STATISTICAL ANALYSIS PLAN

Assessment of the Antimicrobial Efficacy of 3M™ SoluPrep™ Preoperative Skin Preparation against Resident Human Skin Flora on the Inguinal Region

Clinical Study Number: EM-05-014624
Microbac

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1. INTRODUCTION

The text in the Statistical Analysis Plan (SAP) reflects the analysis methods planned as of the date of the SAP. It contains more details for the analyses mentioned in the Clinical Investigational Plan (CIP) and may be amended to reflect any additional analyses planned prior to unblinding the data.

2. STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to evaluate the antimicrobial efficacy of 3M™ SoluPrep™ Preoperative Skin Preparation (SoluPrep) by demonstrating non-inferiority to 3M™ SoluPrep™ Film-Forming Sterile Surgical Solution (SoluPrep FF), an FDA-approved active control, with a 0.5 margin (log_{10} scale), and demonstrating a superiority to saline, the negative control, with a superiority margin of 1.2 log_{10}/cm^2. In addition, both active products should demonstrate persistence of effect where the 6-hour post-treatment measurement is lower than or equal to the observed study day baseline measurement for 100% of the subjects.

2.2 Secondary Objectives

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

3. INVESTIGATIONAL PLAN

3.1 Study Design

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

3.2 Randomization

The left and right test areas on the subject's inguinal region will be assigned according to a computer-generated randomization schedule where each will receive 1 of the 3 study products (see Table 1). Treatment randomization will be balanced between left and right sides. There are 3 different possible combinations of treatments. These are shown below in Table 2.

To provide enough subjects to meet study objectives, the treatment combinations will be randomized in a 6:1:1 ratio for combinations 1 through 3, respectively, at the single recruiting clinical facility. The details of block size will be documented in the final study report.

Baseline and post-prep samplings at 10 minutes and 6 hours will be randomized to three sites within each test area. The same test site randomization will be used on the left and right sides of a subject's body to reduce the possibility of sampling errors.

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

Table 1 Study Products

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

Table 2 Subject Treatments

Treatment Combination	Left and Right Treatment
1	SoluPrep 10.5-mL and SoluPrep FF 10.5-mL (SP + SPFF)
2	SoluPrep 10.5-mL and saline (SP + S)
3	SoluPrep FF 10.5-mL and saline (SPFF + S)

Randomization Plan:

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

The Data Manager will strip the treatment assignment from the programs that are used to download the data from Clindex®. This will keep the statistician assigned to the study blinded until data lock.

Randomization Process:

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

- Retrieve a randomization envelope with smallest available randomization envelop number.
- Record randomization number onto a Data Collection Sheet (DCS) and/or the electronic Case Report Form (eCRF).
- When the treatments are applied, record onto a DCS and/or the eCRF; left side treatment applied, right side treatment applied, baseline sample site, 10-minute sample site and 6-hour sample site.
- Subjects will continue to be randomized until a minimum of 100 observations (with a baseline that meets the Treatment Day requirement) from each of the active treatments and 30 observations from the saline control (with a baseline that meets the Treatment Day requirement) have been collected.

3.3 Blinding

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

The biostatistician will remain blinded to treatment assignment before database lock and final study data analysis. The treatment assignments will be removed from the relevant databases and random treatment assignments given to each subject. The final data review

and data classification meeting will be conducted before data lock and statistical programming will occur using the dummy treatment assignments.

4. EFFICACY ENDPOINTS

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

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

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

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

5. ANALYSIS POPULATIONS

5.1 Safety Population

The intent-to-treat (ITT) data set will be the primary data set for safety and will include all subjects randomized to a treatment group. Subjects will be analyzed in the group to

which they were randomly assigned.

5.2 Modified Intent-To-Treat Population (MITT)

A MITT data set will be used for primary efficacy analyses. All subjects who meet baseline requirements on a bilateral side will be included in the analysis for that bilateral side. However, the primary analyses involve a linear regression at the 10-minute post-treatment time point and calculation of return to baseline at 6 hours. These analyses require both baseline and 10-minute recovery for treatment effect or baseline and 6-hour recovery data for return to baseline percentage calculation. There is no scientific basis for imputation of these missing values. Fortunately, missing values are not expected. There was no missing data for the 2346 microbiological samples taken in the pivotal study conducted at this site for SoluPrep FF. The exclusion of subjects who do not meet baseline requirements and who are missing recovery data at either the 10-minute sampling time point or the 6-hour sampling time point (post-treatment) times makes this data set a modified intent-to-treat data set.

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

5.3 Per-Protocol Population

A second per-protocol (PP) data set will be defined excluding any subjects with major deviations including lack of compliance

A treatment side will be excluded from the modified intent-to-treat population that has one of the following:

- A treatment side with a compromised dressing at 6 hours
- A subject who was not compliant to study requirements during the time between the 10 minute and 6-hour sample collection

A treatment side might also be excluded for scrub time or sample times outside of the approved window.

All deviations not captured above that will result in exclusion from the PP analysis will be defined and justified in a blinded fashion. In addition, documentation will be made of these data classification decisions prior to data lock.

6. GENERAL STATISTICAL CONSIDERATIONS

All statistical analyses will be performed using SAS version 9.4 or above (SAS Institute, Cary, NC). Any changes to the methods proposed in this SAP will be documented in the "Changes to Planned Analyses" section of the final clinical investigation report.

Reporting Precision:

- Age will be reported as whole years.
- Log_{10} CFU/cm^2 and reduction log_{10} will be reported to 2 decimal places.
- Means and medians will be presented to one more degree of precision than the raw data; except when specified otherwise.
- Standard deviations and standard errors will be presented to two more degrees of precision than the raw data except when otherwise specified.
- For log recovery and log reduction, means and medians will be reported to 2 decimal places and the standard deviation to 3 decimal places.
- Minimums and maximums will be presented to the same degree of precision as the raw data.
- Confidence limit boundaries will be presented to one more degree of precision than the raw data.
- P-values will be rounded to 3 decimal places and presented as 0.xxx. P-values smaller than 0.001 will be presented as "<0.001." P-values greater than 0.999 will be presented as ">.999".

- Fractional numeric values will be presented with a zero to the left of the decimal point (for example, 0.12 – 0.30).
- Percentages (such as return to baseline percentage) will be presented to one decimal place.

Other Data Handling Issues:

- On treatment day, when a plate at the lowest dilution (highest concentration of bacteria) has no observed colonies, a value of "1" will be used, consistent with standard microbiology.

Process time limits:

- 10-minute post-prep sampling time (post-prep and 3-minute dry) 10 minutes $\pm$ 30 second.
- 6-hour post-prep sampling time (post-prep and 3-minute dry) 6 hours $\pm$ 30 minutes.
- The microbiological samples must be plated within 30 minutes of sample collection.

6.1 Interim Analysis

No interim analysis will be conducted.

6.2 Determination of Sample Size

The Final Monograph (21 CFR Part 310 Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use) calls for a minimum of 100 observations per treatment. A standard deviation of 1.0 $\log_{10}/cm^2$ was observed in study EM-05-012760. One-hundred subjects per treatment arm

provides 94% power using a non-inferiority margin of 0.5 log_{10}/cm^2 with a 1-sided alpha of 0.025.

In the same study, a superiority to the negative control was observed to be approximately 2.5 log_{10}/cm^2, with a lower 95% confidence bound of 2.26 log_{10}/cm^2. A sample size of 30 for the negative control will ensure that the mean for log_{10}/cm^2 reduction of skin flora relative to Treatment Day baseline will be normally distributed. With 100 observations for the active products and 30 observations for the negative control, the mean observed difference must be greater than 1.62 log_{10}/cm^2 for the lower 95% confidence bound to be greater than 1.2 log_{10}/cm^2. At these sample sizes and the lower 95% confidence bound of 2.26 log_{10}/cm^2, there is 79% power to show superiority to the negative control with a 1-sided alpha of 0.025.

6.3 Handling of Dropouts and Missing Data

There will be no imputation of microbiological data because there is no scientific basis for such imputation.

6.4 Validation Plan

Programs were developed for the pivotal studies for SoluPrep FF. Programs were written by both 3M and the Contract Research Organization (CRO) hired by 3M, yielding identical results. The current study is very similar to those studies and the previously developed programs can be used with minor modifications. Validation of statistical programs and output will be through an independent review of the code and output by a statistician not assigned to this study.

7. SUMMARY OF STUDY POPULATION

7.1 Subject Disposition

Subject enrollment, including number of subjects screened, number of subjects randomized and the reasons for screen failures, will be summarized by treatment group and overall.

Subject disposition, including the number and percentage of subjects included in each analysis data set (ITT, MITT or PP), as well as reasons for exclusion from the PP data set, will be summarized by treatment group and overall.

Study completion status (completed the study or discontinued early from the study), as well as the primary reason for study discontinuation, will be summarized by treatment group and overall.

7.2 Protocol Deviations

Protocol deviations are any departure from the protocol. Protocol deviations that affect the evaluability of subjects will be reviewed during a blinded review process and documented before data lock and study analysis. Deviations will be summarized by categories, treatment group and overall. Listing of protocol deviations will also be provided.

7.3 Baseline and Demographic Characteristics

Descriptive summary statistics will be provided for demographic characteristics for each treatment group and overall. For continuous variables, the number of subjects, mean, standard deviation, median, minimum and maximum will be provided. For categorical variables, the number and percentage of subjects in each demographic category will be summarized.

7.4 Treatment Duration and Compliance

Since the treatments will be administered by study staff, subject compliance with respect to treatment compliance is not an issue in this study. Treatment duration is 6-hours, ± 30 minutes. Therefore, treatment exposure and compliance will not be summarized or listed.

The subjects may leave the facility after the 10-minute post treatment microbial samples are taken. They will be queried before the 6-hour sample is taken to determine if they have followed the instructions to avoid showering, tub-bathing, swimming, vigorous physical activity that may cause sweating, and any other activity that may compromise the integrity of the test sites.

8. EFFICACY ANALYSIS

8.1 Primary Efficacy Analysis

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

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

An example code for the analyses specified above is shown below:

```
Proc Mixed data=D_14624.LogMicro;
        Class Treatment;
```

```
        Where Timepoint=1 and Treatment in (1 2);
        Model LogRecovery=Treatment LogBaseline/ddfm=kr;
        Lsmeans Treatment/cl;
Run;
```

The percent of samples for which the 6-hours post treatment recovery is lower than the baseline recovery will be calculated by active product. Thus, if $\log_{10}$ reduction is >0 the sample has not returned to baseline. The Final Monograph requires that these percentages be 100.

An example code for percentage return to baseline is shown below:

```
Proc Format;
        value Return        -5-0='Returned to Baseline'
                             0.0001-8='Did not Return to Baseline';
run;

Proc Freq data=D_14624.LogMicro;
        Where Timepoint=2;
        Tables LogReduction*Treatment/nopercent norow;
        format LogReduction return.;
Run;
```

8.2 Secondary Efficacy Analysis

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

9. SAFETY ANALYSIS

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

9.1 Adverse Events

All adverse events (AEs) occurring after initiation of study treatment (treatment emergent AEs) will be summarized by severity, relatedness, overall and by treatment group. AEs occurring during the screening period will be summarized separately. All AEs will be classified using the MedDRA dictionary.

All verbatim descriptions will be listed for all AEs, along with information regarding onset, duration, severity, relationship to treatment, and action taken.

Serious adverse events will be summarized and presented according to nature, time to onset, duration, relationship to treatment and outcome.

A Fisher's Exact test will be carried out to compare each safety parameter between the treatment groups.

9.2 Other Safety Variables

Skin irritation scores will be summarized by treatment.

APPENDIX

LIST OF ABBREVIATIONS AND DEFINITIONS

AE	Adverse event
ATE	Average Treatment Effect
CFU	Colony forming units
CHG	Chlorhexidine gluconate
CRO	Contract Research Organization
DCS	Data Collection Sheet
eCRF	Electronic Case Report Form
EDC	Electronic data capture
FM	Final Monograph
IPA	Isopropyl alcohol
ITT	Intent to Treat
MITT	Modified Intent to Treat
PP	Per Protocol

Revision History

Revision	**Details:**
1	To establish statistical analysis plan before study enrollment.