MICROBIOLOGICAL METHODS

Evaluation of the Use of Liquid Dishwashing Compounds To Control Bacteria in Kitchen Sponges

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A test procedure for evaluating the effect of adding commercial liquid hand dishwashing detergents to kitchen sponges to control microbial growth is described. Claims for this type of application are being made on dishwashing detergents throughout the world. In this evaluation, commercially available kitchen sponges were stripped of antimicrobial compounds. Sponges were then inoculated with a pool of 7 microorganisms which consisted of Gram positives, Gram negatives, and yeast. Inoculated sponges were treated with the detergent as recommended by the manufacturer and allowed to incubate for 16 h at ambient temperature. Surviving microorganisms were then quantitated using either the spiral or pour plate method. Tests were run using both clean sponges and sponges soiled with 0.5% nonfat dry milk (NFDM). Untreated sponges showed stasis or slightly increased bacterial populations after the incubation period in the absence of NFDM. Significant increases of up to 3 log cfu/mL were observed for untreated sponges when soiled with NFDM. Statistically significant reductions were observed for clean sponges (99.8–99.9998%) and sponges soiled with NFDM (87.6–99.9%) when detergents making "antibacterial sponge" claims were added to the inoculated sponges. Statistically significant differences between detergents making "antibacterial sponge" claims were also observed.

Food poisoning is a common occurrence in the United States, with 5.5–6.5 million cases reported per year (1). The most frequent source of bacterial contamination resulting in food poisoning is the home (2), where the kitchen is reported as the most contaminated area (3, 4). Kitchen sponges and wash cloths used to clean food preparation areas are reservoirs for microorganisms and several studies have documented high bacterial counts on kitchen sponges and dishcloths (3–6). One study identified kitchen sponges and dishcloths as the most contaminated environments in the home, as they retain moisture and offer a favorable environment for bacterial growth once contaminated (4). The common practice of wiping cutting boards, countertops, and general kitchen surfaces tends to spread microbial contamination either picked up by the sponge or already in the sponge (7). Several papers have addressed methods of disinfecting kitchen sponges and wash cloths (8–9). As kitchen sponges and dishcloths are commonly used in the kitchen where raw and ready-to-eat foods are prepared, any effective measure to control or reduce the microorganisms in these items would reduce the public health concerns related to their exposure.

In several countries, including the United States, the United Kingdom, Japan, Thailand, and Taiwan, manufacturers have begun making antibacterial claims on liquid hand dishwashing detergent labels. Antibacterial claims typically are of 2 types. Some products simply state that the detergent is antibacterial. The most prevalent worldwide claim is that use of concentrated detergent on a kitchen sponge or dishcloth is antibacterial or renders the article sanitary or hygienically clean. The specific language is governed by local regulations. The antibacterial sponge claims typically descibe application of a given amount of detergent on a sponge or dishcloth after washing is completed, squeezing to distribute the detergent throughout the washing article, then allowing the article to remain saturated with the detergent until the next use. The most common claim in the United States is that the detergent is also an "Antibacterial Hand Soap." Most commonly, triclosan is the active ingredient used. The "antimicrobial" claim is that washing a consumer's hands with the detergent reduces the residual bacteria on the user's skin. This paper reports only on the antibacterial properties associated with the sponge application type of claim.

Antibacterial activity is not necessarily an inherent feature of liquid dishwashing compounds or any particular ingredient of the formulation. Some formulations will be shown to contribute to the growth of bacteria. Ingredients with known antibacterial properties commonly used in hand dishwashing detergents include preservatives, ethanol, natural oils from fragrance ingredients, and antibacterial hand soap active ingredients such as triclosan. It is unclear, however, as to

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Table 1. Antibacterial effects of unstripped sponges versus stripped sponges

Sponge treatment	Avg. initial inoculum count, log cfu/mL	Avg. final inoculum count, log cfu/mL	Avg. log reduction
Unstripped sponges, preservative present	4.3	1.8	2.5
Stripped sponges, preservative removed	4.3	6.8	–2.3[a]

[a] Sample demonstrated an increase in microbial content rather than a reduction.

whether these ingredients are responsible for the antibacterial activity observed in kitchen sponges.

This paper proposes a test method useful in evaluating the effect of adding liquid hand dishwashing detergents to kitchen sponges to control microbial populations in the sponges. Although the paper deals only with sponges, the techniques are applicable to wash cloths as well.

Experimental

Sponges

Kitchen sponges are generally of 2 types: reticulated open-cell synthetic plastic foam and open-cell cellulosic viscose sponges. Dishcloths can be of various fibers, both synthetic and cellulosic. Sponges are often sold with an abrasive feature for additional scrubbing action. This feature can be an abrasive pad bonded to one side of the sponge or a mesh covering the entire sponge. The test procedure proposed in this paper is applicable to all types of sponges. The sponges used in this test are commercially available 3 4.5 in. synthetic reticulated open-cell sponges with a mesh covering removed (M6069 CLEANEE SCOURING PAD, Arden Cos., Southfield, MI), and a 3 4.5 in. cellulosic viscose sponge with removable mesh covering left in place (SOFT BUDS, Amway Corp., Tokyo, Japan).

Preparation of Sponges

Kitchen sponges are often sold in a plastic wrapper or bag. A small amount of water is often added to the sponge so the consumer will have a soft sponge when the bag is opened. It is a common practice to add preservatives, such as quaternary compounds or zinc pyrrithione, to the sponge to prevent growth after manufacture and prior to consumer use. Once the sponge is in use, the preservative effect of these compounds is short-lived, lasting only until the preservative is washed out of the sponge. The presence of these preservatives can, however, have an effect on bacterial counts observed in the test procedure reported in this paper. For this reason, it was important to remove the preservatives. Prior to an evaluation, all kitchen sponges were subjected to a stripping procedure to remove any preservatives that may have been present.

Sponge Stripping Procedure

(*1*) Sponges, as they were to be tested, were placed into a standard clothes washing machine for the following 3 cycles:

(**a**) *Wash cycle 1*.—Add 40 g triethanol amine salt of lauryl sulfate (Stepan Co., Northfield, IL) and 8 oz sodium hexametaphosphate (Solutia, Inc., St. Louis, MO) to 1 Ldeionized water, heat to dissolve, and add to washing machine.

(**b**) *Wash cycle 2*.—Add 8 oz sodium hexametaphosphate to 1 L deionized water, heat to dissolve, and add to washing machine.

(**c**) *Wash cycle 3*.—Wash sponges in softened water (>50 ppm water hardness as $CaCO_3$).

(*2*) After the stripping procedure, place sponges on a rack and air dry. Sponges are then stored under dry conditions until used.

The necessity of performing the stripping procedure is illustrated in Table 1, which shows an inoculum increase of 2.3 log in stripped sponges compared with unstripped sponges that contained a preservative initially, which showed a 2.5 log decrease in inoculum levels. Testing reported in this paper was done with sponges stripped in softened water. Other types of water and hardness could be used and would not be expected to alter test results. The inoculum was exposed to stripped and unstripped sponges. Final inoculum counts were obtained after the sponges were dried at room temperature overnight and then rehydrated with sterile deionized water. Dishwashing compounds were not used in this study.

Preparation of Inoculum

The organisms used in the pooled inoculum included 2 species of Gram positive, 4 species of Gram negative, and one yeast. Microorganisms were obtained from the American Type Culture Collection (ATCC; Rockville, MD). Strains used include *Staphylococcus aureus* 6538, *Klebsiella*

Table 2. Liquid hand dishwashing detergents making antimicrobial claims

Detergent	Market	Use level, mL	Type of claim
Commercial product—Dish Drops	Worldwide	5	Sponge
Commercial product A	United States	5	Hand soap
Commercial product B	Japan	5	Sponge
Commercial product C	United Kingdom	5	Sponge
Commercial product D	Japan	8	Sponge
Commercial product E	Japan	8	Sponge
Commercial product F	Japan	8	Sponge
Commercial product G	Japan	8	Sponge
Commercial product H	Japan	5	Sponge

Table 3. Bacterial reduction on synthetic foam sponges after treatment with a liquid hand dishwashing detergent

Treatment (use amount)	Mean[a] log cfu/mL	Std. dev.	Confidence level[b] 95%	Avg. log reduction	Avg. % reduction
Dish drops (5 mL)	0.00	0.00	a	4.64	>99.99
Product D (8 mL)	1.34	0.30	b	3.30	99.9
Untreated control (initial count)	4.64	0.11	c	NA[c]	NA[c]
Untreated control (after incubation)	4.77	0.37	c	–0.26[d]	None

[a] $n = 5$.
[b] Letter values are assigned to indicate a significant difference in the mean recovery between products at the specified confidence level.
[c] Not applicable.
[d] Sample demonstrated an increase in microbial concentration rather than a reduction.

terrigena 33257, *Escherichia coli* 11229, *Pseudomonus aeruginosa* 15442, *Enterococcus durans* 19432, *Burkholderia cepacia* 25416, and *Candida albicans* 10231. Dehydrated pellets were resuscitated according to ATCC recommendations. Bacterial cultures were grown on microbial content test agar plates (Difco Laboratories, Detroit, MI) with the exception of *S. aureus*, which was grown on micrococcus agar plates. *Candida albicans* was grown on potato dextrose agar (Difco Laboratories). Cultures were incubated at the ATCC-recommended temperature for recommended times. After propagation, cultures were harvested and placed in cryogenic storage using sterile phosphate buffered saline with 10% glycerol (Sigma Chemical Co., St. Louis, MO). Each culture was quality-checked to ensure organism identity, purity, and count. Stock challenge cultures were combined at equal titers and stored as a mixed inoculum at a final level of 2 10^8 cfu/mL.

Application of Inoculum to and Quantitation of Inoculum on Sponges

Dried, stripped sponges were placed into sterile stomacher bags, 7.5 12 in. One hundred milliliters sterile deionized water was aseptically added to the stomacher bag containing the sponge. A 0.1 mL aliquot of the inoculum was also added to the stomacher bag containing the sponge. A minimum recovery of 1 10^5 cfu/sponge was used in this study. After inoculation, the bagged sponges were massaged by hand 20 times to facilitate mixing the inoculum into the sponge. A 1 mL aliquot was removed, providing an initial quantitation of the inoculum. All inoculated bags, including control bags, were inverted once and squeezed to remove the excess water from the sponge before treatment with detergent.

Application of an Organic Load to Simulate Soiled Sponges

A second variant of this procedure involved simulating conditions found in consumer's homes by adding an organic load to the sponge prior to inoculation. As food residues and other contamination are not completely removed during normal use, these residues can provide a food source promoting greater bacterial growth as compared with a clean sponge inoculated with only the inoculum pool. For this test, 100 mL 0.5% nonfat dry milk (NFDM; Mid America Farms, Springfield, MO) suspended in sterile deionized water was added to the sponge prior to application of the inoculum rather than the 100 mL deionized water used on clean sponges.

Treatment of Sponges with Liquid Dishwashing Detergent

The label-recommended amount of liquid hand dishwashing detergent, 5 or 8 mL, was added to the inoculated sponge in the stomacher bag. The detergent was then massaged 20 times into the sponge in the stomacher bag by

Table 4. Bacterial reduction on synthetic foam sponges after treatment with a liquid hand dishwashing detergent

			Confidence level[b]			
Treatment (use amount)	Mean[a] log cfu/mL	Std. dev.	95%	90%	Avg. log reduction	Avg. % reduction
Product B (5 mL)	3.98	0.26	a	a	0.32	45.1
Product A (5 mL)	4.06	0.79	a	ab	0.24	33.9
Untreated control (initial count)	4.24	0.01	a	ab	NA[c]	NA[c]
Untreated control (after incubation)	4.72	0.37	a	b	–0.52[d]	NA[c]
Commerical product C (5 mL)	6.44	0.05	b	c	–2.14[d]	NA[c]

[a] $n = 5$.
[b] Letter values are assigned to indicate a significant difference in the mean recovery between products at the specified confidence level.
[c] Not applicable.
[d] Sample demonstrated an increase in microbial content rather than a reduction.

Table 5. Bacterial inhibition on soiled[a] synthetic foam sponges after treatment with a liquid hand dishwashing detergent

Treatment (use amount)	Mean[b] log cfu/mL	Std. dev.	Confidence level[c] 95%	Confidence level[c] 90%	Avg. log inhibition	Avg. % inhibition
Dish drops (5 mL)	1.644	0.81	a	a	5.745	99.9998
Product E (8 mL)	2.296	1.12	ab	b	5.093	99.9992
Product F (8 mL)	2.779	1.23	b	b	4.610	99.9975
Product G (8 mL)	2.864	0.71	b	b	4.525	99.9970
Product H (5 mL)	3.601	0.59	c	c	3.788	99.9837
Untreated control (initial count)	4.706	0.09	d	d	NA[d]	NA[d]
Untreated control (after incubation)	7.389	0.20	e	e	NA[d]	NA[d]

[a] Sponges soiled with 0.5% non-fat dry milk (NFDM).
[b] $n = 10$.
[c] Letter values are assigned to indicate a significant difference in the mean recovery between products at the specified confidence level.
[d] Not applicable.

squeezing the bag to evenly distribute the detergent throughout the sponge. Inoculated, treated sponges were allowed to air dry overnight at room temperature (ca 72 F) for 16–24 h in the opened stomacher bag. For the purposes of this test, 5 g dish detergent was used unless the product label specified a different amount. Obviously, the effectiveness of a given concentration of detergent depends on the size and water-holding capacity of the sponge. Detergent use amounts can be adjusted to levels that provide efficacy, if desired.

Quantitation of Treated Sponges

The dried inoculated sponges were rehydrated the following day in the stomacher bag by adding 100 mL sterile deionized water to the sponge, followed by massaging or squeezing of the sponge. A 1 mL aliquot was removed for quantitation by spiral plater and pour plate techniques. Pour plates were used to quantitate values that were below the detection limit of the spiral plater. Plates were incubated for 24 h at 37 C, then enumerated using a laser counter or a Quebec Colony Counter.

Calculation of Microbial Control

Microbial control was determined by subtracting the $\log_{10}$ treated sponge count from the $\log_{10}$ untreated control sponge count. In the case of the NFDM soil tests, the microbial control was determined by subtracting the $\log_{10}$ treated sponge count from the $\log_{10}$ soiled but untreated incubated sponge count, because microbial counts increased significantly after incubation in the control sponges.

Liquid Hand Dish Detergents

Several liquid hand dish detergents were used in this evaluation and are listed in Table 2.

Table 6. Bacterial inhibition on soiled[a] cellulose sponges after treatment with a liquid hand dishwashing detergent

Treatment (use amount)	Mean[b] log cfu/mL	Std. dev.	Confidence level[c] 95%	Confidence level[c] 90%	Avg. log inhibition	Avg. % inhibition
Untreated control (initial count)	4.564	0.25	a	a	NA[d]	NA[d]
Dish drops (5 mL)	4.621	0.92	a	a	2.926	99.88
Product G (8 mL)	4.793	0.54	a	a	2.754	99.82
Product F (8 mL)	5.033	1.09	a	a	2.514	99.69
Product E (8 mL)	5.535	0.68	a	a	2.012	99.03
Product H (5 mL)	6.639	0.83	b	b	0.908	87.65
Untreated control (after incubation)	7.547	0.63	b	c	NA[d]	NA[d]

[a] Sponges soiled with 0.5% nonfat dry milk (NFDM).
[b] $n = 10$.
[c] Letter values are assigned to indicate a significant difference in the mean recovery between products at the specified confidence level.
[d] Not applicable.

Results and Discussion

Five replicates were run on each treatment for unsoiled (clean) sponges. Ten replicates were run on each treatment for sponges with soil. Performance testing without NFDM soil is shown in Tables 3 and 4. Test results were evaluated using log normal analysis of variance, and results are also shown in the 2 tables. For a valid test, sufficient microbial populations in the control sample should survive through the evaluation period. Tables 3 and 4 indicate that the microbial population in the control survived. In both tests, the microbial content of the sponge increased with time but not at a statistically significant level. Table 3 indicates that the dish drops (Access Business Group International, LLC, Ada, MI) formulation used at 5 mL per sponge provided a 99.99% reduction in microbial populations, while commercial product D at 8 mL per sponge provided a 99.9% reduction in microbial population. Both detergents were significantly better than the control and dish drops was significantly better than commerical product D.

There was a range of performance observed among products making "antibacterial" types of claims. As can be seen in Table 4, this range can be from effective to noneffective. Commercial product B, making an "antibacterial sponge" claim, resulted in a 45.1% reduction in microbial content of the sponge and was significantly different from the control. Commercial product A, which makes an "antibacterial hand soap" claim, showed a 33.9% reduction in microbial content of the sponge and was not significantly different from the control. Commercial product C, making an "antibacterial sponge" claim, showed a significant increase in the microorganisms within the sponge.

Results from the test method without additional soil indicate that this method is capable of defining performance differences between commercial products as well as documentation of efficacy of products making "antibacterial sponge" claims. The variance in both tests was only significant between treatments (products) and was not significant between replications. The *F*-ratio of the replicates in both tests was 0.1, indicating that the replicate variance is not statistically significant and the test method is reliable.

Test results with soil load in both synthetic and cellulose sponges are shown in Tables 5 and 6. Test results were evaluated using log normal analysis. As would be expected, the presence of a soil load resulted in a significant increase in microbial population in both the synthetic and cellulose sponges when compared with initial inoculum levels. Average increases were 2.9 log cfu/mL from an initial mean of 4.6 log cfu/mL to an incubated mean of 7.5 log cfu/mL average. These results simulate microbial populations in household dish sponges where high heterotrophic bacterial counts of 8.08 (4) and 7.01 log cfu/mL (6) have been reported. Table 5 shows results of treating soiled synthetic sponges with 5 commercial hand dishwashing products. Microbial growth in soiled synthetic sponges was inhibited by 99.8 to 99.9998%, with significant differences between products. For this data, the microbial inhibition is calculated from the final count untreated control rather than the initial count un-treated control.

Table 6 shows the results of the soiled cellulose sponge testing. As with the synthetic sponge data, significant inhibition of microbial populations was observed (87.6–99.9%). All detergent products were significantly different from the control and differences between products were significant. Total microbial inhibition was not as great with cellulosic sponges as with the synthetic sponges. The synthetic sponges used in this test, typical of consumer sponges worldwide, hold about 5 g water when wrung. Comparably sized cellulose sponges hold about 10 g water. Thus, the hand dishwashing detergent was twice as concentrated in the synthetic sponges as the cellulose sponges and greater microbial control, which would have been expected, was observed. Some variance in label use directions may be warranted on the part of manufacturers regarding sponge type and use level.

Results from the test method run with NDFM soil indicate that the test method is capable of differentiation between commercial products as well as documentation of efficacy of the "antibacterial sponge" use method. The variance in both tests was only significant between treatments or products and not significant between replicates. The *F*-ratio of the replicates for synthetic sponges was 0.5 and, for cellulosic sponges, 1.8, indicating that the replicate variance is not significant and the test method is reliable.

Conclusions

The test procedure presented in this paper has been shown to be capable of demonstrating the efficacy of dishwashing detergent use to control microbial populations in kitchen sponges. Additionally, the proposed method simulates consumer use of a liquid dishwashing compound to control bacteria in kitchen sponges. The procedure is a reproducible and reliable method in both clean and soiled sponges, as noted by the statistically insignificant *F*-ratio values for replicate variance. As kitchen sponges in domestic use carry a natural organic load, the use of the NFDM soil load is more representative of actual use conditions. This test method is the preferred method. However, the use of clean sponges in the procedure may be applicable for screening evaluations. It is also desirable that any test procedure is able to differentiate between the performance of competitive products, and the data presented demonstrates this ability. This test procedure is satisfactory for use in evaluating both product performance and "antibacterial sponge" claims documentation.

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