Persistence of Activity of a Hand Sanitizer

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1.0 OBJECTIVE

The overall purpose of this study is to investigate the ability of a hand sanitizer (my-shield Hand Sanitizer with Aloe Vera) to prevent attachment and growth of a typical skin contaminant (*Staphylococcus aureus*) up to 24 hours after treatment.

2.0 PROTOCOL OVERVIEW

Samples of porcine skin (used as a human skin analog) were prepared and sanitized with 70% ethanol. A test group was also treated with a hand sanitizer (my-shield Hand Sanitizer with Aloe Vera) and left at room temperature for up to 24 hours. At predetermined time intervals, an inoculum of *Staphylococcus aureus* was applied to the samples. After inoculation, samples were enumerated for *S. aureus*. Counts for the samples not treated with the sanitizer were compared to the treated samples to determine the effect of the sanitizer at preventing attachment of the organism. Effectiveness over time from initial treatment was also determined.

3.0 MATERIALS AND METHODS

3.1 Challenge organism and stock solution preparation

The following challenge organism was prepared for this study:

Staphylococcus aureus (ATCC #12600)

The culture was prepared from a lyophilized preparation (KWIK-STIK™, Microbiologics, St. Cloud, MN) according to manufacturer's instructions. The culture was transferred into Tryptic Soy Broth (TSB, Neogen, Lansing, MI) and incubated at 35 ± 2°C for 24 ± 2 hours. This preparation was plated onto Standard Methods Agar (SMA, Neogen) at appropriate dilutions to determine the actual concentration.

3.2 Preparation and treatment of samples

Porcine skin samples were obtained and used as human skin analogs. The skin samples were aseptically cut into pads measuring approximately 3" x 6" inches. Each pad had two holes cut in it to allow for aseptic handling as discussed below. All pads were sanitized using 2.0 mL of a 70% alcohol solution. A total of 16 pads were prepared (treated and untreated pads for each of 7 time points, plus 2 additional uninoculated pads left as negative controls).

Samples in the treatment group were treated with my-shield Hand Sanitizer with Aloe Vera in a manner designed to simulate use on human skin. The skin pad was held using sterile latex gloves, held through the precut holes with the non-dominant hand. Using the dominant hand, 1.0 mL of sanitizer was applied to the interior portion of the pad (an area of approximately 1.5" x 4"), and rubbed onto the surface for 30 to 45 seconds. After treatment, samples were left at ambient temperature. Samples were held for the following time periods before inoculation: 2 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 16 hours, and 24 hours.

3.3 Sample inoculation

After each holding time had elapsed, 2 of the treated samples and 2 of the untreated samples were surface inoculated with *S. aureus*. A 1.0 mL volume of overnight TSB culture (containing approximately 1 x 10^8 cfu) was applied and spread onto the interior portion of the sample, in a manner similar to the sanitizer application. Samples were allowed to air dry for 5 minutes before enumeration of the sample. After the 24 hour samples were inoculated and enumerated, the 2 remaining untreated, uninoculated controls were also enumerated as below.

3.4 Sample plating and enumeration

Samples were rinsed with 100 mL of Butterfield's Phosphate Buffer (BPB) and vigorously hand massaged to remove any remaining viable *S. aureus*. The rinsate was collected and spread plated at appropriate dilutions onto Baird-Parker Agar (BP, Neogen). BP plates were incubated at 35 ± 2°C for 24 ± 2 hours.

After incubation, plates were enumerated using a Quebec colony counter. The number of observed colonies typical for *S. aureus* was multiplied by the dilution factor to determine the total count. Representative isolates were confirmed as *S. aureus* to ensure the recovered counts represent the inoculated culture. Counts for all samples were recorded.

3.5 Data analysis

The raw count observed for each treated sample was compared to the untreated control. The percent reduction between the treated and untreated samples was defined as the efficacy of the treatment at each time point.

4.0 RESULTS

4.1 Challenge organism concentration

Results of the enumeration of the challenge organism are shown in Table 1, below.

Table 1. Challenge organism enumeration

Challenge organism	Sample 1	Sample 2	Sample 3	Average	Log_{10}
S. aureus (cfu/mL)	85,000,000	83,000,000	198,000,000	122,000,000	8.09

The overnight culture suspension was at a concentration of 1.22×10^8 cfu/mL and was used undiluted as the inoculum in the study.

4.2 Porcine skin sample results

Results of the challenge samples are shown in Table 2, below, including holding time, type of sample, sample replicate, amount of *S. aureus* recovered (in cfu/mL of rinsate), and the percent reduction seen between the treated and untreated samples at each time point.

Table 1. Porcine skin sample enumeration

Holding Time (Sample Type)	Sample 1	Sample 2	Average	Log_{10}	% Reduction
2 minutes (Untreated)	22,400,000	16,400,000	19,400,000	7.29	
2 minutes (Treated)	11,200	13,900	12,550	4.10	99.9%
1 hour (Untreated)	5,200,000	18,800,000	12,000,000	7.08	
1 hour (Treated)	87,000	164,000	125,500	5.10	99.0%
2 hours (Untreated)	13,900,000	15,900,000	14,900,000	7.17	
2 hours (Treated)	137,000	380,000	258,500	5.41	98.3%
4 hours (Untreated)	8,200,000	17,200,000	12,700,000	7.10	
4 hours (Treated)	156,000	640,000	398,000	5.60	96.9%
8 hours (Untreated)	9,500,000	13,700,000	11,600,000	7.06	
8 hours (Treated)	1,580,000	1,930,000	1,755,000	6.24	84.9%
16 hours (Untreated)	3,700,000	14,900,000	9,300,000	6.97	
16 hours (Treated)	1,770,000	2,040,000	1,905,000	6.28	79.5%
24 hours (Untreated)	10,500,000	15,400,000	12,950,000	7.11	
24 hours (Treated)	3,400,000	8,500,000	5,950,000	6.77	54.1%
24 hours (Uninoculated)	<1	<1	<1	n/a	n/a

Efficacy of the treatment dropped from a maximum of 99.9% to a minimum of 54.1% of the 24 hours of the study. Representative isolates were confirmed as the inoculum (*S. aureus*). No background *S. aureus* was detected in the uninoculated samples over the course of the study.

5.0 CONCLUSIONS

The data in this study indicates that the hand sanitizer (my-shield Hand Sanitizer with Aloe Vera) was able to prevent the attachment of the test organism (*Staphylococcus aureus* ATCC #12600) for short time periods. The calculated percent efficacy of the treatment peaked at 99.9% (i.e. 3 logs of reduction) 2 minutes after application. Percent efficacy remained above 99.0% (2 logs of reduction) for up to an hour after application, and remained above 90.0% (1 log of reduction) for up to 4 hours after application. Percent efficacy decreased markedly from that point on, indicating that the sanitizer was not able to provide further protection after 4 hours of ambient storage.

6.0 REFERENCES

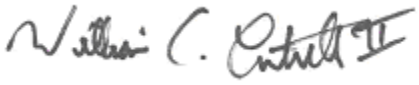
ASTM E2897-12 Standard Guide for Evaluation of the Effectiveness of Hand Hygiene Topical Antimicrobial Products Using *ex-vivo* Porcine Skin

ASTM WK36911 New Guide for Measuring the Inactivation of Persistent Activity of Topical Antimicrobial Products Using *ex-vivo* Porcine Skin

National Advisory Committee on Microbiological Criteria for Foods. 2009. Parameters for Determining Inoculated Pack/Challenge Study Protocols.

7.0 FINAL REPORT APPROVAL

Prepared By:



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