

Microconsult, Inc.

Microbiological Testing Laboratory

Determination of the Antimicrobial Efficacy of a Surgical Hand Scrub Test Product Using a Modified ASTM Standard Test Method for Evaluation of Surgical Hand Scrub Formulation Procedure.

Purpose

The purpose of this study is to evaluate the antimicrobial effectiveness of a surgical hand scrub test product using a Modified ASTM Standard Test Method Procedure.

Scope

The antimicrobial effectiveness of one test product utilizing nine (9) human subjects at each application time over the course of eleven (11) consecutive product applications, with microbial samples taken six hours after first product application, and after scrub four (4), which is on day two, and after scrub eleven (11), which is on day five.

Test Material

Test Product: Hand Sanitizing Solution

Lot Number: 746.052

Manufacturer: Dr.'s Solutions, LLC

Equipment

Pipetter 1.0 mL Capacity

Pipetter 0.1 mL Capacity

Bunsen Burner

Clock with Second Hand

Incubator 25⁰ +/- 2⁰C

Vortex Mixer

Thermometer

Balance

Supplies

Sterile 5.0 mL Capacity Serological Pipettes

Sterile Dilution Tubes

Sterile Polystyrene Petri Dishes

Sterile Powder-Free Surgical Gloves

Sterile 1.0 mL Capacity Pipette Tips

Sterile 0.1 mL Capacity Pipette Tips

Sterile Specimen Cups

Sterile Spatulas

Weigh Boats

Test Tube Racks

70% Ethanol

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Propane Gas Bottles
Bland (Ivory) Soap

Test Solutions and Media

Sampling Solution

Sterile Stripping Fluid with Product Neutralizers (SSF+) Lethicin and Tween 80

Diluting Fluid

Butterfield's Phosphate Buffer Solution with Product Neutralizers (BBP+) Lethicin and Tween 80

Media

Tryptic Soy Agar (TSA) Alpha Biosciences Lot# F04-26 Exp. 07/2007

Test Methods

Nine (9) test subjects used one (1) test product over the course of eleven (11) product application procedures during a five (5) day test period.

Overtly healthy subjects over the age of eighteen (18), but under the age of seventy (70), were admitted to the study. All subjects' hands were free from clinically evident dermatoses, injuries to the hands and forearms, open wounds, hangnails, and/or any other disorders, which would compromise the subjects and the study. Subjects clipped their fingernails to a free edge of 2 mm. All jewelry was removed from the hands and arms prior to scrubbing. All subjects signed an Informed Consent Form with study description before participating in the study. No subject was admitted into the study that was known to be using any topical or systemic antimicrobial, steroids, or any other medication known to affect the normal microbial flora of the skin.

Ten-Minute Hand Scrub Procedure

The subjects dispensed 1.5 grams of the test product into their hands and distributed over hands and lower two-thirds of forearms, via gentle, continuous massage for five (5) minutes. They then rinsed both hands and lower two-thirds of both forearms for thirty (30) seconds. The temperature of the water used for this, and all subsequent rinses, was controlled at $40^{\circ} \pm 2^{\circ}C$. The subjects reapplied 1.5 grams of the test product and distributed over hands and lower one-third of forearms, via gentle, continuous massage for five (5) minutes. Then they performed the final rinse on both hands and lower one-third of both forearms for one (1) minute.

Each subject completed this procedure a total of eleven (11) consecutive times. The hands were sampled for residual microbial flora after product application scrubs one (1), four (4), and eleven (11). All samples were taken using the Glove Juice Sampling Procedure.

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Glove Juice Sampling Procedure

Following the Ten Minute Hand Scrub Procedure, powder-free, loose-fitting sterile latex gloves were donned at the designated sampling times for the Glove Juice Sampling Procedure. Seventy-five (75) mL of Sterile Stripping Fluid with product neutralizers were poured into the glove. The wrist was secured, and an attendant massaged the hand through the glove in a uniform manner for sixty (60) seconds. A 5.0 mL aliquot of the glove juice (dilution 10^0) was removed and serial diluted in Butterfield's Phosphate Buffer with product neutralizers.

Plating

Duplicate serial plates were prepared from appropriate dilutions using Tryptic Soy Agar. The plates were incubated at $35^0 \pm 2^0\text{C}$ for approximately forty-eight (48) hours. All colony-forming units were counted.

Test Period

To provide the baseline recovery levels, two practice scrubs were performed using bland soap to remove dirt and oil from the hands and to familiarize the subjects with the test product hand scrub procedure. The second practice scrub was performed forty-eight (48) to ninety-six (96) hours after the initial practice scrub. After both practice scrubs, the Glove Juice Sampling Procedure was performed on both the left and right hands.

On test day one, each subject was utilized for six (6) to seven (7) hours. The Ten Minute Hand Scrub Procedure was performed once. After the timed two (2) minute air-dry, a powder-free glove was placed on the left hand and six (6) hours later the Glove Juice Sampling Procedure was performed on the left hand.

On test day two, subjects performed the Ten Minute Hand Scrub Procedure three times with a one (1) hour interval between scrubs. After the final one (1) minute rinse and a timed two (2) minute air-dry, a powder-free glove was placed on the right hand and the Glove Juice Sampling Procedure was performed.

On test day three and four, the Ten Minute Hand Scrub Procedure was performed three times with a one (1) hour interval between scrubs. No sampling was performed on day three or four.

On test day five, subjects performed the Ten Minute Hand Scrub Procedure once. After the final one (1) minute rinse and a timed two (2) minute air-dry, a powder-free glove was placed on the right hand and the Glove Juice Sampling Procedure was performed.

Method of Analysis

Data Collection

$$R = \log_{10} B - \log_{10} S$$

$$D = \log_{10} B - \log_{10} T$$

$$C = \log_{10} B - \log_{10} F$$

Surgical Hand Scrub Protocol

3-02-05

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Where for each panelist:

R	=	log reduction at six hours
D	=	log reduction at two days
C	=	log reduction at five days
B	=	average of the four baseline recovery levels
S	=	six hour recovery level
T	=	two day recovery level
F	=	five day recovery level

$$V = \frac{(R1 + R2 + R3 + R4 + R5 + R6 + R7 + R8 + R9)}{9}$$

$$M = \frac{(D1 + D2 + D3 + D4 + D5 + D6 + D7 + D8 + D9)}{9}$$

$$E = \frac{(C1 + C2 + C3 + C4 + C5 + C6 + C7 + C8 + C9)}{9}$$

Where:

V	=	average six hour log reduction of all nine panelists
M	=	average two day log reduction of all nine panelists
E	=	average five day log reduction of all nine panelists

Findings and Conclusions

The study included nine panelists, seven women and two men. To show effectiveness, this product must meet the requirements: bacterial count at six hours does not exceed the baseline, a 2-log reduction is produced at two days, and a 3-log reduction is produced at five days.

The Hand Sanitizing Solution, lot 746.052, utilizing the modified ASTM Surgical Hand Scrub Procedure, showed effective antibacterial activity. The bacterial count at six hours has a log reduction of 0.78, which did not exceed the bacterial baseline count. A 2.30 log reduction was produced at day two, and a 3.06 log reduction at day five.

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Hand Sanitizing Solution Lot 746.052

Initiated 3-2-05

Results 3-15-05

Panelist	Baseline	6 Hour	Log Reduction	Day 2	Log Reduction	Day 5	Log Reduction
1	40,969	5,010	0.91	35	3.07	20	3.31
2	19,467	1,658	1.07	30	2.81	20	2.99
4	48,234	2,812	1.23	210	2.36	5	3.98
5	32,402	2,987	1.04	758	1.63	93	2.54
6	77,008	9,943	0.89	1,355	1.75	88	2.94
7	102,213	18,600	0.74	93	3.04	15	3.83
8	41,708	22,700	0.26	208	2.30	138	2.48
9	38,179	27,750	0.14	833	1.66	90	2.63
11	44,140	8,047	0.74	380	2.07	68	2.82
Total Average Log Reduction		6 Hour	0.78	Day 2	2.30	Day 5	3.06

Completed by:



Date:

3.15.05