MICROBIOTEST PROTOCOL

TEST FOR
PRE OPERATIVE SKIN PREPARATIONS

Prepared for
ENTURIA, INC.
11400 Tomahawk Creek Parkway, Suite 310
Leawood, Kansas 66211

August 24, 2007

MICROBIOTEST Protocol: 371.1a.08.24.07

MICROBIOTEST Project: 371-121

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1.0 INTRODUCTION

Prior to surgery or other invasive procedures, skin must be treated with topical antimicrobial products (Pre-Operative Skin Preparations) to prevent nosocomial infections by reducing the number of microorganisms on the skin. The proposed Tentative Final Monograph (TFM) for *Health-Care Antiseptic Drug Products* (Vol. 59, No. 116, June 17, 1994) describes an *in vivo* procedure for evaluating this type of product as well as expected performance criteria. When a new product is tested a predicate preoperative skin preparation must be included in the study as a positive control.

2.0 OBJECTIVE

The purpose of this study is to compare the antimicrobial effectiveness potential of three investigational test products identified as "ChloraPrep Triple Swabsticks" using two application procedures (three swabsticks at once and the three swabsticks used sequentially). "Hibiclens" will be evaluated to serve as the reference product (positive control). "Sterile Triple Swabsticks Saturated with 5.25 mL of Sterile Deionized Water" ("Sterile Swabsticks with Sterile Deionized Water") using two application procedures (three swabsticks at once and the three swabsticks used sequentially) will be evaluated to serve as a negative control. This evaluation will be conducted based on methodology specified by the Food and Drug Administration Office of Drug Evaluation and Research.

3.0 SPONSOR

ENTURIA, INC.
11400 Tomahawk Creek Parkway, Suite 310
Leawood, Kansas 66211

REPRESENTATIVE: Signature 6 SEP 07 Date

4.0 INVESTIGATIVE ORGANIZATION AND PERSONNEL

MICROBIOTEST
105 Carpenter Drive
Sterling, VA 20164

Contact: M. Hamid Bashir, MD CCRP
Phone: 703-925-0100
Fax: 703-925-9366

4.1 NAME OF THE IRB: MICROBIOTEST INTERNAL IRB

5.0 CLINICAL RESEARCH STANDARDS

The clinical investigation, including the informed consent, will be reviewed by the Institutional Review Board in accordance with Title 21 of the Code of Federal Regulations, Parts 50 and 56. Written approval by the Board must be obtained prior to the initiation of the study. The study will be conducted in accordance with the Good Clinical Practice Regulations, the Standard Operating Procedures of MICROBIOTEST, the study protocol and any protocol amendments.

6.0 SCOPE

This evaluation will measure the antimicrobial effectiveness of a preoperative skin product as specified by the Food and Drug Administration Tentative Final Monograph (TFM) for *Effectiveness Testing of a Patient Preoperative Skin Preparation* (FR 59:116, 17 June 94, pp. 31450-31452).

This study will evaluate the antimicrobial effectiveness of the three test and control articles: "ChloraPrep Triple Swabsticks" using two application procedures (three swabsticks at once and the three swabsticks used sequentially), "Hibiclens", and "Sterile Swabsticks with Sterile Deionized Water" using two application procedures (three swabsticks at once and the three swabsticks used sequentially). A minimum of 278 human subjects will be employed utilizing bilateral product applications assuring that the each test and control articles will be evaluated on sites as described in the following table.

Test Articles (Application)	Number of Evaluations on Abdomen	Number of Evaluations on Groin
ChloraPrep Triple Swabsticks (three swabsticks applied all at once)	111	111
ChloraPrep Triple Swabsticks (three swabsticks applied sequentially)	111	111
Hibiclens (applied according to package directions)	111	111
Sterile Swabsticks with Sterile Deionized Water (three swabsticks applied all at once)	111	111
Sterile Swabsticks with Sterile Deionized Water (three swabsticks applied sequentially)	111	111

Each of the test articles will be evaluated at the groin and the abdominal sites for immediate antimicrobial effect by sampling at ten minutes ± thirty seconds and six hours ± thirty minutes after completion of application. All product applications will be performed using randomized placement.

7.0 TEST ARTICLES

7.1 Test Product:

7.1.1 ChloraPrep Triple Swabsticks (2% Chlorhexidine gluconate (CHG) and 70% isopropyl alcohol).

7.2 Reference Product (Positive control):

7.2.1 Hibiclens (4% CHG).

7.3 Negative control:

7.3.1 Sterile Swabsticks with Sterile Deionized Water.

8.0 LABORATORY SUPPLIES AND EQUIPMENT (PROVIDED BY MICROBIOTEST)

8.1 Equipment
8.1.1 Colony counter.
8.1.2 Incubator - capable of maintaining a temperature of 30 ± 2C may be used.
8.1.3 Sterilizer - gravity steam sterilizer capable of producing the conditions of sterilization.
8.1.4 Stop-clock - One that can be read for hours and minutes.
8.1.5 Water bath - A bath of appropriate size and capable of maintaining temperature ±2C.
8.1.6 Bacteriological pipettes - Sterile pipettes of suitable size.
8.1.7 Dilution bottles - A container that can be sterilized, having a 150 to 200 mL capacity and a tight closure may be used.
8.1.8 Test tubes and closures - Sterile and of suitable size.
8.1.9 Vortex mixer
8.1.10 Sterile hollow stainless steel cylinders 2.2 cm in diameter and approximately 2.54 cm in height.
8.1.11 Sterile rubber policeman.
8.1.12 Sterile polystyrene Petri dishes, approximately 100 mm x 15 mm.
8.1.13 Occlusive sterile catheter dressing – Opsite, or suitable alternative.

8.2 Reagents and Media
8.2.1 Kit products for Washout Period: non-antimicrobial bar soap, shampoo, and deodorant.
8.2.2 Scrub solution – [redacted]

8.2.2.1 Dilution fluid – Butterfield's Phosphate Buffered water containing 1.25 mL AOAC Phosphate Buffer Stock in one liter purified water containing appropriate neutralizers: 10mL of Polysorbate, 3 G of lecithin and 10 G of Tamol. The dilution fluid will have a final pH of 7.2±0.1.
8.2.3 Plating medium - Soybean-Casein Digest Agar (or equivalent) containing 0.5% Polysorbate 80 and 0.07% lecithin to stimulate growth of lipophilic organisms.
8.2.4 Plating medium - Soybean-Casein Digest Agar (or equivalent)

9.0 NEUTRALIZATION

The objective of this evaluation is to determine the ability of the sampling solutions to completely neutralize the active ingredients contained in the test products and positive control when applied to the abdomen of test subjects without exhibiting toxicity to the marker organism. The test microorganism used for the neutralization study will be *Staphylococcus epidermidis*, ATCC 12228, a common skin bacterium. The procedure to be used is shown in Appendix 2.

10.0 SUBJECT SELECTION

10.1 Number of Subjects

The requirement of 111 subjects per test article configuration (test arm) to finish this study was determined by the sample size equation at the recommendation of both the FDA Proposed Rule and ASTM E1173; the following formula was used for group size calculations:

$$n \geq s^2 \frac{(Z_{\alpha/2} + Z_{\beta})^2}{\Delta^2}$$

Where:

- n = minimum number of required samples in each test arm (rounded up).
- s = standard deviation of the acquired data (0.735).
- $Z_{\alpha/2}$ = the Z statistic for a two-sided 95% confidence interval (1.96), where α (0.05) is the probability of allowing a Type I error (predicting significance when there is none).
- Z_{β} = the Z statistic for the power of the test (0.842), where β (0.80) is the power, i.e., the probability of allowing a Type II error (failing to predict significance when it exists).
- Δ = the difference being tested for (0.196).

The best reasonable estimate of the number of subjects needed per test article configuration (study arm), based on available current data is 111.

Since there are five study arms, the minimum number of subjects based on bilateral applications is 278 (Five arms x 111 / 2).

Initial screening baseline counts will be collected from a number of healthy subjects of at least the age of sixteen sufficient to provide at least 278 qualified subjects to enter the treatment phase of the study. (NOTE: As it is anticipated that not all subjects that have qualifying screening-day baseline counts will have qualifying test-day baseline counts, it may be necessary to treat more than 278 subjects.)

The eligibility of each subject will be determined from a medical history collected on the Screening Inclusion/Exclusion Form and Treatment Inclusion/Exclusion Form (to be provided prior to study initiation).

10.2 Criteria for Inclusion

Potential subjects may be included in this study if they meet the following requirements:

10.2.1 Males and/or females, of at least 16 years of age. Subjects less than 18 must have written custodial consent.

10.2.2 Are cooperative and willing to answer questionnaires and sign a consent form (to be provided prior to study initiation).

10.2.3 Are in general good health.

10.2.4 Have skin within 6 inches of the test sites that is free of tattoos, dermatoses, abrasions, cuts, lesions or other skin disorders.

10.3 Exclusions

Individuals cannot be enrolled in the study if they:

10.3.1 Have been exposed to topical or systemic antimicrobials during the two-week pretest-conditioning period. This restriction includes, but is not limited to, shampoos, lotions, soaps, body powders, and materials such as solvents, acids, or alkalis.

10.3.2 Have been medically diagnosed as having a medical condition, which would preclude participation such as: diabetes, hepatitis, an organ transplant, a medical surgical implant or an immune-compromised system.

10.3.3 Have any medical condition that in the opinion of the Investigator would preclude participation.

10.3.4 Have bathed in chemically treated pools or hot tubs two weeks prior to any microbial sampling.

10.3.5 Have used UV tanning lamps two weeks prior to any microbial sampling.

10.3.6 Have bathed or showered less than 48 hours prior to any microbial sampling.

10.3.7 Have a known sensitivity to CHG.

10.3.8 Have a known sensitivity to latex products.

10.3.9 Have a known sensitivity to fragrances.

10.3.10 Are pregnant or nursing.

10.3.11 Are not willing to fulfill the requirements of the protocol.

10.3.12 Subjects who have completed part or all of the study will not be reentered in the study.

11.0 SUBJECT WITHDRAWAL

After admission to the study, a subject may withdraw at any time for any reason, but must report such reason fairly and accurately.

12.0 PROCEDURES

12.1 Pre-Test Period

A period of at least two weeks prior to the first baseline sampling will be designated the "pre-test" period. During this time subjects will be instructed to use only personal hygiene products supplied by the Investigator for personal hygiene (soaps, shampoos, deodorants, etc.) and will be told to avoid skin contact with solvents, acids, and bases. Subjects will be prohibited from using UV tanning lamps, and from bathing in chemically treated pools and/or hot tubs. Additionally, subjects will not be allowed to bathe or shower forty-eight hours prior to being sampled. They will be allowed to take sponge baths; however, they must not disturb the test sites.

Subjects must not shave the anatomical sites within five days prior to being treated with the test products. This regimen will allow for the stabilization of the normal microbial flora of the skin.

12.2 Baseline Week

12.2.1 The week following the pre-test period will constitute the baseline week. Subjects will not shower within forty-eight hours of being sampled. All subjects will be sampled for screening baseline at least seventy-two hours prior to treatment with the Test Articles.

12.2.2 Samples taken from the two contralateral abdominal and groin sites will be collected. Based upon adequate screening baseline count on contralateral abdominal and groin sites, a subject will be eligible to continue the study. Criteria for acceptance of screening counts will be at least 1.0×10^5 CFU/cm^2 on contralateral sites in the groin region and 1.0×10^3 CFU/cm^2 on contralateral abdominal sites. A second and final baseline sample using the same criteria as for screening baseline will be collected at each test area prior to being prepped on the day of treatment.

Post-treatment count data will be excluded from the data analysis if they are derived from any area that fails to exhibit a level of bacteria at the second baseline sampling sufficient (1.0×10^5 CFU/cm^2 on contralateral sites in the groin region and 1.0×10^3 CFU/cm^2 on contralateral abdominal sites) to permit detection of a 3.0 $\log_{10}$ reduction in the groin region and a 2.0 $\log_{10}$ reduction in the abdominal sites.

The table below summarizes the minimum baseline criteria for each of the test sites along with the minimum effective $\log_{10}$ reduction criteria stipulated by the FDA.

Anatomical Site	Minimum Baseline	Minimum $\log_{10}$ Reduction
Abdomen	1.0×10^3 CFU/cm^2	2.0 $\log_{10}$ @ 10 minutes*
Groin	1.0×10^5 CFU/cm^2	3.0 $\log_{10}$ @ 10 minutes*

*Note: 6 hour samples may not exceed the test day baseline.

12.2.3 All sample sites requiring clipping will be clipped at least forty-eight hours prior to test day.

12.2.4 All sampling will be performed using the cylinder sampling technique (Section 12.4.1).

12.3 Test Period

12.3.1 Prior to being sampled, the subjects will be questioned regarding their adherence to protocol restrictions. Subjects will be visually examined again (by a technician) at the anatomical sampling sites to ensure no evidence of injury or dermatoses is present.

12.3.2 Randomization: According to a computer-generated randomization schedule, test areas on each subject's groin and abdominal regions will be delineated to receive one of the five test articles on each side. Randomization will be balanced between left and right sides. Treatment day baseline and post prep sampling sites will be randomized within each test area.

12.3.3 Blinding: The test material cannot be blinded from the investigator because of the obvious difference in applicator design and color. The investigator treating the subject will not be involved in bacterial enumeration and counting the plates of that particular subject.

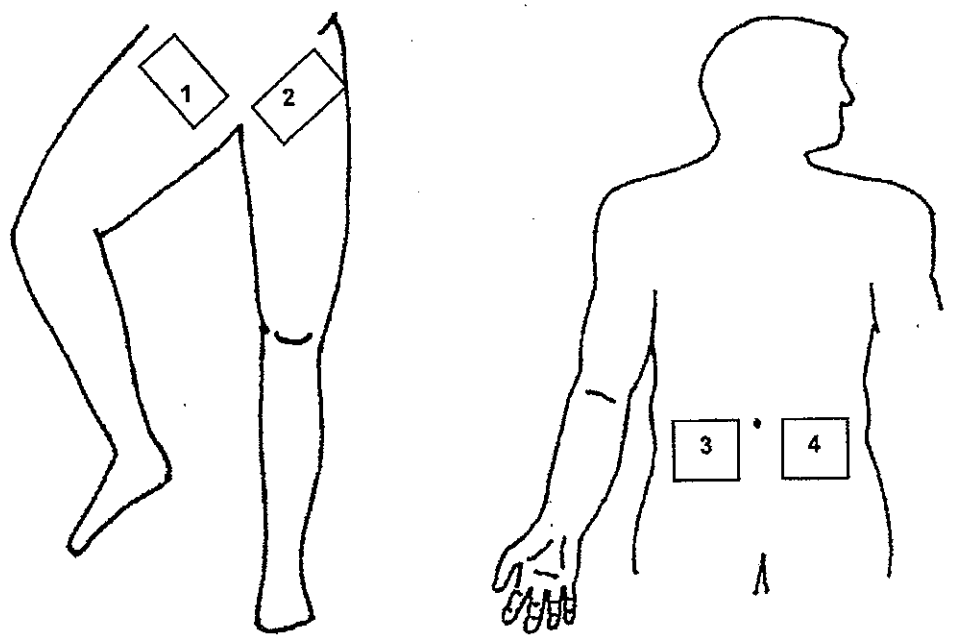
12.3.4 The dimensions of each treatment area will be 2" x 5" on the upper inner part of the thigh below the inguinal ligament. The dimension of each treatment area will be 5" x 5" on side of umbilicus on the abdomen. Four sampling sites will be delineated within each treatment area.

12.3.5 Based upon the design stipulated in the Section 6.0, the groin region and abdomen will be sampled for the final baseline (second baseline). The appropriate areas will be prepped using the test articles (Appendix 1). The sites will be sampled as stipulated in Section 6.0.

12.3.6 For the 6 hour ±30 minutes sites, sterile gauze pads will be placed over the prepped areas to aid prevention of microbial contamination. The gauze pads will be held in place with an occlusive sterile catheter dressing. All samples will be taken from sites using the cylinder sampling technique (Section 12.4.1).

NOTE: Timed sampling will begin when prepping is completed as defined in the instructions for prepping (See Appendix 1).

Diagrams of the treatment regions are provided below:



1-2: Groin (anterior) – 2" x 5" area; 3-4: Abdomen (anterior) – 5" x 5" area

12.4 Microbiological Methods

12.4.1 Sample collection

Quantitative cultures will be obtained by a modification of the cylinder sampling technique of Williamson and Kligman[1]. A sterile scrubbing cup (3.8 cm^2, internal area, height approximately 2.54 cm.) will be held firmly to the skin over the site to be sampled. Three mL of scrub solution containing neutralizers (Section 8.2.2) will be placed into the cup, and the area scrubbed with moderate pressure for one minute using a sterile rubber "policeman." The scrub solution will be aspirated and replaced with 3.0 mL of fresh solution and the scrub repeated. The two aliquots will be pooled. These procedures will be used for all baseline samples and treatment samples. Aliquots of the pooled scrub solutions will be diluted in 10-fold steps, using Butterfield's Phosphate Buffered Dilution water (Section 8.2.2.1) containing neutralizers as the diluent.

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1 Williamson P., Kligman P.M.: A New Method for the Quantitative Investigation of Cutaneous Bacteria. J. Invest. Dermatol. 45:498-503, 1965.

12.4.2 Enumeration of Microorganisms

One mL aliquots of appropriate dilutions will be plated in duplicate in 15-20 mL Soybean-Casein Digest Agar pour plates containing appropriate neutralizers within 30 minutes of the sampling.

After 72±4 hours aerobic incubation at 30±2C, colonies will be counted and viable cells in the original sample will be calculated based on the procedure *Counting Colonies on Plates and Reporting Results* (Section 6.2 K-6.2M), *Standard Methods For Evaluation of Dairy Products*, 14th Ed. American Public Health Association, Washington D.C. The plates may be stored under refrigeration for up to 48 hours prior to counting.

The average number of microorganisms recovered per cm^2 of skin will be determined and reported. To convert the volume of sample collected into CFU/cm^2 of skin, the following formula will be used:

$$R = \log_{10}\left[\frac{F\left(\frac{\Sigma c_i}{n}\right)10^{-D}}{A}\right]$$

Where:

R = the average CFU count in $\log_{10}$ scale per cm^2 of skin.

F = total mL of stripping fluid added to the sampling cylinder; F=6.0 mL

c_i/n =average of the duplicate colony counts used for each sample collected

D = Dilution factor of the plates counted

A = Inside area of the sampling cylinder (3.8 cm^2)

12.4.3 Growth Promotion Control

For each batch of plating medium, Soybean-Casein Digest Agar (or equivalent) containing 0.5% Polysorbate 80 and 0.07% lecithin, fewer than 100 CFU of *Staphylococcus epidermidis*, ATCC 12228 will be inoculated in a single plate pour plate. A 20-26 hours aged culture of *S. epidermidis* will be serially diluted in dilution fluid. The CFU added will be confirmed in duplicate spread plates. The plates will be incubated for 72±4 hours at 30±2C.

13.0 DATA RECORDING

The raw data will be recorded on data collection forms for each subject. The forms will include data for screening baselines, treatment day baselines, treatment application and sampling records, and data for all evaluated sites.

All data collection forms will be provided prior to study initiation.

14.0 ASSESSMENT OF SAFETY

14.1 Skin Irritation

The safety of each test product will be evaluated by observing irritation before samples are taken at baseline and at 10 minutes ± 30 seconds and 6 hours ± 30 minutes after completion of prep.

14.2 Adverse Experiences

14.2.1 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 article. All adverse event/experiences will be recorded and reported according to the Standard Operating Procedures of the laboratory.

All adverse events, regardless of severity or the causal/effect relationship, will be recorded. The severity of the effect will be noted as "*Mild,*" "*Moderate*," 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 article, the relationship will be described as "*None,*" "*Possible,*" "*Probable,*" or "*Definite*." The following definitions will be utilized:

None	No association to the test article. Related to other etiologies such as concomitant medications or conditions or subject's known clinical state.
Possible	Uncertain association. Other etiologies are also possible.
Probable	Clear-cut association with improvement upon withdrawal of the test article. Not reasonably explained by the subject's known clinical state but not an anticipated event.
Definite	An adverse event with a clear-cut temporal association and laboratory confirmation 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 article or the current investigator's brochure. Where test article labeling or investigator's brochure is not available, anticipated experiences will be based on the known pharmacological/toxicological properties of the test article or ingredients.

14.2.2 Follow-up

If an adverse event/experience occurs, the subject under the direction of the Investigator (or designee) 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 (Exhibit A).

14.2.3 Notification

The sponsor will be notified of all adverse event/experiences. Any Serious or Unexpected Adverse Drug Event/Experience that occurs during the study must be reported immediately by the investigator to the sponsor and the reviewing IRB, followed by written notification within 24 hours of the information being reported to the investigative study team.

14.2.4 Anticipated Reactions

The risks associated with this test are primarily related to application of the test article. Tape reactions are also possible. Mild to heavy erythema, swelling, itching, cracking, peeling, or in rare cases, blistering and or an allergic reaction might occur.

Occurrence of any of these events will be considered an "Adverse Event" and will be documented in the study record.

15.0 DATA AND STATISTICAL ANALYSIS

The analyses outlined below will be conducted on the data generated.

15.1 Raw data (CFU/mL) will be converted to Log_{10} CFU/cm^2. Counts of less than 1 CFU /cm^2 will be treated as 1 CFU/cm^2 such that the log transformation will be zero. Log reductions will be calculated by subtracting the post treatment log counts from the treatment day baseline counts obtained.

15.2 Only subjects who meet the minimum inclusion criteria for levels of bacteria on the treatment day of the study will be included in the analysis with the following exception: lab accidents resulting in contaminated samples. If there are missing data at some but not all time points, data from the available times will be included in the analysis.

15.3 Any subject with missing data will not be included in the data analysis. The subject with missing data will be replaced with a new subject.

15.4 Separate statistical analyses will be conducted for data collected from the abdomen and the groin. All hypothesis testing will be conducted at the alpha=0.05 level of significance. Data will be presented in tabular and graphical format.

15.4.1 Microbial Data

15.4.1.1 Source Data

The source data are the CFU counted for each subject at the screening baseline and treatment day baseline evaluations and at 10 minutes and 6 hours following treatment application. The data to be used in the statistical analyses will be the R values on the skin log_{10} of the CFU/cm^2 of skin on the abdomen and groin. Only baseline samples with 0 time CFU counts equal to or greater than 2.0 log_{10} for the abdomen and with 0 time CFU counts (baseline) equal to or greater than 3.0 log_{10} for the groin will be used to determine antimicrobial efficacy.

Data from the screening baseline and treatment day baseline evaluations in each area will be averaged to determine the combined screen and baseline count for that area. Log reductions will be calculated by subtracting the post treatment log count from the average combined screen and baseline log count for that area. Additionally, log reductions will be calculated using only the baseline data from the test day.

15.4.1.2 Analysis of Baseline Data

- The average combined screening baseline and treatment day baseline log_{10} CFU of the test products will be compared using analysis of variance techniques.
- The baseline log_{10} CFU from the test day will be compared using analysis of variance techniques.

15.4.1.3 Within-Treatment Analysis

- The average combined screening baseline and treatment day baseline log_{10} CFU will be compared to the log_{10} CFU at the 10-minute and 6-hour post treatment evaluations utilizing Student's t-test for paired data.
- The log_{10} CFU at the treatment day baseline will be compared to the log_{10} CFU at the 10-minute and 6-hour post treatment evaluations utilizing Student's t-test for paired data.

Summary statistics will consist of mean log_{10} CFU (± 95% CI) and mean log_{10} CFU differences (± 95% CI) expressed as geometric mean log_{10} reductions.

15.4.1.4 Between-Treatment Analysis

- Differences between the test products will be evaluated at the 10-minute and 6-hour post treatment evaluations using analysis of covariance techniques with the average combined screening baseline and treatment day baseline log_{10} CFU as the covariate.
- Differences between the test products will be evaluated at the 10-minute and 6-hour post treatment evaluations using analysis of covariance techniques with the treatment day baseline log_{10} CFU as the covariate.

15.4.2 Skin Irritation Data

15.4.2.1 Source Data

The source data are the erythema, edema, rash, and dryness scores assigned on the test day by the technician immediately before samples are taken at baseline and at 10 minutes and 6 hours after treatment application. The data to be used in the statistical analysis of irritation are the changes from baseline. These data will be sent to the microbiology laboratory site and separate analyses will be conducted for each irritation parameter.

15.4.2.2 Within-Treatment Analysis

Wilcoxon's Signed Rank Test will be used to evaluate changes from baseline at each post treatment evaluation.

15.4.2.3 Between-Treatment Analysis

The changes from baseline will be averaged across the 10-minute and 6-hour evaluation times. The Kruskal-Wallis test will be used to compare the irritation levels of the test products.

16.0 SPECIAL NOTES

16.1 Informed Consent

A written consent form from each subject will be obtained and filed by the Investigator with the subject's records, in accordance with 21 CFR 50 & 56.

16.2 Alteration of the Study

Neither the Investigators nor the Sponsor will modify or alter this protocol without first obtaining the concurrence of the other parties. Approval of the modification by the Investigators' IRB must be obtained before implementation, with two exceptions:

a. When necessary to eliminate an apparent immediate hazard to the subject.
b. When modification does not involve the subject's participation in the study.

17.0 REPORT

The final report will summarize the method, data and conclusions relative to the test materials and the subjects.

18.0 NOTICE

No amendments to the protocol will be permitted without approval from the Study Sponsor, the Investigators, and the Institutional Review Boards. Such changes will be documented in writing. Approval by the Board must be obtained prior to initiation of the amendment.

19.0 PROTOCOL APPROVAL

Submitted for: MICROBIOTEST

By: _____ 9/14/07 _____
M. Hamid Bashir, MD CCRP Date

Accepted for: ENTURIA, INC.

By: _____ 6 SEP 07
Date

EXHIBIT A

Adverse Drug Experience Record

Subject Initials ☐☐☐

Date Form Completed:

Mo. ☐☐ Day ☐☐ Yr. ☐☐☐☐

Screening ID ☐☐☐

Subject Number ☐☐☐

Date of Onset:

Mo. ☐☐ Day ☐☐ Yr.

NOTICE: Contact Sponsor Study Monitor ASAP if the adverse event is serious and unexpected.

*SEVERITY AT ONSET (Check one)	RELATIONSHIP to Investigational Material (Must check one large box in each section)		ACTION TAKEN (Check all that apply)	OUTCOME (Check one)
☐ Mild (subject is aware of signs, symptoms but these are easily tolerated) ☐ Moderate (signs, symptoms are sufficient to restrict but not prevent subject's daily activity) ☐ Severe (subject unable to perform usual activity)	☐ Probably Related (timely and causative relationship; no potential alternative etiology is apparent) ☐ Possibly Related ☐ Probably Not Related (Check one below) ☐ Present before study ☐ Symptoms of primary disease ☐ Concomitant Medications ☐ Intercurrent illness ☐ Intercurrent event ☐ Other (Comment below) ☐ Not Related	☐ Anticipated **(defined in protocol, Investigational Plan/Investigator's Brochure or Informed Consent Form)** ☐ Unexpected in (Check one below) ☐ Nature ☐ Severity ☐ Not Serious ☐ Serious (life threatening or results in death, permanently disabling, requires inpatient hospitalization, a congenital anomaly, cancer or overdose)	☐ None ☐ Withdrawn from Study ☐ Consulted Physician ☐ Procedure (Specify below) ☐ Hospitalized ☐ Study Drug Discontinued ☐ Medications ☐ OTC ☐ Prescription (Specify below) ☐ Other (Comment below)	☐ Recovered Date: Mo. ☐☐ Day ☐☐ Year ☐☐☐☐ ☐ Persistent Effect (Describe below) ☐ Death (Describe below)

Identify Study Drug involved in the Adverse Event:

☐ ☐

Describe the Adverse drug experience; provide chronology of events, actions taken and outcome. Use another form if needed.

***Did the adverse experience increase in severity?** ☐ Yes ☐ No

If yes, to what degree? ☐ Moderate ☐ Severe

Investigator Signature:	**Date:**

APPENDIX 1

TEST ARTICLE PREPPING TECHNIQUES

A. Test Product: ChloraPrep Triple Swabsticks

1. Application Configuration No. 1 (Three swabsticks applied all at once)

On Abdomen:

a. All three swabsticks will be applied to the surface of the treatment area with the flat side down.

b. Using all of the three swabsticks together, the solution will be applied within the designated treatment area using repeated back and forth applications of the sponge for 30 seconds so that a thin even coat is applied.

c. The area will be allowed to air-dry based on visual observations for approximately 30 seconds prior to the initiation of the contact times. The test article exhibit glistening wet shine after application and dry time will be documented, as wet shine will disappear leaving a faint visible film. The patient specific dry time observed will be documented and reported.

On Groin:

a. All three swabsticks will be applied to the surface of the treatment area with the flat side down.

b. Using all of the three swabsticks together, the solution will be applied within the designated treatment area using repeated back and forth applications of the sponge for two minutes so that a thin even coat is applied.

c. The area will be allowed to air-dry based on visual observations for approximately one minute prior to the initiation of the contact times. The test article exhibit glistening wet shine after application and dry time will be documented, as wet shine will disappear leaving a faint visible film. The patient specific dry time observed will be documented and reported.

APPENDIX 1 (continued)

TEST ARTICLE PREPPING TECHNIQUES (continued)

A. Test Product: ChloraPrep Triple Swabsticks (continued)

2. Application Configuration No. 2 (Three swabsticks applied sequentially)

On Abdomen:

a. Each swabstick will be applied to the surface of the treatment area with the flat side down.

b. Using one of the three swabsticks, the solution will be applied within the designated treatment area using repeated back and forth applications of the sponge for approximately 10 seconds so that a thin even coat is applied.

c. The same procedure will be repeated using the second and third swabstick for a total application time of approximately 30 seconds.

d. The area will be allowed to air-dry based on visual observations for approximately 30 seconds prior to the initiation of the contact times. The test article exhibit glistening wet shine after application and dry time will be documented, as wet shine will disappear leaving a faint visible film. The patient specific dry time observed will be documented and reported.

On Groin:

a. Each swabstick will be applied to the surface of the treatment area with the flat side down.

b. Using one of the three swabsticks, the solution will be applied within the designated treatment area using repeated back and forth applications of the sponge for approximately 40 seconds so that a thin even coat is applied.

c. The same procedure will be repeated using the second and third swabstick for a total application time of approximately two minutes.

d. The area will be allowed to air-dry based on visual observations for approximately one minute prior to the initiation of the contact times. The test article exhibit glistening wet shine after application and dry time will be documented, as wet shine will disappear leaving a faint visible film. The specific dry time observed will be documented and reported.

APPENDIX 1 (continued)

TEST ARTICLE PREPPING TECHNIQUES (continued)

B. Reference Control: Hibiclens

On Abdomen and Groin

1. Based on the manufacturer's instructions, five mL of the reference product will be applied onto a sterile gauze pad.
2. The product will be applied to the treatment area using the same area used for the test product for two minutes. The area will be dried with a sterile towel or sterile gauze.
3. Steps 1-2 will be repeated.
4. Contact times will begin after the site has dried a second time.

C. Negative Control: Sterile Swabsticks with Sterile Deionized Water

For both application configurations (No. 1 and No. 2 outlined in Part A), three sterile swabsticks will be placed into a sterile tube containing 5.25 mL of sterile deionized water for 10 seconds before application.

On Abdomen and Groin:
The application procedures and application variations will be the same as used for ChloraPrep Triple Swabsticks (Part A).

APPENDIX 2

NEUTRALIZER VALIDATION PROCEDURE

1.0 OBJECTIVE

The objective of this evaluation is to determine the ability of the sampling solutions to completely neutralize the active ingredients contained in the test and positive control products applied to the abdomen of test subjects without exhibiting toxicity to the marker organism.

2.0 SUBJECT ENTRY CRITERIA

Six subjects will participate in this study to yield six readings per test article, including both application configurations for the ChloraPrep Triple Swabsticks whereas three sites will be delineated on the abdomen, two for the ChloraPrep Triple Swabsticks and one for Hibiclens. Subjects must meet the inclusion and exclusion criteria in Sections 10.0 of the protocol to which this neutralizer validation is to be performed. The neutralization subjects do not require a minimum bacterial count, but they still need to avoid topical and systemic antimicrobials for 14 days prior to the treatment day. Each subject will receive both of the test articles, one product per side.

3.0 TEST ARTICLES

3.1 ChloraPrep Triple Swabsticks
3.2 Hibiclens

4.0 TEST ORGANISM

Staphylococcus epidermidis ATCC 12228. The test organism from the frozen stock culture will be transferred into 10 mL of Tryptic Soy Broth and incubated at 30±2 C. After 24 hours of incubation this culture will be diluted into ten fold dilutions using Butterfield's Phosphate Buffered Dilution water to achieve an appropriate concentration for inoculation of the test samples.

5.0 PROCEDURE [NOTE: All testing described in this section will be performed in triplicate.]

5.1 Serially dilute the overnight suspension with Butterfield's phosphate buffered water (PBW) to achieve an appropriate concentration for inoculation of the test samples.

Note: The density of the test inoculum must be verified by direct plating at beginning and end of this evaluation.

5.2 Neutralization Effectiveness Control: Samples will be taken from the abdomen with scrub solution containing neutralizers. The subject number, location of the prep application, location of the sites sampled within the prep area, and the time of sample collection will be documented on the CRF.

5.2.1 Mark the abdominal test areas using a sterile 2" x 5" template (three sites total).

5.2.2 After the test areas are marked, prep each site with three 70% isopropyl alcohol swabs for a total of one minute and wait site to dry.

5.2.3 Prep the each site with one of the two test articles according to randomization scheme per Appendix 1 (whereas two sites will be used for ChloraPrep Triple Swabsticks, each of which will be used for one of the two application configurations).

5.2.4 Using the cup scrub technique, at 10 minute post prep collect the sample using scrub solution containing neutralizers.

5.2.5 Each pooled sample (approximately 6 mL) will be mixed on a Vortex-type mixer and immediately inoculate this tube with approximately 30-100 CFU per mL.

5.2.6 Immediately (< one minute) and at 30 minutes (± 2 minutes) post-inoculation, pour-plate duplicate 1.0 mL aliquots of the inoculated sample using TSA containing neutralizers. Incubate the plates at 30±2C for 72±4 hours.

5.3 Number Controls: Add diluted inoculum into a tube containing 6.0 mL of sampling solution without neutralizers yield final inoculum concentrations of ~30-100 CFU/mL. Plate duplicate 1.0 mL aliquots immediately (< 1 minute) and 30 minutes (±2 minutes) post inoculation in the same manner as 5.2.6 above except using TSA without neutralizers.

5.4 Toxicity Control: Add diluted inoculum into a tube containing 6.0 mL of sampling solution containing neutralizers yield final inoculum concentrations of ~30-100CFU/mL. Plate duplicate 1.0 mL aliquots immediately (< 1 minute) and 30 minutes (±2 minutes) post inoculation in the same manner as 5.2.6 above.

These controls will provide assurance that the test organism is not adversely affected by the sampling solutions or procedure.

5.5 Enumerate plates and calculate CFU/mL for each sample

5.6 Convert data to Log_{10} CFU/mL.

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

Neutralizer Effectiveness: If the Log_{10} CFU/mL of the antiseptic sample is not more than 0.3 logs less than the Numbers Control, the neutralizer will be considered effective.

Neutralizer Toxicity: If the Toxicity Control (TC) is not more than 0.3 logs less than the Numbers Control sample, the sampling solutions will be considered non-toxic.