ADDRESSING CHALLENGES AND GAPS IN THE HAND HYGIENE LITERATURE USING NOVEL QUANTITATIVE APPROACHES

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ABSTRACT OF THE DISSERTATION

Addressing Challenges and Gaps in the Hand Hygiene Literature Using Novel Quantitative Approaches

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This research was undertaken to develop a cause and effect understanding of the variables that affect hand washing, a critical facet in food safety and public health. Many regulations appear to have been made without sufficient scientific foundational evidence to back up justify these regulations, because of this, many basic aspects of handwashing are still being debated. The studies in this dissertation attempted to clarify several main concepts in hand washing. Specifically, which parts of a hand wash are most important, is soap necessary for a hand wash, handwash communication consistent, what characteristics of the surfactant are important for soap formulation, and what is the current state of hand sanitizer published literature.

Chapter II main finding was that handwash techniques communicated by signs and posters varies greatly. Chapter III is a meta-analysis of the published hand sanitizer literature, and had several key findings. First, a significant difference between ethanol and isopropanol based hand sanitizer effectiveness for bacteria ($p=<0.05$), but not for viruses ($p>0.05$) was observed. Second, alcohol-based hand sanitizers were more effective ($p<0.05$) than those based on other antimicrobials for bacteria, but the same statistical difference was not observed for viruses ($p>0.05$). Finally, different experimental protocols return significantly different results ($p<0.05$), and care must be taken when comparing hand sanitizer studies. Chapters IV and V focused on handwash technique and soap use, which found that hand lathering time, soap volume, and water temperature did not significantly change the microbial reduction from the handwash ($p>0.05$), but that drying method, use of soap, and total wash time did ($p<0.05$). Lastly, Chapter VI results suggest that soap formulations, specifically the type and concentration of surfactant used, has a significant influence ($p<0.05$) on the effectiveness of a soap product. These results from these studies will be used in future risk modeling and soap product development to ideally promote better formulation, better hand wash compliance, and evidenced based hand hygiene regulations.

Dedication

Laryssa,

For being endlessly patient with my graduate work, while still having no idea what it is I exactly do.

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Preface

This dissertation is a compilation of five studies aimed at improving and expanding hand hygiene knowledge. These studies involve collaborations between multiple institutions that have interests in improving hand hygiene, and ultimately food safety. The dissertation is broken down into several chapters, with each chapter focusing on a specific topic. When this research was in the initial stages of planning, the authors of these various manuscripts agreed that a large area of significant knowledge was missing from the hand hygiene field. Chapter I is the literature review, and serves to provide more in depth information that will supplement Chapters II-VI. Chapters II-VI are written in a manuscript style, with each being in the form that they will be submitted to the peer reviewed literature. For all studies, Dane Jensen, at Rutgers University, performed the experiments, data analysis, and wrote the first draft of the manuscript.

Chapters II and III encompass studies in which Donald Schaffner Ph.D. from Rutgers University, is the principle investigator. Chapter II is aimed at studying the hand washing recommendations made by hand washing posters. The results of this study demonstrated the need for variables in Chapters IV-VI to be studied by showing which techniques in hand washing (soap use, drying, lathering, etc.) were being displayed on posters most frequently (highest concern for hand hygiene). These posters were collected from government and private (industry) sources. Chapter III is a meta-analysis of the published hand sanitizer literature, and serves to give a more thorough review of the hand sanitizer literature than what had been

previously done. It also furthers the argument that formulation is key, which is touched upon in Chapters V and VI.

Chapter IV is a collaboration between three land grant institutions; Rutgers University, Department of Food Science; University of Florida, Center for Citrus Research and Education; and University of California Davis, Department of Food Science and Technology. The grant's primary investigator is Michelle Danyluk Ph.D. from University Florida. Linda Harris Ph.D., from UC Davis, and Donald Schaffner Ph,D., from Rutgers University, serve as co-primary investigators on the project. All three investigators have extension appointments from their respective universities, and contributed to overall experimental design and data analysis. Chapter IV aims to understand the necessity of soap in a hand wash and demonstrates the additional microbial reduction benefit of using paper towels to dry hands after a wash.

Chapter V is a collaboration between Rutgers University (NJ) and GOJO Industries, Inc. (Akron OH). Donald Schaffner Ph.D, from Rutgers University, is the principle investigator. Dave Macinga, Ph.D. and Dave Shumaker contributed extensively to overall experimental design. This chapter is focused on understanding several key hand wash techniques, including lather time, volume of soap, and temperature of the wash water.

Chapter VI is a collaboration between Michael Rogers Ph.D. and Schaffner Ph.D, both of which are from the Rutgers Department of Food Science. Donald Schaffner, Ph.D., is the principle investigator. This project focuses on the effect soap formulation (surfactant type and concentration) has on microbial reduction

Chapter I Literature Review

I.1 Microorganisms and hands

I.1.a Resident microflora

Resident microflora, sometimes referred to as resident microbiota, are microorganisms that have colonized skin, but are not necessarily dangerous to human health, and can be considered beneficial (*36*, *95*, *119*). The resident microflora are comprised primarily of Gram-positive coagulase-negative staphylococci, *Corynebacterium* spp., and anaerobes such as *Propionibacterium* spp (*95*, *135*). They rarely cause infection in humans unless they enter the body through damaged skin.

The main determinant of skin microflora concentration is moisture content; with more moist areas of the skin have higher concentrations of bacteria (*135*). While most skin microflora are not pathogenic, roughly 30% of people are colonized with *Staphylococcus aureus*, and about 1% colonized with methicillin-resistant *Staphylococcus aureus (*MRSA) (*88*, *133*). *Staphylococcus aureus* colonization rates are higher in men (37%) than women (28%) (*88*, *133*). Other research suggests that MRSA colonization under nails and in nasal cavities can be as a high as 7% in healthcare settings (*152*). Factors that are associated with being positive for MRSA were prolonged hospital stays, history of surgery, being older than 60 years, previous use of antibiotics, and having open lesions on skin (*152*, *233*).

The human microbiome project is a recent undertaking that is aimed at better

understanding the microbiological ecosystems of the human body (*45*, *95*, *96*, *239*). Environments on the skin (microbiomes) are primarily classified into three groups, sebaceous, moist, and dry (*95*). Sebaceous environments include oily areas of the skin, such as the glabella (between the eyebrows), alar crease (beside the nostril), external auditory canal (inside the ear), occiput (back of the scalp), and the back. *Propionibacteria* and *Staphylococcus* are primarily isolated from these areas. Moist environments include the nares (nostrils), axillary vault (armpit), cubital fossa (inner elbow), interdigital web space (webbing between fingers), gluteal crease (topmost part of the buttocks), popliteal fossa (behind the knee), plantar heel (bottom of the heel), and umbilicus (navel). Moist environments contain both Gram-positive and Gram-negative organisms (*95*, *96*, *135*). Gram negative organism are primarily identified in moist sites, for example the cubital fossa (inner elbow), will most frequently have Proteobacteria, with *Pseudomonas* and *Janthinobacterium,* as the majority. Other sites, such as the interdigital web space and gluteal crease, have Gram-positive bacteria, such as *Corynebacteria* and *Staphylococcus*, and the cubital fossa having smaller concentrations Actinobacteria, Bacteroidetes, and Firmicutes (*95*, *96*). The dry environments include the volar forearm (mid-forearm), hypothenar palm (area of palm closest to the little finger), and buttocks. Dry environments can contain Gram-negative Betaproteobacteria and *Bacteroidetes* (*95*). While the human microbiome project is a recent initiative, it has brought to the light the immense microbiological diversity of the human skin, and advances the complicated subject of determining which bacteria are beneficial to humans and in what ways.

I.1.b Transient microflora

Transient microflora reside in superficial layers of skin, readily transfer between surfaces, and they are more easily removed during a handwash than resident microflora (*36*, *119*, *151*). The transient microflora of interest in microbial food safety includes pathogenic Gram-negative bacteria, such as *Campylobacter, Salmonella spp., Shigella*, and pathogenic *Escherichia coli* (*36*, *119*, *211*, *212*). Transient microflora include microorganisms that are frequently associated with nosocomial infection in hospitals (*36*, *119*). Extreme conditions of skin moisture, ambient relative humidity, and ambient temperature support greater survival of transient organisms on skin (*159*).

I.1.c Populations on hands

Evidence suggest that skin microflora concentrations and profiles can vary widely from person to person, however the transient and resident microflora concentrations tend to remain uniform for an individual (*36*, *119*, *163*). Hands contain anywhere between 2-4 log cfu/cm^2 bacteria on hands, but usually only have as high as 0.5 log cfu/cm^2 of Gram negative organisms (*36*, *160*, *163*). However, the subungal region (under the nail) may have as many gram-negative organisms as 5.5 log cfu/nail, and the palm as high as 4 log cfu/palm depending on exposure to these organisms (*163*). Greater concentrations of bacteria have been correlated with longer fingernail length (*146*).

Studies that observe the impact of ring wearing have found that hands with rings have at minimum a 1 log greater concentration of skin microorganism (*71*, *206*, *238*). Healthcare workers that wear rings have less effective hand wash than those

that do not wearing rings (*206*). A handwashing risk assessment determined that wearing a ring during a wash could decrease the effectiveness of the wash (*173*). Furthermore, wearing a ring will significantly reduced the effectiveness of hand sanitizers (*254*). The type of ring worn (smooth band versus rough band with stones) does not have a significant difference (*254*). Interestingly, one study did not observe a significant difference in microbial concentration between hands with or without rings, but did notice that hands with rings were more likely to carry bacteria from the family Enterobacteriaceae (*66*). A similar finding from another study associated ring wearing with higher risk of contamination with *Staphylococcus aureus*, Gram-negative bacteria, and *Candida* species (*238*). That same study correlated an increased risk of isolating transient organism as the number of rings on the hand increased (*238*).

I.1.d Inoculum levels and reduction in a hand wash

Higher inoculum levels (>6 log cfu/hand) are used to observed large microbial reductions on hands that may be masked by detection limits. Higher inoculum levels have been shown to correlate with a higher percent reduction in handwashing studies (*176*). The hand sanitizer meta-analysis in Chapter III of this dissertation has demonstrated that a higher mean log reduction is correlated with higher initial inoculum levels.

I.1.e Model organism used in handwash experiments

The choice of organism used (resident versus transient) can have an effect on the measured efficacy of soap (*176*). This result was similarly observed in our hand sanitizer meta-analysis (Chapter III) where we noted most hand sanitizers displayed little activity towards spore forming bacteria. Additionally, alcohol based hand sanitizers had the highest efficacy against Gram-positive bacteria, and the lowest log reduction was observed with resident microflora. This highlights the need to carefully choose the model organism, and carefully compare results with other data. The experiments outlined in this proposal use a variety of organisms, which will be discussed briefly.

Enterobacter aerogenes is a bacterium with attachment characteristics similar transient food pathogens, such as *Salmonella* (*259*). The food grade strain used in Chapter IV has been developed to remove free sugars in dried egg products in order to prevent Maillard browning during storage (B199A Vivolac Cultures, Indianapolis, Ind.) (*259*). This organism is used in past experiments as a surrogate for foodborne pathogens (*51*, *112-114*, *175*, *212*, *259*).

Escherichia coli is a Gram negative, rod shaped, facultative anaerobe (*69*, *74*). While some strains of *E. coli*, such as *Escherichia coli* O157:H7, are pathogenic, the strains used in Chapters V and VI are not pathogenic. Both pathogenic and non-pathogenic strains of *E. coli* are found in the gastrointestinal tract of humans and many animals. Some animals carry pathogenic strains of *E. coli* asymptomatically, as some animals lack the receptor for shiga toxin, and are not noticeably harmed by the presence of these bacteria (*67*, *74*, *226*) and *E. coli* O157:H7 is often carried asymptomatically in

intestines of cattle (*67*, *74*, *226*). Foods associated with *E. coli* O157:H7 are undercooked or raw meat, salads, dried salami, raw milk, unpasteurized juice, and unpasteurized cheese (*69*, *210*).

I.2 Technique for quantifying bacteria on hands

There are various techniques for quantifying microbial concentration on skin. One of the key findings from the sanitizer meta-analysis (Chapter III) highlighted that different techniques can result in different observed log reductions for similar initial microbial concentrations. Therefore hand hygiene researchers should take sampling method into consideration when designing experiments and comparing results. Boyce and Pittet best summarized the various test methods in the published hand hygiene recommendations for healthcare settings (2002), and emphasized several factors that need to be addressed when considering bacterial quantification on hands (*36*): w hether hands are purposely contaminated with bacteria before use of test agents; the method used to contaminate fingers or hands; the volume of hand-hygiene product applied to the hands; the time the product is in contact with the skin; the method used to recover bacteria from the skin after the test solution has been used; and the method of expressing the efficacy of the product.

I.2.a Glove juice method

The glove juice method is a type of whole hand measuring protocol that uses buffer inside a glove to recover the bacteria on a hand (*15*, *16*). Many previous studies have used this protocol with reproducible results (*24*, *51*, *77*, *112*, *136*, *140*, *171*, *189*, *191*, *192*, *212*, *227*, *236*). Briefly, a glove is filled with buffer and put over the

subjects' hand, and the hand is massaged for roughly 1 minute. The glove is removed, and the buffer is collected in a vial. The resulting solution contains the bacteria that were removed from the hand. The solution is then plated onto appropriate agar, and the bacterial concentration is quantified from the plate counts.

I.2.b Fingerpad method

The fingerpad method utilizes an inverted vial with buffer to remove and quantify bacteria on a small portion of the fingertip (*11*). A study found no significant difference between the glove juice and fingerpad method (*8*). Our meta-analysis of the published hand sanitizer literature also found no significant difference between the fingerpad method and the glove juice method (Chapter III). Briefly, the fingerpad method involves the target fingerpad being first pressed to the lip of a small jar to create an indent on the fingertip. This is done to mark the spot to be sampled. The fingertip is then rubbed or pressed against a contaminated surface to inoculate the finger. The bacteria on the fingerpad will be removed by placing the mouth of a vial, which contains 1 mL eluent, over the demarcated spot on the finger. The vial is inverted, with the fingerpad still in place, to bring the eluent in contact with the skin. After a 5 s contact, the vial is subjected to 20 full inversions. The fingerpad is lifted off gently and its surface scraped against the inside lip of the mouth of the vial in a downward motion. The eluate is serially diluted and plated onto appropriate agar. Several manuscripts have used the fingerpad method with reproducible results (*8*, *121*, *131*, *149*, *155*, *207*, *237*)

I.2.c Fingertip method or European Norm 1499

European Norm 1499 is a standard that is used to determine the efficacy of antiseptic liquid soaps (*64*). It uses a reference organism, *E. coli* K12 (NTCC 10538), and requires 12-15 volunteers. The protocol is briefly as follows. Hands are washed for 1min with soap, and then dried with paper towels. This prewash is done to remove transient organisms, picked from the environment, on the volunteers' hand. The hands are immersed up to the mid-metacarpals in fluid containing *E. coli* K12 for 5 s. The hands are then allowed to dry for 3 min. The fingertips are then rubbed for 60 s in a petri dish that contains 10 mL liquid broth. This is used to quantify the prewash microbial concentration on the fingertips. After the prewash microbial concentration samples have been taken, the volunteer performs a handwash. A volume of the test product or the reference soap is applied to the hands, and then the hands are rubbed together. The lather time for the reference soap is always 60 s. For the test product, the hands can be lathered for either 30 s or 60 s, and the rinse time under tap water for 15 s. The post wash bacteria concentrations are determined immediately after the wash by rubbing fingertips, up to the mid-metacarpals, for 60 s in petri dishes containing 10 mL liquid broth. Neutralizers are added to all post-wash sampling broths.

I.2.d Swabbing

Using a sterile swab to measure bacteria on hands is more often used for detecting presence, than for quantifying, however this technique does have limited quantifying capabilities (*142*). Several manuscripts have used swabbing to measure

bacteria on hands, but most of such studies were in the 1970's (*5*, *65*, *159*, *216*), with only one recent study in 1998 (*1*).

I.2.e Agar contact-plate method

Pressing or stamping a palm or portion of a hand to an agar plate is considered the least expensive and most simple method to measure bacteria on hand, but this technique has limited quantifying capabilities (*142*). High concentrations (>2-3 log CFU on hands) will overload an agar plate, and make the bacteria concentration too numerous to count. The stamped portion of the hand is measured, and the concentration of bacteria can be extrapolated by using the concentration per area measured (*111*). While this method has been primarily used in older studies, (*29*, *52*, *78*, *128*, *170*, *183*, *184*, *217*) at least one study has used it more recently (*111*).

I.3 Handwashing

I.3.a Surfactant chemistry and solubility parameters

Soap, in its basic form, is an alkali salt of a fatty acid (carboxylic acids) that has a hydrophobic hydrocarbon tail and a hydrophilic carboxylate + salt head group (*147*, *224*, *225*). These fatty acid slats can be either saturated or unsaturated, and derived from natural or synthetic sources. The fatty acid salts are added to water to create the final product. However, additional objects, such as fillers, dyes, or scents, can also be added (*20*). A shear thinning, non-Newtonian fluid is the optimal flow behavior for hand soaps (*20*). During a hand rinse, soaps act to form micelles of the oily debris, either human oils or foreign soil, in order to allow the debris to be washed off (*20*).

A surfactant is a compound that has a lipophilic tail and a hydrophilic head. There exist many kinds of surfactants, and they are classified by their head-groups (*177*). Anionic surfactants have a negatively charged head, cationic surfactants have a positively charged head, zwitterionic surfactants have an overall neutral head-group that contains positively and negatively charged areas, and non-ionic surfactants have a polar, but uncharged head. Surfactant solubility/effectiveness can be measured multiple ways, however HLB value is most commonly used. One method calculates the hydrophilic-lipophilic balance of a molecule (*97*, *98*). The relative static permittivity (dielectric constant) is the ratio of the permittivity (resistance encountered when forming an electric field in a medium) of a substance to that of it stored in a vacuum. The partitioning coefficient is the ratio of the concentrations of a compound (in this case the surfactant) in a mixture of two immiscible phases at equilibrium. The *Dimroth-Reichardt* solvent parameter (Et30) measures the ionizing power of a solvent. Kamlet-Taft parameters measure the hydrogen bond donor (α), hydrogen bond acceptor (β), and the dipolarity or polarizability (π) of a solvent (*177*). The refractive Index (*n)* is used as a quality control in order to confirm identity. It is a measure of how light propagates through a medium, in this case the mixture of surfactant and water (*214*). It is calculated as $n=c/v$. The critical micelle concentration (CMC) is the concentration of a surfactant, that at above which all additional surfactants added can be recruited to form micelles (*110*).

I.3.b Antimicrobial soap

The FDA Model Food Code requires the use of a cleaning agent (colloquially called soap) during a hand wash (2-301.12). The type of soap is not specified in the Model Food Code, however many facilities elect to use an antimicrobial soap. The literature suggests that antimicrobial soaps provide a greater bacterial reduction than plain or bland soap (*72*, *77*, *99*, *211*, *220*), although some papers found minimal difference (*30*, *227*). A meta-analysis of 25 hand washing papers looked at the difference between antimicrobial and bland soaps, and found that antimicrobial soaps tended to have a ~0.5 log CFU greater reduction in microbial concentration than bland soap (*176*).

Product formulation plays a key role in effectiveness of antimicrobial agents and soaps, and many active compounds (antimicrobials) are available to use in soaps (*27*, *232*). Active compounds used in antimicrobial soaps aim to disrupt bacteria cell function or reproduction. Most are antiseptics, and not antibiotics (*36*, *205*). They work by either being bactericidal (destroying the cell) or bacteriostatic (inhibit reproduction). Common active compounds used in soaps include iodophors (*18*, *19*, *99*, *217*, *227*), chloroxylenol (PCMX) (*189*, *191*), chlorhexidine gluconate (CHG) (*8*, *217*, *220*, *227*), and triclosan (*18*, *72*, *77*, *217*, *220*). Iodophors, including povidone, release iodine ions, which penetrate cell walls, and will disrupt protein synthesis (*205*). PCMX (4-chloro-3,5-dimethylphenol) inactivates bacterial enzymes and disrupts cell walls (*36*). CHG disrupts bacterial membranes, but has limited activity against enveloped viruses (*36*). Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether) works by disrupting the cytoplasmic membrane, disrupting lipid and protein

synthesis, and preventing synthesis of RNA (*36*, *115*). The antimicrobial efficacy in the soap is strongly dependent on formulation, and can the efficacy can be reduced or enhanced depending on the surfactant used in the same soap (*27*, *232*). Additionally, the surfactants found in hand lotions can build up in skin and may reduce antimicrobial handwash effectiveness (*75*). While there is a public concern with bacteria forming resistance to these compounds, no studies to date have found resistance at or above bacteriostatic (minimum inhibitory concentrations) concentrations (*3*, *53*, *143*). Furthermore, generating resistance in clinical isolates in a lab setting has not been successful (*68*, *143*). *Pseudomonas* and *Staphylococcus* species commonly show some natural resistance to triclosan (*3*, *53*, *143*), but this resistance has not been connected to residual active compounds in the environment (*143*).

Fuls *et al*. found that using more antimicrobial soap per wash increased observed log reductions by ~0.7 log counts ($p<0.001$), but did not observe the same effect using more plain soap ($p=0.2$) (*77*). Larson *et al.* (1987) found that a control wash with plain soap was not significantly affected by amounts of soap used (1mL versus 3 mL) (*140*), although increased volumes of soap might lead to increased hand irritation (*140*). A recent meta-analysis concluded that the amount of antimicrobial or bland soap used does not appear to influence its effectiveness (*176*). Ultimately the volume of soap used may be particular to a specific product formulation, and therefore the manufacturer may be best suited to determine the optimum volume needed for an effective wash.

I.3.c The effect of soap on skin and the effect of repeated washings

Soaps facilitate the removal of lipids and oily debris from the hand (*10*, *76*, *85*, *125*, *195*, *221*, *252*, *258*), but overuse can cause degradation and eventual irritation the skin by removing excess skin cells, proteins, and lipids essential for skin health (*10*, *76*, *85*, *125*, *129*, *132*, *195*, *213*, *221*, *247*, *252*, *258*). A single wash is capable of damaging the stratum corneum, even if erythema (skin redness) is not immediately observed after the handwash (*221*). Irritation can be measured by water loss (*243*, *245*, *247*), skin tightness (*125*), measuring lipid removal (*76*), and skin capacitance (*252*). Long term affects of over-washing include changes in skin pH and skin microflora (*129*, *213*).

Repeated washing with soap can raise the skin pH by damaging the acid mantle (*33*, *37*, *80*, *82*, *234*). Even repeated washes with tap water can raise skin pH for a short period of time (*33*, *82*). Many studies have been focused on the baseline pH of skin and maintaining a healthy pH (*57*, *61*, *73*, *201*, *213*, *246*, *248*, *261*). Maintaining a healthy skin pH is connected to having a healthy acid mantle (*105*, *181*, *213*). The acid mantle is a layer of the skin that is essential for maintaining skin hydration, and is considered one of the first parts of a strong immune defense (*201*, *255*). This layer is also responsible for neutralizing alkali compounds on the skin (*201*, *213*). High pH soaps have been correlated with skin irritation (*21*). Skin pH is commonly measured using a flat-surface glass electrode (*247*, *261*). The average pH of skin (healthy) is acidic (pH 4-6), but varies throughout the body (*61*, *201*). For the most part, age has not been correlated with changes in skin pH (*57*, *73*). However, some of the published literature suggests that skin starts to become more acidic after the

age of 70 (*246*, *247*), although one study found the pH to become more neutral (*261*). Some studies also suggest men and women have a different skin pH (*61*), and some studies found no difference (*247*, *261*). Studies have recorded cadaver skin as having a mean pH of 6 (*17*, *257*, *260*).

I.3.d Temperature of water

The temperature of water required for an effective handwash is a variable that has been infrequently explored, and still generates significant interest. The common perception is that higher temperature of wash water will both inactivate a greater number of bacteria and help remove greasy/oily debris better. Use of high temperatures that would rapidly destroy bacterial cells would also severely injure human skin and, at extreme temperatures (>55°C), can lead to scalding (*139*, *228*). A 2013 skin care survey determined that comfort of hands and personal beliefs played a key role in choosing the temperature for a handwash (*46*). Two studies by Michaels *et al.* found no difference, in terms of microbial reduction, of a hand wash performed at a range of temperatures (4.4 °C - 48.9 °C) (*168*, *169*), however, these studies only used 4 subjects and only one of the studies (*169*) tested antibacterial soap. Courtenay *et al.* observed a minimal difference in microbial reduction between a cool rinse (26 °C) and a warm rinse (40 °C), but did not use soap as part of the treatments (*54*). A 1980 study of hand sampling methods did not detect a significant difference between bacteria recovered when the sampling solution was at 6 °C or 23 °C (*142*). While these studies would indicate that wash water temperature has limited influence on wash effectiveness, the limited replicates (*54*,

168, *169*) or tangentially related study design (*142*) would indicate more study is needed.

I.3.e Duration of wash

Studies suggest that an extended wash, generally greater than 30 s, may result in a less effective wash (*137*, *169*, *176*). A 2011 meta-analysis found that a 120 s wash had a lower log reduction than a 30 s wash (*176*). A 30 s wash with plain soap averaged a 1.91 ±0.75 log cfu reduction, while a 120 s wash averaged a 0.17 ±0.44 log cfu reduction. Similarly, a 30 s wash with antimicrobial soap averaged a 2.42 ±0.88 log reduction, and the same wash for 120 s averaged a 0.94 cfu log reduction (*176*). Some authors suggest that an extended wash may loosen, but not remove resident microflora from hand, and these loosened microbes are now more easily transferred to other surfaces (including into hand sampling mediums), so an extended wash may also appear less effective at removing organisms (*169*). Additionally, extended and frequent washing can lead to damaged skin (*10*, *76*, *85*, *125*, *132*, *195*, *221*, *252*, *258*), which becomes in turn harder to wash and become more susceptible to colonization by pathogens (*137*, *139*, *141*). 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 (*31*). This finding suggests that total volume of water used or duration of the wash have an effect on the microbial reduction, but does prove which of these two factors (flow rate or total volume) is more essential for an effective handwash.

I.4 Hand Sanitizers

I.4.a Use of hand sanitizers

The definition of hand sanitizers as proposed by the CDC is: *an antiseptic agent that does not require use of exogenous water* [and] *after applying such an agent; the hands are rubbed together until the agent has dried* (*35*). Alcohol, the most commonly used antiseptic in hand sanitizers, is believed to inactivate microbes by disrupting the cell membranes and denaturing proteins (*162*). The alcohol in such hand sanitizers readily evaporates, with minimal amount of alcohol being absorbed through the skin (*38*).

Hand sanitizers are a useful substitute for handwashing, especially when proper hand washing facilities are not present (*104*). Furthermore, hand sanitizers are simple to use (*84*). Alcohol-based hand sanitizers can be effective on visibly soiled hands (*212*). Additionally, hand sanitizers can be more effective than soap at reducing transient organism on the skin (*104*, *123*). Finally, traditional refillable soap dispensers have been shown to allow the growth of bacteria within the dispenser (*40*, *50*, *256*), and growth in refillable soap bottles has been linked to an outbreak of *Serratia marcescens* in a neonatal intensive care unit (*40*). US FDA Model Food Code currently allows hand sanitizers to be used in foodservice establishments only when hand sanitizer use is followed by a proper handwashing (*240*). In contrast, the US CDC has recommended alcohol-based hand sanitizers as an alternative for hand washing in healthcare settings (*35*).

Hand sanitizers are not without their limitations. Hand sanitizers have limited effectiveness against spores (*111*, *161*), and limited effectiveness against non-

enveloped virus (*8*, *25*, *121*, *134*, *149*, *220*). Even without using soap, tap water alone is better than hand sanitizer at removing certain viruses (*220*).

I.5 Cross contamination

Cross contamination rates measure the amount of a substance (in this case bacteria) transferred between two surfaces. While cross contamination is not the focus of this thesis, it is worth noting that hand washing is a practice used to mitigate cross contamination. Cross contamination is a major concern in both the health industry and food industry, and the prevention, or at least mitigation of cross contamination is goal of many risk managers (*41*, *70*, *101*, *200*, *202*, *203*). Numerous routes for contamination of produce or hands exist, and more research on recontamination in the kitchen is needed (*200*). Several manuscripts have *cited* the hands as a critical point for cross contamination (*70*, *144*, *202*, *203*). For the healthcare industry, cross contamination of bacteria from doctors' hands to patient wounds is a well-known, documented problem that is difficult to solve (*128*). Especially due to the numerous reservoirs of bacteria, which includes sink faucets, keyboards, soiled/unwashed clothing, and patients with infections (*41*, *145*, *233*).

Predicting microbial transmission between handling practices and contamination of food can be difficult due to a large number of contamination scenarios that exist (*194*). Outbreaks and their ultimate causes are generally harder to trace because they are more complex and difficult to investigate (*67*, *242*). Multiple field sources, more points of contamination, and variance in vendor handling make tracing the origin and cause of an outbreak difficult. In addition, accusing the wrong producer

or vendor of an outbreak can cause severe economic repercussions and damage the reputation of otherwise safe producers, as evidenced by the *E. coli* O104:H4 sprout outbreak in Europe, during the summer of 2011(*241*).

By aiming to create a thorough and accurate quantification of cross contamination rates, food safety analysts can provide risk analysis in both home and food service kitchens (*51*). Studies that estimate transfer rates between kitchens utensils, raw meat, cutting boards, fresh produce, and hands have been done, but often they try to focus a specific object or action (*51*, *113*, *114*, *154*, *175*, *242*). Patterns that apply to a broad set of scenarios are most useful in creating formulas to assess the risk of cross contamination.

I.6 Handwashing compliance

I.6.a Handwash compliance amongst food workers

Food handler poor hand hygiene practices greatly increase the risk of illness (*93*, *94*, *163*). Observed handwashing compliance by food workers if often poor, with most only completing a thorough handwash (removed gloves, placed hands in running water, used soap, and dried hands) 27% of the time during food preparation activities (*93*, *94*). In a survey of 16 food service operations in a midwestern state, compliance with FDA Model Food Code recommendations for hand washing was only 7% overall in restaurants (*229*). In this study, hands were washed frequently before beginning preparation, but not when changing tasks, touching clothes, or before handling different kinds of foods (*229*). The same study reported zero

compliance with FDA Model Food Code recommendations for hand washing, due to a combination of incomplete lathering of hands, limited time under running water, and non-hygienic means of drying hands (*229*). Restaurant workers are less likely to wash hands correctly when involved in multiple tasks (*93*), and proper handwashing and glove use was more common in chain establishments, in comparison to non-corporate restaurants (*93*, *94*). A notable concern that researchers found is that food service workers rarely complied with handwashing between raw and RTE foods (*229*). However, ~70% of food handlers did wash hands before entering food preparation area (*229*). Investigators noticed improve handwashing regimes in assisted living facilities, schools, and childcare facilities when compared to restaurants (*229*). Guzewich and Ross (1999) determined that, between 1975-1998, 66 of 81 outbreaks had sufficient evidence to track the course of infections to food workers (*101*).

The literature is unclear as to what constitutes an effective guidance to improve handwash compliance (*34*, *58*, *138*). Therefore, as a first step towards generating information on the topic, hand hygiene guides (or posters) intended for food safety, healthcare, and public health were collected, primarily via an Internet-based search, and a quantitative analysis was performed and presented in Chapter II.

I.6.b Handwash compliance in healthcare industry

Handwashing is a critical factor to prevent the spread of infections in healthcare setting, and much of the handwashing peer reviewed research is focused on healthcare. It should be noted that the US Centers for Disease Control and Prevention does make suggestions for appropriate hand wash and handwash

facilities in hospitals (*35*). The main cause for lack of compliance with handwashing is similar to that of the food industry, in that 100% compliance would significantly increase time spent away from their duties, such as treating patients (*32*, *34*, *197*). Measures to increase compliance, such as education, often only improve compliance for a short period of time (*58*, *138*). The intervention that does successfully increases long-term handwashing compliance is ease of access to handwashing facilities and administrative involvement (*34*). One study found that healthcare workers wash hands properly only 10% of the time before seeing a patient, and 22% of the time after seeing patients (*32*). Another study observed an overall 57% handwash compliance, but also noticed that adherence depended heavily on specialty (for example, 87% for internalists versus 23% for anesthesiologist) (*197*).

Chapter II- Quantitative Analysis of Recommendations Made by Handwashing Guides

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Key Words: Handwashing, Sign, Poster, Foodservice

II.1 Abstract

Handwashing is important in preventing microbial cross contamination. The US FDA Model Food Code states that handwashing sinks require a handwashing sign or poster to be visible to employees washing their hands. This research analyzes current handwash guidance by collecting and reviewing existing handwashing signs and posters, and subjects them to a quantitative analysis. An Internet search collected a database of handwashing signs. Lather time, rinse time, overall wash time, water temperature, water use, drying method, technique, and total number of steps were recorded. Eighty-one unique handwashing posters or signs were identified. Every sign had at least one step, with the highest number of steps being thirteen. Thirty-seven signs indicated a specific lather time, with the average time being ~18 s. No sign suggested > 20 s lather, and none suggested < 10 s lather. Twenty-four signs recommended using warm water. Two signs recommended using 100 °F (37.8 °C) water and one recommended using hot water. Sixty-two signs made a recommendation to dry hands and fifty-three suggested using a paper towel. Our analysis reveals that handwashing sign and poster suggestions can vary quite widely. Lack of consistent signage may contribute to a lack of handwashing consistency and compliance.

II.2 Introduction

Handwashing is an important part of preventing microbial cross contamination in the kitchen and elsewhere (*70*, *93*, *94*, *101*, *144*, *174*, *200*, *229*, *250*). The FDA Model Food Code indicates when handwashing is required during food preparation, and the both the CDC and the World Health Organization (WHO) suggest frequent handwashing in healthcare settings (*35*, *240*, *250*). The FDA Model Food Code and the CDC Guideline for Hand Hygiene in Health-Care Settings recommend washing hands for 20 s, under warm running water, with soap, and using either single-use towels or a forced air dryer to dry hands (*35*, *240*). The WHO Guidelines On Hand Hygiene In *Healthcare* recommend washing hands for 40-60 s, with soap, and using a single-use towel to dry hands (*250*).

Research over the past 30+ years has shown that the way hands are washed (including technique, duration, and drying method) can have a significant effect on the microbial reduction. Increased handwashing duration has shown to improve microbial reduction, but the rate of increasing microbial reduction is less after 20 s of handwashing (*113*, *169*, *183*, *227*). Research has also shown that washes below 10 s may be of limited effectiveness (*112*, *169*, *183*, *227*). Moist hands transfer significantly more bacteria than dry hands, and therefore drying, regardless of drying method used, is an essential step to prevent cross contamination (*112*, *188*, *208*, *240*). Using paper towels appears to provides multiple benefits, including faster drying, improved microbial reduction, and the ability to use the towel as a barrier to protect against recontamination from doorknobs and sink faucets (*100*, *112*, *188*, *199*, *200*, *240*, *250*).

The US FDA Model Food Code indicates that handwashing sinks are not considered

fully equipped unless a handwashing sign or poster is clearly visible to any employees washing their hands (section 6-301.14) (*240*) but research is conflicted as to whether these signs improve compliance. Some studies indicate that handwashing reminders, including signs and posters, can improve handwashing compliance in healthcare (*127*, *164*, *179*, *196*) and foodservice (*49*). It has also been suggested that signs are not effective as the sole method to improve handwash compliance in foodservice facilities (*4*). Many studies have noted that even when handwashing sinks are easily accessible and handwashing signs are visible, the workload of the food handler can undermine compliance (*4*, *93*, *94*, *229*), and similar results have been observed in healthcare (*32*, *197*).

Handwashing signs and posters are intended to remind and reinforce the need to wash hands as well and to provide information on proper handwashing technique (*4*, *127*, *164*, *179*, *196*, *240*). Determining what constitutes an effective handwashing sign is difficult, as little research has been done on the subject. This manuscript aims to initiate the discussion by reviewing existing handwashing signs and posters, subjecting them to a quantitative analysis, and comparing those findings to recommendations in the literature.

II.3 Materials and Methods

A search by the authors compiled a comprehensive database of handwashing sign and posters. Keywords used in the Internet search included: handwash, sign, poster, employees, soap, hand hygiene, notice, and guide. A Google (Mountain View, California) search was followed by a targeted search of US state and county health department websites. Although the words sign, guide, and poster can be used

interchangeably, in this study the word “sign” will be used from this point forward.

The data were compiled and analyzed using Excel (Microsoft, Redmond, WA). Specifically, instructions for lather time, rinse time, overall wash time, water temperature, pre-moistening of hands, drying method, technique, and total number of handwashing steps were recorded. The requirements for inclusion were minimal; the sign needed only to mention or show a picture of handwashing. Multiple copies of some signs were located during the search, but only unique entries were compiled for analysis. Some handwashing signs located by the search were translated copies of the 2009 WHO handwashing guide (*250*), but as they provided no new information or figures, they were not added to the database. Signs that utilized the same figures or technique suggestions, but which provided either additional information or removed certain steps were included in the database.

II.4 Results

Eighty-one unique handwashing posters or signs were identified, and split into three groups depending on the target audience. The three groups included healthcare, foodservice, and the general public. Healthcare signs were those specifically intended for healthcare facilities (e.g. hospitals, nursing homes, etc.), while the general public group included signs intended for schools, office buildings, and at-home use. Overall there were 31 (38.3%) unique signs targeted the public, 26 (32.1%) unique signs targeted at foodservice, and 21 (25.9%) unique signs targeted healthcare audiences. A small fraction of signs, 3 (3.7%) targeted both healthcare and foodservice audiences. These 3 signs were added to each group

when the groups were being analyzed separately, but were only counted once when the overall dataset was being analyzed. Sixty (74.1%) signs included figures, which were defined as any graphical representation (e.g. drawings or photographs) of a handwashing step. Twenty-one (25.9%) unique signs identified when to wash hands. Sixty-three (77.8%) unique signs were published by government agencies, and 18 (22.2%) were published by private companies.

Table II.6.a summarizes the number of steps, or directions given in the handwashing sign collection. A step was defined as a direction (either written or pictured) that indicated a specific task to be completed as part of the handwash procedure. Every sign had at minimum one step (e.g. wash your hands), and the highest number of steps observed was thirteen. Sixty-six (81.5%) signs recommend more than one step. The average number of steps per sign was between 5-6, where foodservice signs averaged about 5 steps, and healthcare signs averaged 6 steps.

Table II.6.b summarizes handwashing time recommendations. The handwashing times were grouped into three categories: lather time, rinse time, and total time. The total time group includes the rinse and lather time groups, but 18 signs (22.2%) indicated only a total handwash time, and had no specific breakdown on rinse and lather times. Twenty-three signs (28.4%) did not indicate any duration. Thirty-seven (45.7%) signs indicated a specific lather time, with the average lather time being ~18 s. No sign suggested greater than 20 s lather time, and none suggested less than 10 s lather time. Three (3.6%) signs indicated a specific rinse time, with an average rinse time of ~ 13 s. No sign suggested more than a 20 s rinse, or less than a

10 s rinse. When considering total handwash time, 58 (71.6%) signs gave an average of ~22 s. No total wash time was greater than 60 s, and no total time was less than 10 s.

No foodservice signs suggested a specific rinse time, but 13 (50% of all foodservice signs) indicated a specific lather time, with the average time being ~19 s. Seven foodservice signs (26.9%) did not indicate any handwash duration. No foodservice sign suggested greater than a 20 s lather time, and none suggested less than a 15 s lather time. The average total wash time from the nineteen signs (73.1% of all foodservice sign) was ~21 s. No foodservice total wash time was greater than 60 s, and no foodservice total wash time was less than 10 s. It should be noted, that only one foodservice sign recommended a wash greater than 20 s.

Only one healthcare sign (4.8% of all healthcare signs) indicated a specific rinse time (20 s), and 6 (28.6% of all healthcare signs) signs indicated a specific lather time, with the average lather time being ~18 s. No healthcare sign suggested greater than 20 s lather time, and none suggested less than 15 s lather time. The average overall wash time from sixteen healthcare signs (76.2% of all healthcare signs) was ~27.5 s. Five healthcare signs (23.8%) did not indicate any handwash duration. No healthcare wash time was greater than 60 s, and no wash time was less than 10 s.

Two signs intended for the general public (6.5% of all public signs) suggested a rinse time and both recommended 10 s, and 20 (64.5% of all public signs) suggested a specific lather time, with the average lather time being 18 s. No general public

signs suggested a lather time greater than 20 s, and none less than 10 s. The average total wash time from 26 general public signs (83.9% of general public signs) was 19.2 s. Five general public signs (16.13%) did not indicate any handwash duration. The greatest wash time recommended in the general public signs was 30 s, and the minimum was 10 s.

Table II.6.c summarizes a variety of recommendations made in the handwashing signs relating to water temperature, wetting the hands with water, drying the hands, towel use and various other aspects of handwashing technique. Twenty-four (29.6%) signs recommended using warm water, but did not specify an exact temperature. Two signs (2.5%) recommended using 100 °F (37.8 °C) water. One (1.2%) sign recommended using hot water. Fifty-four (66.7%) signs made no water temperature recommendations. Forty-six signs (56.8%) recommended wetting hands before applying soap, 12 (14.8%) suggest wetting the hands while applying soap, and only 2 signs (2.5%) suggest wetting hands after applying soap. Twenty one signs (25.9%) made no recommendation about when to wet the hands. Not surprisingly, all signs recommended using soap (data not shown). Sixty-two (76.5%) of all signs made a recommendation to dry hands in some manner (data not shown). This is composed of 53 (65.4%) signs that suggested using a paper towel, and 4 (4.9%) that suggested hot air hand dryers. Five (6.2%) signs recommend hand drying, but make no suggestion on how to dry. Interestingly, 31 (38.3%) suggested turning off the tap with a paper towel, and 3 (3.7%) signs suggested opening the door with the same towel used to dry hands.

Table II.6.c also summarizes other various handwashing technique suggestions. We defined these other suggestions as any specific direction on what to do with the hands during the wash. Forty-one (50.6%) signs suggested one or more techniques. Most techniques involved targeting specific areas: 33 signs (40.7%) suggested targeting between fingers, 31 (38.3%) the fingernails, 29 (35.8%) the back of the hands, 27 (33.3%) the palms, 17 (21.0%) the back of fingers, 16 (19.8%) the thumbs, and 14 (17.3%) the wrists. Additionally 6 signs (7.4%) suggested using a fingernail brush, while 2 (2.5%) signs suggested removing jewelry before handwashing.

Table II.6.d summarizes the directions given on the handwashing regarding when a handwash is needed. About 29% (6 of 21) of healthcare signs, ~46% (12 of 26) of foodservice signs, and ~13% (4 of 31) of general public signs described when to wash hands (percentages not shown in Table II.6.d). In total, 21 signs out of 81 indicated when to wash hands. Many "when to wash" suggestion were found, and are all shown in Table II.6.d, but only key aspects will be described here. Almost all signs that did give a "when to wash" suggestion, also indicated the reader to wash their hands after using the restroom, and this was the most common recommendation both overall and within each of the three categories. Aside from washing hands after using the restroom, no other key "when to wash" recommendation are evident from healthcare sign data. Other "when to wash" events appear on 5 of the 6 healthcare signs with "when to wash" recommendations, but they are varied. The four public signs that included specific "when to wash" information all recommended to wash hands after coughing or sneezing as well as

after using the restroom. Three out of four of these signs also recommended washing hands before eating or drinking.

A number of key "when to wash" recommendations occur frequently on the foodservice signs. Following "after using the restroom", the most common recommendation, which appeared on nine signs, was to wash hands after eating or drinking followed by washing hands after coughing or sneezing (7 signs). The next most frequent recommendation to wash hands was after handling dirty utensils or dishes as well as before preparing food (6 signs each). Other recommendations appearing on 5 signs were to wash hands after contact with skin, after using tobacco products, or after handling raw food.

II.5 Discussion

Both the FDA and the CDC currently recommend washing hands for 20 s, under warm running water, with soap, and using either single-use towels or a forced air dryer to dry hands (*35*, *240*). Previous studies suggest a minimal wash (<10 s) are not as effective as a 20 s wash (*112*, *169*, *183*, *227*), and Allwood *et al.* (*4*) noted that one of the most common problems observed with Minnesota state food workers' hand wash regime was failure to wash for 20 s. Almost three quarters of all handwash signs collected (~72%) gave a recommended wash time, and those that do averaged slightly more than 20 s, as do the foodservice specific signs. Healthcare signs have a longer average recommended wash time (27.5 s), while those targeted at the general public have an average recommended wash time of ~ 19 s.

One third of all the signs surveyed (27 or 33.3%) made either qualitative or

quantitative water temperature recommendation. The FDA Model Food Code (section 5-202.12-A) states that a handwash sink must be equipped to provide water at a temperature of at least 100 °F (38 °C) (*240*), and only two signs (one foodservice, one healthcare) specifically mentioned 100 °F as the wash temperature. It should be clarified however, that although the code states a sink must deliver water at 100°F, the code does not mandate that hands be washed at 100°F, only that "clean, running warm water" be used (Section 2-301.12-B-1) (*240*). Twenty-four (29.6%) signs (5 healthcare, 12 food service, and 9 general public) recommend washing hands with warm water. Despite its appearance in the Model Food Code as well as on some handwashing signs, the scientific support for any water temperature for washing does not appear to exist. Two prior research studies have found no correlation between the temperature of water and the microbial reduction (*167*, *169*), and a manuscript in preparation in our lab further confirms this (*112*).

Hand drying plays a significant role in the reduction of microbes on hands after handwashing (*52*, *100*, *112*, *199*, *250*) and in aiding in mitigation of cross contamination risk (*89*, *166*, *188*, *200*, *235*, *240*). Even with the established importance of hand drying as part of a thorough hand wash in the published literature cited above, 19 (23.4%) signs did not make a drying recommendation. Six foodservices signs, 7 healthcare signs, and 6 general public signs did not indicate to dry hands after a wash. Three studies indicated that paper towels provide a ~0.5 log CFU greater microbial reduction than standard air drying (*52*, *100*, *112*), and a majority of handwashing signs surveyed (65.4%) suggest using paper towels. We were surprised to see 31 (38.3%) signs suggested turning off the faucet with a paper

towel as a cross-contamination risk mitigation measure. The use of this mitigation step is supported by one study which showed that ~2% of bacteria present on faucet tap could transfer to the hand (*51*), and another which documented high bacterial population on faucet handles in homes (*118*). The FDA Model Food Code suggests paper towels may be used as a barrier against recontamination "when touching surfaces such as manually operated faucet handles on a handwashing sink or the handle of a restroom door" (2-301.12-C) (*240*). Research has shown that microorganisms may be present over the entire hand, and therefore a wash may only be complete when all areas of the hand are given attention during a wash (*119*, *163*, *182*). The subungal region of fingernails can act as a reservoir for transient Gram-negative organisms, and while 31 (38.27%) signs suggested targeting nails, only 6 (7.41%) suggested using a fingernail brush, which has been suggested to be the most efficient way to remove bacteria from under nails (*4*, *222*). Research has also reported higher microbial counts from hands with artificial nails versus natural nails and that microbial cell numbers were correlated with fingernail length, with greater numbers beneath fingernails with longer nails (*148*). While a nailbrush has shown to provide additional 1-1.5 log microbial reduction over the standard hand wash (*222*), no data currently exist to suggest that targeting the fingernails without a nailbrush provides any additional microbial reduction. Similarly, risk of transfer of bacteria from under the nails to foods or food contact surfaces is also not documented in the literature. The FDA Model Food Code states that a nailbrush can be used, as part of a cross contamination prevention regime, before handling ready to eat foods with bare hands (section 3-301.11-E-6-b)

(*240*).

Only 2 (2.5%) of food service signs suggest removing jewelry during a wash, but this may be because the FDA Model Food Code prohibits all jewelry, except for plain rings, during food preparation (section 2-303.11) (*240*). A risk assessment determined that wearing a ring during a wash could cause the wash to be less effective (*173*), and studies have found that hands with rings have, at minimum, a 1 log greater concentration of skin microorganism (*71*, *206*, *238*). Salisbury *et al*. determined that healthcare workers that were wearing rings had a less effective hand wash than those who were not wearing rings (*206*). Yildirim *et al.* determined that wearing a ring significantly reduced the effectiveness of hand sanitizers (*254*). Fagernes *et al.* did not observe a significant difference in microbial concentration between hands with or without rings, but did notice that hands with rings were more likely to carry bacteria in the family Enterobacteriaceae, which includes *Salmonella* and *E. coli* (*66*).

When to wash recommendation are a detailed part of US hand hygiene guidelines for both healthcare and foodservice (*240*, *250*). The FDA Model Food Code (section 2-301.14-A-I) recommends washing hands after a number of activities (*240*), which are also mentioned to varying degrees in the handwash signs we surveyed. Following the order as presented in the Model Food Code they are: after touching bare human body parts is mentioned in 5 signs; after using the restroom (11 signs), after caring for or handling service animals (3 signs); after coughing, sneezing, using a handkerchief or disposable tissue (7 signs); using tobacco (5 signs), eating, or

drinking (9 signs); after handling soiled equipment or utensils (6 signs); when switching between working with raw food (5 signs, with 4 more mentioning raw meat specifically, and none specifically mentioning raw vegetables/fruits); before donning gloves for working with food (4 signs); and after engaging in other activities that contaminate the hands (3 signs).

Some of these "when to wash" recommendations appear to have scientific support, while others do not. Those that do have scientific support are summarized below. Individuals infected with foodborne pathogens can continue to shed these organisms for extended periods (*9*). *Salmonella* outbreaks in dry pet foods resulted in some human cases arising from handling pets (*47*, *48*). Multiple manuscripts have documented quantifiable cross contamination from dirty cooking utensils to hands (*51*, *87*, *200*, *242*). Likewise, cross contamination to and recontamination of hands have been documented as sources of foodborne outbreaks (*200*, *235*). Cross contamination to hands directly from raw meat (*165*, *175*), and raw meat outbreaks with hands as cross-contamination vehicles (*101*) are well documented.

Given that washing hands can help prevent cross contamination during food preparation (*51*, *62*, *72*, *87*, *194*, *244*), and in many cases, foodborne illness outbreaks can be linked to improper hand hygiene (*28*, *198*, *235*), it is somewhat surprising that more signs did not include details on when to wash hands.

The World Health Organization (WHO) recommends "5 moments" of when to wash hands in healthcare setting (*250*). The five moments are before patient contact, before an aseptic task, after body fluid exposure, after patient contact, and after contact with patient surroundings (*250*). Handwashing signs can provide a

reminder for healthcare employees of what may be mandated by their agreed upon hand hygiene code, however only ~29% of healthcare signs included details on when to wash hands. As with foodservice signs, the suggestions for when to wash hands are discussed in the order presented in the WHO 5 moments. One healthcare sign suggested washing hands after any work break. None suggested washing hands when returning from areas outside the work area. One healthcare sign suggested washing hands after contact with blood, mucous, skin, wound, or body fluid. One sign also mentioned washing hands when visibly soiled. No signs suggested washing hands after contact with vomit, despite the fact that norovirus can be transmitted by vomitus (*157*). One healthcare sign suggested washing hands after changing or removing gloves. Two healthcare signs suggested washing hands after touching animals. Four healthcare signs suggested washing hands after using the restroom. No healthcare sign suggested washing hands after contact with waste/sewage, contact with raw food. No healthcare sign mentioned washing hands after suspected cross contamination, and this includes touching clothing (oneself or other's) or doors.

A hand wash is mandated in several hand hygiene guides (*35*, *240*, *250*). This analysis found 81 unique signs, with the signs varying from simple, one-direction signs, to complex, thirteen-direction signs. What constitutes as an "ideal" handwashing sign is difficult to determine, but signs that contain documented microbial reduction techniques and cross contamination prevention techniques can serve to better educate individuals. This hand hygiene sign analysis highlights that while all signs and posters direct the reader to wash their hands, much of the other

suggestions differ greatly.

II.6 Tables

Table II.6.a Number of steps observed in the handwashing sign collection

	Number of Steps			
	All Data Sets	Foodservice	Healthcare	General public
Mean	5.5	4.7	6.1	5.7
Median	5	5	5.5	5
Minimum	1	1	1	1
Maximum	13	13	12	13

Table II.6.b Summary of handwash duration suggestions in the 81 handwashing signs collected

	Signs Indicating this Step			Average (s)	Median (s)	Min (s)	Max (s)
	Step	Number	Percent				
All Signs							
	Lather	37	45.68%	18.4	20	10	20
	Rinse	3	3.60%	13.3	10	10	20
	Overall	58	71.60%	22.2	20	10	60
	No time Indicated	23	28.40%	-	-	-	-
Food Service Signs							
	Lather	13	50.00%	19.2	20	15	20
	Rinse	0	-	-	-	-	-
	Overall	19	73.08%	21.3	20	15	60
	No time Indicated	7	26.92%	-	-	-	-
Healthcare Signs							
	Lather	6	28.57%	18.3	20	15	20
	Rinse	1	4.76%	20.0	20	20	20
	Overall	16	76.19%	27.5	20	15	60
	No time Indicated	5	23.81%	-	-	-	-
General Public Signs							
	Lather	20	64.52%	18.0	20	10	20
	Rinse	2	6.45%	10.0	10	10	10
	Overall	26	83.87%	19.2	20	10	30
	No time Indicated	5	16.13%	-	-	-	-

Table II.6.c Handwashing procedure or technique suggestions in 81 handwashing signs

Technique area	Technique suggestion	Number of signs
Water temperature	No water temperature indicated	54
	Warm water	24
	100 °F water	2
	Hot water	1
Wetting the hands	Before soap	46
	With soap	12
	After soap	2
	No wetting suggestion indicated	21
Drying method	Drying with paper towel	53
	Drying, not specified	5
	Air drying	4
Towel use besides drying	Turning off tap with towel	31
	Open door with towel	3
Other technique suggestions	Any other suggestions	41
	Target between fingers	33
	Target fingernails	31
	Target back of hands	29
	Target palms	27
	Target back of fingers	17
	Target thumbs	16
	Target wrist	14
	Use fingernail brush	6
	Remove jewelry	2

Table II.6.d Summary of handwashing sign "when to wash" hands recommendations. Values indicate the number of posters that gave a specified "when", and are sorted in descending frequency by frequency over all signs.

When to wash	Specific event	All signs 21	Healthcare 6	Foodservice 12	General Public 4
After	Using restroom	18	4	11	4
	Coughing or sneezing	12	2	7	4
	Drinking or eating	10	1	9	1
	Contact with skin (not hands/arms)	7	1	5	1
	Using tobacco products	7	2	5	0
	Handling dirty utensils or dishes	6	0	6	0
	Handling raw food	6	0	5	1
	Touching animals	6	2	3	2
	Handling raw meat	5	0	4	1
	Contact with body fluids	4	1	1	2
	Any work break	3	1	2	0
	Contact with wound	3	1	2	1
	Handling garbage	3	0	2	1
	Contact with blood	2	1	0	1
	Contact with ill individual	2	0	1	1
	Returning from outside	2	0	0	2
	Answer phone	1	0	1	0
	Contact with mucous	1	1	0	0
	Contact with vomit	1	0	0	1
	Contact with waste water or sewage	1	0	0	1
	Cross contamination	1	0	1	0
	Handling chemicals	1	0	1	0
	Contaminated (not specific)	1	1	0	0
	Removing gloves	1	1	0	0
	Touching clothing	1	0	1	0
	Touching door	1	0	1	0
Before	Resuming work	7	2	4	1
	Preparing Food	6	0	6	1
	Putting on or changing gloves	4	0	4	0
	Drinking or eating	3	0	0	3
	Handling RTE foods	3	0	3	0
	Entering kitchen	2	0	2	0
At the time	If hands are visibly soiled	4	1	1	2
	As needed	3	0	3	0

Chapter III Meta-Analysis of the Published Literature on the Effectiveness of Hand Sanitizers

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Key Words: Hand Sanitizer, Meta-analysis, Antimicrobial, Alcohol

III.1 Abstract

Meta-analyses contrast and combine results from different studies to identify patterns, sources of disagreement, or other interesting relationships that may only come to light in the context of multiple studies. This study was undertaken in an effort to determine those factors contributing to hand sanitizer effectiveness. Between November 2012 and September 2013, a search of hand sanitizer literature was conducted, and the published data on the effects of hand sanitizers on bacteria and viruses were compiled. Twenty-eight publications, containing 336 observations, met the criteria for the study. Data on sample size, experiment protocol used, sanitizing agent, concentration of antimicrobial, exposure time, exposure volume, organism, mean starting microbial concentration, and log reduction were extracted and compiled. There was a significant difference between ethanol and isopropanol hand sanitizer effectiveness for bacteria ($p=0.02$), but not for viruses ($p=0.74$). Isopropanol had a higher mean log reduction (4.2 log cfu) than ethanol (3.7) for bacterial data sets, but not for virus datasets. Log reductions, as measured by fingerpad and glove juice testing protocols (both 1.5 mean log reduction), were significantly lower than those based on European Standard EN 1500 methods (3.5 mean log reduction). Alcohol-based hand sanitizers (3.8 mean log reduction) were more effective ($p=0.005$) than those based on other antimicrobials (2.6 mean log reduction) for bacteria, but the same statistical significance was not observed for viral datasets ($p=0.08$).

III.2 Introduction

Hand sanitizers are defined by the CDC as an antiseptic agent that does not require use of exogenous water and where after applying such an agent, the hands are rubbed together until the agent has dried (*35*). Hand sanitizers are a useful and simple to use substitute for handwashing, especially when proper hand washing facilities are not present (*84*, *104*). The most commonly used antiseptic in hand sanitizers is alcohol, which inactivate microbes by disrupting cell membranes and denaturing proteins (*162*). Alcohol-based hand sanitizers readily evaporate, with a minimal amount of alcohol being absorbed through the skin (*38*). Alcohol-based hand sanitizers have been shown to be effective, even on visibly soiled hands (*212*). Hand sanitizers may be more effective than soap at reducing transient organism on the skin in some circumstances (*123*). The FDA Model Food Code recommends that when hand sanitizers are used in retail and foodservice establishments that they only be used following proper handwashing with soap and water (*240*). In contrast, the US CDC have recommended alcohol-based hand sanitizers as an alternative for hand washing in healthcare settings (*35*).

Hand sanitizers are not without shortcomings, and have limited effectiveness against spores (*111*, *161*) and against non-enveloped virus (*8*, *25*, *121*, *134*, *149*, *220*). Some research has shown that tap water alone can remove more viruses than hand sanitizers can inactivate on hands (*220*).

Meta-analyses contrast and combine results from different studies to identify patterns, sources of disagreement, or other interesting relationships that may come

to light in the context of multiple studies (*223*). This meta-analysis gathered available hand sanitizer data from the published literature, and provides a quantitative analysis as a guide to future policy-making and research efforts.

III.3 Material and Methods

A search of hand sanitizer literature was conducted which included searching for published data on the effects of hand sanitizer on bacteria and viruses. The search was done primarily using online resources and databases, specifically the Rutgers University online library archives (New Brunswick, NJ), Science Direct (Elsevier, Amsterdam, Netherlands), PubMed (The United States National Library of Medicine, Bethesda, MD) American Society for Microbiology website, and Google Scholar (Mountain View, CA). Keywords used in the search include hand sanitizer, hand hygiene, alcohol, and antimicrobial. Specific agent names like ethanol, isopropanol, propanol, chlorhexidine gluconate, iodine, benzalkonium chloride, phosphoric acid, hydrogen peroxide, sodium hypochlorite and chloroxylenol were also used. References in and citation of the collected articles were used to expand the search. No date restriction was placed on the studies collected.

Data were excluded if the results were qualitative (*104*) or not fully quantitative (*153*); did not measure inactivation on hands directly (e.g. measured disease reduction in a population) (*102*, *106*); did not use methods that could differentiate between active and inactive microbes (e.g. PCR) (*92*); were performed *in vitro* and not on hands (*99*, *122*, *186*, *218*, *230*); were applied in a non-standard way (e.g. surgical scrub) (*26*, *84*, *108*, *158*, *189*); if reduction due to sanitizer alone could not be determined (due to other interventions like a hand rinse) (*60*, *90*, *170*); the

intervals between sampling were too long (e.g. days versus minutes) (*6*, *137*); or the data in the manuscript were from another source (*123*).

Data on sample size, experimental protocol used, sanitizing agent, concentration of antimicrobial, exposure time, exposure volume, organism, mean starting microbial concentration, and mean log reduction were extracted from each publication. A dataset was defined as a group of observations with a common set of experimental conditions resulting in a calculable mean log reduction. A database of mean log reduction together with the corresponding experimental conditions was complied using Microsoft Excel (Microsoft, Redmond, WA). When microbial reduction was expressed as percent reduction, this was converted to log reduction.

Studies were grouped into one of three experimental protocol types: European Norm 1500 (*63*), Fingerpad (ASTM E1838-02) (*11*), and Glove Juice (ASTM E1115-11, ASTM E1174-13) (*15*, *16*). If the experimental protocol used in a manuscript was not specifically mentioned, it was assigned to the group it most closely matched (*23*, *107*, *185*, *204*)

Histograms were generated using Excel (Microsoft, Redmond, WA) to characterize the variability in the data (*176*). Relative frequency distributions were generated when comparing two data sets with a differing number of observations, where relative frequency shows the fraction of the total number of observation where a value was observed. Linear regression analysis was performed using Microsoft Excel. A linear regression t-test (Excel, Microsoft, Redmond WA) was used to determine if slopes were significantly different from 0 (*176*). ANOVA and post hoc

Tukey's range test were used to determine if multiple means were significantly different at a 0.05 level of significance using MATLAB (MathWorks, Natick MA). Publication bias was assessed using the funnel plot technique (*59*), where study size is plotted as a function of treatment effect. If no publication bias exists, larger studies will show a treatment effect near the average, and while smaller studies will occur roughly evenly on above and below the average, in a funnel-like shape. Deviation from this shape can indicate publication bias.

III.4 Results

Table III.6.a summarizes the data collected. Of the 466 potential datasets, 130 did not meet our criteria for further analysis and were excluded from analysis. Three hundred and thirty six (336) datasets from 28 publications met our criteria for inclusion in the meta-analysis. Two hundred and fourteen datasets were from experiments with bacteria, 114 from viruses, and 8 from fungi. The 214 bacterial datasets can be subdivided into those using either transient (added) or resident (natural biota) organisms, with 188 and 26 datasets, respectively. The transient observations were further separated into those using added Gram-negative or Gram-positive organisms, with 156 and 32 datasets, respectively. The datasets for the Gram-positive organisms include 6 with a spore-forming bacterium, *Clostridium difficile* (not shown). The majority (56.0%) of the data were collected from experiments done using transient, Gram-negative bacteria or from experiments done using viruses (33.9%).

Table III.6.b provides a summary of the active ingredients used in the datasets. Two hundred eighty-six (85.1%) datasets used hand sanitizer that contained alcohol as

an active ingredient, and 63 (18.8%) used a non-alcohol based antimicrobial as the main active ingredient. One hundred forty-five datasets (43.2%) used ethanol, 116 (34.5%) used isopropanol, 9 (2.7%) used propanol, 14 (4.2%) used a mix of alcohols, and 2 (0.6%) used an unspecified alcohol. The most commonly used non-alcohol based antimicrobial was chlorhexidine gluconate, used in 28 (8.3%) datasets, followed by iodine used in 8 (2.4%) datasets, then benzalkonium chloride, phosphoric acid, and hydrogen peroxide, each used in 3 (0.9%) datasets, and sodium hypochlorite, and chloroxylenol were used in 1 (0.3%) dataset. Sixteen datasets (4.8%) used an unspecified antimicrobial. Thirty-six (10.7%) datasets used a hand sanitizer that contained both alcohol and antimicrobials. Eighteen data sets used a tap water treatment, and five data sets used an untreated hand as a control.

Factors influencing mean log reduction. No publication bias was observed in the meta-analysis (data not shown). Mean log reduction was correlated with publication year, but the low R^2 suggests an inaccurate model (R^2=0.04, slope=0.05, p=0.002). The trend line did not change appreciably for any specific antimicrobial, alcohol, or organism. No clear trend was observed in the relationship between product volume and mean log reduction (R^2=0.01, slope=0.08, p=0.39), and likewise for the relationship between exposure time and mean log reduction (R^2=0.01, slope=-0.003, p=0.11).

Previous studies (*175*, *176*) have observed a positive correlation between increased log reduction or transfer rate and mean starting microbial concentration, suggesting that authors should account of inoculum size when analyzing data. Figure III.6.a shows the effect of mean log starting concentration (inoculum size) on log reduction

for alcohol based hand sanitizer treatment for bacteria and viruses. As the inoculum size increases, the log reduction also increases (R^2=0.14, slope=0.6, p=0.002). The correlation for bacterial inoculum size and log reduction is very strong (R^2=0.65, slope= 1.4, p=$2.12x10^{-10}$), while the correlation for the viral data is not (R^2=0.05, slope=-0.2, p=0.22).

Influence of testing protocol. Figure III.6.b shows the distribution of log reductions observed, as measured by the three commonly used protocols, European Norm 1500 (*63*), fingerpad (*11*), and glove juice (*15*, *16*). An ANOVA revealed a difference between one or more of the variables (p=$9.5x10^{-9}$), and a post hoc Tukey's range test revealed EN 1500 protocols had a significantly higher log reduction compared to both fingerpad and glove juice hand sanitizing protocols. There was no significant difference in mean log reduction between fingerpad and glove juice protocols (p = 0.05). EN 1500-based studies had a mean log reduction of 3.5 log cfu (SD=1.7), while fingerpad-based studies had a mean log reduction of 1.5 log cfu (SD=1.3), and glove juice-based studies had a mean log reduction of 1.5 log cfu (SD=1.5). The maximum log reduction reported by an EN 1500-based study was 7.2 log cfu, while the maximum log reduction reported by the other two protocols was 4.5 log cfu.

Influence of alcohol concentration and type. There was no correlation between percent alcohol versus mean log CFU reduction (R^2=0.02, slope=0.03, p=0.73), so additional analysis pooled all concentration data.

Table III.6.c breaks down alcohol efficacy by specific microorganism and type of alcohol used. There are two key points to take away from this table. The first point is that microorganisms react differently to different treatments, and some treatments, notably mixed alcohols and propanol based hand sanitizers, have not been tested on the same variety of microorganisms as ethanol and isopropanol based hand sanitizers. The second key point is that for some microorganisms, notably the viruses, few datasets were available for analysis, which suggest further study is needed with these organisms.

Figure III.6.c.i-ii compares the of log reduction of two of the most common alcohols used in alcohol based hand sanitizers, ethanol and isopropanol, for virus and bacteria datasets. Overall, isopropanol based hand sanitizers had a significantly ($p=5.0x10^{-7}$) greater effect (3.9 mean log reduction) on bacteria than ethanol based hand sanitizers (2.9 log reduction). Conversely, no such difference ($p=0.74$) was seen for a similar analysis of the viral datasets (Fig III.6.c.i) with essentially similar effectiveness for isopropanol (1.6 mean log pfu reduction) and ethanol (1.5 mean log pfu reduction) based hand sanitizers. While lower than the overall difference, a statistically different ($p=0.02$) 0.5 mean log cfu reduction was observed for bacteria data (Fig III.6.c.ii). Ethanol based hand sanitizers had a 3.7 mean log cfu reduction, and isopropanol had a 4.2 mean log cfu reduction.

Influence of alcohol or antimicrobial. Figure III.6.d.i-ii compares the mean log reduction of alcohol versus non-alcohol based hand sanitizers bacteria datasets ($n=281$) and virus datasets ($n=62$). Looking at both bacteria and virus data sets, alcohol based hand sanitizers had a significantly higher ($p=7.7x10^{-7}$) mean log

reduction (3.2 mean log reduction) than antimicrobial sanitizers (1.9 mean log reduction). An analysis of the viral datasets (Fig III.6.d.i), reveal that alcohol based hand sanitizers (1.5 mean log pfu reduction), and antimicrobial-based sanitizers (1.2 mean log pfu reduction), were not significantly different in their effectiveness ($p=0.08$). The same analysis for bacterial datasets (Fig III.6.d.ii) shows that alcohol based hand sanitizers (3.8 mean log cfu reduction) were significantly ($p=0.005$) more effective than antimicrobial sanitizers (2.6 mean log cfu reduction).

Table III.6.c supports the observation that alcohol based hand sanitizers are more effective against bacteria (0.63 to 6.6 mean log cfu reduction) than for viruses (0.43-3.25 mean log pfu reduction), but as the wide ranges indicate, the effect is dependent on the specific organism.

Effect of Bacteria Type. Figure III.6.e summarizes the effect of alcohol based hand sanitizer efficacy by its effect on Gram negative, Gram positive, and Resident flora. Alcohol was most effective against added Gram positive bacteria (4.7 mean log cfu reduction), and least effective against resident flora (1.8 mean log cfu reduction), with an intermediary effect against added Gram negative bacteria (4.0 mean log cfu reduction). An ANOVA, and then a post hoc Tukey's range test, demonstrated that the mean log reductions are all significantly different from one another (ANOVA $p=0.0002$). A similar analysis for the antimicrobial non-alcohol-based hand sanitizers was not possible due to a limited number of observations.

III.5 Discussion

The published literature on the effectiveness of hand sanitizer contains conflicting claims, with some cases noting that it has only a limited effect (*134*, *207*, *220*) and in other case that it is highly effective (*55*, *124*, *130*). Figure III.6.b indicates the testing methodology may have some influence on claims of efficacy. The EN 1500 protocol shows a significantly greater ($p=9.5x10^{-9}$) log reduction than fingerpad or glove juice methods. An examination of the details of the EN 1500 protocol may provide insights into this effect (*63*). In the EN 1500 protocol, the hands are inoculated up to the meta-carpals, and allowed to dry. The pre-treatment concentration value is taken via dipping fingertips in a petri dish containing tryptic soy broth. The fingers are then allowed to dry, and the treatment is applied. The fingertips are then sampled again in the tryptic soy broth for the post treatment values. Since the fingers are not re-inoculated with the test microorganism, the protocol is actually measuring the combined effects of the first sampling and the treatment. This is in contrast to the glove juice method (*15*, *16*) where the hands are sampled for the pre-treatment concentration (baseline), and the hand is re-inoculated after the baseline sampling. Similarly, the fingerpad method (*11*) samples one of the fingers as the pre-treatment concentration, and uses the others for post treatment concentration. These protocol differences would not cause an issue when comparing different treatments obtained using the same testing protocol, but could lead to concerns when comparing between protocols. It should be noted that head-to-head comparisons between the fingerpad and glove juice methods found no statistical difference between the two (*8*), which is consistent with our findings.

The meta-analysis brings together 336 datasets from 28 publications in order to better understand the hand sanitizer literature. Alcohol based hand sanitizers had a significantly higher mean log reduction than antimicrobial sanitizers for experiments with bacteria ($p=0.005$), but not for viruses ($p=0.08$). Isopropanol was significantly more effective against bacteria ($p=0.02$) than ethanol but no difference in efficacy was seen against viruses ($p=0.74$). As we have observed previously, inoculum size or starting concentration appears to have a highly significant effect on measured log reduction (*175*, *176*), although in the case of our hand sanitizer analysis here this effect is only seen for bacteria and not viruses. Our analysis confirms reports in the literature that alcohol based hand sanitizers have limited effectiveness against viruses when compared to vegetative bacterial cells (*8*, *121*, *134*, *149*, *155*, *207*, *220*, *249*). Finally, and perhaps most significantly, the European Norm 1500 testing protocol (*63*) produces a significantly greater measured log reduction, likely due to the fact that it measures both the effect of the treatment as well as an additional effect due to sampling the hand to be treated before treatment. Our comparisons showed no significant difference between the finger pad and glove juice testing protocols for measuring hand sanitizer effectiveness. Although comparison between hand sanitizing agents within a testing protocol is likely valid, great care must be taken when comparing results between studies.

III.6 Tables and Figures

Table III.6.a: Summary of datasets by organism type.

	n	%
Total Included Data Sets	**336**	
Bacteria	**214**	**63.7%**
Transient	188	56.0%
Gram (-)	156	46.4%
Gram (+)	32	9.5%
Resident	26	7.7%
Virus	**114**	**33.9%**
Fungus	**8**	**2.4%**

Table III.6.b: Summary of treatments included in the analysis by active ingredient.

Treatment	Number of data sets	Percent of datasets
Alcohol	**286**	**85.1%**
Ethanol	145	43.2%
Isopropanol	116	34.5%
Mixed Alcohol	14	4.2%
Propanol	9	2.7%
Unspecified Alcohol	2	0.6%
Antimicrobial	**63**	**18.8%**
Chlorhexidine gluconate	28	8.3%
Iodine	8	2.4%
Benzalkonium chloride	3	0.9%
Phosphoric acid	3	0.9%
Hydrogen peroxide	3	0.9%
Sodium hypochlorite	1	0.3%
Chloroxylenol	1	0.3%
Unspecified antimicrobial	16	4.8%
Tap Water	**18**	**5.4%**
Dry Control	**5**	**1.5%**

Table III.6.c: Mean log reduction of alcohol by specific microorganisms, where n is the number of datasets from which the value was calculated from and "-" indicates no available data for analysis.

	Ethanol		Isopropanol		Propanol		Mixed alcohols	
Organism	**mean log reduction**	**n**	**mean log reduction**	**n**	**mean log reduction**	**n**	**mean log reduction**	**n**
Virus								
Adenovirus	3.25	3	-		-		-	
Poliovirus type 1	2.98	1	1.28	2	-		3.09	2
Rotavirus	2.82	8	1.85	7	-		-	
Murine Norovirus	2.51	4	-		-		-	
Norovirus	1.34	12	-		-		-	
Rhinovirus	1.33	1	-		-		-	
Hepatitis A virus	1.32	1	-		-		-	
Feline calcivirus	1.30	15	0.41	4	1.27	3	-	
Snow Mountain Virus	1.26	3	-		-		-	
MS2 bacteriophage	0.43	10	0.25	1	-		-	
Bacterium								
Pseudomonas aeruginosa	6.63	4	5.89	6	-		-	
Enterococcus faecalis	6.16	4	5.32	6	-		-	
Staphylococcus aureus	5.39	8	5.60	6	-		-	
Escherichia coli	3.45	44	4.24	74	4.43	2		
Serratia marcescens	2.54	15	-		-		-	
MRSA	2.05	2	-		-		-	
Natural Flora	1.26	3	2.12	9	2.27	4	1.50	8
Clostridium difficile	0.63	4	1.41	1	-		-	
Fungus								
Candida albicans	4.27	5	-		-		-	

Figures

Figure III.6.a: The influence of inoculum size (CFU or PFU) on measured mean log reduction for alcohol based hand sanitizers on viruses (●) or bacteria (○).

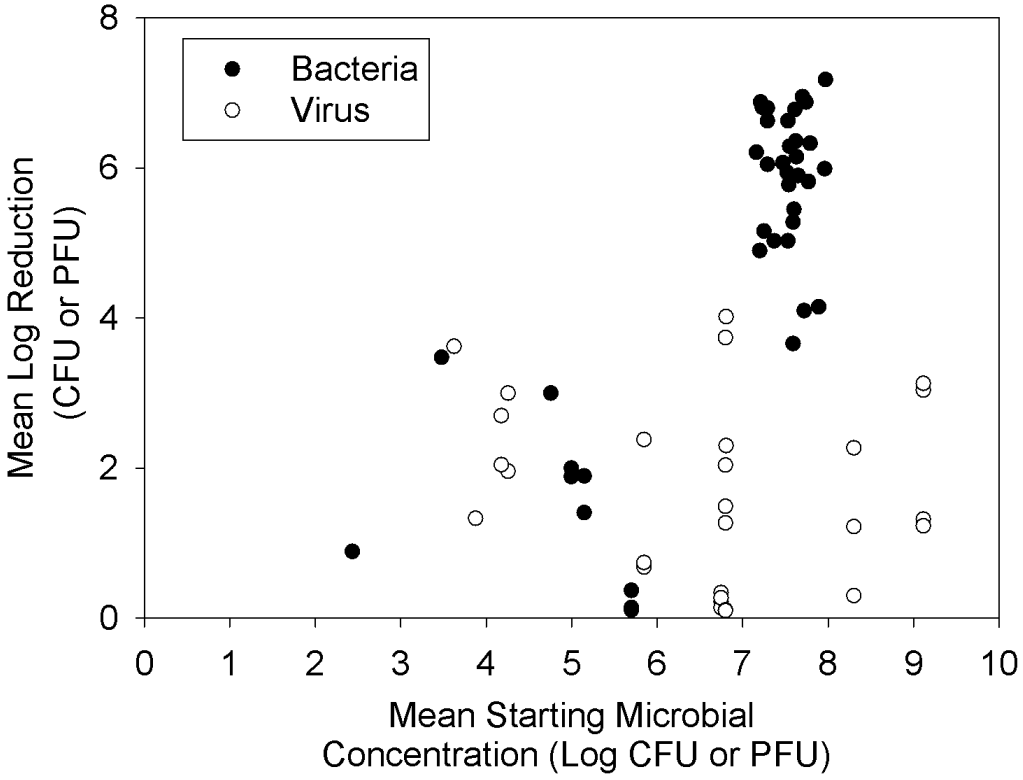


Figure III.6.b: Relative frequency of log reduction by testing protocols: European Norm 1500 (□), fingerpad (▨), and glove juice (■).

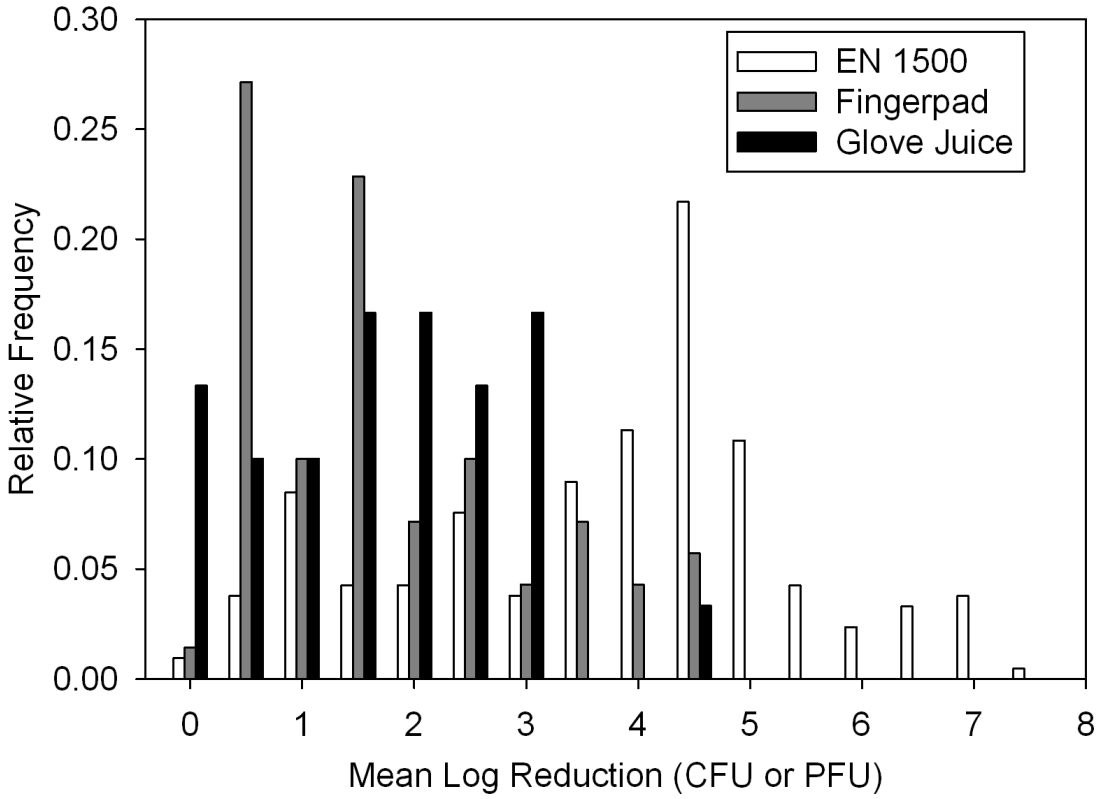


Figure III.6.c.i-ii: Relative frequency of log reduction by ethanol (■) or isopropanol based (□) hand sanitizer on viruses (i) or bactria (ii)
Figure III.6.c.i

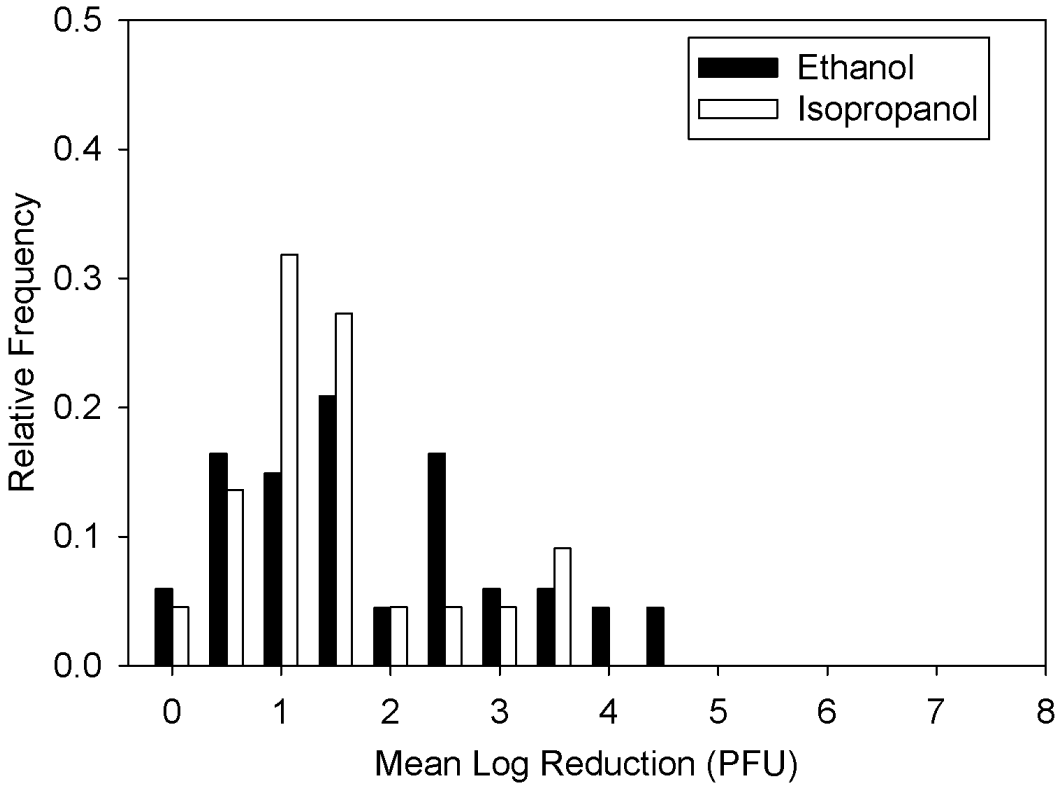


Figure III.6.c.ii

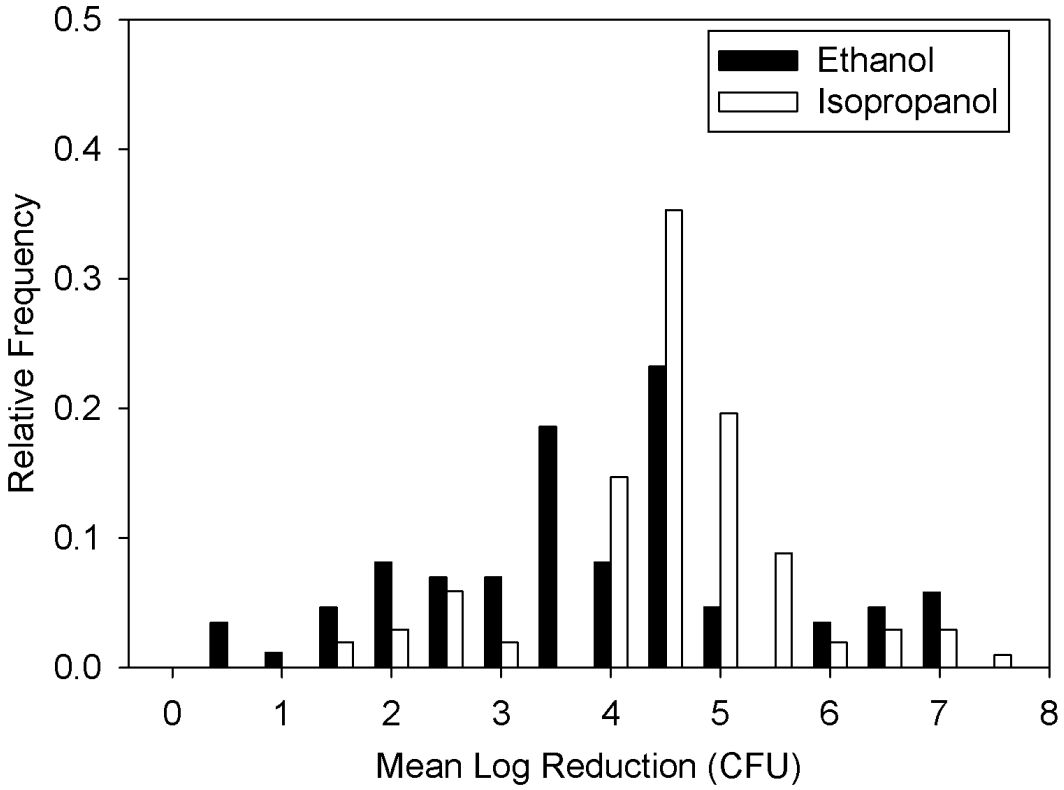


Figure III.6.d.i-ii: Relative frequency of log reduction by alcohol (■) or non-alcohol based (□) hand sanitizer on viruses (i) or bacteria (ii).
Figure III.6.d.i

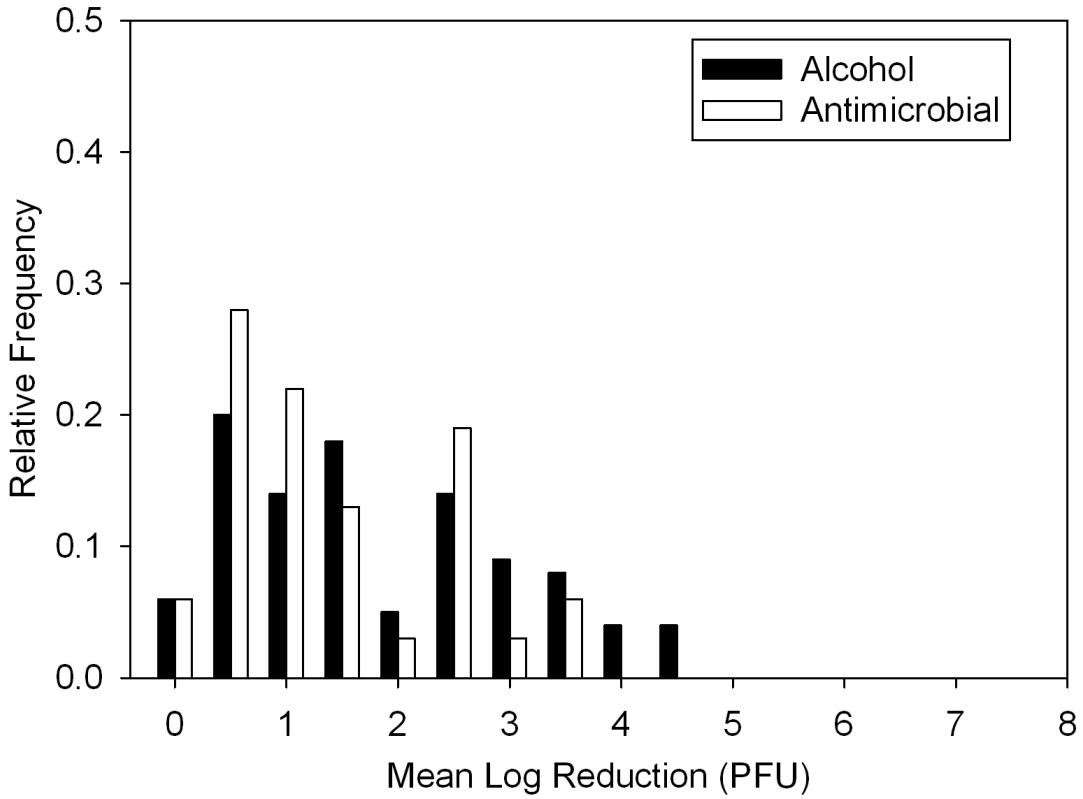


Figure III.6.d.ii

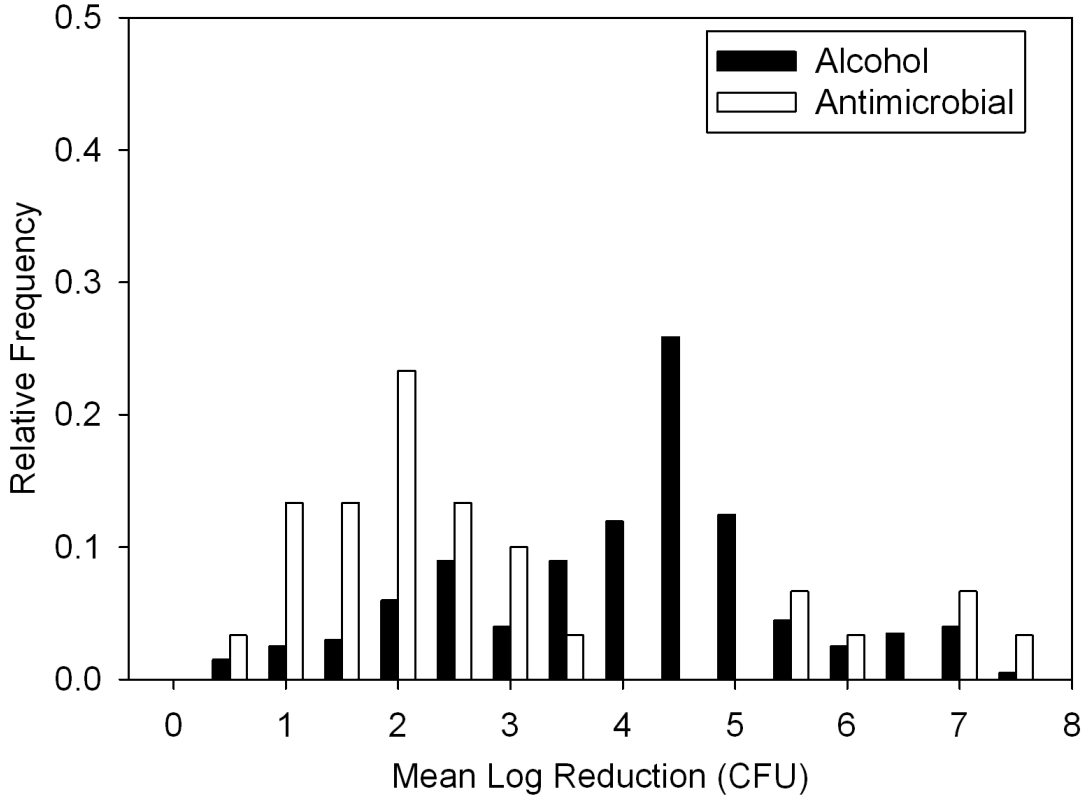
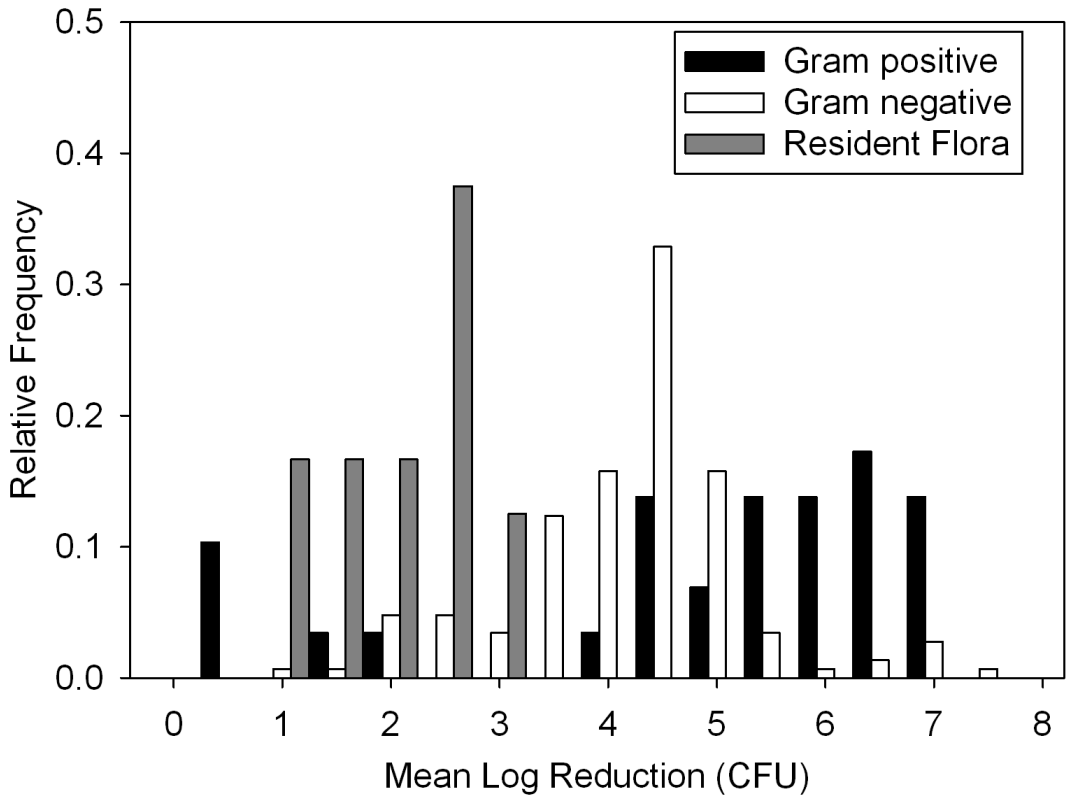


Figure III.6.e: Relative frequency of log reduction by alcohol based hand sanitizer on Gram-positive (■), Gram-negative (□) or resident bacteria (▨) on hands



Chapter IV – Quantifying the Effect of Handwash Duration, Soap Use, and Drying Methods on the Removal of *Enterobacter aerogenes* on Hands

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Key Words: Hand washing, Soap, Wash Duration, Paper Towel

IV.1 Abstract

This research was undertaken to establish the importance of several keys factors (soap, soil, time, and drying method) in reducing microorganisms during handwashing. A nonpathogenic nalidixic acid–resistant *Enterobacter aerogenes* surrogate for Salmonella was used to assess the efficacy of using soap or no soap for 5 or 20 s on hands with or without ground beef debris and drying with paper towel or air. Each experiment consisted of 20 replicates, each from a different individual with ~6 log CFU/ml *E. aerogenes* on their hands. A reduction of 1.0 ±0.4 and 1.7 ±0.8 log CFU of *E. aerogenes* was observed for a 5-s wash with no soap and a 20-s wash with soap, respectively ($p<0.05$). When there was no debris on the hands, there was no significant difference between washing with and without soap for 20 s ($p<0.05$). Likewise, there was no significant difference in the reductions achieved when washing without soap, whether or not debris was on the hands ($p<0.05$). A significantly greater reduction ($p>0.05$) *in E. aerogenes* (0.5 log CFU greater reduction) was observed with soap when there was ground beef debris on the hands. The greatest difference (1.1 log CFU greater average reduction) in effectiveness occurred when ground beef debris was on the hands and a 20-s wash with water was compared to a 20-s wash with soap. Significantly greater ($p>0.05$) reductions were observed with paper towel drying compared to air (0.5 log CFU greater reductions). Used paper towels may contain high bacterial levels (>4.0 log CFU per towel) when hands are highly contaminated. Our results support future quantitative microbial risk assessments needed to effectively manage risks of foodborne illness in which food workers' hands are a primary cause.

IV.2 Introduction

Handwashing is recognized as a crucial step in preventing foodborne disease transmission, by mitigating cross-contamination among hands, surfaces, and foods. It is considered a significant point of control for enteric pathogen transmission, especially for individuals who are shedding the pathogens asymptomatically (*70*, *93*, *94*, *101*, *144*, *174*, *200*, *229*). The FDA Model Food Code recommends washing hands at several occasions during food preparation (*240*). This includes, but is not limited to, before starting a food service task, in between handling ready-to-eat and non–ready-to-eat foods, after using the lavatory, and after handling soiled dishes or equipment. The FDA Model Food Code recommends washing hands for 20 s, under warm running water, with soap, and using either single-use towels or a forced air dryer to dry hands. Although the factors that influence handwashing effectiveness have been studied, these studies may not be comparable due to methodological differences (*52*) or statistical flaws (*54*, *100*, *169*). Evidence of the efficacy of air-drying versus paper towel drying is contradictory. Some studies show that air-drying is more effective (*7*), others show that towel drying is more effective (*199*, *231*), and some show no difference (*39*, *100*).

Microorganism concentration on a hand can vary from 10^2 to $10^6/cm^2$ depending on skin condition, whether or not the individual has recently handled raw foods, and frequency of handwashing (*119*, *182*). The resident microflora of skin consists mainly of gram-positive microorganisms, including coagulase-negative *Staphylococcus*, *Corynebacterium* spp., and anaerobes such as *Propionibacterium* (*119*). These resident organisms rarely cause foodborne illness. Unlike the resident

organisms, transient bacteria colonize the superficial layers of a hand (*36*, *119*, *212*). Transient bacteria are often transferred to and from hands by cross-contamination from touching or handling raw foods or dirty surfaces; these transient bacteria frequently cause foodborne illnesses as well as nosocomial infections in hospitals (*36*, *51*, *119*).

Guzewich and Ross studied 66 outbreaks that occurred in the United States between 1975 and 1998; they found that 82 % of these outbreaks implicated food workers as the source of contamination and that hands were the source of pathogen transmission in 34 (~50 %) (*101*). Compliance with handwashing guidelines varies depending on handwashing training, ease of access to washing facilities, and workload (*4*, *93*, *229*)

Published research indicates that handwashes lasting longer than 20 s have little additional benefit but that washes for less than 10 s may not efficiently remove soil (*169*, *183*). A quick wash (5 s) without soap has been commonly observed in busy environments (*32*, *93*, *197*, *229*).

Drying hands is regarded as a crucial step in handwashing because moist surfaces transfer bacteria more readily than dry surfaces (*89*, *113*, *166*, *188*, *208*, *231*) and drying is stipulated by the U.S. FDA Model Food Code (*240*). Paper towels dry hands quickly and can be used as a barrier to protect against recontamination from doorknobs and sink faucets (*188*, *199*, *200*) but they may not remove bacteria from palms and fingers as well as from fingertips (*253*). Recontamination of up to1 log CFU per hand is possible by transfer from a jammed paper towel dispenser (*103*).

This research was undertaken to establish the importance of several keys factors, using methods that are robust, sufficiently replicated, and statistically valid. This study provides a quantitative measurement of the effectiveness of a minimal 5-s wash and a longer FDA Model Food Code – compliant handwash (20 s) with and without food debris. The amount of bacteria removed by paper towels during hand drying was also quantified.

IV.3 Materials and methods

Bacterial strain and growth conditions. A nonpathogenic nalidixic acid–resistant *Enterobacter aerogenes* surrogate for *Salmonella* (*51*, *113*, *175*, *212*) was grown overnight at 37°C in tryptic soy broth containing 50 μg /ml nalidixic acid. Cells were harvested by centrifugation (Micro 12, Thermo Fisher Scientific, Waltham, MA) at 5,000 x g for 5 min and then were washed in phosphate- buffered saline (PBS; 0.1 M, pH 7.2). This process was repeated three times. The cell pellets were re-suspended in PBS to form a solution of ~8 log CFU/ml.

Inoculation solutions. The inoculation solution was created by serially diluting the harvested cells in PBS until a ~6 log CFU/ml solution was formed. For the soiled-hand inoculation solution, 5 ml of the ~6 log CFU/ml solution was added to 25 g of 80:20 ground beef purchased from a local supermarket in New Brunswick, NJ. The ground beef and bacteria solution were kneaded by gloved hands in a stainless steel bowl that had been sanitized with 60% ethanol. The resulting mixture was split into ~5 g samples using a top-loading balance (Ohaus Corporation, Parsippany, NJ).

Participants. Twenty volunteers were asked to participate in the handwashing experiments. Participants were rejected if any open cuts or wounds were present on

their hands, if they were ill or self-identified as immunocompromised, or if they were uncomfortable with any aspect of the experiment. Before each wash scenario, the participants were instructed to wash their hands with plain soap and dry them with paper towels. After the experiment, the volunteers were instructed to wash their hands, dry them with paper towels, and then apply hand sanitizer.

Quantification of *E. aerogenes* on hands. The glove juice method is a type of whole-hand measuring protocol that uses buffer inside a glove to recover the bacteria on a hand (*15*, *16*). The glove juice method has been used in previous studies to determine the bacterial concentration on volunteers' hands and has proven to be reproducible (*24*, *51*, *77*, *136*, *140*, *189*, *191*, *192*, *212*, *227*, *236*). Briefly, a nitrile glove (Fisherbrand powder-free nitrile examination gloves, Thermo Fisher Scientific) is filled with 20 ml of PBS. The loose-fitting glove is put over the volunteer's hand, and the hand is massaged for 1 min. The glove is carefully pulled off, and the buffer is collected in a vial. The resulting solution contains the bacteria that were removed from the hand.

Handwashing scenario protocols. Several handwashing scenarios were studied in this experiment. Each experiment consisted of 20 replicates, each from a different individual. Each of the 20 individuals participated in each scenario once. The participants were given very basic instructions on how to wash their hands, and, to reduce bias, only the time and drying method were communicated. With the exception of the 5 s wash, all volunteers were asked to wash their hands for 20 s with warm (18 to 35 °C) municipal tap water. Volunteers who used soap used 1 ml of unscented, plain liquid hand soap (Up and Up, Target Brand, Minneapolis, MN) to

wash their hands. Volunteers' hands were either air-dried, without the aid of a mechanical dryer, or were dried using autoclaved paper towels (White Multifold Towel, Oasis Brand Inc., Winchester, VA). Volunteers participated in no more than one handwash experiment per day.

The following scenarios were tested:

(i) Soap versus no soap on nonsoiled hands. Two 0.5 ml aliquots of the inoculation solution that contained ~6 log CFU/ml *E. aerogenes* was placed in each hand of volunteers, and volunteers evenly dispersed the inoculum by rubbing their hands together. The hands were allowed to air-dry until visibly dry (~60 s) before continuing. A volunteer's nondominant hand was sampled using the glove juice method to determine the bacterial concentration on that hand. This sample was used as the prewash bacterial concentration on the hands. After waiting for their nondominant hands to dry, the volunteers washed their hands once with plain soap, under running water for 20 s, and let their hands air-dry. Wash time was measured using a timer. After the hands dried, the microorganisms were recovered using the glove juice method described above for both hands. These samples were used for the postwash bacterial concentration on the hands. The same scenario was repeated with the same individual, without soap, on a different day.

(ii) Soap versus no soap use on soiled hands. The volunteers followed the same protocols as in the previous scenario, except that, to inoculate their hands, volunteers picked up and spread 5 g of 80:20 ground beef inoculated with ~6 log CFU/5 g of *E. aerogenes* over their hands and waited 30 s. The ground beef remained

visibly moist after it was spread on the hands. Scenarios with and without soup use were performed as above.

(iii) Paper towel versus air-drying. The volunteers' hands were inoculated with 1 ml of the inoculation solution (~6 log CFU/ml of *E. aerogenes*). After the volunteers' hands were visibly dry (~60 s), their nondominant hands were sampled using the glove juice method. This sample was used for prewash bacterial concentration on the hands. The volunteers then washed their hands, without soap, for 20 s under running water, and then dried their hands with paper towels. Volunteers were given one paper towel at a time to dry their hands until they felt that their hands were sufficiently dried. No volunteer used more than two paper towels. Each paper towel was collected and put in a Whirl-Pak 7 oz (207 ml) sterile filter bag with 25 ml of buffer. The paper towel and buffer were then homogenized using a stomacher (Dynatech Laboratories, Alexandria, VA). The homogenized samples were plated onto agar to determine the bacterial concentration on the paper towels. After the wash and the drying, both hands were sampled using the glove juice method. These samples were used for the postwash bacterial concentration on the hands.

(iv) Minimal (5 s) wash. The 5 s wash followed the same method as described above, except that the hands were only washed without soap and for 5 s. The effects of soap and debris were not studied for the 5 s wash.

Bacterial quantification and data analysis. After the washing scenarios were completed, the samples collected were serially diluted with PBS and were plated onto MacConkey agar (BBL, BD, Franklin Lakes, NJ) with 50 μg/ml nalidixic acid added. The plated samples were incubated overnight (18 to 24 h) at 37 °C. The CFU

were counted the next day to enumerate the bacterial concentration on the prewash hands, postwash hands, and on the paper towels used for drying. All counts were expressed as CFU per hand or per paper towel.

The prewash concentration was determined by taking the arithmetic count from the nondominant hand and multiplying by 2 (to estimate the concentration on both hands). The log reduction was determined by taking the difference between the logarithm of the estimated prewash concentration and the logarithm of the sum of the postwash concentration on both hands.

A frequency histogram of the data was assembled using Excel (Microsoft, Redmond WA) for each scenario. The frequencies for each wash scenario were plotted to visualize variability in log reduction rates and to compare the different washing scenarios. The frequency is the instance a particular volunteer(s) had a specific log reduction. A paired t-test using Excel was used to determine significant differences between samples. A P value less than 0.05 was considered significant. When more than two comparisons were being made, an analysis of variance and a Tukey's range test (MATLAB, Natick, MA) were used to determine whether multiple means were significantly different at a 0.05 level of significance.

IV.4 Results

Although washing hands for 20 s with soap is the recommended practice, studies that observed handwashing in normal practice suggest that most people wash hands for considerably less time (*32*, *93*, *197*, *229*). Figure IV.6.a shows a frequency diagram comparing minimal handwashing with Model Food Code handwashing, where the y-axis shows the frequency, or number of observations corresponding to

a given log reduction on the x-axis. Our results show a statistically significant difference ($p \sim 0.003$) between the reduction of the inoculated *E. aerogenes* that was achieved using the FDA Model Food Code recommended wash (20 s, with soap) and air-drying and that achieved using a minimal wash (5 s, no soap) with air-drying (Fig. IV.6.a). The recommended wash had an average reduction of 1.7 ±0.8 log CFU, and the minimal wash had an average reduction of 1.0 ± 0.4 log CFU. The greater variability in the 20-s wash time with soap is also apparent from Figure IV.6.a. Four separate washing regimes were compared in Figure IV.6.b: washing hands for 20 s, without soap and with no debris added to the hands; washing hands for 20 s, with soap and with no debris; washing hands for 20 s, without soap and with ground beef debris on the hands; and washing hands for 20 s, with soap and with ground beef debris. The reductions observed ranged from no observed reduction to ~4 log CFU reduction (Fig. IV.6.b). The least log reduction was seen when no soap was used with ground beef debris on the hands (1.1 ±0.6 log CFU reduction). The next greatest log reduction was seen when no soap was used and no debris was present on the hands (1.4 ± 0.4 log CFU reduction), followed by that seen when hands were washed with soap and without debris present (1.7 ±0.8 log CFU reduction). The greatest log reduction was observed when soap was used and debris was present on the hands (2.2 ± 0.5 log CFU reduction). There was only a slight difference in the effect of ground beef debris on the hands when soap was not used (0.3 log CFU difference in reduction), and this difference was not significant. Similarly, the effect of using soap when no debris was on the hands was slight (0.3 log CFU difference in reduction), and this difference was not significant. When the

two soap treatments (ground beef debris and no debris) were compared, the difference was significantly greater (0.5 log CFU difference in reduction; $p<0.01$), with the greater mean reduction observed when ground beef debris was present on the hand. Statistically significant differences were also observed between other treatments, with the greatest difference (1.1 log CFU difference in reduction) seen when ground beef was present on the hands; this was the case whether soap was used or not, although the greater reduction was seen when soap was used.

The effect of using paper towels to dry hands after washing or letting the hands air-dry (i.e., evaporation) on the frequency of log reductions of *E. aerogenes* achieved per wash is shown in Figure IV.6.c. Using a paper towel to dry hands resulted in a 1.9 ±0.9 CFU per wash reduction of *E. aerogenes*, which was a significantly ($P \sim 0.03$) greater reduction than that achieved with air-drying (1.4 ±0.4 CFU per wash reduction). The greater person-to-person variability seen when paper towels are used is apparent from the wide spread seen in the paper towel data in Figure IV.6.c as well as the standard deviations reported above.

Figure IV.6.d shows the amount of *E. aerogenes* (log CFU per towel) recovered on the first and second paper towels used by study participants to dry hands after the 20 s washing regime, without soap. One of the first 20 towels used was below the *E. aerogenes* detection limit (2.0 log CFU per paper towel). Five of the second group of towels used had bacterial concentrations below the detection limit, and three volunteers did not use a second paper towel. The mean log CFU per towel for the countable first paper towels used was 3.8 ±0.6 log CFU per paper towel and for the countable second paper towel used was 3.5 ± 0.6 log CFU per paper towel.

Our results manifest a greater variability in log reduction for a 20-s wash time with soap versus a 5 s wash time with no soap (Fig. IV.6.a). If there is person-to-person variability in handwashing technique and effectiveness, it logically follows that this effect is smaller when duration is shorter, but, as wash duration lengthens (and as soap is added), the variability will increase. We have previously observed less variability for an intervention with hand sanitizer versus handwashing (*212*), which may be because sanitizer effectiveness depends less on technique than handwashing effectiveness does. Clearly, more research on the possible causes of person-to-person handwashing variability is needed.

IV.5 Discussion

Our results show no significant difference between washing for 20 s with or without soap when no debris is present on the hands (Fig. IV.6.b). This is in contrast to a study by Coates *et al*., who examined the reduction of *Campylobacter* on the fingertips when using rinses with and without soap (*52*). These authors concluded that a wash with soap and water was more effective at removing *Campylobacter* than a wash with only water, but they did not do a statistical analysis or report standard deviations. Although our results show that the average log reductions are not significantly different whether or not soap is used when there is no debris on the hand, the 1.1 log CFU greater average effectiveness of soap was statistically significant ($p<0.05$) when ground beef was present. Although the reasons for this are unclear, we speculate that, because the individuals can see and feel the ground beef on their hands, they are more effective in their handwashing technique when trying to remove it. Soap adds to this effectiveness because of its surfactant

properties, allowing the insoluble ground beef particles to become soluble in water and then to be rinsed away (*76*).

Two separate studies, Michaels *et al.* (*169*) and Courtenay *et al. (54)*, used inoculated ground beef as debris and found log reductions of an inoculated surrogate similar to those in our study. Michaels *et al.* reported a 1.5 to 2.5 log CFU reduction of *Serratia marcescens*, with a 15 s wash using antimicrobial soap (*169*); Courtenay *et al*. observed a 2.7-log reduction (*54*). This is consistent with our study (Fig. IV.6.b), which shows an average log reduction of 2.2 log for a wash with soap and debris on the hand, with individual handwashing effectiveness varying from a low of a 1.1 log reduction to a high of 3.0 log reduction.

A study by Gustafson *et al.* used 99 volunteers and tested four different methods of drying hands and their effect on the microbial reduction of bacteria during a hand wash (*100*). They tested cloth towels from a rotary dispenser, paper towels in a stack, a forced air dryer, and air-drying (evaporation). Although these researchers indicated that there was no difference between the drying methods examined, they reported their data as differences in CFU rather than differences in log CFU. If the correct statistical transformation (logarithmic) is used on the reported data and a log reduction is calculated, it can be inferred that drying hands with paper towels provides a 0.5 log CFU greater reduction than evaporation (air-drying) or drying with warm air. Similarly, Coates *et al.* determined that *Campylobacter* is more readily removed from fingertips if a paper towel is included in the handwashing regime (*52*). These studies agree with our finding (Fig. IV.6.c) that using a paper

towel provides a statistically significant greater log reduction (1.9 ±0.9 log reduction) versus air-drying by evaporation (1.4 ±0.4 log reduction).

A minimal handwash (5 s, no soap) can reduce bacterial populations on the hands by 90%, but an FDA Model Food Code –compliant handwash (20 s, with soap) is significantly more effective. When hands are not contaminated by food debris, our results show that a 20 s hand wash is equally effective with or without soap. Soap is more effective when debris is present on the hands, likely because of the effect of the soap in removing debris, and perhaps by the sensory cues from the presence of ground beef. Paper towels appear to offer a measurably significant benefit (i.e., 0.5 log CFU greater reduction) when used after handwashing. Used paper towels may contain high bacterial levels when hands are highly contaminated. Our results, in conjunction with data on cross- contamination between hands and food (*51*) and data on the microbial contamination of foods and food worker hands, form the basis for future quantitative microbial risk assessments needed to effectively manage risks of foodborne illness in which food workers' hands are a primary cause.

Acknowledgement

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IV.6 Figures

Figure IV.6.a. Reduction of *Enterobacter aerogenes*, comparing a minimal hand wash (5 s wash, no soap;●) versus the USFDA style model food code wash (20 s wash, with soap; ○). In both scenarios the hands were air-dried.

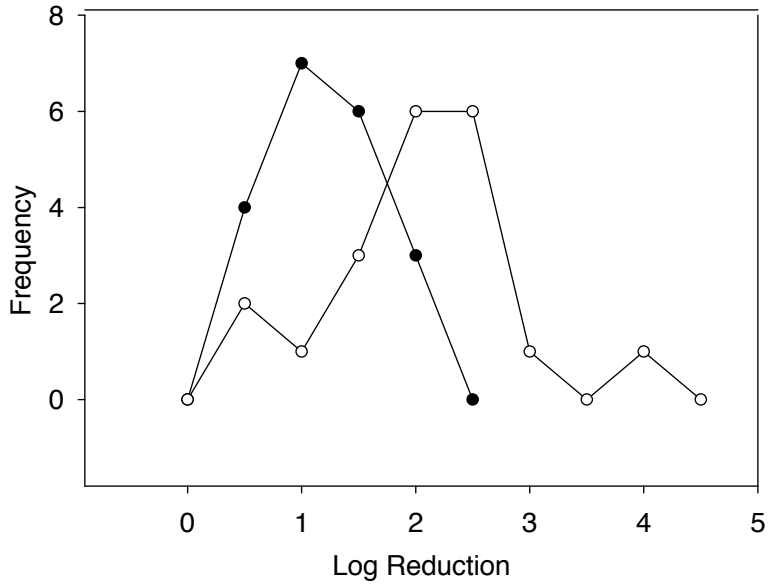


Figure IV.6.b. Reduction of *Enterobacter aerogenes*, comparing a hand wash without soap (solid) and with soap (open), and with debris (triangle), and without debris (circle). A 20 s wash without soap or debris, (△), 20 s wash with soap, and no debris (○), 20 s wash without soap, but with debris (▲), 20 s wash with soap, and debris (●)

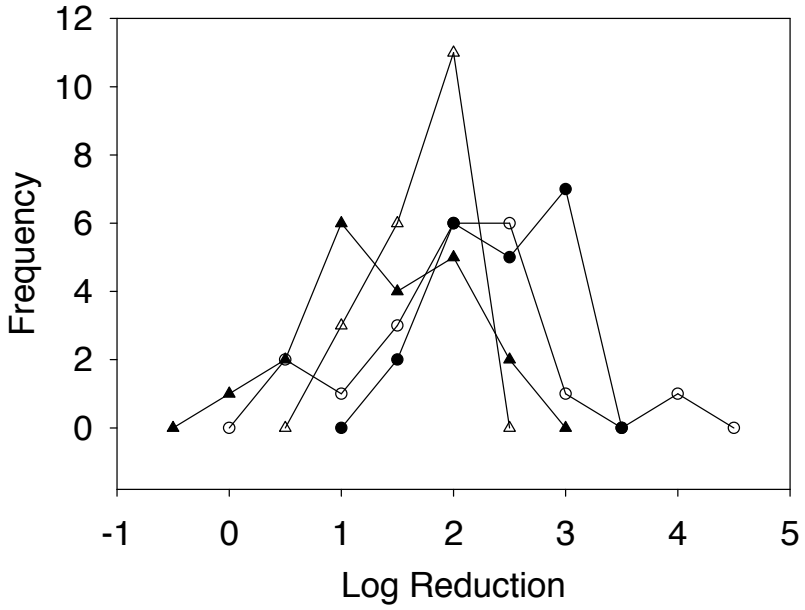


Figure IV.6.c. Reduction of *Enterobacter aerogenes*, comparing a hand wash in which a paper towel is used to dry hand afterwards (●), and a hand wash in which the hands are air-dried afterwards (○). Both hand washes were a 20 s wash, without soap.

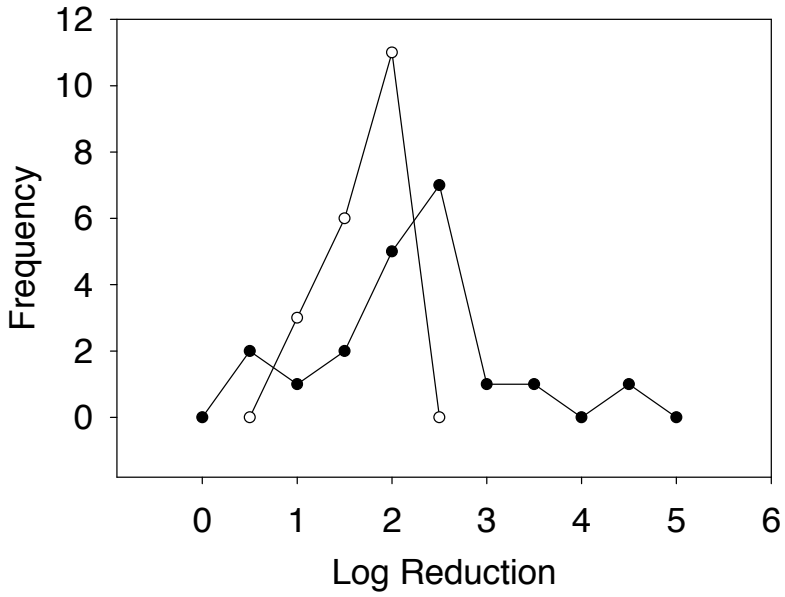
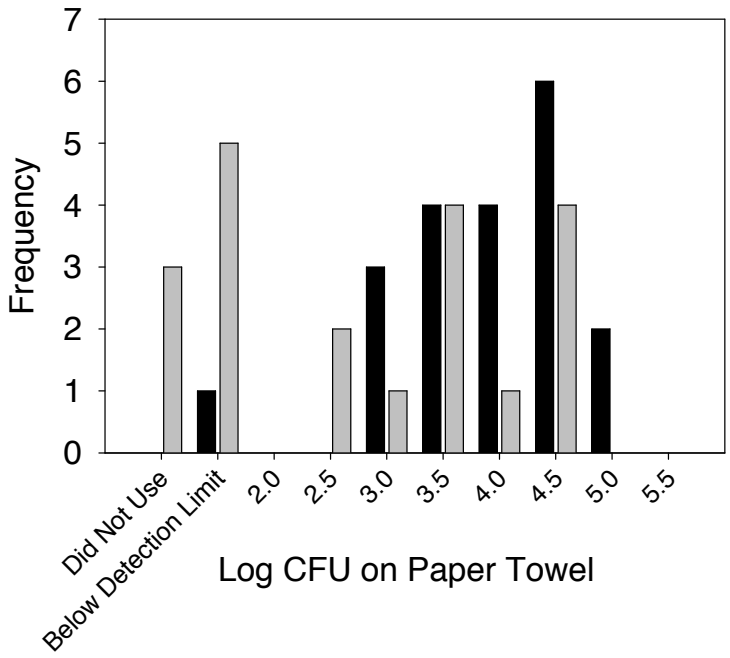


Figure IV.6.d. Recovery of *Enterobacter aerogenes* from the first (black) and second (gray) paper towels used during the wash in which the hands were dried with paper towels. In some cases a volunteer did not use a second a paper towel; no volunteers used more than two towels.



Chapter V 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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Key Words: Hand washing, Antimicrobial Soap, Water Temperature, Soap Volume

V.1 Abstract

The scientific support for many handwashing recommendations made in the FDA model food code is not evident or in disagreement. This research was done to address a few key variables that are thought to affect the efficacy of a hand wash. *Lather time, Soap Volume, Water Temperature, and Product Formulation* were tested using a fractional design study. A set of conditions (5 s lather, 38 °C (100 °F) water temperature, and 1 mL product volume) served as the baseline. A nonpathogenic strain of *Escherichia coli* ATCC 11229 was used as the model microorganism. Each condition had 20 replicates, and 20 volunteers (10 men, 10 women) were used for the study. The glove juice method was used to recover bacteria from the volunteer's hands. The results of this study demonstrated that a 1% chloroxylenol soap was not statistically different from a plain soap formulation at removing *Escherichia coli* ATCC 11229 under a variety of treatment conditions. Overall, antimicrobial soap had a mean 1.94 log CFU reduction, and ranged from 1.83 mean log reduction to 2.10 mean log reduction. Plain 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. Overall, the length lather time did not make a large difference, but a ~0.5 log greater reduction was observed for a 20 s plain soap vs a baseline wash. No statistical difference was observed for mean log reductions of men and women (men= 2.08 mean log reduction, women=2.08 mean log reduction, p=0.99). One of the key findings from this study is that there exists variability between people in microbial reduction from a handwash, and also hand wash behavior. Understanding what behaviors influence

hand washes the most may help future studies find which techniques can optimize the effectiveness of a hand wash.

V.2 Introduction

The FDA Model Food Code makes recommendations regarding handwashing frequency, duration and technique (*240*), however, the scientific support for many of those recommendations is not always evident. This project's goals were centered around designing experiments to address a few key variables in hand washing.

The FDA Food Code (section 2-301.12) requires the use of a cleaning agent (colloquially "soap") during a hand wash (*240*). The type of soap is not specified, and facilities may elect to use either bland or antimicrobial soap. Active compounds used in antimicrobial soaps aim to disrupt bacteria cell function or reproduction. Most are antiseptics, and are not antibiotics (*36*, *205*). They work by either being bactericidal (destroying the cell) or bacteriostatic (inhibit reproduction). The literature suggests that antimicrobial soaps provide a greater bacterial reduction than plain (or bland soap) by both inactivating and removing bacteria on hands (*72*, *77*, *99*, *176*, *211*, *220*). However some papers found minimal difference (*30*, *227*). A hand soap meta-analysis found that antimicrobial soaps, when accounting for all types of bacteria, tended to have a ~0.5 log CFU greater microbial reduction than bland soap (*176*). Product formulation plays a key role in effectiveness of antimicrobial agents and soaps, as many active compounds (antimicrobials) are available to use in soaps, and many surfactants used in soaps and lotions can inhibit (or enhance) these compounds (*27*, *75*, *232*).

The combined literature on soap volume shows no significant trends in terms of strong interactions between soap volume and effectiveness of soap (*77*, *140*, *176*).

The data can become confusing, and often conflicting if too many brands and formulations are compared to each other. Fuls *et al*. found a increasing antimicrobial soap volume increased their observed log reductions by ~0.7 log counts ($p<0.001$), but did not observe any significant increase in microbial reduction when using more plain soap ($p=0.2$) (*77*). Larson *et al.* (1987) found that a control wash with plain soap was not significantly affected by amounts of soap used (1mL vs 3 mL) (*140*). However, Larson *et al.* (1987) also suggested that greater volume of soap could contribute to increase skin damage after a wash, and suggested that the minimal amount of soap required for a thorough wash be measured in order to reduce skin damage (*140*).

The temperature of water required for an effective handwash is a variable that has been sparsely explored, and still generates interest. However 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 and, at extreme temperatures (>55°C), can lead to scalding (*139*, *228*). A handwashing survey determined that comfort of hands and personal beliefs played a key role when choosing the temperature for a handwash (*46*). Two studies by Michaels *et al.* found no difference, in terms of microbial reduction, of a hand wash performed at a range of temperatures (4.4 °C - 48.9 °C) (*168*, *169*). However, the two studies did not use a large pool of volunteers (4 subjects), and only one study (*169*) tested antibacterial soap. Courtenay *et al.* observed a minimal difference in microbial reduction between a cool rinse (26 °C) and a warm rinse (40 °C), but did not use soap as part of the treatments (*54*). Courtenay *et al.* did use a ServSafe recommended wash,

which uses soap, in conjunction with warm water, but did not do a ServSafe wash with cool water. A study that tested different 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 (*142*). While these studies would indicate that the temperature of a wash would have no effect, the limited repetitions of the studies (*54*, *168*, *169*), performing handwashes without soap (*54*), and the lack of an actual hand wash (*142*) indicated further need to study effect of temperature.

No manuscripts have been recovered that specifically measure lather time as a variable. A meta-analysis of the published handwashing literature suggested that more studies were needed to understand the importance of wash duration (*176*). However, many manuscripts have explored wash time and, and suggest greater wash times are correlated with greater microbial reduction (*72*, *77*, *112*, *150*, *183*). More research is needed to determine what part of the hand wash can be lengthened that will result in an increase in microbial reduction. Studies suggest that an extended wash, generally greater than 30 s, may result in a less effective wash (*137*, *169*, *176*). Some authors suggest that an extended wash (30 s+) may loosen, but not remove resident flora from hand, and these loosened microbes are now more easily transferred to other surfaces (*169*). Because of this, an extended wash may also appear less effective at removing organisms (*169*). Additionally, extended washes, alongside frequent washing, can lead to damaged skin (*10*, *76*, *85*, *125*, *129*, *132*, *195*, *213*, *221*, *247*, *252*, *258*), which is harder to wash and can promote colonization by more dangerous microbes (*137*, *140*, *141*). 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 greater amounts of water may play a key role in hand washing (*31*).

V.3 Materials and Methods

Experimental Design: Four variables (lather time, soap volume, water temperature, and product formulation) were tested 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 plain and antimicrobial soap, resulting in 320 total hand washes.

Lather time: Lather times of 5, 10, 20, 40 s were evaluated. When lathering, the volunteer did not have their hands under the running water. Lather time was defined as the amount of time a person 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 drying. For lathering directions, the volunteers were told to lather their hands how they felt most comfortable. No direction was given other than to "lather your hands for x seconds, away from the water".

Water Temperature: Water temperatures of 38 °C (100 °F), 26 °C (80 °F), 15 °C (60 °F) were used. The temperature of the water 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 determined to be at a constant temperature for at least 60 s. The highest temperature to be used (38 °C) was selected because the FDA Model 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 Model Food Code (*240*). The lowest temperature used (15 °C) was both deliverable by the existing plumbing, and judged by the authors to be the lower tolerable temperature for comfort.

Product Volume: Three volumes were measured (0.5 ml, 1.0 ml and 2.0 ml soap). An automatic soap dispenser (GOJO Industries, Inc., Akron, OH) was used that pumped out soap in 0.5 mL increments.

Product Formulation: Foaming soap was used for all experiments. Two formulations, bland and antibacterial were used. The bland soap was a commercially available soap, and the antimicrobial soap was test product (GOJO Industries, Inc., Akron, OH). The antimicrobial agent used was chloroxylenol at a 1% concentration. The soaps were identical in formulation except for the antimicrobial agent.

Bacteria Strain: A non-pathogenic strain of *Escherichia coli* (ATCC 11229) was used as the model organism for this experiment. Use of this strain is in accordance with current ASTM International handwashing protocols (*12*, *14*, *86*). This strain serves as a surrogate for transient bacteria transferred to hands during handling of raw foods. Culture methods were used as indicated in ASTM protocols (*12*, *14*, *86*), but briefly, a homogenous culture was used to inoculate the hands. The *E. coli* was

cultured in 5 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). This process was repeated three times. The cell pellets were re-suspended in PBS to form a solution of ~8 log CFU/mL. This final solution was used to inoculate hands.

Volunteers: Twenty-one volunteers were selected from the Rutgers University and related communities. The volunteers were asked to refrain from using antimicrobial hand soap and non-alcohol based hand sanitizers products for the duration of the study to avoid antimicrobial build-up in the skin, which could have interfered with the results (*6*, *24*, *77*, *180*, *190*, *219*). A volunteer was dismissed from the experiment if they were uncomfortable with the experiment, had cuts or abrasions on their hands, or appear to be or self-identified as immuno-compromised. Rutgers IRB approval had been given for this study. One volunteer 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). No volunteer had taken antibiotics or were self-described as ill during the previous six weeks before starting the study, nor during the study.
Questionnaire: The volunteers were asked to fill out a questionnaire. The questionnaire was used by the researchers to account for extraneous variables that would affect skin quality and skin bacterial profiles. The questionnaire was given before the volunteers began participation in the experiments.

Sampling procedure: The glove juice method (*15*, *16*) was used to sample bacteria on the volunteers hands. It has been used in previous studies and has proven to be reproducible (*24*, *51*, *77*, *136*, *140*, *189*, *191*, *192*, *212*, *227*, *236*). A nitrile glove (Fisherbrand Powder-free Nitrile Examination Gloves, Thermo Fisher Scientific, Waltham, MA), filled with 20 mL of phosphate buffer saline, was placed over the volunteer's hand. The hand was massaged for ~ 60 s to dislodge the bacteria on 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). The amount of buffer recovered was noted and used in the final calculations of bacterial concentration. An appropriate neutralizer, tween 80 (10%), was used in the sampling buffers for the antimicrobial soap experiments (*13*). The evaluation of the antimicrobial inactivator effectiveness was tested using ASTM method designated E 1054-08, section 9 (Neutralization Assay with Recovery in Liquid Medium) (*13*).
Plating and dilutions: Samples were plated onto BBL™ MacConkey agar and the colonies forming units were counted to enumerate bacterial concentrations in the samples. Phosphate buffer saline (pH 7.2 ±0.1) was used for serial dilutions, and contained an appropriate antimicrobial quenching agent if necessary. The media contained MUG (4-Methylumbelliferyl-β-D-glucuronide) (Sigma-Aldrich Corporation, Saint Louis, MO) to help identify *E. coli* without affecting colony morphology or viability *(172)*.

Handwashing area: A standard sink was used for the experiment. Before and after each experiment, the sink was sanitized with 70 % ethanol. The sink was free of

debris, and municipal tap water was used. The water was turned on before the experiment, and remained on for the duration of the experiment.

Experimental variables: There were 16 unique sets of handwashes in each dataset, and each volunteer performed one handwash for each dataset. The target variables to be tested were randomly selected for each experiment.

Prewash procedure: Volunteers were asked to wash their hands before beginning the experiment. No direction was given on how to wash their hands. The researcher simply asked the volunteer to wash their hands. A researcher discretely recorded the amount of soap used, lather time, rinse time, and total wash time. The volunteers were handed paper towels to dry their hands.

Inoculation of hands: *E. coli* ATCC 11229 were distributed over the volunteer's hands by adding 1 mL of the inoculation solution (~8 log CFU/mL) and having the volunteer rub their hands evenly to coat them. The volunteers were asked to rub their hand parallel to the floor so as to avoid unnecessary contamination of the forearms or elbows. This differs from the ASTM (*14*) in which 3 series of 1.5 mL is used. The hands were allowed to dry until the hands 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.

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 when to wet their hands, and to rinse for 10 seconds after the lathering is complete. The volunteers did not dry their hands.

This was done to avoid adding complications of the data due to drying (*52*, *100*, *103*, *112*, *253*).

Post wash sampling: The hands were sampled immediately after the wash (<5 s). Both hands were sampled using the glove juice method (*15*, *16*). These samples were used to the post-wash bacterial concentration.

Post-experiment decontamination protocol: Volunteers washed their hands under running water for 20 s using plain soap, and dried their hands with a paper towel. Alcohol based hand sanitizer (Purell, GOJO Industries, Inc., Akron, OH) was used after the hand wash and drying.

Data Analysis: Microbial reduction data gathered from the experiment were log transformed to normally distribute the data (*209*). 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 frequency histogram of the data was assembled using Excel (Microsoft, Redmond Washington) for each scenario. The frequencies for each wash scenario were plotted to visualize variability in log reduction rates and compare the different washing scenarios. The frequency is the instance a particular volunteer(s) had a specific log reduction. The p-values were calculated using Excel to determine significant differences between samples. A p-value less than 0.05 was considered significant. When more than two comparisons where being made, an ANOVA and a Tukey's range test (MATLAB, Natick, Massachusetts) were used to determine if

multiple means were significantly different and if any significant interactions existed between the variables.

V.3 Results

Table V.6.a shows the mean log reductions for the treatment conditions tested, as well as the average antimicrobial soap mean log reduction and the average plain soap mean log reduction. Overall, antimicrobial soap 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). Plain 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).

An ANOVA analysis, that tested *Lather time, Water Temperature, Soap Volume, Soap Formulation, and Volunteer* as independent variables, revealed statistically significant differences ($p<0.05$) for both the *Soap Formulation* ($p=0.0003$) and *Volunteer* ($p=0.0002$) variables. However, the authors want to point out that the difference observed between antimicrobial and plain soap mean log reductions (Table V.6.a) is ~0.3 log CFU and can be considered within the range of error for microbiology data (clinically insignificant). A post-hoc HSD on the individuals volunteer's mean log reduction data revealed statistically significant 0.5 log CFU or greater mean log reductions. This suggests a large part of the variability observed in the data sets were due to variability amongst the volunteers.

A second ANOVA was done on the just results for the plain soap data. Significant differences were observed for *Lather time* ($p=0.01$) and *Volunteer* ($p=0.0002$), and while not statistically significant, *Water Temperature* did have a $p=0.08$. 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.

A third ANOVA was done for just the results for the antimicrobial soap data. The only significant difference observed by the ANOVA was the differences between the *Volunteer* variable ($p=3.03x10^{-13}$). Interestingly, within the antimicrobial soap data, the p values from the ANOVA were higher for *Lather time* and *Water Temperature* in these groups (Lather p=0.86, Temperature p=0.98, Volume p=0.21), than in the plain soap data (Lather p=0.01, Temperature p=0.08, Volume p=0.23).

Questionnaire Results

No differences were observed for volunteers that did/did not use acne medication (p=0.14) or facial cleanser (p=0.62). No difference was observed between the age groups ($R^2=0.009$, p=0.09).

Lotion Use: While statistically significant (p=0.02), the difference in microbial reduction between volunteers that used lotion, and those that did not was not clinically relevant (~0.2 log cfu difference, High lotion use=2.15 mean log reduction, low lotion use=1.95 mean log reduction).

Hand washing frequency: There were 16 volunteers that indicated a high frequency of hand washing (>4 times per day), and 4 volunteers indicated low hand washing frequency (<4 times per day). The two groups did differ by mean total wash time (p=0.01, HF= 18.2 s, LF=15 s), but closer analysis revealed that the difference was in lather time, and not rinse time. There was not a significant difference between mean rinse times (p=0.71, HF= 11.4 s, LF=11.0s), but there was a difference in mean lather time (p=0.00002), HF=6.8s, LF=4 s). The four lower

frequency hand washers did show a statistically significant higher microbial reduction than the high frequency hand washers (p=0.00001, HF= 2.01 mean log reduction, LF= 2.37 mean log reduction), but the four low-frequency hand washers also all reported the highest usage of lotion use (more than twice a day), which was previously shown in this analysis to marginally improve the microbial reduction of the hand wash.

Men vs Women: No statistical difference was observed for mean log reductions of men and women (men= 2.08 mean log reduction, women=2.08 mean log reduction, p=0.99). The value did not change for either antimicrobial or plain soap data. However, there was almost a ~0.5 mean log CFU reduction difference (p=0.0003) between men that did use lotion (2.34 mean log CFU reduction) and 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.

Pre-wash Data: The mean recorded lather time was 6.2 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= 5.3 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). While having a low p-value (p=0.01), there was a minimal correlation between

length of lather time and rinse time (R^2=0.03). The average volume of soaps used was 0.6 mL of soap (SD=0.25, ~1 pump of soap), with both men and women averaging 0.6 mL of soap. While no statistical difference was observed between men and women for volume of soap used (p=0.39), an ANOVA revealed that there was a significant difference in volume of soap used between all the volunteers ($p=1.4x10^{-7}$), 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 percentages did not 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). Roughly 43.4% of volunteers used water before soap, and 56.6% of volunteers used soap before 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.

V.4 Discussion

Lather time- (length of wash). It was somewhat surprising that only a 30 s wash (20 s lather time, 10 s rinse) with plain soap provided a statistically different mean log reduction from the baseline. Several studies have suggested that a longer wash time will provide a greater microbial reduction benefit (*72*, *77*, *112*, *150*, *183*). 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 a 120 s washes had a lower log reduction than a 30 s wash (*176*). Suggesting wash time greater than 30 s may not be more effective. This may explain why we did not see a greater microbial reduction after 10-20 s lather time, because the total wash time was above 30 s.

Water Temperature. Our study found no significant difference in washes done at different temperature ranges, which agrees with the findings Michaels *et al.* 2001 and Michaels *et al.* 2002 studies. They tested a wider rage of temperatures (4.4 °C - 48.9 °C), than we did (15 °C -38 °C), and found ~2-2.5 mean log reductions, which was similar to our range of 1.9-2.3 mean log reductions. Unlike Michaels *et al.,* we did not find a non-statistically significant trend of increasing microbial reduction with increasing temperature for plain soap (p=0.08) or antimicrobial soap (p=0.99). Courtenay *et al.* did observe a small difference in microbial reduction between a cool rinse (26 °C) and a warm rinse (40 °C), but the fact that they did not use soap as part of the treatment suggest that soap used during a hand wash may negate any slight microbial reduction benefits due to using warmer water (*54*). However it is worth noting that Courtenay *et al.* did use volunteers whose hands were covered inoculated ground beef, and the saturated fats in the ground beef may be more easily removed with warmer water temperatures.

Volume of Soap. We did not observe a statistically significant difference with any volume of soap used (p=0.48 plain soap, p=0.41 antimicrobial soap). Both Fuls *et al.* (*77*) and Larson *et al.* (*140*) did not observe any significant increase in microbial reduction when using more plain/bland soap. Unlike in our findings, Fuls *et al*. an Larson *et al.* did find that increasing antimicrobial soap volume increased their

observed log reductions. Both authors suggest increased exposure to more antimicrobial agent as the explanation for increased microbial reduction. The difference in observed 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 (*27*, *232*). 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.

Antibacterial and Bland Soaps. Several studies have shown greater microbial reductions when using antimicrobial soaps than when using bland soaps (*72*, *77*, *99*, *211*, *220*). Many studies have showed that effectiveness of antimicrobial soaps increased with repeated use by building up the antimicrobial in the skin (*6*, *24*, *77*, *180*, *190*). This affect can also be seen in hand sanitizers based on antimicrobials other than alcohol (*219*). Future work with the antimicrobial soap used in this study should take into consideration the need for buildup in the skin to improve efficacy. The meta-analysis of hand soaps by Schaffner and Montville suggested that overall, accounting for all types of bacteria, antimicrobial soap (mean 1.91) should have a ~0.5 log greater reduction than plain soap (mean 2.4)(*176*). We did not see a grater difference, but the plain soap data and the antimicrobial soap data both fell within this range of mean log reductions (*176*), and were not tested using repeated applications.

Other Observations. Much like we did, Larson *et al.* also recorded the mean amount (mL) of soap used by *healthcare* workers (*140*). They observed that healthcare 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. Our volunteers (who were not attending to patients) used an average of 0.6 mL. 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, much like us, Larson *et al.* did observe significant differences between the amounts of soap the individual volunteers used, and also observed that a volunteer would routinely use the same volume of soap.

V.5 Conclusions

Our study has demonstrated that a 1% chloroxylenol soap is not statistically different from a plain soap formulation at removing *Escherichia coli* ATCC 11229 under a variety of treatment conditions. The study has also shown that water temperature is not a critical factor for the removal of transient organisms, however factors such as skin damage and skin comfort should be taken into consideration to prevent damage by hot water (*139*, *228*). Overall, the length lather time and volume of soap used did not make a large difference. One of the key findings from this study is that there exists a variability between people not only in observed microbial reduction, but also hand wash behavior. Understanding what behaviors influence hand washes the most may help future studies find which techniques can optimize the effectiveness of a hand wash.

V.6 Tables

Table V.6.a: Mean log reductions, median log reduction, and range of log reductions observed in the various treatments

Variable	Formulation	Mean log CFU reduction	Standard deviation	Median	Maximum	Minimum	Range
All	Antimicrobial	1.94	0.78	1.92	4.42	0.06	4.36
	Plain	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
	Plain	1.91	0.64	1.76	2.99	0.82	2.17
Lather 10s	Antimicrobial	2.03	0.64	2.00	3.30	0.89	2.41
	Plain	2.16	0.74	2.22	3.60	1.03	2.58
Lather 20s	Antimicrobial	1.95	1.00	1.82	4.39	0.35	4.03
	Plain	2.54	0.62	2.48	3.75	1.63	2.12
Lather 40s	Antimicrobial	1.91	0.98	2.00	3.47	0.13	3.34
	Plain	2.43	0.71	2.25	4.09	1.57	2.52
Temp 60 F	Antimicrobial	1.88	0.62	1.91	3.34	0.76	2.57
	Plain	2.34	0.54	2.33	3.22	1.08	2.15
Temp 80F	Antimicrobial	1.90	0.89	1.77	4.42	0.28	4.14
	Plain	1.98	0.71	1.99	3.07	0.80	2.27
Vol 0.5 mL	Antimicrobial	2.10	0.77	2.18	3.24	0.06	3.18
	Plain	2.25	0.86	2.25	4.03	-0.04	4.07
Vol 2.0 mL	Antimicrobial	1.83	0.65	1.81	3.34	0.64	2.69
	Plain	2.15	0.93	1.97	4.40	0.70	3.70

Chapter VI: Does the Choice of Surfactant Concentration and Type Affect *Escherichia coli* Removal from Pig Skin During a Simulated Handwash?

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VI.1 Significance and Impact of Study

This study aims to understand how surfactants play a role in removing microbes during a handwash. While numerous studies exist that look at surfactants in soaps, these studies are primarily focused on how these surfactants support antimicrobials in the soap, or how specific surfactants irritate skin. No study currently exists to show which surfactant is best for removing microbes. This study uses pigskin as a model for human skin and a lathering device to simulate a hand wash. Using this method allowed for testing of surfactants at high concentrations (10%), without risk of irritating a human subject's hand.

VI.2 Abstract

While a few studies have examined the role of surfactants in antimicrobial soaps, the effect of surfactant type and concentration on a bland soap formulations ability to remove bacteria from hands remains largely unstudied. Several combinations of surfactants and water were combined to test bacterial removal efficacy using a simulated handwashing device. The device consisted of two plates which were mechanically rubbed together using a simple rotational motor, and pigskin could be attached to these plates. A nalidixic acid resistant mutant of a non-pathogenic strain of *Escherichia coli* (ATCC 11229) was used. Four surfactants were selected for study: two anionic surfactants, sodium lauryl sulfate and sodium stearoyl lactylate, and two nonionic surfactants, poloxamer 407 and sorbitan monostearate, each in concentrations of 2%, 5%, and 10%. A slight positive correlation (R^2=0.17, p=0.03) was observed between hydrophilic-lipophilic balance value (HLB, the degree to which the surfactants are hydrophilic or lipophilic) and mean log reduction. No correlation was observed between pH of the treatment solution and mean log reduction (R^2=0.05, p=0.25). A 10% sodium lauryl sulfate mixture showed the highest log reduction ($\bar{x}$= 1.1 log cfu reduction, SD=0.54), and was the only treatment that was significantly different from washing with only water (p=0.0005). There was a strong correlation between increasing surfactant concentrations above the critical micelle concentration, and mean microbial reduction (R^2=0.62, p=0.001).

VI.3 Introduction

Soap, in its basic form, is an alkali salt of a fatty acid (carboxylic acids) that has a hydrophobic hydrocarbon tail and a hydrophilic carboxylate head group (*147*, *224*, *225*). These fatty acids can be saturated or unsaturated, and derived from natural or synthetic sources. The fatty acid salts are added to water to create the final product (soap). However, additional objects, such as fillers, dyes, or scents, can also be added (*20*). The mechanism of action for the surfactants in hand soaps is to facilitate the formation of micelles which can surround oily debris, either human oils or foreign soil, in order to allow the debris to be more readily solubilized in water and be removed during a wash (*10*, *20*, *76*, *85*, *125*, *195*, *221*, *252*, *258*).

Proper hand hygiene is an important intervention to prevent pathogen transmission (*2*, *42*, *70*, *93*, *94*, *101*, *112*, *144*, *174*, *200*, *229*), and washing hands with soap produces a better microbial reduction than washing hands with just water (*42*, *52*, *112*), especially if debris is present on hands (*112*). Many studies focus on antimicrobial soap formulation, and how the additional of an antimicrobial to soap can provide a greater bacterial reduction than plain or bland soap (*72*, *77*, *99*, *176*, *211*, *220*). Very few studies have examined what types of surfactants and what surfactant concentrations are key for optimal effectiveness in removing microbes from hands, with only a limited number of studies examining the link between antimicrobial effectiveness and surfactant concentration (*27*, *232*).

The choice of which surfactant to use in a soap is not always directly connected to microbial removal, and often skin health and sensory aspects also play a role (*10*, *20*, *76*, *85*, *125*, *132*, *221*). Surfactant skin penetration is dependent on exposure time, temperature, and surfactant head group (*156*), and repeated use of surfactant

solutions (soap) to wash hands can lead to damage to the stratum corneum, which often will manifest in dry, cracked, and red skin (*10*, *76*, *85*, *125*, *132*, *195*, *221*, *252*, *258*). Anionic surfactants often cause the most significant damage to skin, with the surfactant sodium lauryl sulfate reportedly causing the most irritation (*10*, *85*, *116*, *117*, *125*, *195*). Irritation can be measured by water loss (*243*, *245*, *247*), skin tightness (*125*), measuring lipid removal (*76*), and skin capacitance (*252*). Anionic surfactants may irritate the skin because they cause more of the stratum corneum to be removed during a hand wash, while cationic surfactants are gentler on the hands during a hand wash, but are less effective at removing the stratum corneum (*83*, *109*, *125*, *126*, *132*, *215*). An argument could be made that since transient microflora reside in superficial layers of skin (*36*, *119*, *151*), a surfactant that is better at removing this layer would provide a greater microbial reduction.

Sodium lauryl sulfate can be found in more than 75% of personal hygiene products (soap, toothpaste, shampoo, etc.), and is used in 70% of commercially available soaps (Table VII.1). Sodium lauryl sulfate is used because of its high HLB value, or the degree to which the surfactant is hydrophilic or lipophilic, which allows it to readily dissolve in aqueous solutions (*193*), and remove lipids and oily debris from hands easily during a wash (*125*, *126*).

VI.4 Methods and Materials

Handwashing efficacy varies from person to person due to differences in skin, skin care, technique, etc. so pigskin was used as a model system. Several studies have used pigskin as a suitable substrate to test topical surfactant and antimicrobial efficacy (*22*, *27*, *43*, *44*, *56*, *79*, *81*, *120*, *161*). The pig skin *stratum corneum* is >90%

cermaide, cholesterol, and fatty acids, and shows no changes with each layer of strateum corneum removed, much like human skin (*91*). Details on preparation of the pig skin test substrate are available elsewhere (*27*, *43*). Briefly, pigskin hides were obtained from a local butcher in New Brunswick, NJ, and washed with tap water, placed inside plastic bags and frozen for later use. The skin was homogenized, and antimicrobial activity was tested using ASTM method designated E 1054-08, section 9 (Neutralization Assay with Recovery in Liquid Medium) (*13*). This was done to ensure no antibiotic was present in the pig skin which could have affected the microbial recovery and survival on the pig skin. Briefly, a pigskin free tryptic soy broth (Remel, Thermo Fisher Scientific, Waltham, MA), and tryptic soy broth with homogenized pigskin was tested to ensure that there was no significant difference in growth between the two samples.

Preparation of pigskin substrate samples: Frozen pigskin was thawed and defatted with a sterilized knife. The pigskin was cut into 3x3 cm and 8x3 cm sections. Pigskin pH was measured using an Accument flat surface pH probe (Fisher Scientific, Waltham, MA). Pigskin had a mean pH of 6.9, and for reference human skin has a mean pH between 4-6 (*61*, *201*), and cadaver skin has a mean pH of 6 (*17*, *257*, *260*).

Bacteria Strain: A nalidixic acid resistant mutant of the non-pathogenic strain of *Escherichia coli* (ATCC 11229) commonly used in hand washing experiments was used (*12*, *14*). *Escherichia coli* has been shown to be able to survive for several hours on pigskin (*43*). The *E. coli* strain was made resistant to 50 µg nalidixic acid/g nalidixic acid by stepwise exposure (*187*). Nalidixic acid resistance facilitates

recovery of the *E. coli* amidst the natural pigskin microbiota. Culture of *E. coli* was as indicated in ASTM protocols (*12*, *14*). Briefly, *E. coli* was cultured in 5 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 was washed in phosphate buffer saline (PBS; 0.1M, pH 7.2). The centrifugation and washing process was repeated three times. The cell pellets were re-suspended in phosphate buffer saline to form a solution of ~8 log CFU/mL used to inoculate the pigskin.

Surfactants: Surfactant used in study are all commonly used in hand soap, shampoo/conditioner, body wash, body soap, lotion, or toothpaste (Table VII.1). Two anionic surfactants, sodium lauryl sulfate (Sigma-Aldrich, St. Louis, MO) and sodium stearoyl lactylate (Spectrum Chemical MFG Corp, New Brunswick, NJ), and two nonionic surfactants, poloxamer 407 (Sigma-Aldrich, St. Louis, MO), and sorbitan monostearate (Span 60) (Sigma-Aldrich, St. Louis, MO) were each used in concentrations of 2%, 5%, and 10%. The solutions were prepared by combining appropriate amounts of the surfactant and distilled water as follows. Water was boiled in a covered glass beaker for 5 minutes to thermally inactivate any vegetative bacteria cells, and then allowed to cool to ~25 °C. With the exception of sorbitan monostearate (see below), a given surfactant at the required concentration was added, the cover replaced on the beaker, and the solution was mixed for another 5 minutes. Sorbitan monostearate was not miscible in room temperature water, and was dispersed by warming the water to ~70 °C while mixing (*251*). The solutions of

sorbitan monostearate solutions stayed dispersed for the duration of the experiment (~ 1 hr). The critical micelle concentration of surfactants in water (% solution) was determined via the pendant drop method using a goniometer (Ramé-hart, Succasunna, NJ) and surface tension software (KSV Surface Tension Software, Biolin Scientific, Stockholm, Sweden) at room temperature (~25 °C) (*178*), and the calculated critical micelle concentration values were verified using the published literature when possible.

Lathering device: A mechanical lathering device, fabricated by Rutgers Food Science facilities staff, was used to simulate handwashing under controlled pressure and shear stress. The device consists of two horizontal stainless steel metal plates, where the bottom plate (2.5x3 cm) can be moved back and forth (18 RPM) by a simple rotational motor, and the top plate (5x3 cm) remained fixed. The two plates are pressed together by the force of gravity and a 500g weight. Each plate has four spikes to aid in the attachment of the pigskin.

Inoculation of test substrate: The bottom pigskin piece was inoculated using a pipette with 1mL of ~7 log CFU/mL solution of *E. coli*, and rubbed against the other pigskin piece for 30 s evenly distribute the *E. coli*. The pigskin was allowed to dry for ~60 s, until no moisture was visible.

Prewash sampling: A single sample of pigskin (top and bottom) was put into a Whirl-Pak filter bag (Nasco, Fort Atkinson, WI, US) along with phosphate buffer saline. The substrate and buffer were homogenized using a stomacher (Dynatech Laboratories, Alexandria, VA) for 2 min. The solution was then serially diluted in phosphate buffer saline, and plated onto BBL™ MacConkey agar containing 30 µg

nalidixic acid /mL media.

Test substrate wash: Two sections of inoculated pigskin were attached to the pigskin lathering device. One mL of tap water (control) or surfactant solution were put onto the pigskin and the device oscillated [18 RPM] for 10 s. Both pigskin sections were removed from the device using sterile forceps and rinsed for 10 s with plain tap water at ~26 °C. The wet rinsed pigskins were immediately put into a 207 mL Whirl-Pak filter bag (Nasco, Fort Atkinson, WI, US), along with phosphate buffer saline, stomached for 2 min, and the solution was serially diluted with phosphate buffer saline (pH 7.2 ±0.1) and plated on BBL™ MacConkey agar containing 30 µg nalidixic acid /mL media, incubated at 37°C for 24 h and enumerated.
The log reduction was calculated by taking the difference of the logarithm of pretreated pigskin and the logarithm of treated pigskin. Frequency histograms were constructed using Excel (Microsoft, Redmond Washington). ANOVA and Tukey's HSD (MATLAB, Natick, Massachusetts) were used to determine if multiple means were significantly different and if any significant interactions existed between the variables.

VI.5 Results and Discussion

Figure VI.6.a compares the mean log reductions of a water treatment to the treatments with different surfactants at each different concentration (2%, 5%, and 10%). The control treatment with water showed a 0.6 ± 0.4 mean log cfu reduction. A 10% sodium lauryl sulfate solution showed the highest log reduction (1.1 ± 0.5 log cfu), and was the only treatment that was significantly different from water

(p=0.0005). The remaining treatments mean log cfu reduction values are sodium lauryl sulfate 2% ($\bar{x}$= 0.57), sodium lauryl sulfate 5% ($\bar{x}$=0.86), sodium stearoyl lactylate 2% ($\bar{x}$=0.75), sodium stearoyl lactylate 5% ($\bar{x}$=0.62), sodium stearoyl lactylate 10% ($\bar{x}$=0.49), Span 60 2% ($\bar{x}$=0.62), Span 60 5% ($\bar{x}$=0.64), Span 60 10% ($\bar{x}$=0.89), Poloxamer 407 2% ($\bar{x}$= 0.60), Poloxamer 407 5% ($\bar{x}$= 0.66), Poloxamer 407 10% ($\bar{x}$=0.87).

Since surfactants in soaps are capable of removing of lipids from the stratum corneum of the skin (*10*, *76*, *85*, *125*, *195*, *221*, *252*, *258*), a reasonable argument could be made that a greater concentration of bacteria on the hand can be removed with increasing concentration of surfactants, which was seen with a 10% sodium lauryl sulfate solution (Figure VI.6.a), and is also supported by Figure VI.6.b. Most surfactant solutions in this study did show a non-statistically significant increasing microbial reduction trend with increasing surfactant concentrations (Figure VI.6.b). It was surprising to see the opposite trend for sodium stearoyl lactylate. Studies that looked at oily debris and skin removal would suggest that the anionic surfactants tested would remove microbes slightly better than nonionic surfactants tested (*83*, *109*, *132*, *215*). In this study, sodium lauryl sulfate outperformed Span 60 and poloxamer 407, but sodium steroyl lactylate did not outperform the nonionic surfactants. A possible explanation may be the sodium stearoyl lactylate HLB value. At HLB=10 the surfactant could be water-in-oil emulsifier, and the action of applying and lathering the sodium stearoyl lactylate solution could create a barrier on the skin that prevents the superficial layers of the pigskin from being washed away.

A summary of HLB, pH, and CMC values for the surfactants can be found in Table VI.6.a. A slight, yet significant positive correlation was seen between HLB value and mean log reduction (R^2=0.17, p=0.03). No correlation was seen between pH of the treatment solution and mean log reduction (R^2=0.05, p=0.25). The critical micelle concentration of sodium stearoyl lactylate was reached at a 7% solution, sorbitan monostearate was reached at 2% solution, poloxamer 407 was reached at a 0.5% solution, and sodium lauryl sulfate at a 0.2% solution (Table VI.6.a). Figure VI.6.b is a scatter plot of free grams of surfactant in solution, and the observed microbial reduction and shows a strong positive correlation (R^2=0.62, p=0.001) between the two variables. Free grams refers to the amount of surfactants available in 1 mL of surfactant solution that are able to be recruited to form micelles. The higher the value of free surfactants, the grater microbial reduction observed. If accounting for the critical micelle concentration and free grams in the surfactant solutions, the concentration of surfactant plays an important role. Froebe *et al.* did not measure microbial reduction, but they did observe a concentration dependent lipid removal with the surfactant solutions above the critical micelle concentration (*76*). A study by Krawczyk determined that a ionic surfactant (sodium lauryl sulfate) concentration dependent interaction between the liquid molecules and the skin surface once the break point (critical micelle concentration) of the surfactant solution was reached (*132*). An argument could be made that increasing the concentrations of surfactants does have benefit, as long as the concentration is increased to or above the critical micelle concentration (Table VI.6.a), as observed in Figure VI.6.b.

The fact that many of the tested surfactant solutions did not show a significant difference from a water treatment is not surprising, as a study done in our lab previously showed no statistical difference between a wash with water or a wash with water+ soap when no oily/fatty debris was present on the subjects' hands (*112*). It is important to point out that the maximum log reduction observed on the treated pigskin when a surfactant was used was ~2.5 log reduction, while the maximal log reduction observed with a water treatment was 1.3 log reduction. Similarly, in the Jensen *et al*. study, the maximal log reduction observed when soap was used was higher (~4 log CFU) when soap was used versus when no soap was used (~2 log reduction).

The lack of answers in the literature for phenomena observed in this study suggests much future research is needed in this area. While we did not observe a significant difference between plain water or a surfactant solution for most of the treatments, the findings from the Jensen *et al.* study would suggest that further research done with pigskin rubbed in oily/greasy debris contaminated with bacteria is needed to see the full efficacy of a surfactant solution (*112*). This study would suggest that a high concentration of sodium lauryl sulfate is ideal for microbial reduction, which may be due to the anionic molecules' repulsion to skin (*83*, *109*, *132*, *215*), but care must be taken, as sodium lauryl sulfate can be damaging to skin (*10*, *85*, *116*, *117*, *125*, *195*). Finally, there was a strong correlation between surfactant concentrations above the critical micelle concentration, and mean microbial reduction.

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VI.6 Figures and Tables

Table VI.6.a: Breakdown of pH of surfactants and surfaces used in this study. HLB and CMC values are for surfactant solutions only. *Data obtained from (*61*, *201*), † Data obtained from (*17*, *257*, *260*).

Surfactant or Surface	pH	HLB	CMC (% solution)	free grams in 1mL solution
Sodium lauryl sulfate 2%	9.2	40	0.2	0.018
Sodium lauryl sulfate 5%	9.7	40	0.2	0.048
Sodium lauryl sulfate 10%	9.9	40	0.2	0.098
Sodium stearoyl lactylate 2%	4.5	10	7	0
Sodium stearoyl lactylate 5%	4.5	10	7	0
Sodium stearoyl lactylate 10%	4.3	10	7	0.03
Polaxamer 407 2%	6.7	23	0.5	0.015
Polaxamer 407 5%	6.9	23	0.5	0.045
Polaxamer 407 10%	6.9	23	0.5	0.095
Sorbitan monostearate 2%	6.6	4.7	2	0
Sorbitan monostearate 5%	6.3	4.7	2	0.03
Sorbitan monostearate 10%	5.9	4.7	2	0.08
Water (control)	6.7	-	-	-
Pig skin	6.9	-	-	-
Human skin*	4-6	-	-	-
Cadaver Skin†	5.9	-	-	-

Figure VI.6.a: Comparison of the mean log reduction from treatments with different surfactant type and concentration. The black brackets represent the standard error of the mean. Each surfactant is presented in order of increasing surfactant concentration.

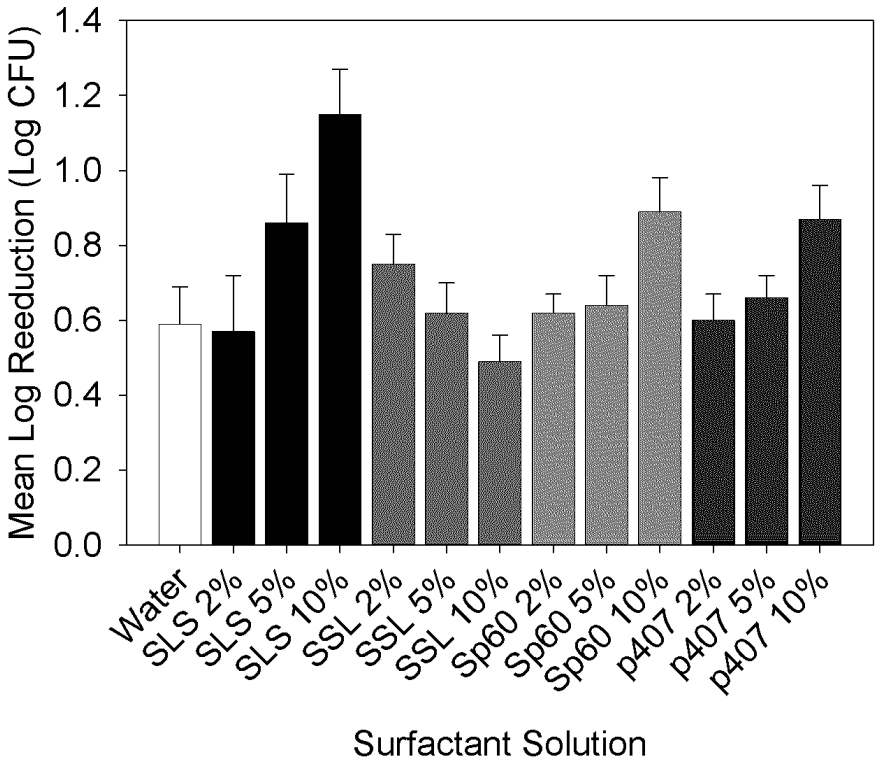
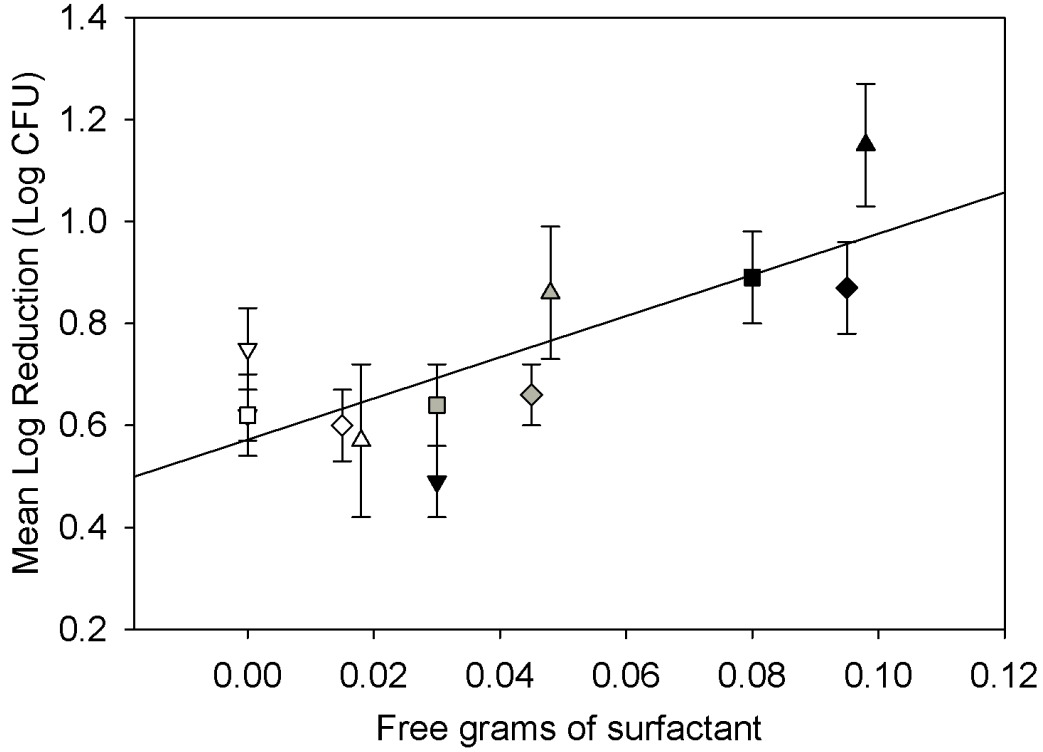


Figure VI.6.b. Correlation between free grams of surfactant (i.e. surfactant available to form micelles) and mean log reduction. Surfactants in solution that were below the critical micelle reported as zero free grams of surfactant. Open shapes are 2% solutions, solid gray are 5% solutions, and solid black shapes are 10% solutions. Sodium lauryl sulfate (△), Sodium stearoyl lactylate (▽), Poloxamer 407 (◇), Sorbitan monostearate (□).



VII Appendix

Food Code: Sections concerning hand washing, Numbers, and Capacities

5-203.11 Handwashing Facilities.*

- (A) Except as specified in ¶¶ (B) and (C) of this section, at least 1 handwashing lavatory, a number of handwashing lavatories necessary for their convenient use by Employees in areas specified under § 5-204.11, and not fewer than the number of handwashing lavatories required by law shall be provided.

- (B) If approved and capable of removing the types of soils encountered in the food operations involved, automatic handwashing facilities may be substituted for handwashing lavatories in a food establishment that has at least one handwashing lavatory.

- (C) If approved, when food exposure is limited and handwashing lavatories are not conveniently available, such as in some mobile or temporary food establishments or at some vending machine locations, Employees may use chemically treated towelettes for handwashing.

2-301.11 Clean Condition.

FOOD EMPLOYEES shall keep their hands and exposed portions of their arms clean.[P]

2-301.12 Cleaning Procedure.

1. (A) Except as specified in ¶ (D) of this section, FOOD EMPLOYEES shall clean their hands and exposed portions of their arms, including surrogate prosthetic devices for hands or arms for at least 20 seconds, using a cleaning compound in a HANDWASHING SINK that is equipped as specified under § 5-202.12 and Subpart 6-301. [P]
2. (B) FOOD EMPLOYEES shall use the following cleaning procedure in the order stated to clean their hands and exposed portions of their arms, including surrogate prosthetic devices for hands and arms:
    1. (1) Rinse under clean, running warm water; [P]
    2. (2) Apply an amount of cleaning compound recommended by the cleaning compound manufacturer; [P]
    3. (3) Rub together vigorously for at least 10 to 15 seconds while:

1. (a) Paying particular attention to removing soil from underneath the fingernails during the cleaning procedure, P and
2. (b) Creating friction on the surfaces of the hands and arms or surrogate prosthetic devices for hands and arms, finger tips, and areas between the fingers; P

4. (4) Thoroughly rinse under clean, running warm water; P and
5. (5) Immediately follow the cleaning procedure with thorough drying using a method as specified under § 6-301.12. P

3. (C) To avoid recontaminating their hands or surrogate prosthetic devices, FOOD EMPLOYEES may use disposable paper towels or similar clean barriers when touching surfaces such as manually operated faucet handles on a HANDWASHING SINK or the handle of a restroom door.
4. (D) If APPROVED and capable of removing the types of soils encountered in the FOOD operations involved, an automatic handwashing facility may be used by FOOD EMPLOYEES to clean their hands or surrogate prosthetic devices.

2-301.13 Special Handwash Procedures.

Reserved.

2-301.14 When to Wash.

FOOD EMPLOYEES shall clean their hands and exposed portions of their arms as specified under § 2-301.12 immediately before engaging in FOOD preparation including working with exposed FOOD, clean EQUIPMENT and UTENSILS, and unwrapped SINGLE-SERVICE and SINGLE-USE ARTICLES[P] and:

1. (A) After touching bare human body parts other than clean hands and clean, exposed portions of arms; P
2. (B) After using the toilet room; P
3. (C) After caring for or handling SERVICE ANIMALS or aquatic animals as specified in ¶ 2-403.11(B); P
4. (D) Except as specified in ¶ 2-401.11(B), after coughing, sneezing, using a handkerchief or disposable tissue, using tobacco, eating, or drinking; P
5. (E) After handling soiled EQUIPMENT or UTENSILS; P
6. (F) During FOOD preparation, as often as necessary to remove soil and contamination and to prevent cross contamination when changing tasks; P
7. (G) When switching between working with raw FOOD and working with READY-TO-EAT FOOD; P
8. (H) Before donning gloves for working with FOOD; P and
9. (I) After engaging in other activities that contaminate the hands.P

2-301.15 Where to Wash.

FOOD EMPLOYEES shall clean their hands in a HANDWASHING SINK or APPROVED automatic handwashing facility and may not clean their hands in a sink used for FOOD preparation or WAREWASHING, or in a service sink or a curbed

cleaning facility used for the disposal of mop water and similar liquid waste. Pf

2-301.16 Hand Antiseptics.

1. (A) A hand antiseptic used as a topical application, a hand antiseptic solution used as a hand dip, or a hand antiseptic soap shall:
    1. (1) Comply with one of the following:
        1. (a) Be an APPROVED drug that is listed in the FDA publication **Approved Drug Products with Therapeutic Equivalence Evaluations** as an APPROVED drug based on safety and effectiveness; Pf or
        2. (b) Have active antimicrobial ingredients that are listed in the FDA monograph for OTC Health-Care Antiseptic Drug Products as an antiseptic handwash, Pf and
    2. (2) Comply with one of the following:
        1. (a) Have components that are exempted from the requirement of being listed in federal FOOD ADDITIVE regulations as specified in 21 CFR 170.39 - Threshold of regulation for substances used in food-contact articles;Pf or
        2. (b) Comply with and be listed in:
            1. (i) 21 CFR 178 - Indirect Food Additives: Adjuvants, Production Aids, and Sanitizers as regulated for use as a FOOD ADDITIVE with conditions of safe use, Pf or
            2. (ii) 21 CFR 182 - Substances Generally Recognized as Safe, 21 CFR 184 - Direct Food Substances Affirmed as Generally Recognized as Safe, or 21 CFR 186 - Indirect Food Substances Affirmed as Generally Recognized as Safe for use in contact with food, Pf and
    3. (3) Be applied only to hands that are cleaned as specified under § 2-301.12. Pf
2. (B) If a hand antiseptic or a hand antiseptic solution used as a hand dip does not meet the criteria specified under Subparagraph (A)(2) of this section, use shall be:
    1. (1) Followed by thorough hand rinsing in clean water before hand contact with FOOD or by the use of gloves; Pf or
    2. (2) Limited to situations that involve no direct contact with FOOD by the bare hands. Pf
3. (C) A hand antiseptic solution used as a hand dip shall be maintained clean and at a strength equivalent to at least 100 MG/L chlorine. Pf

6-301.10 Minimum Number.

HANDWASHING SINKS shall be provided as specified under § 5-203.11.

6-301.11 Handwashing Cleanser, Availability.

Each HANDWASHING SINK or group of 2 adjacent HANDWASHING SINKS shall be provided with a supply of hand cleaning liquid, powder, or bar soap. Pf

6-301.12 Hand Drying Provision.

Each HANDWASHING SINK or group of adjacent HANDWASHING SINKS shall be provided with:

1. (A) Individual, disposable towels; Pf
2. (B) A continuous towel system that supplies the user with a clean towel; Pf or
3. (C) A heated-air hand drying device; Pf or
4. (D) A hand drying device that employs an air-knife system that delivers high velocity, pressurized air at ambient temperatures. Pf

6-301.13 Handwashing Aids and Devices, Use Restrictions.

A sink used for FOOD preparation or UTENSIL washing, or a service sink or curbed cleaning facility used for the disposal of mop water or similar wastes, may not be provided with the handwashing aids and devices required for a HANDWASHING SINK as specified under §§ 6-301.11 and 6-301.12 and ¶ 5-501.16(C).

6-301.14 Handwashing Signage.

A sign or poster that notifies FOOD EMPLOYEES to wash their hands shall be provided at all HANDWASHING SINKS used by FOOD EMPLOYEES and shall be clearly visible to FOOD EMPLOYEES.

6-301.20 Disposable Towels, Waste Receptacle.

A HANDWASHING SINK or group of adjacent HANDWASHING SINKS that is provided with disposable towels shall be provided with a waste receptacle as specified under ¶ 5-501.16(C).

Table VII.1: Surfactants commonly used in hygiene products. Products include hand soap, body wash, toothpaste, shampoo, condition, and lotion

Surfactants	**% Used in products**	**(n)**	**% Used in handsoap**	**(n)**
Sodium lauryl sulfate	76.3%	(29)	69.2%	(9)
Cocamidopropyl betaine	57.9%	(22)	61.5%	(8)
Cocamide MEA	23.7%	(9)	38.5%	(5)
Gylcol Distearate	13.2%	(5)	7.7%	(1)
Decyl glucoside	10.5%	(4)	30.8%	(4)
PEG-150 distearate	10.5%	(4)	15.4%	(2)
Lauryldimethylamine oxide	7.9%	(3)	23.1%	(3)
PEG-7 Gylcerol cocoate	7.9%	(3)	15.4%	(2)
Cocamide MIPA	5.3%	(2)	7.7%	(1)
Glyceryl caprylate	5.3%	(2)	15.4%	(2)
Linoleamidopropyl pg-dimonium chloride phosphate	5.3%	(2)	-	
Poloxamer 124	5.3%	(2)	15.4%	(2)
Poloxamer 407	5.3%	(2)	-	
Sodium monoflourophosphate	5.3%	(2)	-	
Stearamidopropyl dimethylamine	5.3%	(2)	-	
Cetostearyl alcohol	2.6%	(1)	-	
Cocamidopropyl hydroxysultaine	2.6%	(1)	-	
Coco-glucoside	2.6%	(1)	7.7%	(1)
Glyceryl monostearate	2.6%	(1)	-	
Gylceryl oleate	2.6%	(1)	7.7%	(1)
Laureth-4	2.6%	(1)	-	
Laureth-23	2.6%	(1)	-	
Methyl cocoate	2.6%	(1)	-	
PEG-60 hydrogenated castor oil	2.6%	(1)	-	
PEG/PPG-116/66 COPOLYMER	2.6%	(1)	-	
PEG-200 Hydrogenated Gylcerol Palmat	2.6%	(1)	-	
Phospholipid	2.6%	(1)	7.7%	(1)
polysorbate 20	2.6%	(1)	-	
PPG-38-BUTETH-37	2.6%	(1)	-	
Sodium dodecylbenzenesulfonate	2.6%	(1)	-	
Sodium Hydroxypropyl Starch Phosphate	2.6%	(1)		
Sorbitan monostearate	2.6%	(1)	7.7%	(1)
Glycerin	42.1%	(16)	53.8%	(7)
Sodium xylenesulfonate	7.9%	(3)	7.7%	(1)

Questionnaire form for volunteers from Chapter V

Date _____

Subject #_____ Age _____ Male____ Female_____

1) How often do you apply moisturizer to your hands?

___ More than twice a day

___ Once a day

___ Once a week

___ Once a month

___ I don't use moisturizer

2) How often do you currently use facial cleansers?

___ More than twice a day

___ Once a day

___ Once a week

___ Once a month

___ I don't use facial cleansers

3) Do you currently use acne products?

___ yes ___ no

4) Do you currently take any acne medication that requires prescription?

___ yes ___ no

5) Do you use any products for dandruff?

___ yes ___ no

6) If female, when was the first day of your last period?

_____

7) Estimate how many times a day you normally wash your hands.

___ more than 5 times a day

___ 4-5 times a day

___ 3-4 times day

___ 2-3 times a day

___ 1-2 times a day

___ I don't wash my hands

8) Estimate how many times you wash your hands **today**.

___ more than 5 times

___ 4-5 times

___ 3-4 times

___ 2-3 times

___ 1-2 times

___ I did not wash my hands yet today

9) Were you recently ill within the last two weeks (e.g. cold or allergies)?

___ yes ___ no

10) Have you taken any antibiotics within the last six weeks?

___ yes ___ no

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