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**Part 1: Antibacterial Efficacy of a Novel Antimicrobial Skin Cleanser against Food-borne Enteric
Pathogens on Inanimate Surfaces**

Northern Filter Media Inc. and IPRT Company Assistance Project

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Part 1: Antibacterial Efficacy of a Novel Antimicrobial Skin Cleanser against Food-borne Enteric Pathogens on Inanimate Surfaces

INTRODUCTION

Sanitization of hands is a major intervention for preventing the transmission of pathogens to food, food contact surfaces or other areas that must be kept sanitary in food processing, food service, clinical or veterinary environments. Of late, there has been a growing trend in the demand for sanitizers that may be applied to hands without the need for subsequent rinsing. Many hand sanitizers contain ethanol as the active antimicrobial, along with moisturizers and emollients to keep the skin from becoming dry. While ethanol will exert an antimicrobial effect, its rapid evaporative loss from the skin reduces the effectiveness of many hand sanitizers in providing residual antimicrobial protection. Recently, hand sanitizers (Germ Free Ultra and Germ Free 24) containing silylated quaternary ammonium compounds (QAC's) have been developed. QAC's are well known to have antimicrobial activity that is manifested by disruption of microbial cell membranes, resulting in leakage of intracellular metabolites and cell death (1). Silylated QAC's have the ability to react with and chemically bond to inanimate surfaces such as cellulose fabrics (2, 3, 4), glass (3, 6), zeolite (1), etc., with retention of antimicrobial activity. They can also bond to skin (5). When applied to the hands, quaternary ammonium organosilanes bind to the skin via covalent-, electrostatic-, or hydrogen bonds, forming an antimicrobial layer to inactivate existing skin pathogens as well as pathogens that contaminate the skin long after application of the sanitizer. In this communication, we report the effectiveness of these sanitizers for killing human enteric pathogens on inanimate surfaces (polystyrene Bioscreen plates) with retention of antimicrobial activity for extended periods in a rapid microtiter plate assay.

MATERIALS AND METHODS

Strains and media. All aseptic techniques and handling of microbial cultures were performed in a Nuair Biological Safety Cabinet (Model #NU-425-400). Five strains each of *Escherichia coli* O157:H7 and *Listeria monocytogenes* were used in this study. Each strain was isolated either from infected processed food or human infection (Table 1). *E. coli* O157:H7 was grown at 35°C on tryptic soy broth + 0.6% yeast extract (TSB/YE) (Difco Laboratory, Detroit, MI); *L. monocytogenes* was grown at 30°C on brain heart infusion (BHI) (Difco Laboratory). *E. coli* O157:H7 and *L. monocytogenes* cell counts were made on sorbitol-MacConkey agar (SMA) (Difco Laboratory) and modified Oxford medium (MOX) (Difco Laboratory), respectively.

For all experiments, a 5-strain mixture ("cocktail") of the specific test organism was used. Five ml of an overnight culture of each strain were aseptically combined in a sterile 50-ml polypropylene centrifuge tube. The cells were harvested by centrifugation (10,000 x g at 4°C for 10 min) in a Sorvall Super T 21 centrifuge with an SL-50T rotor. The supernatant was discarded and the pelleted cells were resuspended and washed in 25 ml of either sterile 0.85% saline or

sterile distilled water. The washed cells were harvested as previously mentioned and the supernatant discarded. The cells were resuspended in 25 ml of either sterile 0.85% saline or sterile distilled water and used as inocula for sanitizer evaluations.

Table 1. Pathogens used for the evaluation of Northern Filter Inc. sanitizers^a.

Organism	Isolate	Source
<i>L. monocytogenes</i>	FSL J1-177	Human sporadic case (1997)
"	FSL C1-056	Human sporadic case (1998)
"	FSL N3-013	Food isolate from listeriosis epidemic (UK, 1988-2000)
"	FSL R2-499	Human isolate from sliced turkey outbreak (USA, 2000)
"	FSL N1-227	Food isolate (US, 1998-1999)
<i>E. coli</i> O157:H7	FRIK 125	Dr. C. Kaspar, Univ of Wisconsin, Madison
"	ATCC 43890	Human feces isolate, ATCC ^b
"	ATCC 43895	Ground beef isolate, ATCC
"	C-467	Dr. C. Kaspar, Univ of Wisconsin, Madison
"	93-062	Culture collection, Microbial Food Safety Lab. FSHN

a) Stock cultures were maintained at -75°C in sterile culture broth with 40% glycerol.

b) American Type Culture Collection

Sanitizers. The sanitizers Germ Free 24 (GF 24) and Germ Free Ultra (GFU) were provided by Northern Filter Media, Inc., Muscatine, IA. These contain silylated quaternary ammonium compounds as their active ingredients. Also included was a Silicate Test Solution containing an inert organosilane compound that served as a control. For comparison, 3 commercially-available sanitizers were also provided: 1) 2 in 1 HandClens; 2) Dial Hand Sanitizer; 3) Purell Instant Hand Sanitizer. Other solutions used in the trials were sterile distilled water and 62% (w/w) ethanol. The sanitizers tested and their ingredients are listed in Table 2.

Table 2. Sanitizers evaluated for Northern Filter Media, Inc.

Sanitizer	Active Ingredient	% Ethanol
Silicate Test Solution	Methyl-Trihydroxy-Silane	18
Germ Free 24	0.5% AMOSILQ ^a	18
Germ Free Ultra	0.5% AMOSILQ ^a	62
2 in 1 HandClens	0.13% Benzalkonium Chloride	0
Dial Hand Sanitizer	Ethanol	62
Purell Hand Sanitizer	Ethanol	62

^a Contains 3-(trihydroxysilyl) propyl-dimethyl-octadecyl ammonium chloride

Rapid Bioscreen assay for antimicrobial activity. The retention of antimicrobial activity of sanitizers on inanimate surfaces was tested using a Microbiology Reader Bioscreen C (Oy Growth Curves AB Ltd., Helsinki, Finland). This instrument continuously monitors absorbance changes of bacterial cultures at specific wavelengths, temperature and shaking speeds in polystyrene plates containing 100 microtiter wells. Evaluations were performed as follows: Sixty μL of each sanitizer were added to separate wells of sterile Bioscreen plates. Plates were dried in a 36-38°C incubator overnight, after which 10 μL of a 1:100 dilution of washed cells of a 5-strain mixture of *E. coli* O157:H7 or *L. monocytogenes* were added to the Bioscreen wells, followed by 250 μL of sterile media. The plates were inserted in the Bioscreen and incubated at 30°C for periods of 2-3 days. The plates were shaken at low speed for 10 sec before hourly absorbance readings (A_{600}) were made. All sanitizers were evaluated in replicates of 5. Growth curves were obtained from the Bioscreen data using Excel.

RESULTS AND DISCUSSION

Part 1. Antimicrobial effect of sanitizers to pathogens on inanimate surfaces.

Figure 1 compares the growth of *E. coli* O157:H7 and *L. monocytogenes* in Bioscreen plate wells with and without exposure to sanitizers. In this experiment, sanitizers were added to the Bioscreen plate wells, and the plate dried at 48-49°C. After 19 hours, inocula were added to the plate wells and the plate incubated at 37°C and ~8 hr to dry the inocula, followed by adding 250 μL of sterile media to each wells. The plate was then incubated in the Bioscreen at 30°C for 62 hr.

Figure 1 compares the growth of *E. coli* O157:H7 and *L. monocytogenes* in Bioscreen plate wells with and without exposure to sanitizers. For both organisms, normal growth was observed in the absence of sanitizers and with cells exposed to distilled water. Cells exposed to wells pre-treated with 62% ethanol, Dial- and Purell sanitizers also grew normally. The latter contain ethanol as their active ingredient. Evaporative loss of ethanol from these wells eliminated the anti-microbial efficacy of these treatments.

Normal growth of both organisms was observed in wells treated with the Silicate Solution. This is identical to GF 24, but contains an inert organosilane derivative. Neither organism was able to grow following exposure to GF 24, GFU and 2 in 1 HandClens. The active ingredients in these products (Table 2) are not volatile and, after the drying step, remain to coat the wells and prevent growth of the microbes following inoculation. At the end of the incubation period, 50 μL aliquots from wells treated with these sanitizers were aseptically transferred to tubes of sterile broth and incubated at a suitable temperature. After 2 days, no growth was observed, demonstrating that absence of growth in the Bioscreen plate was not due to inhibition, but to death of the cells exposed to the sanitizers coating the wells. The results also demonstrate that

the active ingredient in GF 24 and GFU (3-(trihydroxysilyl)-propyl-dimethyl-octadecyl ammonium chloride) is active against Gram positive and Gram negative bacteria. This is in agreement with the findings of others (3, 4).

Profiles obtained with 2 in 1 HandClens show an initial high absorbance, followed by a decrease over the incubation period. This is especially apparent in wells containing *L. monocytogenes* in BHI medium. This was tested and found to be due to some interaction between one or more of the sanitizer components with the medium, and not to an effect on the inocula.

To preclude the possibility that drying the Bioscreen plate at ~49°C prior to inoculation caused thermal decomposition of the sanitizers, the experiment described in Figure 1 was repeated, with the following modifications: (a) the sanitizers were dried in the Bioscreen plate for 23 hrs at ~37°C; (b) the inocula were not taken to dryness prior to addition of media, but instead were incubated in the Bioscreen plate for only 1 hr at 37°C prior to addition of 250 µL of growth media. The results are shown in Figure 2.

The results from this trial were essentially identical to those obtained from the first experiment, confirming the observations from the latter. Thus, exposure of the sanitizers to ~50°C does not cause thermal decomposition. Moreover, the active ingredients in GF24, GFU and 2 in 1 HandClens were biocidal even though the inocula did not go to dryness. This shows that failure of the pathogens to grow in wells treated with these sanitizers was not due desiccation-induced death of the cells.

Summary of Results

E. coli O157: H7 and *L. monocytogenes* grew normally (growth similar to control) in broth medium added to polystyrene wells that were pre-treated with 62% ethanol, Dial or Purell sanitizers. The observed lack of growth inhibition is likely due to the evaporative loss of ethanol from these treatment solutions.

Normal growth of both pathogens also occurred in wells pre-treated with silicate solution.

No growth of either pathogen was observed in wells that were pre-treated with GF24, GF Ultra, or 2 in 1 HandClens. The active antimicrobial agents in these sanitizers are not volatile and persisted in the wells (following the drying step) to inhibit growth of the pathogens.

GF 24, GF Ultra and 2 in 1 HandClens exerted a bactericidal effect on both pathogens. Germ Free 24 and Germ Free Ultra contain 3-(trihydroxysilyl)-propyl-dimethyl-octadecyl ammonium chloride as their active ingredient. Data from Bioscreen and pork skin evaluations show that the Germ Free formulations are active against Gram positive and Gram negative organisms. This is in agreement with findings of others (3,4).

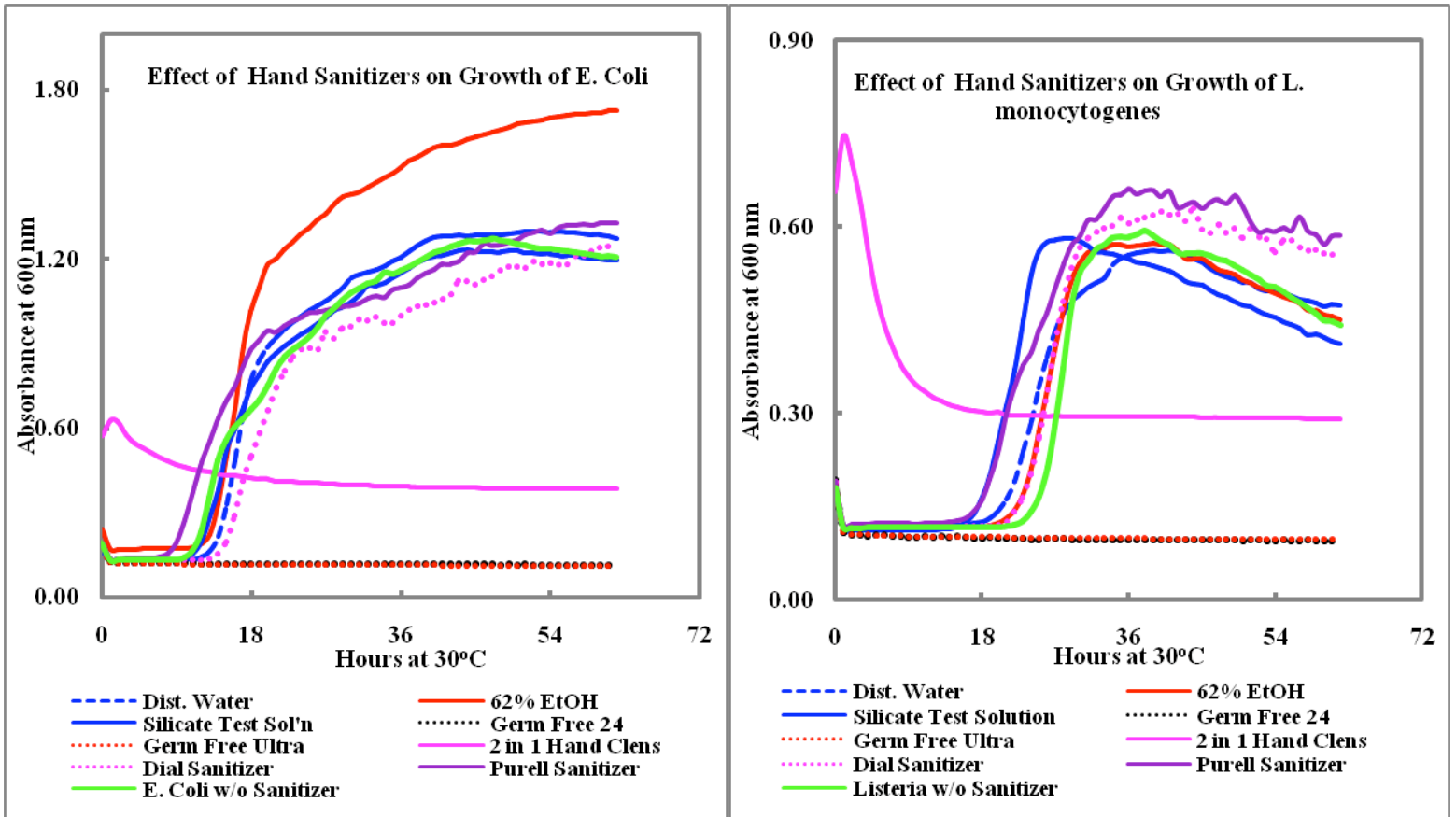


Fig. 1. Growth of *E. coli* O157:H7 and *L. monocytogenes* in Bioscreen plates with and without sanitizer treatment. Sanitizers were added to wells and the plate dried overnight at $\sim 49^{\circ}\text{C}$. Five-strain “cocktails” of *E. coli* O157:H7 and *L. monocytogenes* were used as inocula. Cells were harvested and washed as described in Methods, resuspended in sterile water to one-half their original volume, then diluted 1:100 with sterile water. Inocula ($10\ \mu\text{L}$) were added to wells, then dried for 8 hrs at $37\text{-}38^{\circ}\text{C}$. Next, $250\ \mu\text{L}$ of sterile media were added to all wells. The plate was incubated in the Bioscreen at 30°C for 62 hrs ($n=5$).

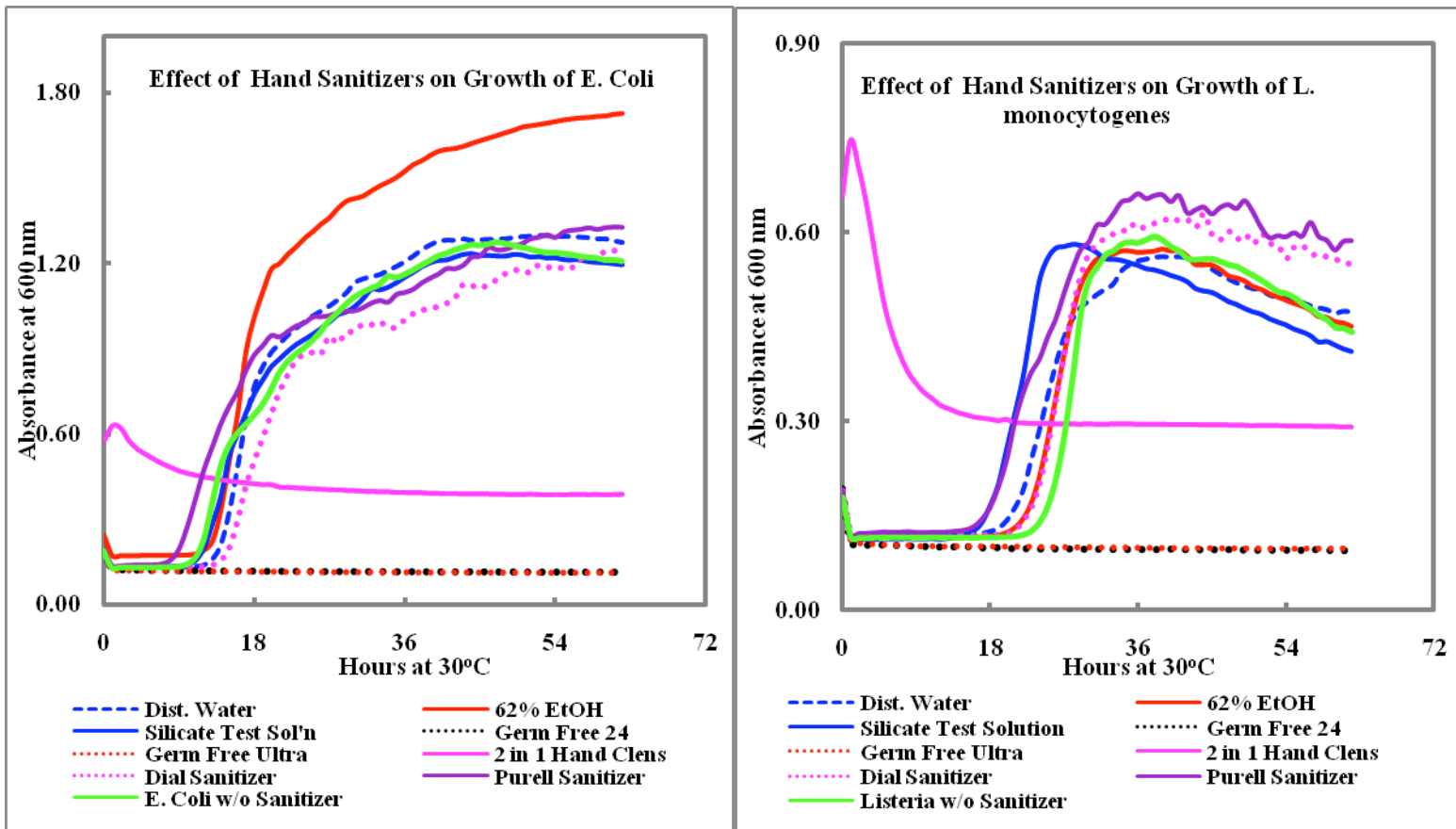


Fig. 2. Growth of *E. coli* O157:H7 and *L. monocytogenes* in Bioscreen plates with and without sanitizer treatment. Sanitizers were added to wells and the plate dried overnight at ~37°C. Five-strain “cocktails” of *E. coli* O157:H7 and *L. monocytogenes* were used as inocula. Cells were harvested and washed as described in Methods, resuspended in sterile water to one-half their original volume, then diluted 1:100 with sterile water. Inocula (10 µL) were added to wells, after which the plate was incubated at 37°C for 60 min. Next, 250 µL of sterile media were added to all wells. The plate was incubated in the Bioscreen at 30°C for 52 hrs (n=5).

ACKNOWLEDGMENTS

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REFERENCES

1. **Abbaszadegan, M., P. Monteiro, R. N. Ouwers, R. Hodon and A. Alum.** 2006. Removal and inactivation of *Cryptosporidium* and microbial indicators by a quaternary ammonium chloride (QAC)-treated zeolite in pilot filters. *J. Environ. Sci. and Hlth.* **A41**:1201-1210.
2. **Eudy, W. W.** Aug. 1981. Organosilicon quaternary ammonium antimicrobial compounds. U. S. patent 4,282,366.
3. **Isquith, A. J., E. A. Abbott, and P. A. Walters.** 1972. Surface-bonded antimicrobial activity of an organosilicon quaternary ammonium chloride. *Appl. Microbiol.* **24**:859-863.
4. **Murray, P. R., A. C. Niles, and R. L. Heeren.** 1988. Microbial inhibition on hospital garments treated with Dow Corning 5700 antimicrobial agent. *J. Clin. Microbiol.* **26**:1884-1886.
5. **Peterson, W. R., D. Giaccio, and R. E. Berman.** Sept. 2003. Antimicrobial skin preparations containing organosilanes quaternaries. U. S. patent 6,613,755 B2.
6. **Walters, P. A., E. A. Abbott, and A. J. Isquith.** 1973. Algicidal activity of a surface-bonded organosilicon quaternary ammonium chloride. *Appl. Microbiol.* **25**:253-256.