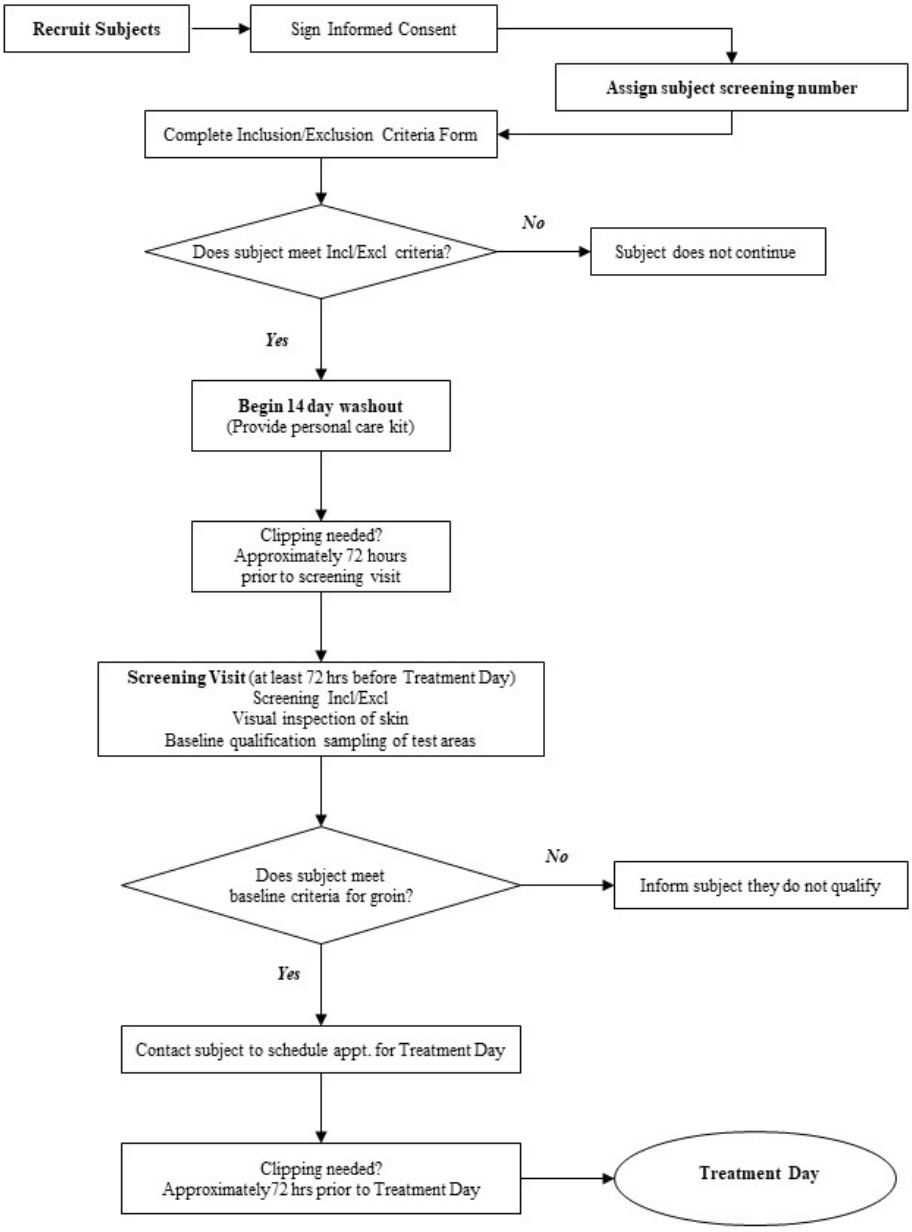
1 Butterfield, CT. The selection of a dilution water for bacteriological examinations. J Bacteriol 1932; 23: 355–68.
2 ASTM International. ASTM E1173, standard test method for evaluation of preoperative, precatheterization, or preinjection skin preparations. West Conshohocken [PA]: ASTM Int'l; 2015.

3 Williamson P, Kligman PM. A new method for the quantitative investigation of cutaneous bacteria. J Invest Dermatol 1965; 45:498-503.
4 ASTM International. ASTM E1054, standard test methods for evaluation of inactivators of antimicrobial agents. West Conshohocken [PA]: ASTM Int'l; 2013.
5 US Food and Drug Administration. Safety and effectiveness of health care antiseptics; topical antimicrobial drug products for over-the-counter human use. Final rule. Federal Register 2017; 82:60474-503.

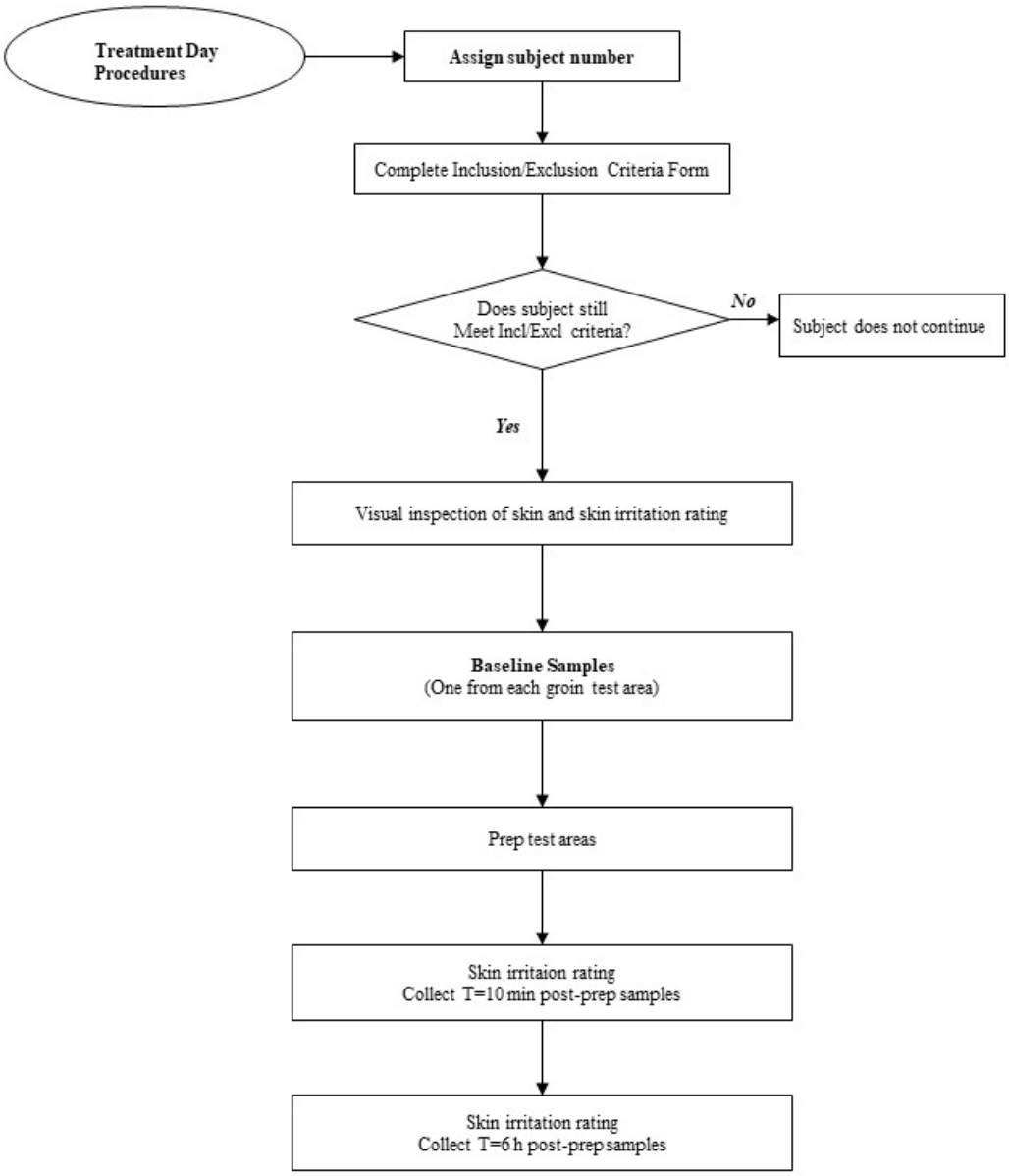
14. APPENDICES

14.1 Study Flow Diagrams

Screening



Treatment


Treatment Day Procedures
Assign subject number
Complete Inclusion/Exclusion Criteria Form
Does subject still Meet Incl/Excl criteria?
No
Subject does not continue
Yes
Visual inspection of skin and skin irritation rating
Baseline Samples
(One from each groin test area)
Prep test areas
Skin irritaion rating
Collect T=10 min post-prep samples
Skin irritation rating
Collect T=6 h post-prep samples

14.2 Study Product Application Instructions

Study personnel must use sterile gloves to apply the solutions.

Treatment Code: SP (SoluPrep)

1. Aseptically remove investigational product from sterile packaging and maintain sample in a sterile environment prior to sample application.
2. With the sponge in a downward position, snap the lever on the applicator allowing the fluid to flow into the sponge. Wait until the sponge fills completely.
3. Apply the product with repeated back-and-forth strokes for 2 minutes on the groin.
4. Do not allow solution to pool. Use sponge applicator to absorb excess solution and continue to apply.
5. Allow the test area to dry for 3 minutes.
6. Contact time begins after step 5.
7. Residual product should be removed from test areas after the final sample collection is complete using alcohol wipes or soap and water.
8. Record the subject number on the package label and retain for inventory purposes. Discard used applicators.

Treatment Code: SPFF (SoluPrep FF)

1. Aseptically remove investigational product from sterile packaging and maintain sample in a sterile environment prior to sample application.
2. With the sponge in a downward position, snap the lever on the applicator allowing the fluid to flow into the sponge. Wait until the sponge fills completely.
3. Apply the product with repeated back-and-forth strokes for 2 minutes on the groin.
4. Do not allow solution to pool. Use sponge applicator to absorb excess solution and continue to apply.
5. Allow the test area to dry for 3 minutes.
6. Contact time begins after step 5.
7. Residual product should be removed from test areas after the final sample collection is complete using alcohol wipes.
8. Record the subject number on the package label and retain for inventory purposes. Discard used applicators.

Treatment Code: S (Sterile 0.9% saline – 20 mL bottle, with sponge applicator)

1. Remove petri plate from sleeve. Place lid sterile side up on bench.
2. Pour 20 mL sterile saline into lid.
3. Aseptically remove applicator from sterile packaging.
4. Holding applicator handle place one side of sponge into saline, allowing fluid to flow into sponge. Gently press sponge against lid until saturated. Flip sponge and repeat, ensuring both sides of sponge are completely saturated with fluid.
5. Apply the product with repeated back-and-forth strokes for 2 minutes on the groin, flipping applicator as needed to ensure the test area remains wet during product application.
6. Allow the test area to dry for 3 minutes.
7. Contact time begins after step 6.
8. Record the subject number on the package label and retain for inventory purposes. Discard used applicators.

14.3 Confirmation of Release & Receipt of Clinical Supplies

CONFIRMATION OF RELEASE & RECEIPT OF CLINICAL SUPPLIES

Investigator: M. Hamid Bashir, MD
Study No: EM-05-014264, MBT #109-296
Test Site: Microbac

Qty	Item Name	ID/Lot Number	Exp. Date

Supplies Released to Site by: _____
Monitor's Signature

Supplies Sent to Site (Date): _____

Supplies Checked and Verified by: _____
Signature Date

_____
Print Name, Title

Once the supplies have been verified and this form is signed / dated, please scan and email a copy to Linda Olson at lkolson2@mmm.com.

14.4 Study Product Disposition Form

As an investigator, you are required to record disposition of clinical study products. This form is supplied for your convenience in fulfilling this obligation. Use one form for each study product.

Study Number: EM-05-014264, MBT #109-296	
Protocol Title: Assessment of the antimicrobial efficacy of 3M™ SoluPrep™ Preoperative Skin Preparation against resident human skin flora on the inguinal region	
Investigator: M. Hamid Bashir, MD	**Investigator Site:** Microbac

Investigational Material ID:	Date Received:	Quantity Received:	Lot Number/ Serial Number:	Date Returned to Sponsor:

Date Dispensed/Distributed	Subject Number	Quantity Dispensed	Quantity Remaining

14.5 Subject Instructions

Subject Instructions during Washout, Screening and Treatment Phases

The instructions below are to be followed until the completion of the study.

1. Use only the soap provided for all sponge-bathing and hand-washing.
2. Use only the shampoo provided when washing your hair.
3. Do not use antiperspirants or deodorants (other than those provided to you in the kit), lotions, colognes, perfumes, after shaves or powders.
4. Do not come in contact with solvents, acids, bases, fabric softener-treated clothing or other household chemicals on the upper thigh body regions.
5. Do not swim in chemically treated pools or bathe in hot tubs, whirlpools or spas.
6. Do not use tanning beds.
7. Do not shave or use depilatories or hot waxes on the upper thigh body regions. If hair is present, allow study staff to clip hair at a designated time.
8. Do not apply any medicated creams or ointments to any area of your skin, nor should you take any antibiotics. If antibiotics are necessary due to illness, please report this to M. Hamid Bashir, MD at 703-925-0100 or 571-435-6371 immediately.
9. Do not use any topical or oral antihistamines within 48 hours of or during your Treatment Day Visit (eg, Claritin®, Benadryl®, Zyrtec®, Singulair®, Cromolyn, Nyquil™, Tylenol® PM).

DO NOT BATHE OR SHOWER IN THE 72-HOUR PERIOD BEFORE YOUR SCREENING VISIT (A sponge-bath may be taken but avoid the areas of the upper thigh.)

Clipping visit (if necessary):_____(Date)

Screening visit :_____(Date)

You will be contacted by telephone on _____to let you know if you have been selected to complete the study or if you have been eliminated. Please continue to use your kit products until you have been eliminated or complete the study.

If you are selected to complete the study:

DO NOT BATHE OR SHOWER IN THE 72-HOUR PERIOD BEFORE YOUR TREATMENT DAY VISIT (A sponge-bath may be taken but avoid the areas of the upper thigh.)

Clipping visit (if necessary):_____(Date)

Application visit :_____(Date)

Return to the test facility for the 6 hour ± 30-minute skin sampling (as scheduled). Avoid sweating or getting the test sites wet during this time period.

If you have questions about this study or in case of emergency, contact M. Hamid Bashir, MD, Principal Investigator, at 703-925-0100 at any time during business hours or 571-435-6371 after business hours.

14.6 Elements of Informed Consent

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

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

ADDITIONAL ELEMENTS OF CONSENT

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

1. A statement that the particular treatment or procedure may involve risks to the subject (or embryo or fetus, if subject became pregnant) which are currently unforeseeable.
2. Anticipated circumstances under which subject's participation may be terminated by the Investigator without regard to subject's consent.
3. Any additional costs to the subject that may result from participation in the research.
4. A statement explaining the consequences of subject's decision to withdraw during the course of the research which may relate to subject's willingness to continue participation will be provided to the subject.
5. A statement that significant new findings developed during the course of the research which may relate to subject's willingness to continue participation will be provided to the subject.
6. Approximate number of subjects involved in the study.

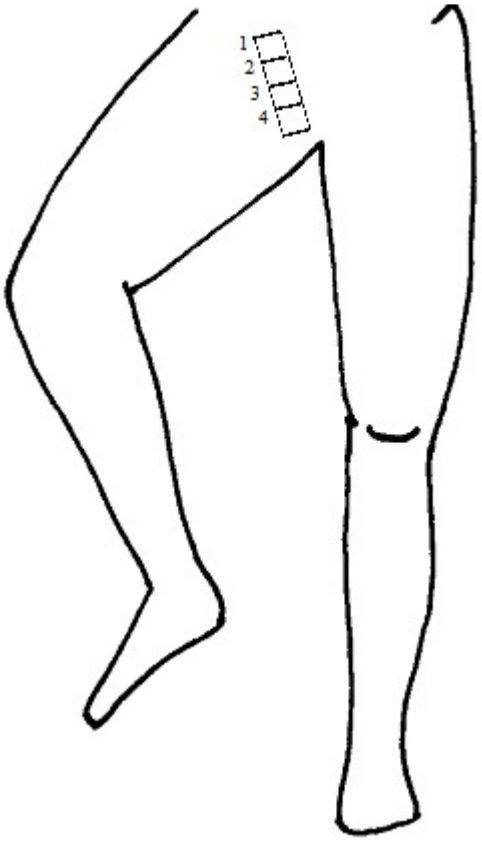
Nothing in these regulations is intended to limit the authority of a physician to provide emergency medical care to the extent the physician is permitted to do so under applicable federal, state, or local laws.

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

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

14.7 Test Site Diagram

Follow the randomization schedule for each subject for the exact placement of test materials.



14.8 Skin Irritation Rating Scale

Skin Irritation Rating Scale (Modified Draize)		
Condition	**Rating**	**Description**
Erythema	0	No reaction
	1	Mild and/or transient redness limited to sensitive area
	2	Moderate redness persisting over much of the product-exposed area
	3[a]	Severe redness extending over most or all of the product-exposed area
Edema	0	No reaction
	1	Mild and/or transient swelling limited to sensitive area
	2	Moderate swelling persisting over much of the product-exposed area
	3[a]	Severe swelling extending over most or all of the product-exposed area
Rash	0	No reaction
	1	Mild and/or transient rash limited to sensitive area
	2	Moderate rash persisting over much of the product-exposed area
	3[a]	Severe rash extending over most or all of the product-exposed area
Dryness	0	No reaction
	1	Mild and/or transient dryness, limited to sensitive area
	2	Moderate dryness persisting over much of the product-exposed area
	3[a]	Severe dryness extending over most of the product-exposed area

[a] = A rating of 3 on the skin irritation scale will be recorded as an Adverse Event.

14.9 Protocol for Neutralization Validation

14.9.1 Background

In order to accurately assess the efficacy of an antimicrobial product, it is necessary to completely inactivate the antimicrobial agent at the time point being evaluated. Inadequate neutralization will allow killing or inhibition of the microorganisms to continue beyond the specified contact time, resulting in an overestimation of antimicrobial activity.

Standard sampling solution (SSS) 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 antimicrobial, CHG, present in the investigational and marketed active study products. Modified Sampling Solution (MSS) is a 1 to 6 ratio of Triacetin to SSS. Triacetin is a fully saturated triglyceride, commonly used as an emollient, and is required to dissolve the film formed by SoluPrep FF. The MSS is used to neutralize SoluPrep and SoluPrep FF. The effectiveness and nontoxicity of these neutralizer systems must be assessed to demonstrate that there is no effect on the growth of microorganisms and that the antimicrobial ingredient is inactivated.

The density of normal human skin flora generally ranges from 10^2 to 10^8 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 these neutralizers will be assessed against a lower bacterial concentration.

This is an *in vivo* test where the active study products are applied to the skin using the same application instructions used for the inguinal region during the study (Appendix 14.2). Scrub cup samples are collected and inoculated with low levels of methicillin-resistant *Staphylococcus epidermidis* (MRSE) or methicillin-sensitive *Staphylococcus epidermidis* (MSSE). Two contact times after inoculation will be evaluated: immediately (<1 minute) and 40 minutes (to test for residual antimicrobial activity between sample collection and the time of plating).

14.9.2 Objective

The objective of this assay is to determine the ability of the sampling solutions to completely neutralize the active ingredients contained in SoluPrep and SoluPrep FF when applied to the abdomen of test subjects without exhibiting toxicity to the test organisms.

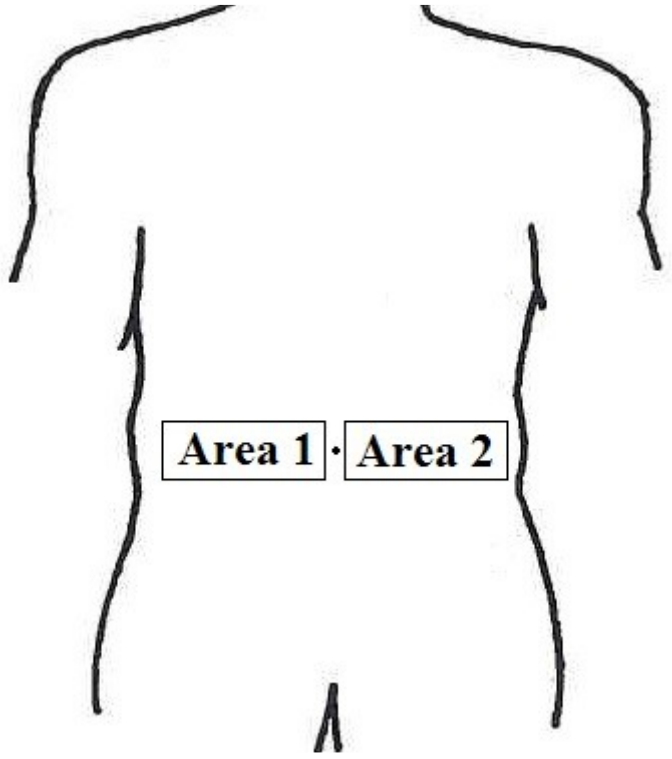
14.9.3 Subject Entry Criteria

Six subjects will participate in this study. Subjects must meet the inclusion and exclusion criteria in Sections 4.1 and 4.2 of the protocol to which this neutralizer validation is attached, except for the baseline bacterial count, the 72-hour exclusion from showering/bathing and the length of the washout period. The neutralization subjects do not require a minimum baseline count and they only need to avoid topical and systemic antimicrobials for 7 days (not 14 days) prior to

Treatment Day. Subjects will be asked to provide information on demographics and inclusion/exclusion criteria and sign the Informed Consent and Authorization Forms before beginning the 7-day washout period. When subjects return to begin their participation in the study they will again be asked to provide information relative to inclusion/exclusion criteria. If they meet all inclusion/exclusion criteria, they may be enrolled. These subjects will be identified by the letter "N" for neutralization and a subject number starting with 001.

Each subject will receive both active study products (SoluPrep and SoluPrep FF), which will be applied to 2 separate test areas on the abdomen. One area will be located on each side of the body as indicated in the diagram below.

Neutralization Test Area Diagram



14.9.4 Test Organism

The test organisms for this study are methicillin-resistant *Staphylococcus epidermidis*, ATCC 51625, and methicillin-sensitive *Staphylococcus epidermidis*, ATCC 12228.

14.9.5 Study Products

- SoluPrep, 10.5-mL, tinted
- SoluPrep FF, 10.5-mL, tinted

14.9.6 Materials, Supplies and Equipment

- Product kits (toiletry items to be used by subjects during study)
- Subject Informed Consent / Authorization forms, IRB-approved
- Standard Sampling Solution (SSS), 75mM phosphate buffer (0.04% monobasic potassium phosphate, and 1.01% dibasic sodium phosphate) containing 0.1% Triton® X-100, 0.3% lecithin, 3.0% polyoxyethylene sorbitan monooleate (Tween® 80), and 1.0% Tamol™ SN; pH 7.9 $\pm$ 0.1, sterile
- Triacetin (provided by sponsor)
- Butterfield's sterile phosphate buffered water[1] (PBW), 312 µM KH_2PO_4, pH 7.2 ± 0.1
- 70% Isopropyl alcohol (w/w) swabs/wipes
- High-purity deionized water, sterile
- Trypticase soy broth (TSB)
- Trypticase soy agar (TSA)
- Trypticase soy agar containing 0.5% Tween 80 and 0.07% lecithin (TSA+N)
- Methicillin-resistant *Staphylococcus epidermidis* (MRSE), ATCC 51625
- Methicillin-sensitive *Staphylococcus epidermidis* (MSSE), ATCC 12228
- Alcohol wipes
- Transfer pipettes, polyethylene, sterile
- Serological pipettes, 1 mL, 2 mL, and 10 mL, sterile
- Pipettor, calibrated to accurately deliver volumes of 0.5 mL, 2.5 mL and 3.0 mL, and sterile tips
- Pipetting, calibrated to accurately deliver 20-200 µL, and sterile tips
- Tubes with sealable caps, polypropylene or glass, sterile
- Petri dishes, 100 mm, sterile
- Gloves, sterile
- Marking templates, 1.5" x 5", sterile (for marking test sites)
- Non-toxic marking pen (Sharpie or equivalent), sterile (Note: Do not use markers containing crystal violet)
- Scrub cups (2.20 cm I.D., 3.80 cm^2), sterile
- Teflon or rubber policemen, sterile
- Timers or stopwatches
- Pipet Aid or similar apparatus
- Vortex mixer
- Water bath (45 ± 2C°)
- Incubator (30 ± 2C°)
- Surgical clippers with blades

14.9.7 Study Procedures

14.9.7.1 Preparation of Inoculum

Prepare an overnight (24 $\pm$ 4 hours) broth culture of each of the test organisms at 35 $\pm$ 2°C in TSB, to contain approximately $10^8 - 10^9$ CFU/mL.

Dilute the overnight cultures with PBW to achieve an appropriate concentration (approximately 10^4 CFU/mL) for inoculation of the test samples. The density of the test inocula must be verified by direct plating at the beginning and end of the study.

14.9.7.2 Preparation of Test Areas and Post-Prep Sampling

Neutralization samples will be evaluated on the abdomen. The test areas on each subject's abdomen will be assigned according to a computer-generated randomization schedule to receive either the investigational product on the left and the active control on the right or the investigational product on the right and the active control on the left. The subject number, location of the product application, location of the sites sampled within the test area, and the time of sample collection will be documented. Each subject will be treated with both study products, one in each test area.

Abdominal test areas will be marked using a sterile 1.5" x 5" template. One 1.5" x 5" area will be delineated on each side of the body with each area containing two 1" x 1" sampling sites (one sampling site will be used for each test organism) to yield 4 sampling sites for each subject (see neutralization test area diagram in Section 14.9.3 of the neutralization validation). One tube from each test area will be inoculated with MRSE and the other will be inoculated with MSSE.

After the test areas are marked, each area will be prepped with three 70% isopropyl alcohol swabs for a total of 1 minute and allowed to dry for 3 minutes.

Each test area will be treated with 1 of the 2 study products, according to the instructions for the inguinal region provided in Appendix 14.2 of the protocol to which this neutralizer validation is attached.

Samples will be collected, using the scrub cup technique, at 10 minutes (± 30 sec.) post-prep. Post-prep timing begins upon completion of study product application, including the 3-minute drying time. For the two sites within each 1.5" x 5" area; the samples may be collected simultaneously by two technicians. The sampling technique is described in Section 5.3.4.1 of the protocol to which this neutralizer validation is attached.

Residual product should be removed from test areas after sample collection is complete using alcohol wipes.

Each pooled sample (~6 mL) will be capped tightly and pulsed 3 times on a vortex mixer. Sample tubes will be sonicated 2 minutes followed by vortex mixing for 30 seconds. Five mL will be transferred to a clean tube and immediately inoculated with 100 µL of the appropriate diluted inoculum (Section 14.9.7.1 of the neutralization validation). This will yield an organism concentration of ~20 – 200 CFU/mL.

Immediately (<1 minute) and at 40 minutes (± 2 minutes) post-inoculation, 1.0-mL aliquots of the inoculated samples will be pour-plated in triplicate using TSA+N. Plates will be incubated inverted at 35 ± 2°C for 48 ± 4 hours.

14.9.7.3 Numbers Control and Toxicity Control

This testing will be performed in triplicate.

Numbers Control (NC):

Add 100 uL of the appropriate diluted inoculum to a tube containing 5 mL PBW to yield final inoculum concentrations of ~20-200 CFU/mL. Sonicate 2 minutes followed by vortex mixing for 30 seconds. Pour-plate triplicate 1.0 mL aliquots immediately (<1 minute) and 40 minutes (+ 2 minutes) post-inoculation in the same manner as 14.9.7.2, except using TSA without neutralizers.

Toxicity Control (TC-MSS):

Add 1.0 mL Triacetin to 5.0 mL SSS and pulse 3 times on a vortex mixer followed by a 2-minute sonication and 30-second vortex. Transfer 5 mL to a clean tube. Add 100 uL of the appropriate diluted inoculum to the 5-mL tube to yield final inoculum concentrations of ~20-200 CFU/mL. Pour-plate triplicate 1.0 mL aliquots immediately (<1 minute) and 40 minutes (+ 2 minutes) post-inoculation in the same manner as 14.9.7.2.

These controls will provide assurance that the test organisms are not adversely affected by the treatments or the sampling procedures.

14.9.7.4 Enumeration

Plates may be refrigerated for up to 72 hours prior to counting. Enumerate plates and calculate CFU/mL for each sample. Convert data to $\log_{10}$ CFU/mL.

14.9.8 Control of Bias

Bias is controlled by internal controls and triplicate plating.

14.9.9 Data Evaluation

Recovery for each of the samples will be expressed as Log_{10} CFU/mL.

Neutralizer Effectiveness:

If the mean $\log_{10}$ CFU/mL of the sample is not more than 0.3 $\log_{10}$ less than the mean $\log_{10}$ CFU/mL of the NC, the neutralization will be considered effective.

- Mean $\log_{10}$ CFU/mL from NC – Mean $\log_{10}$ CFU/mL from sample (use corresponding time points)

Neutralizer Toxicity:

If the mean $\log_{10}$ CFU/mL of the TC is not more than 0.3 $\log_{10}$ less than the mean $\log_{10}$ CFU/mL of the NC, the sampling solutions will be considered non-toxic.

- Mean $\log_{10}$ CFU/mL from NC − Mean $\log_{10}$ CFU/mL from TC (use corresponding time points).