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Running Head: Influence of water temperature, soap volume, lather time, and product formulation on handwash efficacy

Quantifying the Effect of Water Temperature, Soap Volume, Lather Time, and Antimicrobial Soap as a factor in the Removal of *Escherichia coli* ATCC 11229 from Hands

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Abstract

The handwashing literature, while extensive, often contains conflicting data and key variables are understudied or not studied at all. Some handwashing recommendations are made without scientific support, and there is limited agreement between recommendations. The influence of key variables including soap volume, lather time, water temperature, and product formulation on hand wash efficacy was investigated. Baseline conditions were 1 mL of a bland (non-antimicrobial) soap, a 5 s lather time, and 38 °C (100 °F) water temperature. A nonpathogenic strain of *Escherichia coli* ATCC 11229 served as the challenge microorganism. Twenty volunteers (10 men, 10 women) participated in the study and each test condition had 20 replicates. An antimicrobial soap formulation (1% chloroxylenol, or PCMX) was not significantly different from the bland soap at removing *E. coli* under a variety of test conditions. Overall, the antimicrobial soap used in this study had a mean 1.94 log CFU reduction (range 1.83 to 2.10 mean log reduction), and bland soap had a mean 2.22 log CFU reduction (range 1.91 to 2.54 mean log CFU reduction). Overall, lather time did significantly influence efficacy in one scenario, in which a 0.5 greater log reduction was observed for a 20 s with bland soap compared to the baseline wash (P=0.020). Water temperature as high as 38°C (100°F) vs. a low of 15°C (60°F) did not have a significant effect on the reduction of bacteria during hand washing, however this resulted in an energy usage difference between the temperatures. No significant differences were observed between mean log reductions of men and women (men= 2.08 mean log

reduction, women=2.08 mean log reduction, *P*=0.988). A large part of the variability in the data observed was between the volunteers. Understanding what behaviors and human factors influence hand washes the most may help future studies find which techniques can optimize the effectiveness of a hand wash.

The US FDA Model Food Code makes recommendations regarding handwashing frequency, duration and technique (*71*), however the scientific support for many of those recommendations is not always clear, nor based on recent evidence. Section 2-301.12 of the Food Code requires the use of a "cleaning compound" (soap) during a hand wash (*71*). The type of compound is not specified, and facilities may elect to use either bland (soap without antimicrobial) or antimicrobial soap.

Recently, the FDA Center for Drug Evaluation and Research (CDER) issued a final rule which established that over-the-counter consumer antiseptic washes (soaps), with specific active ingredients may no longer be marketed in the United States after September 6, 2017 (*26)*. The FDA indicated that the companies which produce these antimicrobial soaps have not provided sufficient evidence to prove daily-use safety and better efficacy than bland soap and water. This final rule covers 19 specific active ingredients, including triclosan. However, the FDA has deferred the rule for three ingredients: benzalkonium chloride, benzethonium chloride, and chloroxylenol (PCMX). This rule does not extend to hand sanitizers or antiseptic wipes, not does it address antimicrobial soap sold for use in foodservice or food processing facilities.

Active ingredient compounds used in antimicrobial soaps disrupt bacteria cell function or reproduction, working by either being bactericidal (destroying the cell) or bacteriostatic (inhibiting reproduction). These compounds are antiseptics, and not considered antibiotics (*17*, *61*). The literature suggests that antimicrobial soaps provide a greater bacterial reduction than bland soap (*25*, *29*, *31*, *54*, *63*, *66*), however some studies found minimal difference (*15*, *51*, *68*). A hand soap meta-analysis found that antimicrobial soaps, when accounting for all types of bacteria and formulations, tended to have a ~0.5 log CFU greater microbial reduction than bland soap (*54*). Product formulation plays a key role in the effectiveness of antimicrobial agents and soaps, as many active compounds (antimicrobials) are available to use in soaps, and surfactants, in addition to other ingredients used in soaps or lotions, can impede or enhance these compounds and the overall antimicrobial effect (*14*, *27*, *70*).

The combined literature on soap volume (i.e. the dose or amount used per hand cleaning event) shows no significant trends in terms of strong interactions between soap volume and effectiveness of soap (*29*, *43*, *54*). The data can be confusing, and often conflicting if too many brands and formulations are compared to each other. Fuls *et al*. found increasing amounts of foaming, 0.46% triclosan antimicrobial soap (1.5-3 g or 2-4 pumps of soap) increased their observed log reductions by ~0.7 log counts (P<0.001), but did not observe a significant increase in microbial reduction when using more bland soap (P=0.2) (*29*). Larson *et al* (1987) found that a control wash with bland soap was not significantly affected by amounts of soap used (1mL vs. 3 mL) (*43*). However, Larson *et al* (1987) also suggested that greater volume of soap could contribute to increase skin damage, and suggested that the minimal amount of soap required for a thorough wash should be chosen to reduce the incidence of skin damage (*43*).

The temperature of water required for an effective handwash is a variable that has been minimally explored, and still generates interest. There is an upper limit for the wash water temperatures, as achieving high temperatures that would rapidly destroy bacterial cells would also severely injure human skin (*42*, *69*). The temperature of the water (in of itself) used during a comfortable hand wash would not result in microbial inactivation due to thermal exposure from the water. Higher temperatures may still affect hand washing by increasing solvation or temperature dependent reaction rates. Boyce & Pittet (*17*) recommend avoiding using hot water to wash hands, as repeated exposure may lead to increase risk of dermatitis (damaged skin). Temperatures greater than 55°C can lead to scalding, and the recommended water temp for human skin comfort is ≤43°C (*42*, *69*). A handwashing survey determined that comfort of hands and personal beliefs played a key role when choosing the temperature for a handwash (*19*). Two studies by Michaels *et al* found no difference in the microbial reductions of hand washes performed at a range of temperatures (4.4 °C - 48.9 °C) (*50*, *51*). However, the two studies did not use a large pool of volunteers (4 subjects), and only one study (*51*) tested antibacterial soap. Courtenay *et al.* measured the differences between a ServSafe recommended wash (which includes soap), a cool rinse, and a warm rinse (*21*). Courtenay *et al.* observed a minor difference in microbial reduction between a cool rinse (26 °C) and a warm rinse (40 °C), but the influence between temperature and soap could not be inferred from this data (*21*). A study that analyzed various ways to sample bacteria from hands did not notice a significant difference between bacteria recovered when the sampling solution was at 6 °C or 23 °C (*46*). While all of these studies indicate that the temperature of a wash has no significant antimicrobial benefit, the limited repetitions of the studies (*21*, *50*, *51*), comparisons of a wash without soap (*21*), and the lack of an actual hand wash (*46*) indicates further need to study the effect of wash water temperature.

The Food Code (Section 2-301.12-B-3) requires lathering for 10-15 seconds (*71*). No published studies have specifically measured lather time as a variable. However, the added friction (from a brush) has been previously studied, in two studies (*47*, *60*) with differing results. Price *(60)* showed improved microbial reduction efficacy with more scrubbing (constant, time dependent), but Loeb *et al. (47)* did not observe a difference between a hand wash with a brush and one without. A meta-analysis of the published handwashing literature suggested that more studies are needed to understand the importance of wash duration (*54*). However many manuscripts which have explored total wash time suggest greater wash times are correlated with greater microbial reductions (*25*, *29*, *35*, *48*, *56*). Some studies surprisingly suggest that an extended wash, greater than 30 s, may result in a less effective reduction of transmissible microbes, which would diminish the intended purpose of hand washing (*41*, *51*, *54*). One study hypothesizes that an extended wash (>30 seconds) may loosen, but not remove resident flora from hands, and these loosened microbes are now more easily transferred to other surfaces, which results in less perceived benefit from removing microorganisms (*51*). Extended washes and frequent washing, can lead to damaged skin (*4*, *28*, *30*, *38-40*, *59*, *64*, *67*, *73*, *74*, *77*), which promotes colonization by more dangerous microbes and reduces the ability of a handwash to remove bacteria from the (damaged) skin (*41*, *42*, *44*). Bidawid *et al* observed that when finger pads, inoculated with Hepatitis A, were rinsed with 15 mL water, there was no detectable transfer of virus to lettuce pieces, but when rinsed with only 1 mL water, they observed 0.3% transfer, suggesting exposure to a greater volume of water may play a key role in hand washing (*16*). These conflicting results show that more research is needed to determine what part of the handwash can be lengthened to result in an increase in microbial reduction.

There is a tremendous amount of misinformation and missing data in the handwash literature. Many handwashing recommendations are being made without scientific backing, and there is limited agreement amongst these recommendations, as evidenced by the large inconsistencies amongst hand washing signage (*36*). The goal of this study was to close knowledge gaps in the handwash literature surrounding soap volume, temperature of water, and lather time in a handwash. The findings from this work will help drive some valid, evidence based, helpful conclusions for future personal hygiene policies and practices.

Materials and Methods

Volunteers. Twenty-one volunteers were selected from Rutgers University and surrounding communities. Rutgers IRB approval was obtained per the standard process prior to testing volunteers in this study. Volunteers were asked to refrain from using any type of antimicrobial hand soap and non-alcohol based hand sanitizers products for the duration of the study to avoid build-up of antimicrobial active ingredients on the skin, which could have interfered with the results (*2*, *12*, *29*, *55*, *57*, *65*). Exclusion criteria included taking antibiotics or being ill during the previous six weeks leading up to the start of the experiment, cuts or abrasions on the hands, self-identification as immunocompromised, or indication from a volunteer of being uncomfortable with the experiment and wishing to be removed. One volunteer asked to be removed, and did not complete the study. The remaining volunteers (mean age 24.5 yrs., SD=3.9 yrs.) included 10 men (mean age 26 yrs., SD=2.2 yrs.) and 10 women (mean age 23 yrs., SD=4.7 yrs.).

Questionnaire. Volunteers were asked to fill out a questionnaire before participation in the experiments. The questionnaire included questions that may account for external variables that could affect skin quality and skin bacterial profiles. The answers were used to parse out the volunteers into groups to see if log reduction data differs significantly between the different groups. The demographic variables analyzed included age, sex, moisturizer use, facial cleanser use, medication use, hand washing frequency, recent illnesses, and lotion use.

Experimental Design. Four variables (lather time, soap volume, water temperature, and product formulation) were evaluated using a fractional design. One set of conditions (5 s lather, 38 °C water temperature, and 1 mL product volume) served as the baseline, and the effect of each variable was studied, while holding the other two variables constant. Each unique set of conditions was replicated 20 times such that the total number of experiments was 20 (baseline) + 3*20 (lather time) + 2*20 (water temperature) + 2*20 (product volume) for 160 hand washes. The entire design was repeated for bland and chloroxylenol-containing-antimicrobial soap, resulting in 320 total hand washes. Therefore, each volunteer completed 16 hand washes. The target variables to be tested were randomly selected for each experiment. A volunteer only performed one wash per day. This was continued until there were no more of the 16 sets for a volunteer to perform.

Lather time. Lather times of 5, 10, 20, 40 s were evaluated. Lather time was defined as the length of time the volunteer lathered soap on their hands (by rubbing hands together) during a hand wash. Lather time did not include initial hand wetting (<1 s), soap application, hand rinsing (held constant at 10 s), or hand drying. Volunteers were instructed to lather their hands in a way that felt most comfortable.

Water Temperature. Water temperatures of 38 °C (100 °F), 26 °C (80 °F), 15 °C (60 °F) were evaluated, and the water temperature was verified using a Thermapen with a ± 0.4°C accuracy (ThermoWorks, Lindon, UT). The temperature of the water was set prior to volunteer arrival, and needed to be within +/- 2 °F at the target temperature for at least 60 s. The highest temperature to be used (38 °C) was selected because the FDA food code indicates that a handwashing sink shall be equipped to provide water at a temperature of at least 38 °C (100 °F) (5-202.12 of FDA Food Code) (*71*). The lowest temperature used (15 °C) was deliverable by the existing plumbing, and judged by the authors to be the lowest tolerable temperature for comfort.

Estimation of energy consumption*.* The energy consumption related to the water heating for hand washing was calculated with the following thermodynamic formula:

$Q = M.Cp.dT/\eta$ where

Q = amount of heat (kJ)

M = mass (kg), represents the amount of water used for a hand wash, a flow of 1 gallon per minute has been considered as an average water flow with aerator (*1*), a time of 10 seconds was assumed for rinsing.

Cp = specific heat of Water (kJ/kg.K) = 4.19

dT = temperature difference between the heated and ambient water, an average temperature of 10°C was assumed as the normal temperature for cold tap water. Calculations were done for the three temperatures studied: 38 °C (100 °F), 26 °C (80 °F), 15 °C (60 °F)

ŋ= water heater efficiency, an electric water heater has been taken into account with an average efficiency of 0.92, based on guidance from the US Office of Energy Efficiency & Renewable Energy (*72*).

Soap Volume. Three volumes were evaluated (0.5 ml, 1.0 ml and 2.0 ml soap). An automatic dispenser (GOJO Industries, Inc., Akron, OH) with a 0.5 mL output was used to dispense the soap. The dispenser was nondescript, had no timer, and did not reveal the formulation being used. This soap dispenser was validated before use each day by catching an aliquot of the foam solution from the dispenser and measuring this aliquot with a scale (Ohaus Scout Pro, Parsippany, NJ). This aliquot was compared to a 0.5 mL volume of the soap that was not converted to foam.

Soap Product Formulation. Two foaming soap formulations were used for all experiments, one bland (i.e. no antimicrobial active ingredient) and one antibacterial (1.0% chloroxylenol or PCMX). Both soaps are commercially available products (GOJO Industries, Inc., Akron, OH) used commonly in a variety of settings, including food service markets.

The chloroxylenol was used at a 1.0% concentration. The soaps were typical in formulation except for the antimicrobial agent, primarily containing a blend of amphoteric and anionic surfactants to remove soils, preservatives and skin conditioners to soften the skin and balance the effects from the cleansing agents, which can be drying and irritating to the skin. Both soaps were slightly acidic, with the bland soap pH = 5.2 and the antibacterial soap pH = 5.5.

Prewash procedure. Volunteers performed a prewash before beginning the experiment, as follows. The volunteer was invited into the laboratory and the researcher showed the volunteer where the sink was located, but did not give any directions other than to simply ask the volunteer to wash their hands. No direction was given on how to wash hands or for what length of time to wash. The researcher discretely recorded the amount of soap used, when hands first touched the water, lather time, rinse time, and total wash time. A stop watch was used to measure the times. The volunteer was handed paper towels, one at a time, to dry their hands, after washing their hands and was given as many paper towels as requested.

Challenge Bacteria. A non-pathogenic strain of *Escherichia coli* (ATCC 11229) served as the challenge bacteria for this experiment. Use of this strain is in accordance with current ASTM International handwashing protocols (*6*, *9*). This strain serves as a well-established surrogate for transient bacteria transferred to hands during handling of raw foods. Culturing methods were used as indicated in ASTM E2946. (*6*). The *E. coli* was cultured in 10 mL soybean-casein digest broth for 24 ± 4 h at 35 ± 2 °C. The 24 h *E. coli* culture was harvested by centrifugation (Micro 12, Fisher Scientific) at 7,000 x *g* for 10 min, and then washed in phosphate buffer saline (PBS; 0.1M, pH 7.2). The wash process was repeated three times and cell pellets were re-suspended in PBS to form a challenge suspension of ~8 log CFU/mL.

Hand Contamination. One mL of the *E. coli* challenge suspension was added to the volunteer's hands. Volunteers were instructed to rub their hands together to cover all surfaces of their hands (10-20 s). Hands were held parallel to the floor to avoid unnecessary contamination of the forearms or elbows. The hands were allowed to dry until they did not appear visibly moist (~40-60 s). The non-dominant hand was sampled before the hand wash, and that sample was used to calculate the pre-wash bacterial concentration.

Bacterial recovery procedure. A modification of the glove juice procedure (*10*, *11*) was used to recover bacteria from the volunteers' hands. A nitrile glove (Fisherbrand Powder-free Nitrile Examination Gloves, Thermo Fisher Scientific, Waltham, MA), filled with 20 mL of phosphate buffered saline, was placed over the volunteer's hand. The hand was massaged for 60 s to dislodge the bacteria from the hand. The glove was carefully removed, and the buffer was poured into a collection tube (Falcon™ 50mL Conical Centrifuge Tubes, Corning Inc., Corning, NY). An appropriate neutralizer, Tween 80 (10%), was used in the sampling buffers for the antimicrobial soap experiments (*8*). Antimicrobial neutralizing capability was confirmed using ASTM E 1054-08, section 9 (Neutralization Assay with Recovery in Liquid Medium) (*7*).

Sample dilution and plating. Phosphate buffer saline (pH 7.2 ±0.1) was used for serial dilutions, and contained an appropriate neutralizer when necessary. Samples were plated onto BBL™ MacConkey agar and the colonies forming units were enumerated after incubating for 24 hours at 35°C. The media contained MUG (4-Methylumbelliferyl-β-D-glucuronide) (Sigma-Aldrich Corporation, Saint Louis, MO) to assist with identifying *E. coli* without affecting colony morphology or viability (*53*).

Hand wash. The volunteer carried out a hand wash based on the four variables outlined above (lather time, water temperature, produce volume and formulation). Additional instructions were given as to how much soap to use (number of pumps), when to wet their hands, when to stop lathering, and when to stop rinsing. The volunteers were not told what formulation they were using or the water temperature. The volunteers did not dry their hands to avoid the effect of bacterial removal by paper towel (*20*, *33-35*, *75*).

Post wash sampling. The hands were sampled immediately after the wash (<5 s). Both hands were sampled using the modified glove juice method (*10*, *11*). These samples were used to calculate the post-wash bacterial concentration.

Post-experiment decontamination protocol. Before leaving the testing area, volunteers washed their hands under running water for 20 s using bland soap, and dried their hands with a paper towel. One pump of alcohol based hand sanitizer (Purell, GOJO Industries, Inc., Akron, OH) was then applied to the volunteers' hands and volunteers were asked to rub their hand together until the sanitizer was completely dry. The volunteers were then asked to leave the testing area.

Data Analysis. Microbial reduction data gathered from the experiment were log transformed to normally distribute the data (*62*). The log reduction was determined by taking the logarithm of prewash concentration on the non-dominant hand multiplied by two (to estimate the concentration on both hands), and subtracting from that the logarithm of the sum of the post-wash concentration on both hands.

A repeated measures ANOVA and a Tukey's range test/Tukey HSD (GraphPad Prism, GraphPad Software Inc., La Jolla, CA) were used to determine if multiple means were significantly different and if any significant interactions existed between the variables. A *P*-value less than 0.05 was considered significant. For scenarios in which only two variables were being compared, a two-tailed t-test was used to calculated *P*-values (Excel, Microsoft, Redmond, WA) to determine if significant differences existed between samples. This included scenarios when comparing groups from the questionnaires.

Results

Table 1 shows the overall log reductions for all treatment conditions tested, as well as the overall chloroxylenol-containing-antimicrobial soap mean log reduction and the overall bland soap mean log reduction. Overall, the antimicrobial soap used in this study had a mean 1.94 log CFU reduction, and ranged from 1.83 mean log reduction to 2.10 mean log reduction (SD=0.78). Bland soap had a mean 2.22 log CFU reduction, and ranged from 1.91 mean log CFU reduction to 2.54 mean log CFU reduction (SD=0.74). The analysis revealed statistically significant difference for *Soap Formulation* (P=2.5x10^{-4}).

An ANOVA analysis was performed to observe differences within the data sets and between volunteers (Table 2). The analysis revealed a difference between volunteers (P<0.0001) (person to person variability factors). A post-hoc Tukey HSD Test on the individual volunteer's mean log reduction data revealed statistically significant differences (P<0.05, data not shown). There were multiple >0.5 log CFU or greater mean log reductions differences between the volunteers. This suggests a large part of the variability in the data sets were due to variability between the volunteers. A subsequent Tukey HSD Test was performed for the datasets to determine differences between the individual scenarios (Table 3). This was done to observe differences between scenarios that may be overshadowed by combing the two groups. The analysis included *Lather time, Water Temperature, and Soap Volume* as independent variables. The data was separated by formulation. For the bland soap data, there were significant differences for lather time (P=0.01). A post-hoc HSD revealed that the 20 s lather time was significantly different from the baseline lather time (5 s, P=0.01), but not from the 10 s or 40 s lather time. Bland soap volume (*P*= 0.23) and the water temperature (*P*=0.08) variable did not have a significant effect. For the antimicrobial soap data, lather time (*P*= 0.85), water temperature (*P*=0.97), and volume (*P*=0.22) did not have a significant effect. Interestingly, within the antimicrobial soap data, the *P*-values were higher for lather time and water temperature in these groups (Lather *P*=0.85, Temperature *P*=0.97, Volume *P*=0.22), than in the bland soap data (Lather *P*=0.01, Temperature *P*=0.08, Volume *P*=0.23).

Higher water temperature results in higher energy consumption (see Figure 2). With a temperature of 38 °C (100 °F), the energy consumption related to water heating for 1,000 hand washes is 22.35 kWh, when water temperature is 26 °C (80 °F) the energy consumption for 1,000 hand washes is 12.77 kWh, which results in a reduction of 42% compared to the baseline (38 °C). With a temperature of 15 °C (60 °F) the energy consumption related to water heating for 1,000 hand washes is 3.99 kWh which results in a reduction of 68% compared to the baseline (38 °C).

Questionnaire Results. There were no statistical differences for volunteers that did/did not use acne medication (*P*=0.14) or facial cleanser (*P*=0.62). Age did not have an effect on mean log reduction (r^2=0.009, *P*=0.09).

Lotion Use: The questionnaire results indicate a significant (*P*=0.02) difference between high and low lotion users. The difference in microbial reduction between volunteers that used lotion, and those that did not use lotion is ~0.2 log cfu (high lotion use=2.15 average mean log reduction, low lotion use=1.95 average mean log reduction).

Hand washing frequency. Sixteen volunteers indicated they typically washed their hands >4 times per day, and 4 volunteers indicated they washed their hands <4 times per day. The pre-wash, mean total wash time differed significantly between the two groups (*P*=0.012) with the high frequency hand washers washing for an average of 18.2 s, and the low frequency hand washers washing for an average of 15 s. Further analysis revealed that the difference in wash times was due to lather time, and not rinse time. There was no significant difference between mean rinse times (*P*=0.714), but there was a highly significant difference in mean lather time (P= $2.2x10^{-5}$), with frequent hand washers lathering for 6.8 s, and less frequent hand washers lathering for 4.0 s. Interestingly, the low frequency hand washers were significantly more effective in their washing than the high frequency hand washers (P=$8x10^{-4}$) with an average log reduction of 2.37 CFU for low frequency washers, while the average log reduction for high frequency hand washers was 2.01. This difference was still significant when accounting for formulation (Antimicrobial *P*=0.048, Bland *P*=0.0045). It should be noted that the four low-frequency hand washers also reported the highest usage of lotion (more than twice a day), which was shown above to improve handwashing efficacy.

Men vs. Women. There was no statistical difference for mean log reductions of men and women (men= 2.08 mean log reduction, women=2.08 mean log reduction, *P*=0.988). The value did not change for either chloroxylenol-containing-antimicrobial or bland soap data. However, there was almost a ~0.5 mean log CFU reduction improvement (P=$3.9x10^{-4}$) for men that used lotion (2.34 mean log CFU reduction) vs. men who did not use lotion (1.90 mean log CFU reduction). This same comparison for women could not be made, as all of the women volunteers reported using lotion at least once a day (high usage).

Pre-wash Data. Breakdown of the prewash data may be viewed in Table 4. The mean recorded lather time of the volunteers' pre-wash was 6.3 s, the mean rinse time was 11.4 s, and the mean total wash time was 17.7 s. The temperature of the wash water did not change the observed lather (P=0.76), rinse (P=0.31), and overall wash (P=0.70) times. For both men and women, there was no effect of the temperature on the observed wash times, and the respective P-values remained roughly the same. Men lathered and rinsed their hands for a longer time (~2 s) than that of women (Lather, men= 7.4 s, women= 5.4 s, P=0.006; Rinse men =12.3 s, women=10.5 s, P=0.04), which resulted in a longer overall handwash time for men (P=0.002). There was a minimal correlation between length of lather time and rinse time (R^2=0.03) for all volunteers. The average volume of soaps used was 0.6 mL (Figure 1, SD=0.25, ~1 pump of soap), with both men and women averaging 0.6 mL of soap. While no statistical difference between men and women for the volume of soap used (P=0.39), further analysis revealed that there was a significant difference in volume of soap used between all the volunteers (P= 1.35×10^{-7}), suggesting personal behavior dictated choice of soap volume. Seventy-one percent of volunteers used 1 pump, 26% used 2 pumps, 1 % used three pumps, and 2% used no pumps of soap. These percentage differences did not noticeably change with the temperature of the water. A volunteer did not change the number of pumps of soap used for each prewash, and would routinely use the same amount of soap for each pre-wash. There was a weak correlation (low R^2) between total wash time and pumps of soap used (*P*=0.001, R^2=0.07). 43.4% of volunteers used water before applying soap, and 56.6% of volunteers applied soap before using water. When subdividing the groups by men or women, 56.8% of men used water first and 43.2% of men used soap first, and 31.1% of women used water first, and 68.9% of women used soap first.

Discussion

Lather time (length of wash). It was somewhat surprising that only a 30 s wash (20 s lather time, 10 s rinse) with bland soap provided a statistically different mean log reduction from the baseline 15 s wash. Several studies have suggested that a longer wash time will provide a greater microbial reduction benefit (*25*, *29*, *35*, *48*, *56*). However, these studies looked at an overall wash time less than 30 s, and did not break the wash down into separate parts (lather vs. rinse). Additionally, a handwash meta-analysis found that 120 s washes had a lower log reduction than a 30 s washes (*54*), suggesting wash time greater than 30 s may not be more effective. These results are consistent with our findings, and suggest microbial reduction will not increase significantly beyond 10-20 s lather times. One hypothesis to explain this finding is that easier-to-remove microbes are lifted by the wash in <30 s, however microbes that are embedded in deeper layers / pores or biochemically attached to skin will not be removed regardless of added hand wash time.

Water Temperature. Our study found no significant difference in washing effectiveness at different temperatures. This agrees with studies by Michaels *et al.* (*50*, *51*), in which they tested a wider range of water temperatures (4.4 °C - 48.9 °C), than we did (15 °C -38 °C), but found ~2-2.5 mean log reductions, very similar to our 1.9-2.3 mean log reductions. Courtenay *et al.* did observe a small but significant (P<0.05) difference (94% vs. 99%) in microbial reduction between a cool rinse (26 °C) and a warm rinse (40 °C) respectively, but since none of these experimental washes used soap, the relevance to a Food Code handwash is unclear (*21*). It is worth noting that since Courtenay *et al.* did study hands inoculated with a ground beef matrix, the saturated fats in the meat may have been more easily removed at warmer water temperatures. Given that warmer water does not improve antimicrobial benefits, but does create a negative environmental impact (i.e. energy consumption), policy requirements for warm water should be reconsidered (e.g. the FDA Food Code).

Volume of Soap. We did not observe a statistically significant difference with volume of soap used (P=0.48 bland soap, P=0.41 antimicrobial soap). Both Fuls *et al.* (*29*) and Larson *et al.* (*43*) did not observe any significant increase in microbial reduction when using more bland soap. Unlike in our findings, Fuls *et al*. and Larson *et al.* did find that increasing antimicrobial soap volume increased the log reductions. Both of theses studies' authors suggest increased exposure to more antimicrobial agent as the explanation for increased microbial reduction. The difference in mean log reductions for increasing volume of antimicrobial soap may be due to the types of active agents being tested, as formulation has been shown to effect efficacy (*14*, *70*). This study used a 1% chloroxylenol soap solution, while Larson *et al.* used a 4% chlorhexidine gluconate antimicrobial soap, and Fuls *et al.* used a 0.46% triclosan antimicrobial soap. The minimum volume or dose of soap that should be used should also consider the soil removal required by the users, which is also likely to significantly be effected by soap formulation (especially surfactant choices).

Antibacterial and Bland Soaps. This study did show a statistical difference between soap formulations (*P*=0.0003). However, it should be noted that the difference between the chloroxylenol-containing-antimicrobial and bland soap mean log reductions (Table 1) is only ~0.3 log CFU and can be considered within the range of error for microbiology data (clinically insignificant). Several studies have shown greater microbial reductions when using antimicrobial soaps than when using bland soaps (*25*, *29*, *31*, *63*, *66*). Many studies have showed that effectiveness of antimicrobial soaps increased with repeated use by building up the antimicrobial in the skin (*2*, *12*, *29*, *55*, *57*). This effect can also be seen in hand sanitizers based on antimicrobials that remain on the skin (*65*), unlike alcohol, which is not readily absorbed (*13*, *18*). Given the FDA one-year extension for soaps containing chloroxylenol (*26*), future work with the antimicrobial soap used in this study should take into consideration the need for buildup in the skin to improve efficacy and formulation style. The meta-analysis of hand soaps by Schaffner and Montville suggested that overall, accounting for all types of bacteria, antimicrobial soap (mean 2.4) should have a ~0.5 log greater reduction than bland soap (mean 1.9)(*54*). We did not see a greater difference, but the bland soap data and the antimicrobial soap data both fell within the meta-analysis's range of mean log reductions (*54*). Finally, future studies should take into consideration antimicrobial soap surfactant profile, as this can have a significant effect on the results (*14*, *70*). This study used two formulations that were both commonly used by the public and designed to be mild to the skin and similar in use. Highly efficacious antimicrobial soaps are made by designing the ingredient matrix around the antimicrobial active ingredient, to create a formulation that does not inhibit, and ideally highly activates the antimicrobial agent (*14*, *70*). Future work should take into consideration the variety of antimicrobial soaps available, and the methods of testing these soaps.

Lotion Use. While the mean differences were small (~0.2 log CFU difference) between lotion users and non-lotion users, the authors point out that lotion use could compound several analyses. Skin damage from frequent hand washing is well established (*4*, *28*, *30*, *38-40*, *59*, *64*, *67*, *73*, *74*, *77*), and often lotion is used to repair this damaged skin (*5*, *45*, *49*). Damaged skin is more difficult to wash (*41*, *42*, *44*), so it is not surprising to see a slight, yet higher log reduction for the volunteers that indicated regular lotion use. While all women indicated using lotion more than once a day, not all men used lotion regularly (~0.5 greater mean log CFU reduction for men who were lotion users). This study did not have sufficient evidence to draw a strong conclusion on lotion use. However there is certainly enough evidence to warrant further investigation that involves studies more precisely controlled and designed to measure the effect of hand lotion use on hand washes. Certainly using lotion to improve skin quality (*5*, *45*, *49*) and reduce the instance of pathogen colonization of damaged skin (*41*, *42*, *44*) would be an advantage in and of itself to both healthcare workers and food handlers.

Person to Person Variability. As seen in Table 2, large parts of the variability in the data sets were due to variability between the volunteers. This is not uncommon for *in-vivo* handwashing research, as large variability is seen both within and between hand washing studies (*54*). Greater than 4 log CFU reductions are consistently observed in hand sanitizer research, with limited variability also recorded (*3*, *22-24*, *32*, *37*, *52*, *58*, *76*). Suggesting that hand soap and hand sanitizer effectiveness may be more influenced by human behavior and/or physiological hand differences than soap/ sanitizer effectiveness, which is not surprising considering the number of steps recommended for a proper handwash (*36*). No published work was discovered that links physiological differences, such as skin moisture levels, skin sensitivity, hair density, scar tissue, and hand size, to hand washing outcomes. How these physiological differences affect microbial loads, reductions and risk would be an interesting topic for future hand hygiene research.

Other Observations. Much as we did, Larson *et al* also recorded the mean amount (mL) of soap used by health care workers (*43*). They observed that health care workers used ~ 2.7mL of soap when attending to high-risk patients, ~2 mL when attending to low-risk patients, and ~1 mL when not attending to patients. As seen in Figure 1, our volunteers, who were not healthcare workers, used a much lower amount of soap than the Larson *et al* average (0.6 mL for the pre-wash). Sixty-five percent of men used 1 pump of soap, and 75% of women used 1 pump of soap. Larson *et al* did not use a foaming soap, but used liquid soap in a syringe dispenser, and asked the volunteers to use an amount of soap they would normally use for a handwash, while our soap was released in 0.5 mL increments from a dispenser.

Interestingly, similar to the Larson *et al.* study (*43*), we did observe that an individual volunteer would use a different amount of soap than another volunteer, and each individual would routinely use the same volume of soap for each of their handwashes, i.e. consistently follow their individual habit.

The study has shown that water temperature is not a critical factor for the removal of transient microorganisms. Combining these results with other studies that tested water temperature as a variable (*50*, *51*), it is apparent that water temperature does not have a strong effect on hand washing. Therefore, it may be time to remove water temperature recommendations for hand washing from regulations and promote recommendations aimed at skin comfort (*42*, *69*). Overall, the length of lather time and volume of soap used did not make a large difference, but a minimum of 0.5ml of soap and 10 s of lather time is recommended based on this work. Lotion use by the volunteers had an influence on the results, with volunteers that used lotion regularly showing a greater microbial reduction. One of the key findings from this study is that variability exists between people in both the microbial reduction and hand wash behavior. Understanding which behaviors, human factors, and physiological differences influence hand washes the most may help future studies find which techniques can optimize the effectiveness of a hand wash, and thereby reduce infection transmission risk and improve food safety.

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Figure Legends

Figure 1: Breakdown of the number of pumps used by women (black) and men (gray) during the prewash. Each pump delivered 0.5 mL of soap.

Figure 2: Energy consumption related to water heating for hand washing

Table 1: Mean log reductions, median log reductions, and range of log reductions in various handwashing treatments

Variable	Formulation	Mean log CFU reduction	Standard deviation	Median	Maximum	Minimum	Range
All Data	Antimicrobial	1.94	0.78	1.92	4.42	0.06	4.36
	Bland	2.22	0.74	2.22	4.40	-0.04	4.44
Baseline*	Antimicrobial	1.92	0.68	1.87	3.13	0.69	2.44
	Bland	1.91	0.64	1.76	2.99	0.82	2.17
Lather time 10s	Antimicrobial	2.03	0.64	2.00	3.30	0.89	2.41
	Bland	2.16	0.74	2.22	3.60	1.03	2.58
Lather time 20s	Antimicrobial	1.95	1.00	1.82	4.39	0.35	4.03
	Bland	2.54	0.62	2.48	3.75	1.63	2.12
Lather time 40s	Antimicrobial	1.91	0.98	2.00	3.47	0.13	3.34
	Bland	2.43	0.71	2.25	4.09	1.57	2.52
Water temperature 15 °C	Antimicrobial	1.88	0.62	1.91	3.34	0.76	2.57
	Bland	2.34	0.54	2.33	3.22	1.08	2.15
Water temperature 26 °C	Antimicrobial	1.90	0.89	1.77	4.42	0.28	4.14
	Bland	1.98	0.71	1.99	3.07	0.80	2.27
Soap volume 0.5 mL	Antimicrobial	2.10	0.77	2.18	3.24	0.06	3.18
	Bland	2.25	0.86	2.25	4.03	-0.04	4.07
Soap volume 2.0 mL	Antimicrobial	1.83	0.65	1.81	3.34	0.64	2.69
	Bland	2.15	0.93	1.97	4.40	0.70	3.70

* 5s lather time, 38 °C water temperature, 1mL soap volume. Other conditions are identical to baseline except as noted. N = 20 for all treatments, except all data where n = 160.

Table 2: ANOVA analysis of scenarios and volunteers

Factor	Formulation	Standard Deviation	Degrees of Freedom	Mean Square
Between volunteers	Antimicrobial	0.9985	7	0.1426
	Bland	6.465	7	0.9235
Between scenarios	Antimicrobial	27.37	19	1.441
	Bland	26.2	19	1.379
Residual	Antimicrobial	68.08	133	0.5119
	Bland	54.5	133	0.4098
Total	Antimicrobial	96.45	159	
	Bland	87.17	159	

Table 3: Tukey Multiple Comparison test results. The results are split into Antimicrobial and Bland Soap..

	Antimicrobial Soap			Bland Soap		
Comparison	Mean Diff.	q	95% CI of diff	Mean Diff.	q	95% CI of diff
Baseline vs Lather -10s	-0.110	0.687	-0.8079 to 0.5880	-0.244	1.708	-0.8689 to 0.3800
Baseline vs Lather-20s*	-0.030	0.188	-0.7280 to 0.6679	**-0.628**	**4.384**	**-1.252 to -0.003004**
Baseline vs Lather-40s	0.010	0.064	-0.6877 to 0.7082	-0.521	3.641	-1.146 to 0.1034
Baseline vs Temp-60F	0.033	0.207	-0.6648 to 0.7311	-0.427	2.982	-1.051 to 0.1977
Baseline vs Temp-80F	0.011	0.072	-0.6865 to 0.7094	-0.071	0.497	-0.6956 to 0.5533
Baseline vs Vol-0.5mL	-0.182	1.134	-0.8794 to 0.5165	-0.339	2.369	-0.9635 to 0.2854
Baseline vs Vol-2mL	0.083	0.518	-0.6151 to 0.7808	-0.233	1.625	-0.8571 to 0.3918
Lather -10s vs Lather-20s	0.080	0.500	-0.6180 to 0.7779	-0.383	2.676	-1.008 to 0.2414
Lather -10s vs Lather-40s	0.120	0.752	-0.5777 to 0.8182	-0.277	1.933	-0.9012 to 0.3478
Lather -10s vs Temp-60F	0.143	0.895	-0.5548 to 0.8411	-0.182	1.274	-0.8068 to 0.4421
Lather -10s vs Temp-80F	0.122	0.759	-0.5765 to 0.8194	0.173	1.211	-0.4512 to 0.7977
Lather -10s vs Vol-0.5mL	-0.072	0.447	-0.7695 to 0.6265	-0.095	0.661	-0.7191 to 0.5299
Lather -10s vs Vol-2mL	0.193	1.205	-0.5051 to 0.8908	0.012	0.082	-0.6127 to 0.6363
Lather-20s vs Lather-40s	0.040	0.252	-0.6576 to 0.7383	0.106	0.743	-0.5181 to 0.7308
Lather-20s vs Temp-60F	0.063	0.395	-0.6347 to 0.7612	0.201	1.402	-0.4238 to 0.8252
Lather-20s vs Temp-80F	0.042	0.260	-0.6564 to 0.7395	0.556	3.887	-0.06816 to 1.181
Lather-20s vs Vol-0.5mL	-0.151	0.947	-0.8494 to 0.5465	0.288	2.015	-0.3360 to 0.9129
Lather-20s vs Vol-2mL	0.113	0.706	-0.5850 to 0.8109	0.395	2.758	-0.2296 to 1.019
Lather-40s vs Temp-60F	0.023	0.143	-0.6751 to 0.7209	0.094	0.659	-0.5301 to 0.7188
Lather-40s vs Temp-80F	0.001	0.008	-0.6967 to 0.6992	0.450	3.143	-0.1745 to 1.074
Lather-40s vs Vol-0.5mL	-0.192	1.199	-0.8897 to 0.5062	0.182	1.272	-0.4424 to 0.8065
Lather-40s vs Vol-2mL	0.073	0.454	-0.6253 to 0.7706	0.289	2.015	-0.3360 to 0.9129
Temp-60F vs Temp-80F	-0.022	0.136	-0.7196 to 0.6763	0.356	2.484	-0.2688 to 0.9801
Temp-60F vs Vol-0.5mL	-0.215	1.342	-0.9126 to 0.4833	0.088	0.613	-0.5367 to 0.7122
Temp-60F vs Vol-2mL	0.050	0.311	-0.6482 to 0.7477	0.194	1.356	-0.4303 to 0.8186
Temp-80F vs Vol-0.5mL	-0.193	1.206	-0.8909 to 0.5050	-0.268	1.872	-0.8924 to 0.3566
Temp-80F vs Vol-2mL	0.071	0.446	-0.6266 to 0.7694	-0.162	1.128	-0.7860 to 0.4630
Vol-0.5mL vs Vol-2mL	0.264	1.652	-0.4336 to 0.9623	0.106	0.743	-0.5181 to 0.7309

*Bolded data indicates a *P*<0.05

Table 4: Breakdown of the prewash data. Mean lather, rinse, and total wash times are in seconds. Percentages for the "All Data" group are out of 198. Percentages for the "Men" group are out of 95 and for the "Women" group out of 103. Some of the prewash data was compromised (equipment malfunction), hence the different number of prewashes observed between men and women. Each pump of soap was 0.5 mL of foaming product.

Group	Total Washes in the Group	Mean Lather Time	Mean Rinse Time	Mean Total Wash Time	% use 0 pump	% use one pump	% use two pumps	% use three pumps	% use water first	% use soap first
All Data	198	6.3	11.4	17.7	2.0	70.7	26.3	1.0	43.4	56.6
15°C	31	7.0	10.6	17.6	0.5	11.1	4.0	0.0	6.6	9.1
26°C	47	6.1	12.5	18.6	0.5	16.7	6.1	0.5	9.1	14.7
38°C	120	6.3	11.1	17.4	1.0	42.9	16.2	0.5	27.8	32.8
Men	95	7.4	12.3	19.7	3.0	62.0	29.0	1.0	56.8	43.2
15°C	19	7.6	11.4	19.0	1.0	12.0	6.0	0.0	11.6	8.4
26°C	20	6.2	13.3	19.5	1.0	14.0	5.0	0.0	11.6	9.5
38°C	56	7.8	12.2	19.9	1.0	36.0	18.0	1.0	33.7	25.3
Women	103	5.4	10.5	15.9	1.0	78.0	23.0	1.0	31.1	68.9
15°C	12	6.0	9.3	15.3	0.0	10.0	2.0	0.0	1.9	9.7
26°C	27	6.3	11.9	18.0	0.0	19.0	7.0	1.0	6.8	19.4
38°C	64	4.9	10.2	15.1	1.0	49.0	14.0	0.0	22.3	39.8

Fig. 1

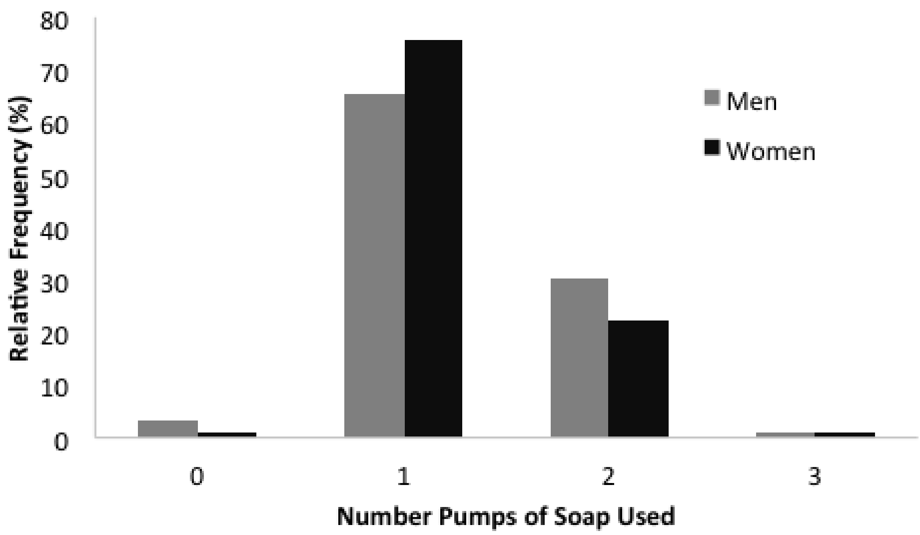

Relative Frequency (%)
80
70
60
50
40
30
20
10
0
Men
Women
0
1
2
3
Number Pumps of Soap Used

Fig. 2

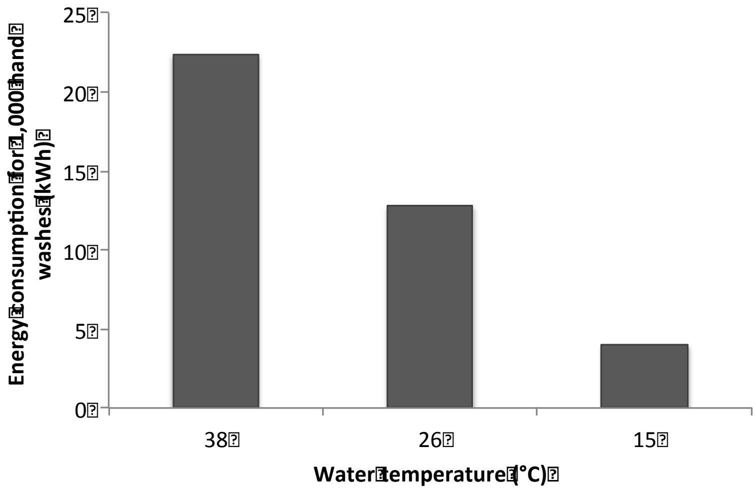

Energy consumption for 1,000 hand washes (kWh)
0
5
10
15
20
25
38
26
15
Water temperature (°C)

Fig 1

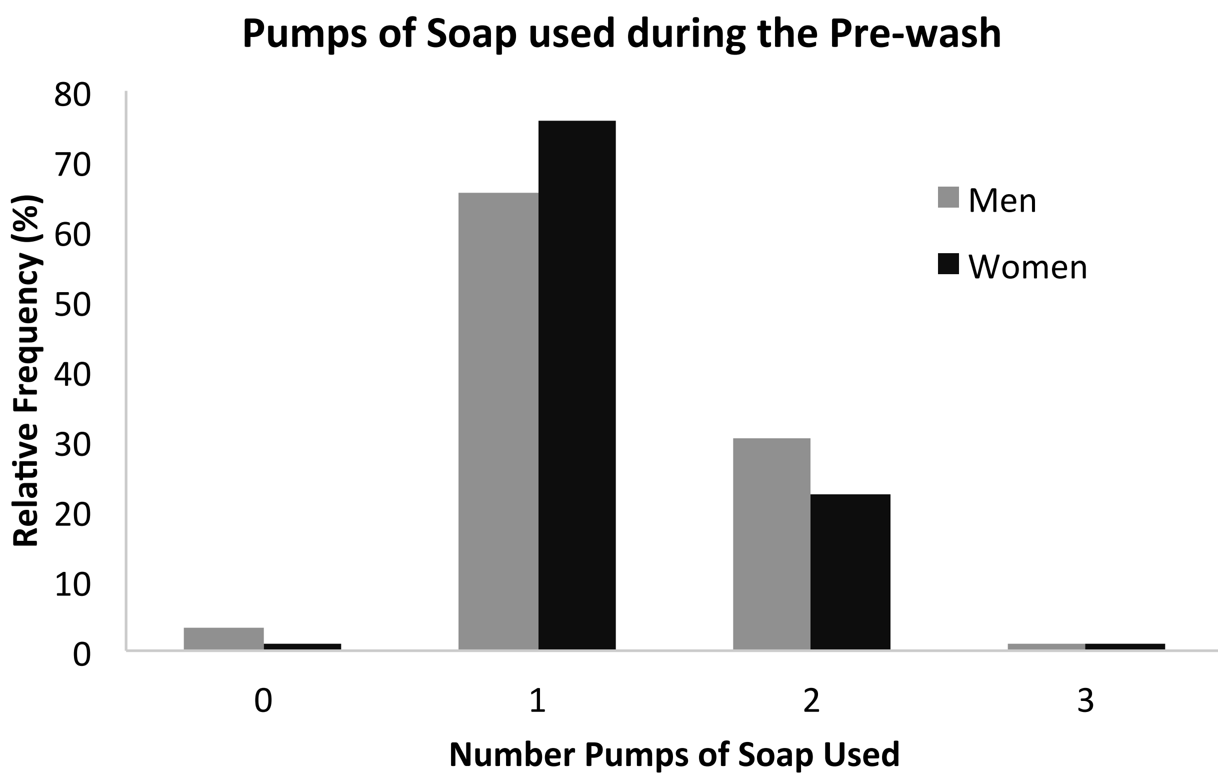
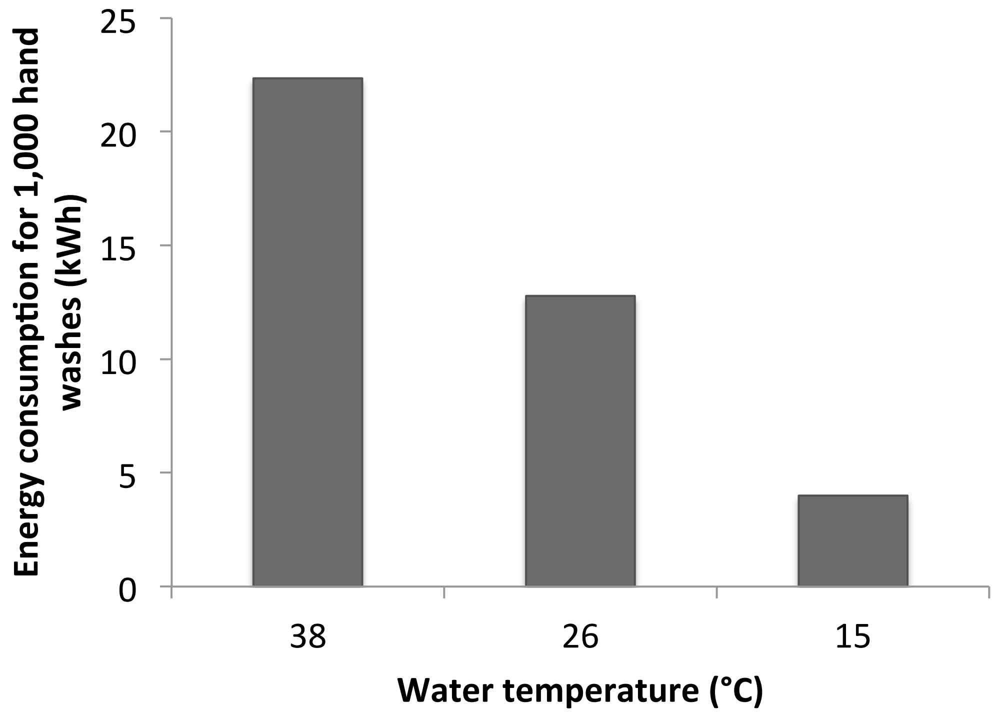

Pumps of Soap used during the Pre-wash
Relative Frequency (%)
80
70
60
50
40
30
20
10
0
Men
Women
0
1
2
3
Number Pumps of Soap Used

Fig 2



Energy consumption for 1,000 hand washes (kWh)
25
20
15
10
5
0
38
26
15
Water temperature (°C)

Table 1: Mean log reductions, median log reductions, and range of log reductions in various handwashing treatments

Variable	Formulation	Mean log CFU reduction	Standard deviation	Median	Maximum	Minimum	Range
All Data	Antimicrobial	1.94	0.78	1.92	4.42	0.06	4.36
	Bland	2.22	0.74	2.22	4.40	-0.04	4.44
Baseline*	Antimicrobial	1.92	0.68	1.87	3.13	0.69	2.44
	Bland	1.91	0.64	1.76	2.99	0.82	2.17
Lather time 10s	Antimicrobial	2.03	0.64	2.00	3.30	0.89	2.41
	Bland	2.16	0.74	2.22	3.60	1.03	2.58
Lather time 20s	Antimicrobial	1.95	1.00	1.82	4.39	0.35	4.03
	Bland	2.54	0.62	2.48	3.75	1.63	2.12
Lather time 40s	Antimicrobial	1.91	0.98	2.00	3.47	0.13	3.34
	Bland	2.43	0.71	2.25	4.09	1.57	2.52
Water temperature 15 °C	Antimicrobial	1.88	0.62	1.91	3.34	0.76	2.57
	Bland	2.34	0.54	2.33	3.22	1.08	2.15
Water temperature 26 °C	Antimicrobial	1.90	0.89	1.77	4.42	0.28	4.14
	Bland	1.98	0.71	1.99	3.07	0.80	2.27
Soap volume 0.5 mL	Antimicrobial	2.10	0.77	2.18	3.24	0.06	3.18
	Bland	2.25	0.86	2.25	4.03	-0.04	4.07
Soap volume 2.0 mL	Antimicrobial	1.83	0.65	1.81	3.34	0.64	2.69
	Bland	2.15	0.93	1.97	4.40	0.70	3.70

* 5s lather time, 38 °C water temperature, 1mL soap volume. Other conditions are identical to baseline except as noted. N = 20 for all treatments, except all data where n = 160.

Table 2: ANOVA analysis of scenarios and volunteers

Factor	Formulation	Standard Deviation	Degrees of Freedom	Mean Square
Between volunteers	Antimicrobial	0.9985	7	0.1426
	Bland	6.465	7	0.9235
Between scenarios	Antimicrobial	27.37	19	1.441
	Bland	26.2	19	1.379
Residual	Antimicrobial	68.08	133	0.5119
	Bland	54.5	133	0.4098
Total	Antimicrobial	96.45	159	
	Bland	87.17	159	

Table 3: Tukey Multiple Comparison test results. The results are split into Antimicrobial and Bland Soap.

Comparison	Antimicrobial Soap			Bland Soap		
	Mean Diff.	q	95% CI of diff	Mean Diff.	q	95% CI of diff
Baseline vs Lather -10s	-0.110	0.687	-0.8079 to 0.5880	-0.244	1.708	-0.8689 to 0.3800
Baseline vs Lather-20s*	-0.030	0.188	-0.7280 to 0.6679	**-0.628**	**4.384**	**-1.252 to -0.003004**
Baseline vs Lather-40s	0.010	0.064	-0.6877 to 0.7082	-0.521	3.641	-1.146 to 0.1034
Baseline vs Temp-60F	0.033	0.207	-0.6648 to 0.7311	-0.427	2.982	-1.051 to 0.1977
Baseline vs Temp-80F	0.011	0.072	-0.6865 to 0.7094	-0.071	0.497	-0.6956 to 0.5533
Baseline vs Vol-0.5mL	-0.182	1.134	-0.8794 to 0.5165	-0.339	2.369	-0.9635 to 0.2854
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26°C	20	6.2	13.3	19.5	1.0	14.0	5.0	0.0	11.6	9.5
38°C	56	7.8	12.2	19.9	1.0	36.0	18.0	1.0	33.7	25.3
Women	103	5.4	10.5	15.9	1.0	78.0	23.0	1.0	31.1	68.9
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26°C	27	6.3	11.9	18.0	0.0	19.0	7.0	1.0	6.8	19.4
38°C	64	4.9	10.2	15.1	1.0	49.0	14.0	0.0	22.3	39.8