Document Cover Page

Official Title of Study:

Preoperative Skin Preparation Study to Evaluate the Antimicrobial Capabilities of Four Test Substances

NCT Number: NCT04218110

Date of Document: November 29, 2019

CLINICAL STUDY PROTOCOL

Study Number:	ER 19/232
Current version:	Version 1.0 of 29/11/2019
Former versions:	***
Study Title:	Preoperative Skin Preparation Study Following ASTM E1173 Methods to Evaluate the Antimicrobial Capabilities of Four Test Substances
Investigational Products:	26 ml Project X (A)
	10.5 ml Project X (B)
	5.1 ml Project X (C)
Positive Control:	Prevantics® Maxi Swabstick 5.1 ml (D)
Principal Investigator:	Rozalia Olsavszky M.D. Dermatologist Registered Number (Romanian Ministry of Health): 461524 (specialist in dermato-venerology doctor, doctor in medical science)
Sub-Investigators:	Elena Chitoiu M.D. Resident Dermatologist
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Sponsor:	Professional Disposables International Inc. 400 Chestnut Ridge Road Woodcliff Lake, NJ 07677
Sponsor Representative:	James Clayton Professional Disposables International Inc. Email: James.Clayton@pdipdi.com
Date	November 29, 2019

SPONSOR (PROFESSIONAL DISPOSABLES INTERNATIONAL INC.) PROTOCOL APPROVAL

The signature of the representative below constitute the approval of this protocol and provide the necessary assurances that this study will be conducted according to all stipulations stated in the protocol, including all statements as to confidentiality. It is also agreed that the study will not be initiated without the approval of an appropriate IRB/IEC.

Approved by the following:

Sponsor Representative	James Clayton
Sponsor	Professional Disposables International Inc. 400 Chestnut Ridge Road Woodcliff Lake, NJ 07677

Signature of Sponsor Representative

December 2nd 2019.
Date

PRINCIPAL INVESTIGATOR SIGNATURE

Principal Investigator	Rozalia Olsavszky M.D. Dermatologist
Research Facility	EUROFINS EVIC PRODUCT TESTING ROMANIA S.R.L. 64-66, Marasesti Boulevard Bucharest 040256 Romania

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

Signature of Principal Investigator

02.12.2019
Date

Synopsis

Study Number:	ER 19/232
Current version:	Version 1.0 of 29/11/2019
Former versions:	***
Protocol Title:	Preoperative Skin Preparation Study Following ASTM E1173 Methods to Evaluate the Antimicrobial Capabilities of Four Test Substances
Planned Sample Size:	This evaluation will be performed with an estimated of a minimum of 50 evaluable sites per product with qualifying treatment day microbial baseline counts.
Overall Study Design and Plan:	This study is designed to determine the antimicrobial effectiveness of three treatments (A, B and C), intended for use as Patient Preoperative Skin Preparation. The testing methods are based on the standardized test method ASTM E1173-15, Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations, and using the requirements specified by in the 2017 FDA 21 CFR Part 310, Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use, Final Rule (December 20, 2017). The immediate antimicrobial efficacy will be evaluated in this study. At least 100 subjects will be treated bilaterally with two of the four test materials (A, B, C and Positive Control), one per each side of the groin test site. Subjects will undergo at least a 14-day washout period before Treatment Day. During Treatment Day subjects who meet the Inclusion Criteria will be treated with the study products and samples will be taken before and after treatment application. The cup scrub technique will be used for sampling baseline, 30-seconds and 10-minutes on the Treatment Day. The immediate antimicrobial efficacy at 30 seconds and 10 minutes post-application will be demonstrated if the non-inferiority of the ATE of the investigational products (A, B and C) compared to Positive Control (D) is met (the upper two-sided 95% confidence bound of the post-application microbial counts corrected for Baseline of the investigational products A, B or C compared to the Positive Control should be less than 0.5 $\log_{10}$).
Study Methodology:	ASTM Standard Test Method E1173-15
Study Population:	Healthy subjects at least 18 years of age and of any race. Treatment Day Baseline microbial count requirements are in the range of 5.00 to 7.50 $\log_{10}$ CFU/cm^2, inclusive, on the groin.
Objectives:	**Efficacy objectives** The objective of this study is to demonstrate immediate (30 seconds and 10 minutes) antimicrobial efficacy of three investigational products (26 ml Project X, 10.5 ml Project X and 5.1 ml Project X) compared to Prevantics® Maxi Swabstick 5.1 ml (active control) on the groin. To demonstrate the immediate antimicrobial efficacy at 30 seconds and 10 minutes post-application the following criteria should be met on the groin: - non-inferiority of the ATE of the product A, B and C compared to product D (the upper two-sided 95% confidence bound of the post-application microbial counts corrected for Baseline of the A-D or B-D or C-D should be less than 0.5 $\log_{10}$). **Exploratory Objectives** Evaluation of the investigational products consumption weight (product weight before application – product weight after application).

<table>
<tr><td></td><td>Safety objectives
Safety will also be evaluated based on the incidence of adverse events reported during the study and assessment of skin irritation ratings.</td></tr>
<tr><td>Study Endpoints:</td><td>- Log_{10} CFU/cm^2 of skin flora at the groin sites before and after each treatment at 30 seconds and 10 minutes.
- Skin irritation scores (Erythema, Edema, Rash, and Dryness) at each time interval and the incidence of AEs reported during the study.</td></tr>
<tr><td>Study Products:</td><td>
<table>
<tr><th>Treatment</th><th>Description</th><th>Body Area</th><th>Application Time</th><th>Drying Time</th><th>Treatment Area</th></tr>
<tr><td>A – 26 ml Project X</td><td>3.15 % w/v CHG / 70% v/v IPA</td><td>Groin</td><td>120 seconds</td><td>min. 180 seconds</td><td>1.5″ x 5.0″ (3.81 cm x 12.7 cm)</td></tr>
<tr><td>B – 10.5 ml Project X</td><td>3.15 % w/v CHG / 70% v/v IPA</td><td>Groin</td><td>120 seconds</td><td>min. 180 seconds</td><td>1.5″ x 5.0″ (3.81 cm x 12.7 cm)</td></tr>
<tr><td>C – 5.1 ml Project X</td><td>3.15 % w/v CHG / 70% v/v IPA</td><td>Groin</td><td>120 seconds</td><td>min. 180 seconds</td><td>1.5″ x 5.0″ (3.81 cm x 12.7 cm)</td></tr>
<tr><td>D – 5.1ml Prevantics® Maxi Swabstick</td><td>3.15 % w/v CHG / 70% v/v IPA</td><td>Groin</td><td>120 seconds</td><td>~ 90 seconds</td><td>1.5″ x 5.0″ (3.81 cm x 12.7 cm)</td></tr>
</table>
</td></tr>
<tr><td>Statistical Methods:</td><td>Subjects with treatment day baseline bacterial count in the range of 5.00 to 7.50 log_{10}/cm^2, inclusive, on the groin will be included in the analysis.

An alpha level of 5% will be used for all analyses.

The following descriptive statistics for log_{10} CFU/cm^2 and for log_{10} CFU/cm^2 reductions from Baseline will be computed for each treatment, grouped by post application sampling time point (30 seconds, 10 minutes and/or Baseline, by case): mean, median, standard deviation, minimum, maximum, and count.

Efficacy Analysis

A linear regression model for each post-treatment sampling time point of immediate efficacy (30 seconds and 10 minutes post-application) will be used. In the model, the dependent variable used will be the post-treatment bacterial count (log_{10} CFU/cm^2) and predictors will be the treatment as a fixed effect and the Baseline as a covariate. The ATE corrected for Baseline will be estimated from the model and compared to non-inferiority criteria.

Safety Analysis

All treated subjects will be considered evaluable for safety. Skin irritation scores will be reported for any subject who is scored with a 1 or more at any observation (treatment day, post-application/prior to 30 seconds and 10 minutes), in any category for any site.

Adverse events (including post treatment skin irritation scores of 3), will also be summarized. Summary tables will present incidence rates of adverse events by treatment group for all subjects who enter the treatment period. Listings of adverse events will be provided.</td></tr>
</table>

Contents

Abbreviations

AE	Adverse event
AIDS	Acquired immune deficiency syndrome
ASTM	American Society for Testing Materials
ATCC	American Type Culture Collection
ATE	Average treatment effect
CFR	Code of Federal Regulations
CFU	Colony Forming Units
CHG	Chlorhexidine gluconate
cm^2	Square centimeter
CRF	Case Report/Record Form
ER	EUROFINS EVIC PRODUCT TESTING ROMANIA S.R.L.
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIV	Human immunodeficiency virus
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Conference on Harmonization
IEC	Institutional Ethics Committee
IPA	Isopropyl Alcohol
IRB	Institutional or Independent Review Board
M.D.	medical doctor
PBW	Phosphate Buffered Water
PDI	Professional Disposables International Inc.
PI	Principal Investigator
SAE	Serious adverse event
SOP	Standard Operating Procedure
SSF++	Stripping Suspending Fluid with product neutralizers
TSA+N	Trypticase Soy Agar containing neutralizers
US	United States of America

1 Introduction

The immediate and persistent antimicrobial efficacy of the investigational products will be evaluated in this study according to US FDA analysis criteria.

2 Study Objectives

2.1 Efficacy objectives

The objective of this study is to demonstrate immediate (30 seconds and 10 minutes) antimicrobial efficacy of three investigational products (26 ml Project X, 10.5 ml Project X and 5.1 ml Project X) compared to Prevantics® Maxi Swabstick 5.1 ml (active control) on the groin.

To demonstrate the immediate antimicrobial efficacy at 30 seconds and 10 minutes post-application the following criteria should be met on the groin:

- non-inferiority of the ATE of the product A, B and C compared to product D (the upper two-sided 95% confidence bound of the post-application microbial counts corrected for Baseline of the A-D or B-D or C-D should be less than 0.5 $\log_{10}$).

2.2 Exploratory Objectives

Evaluation of the investigational products consumption weight (product weight before application – product weight after application).

2.3 Safety objectives

Safety will also be evaluated based on the incidence of adverse events reported during the study and assessment of skin irritation ratings from Appendix 17.6.

3 Study Design

3.1 Overall Study Design and Plan

This study is designed to determine the antimicrobial effectiveness of three treatments (A, B and C), intended for use as Patient Preoperative Skin Preparation. The testing methods are based on the standardized test method ASTM E1173-15, Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations, and using the requirements specified by in the 2017 FDA 21 CFR Part 310, Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use, Final Rule (December 20, 2017). The immediate antimicrobial efficacy will be evaluated in this study.

At least 100 subjects will be treated bilaterally with two of the four test materials (A, B, C and Positive Control), one per each side of the groin test site. Subjects will undergo at least a 14-day washout period before Treatment Day. During Treatment Day subjects who meet the Inclusion Criteria will be treated with the study products and samples will be taken before and after treatment application. The cup scrub technique will be used for sampling baseline, 30-seconds and 10-minutes on the Treatment Day.

The immediate antimicrobial efficacy at 30 seconds and 10 minutes post-application will be demonstrated if the non-inferiority of the ATE of the investigational products (A, B and C) compared to Positive Control (D) is met (the upper two-sided 95% confidence bound of the post-application microbial counts corrected for Baseline of the investigational products A, B or C compared to the Positive Control should be less than 0.5 $\log_{10}$).

3.2 Study Type

This is a randomized, single-center, single blind study in healthy subjects. The staff member(s) performing bacterial enumeration will be blinded from the identification of treatment assignment.

3.3 Endpoints

- Log_{10} CFU/cm^2 of skin flora at the groin site before and after each treatment at 30 seconds, 10 minutes.
- Skin irritation scores (Erythema, Edema, Rash, and Dryness) at each time interval and the incidence of AEs reported during the study.

3.4 Randomization and Blinding

The randomization scheme for the study will be provided by the Research facility.

Each subject will receive two treatments. Assignment of products to the left or right side for each body area will be per a computer-generated randomization schedule.

Specific sites of sampling for baseline and post application sampling time points will be randomized.

Randomization will continue until required number of subjects with required treatment day baseline counts is enrolled.

The PI is responsible for ensuring that the randomization is followed. The randomization schedule for the groin sites will be provided in a separate document. The study products will be labeled with the appropriate codes provided in Table 1.

Each subject will be identified by their initials and a subject number for which they qualify. Inclusion subject numbers will be assigned at Treatment day.

- Washout subjects will be assigned numbers starting with 001 and the letter "P": P001
- Treatment subjects will be assigned numbers with 001 and the letter "I": I001

The study products will not be blinded from the PI or other study staff performing the study material application or bacterial sample collections. The staff member(s) performing bacterial enumeration and the statistician will be blinded from the identification of treatment assignment. The study staff performing the bacterial enumeration will not be involved in the study products application or the collection of samples. The Raw Data Sheet sections of the CRF will be maintained separately (from the pages within the CRF which include study treatment identifications) during the conduct phase of the study. The study staff performing the bacterial enumeration will record counts directly onto the Raw Data Sheet pages of the CRF without accessing the subject study documentation folder containing the other CRF pages. The Raw Data Sheets will be compiled with the entire CRF after all data recording have been completed.

The generated randomization scheme will contain the following treatment codes: P1, P2, P3 and P4 to ensure the blinding for the study statistician. The assignment between the treatment codes (P1, P2, P3 and P4) from the randomization scheme and the label treatment codes (A, B, C and D) will be made by the Sponsor in a separate document.

In this study four products will be tested and each subject will receive two products in the same time, one on the left side and the other on the right side. More than 100 randomization schemes will be generated to compensate for the baseline requirement failure and to provide enogh evaluable sites. The block randomization method will be used to ensure equal sample size groups over time. Products will be randomized in blocks of 12 and sampling time points will be randomized in blocks of 6. Randomization scheme will be generated using an electronic online randomizer provided by Urbaniak et al. (2013).

3.5 Study Products

The products identified in Table 1 will be used in the study. Specific product identification codes and lot numbers will be included with the clinical supplies when they are shipped to the Research facility.

Table 1: Study Products

Treatment	Denomination	Volume	Description
A	26 ml Project X	26 ml	3.15 % w/v CHG / 70% v/v IPA
B	10.5 ml Project X	10.5 ml	3.15 % w/v CHG / 70% v/v IPA
C	5.1 ml Project X	5.1 ml	3.15 % w/v CHG / 70% v/v IPA
D	Prevantics® Maxi Swabstick	5.1 ml	3.15 % w/v CHG / 70% v/v IPA

PDI is responsible for analytically testing (content and purity) the study products to ensure they comply with their specifications and both released as per approved specifications of the marketed control product.

3.5.1 Study Products Labeling

PDI will label, package, and ship the study products to the research facility. ER personnel will document receipt and storage of the study materials.

3.5.2 Product Incidents

A Product Incident is defined as any problem or issue involving the investigational product(s). PDI and Study Monitor should be contacted immediately when site becomes aware of or suspects:

- Product damaged upon visual inspection
- Product damaged upon removal/preparation
- Product packaging damaged/broken
- Product failure or malfunction
- Product handling issue
- Product lacking liquid antiseptic

Product considered as Product incident should be quarantined and returned with appropriate documentation to the PDI unless instructed otherwise by PDI

3.6 Study Supplies

3.6.1 Study Supplies Provided by Sponsor

- Study Products

3.6.2 Study Supplies Provided by Research facility

- Product kits (toiletry items to be used by subjects during study)
- Stripping Suspending Fluid with product neutralizers (SSF++) containing 1.01% Na_2HPO_4, 0.04% KH_2PO_4, 0.1% Triton X-100, 1.167% Lecithin, 10% Polysorbate 80, 0.5% $Na_2S_2O_3 \bullet 5H_2O$, and 1% Tamol™ (pH 7.9 ± 0.4)
- Butterfield's sterile phosphate-buffered water (PBW), 312 µM KH_2PO_4 (pH 7.2 ± 0.4)
- Trypticase soy agar containing 0.5% Polysorbate 80 and 0.07% lecithin (TSA+N)
- Disposable Pasteur Pipettes, sterile
- Tubes with sealable caps, polypropylene or glass, sterile
- Petri dishes, 90 mm, sterile
- Gloves, sterile
- Gauze, sterile
- Non-toxic marking pen: Chemo Skin Marker- Regular™
- Rubber policemen, sterile
- Scrub cups (2.20 cm I.D., 3.80 cm^2), sterile
- Timers
- Pipette Aid or similar apparatus
- Vortex mixer
- Surgical Clipper & clipper blades: 3M Surgical Clipper
- Culture Media Preparator
- Peristaltic Pump
- Incubator (30 ± 2^0C)
- Disposable underwear for subjects
- urine pregnancy test (UPT)
- Biological safety cabinet

- Manual colony counter
- pH-meter

3.7 Study Duration

The expected duration of this study for each subject is up to 3 weeks. Subjects will undergo at least a 14-day washout period before Treatment Day. The treatment day will be scheduled no sooner than 14 days from the washout visit. Each subject who chooses to participate in this study will be required to stay in the test facility for one scheduled Treatment Day.

3.8 Study Termination/Subject Discontinuation or Withdrawal

3.8.1 Study Termination

PDI or the PI has the right to discontinue the study at any time for medical and/or administrative reasons. As much as possible, this should occur after mutual consultation.

3.8.2 Subject Discontinuation and Withdrawal

The PI may discontinue individual subjects from the study at any time. Subjects may voluntarily withdraw from the study at any time without reason or consequence. A reason will be reported, if provided. The PI will provide a written report on the appropriate CRF including the date and reason for discontinuance.

Additional subjects will be recruited, as necessary, to meet the required number of evaluable treatment sites per treatment, per collection time.

3.9 Study Product Accountability

PDI requires PI to maintain accountability and adequate inventory security of the study material at all times. The PI or designated sub-investigator trained by the PI will:

- complete the Confirmation of Receipt form upon receipt of the shipment and maintain and account for inventory on the Study Material Disposition form.
- keep study materials in a secure storage area, accessible only to authorized individuals.
- dispense study material only to subjects properly enrolled into the study.
- destroy all used study materials during the technical execution of the study
- return all study materials involved into Product incident and/or AE/SAE to PDI upon Sponsor agreement.
- return or destroy all unused study materials to PDI at the end of technical execution of the study upon Sponsor agreement.

Before starting the study, investigational products and positive control product will be supplied and shipped to the site by PDI and stored at controlled room temperature avoiding freezing and heat above 40°C. A sample from each product will be kept in the sample storage area of the Research facility for at least 3 years after the end of the study, then destroyed, according to the corresponding procedure of the Research facility.

3.10 Source Data

Source data includes any original documents, data and records where any data are first recorded (e.g., ICFs, laboratory notes, etc) used for study traceability. If data are recorded for the first time directly onto the CRF, then the CRF is considered the source document for these data.

3.11 Protocol Modifications

3.11.1 Protocol Amendments

Protocol Amendments will not be implemented without prior agreement between the PI and PDI and prior submission and approval from the IRB/EC, except when necessary to eliminate an immediate hazard to the subject and to protect the safety and well-being of the subject.

Will constitute Protocol Amendments for administrative inputs and changes: correction of typing mistakes, changes in study personnel (other than the PI) or contact information. Protocol Amendments for administrative inputs and changes has to be agreed between the PI and PDI and will be submitted to the IRB/EC but implementation may proceed without prior IRB/EC approval, unless so required by the IRB/EC or site SOPs.

3.11.2 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, the integrity of the data or the scientific quality of the study. 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 communication to PDI at the earliest convenience.

A Protocol Deviation Form must be completed by the PI or designated sub-investigator trained by the PI and checked and approved by PI and include a description of the circumstances surrounding and the reason for the deviation, any actions taken, and whether or not the subject was allowed to continue in the study.

If during monitoring visits a deviation is identified, the Study Monitor will report it to the PI and PDI.

4 Study Population

The trial will utilize ER's subject database and would not require the recruitment of subjects through study-specific advertisement.

Subjects must satisfy all Treatment Day Inclusion/Exclusion Criteria prior to Treatment Day procedures.

Treatment Day Baseline microbial count requirements are in the range of 5.00 to 7.50 $\log_{10}$ CFU/cm^2, inclusive, on the groin.

Additional subjects will be recruited and treated as necessary, to meet the required number of evaluable treatment sites per collection time.

All subjects will be given verbal and written information about the study procedures - Subject Instructions for Main Study (Appendix 17.5) will be provided to each subject for the Washout phase of the study.

4.1 Subject Inclusion Criteria

Subjects will be included in this study if they meet the following requirements:

1. Male or female at least 18 years of age and of any race
2. In good general health
3. Read, understand and sign the ICF
4. If female of child-bearing potential, are willing to use an acceptable method of contraception to prevent pregnancy (i.e. oral contraceptive, intra-uterine device, diaphragm, condom, abstinence, bilateral tubal ligation, or are in a monogamous relationship with a partner who has had a vasectomy)
5. Female subjects of child-bearing potential, must have a negative Urine Pregnancy Test (UPT) on Treatment Day prior to any applications of the study products

4.2 Subject Exclusion Criteria

Potential subjects will be excluded from participation if any one of the following criteria apply to them.

1. Exposure of test sites to antimicrobial agents, medicated soaps, medicated shampoos, or medicated lotions, use of biocide-treated pools or hot tubs, use of tanning beds, or sunbathing during the 14-day washout conditioning period and during the test period
2. Exposure of the test sites to strong detergents, solvents, or other irritants during the 14-day washout conditioning period and during the test period
3. Wear fabric softener, bug repellent or UV-treated clothing during the 14-day washout conditioning period and during the test period
4. Receive an irritation score of 1 (any redness, swelling, rash, or dryness present at any treatment area) for any individual skin condition prior to the Treatment Day baseline sample collection
5. Use of systemic or topical antibiotic medications, steroid medications (other than for hormonal contraception or post-menopausal reasons), or any other product known to affect the normal microbial flora of the skin during the 14-day washout conditioning period and during the test period
6. Known allergies or sensitivities to vinyl, latex (rubber), alcohols, metals, inks, or tape adhesives, or to common antibacterial agents found in soaps, lotions, or ointments, particularly isopropyl alcohol or chlorhexidine gluconate
7. A medical diagnosis of a physical condition, such as a current or recent severe illness, asthma, diabetes, hepatitis, an organ transplant, mitral valve prolapses, congenital heart disease, internal prostheses, or any immunocompromised conditions such as AIDS (or HIV positive)
8. Pregnancy, plans to become pregnant within the washout and test periods of the study, or nursing a child
9. Any tattoos or scars within 2" (5.08 cm) of the test sites
10. Dermatoses, cuts, lesions, active skin rashes, or breaks or other skin disorders within 6" on or around the test sites
11. A currently active skin disease or inflammatory skin condition (for example, contact dermatitis) anywhere on the body that, in the opinion of the Consulting Physician or PI, would compromise subject safety or study integrity
12. Showering, bathing, or swimming within the 72-hour period prior to sampling for Treatment Day, and throughout the test period
13. Participation in another clinical study in the past 30 days or current participation in another clinical study at time of signing informed consent
14. Any medical condition or use of any medications that, in the opinion of the PI, should preclude participation
15. Unwillingness to fulfill the performance requirements of the study.

4.3 Subject Consent

The PI or designated sub-investigator trained by the PI must ensure that written informed consent to participate in the investigation is obtained before including any individual as a subject in the investigation. The PI or designated sub-investigator trained by the PI must provide the prospective subject 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 needed,
2) ensure that the subject understands the information,
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 PI or designated sub-investigator trained by the PI is to explain to each subject all elements of ICF as specified in 21 CFR 50.25. After the explanation, subjects will voluntarily sign and

date the ICF if they wish to participate in the study. A copy of the ICF must be provided to the subject. A signed and dated ICF must be maintained in the PI study documentation file at all times.

5 Study Treatment

These study procedures are based on the American Society for Testing and Materials (ASTM) "Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations" (E1173-15).

5.1 Study Procedures

5.1.1 Washout Period

The Inclusion/Exclusion Criteria will be reviewed with each subject to ensure eligibility for the study after subjects has signed the ICF.

A skin irritation assessment using the scale from Appendix 17.6 will be performed by the PI or designated sub-investigator trained by the PI. If an irritation score of 1 for any individual skin condition is observed, the subject will be excluded from the study.

Prior to the scheduled Treatment Day, subjects will undergo a minimum 14-day restriction. The subjects will be instructed to avoid contact with any topical or systemic antimicrobial products or any other product know to affect the normal microbial flora of the skin for the duration of their involvement in the study as written in the Subject Instructions for Main Study (Appendix 17.5.1).

If it becomes necessary to take systemic antibiotics or to apply topical medications to the test areas within this Washout period, the subject must contact the PI or designated sub-investigator whom will inform the PI as soon as reasonably possible so that another volunteer may be recruited.

Restrictions include, but are not limited to:

- Use of antimicrobial soaps, shampoos, lotions, perfumes, after shaves, colognes, antiperspirants, deodorants
- Contact with materials such as acids, bases, solvents
- Swimming in chemically treated pools and bathing in hot tubs, spas and/or whirlpools
- Use of tanning beds, hot waxes or depilatories (including shaving)

Subjects will be provided a kit with non-antimicrobial personal care products for exclusive use during the study.

If subjects require hair removal to facilitate sample collection, the subject will be asked to return to the test facility at approximately 48-96 hours prior to Treatment Day for clipping/re-clipping.

Prior to performing the clipping/re-clipping before the Treatment Day, a skin irritation assessment using the scale from Appendix 17.6 will be performed by the PI or designated sub-investigator trained by the PI. If an irritation score of 1 for any individual skin condition is observed, the subject will be excluded from the study.

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

5.1.2 Treatment Day

The PI or a designated sub-investigator will complete the Treatment Day Inclusion/Exclusion Criteria CRF. If these criteria are satisfied, a skin irritation assessment using the scale from Appendix 17.6 will be performed prior to performing the Treatment Day baseline sample collection, by the PI or designated sub-investigator trained by the PI. If an irritation score of 1 for any individual skin condition at the Treatment Day baseline is observed, the subject will be excluded from the study.

5.1.2.1 Preparation of Test Areas on Treatment Day

A Test Site Diagram for the groin test area is shown in Appendix 17.3.

5.1.2.1.1 Preparation of Groin Test Area

The test site within the groin region (groin test area) is defined as the inner aspect of the uppermost thigh, one inch parallel distance to the inguinal crease and where skin to skin contact is expected. Where subjects present with deep inguinal folds at the crease, the sampling distance from the crease may be increased to avoid skin folds in the sampling area. The corners of each groin test area will be marked directly on the skin using a non-toxic skin marker. Three sampling sites will be numbered within each groin test area, on each side of the groin region. The positioning and numbering of the groin sampling sites are standard for all subjects. Sampling sites on the contra-lateral side of the groin will be numbered in a mirror-image orientation. The three sampling sites within each test area represent one baseline (pre-prep) site and two post-prep sample sites (30 seconds and 10 minutes).

Test sites for the investigational product and active control applicator on the groin will be 1.5" x 5" (3.81 cm x 12.7 cm) in area, occur at the uppermost inner aspects of both thighs, and appear to be similar in condition.

After groin test areas are marked and sample sites numbered, baseline samples will be collected from the appropriate site per the randomization schedule in each test area using the scrub cup technique (section 5.2.1).

5.1.2.2 Study Products Application

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

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

The study products will be applied and the sampling configurations will be performed per the randomization scheme and the Treatment Application Instructions (Appendix 17.6). The duration of each application procedure will be recorded on the appropriate CRF. The study products, body area, application and dry times and treatment area are summarized in Table 2.

Table 2: Study products, body area, application and dry times and treatment area

Products	Body Area	Application Time	Drying Time	Treatment Area
26 ml Project X (A)	Groin	120 seconds	min. 180 seconds	1.5" x 5.0" (3.81 cm x 12.7 cm)
10.5 ml Project X (B)	Groin	120 seconds	min. 180 seconds	1.5" x 5.0" (3.81 cm x 12.7 cm)
5.1 ml Project X (C)	Groin	120 seconds	min. 180 seconds	1.5" x 5.0" (3.81 cm x 12.7 cm)
Prevantics® Maxi Swabstick 5.1 ml (D)	Groin	120 seconds	90 seconds	1.5" x 5.0" (3.81 cm x 12.7 cm)

5.1.2.3 Timing of Post Application Sample Collection

Microbial samples will be collected at 30 seconds (+5 seconds) and 10 minutes (± 30 seconds), post treatment application for both left side and right side. Post application timing begins upon completion of the study product application, including drying time. Microbial samples will be collected using the scrub cup technique (section 5.2.1).

A skin irritation assessment using the scale from Appendix 17.7 will be performed by the PI or designated sub-investigator trained by the PI prior to collection of each post treatment microbial sample

collection (30 seconds and 10 minutes) and a corresponding rating score for each individual skin condition will be recorded in the subject's CRF.

If an irritation score of 3 for any individual skin condition at any post treatment observation is assigned, the subject will be discontinued from the study and an adverse event will be recorded. See Section 8.3 (Adverse Events).

Following final sample collection, the remaining study material will be removed from the subjects' skin with water and soap as necessary and dried with paper towels.

5.2 Microbiological Methods

5.2.1 Microbial Sample Collection / Cup Scrub Technique

Quantitative cultures (treatment day baselines and post treatment samples) will be obtained by a modification of the cylinder sampling technique of Williamson-Kligman cup scrub technique following ASTM E1874-14 "Standard Test Method for Recovery of Microorganisms from Skin using the Cup Scrub Technique". To collect the samples, a sterile scrub cup (2.20 cm I.D.) will be placed on the site and held firmly to the skin. 3.0 ml of SSF++ will be pipetted into the cup and the skin will be scrubbed in a circular motion with moderate pressure for 1 minute using a sterile rubber policeman. Using a sterile transfer pipette, the SSF++ will be removed and placed in a sterile test tube. An additional 3.0 ml of fresh SSF++ will be pipetted into the cup and the scrub procedure will be repeated. This solution will be pooled with the first solution collected.

5.2.2 Bacterial Enumeration Methods

Following sample collection, vortex the sample for 15 seconds and 10-fold serial dilutions will be prepared using PBW. One ml aliquots of appropriate dilutions will be pour-plated in TSA+N. Samples must be plated within 30 minutes of collection.

Serial dilutions of collected treatment day samples as follows:

for groin sites:

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

After 72 ± 4 hours of aerobic incubation at 30 ± 2°C, colonies will be manually counted and viable cells in the original sample will be calculated according to SOPs. After incubation, plates may be refrigerated up to 48 hours prior to counting.

Bacterial colonies enumerated manually within the countable range 25-250 from each dilution will be recorded on the appropriate CRFs for each subject. The average number of microorganisms recovered (CFU/cm^2) from the skin during treatment day will be calculated using the formula to convert the volume of sample collected into $\log_{10}$CFU/cm^2 of skin:

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

Where:

R = the average colony-forming unit count in $\log_{10}$ scale per cm^2 of sampling surface
F = total number of ml of stripping fluid added to the sampling cylinder; in this study, F = 6 ml
$\frac{\sum_{i=1}^{3} c_i}{n}$ = average of the triplicate colony counts used for each sample collected
D = dilution factor of the plate counts

A = inside area of the cylinder in cm^2; in this study, A = 3.80 cm^2 for the 2.2-cm diameter cylinder.

6 Assessment of Efficacy

6.1 Efficacy Parameters

The measure of antimicrobial efficacy will be the log_{10} CFU/cm^2 of skin flora at the groin site 30 seconds and 10 minutes following study products application relative to the Treatment Day baseline log_{10} counts.

6.2 Assessment Methods

Efficacy will be assessed by sampling the skin using the cup scrub method and analysis methods described in Section 5.2.

7 Risk / Benefit Assessment

7.1 Potential Risks

Subjects participating in this study could experience side effects to the skin such as:

- Redness
- Swelling
- Burning, stinging and itching
- Cracking
- Peeling
- Small blisters or sores (in uncommon cases)
- Rash or other allergic reaction including in rare cases anaphylaxis
- Irritation
- Pain
- Localized rash or inflammation (dermatitis)
- Impetiginized eczema (infected eczema or infected dry skin and recurring skin rashes)
- Application site hypopigmentation (lightening of skin) or hyperpigmentation (darkening of skin).

Subjects may also experience folliculitis from clipping.

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

7.2 Potential Benefits

There are no direct benefits to the subject for participation in this study.

8 Assessment of Safety

8.1 Safety Parameters

The principal measures of safety will be skin irritation scores and the incidence of adverse events reported during the study.

Adverse events will be captured from the time the subject have signed the Informed consent form to the time of subject discharge from the study. Adverse events will be categorized in relationship to the product that was applied to the specific skin site. All local and systemic adverse events observed or reported to the investigator will be evaluated.

The severity, duration, causal relationship to the study product and study procedure, action taken (study related and subject related), study impact, outcome of the event; will be described for all adverse events.

8.2 Assessment Methods for Skin Irritation

All randomized subjects will be considered evaluable for safety. Skin irritation scores based on the scale from Appendix 17.6 will be reported for any subject who is scored with a 1 or more at any observation (baseline treatment day, clipping/re-clipping and prior/post-application to 30 seconds and 10 minutes sampling procedures), in any category for any site.

Adverse events (including post treatment skin irritation scores of 3), will also be summarized. Summary tables will present incidence rates of adverse events by treatment group for all subjects who enter the treatment period. Listings of adverse events will be provided.

A corresponding rating score for each individual skin condition, for each site will be recorded in the subject's CRF. (See Appendix 17.6, which includes the following four independent evaluation categories: Erythema, Edema, Rash, and Dryness).

If an irritation score of 1 or greater for any individual skin condition prior to the baseline sample collection (at treatment day phase) is assigned, the subject will be excluded from the study (no study treatment will be applied).

If an irritation score of 3 for any individual skin condition at any observation period is assigned, the subject will be discontinued from the study and an adverse event will be recorded. See Section 8.3 (Adverse Events).

8.3 Adverse Events

The PI is responsible for identifying adverse events that occur to each subject throughout the study and follow-up period. An adverse event can occur at any time during the conduct of the study, in any phase of the study or after the study is completed. An adverse event can be identified by the PI or sub-investigators or reported by the subject.

Definitions:

Adverse Event/Experience

An Adverse Event/Experience is any unexpected or undesirable experience occurring to a subject during a study, which may or may not be related to the test product.

All adverse events, regardless of severity or the causal/effect relationship, will be recorded. The severity of the effect will be noted as *Mild, Moderat*e, or *Severe* according the following definitions:

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

Causal Relations of Adverse Event/Experience

When determining the causal/effect relationship to the test product, the relationship will be described as *Not related, Unlikely related, Possibly related* or *Related*.

The following definitions will be utilized:

Not related	No association to the test product. Related to other etiologies such as concomitant medications or conditions or subject's known clinical state.
Unlikely related	Uncertain association. Other etiologies are also possible.
Possibly related	Clear-cut association with improvement upon withdrawal of the test product. Not reasonably explained by the subject's known clinical state.
Related	An adverse event with a clear-cut temporal association with exposure to study materials and cannot reasonably be explained by the subject's known clinical state. Association with study material is confirmed by laboratory testing if possible.

Serious Adverse Event/Experience

A Serious Adverse Event/Experience is any adverse experience occurring at any dose that results in any of the following outcomes:

- Death;
- A life-threatening adverse drug experience;
- Inpatient hospitalization or prolongation of existing hospitalization;
- A persistent or significant disability/incapacity;
- Congenital anomaly/birth defect;
- An important medical event that may require medical or surgical intervention to prevent one of the previously listed outcomes.

Unexpected Adverse Event/Experience

An Unexpected Adverse Event/Experience is any adverse drug event/experience not listed in the current labeling for the test product or the current IB. Where test product labeling or IB is not available, anticipated experiences will be based on the known pharmacological/toxicological properties of the test product or ingredients.

Follow-up:

If an adverse event/experience occurs, the subject under the direction of the PI or designated sub-investigator trained by the PI may be referred to the nearest acute care facility for treatment. Serious or Unexpected Drug Event/Experience will be followed to resolution. Any adverse event will be documented on an Adverse Event Report Form.

Recording and Reporting

The PI or designee records all adverse events on an Adverse Event Form in the subject's CRF.

The PI must promptly report all treatment related adverse events to the PDI study monitor.

All SAE must be reported to the:

- Sponsor within 24 hours of the PI awareness/notification of the event.
- IRB/IEC within 10 working days of the PI awareness/notification of the event.

If a subject has no adverse event during the study, the absence of such must be recorded on the CRF.

9 Statistics

9.1 Data sets analyzed

A modified intent to treat data set will be used for efficacy analysis. Subjects with treatment day baseline bacterial count in the range of 5.00 to 7.50 $\log_{10}/cm^2$, inclusive, on the groin will be included

in the modified intent to treat data set. The acceptability of each bilateral baseline site (left or right) will be assessed separately for inclusion in the modified intent to treat data set. The exclusion of subjects who do not meet treatment day baseline requirements and the exclusion of either left or right groin sites without exclusion of the subject as a whole makes this data set a modified intent to treat data set.

A per protocol data set will also be used for efficacy analysis. Subjects included in the modified intent to treat data set with no major protocol deviations and no missing for one or more of the 30 minutes and 10 minutes time points will be included in the per protocol data set. The acceptability of each bilateral site (left or right) will be assessed separately for inclusion in the per protocol data set.

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

9.2 Sample Size

This evaluation will be performed with an estimated of a minimum of 50 evaluable sites per product with qualifying treatment day microbial baseline counts. No sample size calculation was performed to provide statistically-powered evidence of efficacy as part of this study.

9.3 Efficacy Analyses

An alpha level of 5% will be used for all analyses.

The following descriptive statistics for $\log_{10}$ CFU/cm^2 and for $\log_{10}$ CFU/cm^2 reductions from Baseline will be computed for each treatment, grouped by body area and each post application sampling time point (30 seconds, 10 minutes and/or Baseline, by case): mean, median, standard deviation, minimum, maximum, and count.

The statistician will be blinded to the treatment during the analysis phase.

9.3.1 Efficacy Analysis

A linear regression model for each post treatment sampling time point of immediate efficacy (30 seconds and 10 minutes post-application) will be used. In the model, the dependent variable used will be the post treatment bacterial count ($\log_{10}$ CFU/cm^2) and predictors will be the treatment as a fixed effect and the Baseline as a covariate. The ATE corrected for Baseline will be estimated from the model and compared to non-inferiority criteria.

9.4 Safety Analysis

All treated subjects will be considered evaluable for safety. Skin irritation scores will be reported for any subject who is scored with a 1 or more at any observation (baseline treatment day, post-application/prior to 30 seconds and 10 minutes sampling procedures), in any category for any site.

Adverse events (including post treatment skin irritation scores of 3), will also be summarized. Summary tables will present incidence rates of adverse events by treatment group for all subjects who enter the treatment period. Listings of adverse events will be provided.

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

Missing microbiological data at 30 seconds, or 10 minutes, such as due to laboratory error or subject lost to follow-up, will be reported as missing and will not be imputed. Inclusion of these subjects in the per-protocol data set will be based on the criteria defined above. Details of any missing data and rationale for inclusion/exclusion in the per-protocol data set will be described in the study report.

10 PI Responsibilities

The PI is responsible for ensuring that this clinical investigation is conducted according to this protocol; protecting the rights, safety, and welfare of subjects; and controlling the study products under investigation. The PI has overall responsibility for the technical conduct of the study, as well as for the interpretation, analysis, documentation and reporting of results, and represents the single point of study control.

11 Monitoring

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

- Periodic on-site review
- Telephone communications and e-mail
- Review of CRFs and source documents

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

12 Quality Control and Quality Assurance

PDI, and the PI are responsible for implementing and maintaining Quality Assurance and Quality Control systems through written 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 14 of this protocol. Study monitoring may be carried out to accomplish this.

13 Audits and Inspections

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

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

14 Ethical and Regulatory Standards

The study will be conducted in compliance with this protocol, the regulatory guidelines of the US FDA regulations, 21 CFR 50 (Protection of Human Subjects), 56 (IRB), the ethical principles of the Declaration of Helsinki, the ICH-GCP Guidelines as currently amended, and all applicable SOPs of ER

The study will start only after approval of the protocol and ICF including Subjects instruction and Subjects calendar by the IRB/IEC. The approval letter or notice must contain the IRB name and identification number, meeting date, and sufficient information to identify the protocol and ICF form by name and number that were reviewed. PDI prior to study initiation, must receive a copy of the IRB/IEC approval letter.

The IRB used for this study will be:

Name: Advarra, Inc

Address: 6940 Columbia Gateway Drive, Suite 110, Columbia, MD 21046, USA

The IEC used for this study will be:

Name: Institutional ethics committee

Address: 64-66, Marasesti Boulevard, 040256 - Bucharest, Romania

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

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

PDI will maintain the security and confidentiality of all clinical study data received.

PDI clinical study databases will not be shared with any third party without the express written consent of the PI and/or Research facility.

15 Data Handling and Record Keeping

15.1 Completion of Case Report Forms

The PI or sub-investigator will review all CRF entries for completeness and accuracy. 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).

The PI or sub-investigator must review and sign each CRF in a timely fashion following completion and make them available to the PDI study monitor for monitoring. Before acceptance, the monitor will review the CRFs to ensure accuracy and completeness. The original CRFs will be kept at the Research facility and electronic/scanned copies will be sent to PDI In addition, any data queries prepared after the original CRFs have been completed must be answered promptly.

15.2 Final Clinical Study Report

Final Clinical Study report will be produced by Research facility in collaboration with PDI team.

15.3 Records, Reports and Retention Requirements

The PI will maintain study records for a minimum of 10 years following completion of the study. Sponsor will be notified in written regarding the due date of the record retention time.

Records that must be maintained by the PI include, but are not restricted to:

- Signed study protocol, amendments, deviations
- IRB/IEC approval of protocol, ICF including Subjects instruction and Subjects
- Applications to the IRB/IEC
- Signed ICFs
- CRFs
- Adverse event reports
- Records of receipt, use or disposition of the study material
- Correspondence relating to the study
- Investigator Final Report
- Sponsor Final Report (if provided)

No formal presentation or publication of data from this study can be initiated without the sponsor's explicit written agreement and in direct collaboration with the sponsor.

16 References

1. ASTM E 1874-14, Standard Test Method for Recovery of Microorganisms from Skin using the Cup Scrub Technique, ASTM International, West Conshohocken, PA. 2014, www.astm.org
2. ASTM E1173-15, Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations, ASTM International, West Conshohocken, PA, 2015, www.astm.org
3. Butterfield, C.T. The Selection of a Dilution Water for Bacteriological Examinations. J. Bacteriol. 23: 355-368, 1931.
4. Food and Drug Administration (FDA). Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Final Rule. December 2017. Accessed at https://www.gpo.gov/fdsys/pkg/FR-2017-12-20/pdf/2017-27317.pdf
5. Williamson, P., Kligman, A.M. A New Method for the Quantitative Investigation of Cutaneous Bacteria. J. Invest. Dermatol. 45:498-503, 1965.
6. Urbaniak, G. C., & Plous, S. (2013). Research Randomizer (Version 4.0) [Computer software]. Available from http://www.randomizer.org/

17 Appendices

17.1 Study Summary

Study Procedures	Washout phase	Treatment Day
Initiation of consenting process	x	
Review study ICF	x	
Review Inclusion/ Exclusion Criteria	x	x
Review subject's instructions	x	
Subject signs ICF	x	
Visual skin assessment (groin region) and Inclusion/ Exclusion Criteria	x	x
Dispense subject kits	x	
Schedule for clipping/re-clipping, if needed, approximately 48-96 hours prior to treatment visit	x	
No bathing / showering 72 hours prior to treatment visit	x	
Mark test area, Collect baseline samples (treatment)		x
Apply test products		x
Visual skin assessment, within 30 seconds post-prep sample		x
Visual skin assessment, 10 minutes post-prep sample		x
Count Baseline (treatment) plates, determine qualification		x

17.2 Groin Diagram

17.3 Randomization Scheme Example

The randomization will be provided in a separate document.

Randomization number	Body Area	Product Left Side	Product Right Side	Site 1	Site 2	Site 3

17.4 Subject Instructions

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

- Use only the soap provided for all bathing, sponge bathing and hand washing.
- Use only the shampoo provided when washing your hair.
- Do not use antiperspirants or deodorants (other than those provided to you in the kit), lotions, colognes, perfumes, after shaves or powders.
- Do not come in contact with solvents, acids, bases, fabric softener-treated clothing or other household chemicals in the groin and upper thigh areas.
- Do not swim in chemically treated pools or bathe in hot tubs, whirlpools or spas.
- Do not use tanning beds.
- Do not shave, use depilatories or hot waxes on the groin or upper thigh areas. If hair is present, allow study staff to clip hair at a designated time.
- Do not apply any medicated creams or ointments to any area of your skin, nor should you take any antibiotics. If antibiotics are necessary due to illness, please report this to Chitoiu Elena, Resident Dermatologist or Monica Grigore, Dermatologist at Phone: +40213357090 (work), or Mobile: +40721322073 (home) immediately.

<u>Additional Instructions for Treatment Day Visit</u> **dd/mm/yyyy** **_ _ / _ _ / _ _**

- Do not bathe or shower in the 72-hour period before your scheduled appointment and for the rest of the study. A sponge bath may be taken, but avoid the areas of the lower groin, and/or upper thigh.
- You may be required to return to the Research facility at least approximately 48-96 hours before your Treatment Day Visit for hair clipping.
- On the Treatment Day Visit, you will return to the Research facility for treatment and the initial sampling.

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

CHITOIU ELENA, Resident Dermatologist / MONICA GRIGORE, Resident Dermatologist

Phone: +40213357090 (work)
Mobile: +40721322073 (home)

17.5 Treatment Application Instructions

Investigational Product: Treatment A (Project X 26 mL)

Treatment Site Application Instructions – Groin

1. Weigh the applicator package and record the weight
2. Peel open package and remove applicator – do not touch swab.
3. Hold the applicator with swab down.
4. Press down the antiseptic reservoir to release the antiseptic to the swab.
5. Wet the swab by repeated blotting against the treatment area until antiseptic is visible on the skin.
6. Thoroughly wet the treatment area with antiseptic using gentle back-and-forth strokes for two minutes.
7. Weigh the applicator package and record the weight
8. Allow antiseptic to completely dry (minimum of 3 minutes).
9. Do not blot or wipe dry.

Investigational Product: Treatment B (Project X 10.5 ml)

Treatment Site Application Instructions – Groin

1. Weigh the applicator package and record the weight
2. Peel open package and remove applicator – do not touch swab.
3. Hold the applicator with swab down.
4. Press down the antiseptic reservoir to release the antiseptic to the swab.
5. Wet the swab by repeated blotting against the treatment area until antiseptic is visible on the skin.
6. Thoroughly wet the treatment area with antiseptic using gentle back-and-forth strokes for two minutes
7. Weigh the applicator package and record the weight
8. Allow antiseptic to completely dry (minimum of 3 minutes).
9. Do not blot or wipe dry.

Investigational Product: Treatment C (Project X 5.1 ml)

Treatment Site Application Instructions – Groin

1. Weigh the applicator package and record the weight
2. Peel open package and remove applicator – do not touch swab.
3. Hold the applicator with swab down.
4. Press down the antiseptic reservoir to release the antiseptic to the swab.
5. Wet the swab by repeated blotting against the treatment area until antiseptic is visible on the skin.
6. Thoroughly wet the treatment area with antiseptic using gentle back-and-forth strokes for two minutes.
7. Weigh the applicator package and record the weight
8. Allow antiseptic to completely dry (Minimum of 3 minutes).
9. Do not blot or wipe dry.

Positive Control: Treatment D (Prevantics® Maxi Swabstick)

Treatment Site Application Instructions – Groin

1. Weigh the swabstick package and record the weight
2. Peel open package and remove swabstick – do not touch swab.
3. Place one flat side of the foam tip within the pre-measured treatment area, and prep with back-and-forth repeated strokes for 60 seconds.
4. Turn the Maxi-swabstick over and repeat the procedure for 60 seconds
5. Do not apply swab beyond borders of marked skin areas by more than one swab width.
6. Weigh the swabstick package and record the weight
7. Allow the area to completely air-dry; approximately 90 seconds.
8. Do not blot or wipe dry.

17.6 Skin Irritation Rating Scale

Skin Irritation Scoring System		
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*	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*	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*	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*	Severe dryness extending over most of all of the product-exposed area

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

17.7 Confirmation of Release and Receipt of Study Materials Form

CONFIRMATION OF RELEASE AND RECEIPT OF STUDY MATERIALS

Investigator: Rozalia Olsavszky M.D.

Study No: ER 19/232

Research facility: EUROFINS EVIC PRODUCT TESTING ROMANIA S.R.L.

Quantity (Units)	Description	ID/Lot Number	Expiration Date

Supplies Released to Research facility by: _____

Sponsor Signature

Supplies Sent to Research facility (Date):_____

Supplies Checked and Verified by:_____

Signature Date

_____

Print Name, Title

Once the supplies have been verified and this form is signed / dated, a signature copy will be sent to the sponsor representative: James.Clayton@pdipdi.com.

17.8 Study Material Disposition Form

Use one form for each study material.

Study Number: ER 19/232	
Principal Investigator: Rozalia Olsavszky M.D.	**Research facility:** EUROFINS EVIC PRODUCT TESTING ROMANIA S.R.L.

Study Material ID	Date Received	Quantity Received	Lot Number/ Serial Number	Expiration date

Date Dispensed/ Distributed	Subject Number	Quantity Dispensed	Quantity Remaining