

HTR Study No.: 01-109127-11

## APPENDIX I

Total number of pages = 12

**IRB Approval Letter, Approved Consent Forms  
And Subject Instructions**

**INSTITUTIONAL REVIEW BOARD  
OF  
HILL TOP RESEARCH, INC.**

*Nancy J. Pelc, M.D., Chairman*

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July 31, 2001

Gayle K. Mulberry, M.S.  
Hill Top Research, Inc.  
Main and Mill Streets  
Miamiville, OH 45147

Ref: 01-109127-11  
Title: Efficacy Evaluation of Health Care Personnel Handwash Products  
Protocol Date: July 25, 2001  
Sponsor: Ciba Specialty Chemicals Corporaton

Dear Mr. Mulberry:

The Institutional Review Board of Hill Top Research, Inc. has reviewed and approved the above referenced study by the expedited review procedure. Documents included in this review were: protocol, consent forms, subject instructions, safety information and Form FDA 1572. Approval of this study has been granted for one year from the date of this letter.

Please remember that the FDA requires you to receive approval from the IRB for any amendments or changes in the protocol or consent form and for any new advertisements. Serious and unexpected adverse experiences and unanticipated problems involving risk to subjects must be reported promptly to the IRB. Progress reports on the research activity are to be submitted every six months.

The Institutional Review Board of Hill Top Research, Inc. is a duly constituted institutional review board under CFR, Title 21, Parts 50 and 56.

Sincerely,



Nancy J. Pelc, M.D.  
Chairman

7-31-01

Date

NJP/sll

Institution: Hill Top Research, Inc.  
Investigator: Gayle K. Mulberry, M.S.  
Study Title: "Efficacy Evaluation of Health Care Personnel Handwash Products"

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Page No. I-2

### **CONSENT FORM**

**INTRODUCTION:** You are being asked to take part in a research study. Before you give your consent to be a subject, it is important that you take enough time to read and understand what your participation would involve. In preparing this consent form, it has been necessary to use some technical language. Please ask questions if there is anything you do not understand.

You will be given a signed copy of this consent form and any other necessary written information prior to the start of the study.

**PURPOSE:** The purpose of this research study is to measure the ability of two liquid soap products to reduce the number of bacteria on the hands after repetitive use. Approximately ninety (90) people between and including the ages of 18 – 65 will be screened as potential subjects in this study. Forty-five (45) subjects are expected to complete the three-visit study.

**TEST ARTICLES:** One of the test articles is an experimental liquid soap product. The other test article is a marketed antibacterial liquid soap product. One product will be randomly assigned to each participating subject. Two of every three subjects will receive the experimental product.

**STUDY PROCEDURES:** Prior to enrollment in the test, you will be asked to complete a brief medical history questionnaire. It is possible that you may not be able to participate based on your answers to these questions. If you qualify, you will be given a kit containing non-antibacterial bar soap, shampoo, Ban® antiperspirant/deodorant, and rubber and poly gloves to be used at least one week prior to the start of the actual study. You will be given written instructions on how to use the kit.

After at least one week, you will be required to return to the lab. You will be asked to complete another brief medical history questionnaire. It is possible that you may not be able to participate based on your answers to these questions or the condition of the skin on your hands and wrists. If you qualify, you will wash your hands with a non-medicated soap. Then, your hands will be contaminated with a watery liquid containing a non-harmful bacteria (*Serratia marcescens*). This liquid containing the bacteria will be spread over the surfaces of the hands, and the hands will be allowed to air dry. Following air drying, the hands will be sampled. Sampling is accomplished by having you place your hands into large plastic bags to which will be added a mild soap-like solution. A laboratory technician will massage each bagged hand for one minute. The hands will be removed from the bags and the solution from each bag will be tested to determine the number of test bacteria added to the hands. Following the baseline sampling, your hands will be rinsed with tap

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water and washed with a non-medicated soap and dried. You will then begin the treatment part of the study. Prior to each treatment, your hands will be contaminated with bacteria as described above. Your hands and wrists will then be treated (washed) with the test material, following specific instructions. Your hands will be contaminated and treated 11 times. Your hands will be sampled (to determine the number of bacteria removed or killed by treatment) after the 1<sup>st</sup> and 11th washes. After the 1<sup>st</sup> wash and sampling, the hands are rinsed with tap water. Following the last sampling, your hands will be rinsed with water, washed with Hibiclens®, an antimicrobial soap and treated with alcohol, prior to leaving the lab.

After completing the treatment visit and until your follow-up visit, you will need to check the skin on your hands each day for any pimples, bumps or rashes. Within four to eight days after you have completed treatment, you will be required to return to the lab for a follow-up visit. Your hands will be checked for infection by a Dermatologist trained in observing infection.

**FEMALES OF CHILDBEARING POTENTIAL:** You may not participate in this study if you are pregnant or nursing. As part of giving your consent you must agree to have a urine pregnancy test at the start of the study.

**RISKS:** The risks associated with this test are primarily related to contamination with the test bacteria. For healthy persons, the possibility of a skin infection exists; however, this possibility is remote because, (1) test bacteria are applied only to intact skin, and (2) the skin is cleansed with antibacterial products following contact with the test bacteria.

You may also develop a reaction on your hands from the test materials. A reaction could be redness, swelling, itching, cracking, peeling, or in rare cases, blistering.

No risks to you as a study participant, other than those described above, are anticipated during the study. Reactions are usually due to irritation, although an allergic reaction might occur. If you become allergic, it is possible that future exposures to the same ingredient may cause a skin reaction. If this occurs, you will be provided with information to minimize the chance for future exposures.

You may experience risks or side effects that are not known at this time. You will be informed in a timely manner if new information becomes available that may influence your willingness to continue in this study.

**BENEFITS:** You will not benefit from the applications of test article but the study results may allow a new or improved product to be marketed.

**ALTERNATIVE PROCEDURES/TREATMENTS:** Because you are not being treated for a medical condition, alternative treatments do not apply to this study.

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**CONFIDENTIALITY:** Information concerning you that is obtained in connection with this study will be kept confidential by Hill Top Research, except that the sponsoring company whose product is being tested will receive a copy of the study records. The records will be coded to protect your identity. In addition, the sponsoring company, the Institutional Review Board (IRB) and government regulatory agencies, including the U.S. Food and Drug Administration (FDA), may inspect the records of the study. Information obtained in the study may be used for medical or scientific publication, but your identity will remain confidential.

**MEDICAL TREATMENT:** If in the course of this study you experience illness, discomfort or injury that appears to be a result of the study, Hill Top Research will provide you with medical care at no cost to you. Providing such medical care is not an admission of legal responsibility. If such illness, discomfort or injury does occur, ask any staff member to arrange a meeting for you with the appropriate personnel.

In certain cases of illness or injury resulting from this study, workers' compensation coverage may be available. In accordance with Ohio law, Hill Top Research has secured workers' compensation coverage for participants in its studies and tests, and has paid and will pay appropriate premiums into the State Insurance Fund on behalf of such participants.

**WHO TO CONTACT:** If you have any questions about this study or in case of an emergency, contact Emilie, Study Coordinator, at 513-831-3114 ext. 2324 during business hours (M-F, 8:00 A.M. - 5:00 P.M.) or Ann Brady, Study Manager, at 513-831-3354 after hours. In addition, if you have any questions as to your rights as a research subject, contact the Institutional Review Board of Hill Top Research, Nancy J. Pelc, M.D., Chairman, at 1-513-831-3114.

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**VOLUNTARY PARTICIPATION/WITHDRAWAL:** Your participation in this research study is strictly voluntary. You may refuse to participate or may discontinue participation at any time during the study without penalty or loss of benefits to which you are otherwise entitled.

If you agree to participate in this study, you are also agreeing to provide Hill Top Research with accurate information and to follow study instructions as given to you. If you fail to follow study instructions, your participation may be ended.

Your participation in the study may be discontinued at any time without your consent by the Investigator, the IRB, the FDA, or the sponsoring company.

**COMPENSATION:** You will be paid \$80.00 for the completion of this study. You will be compensated according to the following schedule:

If you complete	Visit 1	You will receive	\$0*
If you do not qualify	Visit 2	you will receive	\$10.00
If you qualify but are eliminated as an extra subject	Visit 2	you will receive	\$20.00
If you complete	Visit 2	you will receive	\$50.00
If you complete	Visit 3	you will receive	\$80.00

\*No payment-kit products given.

Payments will be made at the end of the study.

There are no anticipated expenses to you for participating in this study. All test related materials will be provided at no cost to you. (Soap, shampoo, antiperspirant/deodorant and gloves)

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Institution: Hill Top Research, Inc.  
Investigator: Gayle K. Mulberry, M.S.  
Study Title: "Efficacy Evaluation of Health Care Personnel Handwash Products"  
Neutralizer Validation Study

HTR Study No. 01-109127-11  
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### **CONSENT FORM-2**

**INTRODUCTION:** You are being asked to take part in a research study. Before you give your consent to be a subject, it is important that you take enough time to read and understand what your participation would involve. In preparing this consent form, it has been necessary to use some technical language. Please ask questions if there is anything you do not understand.

You will be given a signed copy of this consent form and any other necessary written information prior to the start of the study.

**PURPOSE:** The purpose of this research study is to assure that the materials used in the main study, for growing and counting bacteria recovered from the hands of subjects, will allow the growth of the bacteria. Approximately two (2) people between and including the ages of 18 - 65 will be screened as potential subjects in this study. Two (2) subjects are expected to complete the one visit study.

**TEST ARTICLES:** One of the test articles is an experimental liquid soap product and the other test article is a marketed antibacterial liquid soap product. One product will be randomly assigned to each participating subject.

**STUDY PROCEDURES:** As a participant, your hands and wrists will be washed eleven times following specific directions. Your hands will be sampled after the first and eleventh wash. Sampling is accomplished by having you place your hands into large plastic bags to which will be added a mild soap-like solution. A laboratory technician will massage each bagged hand for one minute. Your hands will be removed from the bags and the solution from each bag will be taken to the laboratory. The solution collected after the 1<sup>st</sup> wash will be discarded. The solution collected after the 11<sup>th</sup> wash will be tested to determine if it can be neutralized to allow growth of bacteria, which the laboratory will add to it. Following the sampling, you will rinse your hands and forearms in tap water.

**FEMALES OF CHILDBEARING POTENTIAL:** You may not participate in this study if you are pregnant or nursing. As part of giving your consent you must agree to have a urine pregnancy test at the start of the study.

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**RISKS:** Your hands may show a "reaction." A "reaction" could be redness, swelling, itching, cracking or peeling, or in rare cases, small blisters. It is unlikely, but possible, that a rash could develop. No risk to study participants, other than those described above as "reactions" are anticipated during the study. Reactions are usually due to irritation, although an allergic reaction might also occur. If you become allergic, it is possible that future exposures to the same ingredient may cause a skin reaction. If this occurs, you will be provided with information to minimize the chance for future exposures.

**BENEFITS:** You will not benefit from the applications of test article but the test results may allow a new or improved product to be marketed.

**ALTERNATIVE PROCEDURES/TREATMENTS:** Because you are not being treated for a medical condition, alternative treatments do not apply to this study.

**CONFIDENTIALITY:** Information concerning you that is obtained in connection with this study will be kept confidential by Hill Top Research, except that the sponsoring company whose product is being tested will receive a copy of the study data. The data will be coded to protect your identity. In addition, the sponsoring company, the Institutional Review Board (IRB) and government regulatory agencies, including the U.S. Food and Drug Administration (FDA), may inspect the records of the study. Information obtained in the study may be used for medical or scientific publication, but your identity will remain confidential.

**MEDICAL TREATMENT:** If in the course of this study you experience illness, discomfort or injury that appears to be a result of the study, Hill Top Research will provide you with medical care at no cost to you. Providing such medical care is not an admission of legal responsibility. If such illness, discomfort or injury does occur, ask any staff member to arrange a meeting for you with the appropriate personnel.

In certain cases of illness or injury resulting from this study, workers' compensation coverage may be available. In accordance with Ohio law, Hill Top Research has secured workers' compensation coverage for participants in its studies and tests, and has paid and will pay appropriate premiums into the State Insurance Fund on behalf of such participants.

**WHO TO CONTACT:** If you have any questions about this study or in case of an emergency, contact Emilie, Study Coordinator, at 513-831-3114, ext. 2324 during business hours (M-F, 8:00 A.M. - 5:00 P.M.) or Ann Brady, Study Manager, at 513-831-3354 after hours. In addition, if you have any questions as to your rights as a research subject, contact the Institutional Review Board of Hill Top Research, Nancy J. Pelc, M.D., Chairman, at 1- 513-831-3114.

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**VOLUNTARY PARTICIPATION/WITHDRAWAL:** Your participation in this research study is strictly voluntary. You may refuse to participate or may discontinue participation at any time during the study without penalty or loss of benefits to which you are entitled.

If you agree to participate in this study, you are also agreeing to provide Hill Top Research with accurate information and to follow study instructions as given to you. If you fail to follow study instructions, your participation may be ended.

Your participation in the study may be discontinued at any time without your consent by the Investigator, the IRB, the FDA, or the sponsoring company.

**COMPENSATION:** You will be paid \$10.00 for the completion of this study.

Payment will be made at the end of the study.

There are no anticipated expenses to you for participating in this study. All test related materials will be provided at no cost to you.

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**EXHIBIT B****EVALUATION OF HEALTH CARE PERSONNEL HANDWASH  
SUBJECT INSTRUCTIONS**

Today you will be given a kit of products (bar soap, shampoo, and deodorant/antiperspirant) to use exclusively during this study. Please set aside all products you normally use in these categories and use only the products in the kit. In addition, please refrain from using perfumes, deodorants or antiperspirants (other than the ones furnished), powders and anti-dandruff hair shampoos, and do not swim in a chemically treated pool or hot tub during the study.

Beginning today, no body lotions, medicated creams or ointments should be applied to any area of your skin. Also, do not take any antibiotics. These medications may affect the bacteria of the skin. If antibiotics are necessary due to illness, please report this to Hill Top Research at the phone number below.

Please use the rubber gloves provided with the product kit for all household chores involving detergents, acid, alkalis, and solvents until the completion of the study.

**SUBJECT SCHEDULE****TEST DAY**

\_\_\_\_\_

Time of Visit: \_\_\_\_\_

1. Plan to arrive at the laboratory about 10 minutes before your scheduled time. You are expected to be prompt.
2. Please wear clothing that will allow easy access to your hands.
3. You will be required to remove all jewelry, watches, and bracelets before washing.
4. You will undergo a supervised wash regimen at the laboratory.
5. Approximate time at the laboratory -      hours.
6. Additional instructions will be provided for the Follow Up Visit.

**FOLLOW UP VISIT**

\_\_\_\_\_

Time of Visit: \_\_\_\_\_

1. A Dermatologist will check your hands for infection
2. Approximate time at the lab - 1/2 hour.

If you have any questions regarding this study, please contact Emilie, Study Coordinator, at 513-831-3114 ext. 2324 between 8:00 a.m. - 5:00 p.m. or Ann Brady, Study Manager, after hours and on weekends at 831-3354.

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**JUL 31 2001**

**Approved**

**EXHIBIT C**

**SUBJECT'S INSTRUCTIONS FOLLOWING STUDY COMPLETION**

You have just completed participation in a clinical study, "Efficacy Evaluation of Health Care Personnel Handwash Products". During this study, your hands were in contact with a liquid containing bacteria (*Serratia marcescens*). Although we do not expect you to have any adverse experience as a result of participation in this study, there is a remote possibility that an infection may develop on your hands.

To determine whether you have developed an infection from the test bacteria, we would like you to examine your hands and wrists daily. If you notice the appearance of any pimples, blisters or raised bumps surrounded by redness and/or swelling, please contact Emilie, Study Coordinator at (513) 831-3114 ext. 2324 during normal business hours (8:00 am- 5 pm) or Ann Brady at (513) 831-3354 after hours.

You are required to return to the test site for a follow-up visit. Your follow-up is scheduled for:

---

Date

Time

Thank you for your cooperation.

**IRB of  
Hill Top Research**

**JUL 31 2001**

**APPROVED**

HTR Study No.: 01-109127-11

## APPENDIX II

Total number of pages = 35

**Protocol**

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HILL TOP RESEARCH, INC.

**PROTOCOL FOR  
EFFICACY EVALUATION OF  
HEALTH CARE PERSONNEL HANDWASH PRODUCTS**

**FOR: CIBA SPECIALTY CHEMICALS CORPORATION**

**HTR STUDY NO.: 01-109127-11**

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## EXHIBITS

- Exhibit A: Sample Consent Form
- Exhibit B: Subject Instructions - Handwash Study
- Exhibit C: Subject Instructions Following Study Completion

## DATA COLLECTION FORMS

- 1 Demographics/Dermatological/Medical History Form
- 2 Inclusion/Exclusion Form
- 3 Intercurrent Illness/Concomitant Medication Form
- 4 Health Care Personnel Handwash Bacterial Counts
- 5 Adverse Event Report
- 6 Follow up Visit

## 1.0 INTRODUCTION

The FDA issued a tentative final monograph (Federal Register, Vol. 59, pp. 31402 to 31452, June 17, 1994) prescribing the use of a health care personnel handwash method to demonstrate the antimicrobial efficacy of cleansing products containing antimicrobial ingredients for frequent use. The method presented in the Monograph is based on an American Society for Testing Materials Standard Method for Evaluation of Health Care Personnel Handwash Formulation E1174-87 published in 1987. A revision of the ASTM Method was published in 2000 entitled Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel or Consumer Handwash Formulations, E1174-00. This protocol is aligned with a revised version of the ASTM Method.

The procedure is designed to simulate routine hand washing conducted for the purpose of reducing the level of hand contamination of health care personnel under conditions of frequent use. For this procedure a broth culture of *Serratia marcescens*, ATCC 14756, is used as an artificial contaminant bacteria on the hands. Activity is measured by comparing the number of marker bacteria removed from artificially contaminated hands after a single use of the hand washing formulation to the baseline number, the number recovered from contaminated unwashed hands. A similar comparison is made following the 11th wash of a multiple (11) wash procedure. Prior to each of the washes, the hands are artificially contaminated with the *S. marcescens*.

The method described in this protocol eliminates a shortcoming common to the Proposed Monograph version of the method. This method fails to provide procedures to assure adequate rapid neutralization of the antimicrobial in the handwash formulation. A neutralizer is only included in the hand sampling fluid used to sample the last wash and is omitted from the hand sampling fluid used to sample washes preceding the final wash. This failure to include neutralizers in the hand sampling fluid may provide data that falsely exaggerates the effectiveness of the antimicrobial handwash formulation. This issue is resolved in this protocol by requiring immediate neutralization in the hand sampling fluid at all sampling points.

## 2.0 OBJECTIVE

The purpose of this study is to determine the ability of hand-washing agents to give reduction of transient microbial flora (contaminants) when used in a hand washing procedure with a marker organism.

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### 3.0 STUDY SPONSOR AND MONITOR

Ciba Specialty Chemicals Corporation  
4090 Premier Drive  
High Point, NC 27265  
Telephone No.: (888) 396-2422  
Fax No.: (336) 801-2086

REPRESENTATIVE: Stephen Spainhour

### 4.0 INVESTIGATIVE ORGANIZATION AND PERSONNEL

Hill Top Research, Inc.  
Main and Mill Streets  
Miamiville, Ohio 45147  
Telephone No.: (513) 831-3114  
Fax No.: (513) 831-1217

Investigator: Gayle K. Mulberry, M.S.  
Technical Director  
Microbiological Services

Sub-Investigators: Kathleen A. Baxter, B.S.  
Ann R. Brady, B.A.G.S.

Medical Consultant: Joseph G. Daddabbo, M.D.

### 5.0 CLINICAL RESEARCH STANDARDS

The clinical investigation, including the informed consent, will be reviewed by an 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 compliance with the Good Clinical Practice Regulations, the Standard Operating Procedures of Hill Top Research, Inc., the Sponsor's protocol and protocol amendment(s).

## 6.0 EXPERIMENTAL DESIGN

This will be a two sample (test article) study utilizing a direct paired comparison test design of baseline bacterial populations vs. post treatment bacterial populations. The study will consist of a one-week pretest conditioning period and one day of treatment. Forty-five (45) subjects are expected to start and complete the study, thirty (30) using HTR Code A and fifteen (15) using HTR Code B.

## 7.0 STUDY MATERIAL

### 7.1 Test Article

HTR Code	Code and Description	
A	<u>Test Formulation:</u>	Foaming handwash
	<u>Lot Code:</u>	3456-38
	<u>Description:</u>	thin colorless liquid
B	<u>Test Formulation:</u>	Hibiclens
	<u>Lot Code:</u>	4563C Exp. 12/02
	<u>Description:</u>	clear red liquid

### 7.2 Equipment

- 7.2.1 Colony Counter - Quebec colony counter.
- 7.2.2 Incubator - Any incubator capable of maintaining a temperature of  $25 \pm 2^\circ\text{C}$  may be used.
- 7.2.3 Sterilizer - Any suitable steam sterilizer capable of producing the conditions of sterilization.
- 7.2.4 Timer (stop-clock) - One that can be read for hours, minutes and seconds.
- 7.2.5 Plastic Bags to Sample Hands - Low bioburden – such as Glad Food Storage Bags or equivalent, 29.2 cm x 31.8 cm. (Note: Bioburden is determined according to Hill Top Microbiology Department SOP No. 11-TOPC-20-0016A.)
- 7.2.6 Bacteriological Pipettes, Sterile - 10.0 mL, 5.0 mL, 2.0 mL and 1.0 mL capacity.
- 7.2.7 Water Dilution Bottles - Any container that can be sterilized, having a 150 to 200 mL capacity and a tight closure may be used.
- 7.2.8 Test Tubes and Closures - Any of suitable size.
- 7.2.9 Handwashing Sink - A sink of sufficient size to permit subjects to wash without touching hands to sink surface or other subjects.

## 7.0 STUDY MATERIAL (CONT.)

### 7.2 Equipment (Cont.)

- 7.2.10 Water faucets - located above the sink at a height, which permits the hands to be held higher than the elbow during the washing procedure.
- 7.2.11 Tap Water Temperature Regulator and Temperature Monitor - To monitor and regulate water temperature of  $40 \pm 2^{\circ}\text{C}$ .
- 7.2.12 Erlenmeyer Flask - 2 L capacity for culturing test organism.

### 7.3 Reagents and Materials

- 7.3.1 Kit Products for Washout Period: non-antimicrobial bar soap and shampoo, roll on antiperspirant/deodorant, rubber gloves, and disposable poly gloves.
  - 7.3.2 Baby San® liquid castile soap. Ecolab Inc.
  - 7.3.3 Stripping Fluid with Neutralizer - 0.075M phosphate buffer with 0.1% Triton X-100 (dissolve 0.41 g  $\text{KH}_2\text{PO}_4$ , 10.3 g  $\text{Na}_2\text{HPO}_4$  and 1.0 g Triton X-100 in 1-L distilled water containing an inactivator which rapidly quenches the antimicrobial activity of the test article(s). Final pH  $7.8 \pm 0.1$ . Final volume  $75 \pm 1.0$  mL).
  - 7.3.4 Dilution Fluid - Butterfield's phosphate buffered water (or other suitable diluent) containing an antimicrobial inactivator specific for the test formulation.
  - 7.3.5 Plating Medium - Trypticase Soy Agar
  - 7.3.6 Tryptic Soy Broth (BBL or Difco)
- 7.4 Test Microorganism  
*Serratia marcescens*, ATCC 14756 is to be used as a marker organism.

## 8.0 STUDY POPULATION

An adequate number of potential subjects will be enrolled into the pre-test conditioning period in order to provide 45 subjects who fulfill the criteria described below and who complete the study. The subjects will be randomly assigned to two treatment groups, one for each test article. Subject eligibility will be based upon information provided in the Demographics/Dermatological/ Medical History Form (DCF 1) and the Inclusion/Exclusion Form (DCF 2); and completion of a written informed consent (Exhibit A).

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## 8.0 STUDY POPULATION (CONT.)

### 8.1 Subject Inclusion Criteria

Subjects will be eligible for enrollment if they:

- 8.1.1 Are a male or female, 18 through 65 years old;
- 8.1.2 Have signed a written informed consent (Exhibit A);
- 8.1.3 Are in good health, as evidenced by response to the Demographics/Dermatological/Medical History Form (DCF 1);
- 8.1.4 Have hands and wrists that are free of dermatoses, cuts, lesions, and other skin disorders;
- 8.1.5 Have fingernails that are clean and extend no longer than approximately one (1) mm past the nail bed;
- 8.1.6 Are willing to refrain from using antimicrobial soaps (liquids and/or bars) for bathing, showering, and hand washing during the entire study;
- 8.1.7 Are willing to refrain from using anti-dandruff shampoo during the entire study;
- 8.1.8 Are willing to refrain from using medicated/antibacterial lotions and creams during the entire study, unless prescribed by a physician for an intercurrent illness;
- 8.1.9 Are willing to refrain from using topical steroids during the entire study, unless prescribed by a physician for an intercurrent illness;
- 8.1.10 Are willing to refrain from using topical or systemic antibiotic medication during the entire study, unless prescribed by a physician for an intercurrent illness; and
- 8.1.11 Are willing to comply with all study protocol requirements.

### 8.2 Subject Exclusion Criteria

Subjects will not be enrolled in the study if they:

- 8.2.1 Are currently participating in another clinical study at this or any other facility;
- 8.2.2 Have participated in any type of arm or hand wash study within the past seven (7) days;
- 8.2.3 Have cuts, scratches, or other skin disorders on their hands or wrists;
- 8.2.4 Have soap, detergent, and/or perfume allergies;
- 8.2.5 Have eczema or psoriasis on their hands or wrists;
- 8.2.6 Are currently pregnant;
- 8.2.7 Are currently lactating;
- 8.2.8 Have been medically diagnosed as having a medical condition such as: diabetes, hepatitis, an auto-immune disorder, an organ transplant, or AIDS (or HIV positive); and/or
- 8.2.9 Have any other medical condition, which in the opinion of the Investigator(s) would preclude participation.
- 8.2.10 Have artificial nails or nail tips.

## 8.0 STUDY POPULATION (CONT.)

### 8.3 Other Study Restrictions

- 8.3.1 Subjects should not use any other personal cleansing products.
- 8.3.2 Subjects should avoid chemically treated pools and hot tubs.
- 8.3.3 Subjects should avoid exposing their hands to harsh cleaning products, chlorine, or solvents.

## 9.0 SUBJECT WITHDRAWAL

After admission to the study, the subject may withdraw at any time for any reason. If possible, the reason for withdrawal will be recorded.

## 10.0 PROCEDURE

The study will be divided into three phases; subject enrollment period, a pre-test washout (conditioning) period of at least one week duration, and a one day test period.

### 10.1 Subject Qualification and Enrollment

Prospective subjects will visit the test facility to be screened for their eligibility to participate in the study. Eligibility will be based upon information provided in the Demographics/Dermatological/ Medical History Form (DCF 1) and the Inclusion/Exclusion Form (DCF 2); and completion of a written informed consent (Exhibit A). Qualified subjects will be given non-antibacterial containing soap, shampoo, roll-on-antiperspirant/deodorant, several pairs of disposable poly gloves, a pair of rubber gloves, a copy of the Subject's Study Instructions (Exhibit B). They will be instructed to use the soap, shampoo, antiperspirant/deodorant, poly gloves and rubber gloves and to follow the written instructions for the entire study period.

### 10.2 Washout Period

This period will last at least seven (7) days. Subjects will continue to follow the special study restrictions, use the non-antibacterial soap, shampoo, and antiperspirant/deodorant, rubber gloves and poly gloves.

## 10.0 PROCEDURE (CONT.)

### 10.3 Test Day Schedule

On the day of the test period, subjects will return to the test facility. Their hands and wrists will be re-examined to ensure that they are still free of cuts, lesions, and other skin disorders. They will also be asked if they have had any illnesses or taken any medications (proprietary or prescribed) ordered by a physician since the last visit (DCF 3). Subjects who still meet the study criteria will be eligible to continue on the study. Subjects continuing on the study will be assigned a permanent subject number.

**The following outlines the schedule of procedures for the test day:**

- a. Subjects will wash with a mild soap for 15 seconds. (Section 10.3.1)
- b. Subjects' hands will be contaminated and baseline sampling performed. (Section 10.3.2)
- c. Subjects' hands treated with the test articles, each treatment preceded by hand contamination. (Section 10.3.3)
- d. Following treatments 1 and 11, subjects hands are sampled for post-treatment count and the *S. marcescens* in the sampling fluid enumerated. (Sections 10.3.5 and 10.3.6).
- e. Following each sampling of subject's hands the hands are rinsed with warm water.
- f. After the hand sampling following treatment 11, the Subjects' hands will be rinsed with water, washed with Hibiclens and treated with 70% Isopropyl Alcohol (Section 10.3.7) for at least 30 seconds and allowed to air dry.

#### 10.3.1 Conditioning Wash

All subjects, prior to the baseline sampling perform a 15-second wash using a non-antimicrobial liquid soap, Baby San® (Section 7.3.2). This procedure, described below, removes oil and dirt and familiarizes the subjects with the treatment procedure.

- 10.3.1.1 Five mL of Baby San® Soap is dispensed into cupped hands and distributed over all surfaces of the hands taking care not to lose the substance.
- 10.3.1.2 After the material is spread, a small amount of tap water  $40 \pm 2^\circ\text{C}$  is added, and the hands and lower third of the forearms are completely lathered for 15 seconds in a vigorous manner.
- 10.3.1.3 The hands and forearms are then rinsed under running tap water  $40 \pm 2^\circ\text{C}$  for 30 seconds.
- 10.3.1.4 The hands are thoroughly dried with a disposable paper towel.

## 10.0 PROCEDURE (CONT.)

### 10.3.2 Baseline Bacteria Count

After completing the conditioning wash, a total volume of 4.5 mL of *S. marcescens*, ATCC 14756 suspension (Section 10.4), (minimum of  $10^8$  organisms per mL) is added into the subjects' cupped hands in 1.5 mL increments. After each 1.5 mL aliquot is added, the suspension is rubbed thoroughly over the surface of both hands, not going above the wrist. Each application and spreading should last approximately twenty (20) seconds. Between each aliquot the hands will be held away from the body and allowed to air dry for approximately thirty (30) seconds. Following the third 1.5mL aliquot the hands are held motionless away from the body and allowed to air dry for  $90 \pm 5$  seconds.

(NOTE: The hands may not be completely dry at this time.)

Plastic bags having documented low bioburden, (Section 7.2.5) are placed on the subject's right and left hands. A 75 mL aliquot of stripping solution (Section 7.3.3) is aseptically added into each bag and the bacterial sampling procedure is carried out as described under Section 10.3.5 (Bacterial Sampling Procedure). The hands and forearms are then washed thoroughly with castile soap (Section 7.3.2) and dried.

### 10.3.3 Multiple Treatment Procedure

Prior to each treatment, the subject's hands will be contaminated with 4.5 mL of the *S. marcescens* suspension as described in Section 10.3.2

After completing the contamination step, the subjects perform a treatment with the assigned test article, under close supervision. The treatment procedure follows that described in the Section 10.3.4 (Method for Treating Hands). The lower third of the forearm is to be included in the wash procedure.

This procedure is repeated a total of 11 times with at least five minutes between each treatment. Immediately after completing the 1st and 11th treatments, the hands are sampled as described in Section 10.3.5 (Bacterial Sampling Procedure).

### 10.3.4 Method for Treating Hands

10.3.4.1 Test Article HTR Code A - Dispense two (2) pumps (3.2 mL) from the test article container into cupped palm of one hand and distribute over all surfaces of both hands. The material is worked vigorously over all surfaces of the hands and lower third of the forearm for thirty (30) seconds. (A small amount of water may be added to moisten the hands if necessary after approximately 15 seconds.) Particular attention is to be paid to the area between the fingers, beneath the nails and around the thumb. Rinse hands under running tap water for 30 seconds.

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## 10.0 PROCEDURE (CONT.)

10.3.4.2 Test Article HTR Code B - Immediately prior to treating the hands are to be wetted with small amount of water by passing hands rapidly under the tap. Dispense 5.0 ml from a syringe into cupped palm of one hand and distribute over all surfaces of both hands. The material is worked vigorously over all surfaces of the hands and lower third of the forearm for fifteen (15) seconds. Particular attention is to be paid to the area between the fingers, beneath the nails and around the thumb. Rinse hands under running tap water for 30 seconds.

**NOTE:** After treatments that are not followed by a sample collection, the subjects will thoroughly dry their hand and forearms with a disposable paper towel.

### 10.3.5 Bacterial Sampling Procedure

Plastic bags having low bioburden (Section 7.2.5) will be placed on the subject's right and left hands. A 75 mL aliquot of stripping fluid with neutralizer (Section 7.3.3) is aseptically added into each bag. The bag on each hand is secured and massaged for one minute in a uniform manner by a lab technician. An aliquot of the fluid is aseptically obtained directly from the bagged hands within one minute of completing the massaging and immediately placed into tubes containing sterile Dilution Fluid (Section 7.3.4).

Fluid samples for bacteria counts are to be labeled by an Investigator derived code so that the individuals who prepare the plates and count the colonies are unaware of the sources of the sampling solution.

After each bacterial sampling, subjects will rinse their hands under running warm tap water  $40 \pm 2^{\circ}\text{C}$  to help remove residual stripping fluid.

## 10.0 PROCEDURE (CONT.)

### 10.3.6 Bacterial Counts of Sampling Solution

Aliquots of the stripping fluid or dilutions of the fluid are spread plated in duplicate on Trypticase Soy Agar plates (Section 7.3.5).

The dilutions of the baseline sample plated represent dilutions of  $10^{-4}$  through  $10^{-6}$  of milliliter aliquots of the stripping fluid. The aliquots or dilutions of the treatment sample fluid plated represent dilutions of  $10^{-1}$  through  $10^{-4}$  milliliter aliquots of the stripping fluid.

The prepared plates are to be incubated for  $48 \pm 4$  hours at  $25 \pm 2^{\circ}\text{C}$ . Standard plate counting procedures are used to count only red pigmented colonies. The actual plate counts are recorded on the form entitled Handwash Bacterial Count Form (Data Collection Form 4).

### 10.3.7 Disinfection of Hands

After the final sampling is completed, subjects' hands and wrists will be rinsed with water, washed for at least sixty (60) seconds with 5mL of [REDACTED] then treated for at least thirty (30) seconds with 70% Isopropyl Alcohol and allowed to air dry.

To ensure that any delayed adverse events, such as primary skin infections, are reported to the Study Investigator, all test subjects will be given a copy of Subjects' Instructions Following Study Completion (Exhibit C) before leaving the clinical site after they have completed the study. This sheet will instruct the subjects to examine their hands daily until the final scheduled visit for the presence of pimples, blisters, or raised, red itching bumps surrounded by erythema and/or edema that may be indicative of a skin infection. Subjects, who notice such lesions, will be instructed to call the clinical test site. The subjects will return to the clinical test site within four (4) to eight (8) days after the study procedures have been completed to have their hands examined by the Medical Consultant. The Medical Consultant will complete Data Collection Form 6 for each subject on their follow-up visit.

## 10.0 PROCEDURE (CONT.)

### 10.4 Marker Organism and Preparation

*S. marcescens*, ATCC 14756 will be used to challenge the efficacy of the test materials.

A stock culture of *S. marcescens*, ATCC 14756 is prepared by aseptically transferring at least three isolated colonies from an agar plate to 10 mL of sterile Tryptic Soy Broth (TSB) (Section 7.3.6) which is then incubated at  $25 \pm 2^\circ\text{C}$  for  $24 \pm 4$  hours. A series of at least three but no more than ten 24 hour broth transfers are made in 10 mL of TSB from this broth culture. If testing occurs on multiple days, it is desired to use a test culture the same number of transfers from the source.

A 2-liter flask containing 1000 mL of TSB is inoculated with 1.0 mL of a 24-hour broth transfer. The flask is incubated for  $24 \pm 4$  hours at  $25 \pm 2^\circ\text{C}$ . Prior to any withdrawal of culture, whether for hand contamination or for numbers assay, the suspension is stirred or shaken. A suspension is not used for more than eight hours.

The suspension is assayed for the number of organisms at the beginning and end of the use period.

## 11.0 DATA EVALUATION

The number of colony forming units (CFU) recovered per sample dilution will be tabulated. The total number of CFU per mL of sampling solution will be calculated as well as the number per hand.

The data will be evaluated using parametric statistical analyses as follows:

Bacterial counts recovered from the hands will be transformed into log counts. The log count of each subjects left and right hand will be averaged. The changes from baseline counts at each sampling interval will be obtained for each test article.

An analysis of variance will be performed on the data to:

Compare baseline counts of subjects assigned different test articles.

Evaluate the effectiveness of each treatment as a function of the number of treatments (within treatment analysis).

## 12.0 ADVERSE EXPERIENCES

### 12.1 Definitions

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 (Data Collection Form 5) and reported according to the Standard Operating Procedures of Hill Top Research, Inc.

A **Serious Adverse Drug Event/Experience** is any adverse drug 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;
- a congenital anomaly/birth defect

Important medical event/experiences that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

An **Unexpected Adverse Drug Event/Experience** is any adverse drug event/experience not listed in the current labeling for the test article or the current investigator's brochure

### 12.2 Follow-up

If an **Adverse Event/Experience** occurs, the subject under the direction of the Investigator (or designee), may be referred to Hill Top's consultant physician for treatment.

**Serious or Unexpected Drug Event/Experience** will be followed to resolution to the extent possible (e.g., medical attention by subject's primary care physician).

### 12.3 Notification

The sponsor will be notified of all adverse event/experiences. Any **Serious or Unexpected Adverse Drug Event/Experience** which occurs during the study must be reported promptly by the investigator to the sponsor and the reviewing IRB, where applicable, within 24-hours of the information being reported to Hill Top Research, Inc.

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**13.0 INTERCURRENT ILLNESS REPORTING**

If a subject reports that he/she has had an intercurrent illness during the wash-out period or during the one (1) day test period, the illness and any new medication taken will be documented on DCF 3. The subject may be discontinued from the study at the discretion of the Investigator(s).

**14.0 CONCOMITANT MEDICATION**

If the subject has taken any medication (proprietary or prescribed) ordered by a physician, information pertaining to that medication intake will be recorded appropriately on either DCF 3 or DCF 5.

**15.0 DEVIATIONS FROM PROTOCOL**

Any minor deviations from the protocol, not previously agreed to by the Sponsor and Investigator(s), that occur during the conduct of the study will be documented.

**16.0 REPORT**

The final report will summarize the method, data and conclusions relative to the test articles and the subjects. Source data will be retained by the testing facility on microfilm. The original source data will be maintained according to the investigator's standard operating procedure. A copy of the source documents may be obtained upon request of the Study Sponsor. Copies of transcribed data will be incorporated in the report.

**17.0 NOTICE**

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

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**18.0 PROTOCOL APPROVAL**

**HILL TOP RESEARCH, INC.**

By: *Gayle K. Mulberry* 7-26-01  
 Gayle K. Mulberry (Date)  
 Investigator

By: *Kathleen A. Baxter* 7-26-01  
 Kathleen A. Baxter (Date)  
 Sub-Investigator

By: *Ann R. Brady* 7-26-01  
 Ann R. Brady (Date)  
 Sub-Investigator

**CIBA SPECIALTY CHEMICALS CORPORATION**

By: *John Brinkman* 7-27-01  
 (Date)

**EXHIBIT A**  
**SAMPLE CONSENT FORM**

PRO NO.	01-109127-11
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Institution: Hill Top Research, Inc.  
Investigator: Gayle K. Mulberry, M.S.  
Study Title: "Efficacy Evaluation of Health Care Personnel Handwash Products"

HTR Study No. 01-109127-11  
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### CONSENT FORM

**INTRODUCTION:** You are being asked to take part in a research study. Before you give your consent to be a subject, it is important that you take enough time to read and understand what your participation would involve. In preparing this consent form, it has been necessary to use some technical language. Please ask questions if there is anything you do not understand.

You will be given a signed copy of this consent form and any other necessary written information prior to the start of the study.

**PURPOSE:** The purpose of this research study is to measure the ability of two liquid soap products to reduce the number of bacteria on the hands after repetitive use. Approximately ninety (90) people between and including the ages of 18 – 65 will be screened as potential subjects in this study. Forty-five (45) subjects are expected to complete the three-visit study.

**TEST ARTICLES:** One of the test articles is an experimental liquid soap product. The other test article is a marketed antibacterial liquid soap product. One product will be randomly assigned to each participating subject. Two of every three subjects will receive the experimental product.

**STUDY PROCEDURES:** Prior to enrollment in the test, you will be asked to complete a brief medical history questionnaire. It is possible that you may not be able to participate based on your answers to these questions. If you qualify, you will be given a kit containing non-antibacterial bar soap, shampoo, Ban® antiperspirant/deodorant, and rubber and poly gloves to be used at least one week prior to the start of the actual study. You will be given written instructions on how to use the kit.

After at least one week, you will be required to return to the lab. You will be asked to complete another brief medical history questionnaire. It is possible that you may not be able to participate based on your answers to these questions or the condition of the skin on your hands and wrists. If you qualify, you will wash your hands with a non-medicated soap. Then, your hands will be contaminated with a watery liquid containing a non-harmful bacteria (*Serratia marcescens*). This liquid containing the bacteria will be spread over the surfaces of the hands, and the hands will be allowed to air dry. Following air drying, the hands will be sampled. Sampling is accomplished by having you place your hands into large plastic bags to which will be added a mild soap-like solution. A laboratory technician will massage each bagged hand for one minute. The hands will be removed from the bags and the solution from each bag will be tested to determine the number of test bacteria added to the hands. Following the baseline sampling, your hands will be rinsed with tap

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water and washed with a non-medicated soap and dried. You will then begin the treatment part of the study. Prior to each treatment, your hands will be contaminated with bacteria as described above. Your hands and wrists will then be treated (washed) with the test material, following specific instructions. Your hands will be contaminated and treated 11 times. Your hands will be sampled (to determine the number of bacteria removed or killed by treatment) after the 1<sup>st</sup> and 11th washes. After the 1<sup>st</sup> wash and sampling, the hands are rinsed with tap water. Following the last sampling, your hands will be rinsed with water, washed with Hibiclens®, an antimicrobial soap and treated with alcohol, prior to leaving the lab.

After completing the treatment visit and until your follow-up visit, you will need to check the skin on your hands each day for any pimples, bumps or rashes. Within four to eight days after you have completed treatment, you will be required to return to the lab for a follow-up visit. Your hands will be checked for infection by a Dermatologist trained in observing infection.

**FEMALES OF CHILDBEARING POTENTIAL:** You may not participate in this study if you are pregnant or nursing. As part of giving your consent you must agree to have a urine pregnancy test at the start of the study.

**RISKS:** The risks associated with this test are primarily related to contamination with the test bacteria. For healthy persons, the possibility of a skin infection exists; however, this possibility is remote because, (1) test bacteria are applied only to intact skin, and (2) the skin is cleansed with antibacterial products following contact with the test bacteria.

You may also develop a reaction on your hands from the test materials. A reaction could be redness, swelling, itching, cracking, peeling, or in rare cases, blistering.

No risks to you as a study participant, other than those described above, are anticipated during the study. Reactions are usually due to irritation, although an allergic reaction might occur. If you become allergic, it is possible that future exposures to the same ingredient may cause a skin reaction. If this occurs, you will be provided with information to minimize the chance for future exposures.

You may experience risks or side effects that are not known at this time. You will be informed in a timely manner if new information becomes available that may influence your willingness to continue in this study.

**BENEFITS:** You will not benefit from the applications of test article but the study results may allow a new or improved product to be marketed.

**ALTERNATIVE PROCEDURES/TREATMENTS:** Because you are not being treated for a medical condition, alternative treatments do not apply to this study.

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**CONFIDENTIALITY:** Information concerning you that is obtained in connection with this study will be kept confidential by Hill Top Research, except that the sponsoring company whose product is being tested will receive a copy of the study records. The records will be coded to protect your identity. In addition, the sponsoring company, the Institutional Review Board (IRB) and government regulatory agencies, including the U.S. Food and Drug Administration (FDA), may inspect the records of the study. Information obtained in the study may be used for medical or scientific publication, but your identity will remain confidential.

**MEDICAL TREATMENT:** If in the course of this study you experience illness, discomfort or injury that appears to be a result of the study, Hill Top Research will provide you with medical care at no cost to you. Providing such medical care is not an admission of legal responsibility. If such illness, discomfort or injury does occur, ask any staff member to arrange a meeting for you with the appropriate personnel.

In certain cases of illness or injury resulting from this study, workers' compensation coverage may be available. In accordance with Ohio law, Hill Top Research has secured workers' compensation coverage for participants in its studies and tests, and has paid and will pay appropriate premiums into the State Insurance Fund on behalf of such participants.

**WHO TO CONTACT:** If you have any questions about this study or in case of an emergency, contact Emilie, Study Coordinator, at 513-831-3114 ext. 2324 during business hours (M-F, 8:00 A.M. - 5:00 P.M.) or Ann Brady, Study Manager, at 513-831-3354 after hours. In addition, if you have any questions as to your rights as a research subject, contact the Institutional Review Board of Hill Top Research, Nancy J. Pelc, M.D., Chairman, at 1-513-831-3114.

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**VOLUNTARY PARTICIPATION/WITHDRAWAL:** Your participation in this research study is strictly voluntary. You may refuse to participate or may discontinue participation at any time during the study without penalty or loss of benefits to which you are otherwise entitled.

If you agree to participate in this study, you are also agreeing to provide Hill Top Research with accurate information and to follow study instructions as given to you. If you fail to follow study instructions, your participation may be ended.

Your participation in the study may be discontinued at any time without your consent by the Investigator, the IRB, the FDA, or the sponsoring company.

**COMPENSATION:** You will be paid \$80.00 for the completion of this study. You will be compensated according to the following schedule:

If you complete	Visit 1	You will receive	\$0*
If you do not qualify	Visit 2	you will receive	\$10.00
If you qualify but are eliminated as an extra subject	Visit 2	you will receive	\$20.00
If you complete	Visit 2	you will receive	\$50.00
If you complete	Visit 3	you will receive	\$80.00

\*No payment-kit products given.

Payments will be made at the end of the study.

There are no anticipated expenses to you for participating in this study. All test related materials will be provided at no cost to you. (Soap, shampoo, antiperspirant/deodorant and gloves)

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Institution: Hill Top Research, Inc.  
Investigator: Gayle K. Mulberry, M.S.  
Study Title: "Efficacy Evaluation of Health Care Personnel Handwash Products"  
Neutralizer Validation Study

HTR Study No. 01-109127-11

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### CONSENT FORM-2

**INTRODUCTION:** You are being asked to take part in a research study. Before you give your consent to be a subject, it is important that you take enough time to read and understand what your participation would involve. In preparing this consent form, it has been necessary to use some technical language. Please ask questions if there is anything you do not understand.

You will be given a signed copy of this consent form and any other necessary written information prior to the start of the study.

**PURPOSE:** The purpose of this research study is to assure that the materials used in the main study, for growing and counting bacteria recovered from the hands of subjects, will allow the growth of the bacteria. Approximately two (2) people between and including the ages of 18 - 65 will be screened as potential subjects in this study. Two (2) subjects are expected to complete the one visit study.

**TEST ARTICLES:** One of the test articles is an experimental liquid soap product and the other test article is a marketed antibacterial liquid soap product. One product will be randomly assigned to each participating subject.

**STUDY PROCEDURES:** As a participant, your hands and wrists will be washed eleven times following specific directions. Your hands will be sampled after the first and eleventh wash. Sampling is accomplished by having you place your hands into large plastic bags to which will be added a mild soap-like solution. A laboratory technician will massage each bagged hand for one minute. Your hands will be removed from the bags and the solution from each bag will be taken to the laboratory. The solution collected after the 1<sup>st</sup> wash will be discarded. The solution collected after the 11<sup>th</sup> wash will be tested to determine if it can be neutralized to allow growth of bacteria, which the laboratory will add to it. Following the sampling, you will rinse your hands and forearms in tap water.

**FEMALES OF CHILDBEARING POTENTIAL:** You may not participate in this study if you are pregnant or nursing. As part of giving your consent you must agree to have a urine pregnancy test at the start of the study.

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**RISKS:** Your hands may show a "reaction." A "reaction" could be redness, swelling, itching, cracking or peeling, or in rare cases, small blisters. It is unlikely, but possible, that a rash could develop. No risk to study participants, other than those described above as "reactions" are anticipated during the study. Reactions are usually due to irritation, although an allergic reaction might also occur. If you become allergic, it is possible that future exposures to the same ingredient may cause a skin reaction. If this occurs, you will be provided with information to minimize the chance for future exposures.

**BENEFITS:** You will not benefit from the applications of test article but the test results may allow a new or improved product to be marketed.

**ALTERNATIVE PROCEDURES/TREATMENTS:** Because you are not being treated for a medical condition, alternative treatments do not apply to this study.

**CONFIDENTIALITY:** Information concerning you that is obtained in connection with this study will be kept confidential by Hill Top Research, except that the sponsoring company whose product is being tested will receive a copy of the study data. The data will be coded to protect your identity. In addition, the sponsoring company, the Institutional Review Board (IRB) and government regulatory agencies, including the U.S. Food and Drug Administration (FDA), may inspect the records of the study. Information obtained in the study may be used for medical or scientific publication, but your identity will remain confidential.

**MEDICAL TREATMENT:** If in the course of this study you experience illness, discomfort or injury that appears to be a result of the study, Hill Top Research will provide you with medical care at no cost to you. Providing such medical care is not an admission of legal responsibility. If such illness, discomfort or injury does occur, ask any staff member to arrange a meeting for you with the appropriate personnel.

In certain cases of illness or injury resulting from this study, workers' compensation coverage may be available. In accordance with Ohio law, Hill Top Research has secured workers' compensation coverage for participants in its studies and tests, and has paid and will pay appropriate premiums into the State Insurance Fund on behalf of such participants.

**WHO TO CONTACT:** If you have any questions about this study or in case of an emergency, contact Emilie, Study Coordinator, at 513-831-3114, ext. 2324 during business hours (M-F, 8:00 A.M. - 5:00 P.M.) or Ann Brady, Study Manager, at 513-831-3354 after hours. In addition, if you have any questions as to your rights as a research subject, contact the Institutional Review Board of Hill Top Research, Nancy J. Pelc, M.D., Chairman, at 1-513-831-3114.

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**VOLUNTARY PARTICIPATION/WITHDRAWAL**: Your participation in this research study is strictly voluntary. You may refuse to participate or may discontinue participation at any time during the study without penalty or loss of benefits to which you are entitled.

If you agree to participate in this study, you are also agreeing to provide Hill Top Research with accurate information and to follow study instructions as given to you. If you fail to follow study instructions, your participation may be ended.

Your participation in the study may be discontinued at any time without your consent by the Investigator, the IRB, the FDA, or the sponsoring company.

**COMPENSATION**: You will be paid \$10.00 for the completion of this study.

Payment will be made at the end of the study.

There are no anticipated expenses to you for participating in this study. All test related materials will be provided at no cost to you.

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**EXHIBIT B****EVALUATION OF HEALTH CARE PERSONNEL HANDWASH**  
**SUBJECT INSTRUCTIONS**

Today you will be given a kit of products (bar soap, shampoo, and deodorant/antiperspirant) to use exclusively during this study. Please set aside all products you normally use in these categories and use only the products in the kit. In addition, please refrain from using perfumes, deodorants or antiperspirants (other than the ones furnished), powders and anti-dandruff hair shampoos, and do not swim in a chemically treated pool or hot tub during the study.

Beginning today, no body lotions, medicated creams or ointments should be applied to any area of your skin. Also, do not take any antibiotics. These medications may affect the bacteria of the skin. If antibiotics are necessary due to illness, please report this to Hill Top Research at the phone number below.

Please use the rubber gloves provided with the product kit for all household chores involving detergents, acid, alkalis, and solvents until the completion of the study.

---

**SUBJECT SCHEDULE****TEST DAY**

Time of Visit: \_\_\_\_\_

1. Plan to arrive at the laboratory about 10 minutes before your scheduled time. You are expected to be prompt.
2. Please wear clothing that will allow easy access to your hands.
3. You will be required to remove all jewelry, watches, and bracelets before washing.
4. You will undergo a supervised wash regimen at the laboratory.
5. Approximate time at the laboratory -      hours.
6. Additional instructions will be provided for the Follow Up Visit.

**FOLLOW UP VISIT**

Time of Visit: \_\_\_\_\_

1. A Dermatologist will check your hands for infection
2. Approximate time at the lab - 1/2 hour.

If you have any questions regarding this study, please contact Stacey, Study Coordinator, at 831-3114 ext. 2324 between 8:00 a.m. - 5:00 p.m. or Ann Brady, Study Manager, after hours and on weekends at 831-3354.

**EXHIBIT C**

**SUBJECT'S INSTRUCTIONS FOLLOWING STUDY COMPLETION**

You have just completed participation in a clinical study, "Efficacy Evaluation of Health Care Personnel Handwash Products". During this study, your hands were in contact with a liquid containing bacteria (*Serratia marcescens*). Although we do not expect you to have any adverse experience as a result of participation in this study, there is a remote possibility that an infection may develop on your hands.

To determine whether you have developed an infection from the test bacteria, we would like you to examine your hands and wrists daily. If you notice the appearance of any pimples, blisters or raised bumps surrounded by redness and/or swelling, please contact Stacey, Study Coordinator at (513) 831-3114 ext. 2324 during normal business hours (8:00 am- 5 pm) or Ann Brady at (513) 831-3354 after hours.

You are required to return to the test site for a follow-up visit. Your follow-up is scheduled for:

---

Date

Time

Thank you for your cooperation.

## Data Collection Form 1

## DEMOGRAPHICS/DERMATOLOGICAL/MEDICAL HISTORY FORM

Visit Code	Date	Subject Initials	Subject Screen #:	Study #
Subject Qualification	____/____/____ mm dd yy	____/____/____ F M L	Permanent #:	01-109127-11

Gender: <input type="checkbox"/> Male <input type="checkbox"/> Female	Age: _____ Years
---	------------------

Does the subject have any of the following at the treatment sites?

I. DERMATOLOGIC DISORDER	No	Yes	Don't Know
1. Psoriasis ?			
2. Eczema ?			
3. Skin Cancer ?			
4. Skin Allergies ? Please specify:			
5. Hives ?			

Does the Subject have any of the following (present and past)?

II. OTHER MEDICAL INFORMATION	No	Yes	Don't Know
1. Allergies.? Please specify.			
2. Hepatitis ?			
3. Heart and Vascular Disease?			
4. Liver Disease ?			
5. Kidney Disease ?			
6. Tuberculosis ?			
7. Diabetes ? Controlled? Diet [ ] Oral [ ] Insulin [ ]			
8. Cancer ?			
9. Auto-immune disease (Lupus erythematosus, thyroiditis, AIDS, etc.) ?			
10. Organ transplant ?			
11. Any other condition not listed ? Please specify:			

Is the subject taking any medication? If yes, please specify below:

III. MEDICATION	No	Yes	Don't Know
1. Antibiotics, oral or systemic ?			
2. Cortisone, Steroids, ACTH, Anti-reaction Drugs ?			
3. Heart Medication ?			
4. Insulin ?			
5. Other ?			

Comments:

Based on the above medical history, the subject is:  Qualified or  Not qualified for the study.

Interviewer's Signature:	Date: ____ / ____ / ____ mm dd yy
--------------------------	--------------------------------------

**Data Collection Form 2  
INCLUSION / EXCLUSION FORM**

HTR Study No.: 01-109127-11

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<b>Visit Code</b>	<b>Date</b>	<b>Subject Initials</b>	<b>Subject Screen #:</b>	<b>Study #</b>
<b>Subject Qualification</b>	____/____/____ mm dd yy	____/____/____ F M L	<b>Permanent #:</b>	<b>01-109127-11</b>

**INCLUSION CRITERIA**

Check one		
<b>YES</b>	<b>NO</b>	<b>Subject:</b>
		1. Is 18 through 65 years ?
		2. Has signed informed consent ?
		3. Is healthy as evidenced by responses on DCF 1 ?
		4. Has hands and wrists that are free of dermatoses, cuts, lesions, and other skin disorders ?
		5. Has fingernails that extend no longer than approximately one (1) mm past the nail bed ?
		6. Is willing to refrain from using antimicrobial soaps (liquids and/or bars) for bathing, showering, and handwashing during the entire study ?
		7. Is willing to refrain from using anti-dandruff shampoo during the entire study ?
		8. Is willing to refrain from using medicated/antibacterial lotions and creams during the entire study, unless prescribed by a physician for an intercurrent illness ?
		9. Is willing to refrain from using topical steroids during the entire study, unless prescribed by a physician for an intercurrent illness ?
		10. Is willing to refrain from using topical or systemic antibiotic medication during the entire study, unless prescribed by a physician for an intercurrent illness ?
		11. Is willing to comply with all study protocol requirements ?

**EXCLUSION CRITERIA**

Check one			
<b>YES</b>	<b>NO</b>	<b>N/A</b>	<b>Subject:</b>
			1. Is currently participating in another clinical study at this or any other facility ?
			2. Has participated in any type of hand or arm wash study within the past 7 days ?
			3. Has cuts, lesions, or other skin disorders on their hands or wrists ?
			4. Has artificial nails or nail tips?
			5. Has soap, detergent, and/or perfume allergies ?
			6. Has eczema or psoriasis on their hands or wrists ?
Female	Female	Male	7. Is currently pregnant ? <input type="checkbox"/> Yes <input type="checkbox"/> No Of child-bearing potential: <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Surgically Sterile, year _____ <input type="checkbox"/> Post-menopausal If of child bearing potential - $\beta$ -HCG Test Results: <input type="checkbox"/> negative <input type="checkbox"/> positive
			8. Is currently lactating ? <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> NA
			9. Has been medically diagnosed as having a medical condition such as: diabetes, hepatitis, an organ transplant, or AIDS (or HIV positive) ?
			10. Has another medical condition which in the opinion of the Investigator would preclude participation ?

Based upon dermatologic evaluation and the information contained in Data Collection Forms 1 and 2, the subject is:  
 **Qualified**     **Not Qualified**    for participation in this study.

Reasons for disqualification: \_\_\_\_\_ Interviewer's Initials/Date: \_\_\_\_\_ / \_\_\_\_\_

Investigator's Signature: _____	Date: _____ / _____ / _____ mm                  dd                  yy
---------------------------------	---

## Data Collection Form 3

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## INTERCURRENT ILLNESS / CONCOMITANT MEDICATION FORM

Visit Code	Date	Subject Initials	Subject Screen #:	Study #
Test Period	____/____/____ mm dd yy	____/____/____ F M L	Permanent #:	01-109127-11

I. Is skin on subject's hands and wrists still free of dermatoses, cuts, lesions, and other skin disorders?  Yes  No

If no, please indicate condition: \_\_\_\_\_

II. Has subject used non-antibacterial soap and followed the instructions in Appendix B?  Yes  No

If no, please explain: \_\_\_\_\_

III. Has subject been ill since the last visit?  Yes (Complete below)  No

IV. Has subject used any oral or topical medication?  Yes (Complete below)  No

Based upon the above responses, the subject is:  Qualified  Not Qualified to continue on the study.

Reasons for disqualification: \_\_\_\_\_

## TO BE COMPLETED IF SUBJECT HAS AN INTERCURRENT ILLNESS

Date of Onset: \_\_\_\_\_ Date Reported: \_\_\_\_\_ Date Resolved: \_\_\_\_\_

Describe condition: \_\_\_\_\_

Was reaction related to treatment?  Not related  Possibly related  Definitely related  Other (explain)

Action Taken:  None  Continued on study  Withdrawn from the study  Consulted physician

Medication taken (Complete below)  Hospitalized  Other (explain)

Additional Comments: \_\_\_\_\_

## CONCOMITANT MEDICATION

Medication (Oral or Systemic)	Total Daily Dose	Start Date mm / dd / yy	Stop Date mm / dd / yy	Indication (Reason for Taking)
		/ /	/ /	
		/ /	/ /	
		/ /	/ /	

Comments:

Interviwer's Signature:	Date: ____/____/____ mm dd yy
-------------------------	----------------------------------

**HEALTH CARE PERSONNEL HANDWASH BACTERIAL COUNTS**

**CFU/mL of Sampling Solution**

Test Date	Subject Initials	Subject Screen #	Study #
<u>   </u> / <u>   </u> / <u>   </u> mm dd yy	<u>   </u> / <u>   </u> / <u>   </u> F M L	Permanent #:	01-109127-11

BASELINE					
LEFT HAND DILUTIONS			RIGHT HAND DILUTIONS		
10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>
CFU/mL _____ Counted by : _____ / _____			CFU/mL _____ Counted by : _____ / _____		

LEFT HAND				WASH 1				RIGHT HAND							
10 <sup>-1*</sup>		10 <sup>-2</sup>		10 <sup>-3</sup>		10 <sup>-4</sup>		10 <sup>-1*</sup>		10 <sup>-2</sup>		10 <sup>-3</sup>		10 <sup>-4</sup>	
CFU/mL _____ Counted by : _____ / _____								CFU/mL _____ Counted by : _____ / _____							

LEFT HAND				WASH 11				RIGHT HAND							
10 <sup>-1*</sup>		10 <sup>-2</sup>		10 <sup>-3</sup>		10 <sup>-4</sup>		10 <sup>-1*</sup>		10 <sup>-2</sup>		10 <sup>-3</sup>		10 <sup>-4</sup>	
CFU/mL _____ Counted by : _____ / _____								CFU/mL _____ Counted by : _____ / _____							

Calculations by: \_\_\_\_\_ / \_\_\_\_\_ Raw data reviewed by \_\_\_\_\_ / \_\_\_\_\_  
 Calculations Verified by: \_\_\_\_\_ / \_\_\_\_\_

\*10<sup>-1</sup> dilution is the sum of 1.0 mL spread across 3 plates in duplicate.  
 TNTC – Too Numerous To Count

Investigator's Signature: _____	Date: _____ / _____ / _____ mm dd yy
---------------------------------	---

Data Collection Form 5A

Subject Initials \_\_\_\_\_ Subject # \_\_\_\_\_ HTR Study No. 01-109127-11  
 Page No. II-33

**ADVERSE EVENTS**

Symptom / Event	Onset Date	End Date	SAE <sup>1</sup> Y/N	Severity	Action Taken	Outcome	Relation-ship	Investigator Signature/Date
Entry Date	Comment/Note:							Initials

Symptom / Event	Onset Date	End Date	SAE <sup>1</sup> Y/N	Severity	Action Taken	Outcome	Relation-ship	Investigator Signature/Date
Entry Date	Comment/Note:							Initials

Symptom / Event	Onset Date	End Date	SAE <sup>1</sup> Y/N	Severity	Action Taken	Outcome	Relation-ship	Investigator Signature/Date
Entry Date	Comment/Note:							Initials

Note: Severity, Relationship and Outcome MUST be determined by principal investigator.

- Severity: 1=Mild 2=Moderate 3=Severe
- Relationship: 1=Definite 2=Probable 3=Possible 4=Unrelated
- Action Taken: 1=None 2=Rx Therapy 3=Discontinued Study 4=Other (specify)
- Outcome: 1=Resolved w/o sequelae 2=Resolved w/ sequelae (describe) 3=Ongoing 4=Death

<sup>1</sup>Serious Adverse Event/Experience



FOLLOW-UP VISIT

Visit Code	Date	Subject Initials	Subject Screen #:	Study #
Follow-up Visit	____/____/____ mm dd yy	____/____/____ F M L	Permanent #:	01-109127-11

Date Subject Entered the Study: ____/____/____ mm dd yy	Follow-Up Visit Date: ____/____/____ mm dd yy
---	---

Does the subject's hands have the presence of pimples, blisters, or raised itching bumps surrounded by erythema and/or edema that may be indicative of a skin infection?

YES     NO    If yes, complete below:

Clinical Observations: (Include date of onset and descriptions/severity/locations, etc.)

---



---



---

Comments:

---



---



---

Has the subject had any health related issues since the treatment procedure?

YES     NO    If yes, complete below

Comments:

---



---



---

Medical Consultant's Signature:		Date ____/____/____ mm dd yy
---------------------------------	--	------------------------------------

HTR Study No.: 01-109127-11

## APPENDIX III

Total number of pages = 1

**Miscellaneous Procedural Information**

## MISCELLANEOUS PROCEDURAL INFORMATION

1. **Stripping Fluid with Neutralizer**  
The stripping fluid with neutralizer used for sampling contained 0.41g  $\text{KH}_2\text{PO}_4$ , 10.3 g  $\text{Na}_2\text{HPO}_4$ , 3.0 g Lecithin, 10 g Tween 80, and 1.0 g Triton X-100 in one liter purified water. The pH was adjusted to  $7.8 \pm 0.1$  prior to dispensing into water dilution bottles, or other suitable containers, to yield a final volume of  $75 \pm 1.0$  mL after autoclaving at  $121^\circ\text{C}$ .
2. **Dilution Fluid: Butterfield's Phosphate Buffer with Neutralizer**  
The dilution fluid contained 1.25 mL AOAC Phosphate Buffer Stock\*, 10.0 g Tween 80, and 3.0 g Lecithin in one liter purified water. The pH was adjusted to  $7.2 \pm 0.2$  prior to dispensing into tubes, to yield a final volume of  $9.0 \pm 0.1$  mL after autoclaving at  $121^\circ\text{C}$ .
3. **Tryptic Soy Broth**  
The broth contained 30g Tryptic Soy Broth powder in one liter purified water. The media was dispensed into sterile tubes, to yield a final volume of  $9 \pm 0.1$  mL after autoclaving at  $121^\circ\text{C}$ .
4. **Tryptic Soy Agar**  
The plating medium contained 40 g Trypticase Soy Agar in one liter purified water. The pH was  $7.3 \pm 0.2$ . The media was autoclaved at  $121^\circ\text{C}$ . After autoclaving and tempering, the media was aseptically dispensed into sterile Petri dishes, approximately 18 mL per plate.

\* The AOAC Phosphate Buffer Stock contained 34 g  $\text{KH}_2\text{PO}_4$  in one liter of purified water. The pH was adjusted to 7.2 prior to dispensing into water dilution bottles, to yield a final volume of approximately 100 mL after autoclaving at  $121^\circ\text{C}$ .

**Note:** Recipes which are given as liter volumes may be prepared in greater or lesser volumes.

**Subjects Excluded/Withdrawn from the Study**

<b>Subject Screening No.</b>	<b>Reason</b>
107, 109, 110, 114, 118, 123, 128, 134, 136, 137, 138, 139, 143, 148, 151, 160, 161, 167, 172, 176, 181	Subjects withdrew prior to test day – personal reasons
112, 130, 146, 179, 180	Subjects excluded – cut/scratch on hand or wrist
113	Subject excluded – positive on pregnancy test
132, 133	Subjects excluded – over age limit
181	Subject excluded – unwilling to participate
122, 175	Subjects excluded – late on test day
102, 121, 177	Subjects excluded – extra subject

HTR Study No.: 01-109127-11

## APPENDIX VI

Total number of pages = 4

**Test for Adequacy of Neutralizer**

HTR Study No.: 01-109127-11

## TEST FOR ADEQUACY OF THE NEUTRALIZER

### 1.0 OBJECTIVE

To determine an appropriate antimicrobial neutralizer system for use in a Health Care Personnel Handwash study, HTR Study No. 01-109127-11.

### 2.0 TEST ARTICLES

The following test article assigned Hill Top Research Code HTR A and identified as follows was received from the [REDACTED] Ciba Specialty Chemicals Corporation on August 2, 2001 for evaluation in this study. The test article assigned HTR B was purchased by Hill Top Research on July 2, 2001 for use in the study:

HTR Code	Sponsor Code	Description
A	3456-38, [REDACTED]	[REDACTED] liquid
B	[REDACTED]®, Lot: 4563C, Exp: 12/02	[REDACTED] quart bottle with [REDACTED] [REDACTED] containing liquid

### 3.0 PROCEDURE

Prior to washing with the test articles, two subjects performed a conditioning wash using Baby San Soap according to the protocol directions. The subjects then washed their hands eleven times, using either HTR Code A or HTR Code B, according to the protocol directions. The subjects' hands were sampled after treatment 1, using stripping fluid with neutralizer<sup>1</sup> according to the protocol directions and the samples were discarded. Following the sampling after treatment 1, the subjects' hands were rinsed with tap water and dried. The subjects also dried after treatments 2 through 10 with at least five minutes elapsing between treatments. One hand was sampled after the 11<sup>th</sup> treatment using stripping fluid with neutralizer. Aliquots from the subjects' stripping fluid were removed as follows and used to test the adequacy of the neutralizer: A 10.0 mL aliquot of the stripping fluid with neutralizer was removed and placed in a sterile tube. An additional 1.0 mL aliquot of the stripping fluid with neutralizer was added to a tube containing 9.0 mL of diluent with neutralizer<sup>2</sup>.

A 0.1 mL aliquot of diluted *Serratia marcescens* ATCC 14756 was added to each of the prepared tubes. The diluted culture was a 24 ± 4 hour Tryptic Soy Broth<sup>3</sup> culture of *S. marcescens* ATCC 14756 serially diluted to 10<sup>-5</sup> in 0.9% saline<sup>4</sup>.

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### **3.0 PROCEDURE (CONT.)**

After mixing, a 1.0 mL aliquot from each inoculated tube was surface plated immediately and again at 30 minutes by distributing the 1.0 mL across three Tryptic Soy Agar<sup>5</sup> plates in duplicate. The plates were incubated at  $25 \pm 2^\circ\text{C}$  for  $48 \pm 4$  hours. After incubation, the numbers of *S. marcescens* colony forming units were enumerated.

Number and toxicity control tubes were also prepared. The numbers control consisted of 10.0 mL 0.9% saline. Two different toxicity control tubes were prepared. One tube contained 10.0 mL stripping fluid with neutralizer and the second tube contained 10.0 mL diluent with neutralizer. These control tubes were inoculated, plated, incubated, and enumerated in the same manner as the collected samples.

### **4.0 RESULTS**

Results are shown in the Table of Results.

### **5.0 CONCLUSIONS**

The neutralizer system is considered effective if recovery is  $\geq 75\%$  of the corresponding numbers control. In this study the neutralizer system adequately neutralized the active ingredients in the test product.

**TABLES OF RESULTS**  
**RECOVERY OF *S. MARCESCENS* ATCC 14756**

ARTICLE	TIME	PLATE COUNTS*						AVG. CFU/mL	% RECOVERY
		63	63	66	61	69	53		
Numbers Control 10.0 mL Saline	0 min.	63	63	66	61	69	53	$1.9 \times 10^2$	NA**
	30 min.	67	75	95	122	78	61	$2.5 \times 10^2$	NA
Toxicity Control 10.0 mL Stripping Fluid with Neut.	0 min.	99	79	168	68	122	81	$3.1 \times 10^2$	163
	30 min.	76	97	99	72	80	85	$2.5 \times 10^2$	100
Toxicity Control 10.0 mL Diluent with Neut.	0 min.	93	84	82	76	90	68	$2.5 \times 10^2$	132
	30 min.	53	66	57	69	75	59	$1.9 \times 10^2$	76
HTR Code A 10. mL Stripping Fluid with Neut.	0 min.	87	87	65	130	82	52	$2.5 \times 10^2$	132
	30 min.	68	69	69	67	96	62	$2.2 \times 10^2$	88
HTR Code A 1.0 mL Stripping Fluid w/ Neut. into 9.0 mL Diluent w/ Neut.	0 min.	66	86	90	59	56	72	$2.1 \times 10^2$	110
	30 min.	68	72	58	65	55	76	$2.0 \times 10^2$	80
HTR Code B 10.0 mL Stripping Fluid w/ Neut.	0min.	65	82	82	87	87	76	$2.4 \times 10^2$	126
	30 min.	74	68	79	50	76	55	$2.0 \times 10^2$	80
HTR Code B 1.0 mL Stripping Fluid w/ Neut. into 9.0 mL Diluent w/ Neut.	0min.	61	70	72	75	72	59	$2.0 \times 10^2$	105
	30 min.	72	58	58	82	59	60	$1.9 \times 10^2$	76

\*CFU/mL is the sum of 1.0 mL spread across three plates.

\*\*NA = Not Applicable

$$0 \text{ Minute \% Recovery} = \frac{\text{Count at 0 min.}}{\text{Numbers Control Count at 0 min.}} \times 100$$

$$30 \text{ Minute \% Recovery} = \frac{\text{Count at 30 min.}}{\text{Numbers Control Count at 30 min.}} \times 100$$

### REFERENCES

1. **Stripping Fluid with Neutralizer**  
The stripping fluid with neutralizer used for sampling contained 0.41 g  $\text{KH}_2\text{PO}_4$ , 10.3 g  $\text{Na}_2\text{HPO}_4$ , 3.0 g Lecithin, 10.0g Tween 80, and 1.0 g Triton X-100 in one liter purified water. The pH was adjusted to  $7.8 \pm 0.1$  prior to dispensing into water dilution bottles, or other suitable containers, to yield a final volume of  $75 \pm 1.0$  mL after autoclaving at  $121^\circ\text{C}$ .
2. **Dilution Fluid: Butterfield's Phosphate Buffer Water with Neutralizer**  
The dilution fluid contained 1.25 mL AOAC Phosphate Buffer Stock\*, 10.0 g Tween 80, and 3.0 g Lecithin in one liter purified water. The pH was adjusted to  $7.2 \pm 0.2$  prior to dispensing into tubes, to yield a final volume of  $9.0 \pm 0.1$  mL after autoclaving at  $121^\circ\text{C}$ .
3. **Tryptic Soy Broth**  
The broth consisted of 30.0 g Tryptic Soy Broth powder in one liter purified water. The pH was  $7.3 \pm 0.2$ . The media was dispensed into tubes and sterilized by autoclaving at  $121^\circ\text{C}$ .
4. **0.9% Saline**  
The saline contained 9.0 g NaCl in one liter purified water. The material was dispensed into tubes to yield a final volume of  $9.0 \pm 0.1$  mL after autoclaving at  $121^\circ\text{C}$ . For the numbers control, the volume of saline was aseptically adjusted to 10.0 mL.
5. **Tryptic Soy Agar**  
The plating medium contained 40.0 g Tryptic Soy Agar powder in one liter purified water. The pH was  $7.3 \pm 0.2$ . The media was autoclaved at  $121^\circ\text{C}$ . After autoclaving and tempering, the media was aseptically dispensed into sterile Petri dishes, approximately 18 - 20 mL per plate.

\*The AOAC Phosphate Buffer Stock contained 34.0 g  $\text{KH}_2\text{PO}_4$  in one liter purified water. The pH was adjusted to  $7.2 \pm 0.1$  prior to dispensing into bottles, to yield a final volume of approximately 100 mL after autoclaving at  $121^\circ\text{C}$ .

**Note:** Recipes which are given as liter volumes may be prepared in greater or lesser volumes.

## APPENDIX VII

Total number of pages = 19

### **Statistical Tables**

Table 1	Summary of CFU counts and log conversions
Table 2A	Mean summary of $\log_{10}$ averages and $\log_{10}$ reductions from baseline
Table 3	Mean $\log_{10}$ reductions from baseline, percent microbial reductions from baseline and the 95% confidence limits
Table 4	Analysis of variance for the comparison of the test article baseline counts
Table 4	Analysis of variance to evaluate the effectiveness of each treatment as a function of the number of treatments

Hill Top Research Study Number 01-109127-11  
 Table 1. Summary of CFU counts and log conversions.

----- HTR Code=A: Lot Code 3456-38 Foaming handwash -----

Subject	Wash	Left Hand			Right Hand			Log10 Average	Log10 Reduction
		CFU/mL	CFU/Hand	Log10 CFU/Hand	CFU/mL	CFU/Hand	Log10 CFU/Hand		
2	Baseline	2.0E+07	1.5E+09	9.1761	2.8E+07	2.1E+09	9.3222	9.2492	
	Wash 1	2.5E+04	1.9E+06	6.2730	3.5E+04	2.6E+06	6.4191	6.3461	2.9031
	Wash 11	3.6E+04	2.7E+06	6.4314	5.3E+04	4.0E+06	6.5993	6.5154	2.7338
4	Baseline	2.5E+07	1.9E+09	9.2730	2.4E+07	1.8E+09	9.2553	9.2641	
	Wash 1	9.7E+03	7.3E+05	5.8618	3.3E+03	2.5E+05	5.3936	5.6277	3.6364
	Wash 11	6.7E+03	5.0E+05	5.7011	2.4E+03	1.8E+05	5.2553	5.4782	3.7859
7	Baseline	1.9E+07	1.4E+09	9.1538	1.7E+07	1.3E+09	9.1055	9.1297	
	Wash 1	6.0E+03	4.5E+05	5.6532	1.3E+04	9.8E+05	5.9890	5.8211	3.3086
	Wash 11	7.0E+04	5.3E+06	6.7202	7.0E+04	5.3E+06	6.7202	6.7202	2.4095
11	Baseline	2.6E+07	2.0E+09	9.2900	2.5E+07	1.9E+09	9.2730	9.2815	
	Wash 1	9.5E+03	7.1E+05	5.8528	1.7E+04	1.3E+06	6.1055	5.9791	3.3024
	Wash 11	1.8E+04	1.4E+06	6.1303	6.1E+04	4.6E+06	6.6604	6.3954	2.8862
12	Baseline	3.9E+07	2.9E+09	9.4661	3.4E+07	2.6E+09	9.4065	9.4363	
	Wash 1	1.7E+04	1.3E+06	6.1055	1.5E+04	1.1E+06	6.0512	6.0783	3.3580
	Wash 11	2.0E+04	1.5E+06	6.1761	1.4E+04	1.1E+06	6.0212	6.0986	3.3377
13	Baseline	2.7E+07	2.0E+09	9.3064	2.8E+07	2.1E+09	9.3222	9.3143	
	Wash 1	9.5E+03	7.1E+05	5.8528	8.7E+03	6.5E+05	5.8146	5.8337	3.4806
	Wash 11	2.3E+04	1.7E+06	6.2368	2.6E+04	2.0E+06	6.2900	6.2634	3.0509
14	Baseline	2.8E+07	2.1E+09	9.3222	3.6E+07	2.7E+09	9.4314	9.3768	
	Wash 1	2.5E+04	1.9E+06	6.2730	2.4E+04	1.8E+06	6.2553	6.2641	3.1127
	Wash 11	6.5E+04	4.9E+06	6.6880	9.6E+04	7.2E+06	6.8573	6.7727	2.6041
16	Baseline	2.3E+07	1.7E+09	9.2368	2.2E+07	1.7E+09	9.2175	9.2271	
	Wash 1	1.5E+04	1.1E+06	6.0512	1.0E+04	7.5E+05	5.8751	5.9631	3.2640
	Wash 11	9.0E+04	6.8E+06	6.8293	1.3E+05	9.8E+06	6.9890	6.9092	2.3180
17	Baseline	2.4E+07	1.8E+09	9.2553	2.5E+07	1.9E+09	9.2730	9.2641	
	Wash 1	3.6E+04	2.7E+06	6.4314	3.1E+04	2.3E+06	6.3664	6.3989	2.8652
	Wash 11	2.8E+04	2.1E+06	6.3222	4.6E+04	3.5E+06	6.5378	6.4300	2.8341
18	Baseline	2.7E+07	2.0E+09	9.3064	2.6E+07	2.0E+09	9.2900	9.2982	
	Wash 1	1.5E+04	1.1E+06	6.0512	2.2E+04	1.7E+06	6.2175	6.1343	3.1639
	Wash 11	8.7E+04	6.5E+06	6.8146	8.5E+04	6.4E+06	6.8045	6.8095	2.4887
19	Baseline	2.6E+07	2.0E+09	9.2900	2.8E+07	2.1E+09	9.3222	9.3061	
	Wash 1	3.7E+04	2.8E+06	6.4433	3.8E+04	2.9E+06	6.4548	6.4491	2.8571
	Wash 11	1.6E+05	1.2E+07	7.0792	1.1E+05	8.3E+06	6.9165	6.9978	2.3083

Hill Top Research Study Number 01-109127-11  
 Table 1. Summary of CFU counts and log conversions.

----- HTR Code=A: Lot Code 3456-38 Foaming handwash -----

Subject	Wash	Left Hand			Right Hand			Log10 Average	Log10 Reduction
		CFU/mL	CFU/Hand	Log10 CFU/Hand	CFU/mL	CFU/Hand	Log10 CFU/Hand		
20	Baseline	3.7E+07	2.8E+09	9.4433	2.5E+07	1.9E+09	9.2730	9.3581	
	Wash 1	6.3E+03	4.7E+05	5.6744	3.1E+03	2.3E+05	5.3664	5.5204	3.8377
	Wash 11	2.0E+04	1.5E+06	6.1761	2.2E+04	1.7E+06	6.2175	6.1968	3.1613
22	Baseline	2.6E+07	2.0E+09	9.2900	3.3E+07	2.5E+09	9.3936	9.3418	
	Wash 1	1.4E+04	1.1E+06	6.0212	1.0E+04	7.5E+05	5.8751	5.9481	3.3937
	Wash 11	2.4E+03	1.8E+05	5.2553	1.3E+03	9.8E+04	4.9890	5.1221	4.2197
24	Baseline	2.3E+07	1.7E+09	9.2368	2.5E+07	1.9E+09	9.2730	9.2549	
	Wash 1	6.6E+04	5.0E+06	6.6946	4.0E+04	3.0E+06	6.4771	6.5859	2.6690
	Wash 11	5.2E+04	3.9E+06	6.5911	2.5E+04	1.9E+06	6.2730	6.4320	2.8229
25	Baseline	2.6E+07	2.0E+09	9.2900	3.0E+07	2.3E+09	9.3522	9.3211	
	Wash 1	6.7E+03	5.0E+05	5.7011	1.2E+04	9.0E+05	5.9542	5.8277	3.4934
	Wash 11	1.4E+04	1.1E+06	6.0212	1.6E+04	1.2E+06	6.0792	6.0502	3.2709
26	Baseline	1.2E+07	9.0E+08	8.9542	1.4E+07	1.1E+09	9.0212	8.9877	
	Wash 1	1.3E+03	9.8E+04	4.9890	9.5E+02	7.1E+04	4.8528	4.9209	4.0668
	Wash 11	7.1E+03	5.3E+05	5.7263	1.4E+04	1.1E+06	6.0212	5.8738	3.1140
28	Baseline	2.4E+07	1.8E+09	9.2553	2.6E+07	2.0E+09	9.2900	9.2727	
	Wash 1	2.6E+04	2.0E+06	6.2900	2.6E+04	2.0E+06	6.2900	6.2900	2.9826
	Wash 11	5.2E+04	3.9E+06	6.5911	4.9E+04	3.7E+06	6.5653	6.5782	2.6945
29	Baseline	1.7E+07	1.3E+09	9.1055	1.5E+07	1.1E+09	9.0512	9.0783	
	Wash 1	8.0E+03	6.0E+05	5.7782	1.0E+04	7.5E+05	5.8751	5.8266	3.2517
	Wash 11	4.8E+04	3.6E+06	6.5563	4.7E+04	3.5E+06	6.5472	6.5517	2.5266
30	Baseline	2.4E+07	1.8E+09	9.2553	2.4E+07	1.8E+09	9.2553	9.2553	
	Wash 1	2.2E+04	1.7E+06	6.2175	9.8E+04	7.4E+06	6.8663	6.5419	2.7134
	Wash 11	7.2E+04	5.4E+06	6.7324	1.2E+05	9.0E+06	6.9542	6.8433	2.4120
31	Baseline	3.0E+07	2.3E+09	9.3522	3.2E+07	2.4E+09	9.3802	9.3662	
	Wash 1	1.4E+04	1.1E+06	6.0212	9.8E+03	7.4E+05	5.8663	5.9437	3.4225
	Wash 11	2.0E+04	1.5E+06	6.1761	2.5E+04	1.9E+06	6.2730	6.2245	3.1417
32	Baseline	3.0E+07	2.3E+09	9.3522	2.4E+07	1.8E+09	9.2553	9.3037	
	Wash 1	2.1E+04	1.6E+06	6.1973	7.2E+03	5.4E+05	5.7324	5.9648	3.3389
	Wash 11	2.9E+04	2.2E+06	6.3375	2.1E+04	1.6E+06	6.1973	6.2674	3.0364
34	Baseline	2.2E+07	1.7E+09	9.2175	2.6E+07	2.0E+09	9.2900	9.2538	
	Wash 1	1.9E+04	1.4E+06	6.1538	2.1E+04	1.6E+06	6.1973	6.1755	3.0782
	Wash 11	6.2E+04	4.7E+06	6.6675	5.3E+04	4.0E+06	6.5993	6.6334	2.6204

Hill Top Research Study Number 01-109127-11  
 Table 1. Summary of CFU counts and log conversions.

----- HTR Code=A: Lot Code 3456-38 Foaming handwash -----

Subject	Wash	Left Hand			Right Hand			Log10 Average	Log10 Reduction
		CFU/mL	CFU/Hand	Log10 CFU/Hand	CFU/mL	CFU/Hand	Log10 CFU/Hand		
35	Baseline	1.9E+07	1.4E+09	9.1538	2.0E+07	1.5E+09	9.1761	9.1650	
	Wash 1	1.8E+04	1.4E+06	6.1303	1.1E+04	8.3E+05	5.9165	6.0234	3.1416
	Wash 11	1.2E+05	9.0E+06	6.9542	9.6E+04	7.2E+06	6.8573	6.9058	2.2592
36	Baseline	2.8E+07	2.1E+09	9.3222	2.4E+07	1.8E+09	9.2553	9.2887	
	Wash 1	8.2E+03	6.2E+05	5.7889	5.7E+03	4.3E+05	5.6309	5.7099	3.5788
	Wash 11	5.2E+04	3.9E+06	6.5911	2.7E+04	2.0E+06	6.3064	6.4487	2.8400
37	Baseline	1.4E+07	1.1E+09	9.0212	1.4E+07	1.1E+09	9.0212	9.0212	
	Wash 1	5.3E+03	4.0E+05	5.5993	8.2E+03	6.2E+05	5.7889	5.6941	3.3271
	Wash 11	3.1E+04	2.3E+06	6.3664	3.8E+05	2.9E+07	7.4548	6.9106	2.1106
38	Baseline	2.7E+07	2.0E+09	9.3064	2.2E+07	1.7E+09	9.2175	9.2620	
	Wash 1	6.8E+04	5.1E+06	6.7076	2.8E+04	2.1E+06	6.3222	6.5149	2.7471
	Wash 11	7.8E+04	5.9E+06	6.7672	8.4E+04	6.3E+06	6.7993	6.7832	2.4787
39	Baseline	2.3E+07	1.7E+09	9.2368	2.0E+07	1.5E+09	9.1761	9.2064	
	Wash 1	2.6E+04	2.0E+06	6.2900	2.6E+04	2.0E+06	6.2900	6.2900	2.9164
	Wash 11	1.2E+05	9.0E+06	6.9542	1.1E+05	8.3E+06	6.9165	6.9353	2.2711
41	Baseline	2.4E+07	1.8E+09	9.2553	2.6E+07	2.0E+09	9.2900	9.2727	
	Wash 1	1.4E+04	1.1E+06	6.0212	2.5E+04	1.9E+06	6.2730	6.1471	3.1256
	Wash 11	5.8E+04	4.4E+06	6.6385	6.3E+04	4.7E+06	6.6744	6.6564	2.6162
44	Baseline	9.9E+06	7.4E+08	8.8707	1.2E+07	9.0E+08	8.9542	8.9125	
	Wash 1	6.7E+03	5.0E+05	5.7011	4.2E+03	3.2E+05	5.4983	5.5997	3.3127
	Wash 11	1.3E+04	9.8E+05	5.9890	2.2E+04	1.7E+06	6.2175	6.1032	2.8092
45	Baseline	2.4E+07	1.8E+09	9.2553	2.4E+07	1.8E+09	9.2553	9.2553	
	Wash 1	3.7E+04	2.8E+06	6.4433	1.9E+04	1.4E+06	6.1536	6.2985	2.9567
	Wash 11	2.2E+04	1.7E+06	6.2175	2.1E+04	1.6E+06	6.1973	6.2074	3.0479

Hill Top Research Study Number 01-109127-11  
 Table 1. Summary of CFU counts and log conversions.

----- HTR Code=B: Lot Code 4563C Hibiclens -----

Subject	Wash	Left Hand			Right Hand			Log10 Average	Log10 Reduction
		CFU/mL	CFU/Hand	Log10 CFU/Hand	CFU/mL	CFU/Hand	Log10 CFU/Hand		
1	Baseline	2.6E+07	2.0E+09	9.2900	2.7E+07	2.0E+09	9.3064	9.2982	
	Wash 1	2.3E+04	1.7E+06	6.2368	5.3E+04	4.0E+06	6.5993	6.4181	2.8802
	Wash 11	4.0E+03	3.0E+05	5.4771	1.0E+04	7.5E+05	5.8751	5.6761	3.6221
3	Baseline	3.4E+07	2.6E+09	9.4065	2.6E+07	2.0E+09	9.2900	9.3483	
	Wash 1	3.2E+04	2.4E+06	6.3802	5.8E+04	4.4E+06	6.6385	6.5094	2.8389
	Wash 11	1.5E+04	1.1E+06	6.0512	2.1E+04	1.6E+06	6.1973	6.1242	3.2241
5	Baseline	2.1E+07	1.6E+09	9.1973	2.2E+07	1.7E+09	9.2175	9.2074	
	Wash 1	1.2E+05	9.0E+06	6.9542	1.0E+05	7.5E+06	6.8751	6.9147	2.2927
	Wash 11	9.2E+03	6.9E+05	5.8388	8.8E+03	6.6E+05	5.8195	5.8292	3.3782
6	Baseline	2.3E+07	1.7E+09	9.2368	3.2E+07	2.4E+09	9.3802	9.3085	
	Wash 1	1.0E+05	7.5E+06	6.8751	1.0E+05	7.5E+06	6.8751	6.8751	2.4334
	Wash 11	1.6E+04	1.2E+06	6.0792	1.9E+04	1.4E+06	6.1538	6.1165	3.1920
8	Baseline	2.5E+07	1.9E+09	9.2730	2.1E+07	1.6E+09	9.1973	9.2351	
	Wash 1	6.7E+04	5.0E+06	6.7011	5.7E+04	4.3E+06	6.6309	6.6660	2.5691
	Wash 11	1.3E+04	9.8E+05	5.9890	4.2E+03	3.2E+05	5.4983	5.7437	3.4915
9	Baseline	2.6E+07	2.0E+09	9.2900	2.7E+07	2.0E+09	9.3064	9.2982	
	Wash 1	5.2E+04	3.9E+06	6.5911	7.5E+04	5.6E+06	6.7501	6.6706	2.6276
	Wash 11	3.0E+03	2.3E+05	5.3522	4.1E+03	3.1E+05	5.4878	5.4200	3.8782
10	Baseline	2.0E+07	1.5E+09	9.1761	1.9E+07	1.4E+09	9.1538	9.1650	
	Wash 1	3.5E+04	2.6E+06	6.4191	6.3E+04	4.7E+06	6.6744	6.5468	2.6182
	Wash 11	5.5E+03	4.1E+05	5.6154	6.3E+03	4.7E+05	5.6744	5.6449	3.5200
15	Baseline	2.6E+07	2.0E+09	9.2900	2.4E+07	1.8E+09	9.2553	9.2727	
	Wash 1	5.8E+04	4.4E+06	6.6385	1.6E+05	1.2E+07	7.0792	6.8588	2.4138
	Wash 11	2.3E+04	1.7E+06	6.2368	1.4E+04	1.1E+06	6.0212	6.1290	3.1437
21	Baseline	2.2E+07	1.7E+09	9.2175	1.7E+07	1.3E+09	9.1055	9.1615	
	Wash 1	4.9E+04	3.7E+06	6.5653	5.5E+04	4.1E+06	6.6154	6.5903	2.5712
	Wash 11	4.3E+03	3.2E+05	5.5085	1.8E+04	1.4E+06	6.1303	5.8194	3.3421
23	Baseline	1.9E+07	1.4E+09	9.1538	2.3E+07	1.7E+09	9.2368	9.1953	
	Wash 1	5.2E+04	3.9E+06	6.5911	2.0E+04	1.5E+06	6.1761	6.3836	2.8117
	Wash 11	7.2E+03	5.4E+05	5.7324	2.8E+03	2.1E+05	5.3222	5.5273	3.6680
27	Baseline	2.1E+07	1.6E+09	9.1973	2.1E+07	1.6E+09	9.1973	9.1973	
	Wash 1	7.8E+04	5.9E+06	6.7672	8.0E+04	6.0E+06	6.7782	6.7727	2.4246
	Wash 11	4.3E+03	3.2E+05	5.5085	5.7E+03	4.3E+05	5.6309	5.5697	3.6275

HTR Study No.: 01-109127-11

Hill Top Research Study Number 01-109127-11  
 Table 1. Summary of CFU counts and log conversions.

----- HTR Code=B: Lot Code 4563C Hibiclens -----

Subject	Wash	Left Hand			Right Hand			Log10 Average	Log10 Reduction
		CFU/ml	CFU/Hand	Log10 CFU/Hand	CFU/ml	CFU/Hand	Log10 CFU/Hand		
33	Baseline	2.0E+07	1.5E+09	9.1761	1.6E+07	1.2E+09	9.0792	9.1276	
	Wash 1	1.4E+05	1.1E+07	7.0212	7.8E+04	5.9E+06	6.7672	6.8942	2.2335
	Wash 11	6.9E+03	5.2E+05	5.7139	7.2E+03	5.4E+05	5.7324	5.7232	3.4045
40	Baseline	3.0E+07	2.3E+09	9.3522	2.5E+07	1.9E+09	9.2730	9.3126	
	Wash 1	3.4E+04	2.6E+06	6.4065	4.7E+04	3.5E+06	6.5472	6.4768	2.8357
	Wash 11	9.6E+03	7.2E+05	5.8573	7.9E+03	5.9E+05	5.7727	5.8150	3.4976
42	Baseline	3.0E+07	2.3E+09	9.3522	2.4E+07	1.8E+09	9.2553	9.3037	
	Wash 1	3.0E+04	2.3E+06	6.3522	4.8E+04	3.6E+06	6.5563	6.4542	2.8495
	Wash 11	2.6E+03	2.0E+05	5.2900	6.8E+03	5.1E+05	5.7076	5.4988	3.8049
43	Baseline	1.8E+07	1.4E+09	9.1303	1.8E+07	1.4E+09	9.1303	9.1303	
	Wash 1	5.7E+04	4.3E+06	6.6309	3.2E+04	2.4E+06	6.3802	6.5056	2.6248
	Wash 11	1.0E+04	7.5E+05	5.8751	5.7E+03	4.3E+05	5.6309	5.7530	3.3773

HTR Study No.: 01-109127-11

Hill Top Research Study Number 01-109127-11  
 Table 2. Means summary of the log 10 averages and log 10 reductions from baseline.

		Baseline		Wash 1		Wash 11	
		Log10 Average	Log10 Reduction	Log10 Average	Log10 Reduction	Log10 Average	Log10 Reduction
HTR Code:							
A: Lot Code 3456-38 Foaming handwash	Mean	9.2442	ND	6.0240	3.2202	6.4372	2.8070
	Std. Dev.	0.1165	ND	0.3605	0.3267	0.4367	0.4644
	N	30	0	30	30	30	30
B: Lot Code 4563C Hibiclens	Mean	9.2374	ND	6.6358	2.6017	5.7593	3.4781
	Std. Dev.	0.0731	ND	0.1860	0.2111	0.2241	0.2156
	N	15	0	15	15	15	15

HTR Study No.: 01-109127-11

Hill Top Research Study Number 01-109127-11  
Table 3. Mean Log 10 reductions from baseline, percent microbial reductions from baseline and the 95% confidence limits.

		Log10 Reduction	Log10 Lower Limit	Log10 Upper Limit	Percent Reduction	Percent Lower Limit	Percent Upper Limit
HTR Code A	Wash 1	3.2202	3.0982	3.3422	99.9398	99.9202	99.9545
	Wash 11	2.8070	2.6336	2.9804	99.8440	99.7675	99.8954
HTR Code B	Wash 1	2.6017	2.4848	2.7185	99.7498	99.6725	99.8088
	Wash 11	3.4781	3.3587	3.5975	99.9667	99.9562	99.9747

HTR Study No.: 01-109127-11

Hill Top Research Study Number 01-109127-11  
Table 4. Analysis of variance for the comparison of the test article baseline counts (using the average of left and right log10 baseline counts).

The GLM Procedure

Class Level Information

Class	Levels	Values
HTRCode	2	HTR Code A HTR Code B

Number of observations 45

HTR Study No.: 01-109127-11

Table 4. Analysis of variance for the comparison of the test article baseline counts (using the average of left and right log10 baseline counts).  
 Hill Top Research Study Number 01-109127-11

The GLM Procedure

Dependent Variable: logavg

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00045039	0.00045039	0.04	0.8399
Error	43	0.46864573	0.01089874		
Corrected Total	44	0.46909612			

R-Square      Coeff Var      Root MSE      logavg Mean  
 0.000960      1.129603      0.104397      9.241924

Source	DF	Type I SS	Mean Square	F Value	Pr > F
HTRCode	1	0.00045039	0.00045039	0.04	0.8399

Source	DF	Type III SS	Mean Square	F Value	Pr > F
HTRCode	1	0.00045039	0.00045039	0.04	0.8399

HTR Study No.: 01-109127-11

Hill Top Research Study Number 01-109127-11

Table 4. Analysis of variance for the comparison of the test article baseline counts (using the average of left and right log10 baseline counts).

The GLM Procedure

Level of HTRCode	N	-----logavg-----	
		Mean	Std Dev
HTR Code A	30	9.24416086	0.11652866
HTR Code B	15	9.23744973	0.07312258

HTR Study No.: 01-109127-11

Hill Top Research Study Number 01-109127-11  
Table 4. Analysis of variance for the comparison of the test article baseline counts (using the average of left and right log10 baseline counts).

The GLM Procedure

Tukey's Studentized Range (HSD) Test for logavg

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	43
Error Mean Square	0.010899
Critical Value of Studentized Range	2.85208
Minimum Significant Difference	0.0666
Harmonic Mean of Cell Sizes	20

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	HTRCode
A	9.24416	30	HTR Code A
A	9.23745	15	HTR Code B

HTR Study No.: 01-109127-11

Hill Top Research Study Number 01-109127-11  
Table 5. Analysis of variance to evaluate the effectiveness of each treatment as a function of the number of treatments  
(using the log10 count differences from baseline for each test article).

----- HTRCode=HTR Code A -----

The GLM Procedure

Class Level Information

Class	Levels	Values
Subject	30	2 4 7 11 12 13 14 16 17 18 19 20 22 24 25 26 28 29 30 31 32 34 35 36 37 38 39 41 44 45
Eval	2	Wash 1 Wash 11

Number of observations 60

HTR Study No.: 01-109127-11

Hill Top Research Study Number 01-109127-11  
 Table 5. Analysis of variance to evaluate the effectiveness of each treatment as a function of the number of treatments  
 (using the log10 count differences from baseline for each test article).

----- HTRCode=HTR Code A -----

The GLM Procedure

Dependent Variable: reduc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	30	9.39831051	0.31327702	3.62	0.0004
Error	29	2.51108323	0.08658908		
Corrected Total	59	11.90939374			

R-Square	Coeff Var	Root MSE	reduc Mean
0.789151	9.764397	0.294260	3.013604

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Subject	29	6.83742819	0.23577339	2.72	0.0044
Eval	1	2.56088232	2.56088232	29.58	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Subject	29	6.83742819	0.23577339	2.72	0.0044
Eval	1	2.56088232	2.56088232	29.58	<.0001

HTR Study No.: 01-109127-11

Hill Top Research Study Number 01-109127-11  
Table 5. Analysis of variance to evaluate the effectiveness of each treatment as a function of the number of treatments  
(using the log10 count differences from baseline for each test article).

----- HTRCode=HTR Code A -----

The GLM Procedure

Level of Eval	N	-----reduc----- Mean	Std Dev
Wash 1	30	3.22019833	0.32669183
Wash 11	30	2.80700892	0.46436506

HTR Study No.: 01-109127-11

Hill Top Research Study Number 01-109127-11  
Table 5. Analysis of variance to evaluate the effectiveness of each treatment as a function of the number of treatments  
(using the log10 count differences from baseline for each test article).

----- HTRCode=HTR Code A -----

The GLM Procedure

Tukey's Studentized Range (HSD) Test for reduc

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGM.

Alpha	0.05
Error Degrees of Freedom	29
Error Mean Square	0.086589
Critical Value of Studentized Range	2.89240
Minimum Significant Difference	0.1554

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Eval
A	3.22020	30	Wash 1
B	2.80701	30	Wash 11

HTR Study No.: 01-109127-11

Hill Top Research Study Number 01-109127-11  
Table 5. Analysis of variance to evaluate the effectiveness of each treatment as a function of the number of treatments  
(using the log10 count differences from baseline for each test article).

----- HTRCode=HTR Code B -----

The GLM Procedure

Class Level Information

Class	Levels	Values
Subject	15	1 3 5 6 8 9 10 15 21 23 27 33 40 42 43
Eval	2	Wash 1 Wash 11

Number of observations 30

HTR Study No.: 01-109127-11

Hill Top Research Study Number 01-109127-11  
 Table 5. Analysis of variance to evaluate the effectiveness of each treatment as a function of the number of treatments  
 (using the log10 count differences from baseline for each test article).

----- HTRCode=HTR Code B -----

The GLM Procedure

Dependent Variable: reduc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	6.65859515	0.44390634	16.49	<.0001
Error	14	0.37691017	0.02692215		
Corrected Total	29	7.03550531			

R-Square	Coeff Var	Root MSE	reduc Mean
0.946427	5.397554	0.164080	3.039890

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Subject	14	0.89735455	0.06409675	2.38	0.0581
Eval	1	5.76124060	5.76124060	214.00	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Subject	14	0.89735455	0.06409675	2.38	0.0581
Eval	1	5.76124060	5.76124060	214.00	<.0001

HTR Study No.: 01-109127-11

Hill Top Research Study Number 01-109127-11  
Table 5. Analysis of variance to evaluate the effectiveness of each treatment as a function of the number of treatments  
(using the log10 count differences from baseline for each test article).

----- HTRCode=HTR Code B -----

The GLM Procedure

Level of Eval	N	-----reduc-----	
		Mean	Std Dev
Wash 1	15	2.60166523	0.21105289
Wash 11	15	3.47811569	0.21558197

HTR Study No.: 01-109127-11

Hill Top Research Study Number 01-109127-11  
Table 5. Analysis of variance to evaluate the effectiveness of each treatment as a function of the number of treatments  
(using the log10 count differences from baseline for each test article).

----- HTRCode=HTR Code B -----

The GLM Procedure

Tukey's Studentized Range (HSD) Test for reduc

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	14
Error Mean Square	0.026922
Critical Value of Studentized Range	3.03319
Minimum Significant Difference	0.1285

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Eval
A	3.47812	15	Wash 11
B	2.60167	15	Wash 1



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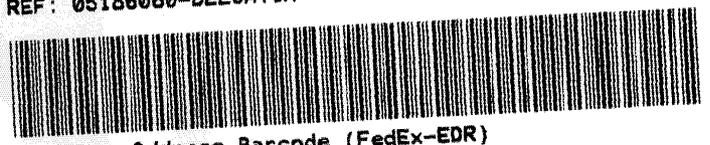
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