3M
Health Care
3M Center
St. Paul, MN 55144-1000

Clinical Study Protocol

Study Number and Protocol Title	EM-05-012926 Evaluation of Microbial Population Reductions Within a Defined Product Coverage Area [redacted]
Principal Investigator	[redacted]
Sub-Investigators:	[redacted]
Research Facility	Eurofins Evic Romania/ S.C. BIO HIGH TECH S.R.L. [redacted] Bucharest 040256 Romania [redacted]
IRB/EC	Chesapeake Institutional Review Board Address: 6940 Columbia Gateway Drive, Suite 110, Columbia, Maryland, 21046, USA

[redacted]

Sponsor	3M Health Care [redacted] 3M Center [redacted] St. Paul, MN 55144-1000
3M Study Monitor	[redacted]
3M Medical Monitor	[redacted]

[redacted]

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[redacted]

[redacted]

[redacted]

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1. BACKGROUND INFORMATION

1.1 Name, Description and Intended Use of the Investigational Product

[redacted] Surgical Solution [redacted] is an investigational antimicrobial skin preparation containing 2% w/v chlorhexidine gluconate (CHG) and 70% v/v isopropyl alcohol (IPA) [redacted] for reducing the bacterial flora on patients' skin prior to surgery. [redacted] he product will be available in the following configurations: 10.5-mL tint and colorless, and 26-mL tint. The 26-mL tint configuration will be evaluated in the current study.

The purpose of this study is to evaluate the ability of a single preoperative preparation product to achieve similar reductions of resident flora at three different sample sites within a defined test area, [redacted] on the skin of the posterior torso (back region). One sample site will be in the center of the application site, a second sample will be taken halfway between (equidistant) the center and the periphery of the application site, and a third sample will be taken on the periphery of the application site.

1.2 Summary of Relevant Studies

[redacted] two studies were conducted to evaluate the coverage area [redacted] on human subjects. In study [redacted] the coverage area for the 26-mL applicator was evaluated and in study [redacted] the coverage area for the 10.5-mL applicator was evaluated. The coverage area determined in study [redacted] is being used as the treatment coverage area for this study. No safety concerns were identified in these studies.

Refer to the Investigator Brochure [redacted] for a summary of studies.

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 investigational product and preclinical safety studies, [redacted], have been completed. [redacted]

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

[redacted]

1.4 Investigational Material Application

This study will be conducted using the tint formulation [redacted] in the 26-mL applicator configuration. The study product will be applied topically to intact skin of the subject's posterior torso (back region) in a defined [redacted] area.

The study product will be applied as intended per instructions for use (Appendix 14.2). [redacted]

1.5 Good Clinical Practices and Regulatory Requirements

[redacted]

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP), Declaration of Helsinki and applicable state, federal and regional 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 in accordance with the European Directive 95/46/EC on the protection of individuals with regard to the processing of personal data and on the free movement of such data and with the Romanian Order No 677/2001 for the protection of persons concerning the processing of personal data and free circulation of such data. And in spirit with Directive of the European Parliament and Council 2001/20/EC, and Romanian Order No. 904/25.07.2006.

1.6 Study Population

Healthy male subjects, 18 years of age or older, who meet the study inclusion/exclusion criteria are eligible for enrollment into this study.

A sufficient number of subjects will be enrolled in the screening phase to provide 18 treatment sites that meet the baseline requirements on treatment day. 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 subjects will be recruited, as needed. Treatment day baseline requirement is greater than or equal to 3.0 $\log_{10}$ CFU/cm^2 on each of the 3 sampling sites in the treatment site.

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

All three sampling sites (center, mid-peripheral and peripheral) on the treatment site must meet the screening baseline requirements stated in the inclusion criteria to qualify for treatment.

[redacted]

2. STUDY OBJECTIVES

The primary objective of this study is to determine whether reductions in resident flora produced by the test product are consistent within a prepped area of skin. Sufficient number of subjects will be included for testing in order to finish with 18 evaluable subjects.

[redacted]

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 prospective, randomized, uncontrolled single-center study in healthy subjects.

[redacted] This study will be conducted using a modification of ASTM E1173-15 Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations.[2] The 2-$\log_{10}$ reduction criteria (dry site) will be used to evaluate efficacy. Time points for sample collection are baseline (pre-prep) and 10 minutes or 13 minutes (post-prep).

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

[redacted]

Table 3.1A Study Summary

14-day Wash Out (Pre-inclusion*) Phase	Screening Phase	Treatment Phase
Perform the Informed Consent process.	Complete Inclusion/Exclusion form; check study constrains. Perform visual skin assessment.	Assign sequential subject number. Complete Inclusion/Exclusion form; check study constrains. Perform visual skin assessment. Record information on demographics.
Assign subject screening number.	Using a [redacted] sterile template mark back test area. Within the test area mark the 3 baseline sampling sites (center, mid-peripheral and peripheral).	Using a [redacted] sterile template mark back test area. Within the test area mark the 3 baseline sampling sites (center, mid-peripheral and peripheral) and the 3 post-prep sampling sites (center, mid-peripheral and peripheral).
Complete Inclusion/ Exclusion Criteria form.	Collect screening baseline samples (center, mid-peripheral and peripheral) from test area.	Perform skin irritation rating.
*Prior to the scheduled Screening Phase, subjects will undergo a minimum 14-day Wash Out (Pre-inclusion) Phase.	Count screening plates to determine which subjects qualify for study.	Collect baseline samples (center, mid-peripheral and peripheral) from test area.
Review subject instructions for wash out and dispense subject personal hygiene kit	Contact eligible subjects to schedule Treatment Day (schedule for clipping 72 hours prior to treatment, if needed).	Weigh product applicator pre-application. Apply study product. Weigh product applicator post-application.
Schedule for clipping 72 hours prior to screening, if needed.		10-minutes [redacted] post-prep perform skin irritation rating.
		Per randomization, collect the post-prep samples [10-minutes [redacted] or 13-minutes [redacted]

[redacted]

3.2 Primary and Secondary Endpoints

Primary Endpoint:

The primary endpoint, coverage area with efficacy, is measured by $\log_{10}$ reductions calculated by subtracting the $\log_{10}$ number of microorganisms recovered at a specified sampling time following product application from the $\log_{10}$ number of microorganisms recovered from the baseline sampling. Baseline samples must satisfy the inclusion criteria to be used in data analysis, $\geq$ 3.0 $\log_{10}$ CFU/cm^2 in all three sampling sites on the treatment site. The sampling time for efficacy evaluation will be specified per the randomization schedule as 10 minutes or 13 minutes post-product application.

[redacted]

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. [redacted]

3.3 Randomization

The three post-product application sampling sites will be randomized so that 2 of the 3 sites will be sampled at one of the post-product application sample times (10 minutes or 13 minutes) and the third sampling is collected at the remaining sample time.

The randomization schedule will be provided to the study site in a separate document.

3.4 Study Products and Labeling

3.4.1 Study Products

Investigational prep: [redacted] Surgical Solution, 2% w/v CHG/70% v/v IPA, tint formulation with 26 mL foam tipped applicator [redacted] will be applied topically to intact skin of the treatment site (back region) of the subject. The prep will be applied with repeated back-and-forth strokes for 30 seconds.

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

3.4.2 Investigational Products Labeling and Accountability

3M will label, package and ship the study products to the research facility. The [redacted] applicators will be provided in individual [redacted] packages, and labeled as below.

[redacted]

Sample Investigational Product Label

CAUTION: New Drug-Limited by Federal Law to Investigational Use For use in study EM 05-012926 3M Health Care, St. Paul, MN 55144-1000 [redacted] Surgical Solution, Tint (26-mL) Product ID: [redacted] **Subject Number:** Lot: TBD 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 product at all times. The Investigator or designee will:

- Document receipt of study product on the Confirmation of Release & Receipt of Clinical Supplies form (Appendix 14.3) and return the completed form to 3M,
- Store study product in a secure facility accessible only to authorized individuals,
- Dispense study product only to subjects properly enrolled into the study,
- Account for inventory and disposition using the Study Product Disposition Form (Appendix 14.4), and
- Dispose of used and unused study product as agreed upon. Once accounted for the study product may be disposed. Documentation shall be maintained concerning the disposal and will contain: the quantity of the study product disposed, the date and manner of disposal, the staff member who conducted the disposal. NOTE: Record the subject number on the package label and retain the label for inventory purposes. The used applicator can be discarded.

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

3.4.3 Additional Study Supplies

Sponsor will provide the following:

- Study Material Disposition forms
- Randomization schedule
- Data Collection Sheets (DCSs)
- Investigational product

[redacted]

Study site will provide the following:

- Subject Informed Consent forms, IRB/EC-approved
- [redacted])

[redacted]

3.5 Study Duration

The expected duration of this study for each subject is up to 3 weeks. Each subject's participation will involve at least a 14-day Wash Out (Pre-inclusion) Phase, a 1-day Screening Phase, and a 1-day Treatment Phase. Following the Wash Out (Pre-inclusion) Phase, each subject will be required to visit the test facility on an arranged date for collection of screening baseline samples from the back 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 collections. Each subject who chooses to participate in this study will be required to return to the test facility for treatment and sample collection. 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/Subject Discontinuation or Withdrawal

3.6.1 Study Termination

Conditions that may warrant the 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 pertinent state, federal or regional regulations or applicable privacy regulations,
- Submission of knowingly false information from the Investigator to 3M,
- Termination of IND or IRB/EC approval, or withdrawal of the IRB/EC approval,
- A decision on the part of 3M to suspend or discontinue evaluation of the investigational product.

3.6.2 Subject Discontinuation or Withdrawal

The Investigator may discontinue individual subjects from the study at any time. Subjects entering the study may withdraw at any time without penalty. The right of each test subject to withdraw from the study and the right of each test subject to privacy, confidential treatment of personal data, and protection from view by others not conducting the study will be respected at all times. The Investigator will provide a written report on the appropriate case report form including the date and reason for discontinuance.

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

All study data will be loaded into the [redacted] data management system.

The current 3M Health Care document storage system, [redacted] will be used to store the study documents.

[redacted] may be used for study documentation and data analysis.

3.9 Protocol Modifications

3.9.1 Protocol Amendments

A protocol amendment is defined as a change in procedure that is identified and documented before initiation. 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.

All protocol amendments must be documented and approved by the Investigator and study monitor prior to implementation. All significant protocol amendments must also be pre-approved by the reviewing IRB/EC prior to implementation.

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

3.9.2 Protocol Deviations

A protocol deviation is defined as a change in procedure that has not been documented before initiation and therefore has not been reviewed and approved with the study sponsor and/or reviewing IRB/EC prior to initiation. All deviations from the study protocol are to be documented and reported to the investigator/study sponsor and reviewing IRB/EC.

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. 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 or designee must contact the 3M study monitor [redacted] 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. A copy is sent to the 3M study monitor [redacted] 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/EC within 5 working days of occurrence.

4. SUBJECT SELECTION

Healthy male subjects will be recruited. A suitable number of subjects will be enrolled in the wash out (pre-inclusion), screening and treatment phase, such that a total of 18 subjects are evaluable for efficacy at completion of the study.

[redacted]

Subjects must follow all Subject Instructions (Appendix 14.5) and satisfy inclusion/exclusion criteria prior to enrollment into the treatment phase of the study.

All subjects will be provided verbal and written information about the study procedures, and subject instructions (Appendix 14.5). The following inclusion/exclusion criteria will be reviewed on wash out (pre-inclusion), screening and treatment day to establish eligibility for participation:

4.1 Subject Inclusion Criteria

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

1. Male subjects of any race who are at least 18 years of age,
2. Subjects must have a [redacted] area on their back region to accommodate the treatment coverage area,
3. Subjects must be in good general health,
4. Subjects who satisfy all inclusion/exclusion criteria and will voluntarily read and sign the Informed Consent Form,
5. Subjects who have good skin condition on the test sites,
6. Subjects who are willing to report to the study facility approximately 72 hours prior to Screening or Treatment Day for clipping, if needed,
7. Subjects who are willing to avoid showering and tub-bathing within 72 hours prior to Screening and Treatment Days, and
8. Subjects who have Screening Day baseline counts of $\geq$ 3.00 $\log_{10}$ per cm^2 in each of the 3 sample sites (center, mid-peripheral and peripheral) in the treatment area (back region).

4.2 Subject Exclusion Criteria

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

1. Participation in another clinical study in the past 30 days, current participation in another clinical study, or previous participation in this study,
2. Has taken antihistamines in the 48 hours prior to Treatment Day,
3. Any tattoos, scars, breaks in the skin, or any form of dermatitis, or other skin disorders (including acne) on the applicable test areas,
4. A history of skin allergies,
5. A history of skin cancer within 6 inches of the test areas,
6. Known sensitivity to acrylate-, chlorhexidine gluconate-, or alcohol-containing products, or to medical tape, metals, natural rubber latex, vinyl, or skin-marking inks,
7. 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),
8. Any medical condition or use of any medications that, in the opinion of the Investigator, should preclude participation,

9. Topical antimicrobial exposure within 14 days prior to Screening Day and throughout the study. Restrictions include, but are not limited to antimicrobial soaps, medicated shampoos, medicated lotions, antiperspirants/deodorants, perfumes, after shaves, and colognes,
10. Use of systemic or topical antibiotic medications, steroid medications, or any other product known to affect the normal microbial flora of the skin within 14 days prior to Screening Day and throughout the study,
11. 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 Day and throughout the study,
12. Swimming in chemically treated pools or bathing in hot tubs, spas, or whirlpools within 14 days prior to Screening Day and throughout the study,
13. Use of tanning beds, hot waxes, or depilatories (in the applicable test areas) within 14 days prior to Screening Day and throughout the study
14. Bathing and showering within 72 hours prior to Screening Day and throughout the study, or
15. Subject has used moisturizers or any topical treatment (e.g. lotion, sunscreen or shaving cream) on the test sites in the 24-hour period prior to Screening Day and Treatment Day participation in the study.

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 the date, must be documented in the subject's record.

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 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 will determine compliance to the requirements of the study.

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 Wash Out (Pre-inclusion) Phase

Perform the Informed Consent process. After the subject has signed the informed consent form assign the subject a screening number and complete the Inclusion/Exclusion form.

If the inclusion/exclusion criteria are satisfied, prior to the scheduled Screening Day, subjects will undergo a minimum 14-day wash out (pre-inclusion) 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 non-antimicrobial 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 area for 14-days before the scheduled Screening Day. If it becomes necessary to take systemic antibiotics or to apply topical medications to the test area within this wash out (pre-inclusion) period, the subject must contact the Investigator or designee as soon as reasonably possible so that another subject 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.

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 area will be performed. The test area within the back region is defined as the uppermost aspect up to the top of the scapula extending down to just above the sacrum as shown in Appendix 14.7. Using a [redacted] sterile template, the corners of each back test area will be marked directly on the skin using a nontoxic skin marker. Three sampling sites will be numbered within the test area. The 3 sampling sites within each back test area represent the 3 screening baseline sites (center, mid-peripheral, and peripheral). The positioning and numbering of the sampling sites are standard for all subjects (Appendix 14.7). The screening baseline samples will be collected using the cup scrub technique described in Section 6.2.1.1.

All three sampling sites (center, mid-peripheral and peripheral) on the test area (back region) must meet the screening baseline requirements stated 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. If subjects require hair removal to facilitate sample collection, the subject will be asked to return to the test facility approximately 72 hours before Treatment Day. Subjects will not be allowed to shower or bathe for 72 hours prior to Treatment Day.

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 at least 18 treatment sites (back region) are evaluable at completion of the study.

The Investigator or a designee will complete the treatment Inclusion/Exclusion form. If these criteria are satisfied, complete information on demographics and perform a visual skin assessment to evaluate the condition of the test area.

5.3.3.1 Preparation of Test Areas

A Test Site Diagram for the test area (back region) is shown in Appendix 14.7.

The test area within the back region is defined as the uppermost aspect up to the top of the scapula extending down to just above the sacrum as shown in Appendix 14.7. Using a [redacted] sterile template, the corners of each back test area will be marked directly on the skin using a nontoxic skin marker. Six sampling sites will be numbered within the test area. The positioning and numbering of the sampling sites are standard for all subjects (Appendix 14.7). The 6 sampling sites within each back test area represent the 3 baseline (pre-prep) sites (center, mid-peripheral, and peripheral) and the 3 post-prep sample sites (center, mid-peripheral, and peripheral). The sampling sites are designated as Site 1 baseline (pre-prep) center, Site 2 baseline (pre-prep) mid-peripheral, Site 3 baseline (pre-prep) peripheral, Site 4 (post-prep) center, Site 5 (post-prep) mid-peripheral and Site 6 (post-prep) peripheral. The three post-prep sampling sites, sites 4, 5 and 6, will be randomized as the 10-minute and 13-minute post-prep sample sites.

After the back region test area is marked and sampling sites are numbered, the 3 baseline (pre-prep) samples will be collected using the cup scrub technique described in Section 6.2.1.1.

5.3.3.2 Study Product Application

Following baseline sample collection, the test area will each be prepped with the study product.

[redacted]

The study product will be applied to the entire test area per the Investigational Product Application Instructions (Appendix 14.2). The duration of the prep application procedure is 30-seconds.

[redacted]

5.3.3.3 Evaluation of Skin Irritation

Prior to collection of the baseline and the [redacted] post-prep samples, the skin in each test area will be evaluated for indications of skin irritation, based on the Skin Irritation Rating Scale

[redacted]

(Appendix 14.8). Skin irritation ratings for each area will be recorded on the Skin Irritation Rating form. [redacted] Any area with a rating of 3 should not be sampled.

5.3.3.4 Timing of Post-Prep Sample Collection

The 3 post-product application sample sites will be randomized so that 2 of the 3 sites will be sampled at one of the post-product application sample times (10 minutes or 13 minutes) and the third within the sample time remaining. The 3 sample locations within the prepped area will be demarcated such that each is taken from the same location on each of the subjects.

Microbial samples will be collected at 10 minutes [redacted] or 13 minutes [redacted] post-prep from the sampling sites within the test area. Post-prep timing begins upon completing the application of the study products, including the 3-minute drying time. Microbial samples will be collected using the cup scrub technique described in Section 6.2.1.1.

Following the final sample collection, residual study products will be removed from the subject's skin [redacted]

6. ASSESSMENT OF EFFICACY

6.1 Efficacy Parameters

The primary measure of coverage area with efficacy [redacted] is measured as ≥ 2 $\log_{10}$ CFU/cm^2 reductions calculated by subtracting the $\log_{10}$ number of microorganisms recovered at a specified sampling time following product application from the $\log_{10}$ number of microorganisms recovered from baseline sampling. Baseline samples must satisfy the inclusion criteria, ≥ 3.0 $\log_{10}$ CFU/cm^2 in each of the 3 sampling sites, to be used in data analysis.

6.2 Assessment Methods

6.2.1 Microbiological Methods

6.2.1.1 Sample Collection and Processing

Quantitative cultures will be obtained from sampling sites using a modification of the cup scrub method of Williamson and Kligman.[3]

[redacted]

A sterile scrub cup will be placed and held firmly on each site. **Scrub 1:** [redacted] 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:** [redacted] fresh SSS will be pipetted into the scrub cup and the scrub procedure will be

[redacted]

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 [redacted] followed by [redacted] 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, as appropriate. One-milliliter (1.0-mL) aliquots of selected dilutions will be pour-plated in duplicate using TSA+N. **Samples must be plated within 30 minutes of collection.** [redacted] After 72 [redacted] 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. [redacted]

6.2.1.2 Collection of Raw Data

Raw colony counts from each of the dilutions will be recorded on the appropriate data collection sheet (DCS) for each subject.

6.2.1.3 Neutralization of Investigational Products

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

6.3 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.

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. [redacted]

7.2 Assessment Methods

The Investigator or designee will assess the subject's skin condition prior to collection of the baseline and post-prep (10-minutes or 13-minutes) 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. An adverse event can be identified by the Investigator or reported by the subject.

[redacted]

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 outcomes.

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/EC 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/EC.

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 Data Sets Analyzed

Subjects with an average treatment day baseline of $\geq$3.0 $\log_{10}$ CFU/cm^2 at each of the sampling sites (center, mid-peripheral and peripheral) within the test site (back region) will be included in the analysis.

8.2 Statistical Methods

- CFU/ml will be converted to CFU/cm^2.
- Summary tables will be produced for all responses. Summary tables for log_{10} recovery, log_{10} baseline and log_{10} reduction from baseline will include n, mean, standard deviation, median, minimum and maximum.

8.2.1 Efficacy Analyses

A mean log_{10} reduction of 2.0 in all 3 sampling locations is needed to support the proposed coverage area.

8.2.2 Safety Analyses

Adverse events, [redacted] will be summarized.

Skin irritation tables will be produced at baseline collection and post-prep sample collection.

8.3 Sample Size Justification

[redacted] The rationale for the number of subjects is based on EN test methods EN1499, EN1500 and EN12791 which require between 12 and 18 subjects.

8.4 Criteria for 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

Any non-use of data will be discussed and justified in the blinded review of data at the data lock meeting and documented in the meeting minutes.

8.6 Deviations to Statistical Plan

Any deviation from the statistical plan discussed above will be documented in the study 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 [redacted]
- Telephone and e-mail communications
- Review of source documents

[redacted]

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/EC, and regulatory personnel for inspection and/or copying.

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 1.5 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/EC. The approval letter or notice must contain the IRB/EC 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/EC 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 and delegation of responsibility log 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/EC approved consent form
- IRB/EC study approval letter
- IRB/EC name, location and membership list
- IRB/EC certification
- Signed Form FDA 1572
- Financial Disclosure
- Signed research agreement

12.3 Completion and Return of Case Report Forms

Electronic data capture (eDC) software will be used for this study. Data may be recorded onto data collections sheets prior to data entry or may be entered directly into the eDC system. Microbiological count data will be recorded onto DCSs prior to entry into the eDC system. If a correction is required, a single line must be drawn through the error. The person making the correction will initial, date, and provide a reason for the error (if not self-evident). Once the forms are completed, the monitor will review the case report form (CRF) to assure accuracy and completeness.

12.4 Final Report

[redacted] The Investigator will submit the Final Report to 3M and the IRB/EC.

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 [see 21 CFR 312.62 (c) and 21 CFR 812.140(d)].

Records to be retained in the Investigator study file include, but are not restricted to:

- Signed study protocol, amendments, deviations
- Applications to the IRB/EC
- IRB/EC approval of protocol, consent form, authorization form and amendments to any of these documents
- Signed consent forms
- Case report forms and/or data collection forms
- Adverse event reports
- Records of receipt, use, or disposition of the investigational drug
- Correspondence relating to the study
- Investigator Brochure
- Statement of Investigator
- Financial disclosure documents
- Final Report

[redacted]

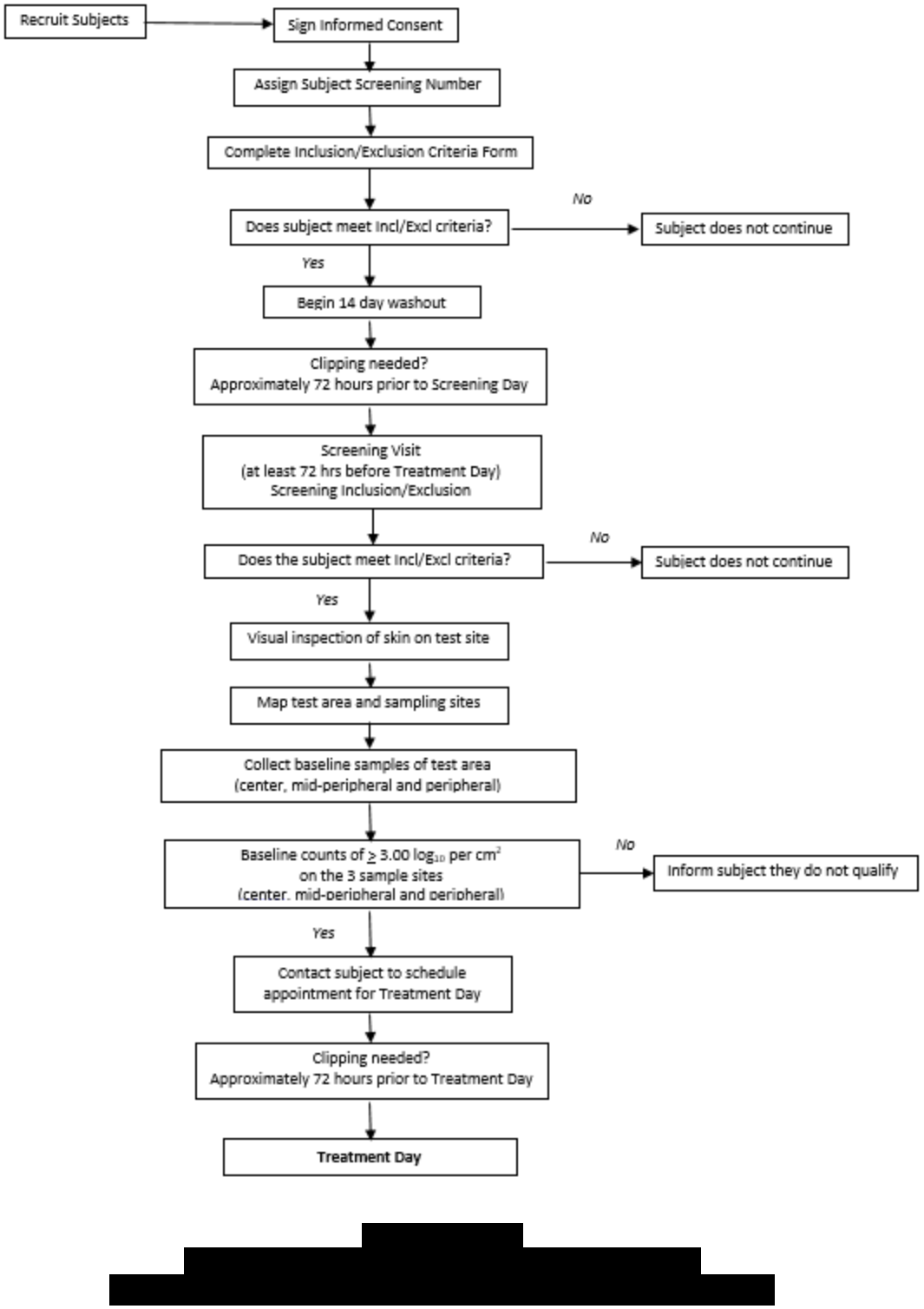
13. REFERENCES

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.

14. APPENDICES

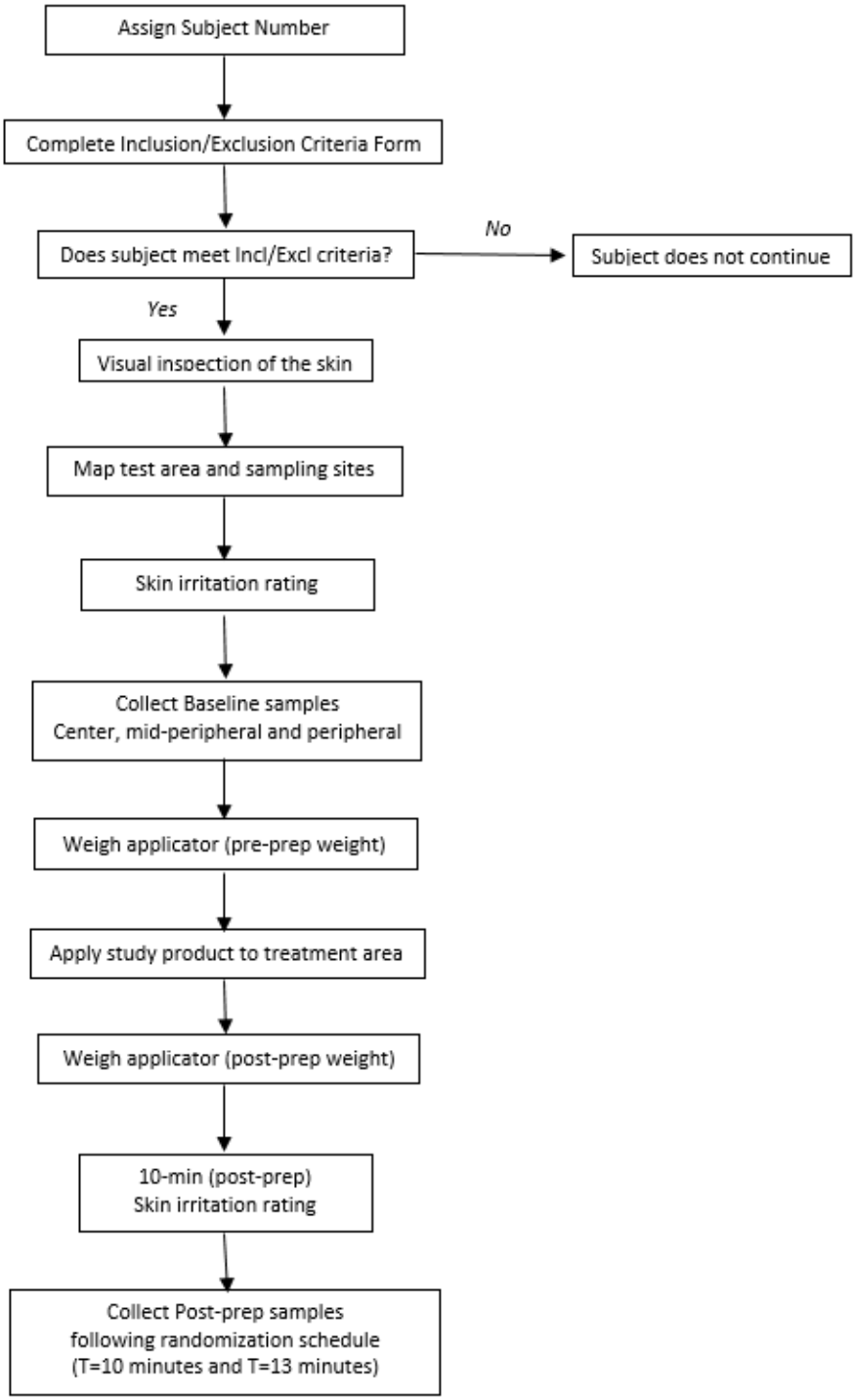
14.1 Study Flow Diagrams

Informed Consent/Wash out and Screening Day



[redacted]

Treatment Day



[redacted]

14.2 Study Product Application Instructions

[redacted]

d.

[redacted]

14.3 Confirmation of Release & Receipt of Clinical Supplies

[redacted]

[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

14.4 Study Product Disposition Form

[redacted]

[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

14.5 Subject Instructions

[redacted]

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 includes 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 any time, 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.

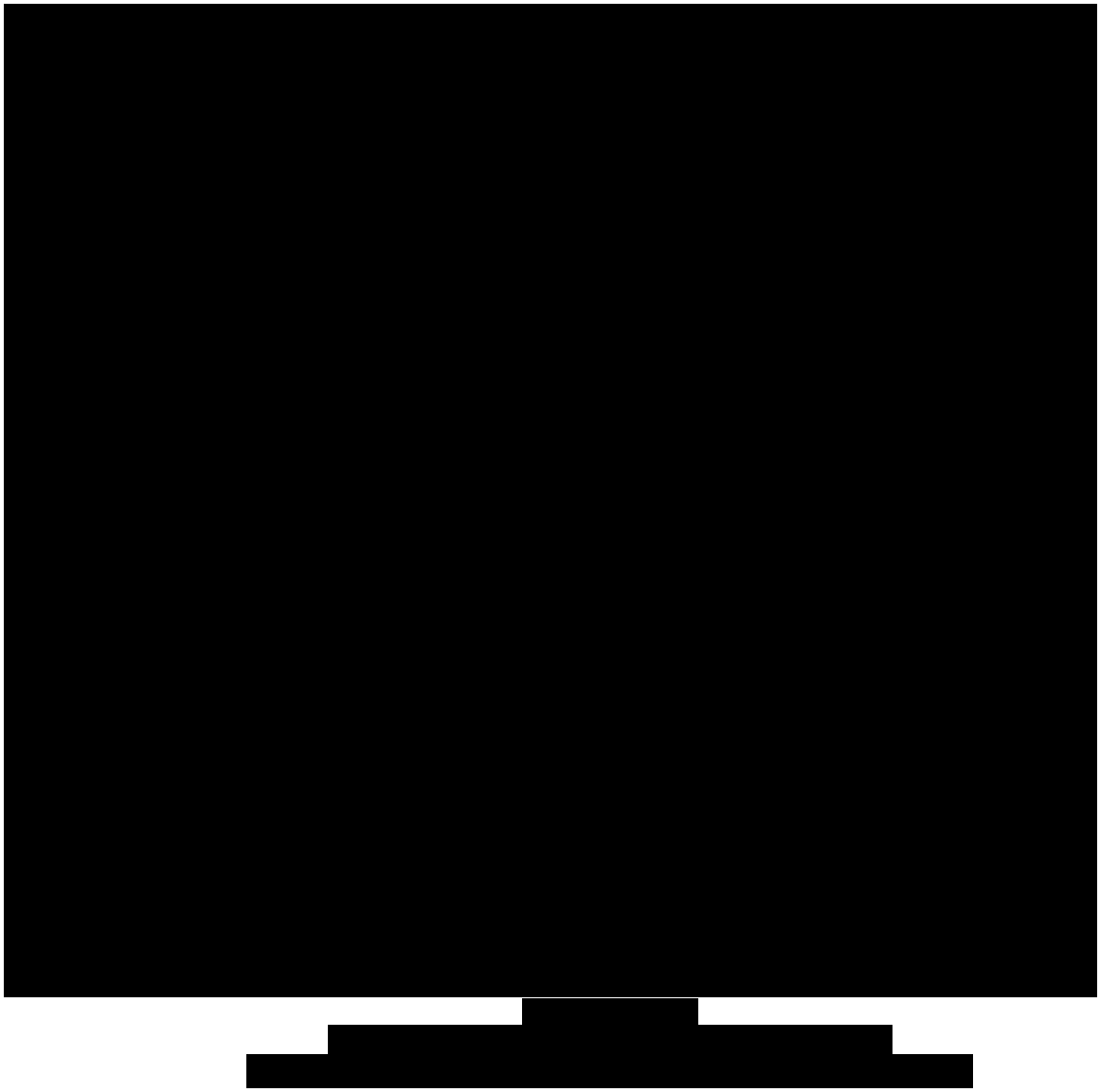
Nothing in these regulations is intended to limit the authority of a physician to provide emergency medical care to the extent the physician is permitted to do so under applicable federal, state, or local laws.

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 Diagrams

[redacted]



14.8 Skin Irritation Rating Scale

[redacted]

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 active study products. [redacted] he 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 10^5 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 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 study (Appendix 14.2). Scrub cup samples are collected and inoculated with low levels of [redacted] Two contact times after inoculation will be evaluated: immediately (<1 minute) and [redacted] 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 [redacted] 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 sign the Informed Consent Form and provide information on demographics and inclusion/exclusion criteria 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 [redacted].

Each subject will receive the active study product, [redacted], which will be applied to the test area on the abdomen. See diagram below.

Neutralization Test Area Diagram

[redacted]

14.9.4 Test Organism

The test organisms for this study are [redacted]

14.9.5 Study Products

[redacted] Surgical Solution, Tint, 26-mL applicator.

[redacted]

14.9.6 Materials, Supplies and Equipment

[redacted]

- Subject Informed Consent forms, IRB/EC-approved

[redacted]

[redacted]

14.9.7 Study Procedures

14.9.7.1 Preparation of Inoculum

One day prior to beginning the neutralization assay, the test organisms from a stock culture slant or from a lyophilized vial will be inoculated on TSA. The plates will be incubated for 24 [redacted] hours at 35° ± 2°C.

Immediately prior to initiating the neutralization assay, an inoculum suspension for each test organism will be prepared in PBW solution from the overnight culture on the TSA plate, and the concentration adjusted to approximately 10^8 – 10^9 CFU/mL.

Dilute the inoculum suspension 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 Area and Post-Prep Sampling

Neutralization samples will be evaluated on the abdomen. Each subject will be treated with the study product in the abdominal test area.

The abdominal test area will be marked using a sterile 1.5" x 5" template [redacted] A 1.5" x 5" area will be delineated on one side of the body containing two 1" x 1" sampling sites, yielding 2 samples per subject. The tube from one sampling area will be inoculated with [redacted] and the other will be inoculated with [redacted]

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

The test area will be treated with the study product, according to the instructions 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 [redacted] post-prep. Post-prep timing begins upon completion of study product application, including the 3-minute drying time. The sampling technique is described in Section 6.2.1.1 of the protocol to which this neutralizer validation is attached.

Residual product should be removed from test area after sample collection is complete [redacted]

Each pooled sample (~6 mL) will be capped tightly [redacted] 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

[redacted]

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 [redacted] minutes [redacted] post-inoculation, [redacted] 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 [redacted] hours.

14.9.7.3 Numbers Control and Toxicity Control

This testing will be performed in triplicate.

Numbers Control (NC):

Add 100 µL of the appropriate diluted inoculum to a tube containing 5 mL PBW to yield final inoculum concentrations of ~20-200 CFU/mL. [redacted] Pour-plate triplicate [redacted] aliquots immediately (<1 minute) and [redacted] minutes [redacted] post-inoculation in the same manner as 14.9.7.2, except using TSA without neutralizers.

Toxicity Control (TC-MSS):

Add [redacted] 5.0 mL SSS [redacted] to a clean tube. Add 100 µL of the appropriate diluted inoculum to the 5-mL tube to yield final inoculum concentrations of ~20-200 CFU/mL. Pour-plate triplicate [redacted] aliquots immediately (<1 minute) and [redacted] minutes [redacted] 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

[redacted] 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.

[redacted]

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).