1 Comparison of two plain soap types for removal of bacteria and viruses from hands with specific
2 focus on food service environments

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Abstract

Handwashing (HW) is a long established and widely accepted method to prevent disease transmission. Ensuring effectiveness of current HW methods is essential for the optimization of HW and enhanced disease prevention. The objective of this research was to determine the difference in microbial reduction between plain foaming and liquid handsoap. The hands of 24 participants were inoculated by the palmar surface method with an average of 1.25×10^8 CFU *Escherichia coli* C3000 or 1.36×10^8 PFU MS2 bacteriophage. Participants washed their hands following a standard protocol with a standardized soap volume and a 10 s HW time. A glove juice method was used to recover microorganisms from hands. Remaining microorganisms were quantified by standard spread plate and plaque assays for *E. coli* and MS2, respectively. Hands inoculated with *E. coli* had an average log reduction of 2.76 ± 0.70 and 2.52 ± 0.58 log CFU for foaming and liquid handsoap, respectively. The mean log reduction for hands inoculated with MS2 was 2.10 ± 0.57 and 2.23 ± 0.51 log PFU for foaming and liquid handsoap, respectively. Data indicate no significant difference in overall microbial removal when comparing the efficacy of plain foaming and liquid handsoap. However, regardless of soap type, the type of microorganism impacted overall log reduction with a greater reduction for *E. coli* when compared to MS2 with a significant difference ($p = 0.0008$) in reduction for foaming handsoap. This study is the first comparison of the efficacy of plain liquid and foaming handsoap for microbial reduction on hands during HW.

Keywords: handwashing, MS2 bacteriophage, *Escherichia coli*, microbial removal, food service, foaming soap

1. Introduction

It is estimated that foodborne pathogens, both major known pathogens as well as unspecified agents, cause 47.8 million illnesses, 127,830 hospitalizations, and 3,037 deaths in the U.S. each year with the leading causes of illness including noroviruses (58%), nontyphoidal *Salmonella* spp. (11%), *Clostridium perfringens* (10%), and *Campylobacter* spp. (9%) (Scallan, Griffin, Angulo, Tauxe, & Hoekstra, 2011). Pathogenic strains of *Escherichia coli* and *Salmonella* are more commonly associated with raw meat (i.e. beef and poultry, respectively) as animals are often hosts for these pathogens (Forsythe 2010). However, cross contamination of pathogens between raw meat and ready-to-eat food products via food handlers' hands is a potential risk; therefore, proper handwashing (HW) is an essential control measure for risk reduction (USFDA 2013). With respect to foodborne viruses, an epidemiologic investigation of foodborne norovirus outbreaks in the U.S. from 2001-2008 found that 53% (473) of the 886 outbreaks were caused by food handler contamination (Hall et al., 2012). Additional analysis of foodborne norovirus outbreaks from 2009 to 2012 confirmed these findings with food workers implicated in 70% of 520 outbreaks, and bare hand contact was identified in 54% of the outbreaks (Hall, Wikswo, Pringle, Gould, & Parashar, 2014). The recommended interventions for preventing norovirus in a food service environment primarily include following US Food and Drug Administration (FDA) *Food Code 2013* guidelines for HW and glove use (Hall et al., 2012; USFDA 2013).

The general population uses HW as an important step in disease prevention, and this is especially critical within a food service environment (Miller, James-Davis, & Milaneis, 1994). The hands of food service employees may become contaminated with foodborne pathogens during critical stages in food service including after using the restroom, handling raw materials

(e.g., meats, vegetables, eggs, etc.) and after touching contaminated surfaces (Miller, James-Davis, & Milaneis, 1994). Therefore, studies on the efficacy of HW agents are essential to ensure that HW procedures are optimized for removal of pathogenic microorganisms from hands during food service.

Numerous soaps (i.e. brands, types, formulations, etc.) are available on the market today, and food service staff and the general population use these soaps daily. Plain (non-antimicrobial) handsoap reduces soil, dirt, and in the case of food service, various physical and biological materials on hands through physical removal with detergents. Meanwhile, antimicrobial handsoap combines physical removal with the inactivation of microorganisms by antimicrobial compounds in the soap that differentially affect viruses and bacteria (Fuls et al., 2008; Sickbert-Bennett et al., 2006). While there have been numerous studies comparing the efficacy of antimicrobial and plain handsoap (Edmonds, McCormack, Zhou, Macinga, & Fricker, 2013; Fuls et al., 2008; Montville & Schaffner, 2011), the soaps used in these studies are typically liquid handsoap. In a recent review by Conover and Gibson (2016), the methodologies and results of 24 HW studies published since 1985 are discussed and despite the vast range of HW agents tested in these studies, only one study evaluated foaming handsoaps (Fuls et al., 2008) and none compare the efficacy of foaming and liquid handsoap. For this reason, the authors of the present study selected to compare plain foaming and liquid handsoaps. One of the primary differences between foaming and liquid handsoap is the level of surfactant. Foaming soaps generally have a lower level of surfactants, and as a result, these soaps do not form micelles as readily as liquid handsoap. Meanwhile liquid handsoaps typically have increased surfactant levels as well as additional salts that allow for the formation of micelles (personal communication provided by M.

Caetta, VCI Formulation Specialist at GOJO Industries, Inc.) that aid in the removal of dirt and oils as well as microorganisms.

With the increasing prevalence of foaming handsoap on the market and within food service establishments, it is critical to determine if the associated microbial reductions are comparable to that of traditional, plain liquid handsoap. For this study, we hypothesized that there would be a significant difference in microbial reduction between foaming and liquid handsoap. More specifically, we hypothesized that reduction of bacteria and virus on hands would differ depending on soap type. Therefore, the overall goal of this study was to determine if a difference exists in the efficacy of plain foaming and liquid handsoap by measuring the reduction of microorganisms on hands inoculated with non-pathogenic *E. coli* and MS2 bacteriophage—a surrogate for the study of human enteric viruses such as norovirus.

2. Materials and Methods

2.1. Study design

The study was based on a Latin square design. The treatment structure was a two by two factorial with microorganisms (*E. coli* C3000 and MS2) and soap type (foaming and liquid) as the two different factors. Each participant visited one time per week over a four week period and was randomly assigned to one of four sequences of treatment. Sequences were selected to alternate exposure of participants to microorganism type and soap type and to adjust for any possible confounding factors (e.g., learning by either the researchers or the experimental participants or any carryover effects that could potentially be present throughout the four weeks of the study).

2.2 Participant recruitment and training

Twenty-four participants (12 men and 12 women), 18 years and older, were recruited from the University of Arkansas (Fayetteville, Arkansas) community. Participants had healthy skin, with no presence of dermatitis, open wounds, cuts, burns, hangnails, or any additional known disorders of the skin (ASTM 2013a). Institutional Review Board and Institutional Biosafety Committee approval were obtained, and participants were informed about the safety of microorganisms used in the study. All participants signed an informed consent form to participate in the study. Sample size was determined based on a minimum power of 0.8 with the following parameters: $\alpha = 0.05$, standard deviation = 0.6, and a difference to detect of 0.5 $\log_{10}$ CFU or PFU.

To employ a standardized HW procedure throughout the study, prior to participating, participants were trained on a standard HW protocol (Singapore Motherhood, 2012). Participants were given 30 s to complete the HW procedure during training as well as throughout the decontamination steps of the study. The actual experimental handwash was completed in 10 s which is considered more representative of actual HW time occurring in daily life (discussed in Section 2.7).

2.3. Preparation of inocula

2.3.1. Preparation of MS2 bacteriophage

A stock of MS2 bacteriophage (ATCC 15597-B1; American Type Culture Collection, Manassas, VA) was prepared through propagation in *E. coli* C3000 followed by chloroform extraction of the infected cell lysate as described previously by Gibson, Crandall, & Ricke (2012). The stock concentration of MS2 bacteriophage was determined to be approximately 10^{11}

PFU/mL by the double agar layer (DAL) method. One milliliter aliquots of MS2 were stored at -80°C. The phage stock was diluted with 0.1% peptone (Becton Dickinson and Company, Sparks, Maryland) to approximately 6.78×10^8 PFU/mL.

2.3.2. Preparation of *E. coli* C3000

Overnight stocks of *E. coli* C3000 (ATCC 15597; ATCC) was prepared in a culture flask containing 25 mL of tryptic soy broth (Acumedia, Lansing, Michigan) incubated at 37°C with shaking at 110 rpm. Stock concentrations were determined by preparing a ten-fold dilution series and plating 1 mL of each dilution in duplicate on 3M Petrifilm™ *E. coli*/coliform count plates (3M, Maplewood, Minnesota). *E. coli* C3000 overnight culture (approximately 10^9 CFU/mL) was diluted with 0.1% peptone (Becton Dickinson and Company) to approximately 6.26×10^8 CFU/mL for inoculation on participants' hands.

2.4. Hand decontamination prior to inoculation

To eliminate resident microorganisms on the hands of participants prior to inoculation with test organisms, hands were treated with a conditioning wash as described by Fuls et al. (2008) with modifications. Modifications included using 1 pump of antibacterial handsoap (The Dial Corporation, Scottsdale, Arizona) with subjects scrubbing hands for 30 s and rinsing hands for 10 s. Hands were also twice soaked in 70% ethyl alcohol and dried thoroughly before inoculation with microorganisms.

2.5. Inoculation of hands

Hand inoculation of *E. coli* C3000 and MS2 was performed by the palmar surface method (PSM) as described in the ASTM Standard Test Method E2870-13 with modifications. One-hundred microliters of prepared *E. coli* or MS2 inoculum were pipetted onto the palm of

each hand (200 µl total) for an average of 1.25×10^8 CFU total (hands combined) or 1.36×10^8 PFU total (hand combined), respectively. The participants were asked to rub the palms and fingers of each hand against each other for 10 ± 1 s in order to spread the inoculum on the palms and fingers of each hand. Following inoculation, the hands were air-dried for 20 ± 5 s.

2.6. Selection of soaps

Two automatic dispensers, one foaming (GOJO Industries, Akron, OH) and one liquid (Epare, Staten Island, New York), were selected for use in this study to limit potential contamination of the soap dispenser from participants and to standardize the volume dispensed each time. Based on the authors' unpublished survey of soap volumes in food service establishments, soap volumes were chosen as follows: one dispense of foaming soap was 0.9 mL (i.e. after allowing 'foam' to subside), and one dispense of liquid handsoap was 1.5 mL.

2.7. Handwashing and drying

After allowing the inocula to dry, the participants were immediately asked to wash their hands with one of the test soaps using the method described in Section 2.2. Following the 10 s handwash, hands were rinsed for 10 s. Participants then dried hands for 10 s with three paper towels, and hands were immediately sampled for recovery of microorganisms using the glove juice method (GJM; Section 2.8.).

2.8. Recovery of microorganisms by GJM

Remaining microorganisms were recovered using the GJM described in ASTM E1174-13 with modifications (ASTM 2013b). Modifications included using 35 mL of 0.1% peptone (Becton Dickinson and Company) sampling solution in each glove. As the soaps used did not contain antimicrobial agents, no neutralizers were added to the sampling solution. An additional

modification included recovering all of the remaining liquid from each glove and transferring to a sterile, glass sample jar (VWR) and using a portion of the remaining solution to dilute and plate. Volumetric adjustments were made based on total volume recovered (Equation 1).

Prior to study commencement, the recovery efficiency of the GJM was determined to be 2.3 and 8.9% for *E. coli* C3000 and MS2, respectively. These values were based on two experimental replicates with samples plated in duplicate. Recovery efficiency was calculated using Equation 2. To ensure the true log reduction was determined, percent recovery efficiency for each microorganism was then incorporated into calculating the recovery of microorganisms (Equation 3). The log reduction that occurred due to HW with foaming and liquid handsoap was then calculated (Equation 4). After the recovery of microorganisms from hands, hands were again decontaminated following the procedure described in Section 2.4.

Equation 1:
$$R_M = \frac{T}{V_A} \times V_R$$

Equation 2:
$$RE = (R_M / I) \times 100$$

Equation 3:
$$I_{RE} = I \times (RE/100)$$

Equation 4:
$$LR = \log_{10} (I_{RE}/R_M)$$

Where: R_M = microorganisms recovered (CFU or PFU); T = total CFU or PFU counted; V_A = volume analyzed (mL); V_R = total volume recovered by GJM (mL); RE = % recovery efficiency; I = initial total inoculum (CFU or PFU); I_{RE} = inoculum with recovery efficiency; LR = $\log_{10}$ reduction (CFU or PFU)

2.9. Detection of microorganisms in recovered sampling solution

For *E. coli* C3000, 1 mL aliquots of each dilution were plated onto 3M Petrifilm™ *E. coli*/coliform count plates (3M) in duplicate and incubated at 37°C for 24 h. After 24 h, CFU

were counted. A negative control of 1 mL of 0.1% peptone was plated as well as a positive control of 1 mL of *E. coli* C3000 at a concentration of 10^1 to 10^2 CFU/mL. For MS2, the DAL method was used as described previously (Gibson, Crandall, & Ricke, 2012). Following incubation for 16-24 h at 37°C PFU were counted, and the PFU/mL were determined. A negative control with 100 μ L of log phase *E. coli* C3000 plus 100 μ L of 0.1% peptone was analyzed in each set of experiments. A positive control of 100 μ L of 1×10^2 PFU/mL MS2 and 100 μ L of log phase *E. coli* C3000 was also performed.

2.10. Statistical Analysis

JMP® Pro 11 (SAS, Cary, NC) statistical analysis software was used for all data analysis. As the study was primarily based on a Latin Square design, with elements of a split plot design, a mixed model was used to test the significance of differences that occurred in reduction of microorganisms for each soap type (foaming or liquid) as well as for each microorganism type (*E. coli* or MS2). Sequence and week were incorporated into the model to account for any variance that may have occurred due to learning by either the participants or researchers throughout the study as well as for any potential cross-over effect that may have occurred between weeks of the study. Gender was also added as an effect in the model to determine any significance. Multiple comparison of the interaction means was performed with Tukey's honest significant difference (HSD) test. Results were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Efficacy of foaming and liquid handsoaps

All 24 participants completed all 4 weeks of the study. The average age of the participants was 31 years of age. As stated in Section 2.5, the initial inoculum on hands was on

average 1.25×10^8 CFU *E. coli* C3000 and 1.36×10^8 PFU MS2. Table 1 reports the average total log reductions for *E. coli* C3000 and MS2 on hands for foaming handsoap, liquid handsoap, and the baseline wash with water. However, this baseline water only wash was not considered a treatment in the mixed model used for statistical analyses (Section 2.10).

3.2. Factors influencing HW efficacy

Statistical analysis of log reductions indicates no significant difference in efficacy of foaming and liquid handsoap for overall microbial removal ($p = 0.56$). However, the reduction of microorganisms on hands was significantly different depending on the type of microorganism with increased reductions occurring for hands inoculated with *E. coli* C3000 as compared to hands inoculated with MS2. Based on Tukey's HSD test, MS2 reduction was significantly less with foaming soap when compared to *E. coli* reductions with foaming soap ($p = 0.0008$). Table 1 shows the comparison of log reduction for each microorganism by treatment. Gender did not have a significant impact on the reduction of microorganisms on hands ($p = 0.44$).

4. Discussion

Even though the results presented here indicate no significant difference in overall microbial removal between foaming and liquid handsoap, the type of microorganism on the hand had a significant effect, with a greater reduction occurring for *E. coli* as compared to MS2. Of particular interest is that while a greater reduction for *E. coli* was observed with foaming soap (2.76 log CFU) as compared to liquid (2.52 log CFU), the opposite was true for MS2 with a 2.10 log PFU reduction using foaming soap and a 2.23 log PFU reduction using liquid soap.

Although the exact reasons behind these differences in reduction are not clear, it is known that MS2 particle size (24-27 nm)—and human enteric viruses in general—is much

smaller than that of bacteria such as *E. coli* (500 nm in diameter) (Abbsazadegan, Mayer, Ryu, & Nwachuku, 2007; Strauss & Sinsheimer, 1963). In addition, MS2 bacteriophage is strongly hydrophobic and this property impacts its attachment to particulates (Bales, Li, Maguire, Yahya, & Gerba, 1993; Shields & Farrah, 2002). With respect to lower reductions in MS2 when using foaming soap when compared to *E. coli*, a possible theory is that the increased surfactant levels of liquid soap create conditions in which the smaller, hydrophobic MS2 particles are surrounded by micelles and are more readily removed from the hands. Conversely, the decreased surfactant concentrations of the foaming handsoap creates conditions that do not allow for the same micelle formation (personal communication provided by M. Caetta, VCI Formulation Specialist at GOJO Industries, Inc.); therefore, limiting the ability to surround the particles with the micelle structure and making it difficult to remove the smaller, hydrophobic MS2 particles present on hands.

The complex physiological state of the skin surface also likely plays a role into how effectively microorganisms are removed from the hand. Numerous researchers have studied skin physiology and have shown that microflora on hands can vary from person to person due to numerous factors including environment, age, and sex (Jumaa 2005; Larson 1985). Each of these variables can cause variation in skin flora, sebum (i.e. oily substance) production, skin flexibility, and skin permeability all of which may impact microbial removal during HW (Leveque, deRigal, Agache, & Monneur, 1980; Noble, 1978).

With respect to MS2 removal reported in previously published studies, Sickbert-Bennett et al. (2005) observed that tap water was actually the most effective for removal of MS2 from hands with 1.89 to 2.56 log PFU reduction followed by plain, liquid soap with 1.54 to 2.03 log PFU reduction. A baseline wash with tap water was completed in the present study, and although this was not technically a ‘treatment’, the results reported here for tap water (1.20 log PFU

reduction) do not corroborate the findings by Sickbert-Bennett et al. (2005) while similar results for plain, liquid handsoap are reported (2.23 log PFU reduction). Soap volume is one difference between the present study and that of Sickbert-Bennett et al. (2005). The authors of that study used 3 mL of liquid handsoap with a 10 s handwash, while the present study used half this amount of soap (1.5 mL) and a 10 s handwash. Although both studies used a 10 s handwash time, the difference in soap volumes may have an effect on the differences in reduction of MS2 by plain, liquid handsoap occurring between the two studies.

Another study by Burton et al. (2011) reported results that echo the findings of the present study and demonstrated that HW with plain soap was more effective than HW with water alone for reduction of naturally acquired bacteria on hands. Several additional studies have investigated the reduction of non-pathogenic *E. coli* on hands via HW and have evaluated the effect of numerous variables including soap type (plain versus antimicrobial) and HW time (Ansari, Sattar, Springthorpe, Wells, & Tostowaryk, 1989; Ayliffe, Babb, Davies, & Lilly, 1987; Fuls et al., 2008; Miller et al. 2011; Paulson et al. 1999; Sasahara, Hayashi, Hosoda, Morisawa, & Hirai, 2014; Snyder 2007; Stiles & Sheena, 1985). In a meta-analysis by Montville and Schaffner (2011), an average reduction of 1.93 ± 0.91 log CFU was reported for studies that inoculated hands with gram-negative bacteria such as *E. coli*. This reduction is comparable to what is reported in the present study for reduction of *E. coli* with plain foaming and liquid handsoaps (2.76 ± 0.70 and 2.52 ± 0.58 log CFU, respectively).

Compared to studies on HW efficacy against bacteria, very few studies have been reported on the reduction of human enteric viruses or viral surrogates achieved through HW with either liquid or foaming handsoaps (Ansari, Sattar, Springthorpe, Wells, & Tostowaryk, 1989; Lin et al., 2003; Liu, Yuen, Hsiao, Jaykus, & Moe, 2010; Mbithi, Springthorpe, & Sattar, 1993;

Sickbert-Bennett et al. 2005; Stowell et al., 2014). Liu, Yuen, Hsiao, Jaykus, & Moe (2010) investigated the efficacy of liquid soap for removal norovirus, using two different finger pad protocols—the standard ASTM method (E 1838-2) and a modifications of this method. The average log reductions achieved with a liquid handsoap containing 0.5% triclosan were 0.67 and 1.10 log for the standard and modified ASTM methods, respectively. Even though the described study used a liquid handsoap containing an antimicrobial agent, the reported log reductions were lower than those reported in the present study and more similar to the baseline water only wash. Numerous differences occurred between the present study and that of Liu, Yuen, Hsiao, Jaykus, & Moe (2010) including microorganisms selected, method of detection (molecular detection versus plaque assay for infectivity), and the HW treatment method.

Lin et al. (2003) compared HW techniques to remove *E. coli* and caliciviruses under natural fingernails. For nails inoculated with *E. coli*, reductions of 1.18 log CFU were reported for both tap water and plain, liquid soap, respectively; meanwhile, reductions of feline calicivirus (FCV) were 1.97 and 1.82 log PFU for tap water and plain, liquid soap, respectively. Reductions reported by Lin et al. (2003) for FCV are comparable to the reductions reported for MS2 in the present study, while the reductions achieved for *E. coli* are quite a bit lower than the reductions reported in the present study. While the palmar surface method of inoculation was applied in the present study, Lin et al. (2003) utilized contact of the underside of the nail with inoculated ground beef to simulate real-life contamination of fingernails which may explain the difference in log reduction values. Additional studies evaluating the efficacy of various HW agents including plain, liquid soap in removal of enteric viruses (i.e. hepatitis A virus, poliovirus type 1, human rotavirus) from hands have reported log reduction values ranging from 1 to 1.2 logs (Ansari, Sattar, Springthorpe, Wells, & Tostowaryk, 1989; Mbithi, Springthorpe, & Sattar,

1993). Compared to the present study, these log reduction values are nearly 1-log lower than those reported for MS2 in the present study and are more similar to the baseline water wash removal. Again, many of these differences can be explained by variations in methodology even though several standard methods for the evaluation of HW agents exist both in the U.S. and the European Union (E.U.) (e.g., ASTM E1174-13, ASTM E2870-13, EN 1499, and EN 1500)

As evidenced by the papers referenced above, researchers test a variety of variables when performing HW studies and numerous variables are changed between studies (especially HW time, soap volume, and soap type). Although there are numerous standard methods for the evaluation of HW efficacy (ASTM (2013a), ASTM (2013b), Ayliffe, Babb, & Quoraishi, 1978, CEN 2013a, CEN 2013b), researchers often change numerous variables based on their experimental design or research objectives. This further complicates direct comparison of data between studies. Additionally, HW is a process that inherently has a great amount of variation each time it occurs, and controlling or standardizing all parts of the HW process is a difficult task.

Here, it is important to acknowledge some of the limitations of the present study. In food service facilities, food handlers' hands are potentially covered with numerous organic materials including oils and food particles as well as both resident and transient microorganisms. However, to eliminate unnecessary variables for the initial evaluation of foaming versus liquid handsoap participant hands were decontaminated at the beginning of each study day and then inoculated with only one test microorganism at a time. Based on this, one limitation of the present study is that the experimental design does not replicate the conditions that would be present in a food service facility. To address this, future research on the effect of additional organic material on hands on the reduction of both viruses and bacteria on hands when washed with foaming and

liquid handsoap would be beneficial. An additional limitation is the use of a short HW time in our study (10 s wash and 10 s rinse). This HW time was selected to be more representative of what has been observed and reported in the peer-reviewed literature as well as based on the authors' experience (Graham 1990; Meengs, Giles, Chisholm, Cordell, & Nelson, 1994; Munger & Harris, 1989; Sickbert-Bennett et al. 2005; Strohbehn, Sneed, & Meyer, 2008). However, it should be noted that the FDA *Food Code 2013* recommends a 20 s HW time with vigorous scrubbing for 10 to 15 s therefore the 20 s may include the rinse time as well. A final, potential limitation is the low recovery efficiency of the GJM. In order to keep the recovery solution in the glove, a volume of 35 mL was chosen for each glove. This lower volume may have had an impact on the recovery efficiency. However, as discussed in Section 2.8, recovery efficiency was incorporated into the overall log reduction that occurred on hands to account for this. Moreover, previously published studies that utilized a GJM rarely report recovery efficiency, and because of this we were unable to accurately compare our recovery efficiency with that of other studies.

5. Conclusions

Results of the present study indicate that no significant difference in overall microbial reduction occurs between foaming and liquid handsoaps. However, a significant difference does exist between the types of microorganisms on hands (bacteria versus viruses). Based on this, future research related to optimization of HW for increased virus removal is warranted. This is especially important since poor hand hygiene is significant to the transmission of human noroviruses in the food service industry as well as within healthcare and other settings commonly implicated in human norovirus outbreaks (Barclay et al. 2014; Hall et al. 2012; Hall, Wikswo, Pringle, Gould, & Parashar, 2014; Moe 2009).

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503 Table 1: Comparison of log reduction of *E. coli* and MS2 by treatment.

Treatment	Total Log ₁₀ CFU or PFU Reduction ($\pm$ SD)		
	Microorganism		p-value
	<i>E. coli</i>	MS2	
Foaming	2.76 (0.70)	2.10 (0.57)	0.0008
Liquid	2.52 (0.58)	2.23 (0.51)	0.079
Water	2.45 (0.93)	1.20 (0.49)	<0.0001

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