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Comparative Efficacy of Foaming and Non-foaming Handsoap in Reduction of Microorganisms in Handwashing

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Comparative Efficacy of Foaming and Non-foaming Handsoap in Reduction of Microorganisms in Handwashing

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Abstract

Handwashing (HW) is a long established method to prevent disease transmission. Ensuring effectiveness of current HW methods is essential for optimal HW and enhanced disease prevention. The objectives of this research were to 1) conduct a survey of soap type and volume in food service establishments in Washington County, Arkansas; 2) investigate how soap type impacts HW behavior; and 3) determine the difference in microbial reduction between foaming (F) and liquid (L) handsoap. For Objective 1, food service establishments in Washington County, AR were selected based on exclusion criteria and random number generations, and handsoap samples were collected to determine soap type and average volume. For Objective 2, 12 volunteers applied 1.0 g of Glo Germ™ (GG) to their hands and washed their hands, and then hands were swabbed in three locations to recover remaining GG. Swabs were eluted and absorbance was measured at OD_{370nm} to quantify remaining GG using a standard curve. For Objective 3, hands of 24 volunteers were inoculated with approximately 10^8 CFU *Escherichia coli* C3000 or 10^8 PFU MS2 bacteriophage. Following completion of a standard HW protocol, microorganisms were recovered using a glove juice method, and culture assays were completed to determine microorganisms remaining. For the Washington County soap survey, the average volume of F and L handsoap was 0.64 ± 0.21 mL and 1.19 ± 0.46 mL, respectively. For Objective 2, no significant difference in behavior was determined in terms of GG remaining, HW time in the baseline HW and post GG HW, and baseline handrinsing time and post GG handrinse. Average time for the baseline handwash was (F) 11.17 ± 3.93 s and (L) 13.83 ± 7.30 s, and for the post GG handwash was (F) 13.33 ± 6.22 s and (L) 14.25 ± 7.70 s. For Objective 3, no significant difference in efficacy of F and L in overall removal of *E. coli* and MS2 combined occurred (p=0.56). However, F handsoap did remove significantly less MS2 when compared to

E. coli (p=0.0008). This research indicates that use of foaming soap in food service may need to be reevaluated for control of foodborne viruses.

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Dedication

I would like to dedicate this thesis to my late grandmother Almeda Faker. Although she was not able to further her education past high school, my grandmother's humble and honest character as well as her passion for knowledge, continuous learning, hard work and service to others has always been an inspiration to me. Although she has not been present throughout my time in graduate school, I know she has been looking over me, and I am forever grateful to her for her love, support, wisdom and encouragement throughout my life.

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List of Published or Under Review Articles:

1. Chapter 2: Conover, D.M., & Gibson, K.E. (2016). A review of methods for the evaluation of handwashing efficacy. *Food Control*, *63*, 53-64.
2. Chapter 5: Conover, D.M., & Gibson, K.E. (2016). Comparison of two plain soap types for removal of bacteria and viruses from hands with specific focus on food service environments. *Food Control*, DOI 10.1016/j.foodcont.2016.04.047.

Chapter One: Literature Review

1. Handy hygiene and its impact on disease transmission

Handwashing (HW) has long been established and accepted as a way to prevent disease and reduce transmission of harmful bacteria and viruses. Hospitals, food industry employees, and the general population require HW to promote a safe and hygienic environment. While HW is accepted as a routine part of everyday life, the importance of this basic activity was not always understood. The importance of hand hygiene has been documented as far back as 1199 by Jewish philosopher and physician, Moses ben Maimon. Maimon wrote the Mishneh Torah, which was a code of Jewish religious law, and included a chapter on hygiene where Mishneh wrote "Never forget to wash your hands after having touched a sick person" (ECJ 2012). Although Mishneh understood that HW was important, his attempt to influence others was limited as the discoveries of Mishneh were primarily disregarded (ECJ 2012). Even though Mishneh's discovery of hand hygiene was essentially disregarded, another important hand hygiene breakthrough was made in 1847.

In 1847 Dr. Ignaz Semmelweis introduced the concept of hand antisepsis. Semmelweis assisted in the maternity ward of a Viennese hospital and discovered that the cause of childbed fever and thus a high mortality rate in a maternity ward was linked to cadaverous particles still attached to the hands of examiners who had worked with cadavers before working in the maternity wing of the hospital. Semmelweis found that the ordinary soap and water hand wash was not sufficient to remove these cadaverous particles, and patients were becoming infected. After introducing a chlorine wash, deaths from childbed fever decreased dramatically (Semmelweis 1861). A breakthrough had been made in the hospital environment, and this

breakthrough continues to have an impact in disease prevention and transmission today. While the importance of hand hygiene and all the variables associated with it have not always been understood, numerous discoveries and breakthroughs have been made which have demonstrated the importance of proper hand hygiene. Today, HW has become widely accepted as the number one method available to prevent transmission of disease.

2. Hand hygiene and its impact on the food industry

Foodborne pathogens are estimated to cause 9.4 million illnesses, 55,961 hospitalizations, and 1,351 deaths in the United States each year (Scallan et al. 2011). Although, foodborne diseases will likely never be completely eliminated, there are certain practices which can be followed to greatly reduce disease incidence. Similar to the medical field, one of the primary prevention strategies is proper and consistent hand washing. Despite common knowledge of the effectiveness of hand washing, the evidence continually shows that consumers and food-preparation employees are failing to follow this simple rule, or are failing to wash hands effectively. A recent report by the U.S. Food and Drug Administration (FDA) found that 38.8% of employees in fast food restaurants are not in compliance with adequate HW, while 75.8% of employees in full service restaurants are not in compliance with adequate HW (FDA 2009). A recent study by Strohbehn et al. (2008) found that only 5% of restaurant employees were compliant with Food Code recommendations in regards to frequency of washing during production, service, and cleaning phases.

Currently, it is estimated that washing hands with soap has the potential to reduce diarrheal disease by 42 to 47% (Curtis et al. 2003). The FDA Food Code, Section 2-301.12, states that proper HW can result in a 2 to 3 log reduction in transient bacteria as well as a 2-log

reduction in transient viruses and protozoa (FDA 2013). Improving the HW of food workers is critical to reducing foodborne illness outbreaks as transmission of pathogens from the hands of food workers to food significantly contributes to the spread of foodborne illnesses (Green et al. 2006; Todd et al. 2010; Michaels et al. 2004; Edmonds et al. 2012; Pragle et al. 2007).

3. Pathogens commonly transferred by hands

Foodborne illnesses can be caused by a wide variety of microorganisms including viruses, bacteria, parasites, fungus, and prions. Symptoms can range from mild (i.e. asymptomatic) to severe (hospitalization or death) (Mead et al. 1999). Scallan et al. (2011) reported that viruses, bacteria, and parasites caused an estimated 59, 39, and 2%, respectively, of foodborne illnesses. While many pathogens can be introduced through natural vectors before a plant or animal is harvested, improper food-handling techniques, more specifically improper HW, leads to a significant percentage of foodborne illnesses. According to the FDA and the CDC, there are five primary pathogens associated with transmission by food workers including norovirus (NoV), Hepatitis A virus, *Salmonella* Typhi, *Shigella* spp., and *Escherichia coli* 0157:H7, or other Shiga toxin producing *E. coli* (FDA 2005).

3.1 Norovirus and handwashing

Of the pathogens associated with transmission by food workers, human noroviruses are the most notable and significant contributors to foodborne illnesses via this transmission route. Noroviruses are a family of non-enveloped, single stranded RNA viruses that causes acute gastroenteritis. With a low infectious dose (as low as 18 to 100 viral particles) and high number of infectious virus particles shed during and after illness, it is relatively easy for an infected food handler to contaminate a ready-to-eat product with NoV (Teunis et al. 2008). The incubation

period of NoV is typically 24 to 48 hours after exposure, with symptoms lasting from 24 to72 hours (Forsythe, 2010). Overall, NoV is the leading cause of foodborne illness in the United States, resulting in an estimated 58% of illnesses, 26% of hospitalizations, and 11% of the deaths attributed to foodborne illness. (Scallan et al. 2011).

NoV infection is exceptionally contagious, with attack rates over 45% (Forsythe 2010). The transmission of NoV can be quite extensive as a contaminated individual can shed NoV during the incubation period before symptoms appear and can continue shedding NoV particles for 10 or more days, while 30% of infected individuals can shed NoV even three weeks after infection and symptoms have subsided (Forsythe 2010). Therefore, this prolonged shedding of infectious virus particles increases the likelihood that a recently infected food worker will contaminate foods. Ready-to-eat foods handled by an ill worker can become contaminated with NoV if the food handler does not take the necessary precautions. While NoV can be transferred to food or food contact surfaces in numerous ways, transmission through food from an infected food handler is one transmission method that can be greatly reduced through proper and consistent HW (Hall et al. 2014).

For instance, in October 2012, a NoV outbreak (GII.4 Sydney strain) occurred among 26 of 103 guests present at a wedding dinner in Austria. As reported in Maritschnik et al. (2012), investigations of the food served at the wedding found that only one food item was linked to NoV. The contaminated dish was a mushroom dish, which was garnished with parsley after the dish was heated. While the mushroom dish was found to be the source of contamination for a large portion of sick wedding guests, a specific food source of contamination could not be found for 57% of those who fell ill with NoV. Based on further environmental investigation, investigators believed handling of silverware by ill food workers likely led to the NoV exposure

in the additional 57% of the cases. Investigations into the source of contamination revealed that no documented food safety training occurred for the kitchen staff. Additionally, it was found the restroom used by the kitchen staff did not have operational hand hygiene facilities. A kitchen worker was found to have been sick with the GII.4 strain. This particular worker assisted in preparation for the wedding, despite being ill, and investigators believe that this symptomatic worker spread the illness through hand contact in the kitchen environment (Maritschnik 2012).

3.2 Enterobacteriaceae and handwashing

Salmonella and *E. coli* are two types of enterobacteriaceae. *Salmonella* and *E. coli* are gram-negative, facultative anaerobic, non-spore forming rods (Forsythe 2010). As previously stated, both *Salmonella* and *E. coli* are among the top five pathogens associated with transmission by food workers. While these pathogens are more commonly inherent to the food rather than to the food handler, cross-contamination is of concern with these particular microorganisms. Inadequate HW can result in cross-contamination of food and food-contact surfaces that can assist in the transmission of these pathogens. Proper and consistent HW is one preventative measure that can assist in reducing the transmission of these two pathogens.

While there are no published examples of the direct transmission of these bacteria from hands to food, the following example related to petting zoos has been provided. Petting zoos have commonly been implicated as a source of *E. coli* infection. These zoos allow direct contact with animals that can often serve as vehicles for *E. coli* and *Salmonella*, but often do not provide proper HW stations. Andrews et al. (2012) reported on an outbreak of *E. coli* O157:H7 from 2004 in which several children became infected attending the petting zoo at the state fair.

Investigations concluded that the animals at the petting zoo were the source of the outbreak. It was concluded that the *E. coli* was transmitted directly from the hands to mouth.

4. Handwashing methods and their effectiveness

While HW is an effective method for disease control, its effectiveness hinges on the ability to follow proper HW methods. There are many variables that determine the effectiveness of HW including frequency, agent used, appropriateness, duration, and technique (Larson et al. 2006). While HW traditionally involves simply using soap and water and rubbing one's hands together, the concept of proper and effective HW has broadened throughout the years. Today there are numerous options available for HW agents: non-antimicrobial handsoap, antibacterial handsoap (e.g. triclosan), foaming and gel-based handsoaps (with or without antibacterial agent), bar soap, and various hand sanitizing agents (typically alcohol based). As stated in the 2013 FDA Food Code (Section 2-301.12), a 10-15 second scrub is necessary to remove transient pathogens from hands. Additionally, the Food Code emphasizes the importance of every step in the cleansing of hands, including scrubbing, rinsing, and drying. Failing to emphasize any of these steps in the HW process can decrease the effectiveness of the HW episode (Food Code 2013).

4.1 Handwashing time

With respect to HW time, 20 seconds is generally considered to be a reasonable amount of time to reduce microorganisms to an acceptable level. The 2013 Food Code (section 2-301.12) states that all food employees must wash hands and exposed portions of the arms for at least 20 seconds, with 10 to 15 seconds of this total time dedicated to rubbing hands vigorously. (Food Code 2013). Numerous organizations have continually shown that effective HW requires a minimum of 20 seconds; however, on average, in both hospital settings as well as in public

restrooms, HW is often under 15 seconds (Soap and Detergent Association 2007). Even still, in a study by Sickbert-Bennett et al. (2005), only a 10 second HW time was utilized as much of the research available stated that people are continually washing hands shorter than the recommended time. Perhaps surprisingly, the authors reported that shorter contact times led to a reduction in transient hand flora which lead to an overall conclusion that more emphasis should be placed on increasing HW compliance rather than increasing HW time as a shortened HW time will likely aid in an increased compliance (Sickbert-Bennett et al. 2005).

4.2 Drying hands

Drying hands after washing is one critical step that can have a significant impact on the overall effectiveness of HW. Bacteria are known to transfer more readily from wet or damp surfaces rather than on dried surfaces (Fuls et al. 2008). A study conducted in 1997 found that the drying of hands after washing has the potential to reduce microbial transfer to skin, tools, and food by up to 99.8% (Patrick 1997). While HW is an effective method to reduce disease transfer, it is essential to combine HW with careful drying of hands to limit the transfer of any remaining microorganisms. A recent study stated that hand hygiene is a two-part process, and adequate hand drying is as imperative as the initial HW (Miller 2011).

Numerous options for hand drying are available. Some common hand drying options include paper towels, a cloth towel on a rotary dispenser, a mechanical air dryer featuring heated air, and simply allowing hands to air dry naturally (Gustafson 2000). While there are numerous methods available for hand drying, the research available on the most effective hand drying technique for bacterial reduction is somewhat inconclusive. A recent study by Gustafson et al. (2000) inoculated hands with *Micrococcus luteus* and then washed hands with a nonantibacterial

soap. Hands were then dried using four different methods: cloth towels on a rotation dispenser, paper towels, a hot air drier, and spontaneous air evaporation. The results of the study indicated that no significant difference in bacterial reduction occurred between the four HW methods. Another study by Yamamoto et al. (2005) compared the effectiveness of paper towel drying with warm air drying. This particular study found that the most effective method for hand drying was the use of a warm air drier with ultraviolet light, while refraining from rubbing hands throughout the drying process (Yamamoto et al. 2005). A recent review of various hand drying methods by Huang et al. (2012) stated that hygienically speaking, paper towels are superior to electric air dryers. Although research is not entirely conclusive as to which hand drying method is more effective, the overall consensus is that handy-drying is essential to prevent the transfer of microorganisms.

4.3 Antibacterial vs. non-antimicrobial handsoap

HW agent used is another variable which can have an impact on overall HW effectiveness. There are two primary types of handsoap available, non-antimicrobial handsoap (handsoap not containing any antimicrobial agents) and antibacterial handsoap. While both are effective at reducing microorganisms found on hands, reports vary on the overall effectiveness of each type of soap.

A recent study by Fuls et al. (2008) focusing on the effectiveness of antimicrobial and non-antimicrobial soap found that the bacterial reductions associated with each type of soap were affected by several variables including wash time, product type, and soap volume. In this study, antimicrobial soap resulted in a greater reduction of bacteria when compared to non-antimicrobial soap. In addition, the bacterial reduction achieved with antimicrobial soap

increased as wash time increased, whereas no additional increase in bacterial reduction occurred for the non-antimicrobial soap (Fuls 2008). The authors stated that a non-antimicrobial soap works primarily through its physical removal of bacteria. At a certain point, maximum removal will be reached, and an increase in soap or wash time will not increase the removal further. Because antimicrobial soaps allow for both physical removal as well as inactivation of the microorganisms, the use of additional soap or added washing time can increase bacterial reduction (Fuls 2008).

While non-antimicrobial soaps physically remove the pathogens and antimicrobial soaps work through both physical removal and inactivation of pathogens, research has indicated that these two soap types impact bacteria and viruses differently. A study by Sickbert-Bennnett et al. (2005) compared the efficacy of hand hygiene agents in the reduction of bacteria and viruses. The authors found that the most effective method to reduce MS2 bacteriophage—a surrogate for the study of enteric viruses—was HW with tap water alone, while the second most effective method was found to be non-antimicrobial soap. The data seemed to indicate that for viruses, physical removal is more beneficial than inactivation of the virus (Sickbert-Bennett et al. 2005). Moreover, most antibacterial and antimicrobial soaps do not use a compound capable of inactivating viruses, specifically NoV (Liu 2009). For example, triclosan—the most common active ingredient found in antimicrobial soap—functions as an antimicrobial agent by either slowing down or inhibiting the growth of bacteria, fungi, and mildew (EPA 2010); however, its effectiveness against viruses (specifically non-enveloped viruses) has been inconsistently reported (Mbithi 1993). A study by Contreras (1999) reported similar findings to Sickbert-Bennett et al. with results indicating that liquid hand dishwashing detergents were 100 times more effective than antibacterial soaps in reducing respiratory syncytial virus.

4.4 Soap volume

In addition to the type of HW agent used, the volume of the HW agent applied can also have an effect on the effectiveness of the HW episode. A study conducted by Larson et al. (1987) focused on the quantity of soap as a variable in HW. The authors stressed the need to investigate the efficacy of HW agents at various volumes since many studies simply utilized a standard 5 mL of HW agent. To address this, the authors used 1 and 3 mL quantities of select HW agents including an antiseptic agent (4% chlorhexidine gluconate), 2 alcohol-based hand-rinses with emollients, and a liquid, non-antimicrobial soap. The results of the study indicated that an antiseptic soap would be beneficial in 3 to 5 mL amounts, while a nonantiseptic liquid soap would likely not be beneficial in volumes exceeding 1 mL per HW (Larson et al. 1987). Similar findings were reported by Fuls et al. (2008) which reported that increasing volumes of antimicrobial soap resulted in increased bacterial reduction, while increased volumes of a non-antimicrobial soap did not have the same result.

In addition to understanding the effectiveness of various volumes of soap, Larson et al. (1987) also surveyed the amounts of soap used by each subject. The results of the study indicated that the amount of soap used by each subject varied from 0.4 mL to 9.0 mL. Palm size of each individual was recorded to account for a possible relationship between palm size and soap volume used. Palm sizes ranged from 58 to 94.5 cm^2, and no significant link between palm size and amount of soap used was determined (Larson et al. 1987).

Mechanistically, non-antimicrobial soap works through the use of surfactants, which reduce bacteria through physical removal. Therefore, a certain maximum amount of bacteria are capable of being removed, and increased soap amount and wash time will not improve the

bacterial removal. Alternatively, antimicrobial soap can benefit from increased volumes as its mechanism of action involves the combination of friction as well as through killing the bacteria (Fuls et al. 2008).

Although soap volume can be an important variable in HW, it is also critical to pay special attention to the time spent HW as indicated previously. A 2011 study by Miller et al. focused on the use of time, hand-to-hand friction, and the use of non-antimicrobial handsoap for hand decontamination. The authors found that the addition of soap in general to HW lead to an initial delay in bacterial reduction in the first 5 to 10 seconds of HW. This delay was not present at 15 or 20 seconds into the HW (Miller et al. 2011). The authors hypothesized that soap served as a sort of lubricant in the HW process and thus the soap initially reduced the hand-to-hand friction resulting in decreased bacterial reduction.

5. Where is research lacking?

Research on HW, the various methods available, and their effectiveness is readily available. There are numerous studies detailing appropriate soaps, the effectiveness of antimicrobial soaps versus non-antimicrobial soaps, and the efficacy of hand sanitizers instead of or in addition to the use of handsoap. While HW has not changed dramatically throughout the years, new technologies are continuing to appear, and the process of HW continues to evolve. In recent years, foaming handsoaps have become increasingly common. Despite the plethora of research available on HW, there is limited research available focusing on the effectiveness of foaming handsoap, and even more limited research in comparing foaming handsoap to traditional gel handsoap. While HW can be extremely beneficial in reducing disease transmission, it is vital that the HW technique is optimized for utmost effectiveness. Considering this, it is important to

understand how people respond to the new developments in HW, as well as to understand how these new developments (particularly foaming handsoap) may alter the proper HW technique which will allow for continued disease prevention.

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Chapter 2: A Review of Methods for the Evaluation of Handwashing Efficacy

Abstract

Handwashing is relied upon in numerous fields as a primary means to prevent transmission of harmful pathogens. While handwashing is a key step in disease prevention, the factors controlling its effectiveness are not always well understood, and there are extensive variations in the methodology used to assess each of these factors. This review summarizes the various factors that can impact handwashing effectiveness as well as the methods and results of studies evaluating each of these factors related to handwashing. Numerous methods are available to inoculate hands as well as to recover microorganisms from hands, and for a given method, experimental variables can be changed between researchers. These variations amongst methods as well as variations in reporting experimental results can make it difficult to compare studies as well as challenging to accurately interpret the results between studies. Standardization of methods and reporting requirements are necessary to allow for comparison of studies so that more accurate conclusions about the handwashing process can be made. Therefore, the need for 1) the development of more standardized handwashing test methods and 2) the formation of guidelines on the minimal information required for publication of handwashing experiments are considered and discussed.

1. Introduction

Handwashing is widely accepted as a primary means to limit the spread of pathogens and aid in the prevention of infectious disease (Larson et al. 2000; Sickbert-Bennett et al. 2005). Numerous fields from medicine to the food industry rely on proper and consistent handwashing to promote a safe and hygienic environment for both employees and clients. While much of the world's population relies on handwashing as a daily method to maintain hygiene and prevent disease transmission, the importance of hand hygiene has not always been understood.

In the food industry, strict guidelines are provided to minimize contamination of food and aid in the production or preparation of a quality and safe product for consumers. The United States Food and Drug Administration's (FDA) Food Code describes in detail the appropriate manner in which hands should be washed as well as the recommended times to wash hands when preparing food, stating that food workers must wash hands immediately before handling: food, clean equipment and utensils, and unwrapped single-service and single-use articles (USFDA 2013). Additionally the Food Code further describes particular situations where handwashing should occur before handling food, including: after using the restroom, switching between raw and ready-to-eat food, after touching bare human body parts, and before putting on gloves when handling food (USFDA 2013).

Despite the vast focus of the food industry on proper handwashing, compliance with proper handwashing often fails, and numerous foodborne disease outbreaks occur each year due to improper handwashing (Green et al. 2006). Understanding and correcting this lack of compliance is key to reduce foodborne illnesses caused by food handlers. In addition, understanding the various factors involved in the handwashing process, and optimizing that

handwashing process are also beneficial in minimizing foodborne illness. Therefore, the objectives of this review are to i) conduct a search of peer-reviewed publications available in the field of handwashing; ii) summarize the different methodologies used in the evaluation of handwashing efficacy; and iii) discuss the need for standardized methodologies and reporting requirements to allow for comparison and consistency between handwashing studies. While a previous meta-analysis by Montville and Schaffner (2011) focused on the effectiveness of antimicrobial soaps along with the factors that may impact study results—including methodologies—the primary focus of the present review is to discuss the experimental steps used to determine the effectiveness of any given handwashing agent and highlight the need for standard approaches and reporting requirements.

2. Background

2.1 Handwashing and Impact on the Food Industry

Proper hygiene and effective handwashing are essential to food safety. It is estimated that foodborne pathogens, both major known pathogens as well as unspecified agents, cause 47.8 million illnesses, 127,830 hospitalizations, and 3,037 deaths in the U.S. each year (Scallan et al. 2011). While eliminating all foodborne disease is unrealistic, certain food safety practices such as handwashing are an effective tool to reduce disease incidence. Section 2-301.12 of the FDA Food Code states that proper handwashing can result in a 2 to 3 log reduction in transient bacteria as well as a 2-log reduction in transient viruses and protozoa (USFDA 2013). Transfer of pathogens from the hands of food workers to food significantly contributes to the spread of foodborne illness, and the improvement of handwashing in food workers is critical to decrease

the amount of foodborne illness outbreaks (Edmonds et al. 2012; Green et al. 2006; Michaels et al. 2004; Pragle et al. 2007; Todd et al. 2010a).

Although handwashing is heavily relied on in the food industry to limit microbial contamination of food, and although clear guidelines on proper handwashing are provided, the transmission of harmful microorganisms from food workers' hands to food remains a significant factor in transmission of foodborne illness (Green et al. 2006). Michaels et al. (2004) conducted a study of 308 outbreaks attributed to ill or asymptomatic food handlers, and 59% of the outbreaks were due to contamination of the food product through hand contact. Not surprisingly, the majority of these outbreaks were due to foods that required a great deal of handling, such as potato salad, salad mixed by hand, and guacamole. While these tasks involving more abundant quantities of food are a significant contributor to foodborne illness, Michaels et al. (2004) also found that much smaller tasks such as handling a slice of tomato or garnishing a dish before serving can have a significant impact on foodborne illness.

One common strategy to aid in safe preparation of food is to utilize gloves when handling foods. While gloves can be a great solution, they must be used properly and not as a substitute for handwashing (Green and Selman 2005; Guzewich and Ross 1999; Michaels et al. 2004). Green et al. (2006) conducted an observational study on the handwashing practices of 321 food workers and found that appropriate handwashing rates decreased at a significant rate when gloves were worn. The use of gloves in food production can present a false sense of security, causing food handlers to practice unsafe food handling techniques such as washing their hands less frequently or less often after high-risk tasks (i.e. handling raw meat) leading to potential microbial contamination of foods (Todd et al. 2010b). Moreover, Montville et al. (2001) conducted a study on glove usage and cross-contamination in food and demonstrated that

bacteria can transfer from food to the hands and from hands to food through the glove. The results of this study further emphasize that gloves are not an ideal solution and can actually cause a false sense of security.

While it has been established that food workers do contribute significantly to foodborne illness outbreaks through improper handling of food, it is important to understand why proper and consistent handwashing is not occurring. Pragle et al. (2007) conducted a study directly asking food handlers about their knowledge, practices and barriers to handwashing in the restaurant environment. The most significant barriers to handwashing included the availability of supplies, accessibility of sinks, time pressure (i.e. not enough time to wash hands between tasks), high volume of business, stress, lack of accountability, type of restaurant, insufficient training at the restaurant, and inadequate food handler training (Pragle et al. 2007).

Even though handwashing is commonly relied on as one of the foremost methods to prevent transmission of pathogens to food, it is clear that food is still frequently contaminated by poor handwashing practices of food handlers. Having a clear understanding of how to make handwashing optimally effective is essential for training employees and preventing contamination of food from food handlers.

2.2 Primary Factors Influencing Effective Handwashing

While handwashing is a beneficial method to aid in disease control, there are a few key variables that can impact the effectiveness of handwashing. Frequency, agent (e.g., soap or sanitizer), appropriateness (i.e. whether the hands were washed when needed to be washed), duration, and technique are all variables that determine the effectiveness of handwashing (Larson et al. 2006). The 2013 FDA Food Code emphasizes the importance of every step in the cleansing

of hands, including scrubbing, rinsing, and drying, noting that failure to perform any of these steps in the handwashing process can have a negative effect on the handwashing episode (USFDA 2013). To improve handwashing, it is essential to understand the factors that make handwashing effective.

2.2.1. Soap Type: Antimicrobial vs. Non-antimicrobial

There are two primary types of hand soap available: antimicrobial and non-antimicrobial hand soap. Both types of hand soap lead to a reduction in microorganisms found on the hands, but reports vary as to the overall effectiveness of each type of soap. Sickbert-Bennett et al. (2005) conducted a study on the efficacy of hand hygiene agents using tap water, non-antimicrobial soap, alcohol solutions, and various antimicrobial agents. Sickbert-Bennett et al. (2005) and others concluded that while antimicrobial handwashing agents were the most effective in bacterial removal, no handwashing agents were significantly superior to non-antimicrobial soap or tap water alone. Edmonds et al. (2013) studied the effectiveness of hand hygiene for removal of *Clostridium difficile* spores from hands. Handwashing agents in this study included: tap water, 4% chlorhexidine gluconate (CHG) hand wash, non-antimicrobial hand soap, 0.3% triclosan hand wash, and peracetic acid wipes which would not be used routinely as they are too harsh on hands. Results of the study found a 0.76 $\log_{10}$ colony forming units (CFU)/mL reduction in *C. difficile* spores with tap water alone. The non-antimicrobial hand soap and the 4% chlorhexidine gluconate hand wash had similar reductions in *C. difficile* when compared to tap water alone. The 0.3% triclosan hand wash had a slightly higher increase in *C. difficile* removal at a 0.99 $\log_{10}$ CFU/mL reduction while the harsh peracetic acid wipes provided the most significant reduction in *C. difficile* spores at a 1.1 $\log_{10}$ CFU/mL reduction.

Another study by Fuls et al. (2008) investigated the bacterial reduction associated with antimicrobial and non-antimicrobial hand soaps as well as the change in bacterial reduction when other variables such as wash time, product type, and soap volume were included. The authors indicated that a greater reduction of bacteria occurred with the use of antimicrobial soap. Additionally, an increase in wash time led to an increase in bacterial reduction with antimicrobial soap while the same was not true for non-antimicrobial soap (Fuls et al. 2008). This lack of correlation between wash time and increased microbial reduction may be explained since non-antimicrobial hand soap relies only on the physical removal of bacteria from hands while antimicrobial hand soap combines physical removal as well as antimicrobial inactivation of bacteria.

Soap type can also impact viruses and bacteria differently. While most hand soaps will aid in the physical removal of microorganisms, antimicrobial soap can inactivate pathogens given a sufficient contact time as discussed previously. However, the majority of antimicrobial soaps do not include compounds that are able to inactivate viruses, most notably human norovirus—the primary cause of foodborne illness in the U.S. (Liu et al. 2009). In addition, some antimicrobials such as CHG, are more effective against bacteria (especially vegetative forms) than viruses and protozoa (McDonnell and Russell 1999). One reason that antimicrobials can have a different effect on viruses, bacteria, and protozoa is microbial structure. Protozoa can develop into their protective cyst forms under certain types of stress such as the presence of antimicrobial compounds thus allowing the protozoa to remain dormant and unaffected by chemicals (Nester et al. 2009). Some bacteria (*Bacillus* and *Clostridium)* are also capable of forming spores that are extremely stable and resistant to stressful conditions such as heat and toxic chemicals (Nester et al. 2009). Viruses are obligate intracellular infectious agents

composed of nucleic acid (either DNA or RNA) surrounded by a protein coat (capsid). Some viruses have a lipid membrane (envelope) which surrounds the capsid, while other viruses—most notably enteric viruses (e.g., human norovirus) transferred by the fecal-oral route—do not have this membrane and are thus naked (Nester et al. 2009). These differences in structure lead to a difference in how each type of microorganism is affected by antimicrobials and handwashing in general.

Sickbert-Bennett et al. (2005) investigated the efficacy of hand hygiene agents against viruses and bacteria. The results indicate that physical removal of non-enveloped viruses either through use of water or a non-antimicrobial soap was more effective than using a hand soap with an antimicrobial agent. Triclosan—one of the most common active ingredients in antimicrobial soap—actually functions by slowing down or inhibiting the growth of bacteria, fungi, and mildew (USEPA 2010). The effectiveness of triclosan against viruses—especially non-enveloped viruses (i.e. enteric viruses)—remains somewhat unclear (Mbithi et al. 1993). Contreras et al. (1999) echoed the findings of Sickbert-Bennett et al. (2005) with findings indicating that liquid hand dishwashing detergents were 100 times more effective than antibacterial soaps in reducing respiratory syncytial virus.

While individual studies have led to a somewhat inconclusive stance with respect to the differences in effectiveness between antimicrobial and non-antimicrobial handsoap, a recent meta-analysis conducted by Montville and Schaffner (2011) reported that antimicrobial soap consistently resulted in a significantly greater reduction of microorganisms on hands than non-antimicrobial handsoap. Although this difference does appear to be small (approximately a 0.5 log CFU reduction difference), this difference does exist and cannot be ignored (Montville and Schaffner 2011). Moreover, Schaffner et al. (2014) showed possible reduction in incidence of

foodborne illness when antibacterial soaps are used; however, this is based on a risk simulation and focused on reduction of bacterial pathogens and not viruses. It is important to take into consideration that the active compounds in antibacterial and antimicrobial soaps are not necessarily antiviral and thus may not have the same effect on viruses—or other pathogens such as protozoa—as they do on bacteria.

2.2.2. Soap Volume

While the type of hand soap used can impact effectiveness, the volume of the handwashing agent applied can also change the effectiveness of the handwashing episode. Larson et al. (1987) conducted a study focused on the volume of soap as a variable in handwashing. At that time, many studies were using a standard volume of 5 mL of handwashing agent, and Larson et al. (1987) stressed the need to investigate the effect of volume on handwashing effectiveness. The authors used two quantities of soap (1 and 3 mL) as well as multiple handwashing agents (4% CHG antiseptic agent, two alcohol-based hand-rinses with emollients, and a liquid non-antimicrobial hand soap). The authors found that a larger volume of 3 to 5 mL was beneficial for the antiseptic soap while a volume no greater than 1 mL was more appropriate for the nonantiseptic, liquid soap (Larson et al. 1987). Fuls et al. (2008) reported similar findings with respect to soap volume indicating an increase in bacterial reduction with larger volumes of antimicrobial soap, while the same did not hold true for non-antimicrobial hand soap. The meta-analysis by Montville and Schaffner (2011) found that while a strong correlation does not exist between soap volume and effectiveness of soap (antimicrobial or non-antimicrobial), there does seem to be an indication that it might be beneficial to use more than 1

mL of antimicrobial soap and that abnormally large volumes of soap (> 5 mL) are potentially less effective.

As discussed previously (Section 2.2.1), non-antimicrobial soaps use physical removal to reduce the level of bacteria on hands. This physical removal of bacteria occurs through the use of surfactants, and because of this, there is a maximum amount of bacteria that are capable of being removed. An increase in soap amount and wash time will not lead to increased bacterial removal after the maximum removal from the use of surfactants is achieved (Fuls et al. 2008). Antimicrobial soap however will benefit from increased volumes of soap as it combines the surfactant abilities of the non-antimicrobial soap with the inactivation of microorganisms, specifically bacteria (Fuls et al. 2008).

Relationships have also been linked between soap volumes, time spent washing, and overall bacterial reduction of handwashing. Miller et al. (2011) completed a study on the use of time, hand-to-hand friction, and the use of non-antimicrobial hand soap for hand decontamination and found that adding soap actually caused an initial delay of bacterial reduction within the first 10 seconds of handwashing. After the initial 10 seconds however, the delay in bacterial reduction was no longer present (Miller et al. 2011). The authors discussed a possible conclusion that the delayed bacterial reduction occurred as a result of a sort of lubricant effect of the soap in the handwashing process, leading to an initial delay in the amount of microorganisms removed from hands.

2.2.3. Handwashing Time

Time spent washing hands is another key variable. Generally, a 20 second hand wash is considered sufficient to reduce microorganisms on hands. The 2013 Food Code (section 2-301.12) requires food employees to wash hands as well as exposed portions of the arm for 20

seconds, designating 10 to 15 seconds of this handwashing to vigorous rubbing of the hands. If performed properly, this handwashing regimen can result in a 2 to 3 log reduction in transient bacteria as well as a 2-log reduction in transient viruses and protozoa (Food Code 2013). Although many organizations including the World Health Organization, the Mayo Clinic, and U.S. Centers for Disease Control and Prevention, recommend a handwashing time of a minimum of 20 seconds for optimal removal of microbes, people in public restrooms as well as in hospitals often wash their hands for 15 seconds or less (Soap and Detergent Association 2007). Munger and Harris (1989) conducted a study testing the social influence on handwashing behavior in a public restroom and found that observed participants washed their hands for an average of 5.2 seconds, while participants who were not observed washed their hands for an average of 4.7 seconds.

Sickbert-Bennett et al. (2005) utilized a 10 second handwash based on the assumption that this amount of time was more representative of what people were actually practicing. The authors of the study concluded that a significant reduction occurred in transient hand flora, and therefore more focus should be placed on increasing handwashing compliance, as a shortened handwashing time could aid in increasing handwashing compliance (Sickbert-Bennett et al. 2005). However, Montville and Schaffner (2011) showed that with a wash time of 30 seconds, a significant difference occurred in reduction of microorganisms with antimicrobial and non-antimicrobial soap. The authors reported that antimicrobial soap resulted in a 2.42 ± 0.88 log reduction while non-antimicrobial soap had a reduction of 1.91 ± 0.75 log. Regardless, there is still a need for additional studies looking at wash time and overall handwashing effectiveness, as the research focusing on this particular factor is somewhat limited (Montville and Schaffner 2011).

Stroehbehn et al. (2008) conducted a study analyzing the handwashing practices of food service employees in operations that serve ready-to-eat food to immunocompromised individuals. The authors found that during food production in schools, of the 69 times out of the 300 times employees should have washed hands, soap was only used 62 times, and actual lathering of soap in hands only occurred 37 times. When compared to employees in the food production area, the employees were found to be more compliant with handwashing when first entering the work area with 12 of 19 participants washing hands (Stroehbehn et al. 2008). However, soap was only used 11 of 12 times, and lathering for 10 seconds only occurred nine times (Stroehbehn et al. 2008). In restaurants, only six of 83 participants washed hands after handling soiled equipment, and while all six participants used soap, only two actually lathered for the 10 seconds recommended by the Food Code (Stroehbehn et al. 2008).

Poor compliance with proper handwashing time also occurs frequently in the medical industry. Graham (1990) conducted a study on the frequency and duration of handwashing in an intensive care unit and found that for observed handwashing episodes, the average handwash duration time was 10 seconds (range of 3 to 45.2 seconds). Meengs et al. (1993) conducted a study on handwashing frequency in an emergency department and found that for the 126 times handwashing occurred with soap and water, the average duration was only 9.5 seconds.

The authors of the present review conducted a study to observe behavioral changes in 12 subjects when using foaming or liquid hand soap. The average wash time for individuals using foaming hand soap was 13.6 seconds with a standard deviation of 6.1 seconds while the average wash time for liquid hand soap was 15.3 seconds with a standard deviation of 6.6 seconds (unpublished data). No significant difference in wash time occurred between foaming and liquid hand soap. However, there was a large wash time range between the two soaps, with the shortest

time spent washing at 6 seconds and the longest at 26 seconds. With research studies indicating that handwashing time is often well below the recommended 20 seconds, it seems that using a more realistic amount of time in studies will produce data that will be more applicable to reductions occurring in everyday handwashing.

2.2.4. Drying

As previously stated, all steps in the handwashing process are critical, and the last step of drying is no less important. Bacteria transfer more efficiently from a wet surface than a dry surface (CDC 2009; Fuls et al. 2008; Patrick et al. 1997). Patrick et al. (1997) observed that drying hands after washing can decrease microbial transfer to skin, tools, and food by as much as 99.8%, or nearly 3-logs.

Various methods are available for hand drying including paper towels, cloth towels on rotary dispensers, mechanical air dryers utilizing heated air, and simple air drying (Gustafson et al. 2000). However, the answer to which drying method is the most effective is not entirely clear. Gustafson et al. (2000) had participants wash hands with a non-antimicrobial hand soap after inoculating one hand of each participant with 1×10^7 bacterial cells of *Micrococcus luteus* and then evaluated four different methods to dry hands including cloth towels on a rotation dispenser, paper towels, a hot air dryer, and spontaneous air evaporation. The authors reported no significant difference in overall bacterial reduction between the four drying methods evaluated. However, Jensen (2015) mentions that Gustafson et al. (2000) reported the data in CFU rather than in log CFU, and that if reported in log CFU, a 0.5 log CFU greater reduction occurred with paper towel drying over air drying or drying with warm air. Yamamoto et al. (2005) evaluated the effectiveness of paper towels and warm air drying and reported that a warm air dryer

combined with a 4 W ultraviolet light without rubbing hands together was the most effective. The authors hypothesized that rubbing hands together could actually allow for an increase in bacteria as bacteria are brought to the surface of skin from the hair follicles (Yamamoto et al. 2005). Conversely, Huang et al. (2012) reported results contrary to Yamamoto et al. (2005) indicating that paper towels are superior to electric air dryers. Despite the fact that there does not seem to be a conclusive answer to which hand drying method is optimum, removing residual moisture from hands is essential to allow for optimum handwashing effectiveness and to prevent unwanted transfer of microorganisms (Jumaa 2004).

3. Methods for Evaluation of Handwashing Efficacy

Since the 1980s, numerous studies have been conducted on handwashing. The focus of these studies spans a wide range of variables from looking at different microorganisms (viruses and bacteria) to handwashing time, soap volume, soap type, etc. While a common goal to optimize handwashing effectiveness is the underlying premise in each study, the methods to evaluate and achieve this goal are inconsistent across studies. Numerous methods to inoculate and recover bacteria from hands are utilized, and different microorganisms are selected for evaluations in each study. Table 1 summarizes the various studies (starting from 1985) along with the inoculation and recovery methods and the microorganisms used. It is important to note here that the authors did not include studies focused on cross contamination as these were out of the scope of this review. In the following sections, the different inoculation and recovery techniques will be discussed as well as the impact of selection of microorganisms on the reported results.

3.1 Hand Inoculation Techniques

Numerous methods are used to inoculate the hands of participants (Table 1). Inoculation methods include: hand contact with inoculated blotting paper, contact with public environmental surfaces (i.e. natural inoculation), pouring of microbial suspension in cupped hands, direct contamination of microbial suspension on fingertips, palmar-surface techniques of contamination, and immersion of hands in microbial suspension.

3.1.1. Palmar Surface Methods

The palmar surface method (PSM) is one of the most common methods used to inoculate hands. There are different variations of this method as highlighted in Table 1. American Society for Testing and Materials (ASTM) Standard Test Method E2870-13 prescribes palmar surface contamination to evaluate effectiveness of antimicrobial handwashing formulations (ASTM 2013a). The standard protocol for this procedure (ASTM E2870-13) is to inoculate each palm with 100 µL of approximately 8 log CFU/mL *Escherichia coli* suspension. Subjects then spread the inoculum across their palms and fingertips for 15 ± 1 seconds, and then hands are air-dried for 30 ± 5 seconds. Bettin et al. (1994) used 100 µL of 6.7 $\log_{10}$ CFU/mL *C. difficile* suspension pipetted onto the right palm and then gently rubbed onto the palmar surface of both hands for 10 seconds Edmonds et al. (2013) completed a similar procedure to Bettin et al. (1994) in which the palms of participants' hands were inoculated with 150 µL of *C. difficile* spore suspension followed by rubbing the palms together for 15 seconds. Fuls et al. (2008) also used a PSM though with significant modifications. Briefly, sterile paper towels were contaminated with 30 mL of a bacterial suspension (6 log CFU/mL), and hands were then inoculated by pressing on the towels for five seconds Based on the sterile bag technique for recovery of microorganisms from

hands (Section 3.2.1), the calculated transfer of bacteria to hands was approximately 5.8 to 6.4 log CFU total depending on the bacteria (Fuls et al. 2008).

Although each individual method is still a variation of the PSM for inoculation of hands, each method leads to different levels of inoculation. While Ansari et al. (1989), Bettin et al. (1994), and Edmonds et al. (2013) used a more direct method of inoculation (Table 1), Fuls et al (2008) used a more indirect method of the palmar surface inoculation technique. More specifically, the paper towels were directly contaminated with the inoculum while the hands were the secondary recipient of this inoculum. When interpreting the data in these studies, it will be important to understand the actual number of microorganisms transferred to hands during the inoculation procedure, so that accurate conclusions on handwashing effectiveness can be determined.

3.1.2. Natural Inoculation

Another common method of inoculation is indirect inoculation (i.e. natural inoculation) or inoculation through contact with everyday surfaces present in either public or controlled environments. Larson et al. (1987) conducted a study that used this particular method (Table 1). In this study hands were initially washed with a control soap, and a baseline hand culture was obtained from each subject. Subjects washed their hands 15 times a day for five days, and hand cultures were taken after the first and last handwash of days one and five to observe the effects of initial and long-term use of a particular soap (Larson 1987). Burton et al. (2011) also used natural inoculation in which participants were either taken to a large, frequently visited museum or were instructed to travel on public transportation (e.g., bus or the “underground”). Participants were instructed to intentionally wipe hands across commonly touched surfaces such as handrails, door handles, and seats to obtain as much bacteria on their hands from the environment as

possible (Burton et al. 2011). The researchers found that washing with water reduced total bacteria on hands from 44% to 23%, while washing with soap and water reduced the total from 44% to 8%. Another study by Amin et al. (2014) also used a type of natural inoculation technique and focused on comparing the removal efficacy of water with barsoap or plain water. Here, the authors did not artificially inoculate hands, but rather recovered microbes from one hand of each participant prior to handwashing (Amin et al. 2014). The participants were then instructed to perform either the wash with barsoap or the wash with water, and microorganisms were recovered from the hand that was not initially sampled to determine the reduction in initial bacterial levels achieved with each soap type (Amin et al. 2014).

Natural inoculation of hands can be beneficial in providing a realistic view of the diversity and concentration of microorganisms that subjects can accumulate on their hands in daily life. However, there are a number of limitations to this method of inoculation if the primary goal is to test the effectiveness of a particular handwashing variable (i.e. handwashing time, soap type, soap volume). For instance, there was an assumption in the study by Amin et al. (2014) that microorganisms would be homogenously distributed between both hands without consideration of possible bias in contamination of the participant's dominant hand. Moreover, depending on the surfaces that subjects are coming in contact with, the types and levels of microorganisms they are coming in contact with have the potential to vary from day to day and even between the hands of a single participant.

In the meta-analysis by Montville and Schaffner (2011), the authors observed that studies using resident microflora on hands as opposed to inoculated transient bacteria (either gram-positive or gram-negative bacteria) as an indicator of hand washing efficacy resulted in significantly different reductions (i.e. 0.31 log CFU resident microflora and approximately 1.9

log CFU transient bacteria) on hands. Initially, these results may indicate that natural inoculation methods would be preferable; however, there is no indication of the number of studies included in each category of microorganism (resident vs. transient) or whether the recovery efficiencies of each method applied were considered when reporting actual log reduction. Overall, if the goal of the research is to understand how one particular variable can impact handwashing, using a more direct and consistent method of contamination such as the PSM or the pouring methods (Section 3.1.3) will likely be more beneficial as they are more controlled and consistent methods.

3.1.3. Pouring Methods

Handwashing studies also utilize the pouring method of inoculation (PMI) which involves pouring a microbial suspension into the participants' hands and instructing the participant to spread the suspension over their hands. Unlike the PSM, the PMI involves dispersing the microorganism suspension over the entire hand (both ventral and dorsal surfaces) rather than just the palmar (ventral) region. Sickbert-Bennett et al. (2005) poured a microbial suspension (volume not specified) containing 8.5 log CFU/mL *Serratia marcescens* and 9.5 log plaque forming units (PFU)/mL MS2 bacteriophage into the subjects' cupped hands and subjects spread the suspension over their hands for 45 seconds. Here, it is interesting to note that while Sickbert-Bennett et al. (2005) referenced ASTM E-1174, stating the only modifications to the procedure included the addition of MS2 bacteriophage and the adjustment to handwash time, additional modification to the ASTM E-1174 occurred. In ASTM E-1174, the procedure states that three 1.5 mL aliquots of the test organisms are to be poured into the subjects cupped hands, with a 20 second spread onto the hands, and a 30 second air dry between each application (ASTM 2013b); however, the PMI utilized by the authors clearly deviates from this standard method (Sickbert-Bennett et al. 2005).

Edmonds et al. (2013) used two different inoculation methods—depending on the microorganism—similar to Sickbert-Bennett et al. (2005). The authors inoculated the hands of participants with 6 $\log_{10}$ spores of either *Bacillus atrophaeus* or *C. sporogenes* by placing a total of 5 mL of the spore suspension onto the hands in three separate aliquots (1.5 mL, 1.5 mL, and 2.0 mL) with the participants spreading each aliquot over the entire hand surface for 45 seconds (Edmonds et al. 2013). Edmonds et al. (2013) also used a PSM of contamination (Section 3.1.1). In this instance, it appears that the different volumes were used to achieve the same inoculum level as opposed to simply diluting the *C. difficile* spore stock to match the concentration of the *B. atrophaeus* and *C. sporogenes*. However, one could speculate that inoculating the hands with two different volumes would likely impact dispersion of the bacteria on the hands. Sasahara et al. (2014) followed a similar procedure to Edmonds et al. (2013) spreading only 2.5 mL of *B. cereus* suspension over the entire surface of each hand. Chamberlain et al. (1996) applied the pouring method as well with slight modifications (Table 1).

3.1.4. Fingerpad Methods

Another technique frequently employed is limitation of contamination to the fingers. Snyder, Jr. (2007) placed 10 µL of 11 $\log_{10}$ CFU/mL *E. coli* on the tips of the second and middle fingers of the hand. Stowell et al. (2014) also completed a similar inoculation procedure, pipetting 400 µL of cytomegalovirus suspension onto the ventral surface of participants' fingers. Ansari et al. (1989) also used a fingerpad method in which the five fingerpads of one hand were each inoculated with 10 µL of rotavirus at approximately 4 log PFU or *E. coli* at approximately 6 log CFU, followed by the drying of hands for 20 minutes. Meanwhile, Miller et al. (2011) adapted a variation on the FPM by inoculating a 25 × 6 cm section of blotting paper with a 6 mL aliquot of *E. coli* and allowed the fingers and thumbs of the participants' hands to contact the

contaminated blotting paper for 10 seconds. Stiles and Sheena (1985) performed an additional variation on the FPM. Here, the finger and thumb tips were pushed into ground beef containing naturally present coliforms, and then the hands were rubbed together until dry. Based on Table 1, it is evident that the FPM of inoculation is one method featuring a large amount of variation throughout the published literature.

3.1.5. Additional Methods

In addition to the methods described in Section 3.1, Jensen et al. (2014) chose two different methods of inoculation not previously described. The first method was selected to compare the efficacy of soap versus no soap (water only) in microbial removal on soiled hands. To inoculate hands, study participants picked up and spread 5 g of ground beef inoculated with 6 log CFU/5 g *Enterobacter aerogenes* over their hands. Here, the researchers also evaluated nonsoiled hands by inoculating the participants' hands with two 0.5 mL aliquots of 6 log CFU/mL *E. aerogenes* followed by dispersion when the subjects rubbed their hands together—a possible variation of PMI though it is unclear. Stiles and Sheena (1985) also used ground beef to inoculate hands. In this particular study, finger and thumb tips were pushed into ground beef (inoculated at 6 to 8 and 7 to 9 log CFU/g ground beef of *E. coli* and *Pseudomonas fluorescens*, respectively) for 5 seconds, and then hands were rubbed together up to the wrists until hands were dry (Stiles and Sheena 1985). Toshima et al. (2001) also used ground beef to contaminate hands, with participants working 200 grams of a ground meat blend in the palms of the hands at two separate times for 30 seconds each time.

3.1.6. Inoculum Level

As evidenced in the preceding sections as well as in Table 1, it is clear that many different techniques are used to inoculate hands with microorganisms in order to test numerous variables and their impact on handwashing. Within these different inoculation methods are many inconsistencies related to reporting inoculum concentrations. While some studies such as Bartzokas et al. (1987), Nicoletti et al. (1990), and Bettin et al. (1994) clearly list the level of microorganisms on inoculated hands, other studies do not make this as clear. For instance, Miller et al. (2011) did not clearly state amount of *E. coli* but simply explained the process of preparing the *E. coli* for suspension in a saline solution and inoculating blotting paper with the bacterial suspension. Even though the baseline level of *E. coli* on participants' is indicated, there is no information provided in relation to the efficiency of the inoculation method used in transferring *E. coli* to fingerpads—how much *E. coli* needs to be added to the blotting paper to result in the baseline levels reported?

In another example, Toshima et al. (2001) inoculated hands by having participants work 200 grams of ground meat in their palms twice for 30 seconds each time. The authors do not mention inoculation of the ground meat, but are studying the effect of handwashing on total coliforms transferred from ground meat. In this particular case, if the ground meat was not artificially inoculated, it is unlikely that coliform contamination in the ground meat will be homogenous or consistent between subjects and replicates, making the full potential for coliform reduction on hands due to handwashing difficult to determine. In Chamberlain et al. (1997) the inoculation process is discussed, and the authors state that early stationary phase cultures of *Micrococcus* sp. were diluted (1:50) into fresh broth which subjects then immersed their hands into for one minute. Once again, the true effectiveness of the handwashing procedure in this

study is difficult to determine because the initial inoculum level was not reported. Not clearly stating inoculum level is a common occurrence in the literature, and makes it difficult to draw accurate conclusions about effectiveness of handwashing methods.

3.2 Methods to Recover Microorganisms from Hands

Numerous methods have been described for recovery of microorganisms from hands to determine handwashing effectiveness. There are five primary methods that have been reported in the published literature including the glove juice method (GJM), sterile bag technique (SBT), swabbing of hands or fingertips (SWT), the glass bead method (GBM), handling surrogate surfaces, and contact on a RODAC™ (Replicate Organism Detection and Counting) plate.

3.2.1. Sterile Bag Technique

Of the recovery methods listed, SBT is one of the most common—applied by five studies evaluating handwashing efficacy (Table 1). Although there is a standard ASTM protocol (ASTM E2870-13) for application of the SBT (ASTM 2013a), this method is often modified in studies reported in the peer-reviewed literature. Larson et al. (1987) utilized the SBT to recover microorganisms from hands. Here, the authors filled a sterile bag with 50 mL of sampling solution and massaged participants' hands for 3 minutes. Fuls et al. (2008) also utilized the SBT, but chose to use 75 mL of a sampling solution in the sterile bag with a massage time of 1 minute. Meanwhile, Amin et al. (2014) applied the SBT for recovery of microbes from hands and utilized 200 mL of sterile Ringer's solution as well as a 30 second massage—participants rubbed their fingers against their palm for 15 seconds and then their hands were massaged for 15 seconds. While all of these studies had slightly different objectives, the overall goal of each study was focused on handwashing, and the effect of a given handwashing variable on microbial

reduction. The slight modification of the SBT between studies makes it difficult to compare the results to one another as differences in buffer type as well as hand massage type have the potential to impact the bacterial recovery; however, to our knowledge, no systematic comparison of these variables has occurred.

Buffer type and volume is a variable that can potentially have a significant impact on the recovery efficiency of the microorganisms. Based on the studies listed in Table 1, numerous sampling solutions are used to recover the microorganisms from hands of participants. Moreover, the addition of a neutralizing solution was included in various studies as well—specifically those evaluating antimicrobial soaps (Benton et al. 1990). Studies that employed the SBT selected a wide range of buffer volumes (50 to 200 mL). This range of buffer volume potentially impacts the ability to remove bacteria from the hands of the participants in order to determine an accurate representation of the efficacy of the handwashing process being evaluated. For instance, the higher the volume of elution buffer, the more dilute the microorganisms will be thus the sensitivity of the chosen detection method is critical. Additionally, massage time ranges from 30 seconds to 3 minutes. While a massage time of 30 seconds may not be enough to remove microorganisms from the hands of participants, a massage time of 3 minutes is likely more time than what is needed. Standardization of this method is essential to provide consistency and allow for more accurate comparison between studies.

3.2.2. Glove Juice Method

The GJM for recovery of microorganisms from hands is another method that varies significantly between studies (Table 1). Sickbert-Bennett et al. (2005) utilized 75 mL of sampling solution per glove and then massaged hands in nonsterile latex gloves for 30 seconds. Edmonds et al. (2013) also used the GJM and followed ASTM standard method E1174-13

(previously ASTM E1174-06) for evaluating the effectiveness of health care personnel and consumer handwash formulations. The ASTM protocol E1174-13 lists procedures for using *S. marcescens* and recommends *E. coli* (American Type Culture Collection [ATCC] 11229) as an alternative challenge organism (ASTM 2013c). After hands are washed and inoculated, the GJM is applied. For this method, gloves (loose-fitting, powder-free, no antimicrobial properties) are put on hands and filled with 75 mL of the ASTM E1174-13 sampling solution with validated neutralizers. Hands are then massaged for 1 min ± 5 seconds, flipping the hands after 30 seconds to ensure the palm as well as the back of the hand are massaged. After massaging, 3 to 5 mL of the fluid in the glove is aseptically retrieved, diluted, and plated within 30 minutes of sampling. Another study by Jensen et al. (2014) utilized the GJM. Here, the authors used 20 mL of phosphate buffered saline (PBS) per glove with a 1 minute massage. Last, Sasahara et al. (2014) used the GJM with 50 mL of sampling solution (0.04% KH_2PO_4, 1.01% Na_2HPO_4, and 0.10% Triton X-100) per glove with a 1 minute massage.

Similar to the SBT, there are several variables that have been modified within the studies applying the GJM. These variations in procedure can impact the resulting data as well as the manner in which the data are analyzed. For instance, the authors of this review experimented with various volumes of recovery solution while evaluating the GJM for use in an ongoing study. It was observed that too low of a volume in the glove (20 to 30 mL) was not sufficient as the volume did not cover the entire surface of the hand (especially a concern on larger hands) and would not be able to adequately recover microorganisms from the entire surface of the hand (unpublished data). Additionally, a larger volume in each glove (more than 40 to 50 mL) was too high of a volume resulting in leakage of the sampling solution from the opening of the glove (a concern for safety as well as for determining an accurate recovery of microorganisms). Based on

this, there is a need to optimize these variables to create consistency and assist with comparison of data between various studies.

3.2.3. Swabbing Techniques

While the GJM and SBT are the two most popular methods used in handwashing research, swabbing techniques (SWT) are also employed. For the SWT methods, various swab types are used as well as swabbing protocols (Table 1). For instance, Chamberlain et al. (1996) used a single, moistened, cotton-wool swab and passed the swab over the skin area (wrists, dorsal and palmar surfaces, fingertips, and the interdigital spaces) in a single continuous movement five times. Meanwhile Burton et al. (2011) used a sodium chloride soaked charcoal swab that was passed only over the fingers of the dominant hand of participants. Another study by Stowell et al. (2014) uniformly swabbed the entire contaminated finger surfaces with cotton-tipped swabs that were premoistened with 100 µL of PBS. While these studies use a variety of swab types, one consistent trend is that the swab is always pre-moistened. In a study investigating the factors that influence recovery of microorganisms from surfaces, Moore and Griffith (2002) found that a pre-moistened swab when used on a wet surface, increased efficiency of the swabbing procedure when compared to a dry swab. Although swabbing is sometimes used in handwashing studies, it is more commonly used in other areas of research such as evaluation of surface sanitation practices and is a valuable tool in the food industry (Davidson et al. 1998; Moore and Griffith 2002).

There are numerous variables involved in optimizing the effectiveness of the SWT, and for this reason, it is possible that it may not be the best method for recovery of microorganisms from hands in handwashing research. While swabbing is often selected based on the ease of sampling, its accuracy depends on the ability of the swab (i.e. buffer and swab composition) to

pick up microorganisms from the sampling surface as well as the ability of the swab to release those microorganisms (Moore and Griffith 2002). Moore and Griffith (2002) conducted a study investigating the factors influencing the recovery of microorganisms from fomite surfaces by use of traditional hygiene swabbing. In this study, stainless-steel squares were inoculated with *Salmonella* spp. and then swabbed with sterile cotton swabs, Dacron swabs, or calcium alginate swabs. Inoculated surfaces were sampled both wet and dry with swabs that were either wet or dry and the results indicated that swabbing efficiency increased when a wet surface was sampled. However, the authors noticed a general trend that if a swab was able to remove a large number of microorganisms from a sampling surface, it was often difficult to then remove those microorganisms from the swab surface (Moore and Griffith 2002). Overall, the many variables present in ensuring efficiency and accuracy of the SWT caused Moore and Griffith (2002) to question the reliability of swabbing as a method to monitor surface cleanliness. If the use of the SWT as a tool for detecting microorganisms in small areas is questioned, then its reliability in swabbing a larger area such as the surface of an entire hand should be considered as well.

3.2.4. Glass Bead Method

Another method less frequently utilized within the past decade is the GBM (Table 1). The GBM typically involves pouring a sampling solution over fingers, and then massaging fingers on glass beads to recover any remaining microorganisms from hands. Ayliffe et al. (1988) used the GBM to compare various handwashing agents in hospital laboratories and wards. In this study, after treatment with the specified handwashing agent, the fingers and thumbs were immersed in a bowl containing 100 mL of nutrient broth with neutralizer and rubbed vigorously over glass beads (3-5mm diameter) for 1 minute (Ayliffe et al. 1988). Nicoletti et al. (1990) utilized the GBM for comparison of CHG detergents and soap. In this study, after treatment with the

specified soap samples, hands were sampled by rubbing fingertips for 2 minutes on 35 g of 3mm glass beads in a bowl with 50 mL of saline (concentration not provided) with 3% Tween 80. The most recent published study using a glass tube variation of the GBM was reported by Guilhermetti et al. (2001) to investigate the effectiveness of hand-cleansing agents for removing methicillin-resistant *Staphylococcus aureus* from contaminated hands. Here, eight fingers were sampled from participants by rubbing for 3 minutes in short, flat-bottomed glass tubes containing 5 mL of 0.1% peptone water with neutralizer to prevent carryover inactivation.

3.2.5. Additional Techniques

In addition to the more commonly used recovery techniques, a few less commonly used techniques are also mentioned in the handwashing literature and have been included in Table 1 as well. Bettin et al. (1994) used TCCFA (tau-rocholate-cycloserine-cefoxitin-fructose agar) RODAC™ plates after hands were inoculated and treated with the particular handwashing method followed by sampling in three locations on the TCCFA RODAC™ plates including the fingertips and thumbtips, the palmar surfaces of the fingers, and the palms. Toshima et al. (2001) used an agar stamping method in which participants stamped the fingers (including thumb and all fingers except the little finger) from the fingertip up to the first phalangeal joint on various selective media. Snyder, Jr. (2007) studied the removal of bacteria from fingertips and the residual amount remaining on a handwashing nailbrush. To recover microorganisms from hands, a rinse of 10 mL of letheen broth was applied to the fingertips in a 1-pint ziplock bag and then participants rubbed the thumb against the second and middle fingers for 20 seconds (Snyder, Jr. 2007). Ansari et al. (1989) eluted microorganisms from the fingertips by inverting fingertips in a vial containing Earle balanced salt solution with 20% tryptose phosphate broth (EBSS-TPB). In this particular method, fingertips were inverted in the vial for 40 seconds with

40 full inversions. In the same study, the authors also applied a technique in which 20 mL of the EBSS-TPB was poured on the hands over a funnel while the hands were rubbed together. Miller et al. (2011) used touch-transfer quantification to recover microorganisms from hands by choosing two items for the participants to handle—licorice to represent a food item and soft synthetic chamois to represent skin. The surrogate surfaces were picked up by the participant and handled firmly for 5 seconds followed by transferring the surrogate surface to sterile 20 mL glass containers containing 9 mL of saline and vortexed for 10 seconds to recover microorganisms (Miller et al. 2011).

3.2.6. Recovery Efficiency and Baseline Inoculum

Although each recovery method has its own set of benefits, there is a need to standardize 1) how long the method should be applied (e.g., how long should hands be massaged in the GJM?) and 2) the appropriate steps and reagents to be used for each method. Standardization of the procedures for the various methods commonly used in handwashing research would allow for easier comparison between studies as well as for more clear and accurate conclusions to be made when comparing handwashing efficacy. Moreover, not only is methodological standardization important, but handwashing researchers could benefit from the formation of guidelines on the minimal information required for publication of handwashing experiments—these types of guidelines have been established for common methods plagued by lack of standardization such as real time, quantitative PCR (Bustin et al. 2008).

For instance, a major concern with handwashing studies is that the recovery efficiency of the microbial recovery methods applied to hands are often not provided. Of the 13 studies covered in Section 3.2, no researchers directly reported the recovery efficiency of the applied method. However, two studies (Fuls et. al, 2008 and Sickbert-Bennett et al.2005) provided

enough information and data to allow for the recovery efficiency to be determined by the authors of the present review. Knowing the recovery efficiency of a particular method is a key detail to determine the true effectiveness of a particular handwashing variable. Variation in a particular method from lab to lab, or even within one particular lab, can be significant, and thus the recovery efficiency of a particular method is vital for determining the true effectiveness of a particular handwashing regimen. Jensen (2015) stressed the importance of taking the sampling method into consideration when designing experiments and comparing results as different techniques can result in different observed log CFU or PFU reductions for similar initial microbial concentrations.

Edmonds et al. (2013) did not detail the recovery efficiency of their method, but they do mention that the baseline population was determined through the GJM prescribed by ASTM E1174-13 (ASTM 2013b). Some methods such as ASTM E2870-13, which uses the PSM for inoculation, do not require a baseline sampling of the hands because the method is designed to determine the difference between handwashing products, rather than an overall reduction (ASTM 2013a). Additionally, the inoculum volume at such a low level (i.e. 100 µL), and the distribution of the small volume of inoculum is controlled and limited to the palms and fingers, thus there is limited opportunity for spillage of inoculum solution. While recovery efficiency is vital to report, the baseline level is also an important detail that needs to be reported. Although baseline sampling is not essential when a small, controlled volume is applied to hands (e.g. PSM of inoculation), it is of more concern in inoculation methods such as the blotting paper method used by Miller et al. (2011) to contaminate hands and the PMI used by other researchers (Table 1). In the blotting paper technique, the hands are not directly inoculated with the microorganisms, rather blotting paper is contaminated and hands then come in contact with the blotting paper,

leading to a lower inoculum level on the hands than on the blotting paper. The baseline level of microorganisms here would be essential to know in order to calculate the recovery efficiency for accurate determination of microbial reduction due to handwashing. Similarly, PMI would result in comparable challenges for calculation of recovery efficiency.

4. Selection of Microorganisms

Numerous types of microorganisms (i.e. species, strains, bacteria [vegetative and spores], viruses) are used in handwashing studies. As shown in Table 1, 14 different microorganisms were used to inoculate hands with most being bacteria (n = 11) followed by one bacteriophage and two viruses. Of the bacteria, *E. coli* was used most frequently (n = 8). The only other organisms used in more than one study were *C. difficile, Micrococcus*, *S. aureus,* and *S. marcescens* (Table 1). Eight of the studies did not artificially inoculate hands with microorganisms, but rather used natural inoculation through environmental microbes which may have included bacteria, viruses, and protozoa though analyses were only chosen for detection of specific types of bacteria.

Some standard methods such as ASTM E1174-13 state the microorganisms that are to be used for the particular procedure—in this case, *S. marcescens* (ATCC 14756) or *E. coli* (ATCC 11229) (ASTM 2013b). Other standard procedures, such as ASTM E2870-13 are more general. ASTM Standard Test Method E2870-13 simply states that the microorganism should have characteristics that allow it to be readily identified (ASTM 2013a). Although some standard methods do list the microorganisms to be used for that particular method, numerous adjustments to the standard protocol are typically made by researchers, and details such as the type of microorganism to be used in a particular method are not always followed. However, this may not necessarily be problematic. For instance, Edmonds et al. (2013) use ASTM E1174-06 which

states that either *S. marcescens* or *E. coli* are the microorganisms to be used in this method; however, the authors used *C. difficile* spores as the test organism to focus on the dynamics of removing spores during handwashing rather than vegetative bacteria. One possible option to allow for consistency between studies would be to create a standardization of microorganisms, where certain surrogate microbes could be chosen as standards to use to represent pathogenic bacteria, viruses, and protozoa of human health concern. Consistency between microorganism types used is not necessarily key, but rather reporting details such as recovery efficiency (which could vary depending on the type of microorganism used) to ensure accuracy when determining reduction of microorganisms on hands in the handwashing process is critical.

5. Conclusions and Recommendations

As evidenced in this review, handwashing research utilizes many different methods to inoculate hands as well as to recover microorganisms from washed hands. In addition to this, numerous microorganisms are used to inoculate hands. While there is a lot of variation in different methods used to test handwashing efficacy, there is also extensive variation within the various methods leading to some confusion in interpretation of published results as well as in study reproducibility to determine handwashing effectiveness. Additionally, the vast amount of variation that is occurring within the numerous studies makes it difficult to compare results of studies and draw definitive conclusions from the data. For this reason, there is a need to create standardized methods that can be routinely used by researchers. These standardized methods will provide consistency and comparability between studies allowing for more transparent conclusions to be drawn. Moreover, the formation of guidelines for the minimal information required for publication of handwashing experiments could be an essential step in allowing accurate comparisons of future published studies.

In preliminary, unpublished data from the authors of this review, the PSM was an ideal method for inoculation. In addition, several commonly used recovery methods were evaluated including the GJM, SWT, and SBT. From both a recovery efficiency standpoint and an ease of use standpoint, the GJM with 35 mL of sampling solution and a 1 minute massage seemed to be the optimum choice (unpublished data). Conversely, the SBT required a much higher volume of sampling solution (around 200 mL), and as the sampling solution was in a larger area (i.e. a plastic bag), it did not seem to thoroughly cover the hands. One issue observed with both the SBT and the GJM was that the sampling solution would often leak and those recovered microorganisms would be lost. Because the opening of the glove or the sterile bag was not entirely closed around the participants' lower arm, a slight change in angle, or an increase in sampling solution volume often led to leakage at the opening of the glove or bag (both a concern for subject safety and accuracy of recovery of microorganisms). For this reason, keeping the sampling solution to a minimum volume of no more than about 50 mL is also optimal. With respect to the SWT, the recovery efficiency of *E. coli* from hands was much lower at 0.47% when compared to the GJM at 2.3% (unpublished data). As discussed previously, swabbing the entire hand is not an ideal recovery efficiency method as the area is far too great compared to the surface area of the swab.

Although the appropriate inoculation and recovery methods as well as the microorganism(s) chosen will vary based on the objectives of a particular study, in the future, consistency within an experimental method should occur to allow for consistency and assist in ease of study comparisons. Additionally, details such as efficiency of the recovery method are essential to report in the future as these types of details allow for a clear understanding of the true reduction in microorganisms that is occurring due to handwashing. Reporting of essential details

such as recovery efficiency by researchers will aid in the determination of accurate conclusions about the handwashing process to be made. Our conclusions support Jensen (2015) who stated that although the factors that influence handwashing have been studied, comparing these studies is difficult because of both methodological differences as well as statistical flaws in studies. It is clear that inconsistencies between methods and between researchers is causing confusion in interpreting dating and is making it difficult to develop accurate conclusions about what details and methods will make handwashing most effective. Creating standardization in handwashing research methodology as well as reporting guidelines is essential to allow for comparison between studies so that researchers can accurately draw conclusions about the handwashing process. Advancements in handwashing research will then allow for optimization of handwashing and proper training of food-handlers so that handwashing effectiveness can be maximized, and foodborne illness caused by improper handling due to food-handlers can be minimized.

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Table 1. Review of methods selected for evaluating handwashing efficacy.

Reference	Purpose of the Study	Inoculation Method	Recovery Method	Microorganisms Used (Concentration or Total)
Stiles and Sheena (1985)	To compare the efficacy of iodophor products, CHG detergents, and non-germicidal soap for reducing microorganisms on hands	1) NI 2) FPM: Finger and thumb tips pushed into ground beef inoculated with bacteria; hands rubbed together until dry	GBM: one hand immersed in 100 mL letheen broth in plastic bag containing 35 g of 4 mm sterile glass beads; rubbed glass beads 20 times over palms	Bacteria naturally present; *Escherichia coli* (6 and 8 log CFU/g beef), *Pseudomonas fluorescens* (7 and 9 log CFU/g beef)
Bartzokas et al. (1987)	To evaluate initial and cumulative efficacy of two antiseptic HW preparations in eliminating enterobacteria from hands	PMI: 5 mL of bacterial suspension poured into cupped hands, rubbed over hands, and air-dried for 60 s	GJM: 75 mL of sterile tryptone water and 0.075 M phosphate buffer in each glove; hands massaged 60 s	*Serratia marcescens* (9 log CFU/mL in suspension; 6.6 log CFU total baseline)
Larson et al. (1987)	To determine effect of soap volume on microbial reduction during HW	NI	SBT: 50 mL of SSN in in bag, hands massaged for 3 min	Bacteria naturally present

Table 1. Review of methods selected for evaluating handwashing efficacy. (Cont.)

Reference	Purpose of the Study	Inoculation Method	Recovery Method	Microorganisms Used (Concentration or Total)
Ayliffe et al. (1988)	To test the efficacy and residual activity of 14 HW or disinfectant preparations on hands	FPM: 0.02 mL of bacteria on fingers and opposing fingers; thumbs rubbed 40 s and air dried 80 s	GBM: fingers and thumbs immersed in 100 mL of nutrient broth + neutralizer and rubbed over glass beads for 60 s	*E. coli* (concentration not provided)
Ansari et al. (1989)	To evaluate a protocol using finger pads for testing the microbial-eliminating efficacy of HW agents	1) FPM: fingerpads on one hand each inoculated with 10 µL of rotavirus or *E. coli* and air dried 20 min 2) PSM: 0.5 mL rotavirus or *E. coli* suspension placed on palm of one hand and palms rubbed together; 20 min air dry	Microorganisms eluted with 1 mL EBSS + 20% tryptose phosphate broth for 40 s with 40 full inversions; 20 mL of eluate poured on hands while rubbed together over a plastic funnel	Rotavirus (4.2 log PFU total), *E. coli* (5 log CFU total)
Larson et al. (1989)	To compare the antimicrobial effects of three products containing CHG, PCMX, or TRI on normal hand flora after HW at different frequencies over 5 days	NI	SBT: dominant hand inserted into a sterile bag with 50 mL SSN; hand massaged for 60 s	Bacteria naturally present

Table 1. Review of methods selected for evaluating handwashing efficacy. (Cont.)

Reference	Purpose of the Study	Inoculation Method	Recovery Method	Microorganisms Used (Concentration or Total)
Nicoletti et al. (1990)	To compare two CHG HW detergents and liquid soap for removal of bacteria contaminated fingers	FPM: 10 µL applied to each of 5 fingertips and rubbed in 10 s per finger with the thumb and then dried	GBM: fingertips rubbed for 2 min on 35 g of 3 mm glass beads in a bowl of 50 mL of saline with 3% Tween 80	*S. marcescens* (9.3 log CFU total on 5 fingertips), *Micrococcus* sp. (8.6 log CFU total on 5 fingertips)
Bettin et al. (1994)	To compare liquid soap versus 4% CHG in 4% alcohol for the decontamination of bare or gloved hands	PSM: 100 µL of bacterial suspension placed on right palm; palms rubbed together for 10 s	RODAC™ plate with TCCFA imprinted by finger/thumb tips, palmar surfaces of fingers, and palm	*Clostridium difficile* (6.7 $\log_{10}$ CFU/mL)
Miller et al. (1994)	To compare the bacterial reduction of plain and various antimicrobial handsoaps and instant hand sanitizers	NI: determination of transient flora prior to washing; determination of resident flora after washing	RODAC™ plate with Difco D/E Neutralizing imprinted by fingertips	Bacteria naturally present
Paulson (1994)	To evaluate the immediate, persistent, and residual efficacy of five surgical hand scrub products	NI: baseline samples of hands obtained after condition wash to remove transient microflora	GJM: 75 mL 0.1M PBS with 0.1% Triton X-100; 60 s massage	Resident microflora

Table 1. Review of methods selected for evaluating handwashing efficacy. (Cont.)

Reference	Purpose of the Study	Inoculation Method	Recovery Method	Microorganisms Used (Concentration or Total)
Chamberlain et al. (1997)	To investigate the effectiveness of a HW procedure	PMI: Hands immersed in bacterial suspension for 60 s	SWT: premoistened, cotton-wool swab passed over skin five times)	*Micrococcus* sp. (concentration not provided)
Paulson et al. (1999)	To examine four HW regimens for reduction of transient microorganisms on hands	PMI: 5 mL of *E. coli* poured into cupped hands in two 2.5 mL aliquots and spread over both hands for 45 s; 2 min air dry	GJM: sterile latex gloves on each hand with 75 mL of SSN in each glove, wrists secured and hands massaged for 60 s	*E. coli* (8 log CFU/mL)
Guilhermetti et al. (2001)	To investigate the effectiveness of hand-cleansing agents for removal of bacteria from hands	FPM: 0.02 mL of bacterial suspension on 4 fingertips of left hand and opposing fingertips rubbed together for 40 s and air dried 80 s	GBM: 8 fingers rubbed 3 min against 10 g of 3-5 mm sterile glass beads in 5 mL of 0.1% peptone + neutralizer	methicillin-resistant *Staphylococcus aureus* (3.8 and 6.8 log CFU/fingertip)
Toshima et al. (2001)	To investigate the efficacy of a commercial antibacterial soap	NI: Ground meat (200 g) massaged in the palm, two times, 30 s each time	GJM: 30 mL SSN poured into gloved hand and hand massaged, covering each side of the hand 10 times	Total coliforms naturally present in ground meat

Table 1. Review of methods selected for evaluating handwashing efficacy. (Cont.)

Reference	Purpose of the Study	Inoculation Method	Recovery Method	Microorganisms Used (Concentration or Total)
Sickbert-Bennett et al. (2005)	To evaluate efficacy of antimicrobial agents in soap.	PMI: Microbial suspension (volume not listed) poured into the subject's cupped hands and spread over hands for 45 s	GJM: 75mL of SSN in one nonsterile glove per hand; massaged for 30 s	*S. marcescens* (8.5 log CFU/mL), MS2 bacteriophage (9.5 log PFU/mL)
Snyder, Jr. (2007)	To determine efficacy of a double HW procedure for removal of bacteria from fingertips	FPM: 10 µL of *E. coli* spread on the tips of the second and middle fingers	Modified SBT: Fingertips rinsed in 10 mL letheen broth in a plastic bag; thumb rubbed against second and middle fingers for 20 s	*E. coli* (11 log CFU/mL)
Fuls et al. (2008)	To optimize ASTM E1174 method of hand contamination technique and to measure effect of time and soap volume on antimicrobial and non-antimicrobial soap effectiveness	PSM: sterile paper towels inoculated with 30 mL of bacterial suspension and hands pressed on towels for 5 s	SBT: 75 mL of SSN in bag, hands massaged vigorously for 60 s	*S. marcescens*, *S. aureus*, *E. coli*, *Shigella flexneri* (variable: 6 to 9 log CFU/mL)

Table 1. Review of methods selected for evaluating handwashing efficacy. (Cont.)

Reference	Purpose of the Study	Inoculation Method	Recovery Method	Microorganisms Used (Concentration or Total)
Burton et al. (2011)	To determine whether non-antibacterial soap reduces enteric bacteria better than water alone	NI	SWT: NaCl soaked charcoal swab wiped across fingers of dominant hand	Bacteria naturally present
Miller et al. (2011)	To evaluate HW time, friction, and soap for reduction in bacterial translocation to skin and food surfaces	Modified FPM: Fingertip and thumb contact with blotting paper inoculated with 6 mL of E. coli	Handling Surrogate Surfaces: surrogate placed in sterile 20 mL glass containers with 9 mL of saline; vortexed for 10 s to elute *E. coli*	*E. coli* (concentration not provided)
Edmonds et al. (2013)	To compare the efficacy of hand hygiene agents against C. *difficile*	1) PMI: 5 mL of microbial suspensions in 3 aliquots (1.5, 1.5, and 2.0 mL) placed in hands and rubbed all over for 45 s 2) PSM: 150 µL of spore suspension rubbed into palms of hands for 15 s	GJM: 75 mL of SSN in each glove, hands massaged uniformly 1 min ± 5 s, flipping hands after 30 s	*C. difficile* (7 log CFU/mL), *Bacillus atrophaeus* (6 log CFU/mL), *C. sporogenes* (6 log CFU/mL)

Table 1. Review of methods selected for evaluating handwashing efficacy. (Cont.)

Reference	Purpose of the Study	Inoculation Method	Recovery Method	Microorganisms Used (Concentration or Total)
Amin et al. (2014)	To compare microbial efficacy of soapy water with bar soap and water alone	NI	SBT: 200 mL sterile Ringer's solution; participant rubbed fingers against palm for 15 s; hands massaged for 15 s	Naturally present thermotolerant coliforms and *C. perfringens*
Jensen et al. (2014)	To determine effectiveness of a minimal 5 s wash and a longer U.S. FDA Model Food Code compliant hand wash (20 s) with and without food debris	1) Spread inoculated ground beef on hands 2) Two 0.5 mL aliquots of bacteria placed in each hand and evenly dispersed by rubbing hands together	GJM: 20 mL of PBS per glove; 60 s hand massage	*Enterobacter aerogenes* (6 log CFU/5 g beef or 6 log CFU/mL)
Sasahara et al. (2014)	To determine the appropriate HW procedure for removing *B. cereus* spores	PMI: 2.5 mL of microbial suspension spread on each hand	GJM: 50 mL of sampling solution in each glove; hands massaged for 60 s	*B. cereus* (6 log CFU/mL) , *E. coli* (6 log CFU/mL)
Stowell et al. (2014)	To evaluate the removal of cytomegalovirus (CMV) through HW	Modified FPM: 400 µL of virus suspension applied to ventral surface of fingers	SWT: ventral surface of fingers swabbed with cotton-tipped swabs premoistened with 100 µL of PBS	CMV (5 log PFU/mL)

Table 1. Review of methods selected for evaluating handwashing efficacy. (Cont.)

ASTM = American Society for Testing and Materials; CFU = colony forming unit; CHG = chlorhexidine gluconate; EBSS = Earle's balanced salt solution; FPM = fingerpad method; GBM = glass bead method; GJM = glove juice method; HW = handwashing; NI = natural inoculation via contact with daily surfaces; PBS = phosphate buffered saline; PCMX = parachlorometaxylenol; PFU = plaque forming unit; PMI = pouring method of inoculation (i.e. microorganisms poured into cupped hands and distributed over the entire hand surface) ; PSM = palmar surface method of inoculation (i.e. microorganisms placed in palm of hand and spread of microorganisms limited to the palmar surface of the hand) ; RODAC™ = Replicate Organism Detection and Counting plate; RTE = Ready to eat; SBT = sterile bag technique; SSN = sampling solution and neutralizer; SWT = swabbing technique; TCCFA = tau-rocholate-cycloserine-cefoxitin-fructose agar; TRI = triclosan; US FDA = United States Food and Drug Administration

Chapter 3: Survey of Soap Volume and Type in Washington County, Arkansas

Abstract

Handwashing (HW) is one of the most significant methods used to prevent the spread of disease, and numerous variables are present in the HW process that can impact the overall effectiveness of a HW episode. Soap volume and soap type are two such variables, and numerous soap types as well as soap volumes exist on the market today. The objectives of this chapter were to conduct a survey of soap type and soap volume in food service establishments in Washington County, Arkansas, to better understand the types of soap as well as the average soap volume used in food service. The data from this chapter will then be used to determine representative volumes of soap selected for use in chapters 4 and 5. First, a list of food service establishments in Washington County, AR was obtained, and the list was narrowed down based on exclusion criteria. Specific locations were selected for sampling through the use of random number generations. Handsoap samples from restrooms in sampling locations were collected in triplicate from 68 of the 75 selected food service locations to determine soap type and average volume. One of the 68 restaurants had both foaming (F) and liquid (L) handsoap giving an overall distribution of 54.4% (n = 37) and 47.06% (n = 32), respectively. The average volume of F and L handsoap was 0.64 ± 0.21 mL and 1.19 ± 0.46 mL, respectively. This information was then used for the selection of representative F and L handsoap volumes used in chapters 4 and 5.

1. Introduction:

Soap volume is a variable that can vary both between and within soap manufacturing companies. As evidenced by my literature review (Chapter 2), soap volume is a variable that has been shown to impact handwashing (HW) effectiveness (Fuls et al. 2008, Larson et al. 1987, Montville and Schaffner 2011. The goal of this study was to conduct a survey of both soap type and soap volume in food service environments to determine the range of soap volumes available as well as the distribution of soap types. Soap volume was sampled from the restrooms of licensed food service establishments in Washington County, Arkansas. I predicted that there would be a similar ratio of foaming to liquid handsoap across restaurant restrooms in Washington County, Arkansas, with an average soap volume of 1 mL for both foaming and liquid handsoap. The data obtained from this soap survey were used for selection of representative soap volumes for studies described in Chapters 4 and 5.

2. Materials and Methods:

2.1 Soap type and volume in Washington County, Arkansas

To determine the distribution of soap types available, as well as the range and average amount of soap dispensed, 75 locations in Washington County, Arkansas were randomly selected for sampling using a random sample generator. Briefly, for sample location selection, a list of all licensed food service facilities in Washington County, Arkansas was obtained from the Washington County Health Unit of the Arkansas Department of Health. The list of facilities was then narrowed down through a set of exclusion criteria (Table 1), and 470 locations remained. From these locations 10% plus an additional 30% (to account for any complications that could

arise in the initial location selections being unavailable for sampling) were chosen by random sampling through the use of JMP® Pro 11.0 (SAS Institute, Cary, NC). Based on this, 75 locations were selected for sampling. At each food service location, one pump of the soap available in the women's restroom was dispensed in 50 or 15 mL conical tubes for foaming soap and liquid soap, respectively. The soaps were collected in triplicate (three "pumps" from the same soap dispenser on the same day) using this method. Following sample collection, the samples were transported to the lab to quantify the volume of soap per pump. The soaps were allowed to settle in their vials with caps on (to prevent evaporation) until all air bubbles settled. Foaming soaps were transferred to 1.5 mL microcentrifuge tubes, and the volumes were estimated using the graduations on the microcentrifuge tubes. Liquid soaps were left in their original 15-mL conical tubes, and 2 mL of water were added to each vial to bring the volume up to a readable level. The volume was then estimated by subtracting the additional 2 mL of water from the total volume in the tube.

2.2 Retail soap type and volume

In addition to determining the volume and types of soap available at food service establishments, the dispense volumes of commercial soaps were also determined. Three brands of foaming soap and 3 brands of liquid soap were purchased from a local grocery store in Fayetteville, Arkansas. Soaps were transported back to the lab, and one pump of each soap was dispensed in 50 or 15 mL conical tubes for foaming soap and liquid soap, respectively. The soaps were sampled in triplicate using this method. Similar to soap samples from food service establishments, soaps were allowed to settle in the tubes with caps on (to prevent evaporation) until all air bubbles settled. Foaming and liquid soaps were processed the same as described in

Section 2.1. The range of soap volumes was recorded, and the average soap volume for each type of soap (foaming versus liquid) was calculated.

3. Results:

Of the 75 locations chosen for sampling, 68 were sampled while the remaining locations were either closed, did not have consumer restrooms, or were not a traditional restaurant (some were determined to be primarily bars or nightclubs rather than food service establishments). One of the 68 restaurants had both foaming and liquid handsoap. Of the food service establishments sampled, 54.4% (n = 37) had foaming handsoap and 47.06% (n = 32) had liquid handsoap. The volume of foaming handsoap ranged from 0.2 to 1.21 mL with an average of 0.64 ± 0.21 mL, and the volume of liquid handsoap ranged from 0.33 to 2.0 mL with an average volume of 1.19 ± 0.46 mL (Figure 1). One-way analysis of variance (ANOVA) of the soap volumes was performed with a p-value of ≤ 0.05 considered significant. The difference between foaming and liquid soap volume was significant ($p = < 0.001$). For the foaming and liquid soaps purchased at retail, the volume of foaming soap ranged from 0.2 to 1.21mL with an average of 0.99 ± 0.48 mL. The volume for retail liquid soap ranged from 0.33 to 2.0 mL with an average of 1.83 ± 0.29 mL.

4. Discussion:

Although the significance of soap volume on HW effectiveness has been somewhat disputed in the literature, some researchers have reported an increased efficacy with an increase in soap volume, especially when using antimicrobial handsoap. In a study conducted with hospital personnel, Larson et al. (1987) concluded that when using antiseptic soap, 3 to 5 mL per

HW should be used to reduce both colonizing and transient hand flora; however, using more than 1 mL of nonantiseptic liquid soap per HW is likely not advantageous. It is important to begin understanding the range of soaps used to assist in understanding the impact of soap volume on HW effectiveness. A meta-analysis on antimicrobial soaps conducted by Montville and Schaffner (2011) found that while there is some evidence that a very small volume (e.g. < 1 mL of antimicrobial soap) is less effective than a larger volume of antimicrobial soap, there were no strong interactions between volume of soap and effectiveness of antimicrobial and non-antimicrobial soap. Overall, research into soap volume as a variable in HW is somewhat limited.

Larson et al. (1987) discuss that the volume of antiseptic soap has the potential to be an important determinant on the reduction of microorganisms in HW, noting that antibacterial efficacy can vary between products; thus product manufacturers should provide specific instructions regarding proper soap amount to be used to achieve maximum HW effectiveness. This however may be a conflict of interest as a soap manufacturer would benefit financially from recommending an increased soap volume. As evidenced by the data collected in Washington County, Arkansas, soap volume does have a wide range depending on the soap type. Additional research into optimal soap volume for microorganism removal for both antimicrobial and non-antimicrobial soap as well as foaming and liquid soap types should be conducted to create a more standardized soap volume on the market and to aid in maximizing HW effectiveness for not only the food service industry, but also for the general population.

As evidenced by the results of my sampling survey, the volumes for liquid and foaming soap (a mix of antimicrobial and non-antimicrobial soaps) in Washington County, Arkansas were

quite variable, with a significant difference in average soap volume between foaming and liquid handsoap. The results of this preliminary study provided insight into the range of volumes of soaps used in Washington County, Arkansas. This information was then used for the standardization of soap volumes used in the studies described in Chapters 4 and 5.

References:

Fuls, J. L., Rodgers, N. D., Fischler, G. E., Howard, J. M., Patel, M. Weidner, P. L., et al. (2008). Alternative hand contamination technique to compare the activities of antimicrobial and nonantimicrobial soaps under different test conditions. *Applied and Environmental Microbiology, 74*, 3739-3744.

Larson, E. L., Eke, P. I., Wilder, M. P., & Laughon, B. E. (1987). Quantity of soap as a variable in handwashing. *Infection Control*, *8,* 371-375.

Montville, R., & Schaffner, D.W. (2011). A meta-analysis of the published literature on the effectiveness of antimicrobial soaps. *Journal of Food Protection, 27*, 1875-1882.

Table 1: Exclusion criteria of food service locations in Washington County, Arkansas

Locations to be excluded
Schools
Churches
Camps
Veterans affairs
Lodges
Nightclubs that are mainly a bar (don't sell food)
Hotels
Bowling alleys
Sports stadiums
Bookstore
Flea market
Self-pick orchard
Gym snack bars
Golf Courses

Figure 1: Boxplot of Average Volume (mL) by Soap Type in Food Service establishments in Washington County, Arkansas

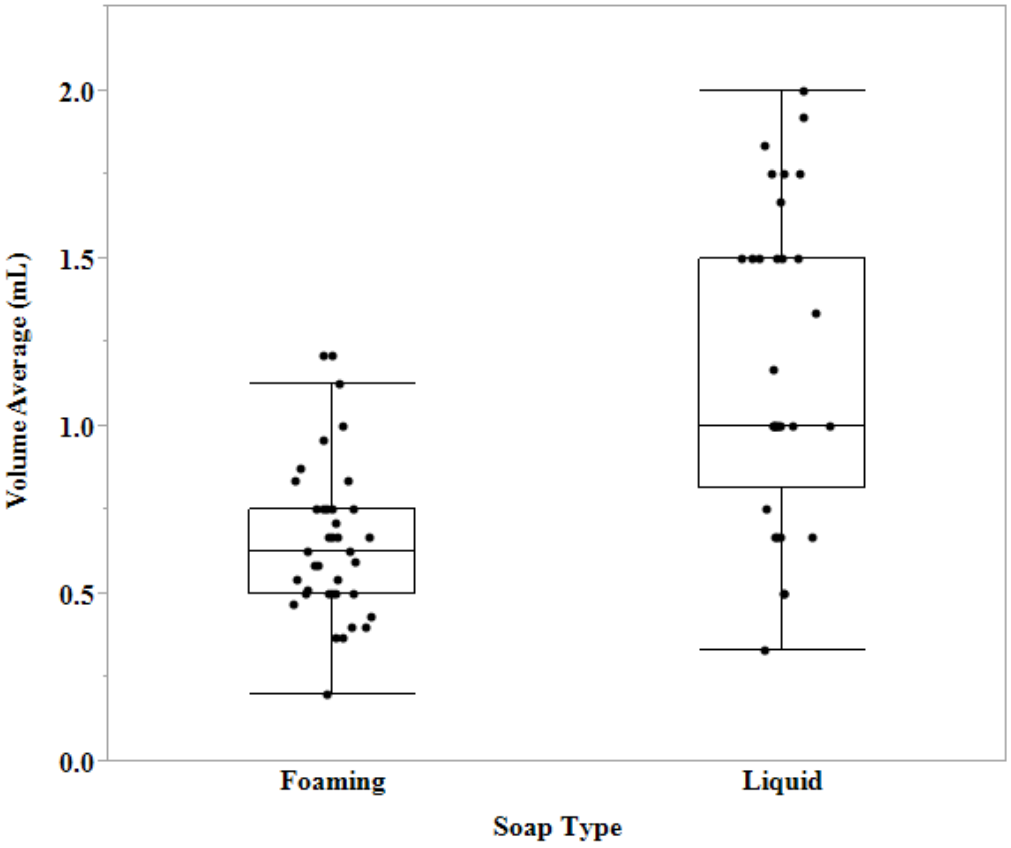

Figure 1 is a boxplot representing the distribution of both foaming and liquid handsoap collected at the 68 different locations in Washington County, Arkansas. The horizontal line in the box represents the median sample values in both foaming and liquid handsoap. The ends of the box represent the 75th and 25th percentiles, or 3rd and 1st quartiles. The interquartile range (IQR) is the difference between the 1st and 3rd quartiles. The whiskers (the area outside the box) extend to the outermost data point within the computed ranges as follows: 3rd quartile + 1.5 × IQR and 1st quartile + 1.5 × IQR. Any points beyond the whiskers are outliers in the data set.

Chapter 4: Investigation of the Impact of Soap Type and Volume on Handwashing Behavior

Abstract

Handwashing (HW) is a tool used daily by the general population as well as the health care and food industries to prevent the spread of disease. Although clear HW guidelines have been established for HW in the food industry, numerous outbreaks due to improper handling by food workers still occur each year. A variety of soaps are used in the food service industry, and more recently, foaming soap was introduced onto the market. However, limited research into the effectiveness of foaming (F) handsoap exists in the published literature when compared to liquid (L) handsoap. Understanding how this soap may effect HW by food service workers is critical to maximizing HW effectiveness and preventing transmission of foodborne illness. The primary objective of this chapter is to understand how soap type impacts HW behavior. To complete this objective, 12 volunteers completed a baseline HW, after hands were dry, volunteers then applied a known amount of Glo Germ™ (GG) fluorescent lotion to their hands and washed their hands without training in proper HW. Following both the baseline and GG HW, hands were swabbed in three locations to recover remaining GG. Swabs were eluted and absorbance was measured at OD_{370nm} and remaining GG was quantified using a standard curve. No significant difference in behavior was determined in terms of GG remaining, HW time in the baseline handwash and post GG handwash, and baseline handrinsing time and post GG handrinse. Average HW time for the baseline handwash was (F) 11.17 ± 3.93 s and (L) 13.83 ± 7.30 s, and for the post GG handwash (F) 13.33 ± 6.22 s and (L) 14.25 ± 7.70 s. While no significant difference in behavior occurred between F and L handsoap, a consistent increase in both wash time and rinse time for L handsoap did occur, indicating that there may be a possible benefit to using liquid handsoap in food service establishments.

1. Introduction:

Handwashing (HW) is one of the primary means to prevent transmission of infectious diseases. While the general population uses HW as a daily tool to limit the spread of disease, it is especially critical within a food service environment (Miller et al. 1994). Throughout the production and preparation of food, food workers are presented with a variety of scenarios in which hand contact (e.g. direct or indirect) can result in the contamination of food with harmful microorganisms. For example, food worker hands' may be contaminated with pathogens from their own gastrointestinal tracts or through contact with objects or food that are contaminated with pathogens (Paulson 2000). Despite the focus on proper HW in the food industry and the establishment of clear guidelines for proper HW via the U.S. Food and Drug Administration (USFDA) 2013 Food Code, food workers are still responsible for the transfer of harmful pathogens to food resulting in a significant contribution to the incidence of foodborne illnesses, especially norovirus (NoV) —the primary cause of foodborne outbreaks (Green et al. 2006, Hall et al. 2012, Scallan et al. 2011). Recently an epidemiologic review of foodborne NoV outbreaks occurring in the U.S. between 2001 and 2008 was conducted, and the authors found that 82% of cases in which at least one food item was implicated involved food handler contact with ready-to-eat (RTE) food, and a food handler was identified as the source of contamination in 53% of outbreaks (Hall et al. 2012).

The 2013 Food Code (section 2-301.12) states that food employees must wash hands as well as exposed portions of the arm for 20 seconds, designating 10-15 seconds of this HW process to vigorous rubbing of the hands (USFDA 2013). Although the U.S. Food Code requires

a 20 second handwash and a minimum of 20 seconds is recommended by numerous other organizations including the World Health Organization (WHO), the Mayo Clinic, and U.S. Centers for Disease Control and Prevention (CDC), people in public restrooms as well as in hospitals often wash hands for 15 seconds or less (Soap and Detergent Association, 2007). A recent study conducted by Burton et al. (2011) found that when participants were instructed to wash their hands as long and as thorough as they normally would do, participants averaged 12 ± 2.8 s for a handwash with water alone and 14 ± 2.3 s for a handwash with water and soap. Meengs et al. (1994) studied HW in an emergency room department and found that the average soap and water handwash was 9.5 s (n = 132). Strohbehn et al. (2008) conducted a study on HW frequencies and procedures used in retail food services and reported that when restaurant employees washed hands before engaging in food preparation, soap was used 61% of the time. Moreover, when using soap, hands were not lathered for the full 10 s, and employees did not dry hands properly 86% of the time.

In order for HW to be as effective as possible, it is essential to understand how all of the different variables associated with HW will affect the end result. Numerous soaps are available on the market today, and food service staff and the general population use these soaps daily. Although liquid handsoaps have been used for a while, foaming handsoaps are relatively new. In 1999, Deb Group Limited introduced the first generic foaming soap system (Deb Group Ltd. 2014) and was described as having a greater convenience, efficiency, and reduced environmental impact when compared to gel-based, or liquid, handsoaps. While these claims may be true, it is important to understand if foaming handsoaps are equally as effective when compared with traditional, liquid handsoaps. Additionally, it is important to determine how people respond to

these foaming handsoaps and to determine if HW behavior changes when different handsoap types are used. Therefore, the primary goal of this objective was to determine if handsoap type (foaming versus liquid) affects HW behavior. I hypothesized that HW behavior will change between foaming and liquid handsoap and predict that participants will wash hands for a shorter period of time when using foaming handsoap as compared to liquid.

2. Materials and Methods:

2.1. Study design

Studies were arranged in a paired t test design. To account for any possible confounding factors, two blocking factors were incorporated into the statistical model. These included sequence and participant. Twelve participants were recruited and completed the study. Two experimental sequences occurred to alternate exposure of participants to soap type and to adjust for any possible confounding factors (e.g., learning by either the researchers or the study participants over the two weeks of the study).

2.2 Participant recruitment

Twelve participants (six men and six women), 18 years and older from the University of Arkansas (Fayetteville, Arkansas) community were recruited as volunteers to wash their hands. Participants were informed that in order to participate, they should have no known conditions of the skin, and also should not have any broken skin. University of Arkansas Institutional Review Board approval was obtained prior to participant recruitment, and all participants signed an informed consent form prior to participation in the study. As this objective was behavioral based,

participants did not receive any training prior to participation, but rather were simply instructed to wash hands as they normally would.

2.3 Selection of soaps

Two unscented, non-antimicrobial soaps were chosen for use in the study. The soaps did not have identical formulations, but were determined to be representative of handsoaps used on the market daily. Two automatic dispensers, one foaming (GOJO Industries, Akron, OH) and one liquid (Epare, Staten Island, New York), were chosen to standardize the soap volume dispensed and allow for ease of observation for the researcher. One dispense of foaming soap was 0.9 mL (after foaming subsided), and one dispense of liquid handsoap was 1.5 mL. Data collected in Chapter 3 determined that the average soap volume at 68 food service locations in Washington County, Arkansas was 0.64 ± 0.22 mL and 1.19 ± 0.46 mL for foaming and liquid handsoap, respectively. Based on these preliminary data, the soap dispensers selected for this study were determined to be representative of the average soap volume used in food service facilities.

2.4 Baseline handwash

On each study day, participants' hands were inspected for any broken skin. Any jewelry or items present on the wrist and hand were removed. Participants then completed a preliminary handwash to remove any possible physical contamination (e.g., residues from hand lotions, biological materials, etc.) present on their hands. Participants did not receive any direction as to how to properly wash their hands. Participants first briefly wet their hands and then they were provided the designated handsoap (either foaming or liquid) to wash their hands. Participants

were instructed to dispense the desired amount of soap into their hands, and the researcher recorded how many pumps of soaps the participant dispensed. After briefly wetting their hands, and then dispensing the soap into the hand, the participant then began lathering their hands. Following the lathering of hands, the participants asked the researchers to turn on the water (distilled tap water) and began rinsing their hands. As one researcher controlled the water for the participant, the second researcher used a stopwatch to track the amount of time it took for the participant to wash and rinse their hands. Following the handwash and rinse, participants were instructed to flick their hands 10 times to remove excess water. Hands were then immediately swabbed in the three locations discussed in Section 2.6, and the swabs were then processed (Section 2.7). Swabs from this step were considered "baseline" swabs.

2.5 Glo Germ™ Handwash

Following the preliminary HW and drying, hands were air-dried for 30 s or until hands appeared visibly dry. Participants were then provided with Glo Germ™ lotion which contains a fluorescing compound approximately the size of bacteria (about 5 microns) (Glo Germ Company, Moab, UT). The lotion is designed to be spread on hands to simulate the presence of microorganisms and serves as a useful tool in training for both the healthcare and food industries (Kilbride et al. 2003, Michaels 2002). Participants were provided with 1.0 ± 0.01 g (i.e. approximately the size of a quarter) of Glo Germ™ lotion as recommended by the manufacturer. Pre-aliquoted lotion on pieces of weigh paper was applied to the hands of participants, and participants thoroughly rubbed the lotion into their hands (both palmar and dorsal sides) for 1 min until evenly distributed and absorbed. Next, hands were air dried for 30 s or until hands

appeared visibly dry. Once dry, participants completed a second handwash as described previously in Section 2.4.

2.6 Swabbing Participant Hands

A study evaluating the HW technique of nurses by Taylor (1978) found that 89% of participants missed some parts of the hand when HW with 56% of participants missing some part of the thumb. Figure 1 illustrates the parts of the hands most frequently missed during HW. Based on these findings, the skin between the thumb and index finger was chosen for swabbing as well as the lower nail bed/skin region of the middle finger and the palmar side of the wrist. After washing, the participants' hands were swabbed using methods described in Gibson et al. (2013) with modifications. To quantify the amount of Glo Germ™ remaining on hands after washing, sterile, foam tipped swabs (VWR, Radnor, PA) were placed in a 15 mL centrifuge tube containing 2 mL of 95% ethanol, and both hands were swabbed in 3 locations (Figure 2A-C) using one swab for each location (i.e. 3 swabs total for each participant). Samples were then processed within 30 min of sampling (Section 2.7).

2.7 Determination of Sample Absorbance

The three swabs were placed in their respective tubes and vortexed for 60 s to elute any Glo Germ™ recovered from the participants' hands. The sample was placed in a disposable acrylic cuvette (VWR), and the absorbance of each swab was measured at an optical density of 370 nm by a DU® 640 spectrophotometer (Beckman Coulter, Inc., Brea, CA). The values from each of the three swabs were combined to create a total fluorescence concentration for each participant.

Prior to conducting this study, a standard curve of the Glo Germ™ (Figure 3) fluorescent lotion was created by preparing a two-fold dilution of the fluorescent lotion in 95% ethanol and subsequently measuring the absorbance at 370 nm with a DU® 640 spectrophotometer (Beckman Coulter, Inc.). Concentration (y) of fluorescent compound remaining on participants hands was determined by using the absorbance (x) measurement for each participant, and the slope (m) and intercept (b) of the fit line of the standard curve through use of the slope intercept equation ($y = mx + b$).

2.8 Statistical Analysis

JMP® Pro 11 (SAS, Cary, NC) statistical analysis software was used for all data analysis. Difference in concentration between the post Glo Germ™ handwash and the baseline handwash was calculated by first subtracting the total absorbance for the baseline absorbance from the total absorbance for the post Glo Germ™ handwash. This value was then inserted into the slope-intercept equation (Section 2.7) to solve for the total concentration. As the study was based on a paired t test design, a fit model was used to test for significant differences in total concentration of Glo Germ™ remaining. Sequence and week were incorporated into the model to account for any variance that may have occurred due to learning by either the participants or researchers throughout the study. Gender was also added as an effect in the model. Results were considered statistically significant at $p \leq 0.05$.

3. Results

All 12 participants completed the study within a two-week period. Participant age ranged from 19 to 72 years with an average age of 30 years old. With respect to concentration of Glo

Germ™ fluorescent compound remaining on hands, no significant difference between foaming and liquid handsoap occurred when the mean differences of the concentration of Glo Germ™ remaining between the post Glo Germ™ and baseline handwash were compared ($p = 0.35$). Gender also did not have a significant impact on the difference of the concentration of Glo Germ™ remaining between the post Glo Germ™ and baseline handwash ($p = 0.40$).

Wash time data are summarized in Table 1. No significant difference between foaming and liquid handsoap occurred in terms of wash time for both the baseline ($p = 0.29$) and the post Glo Germ™ handwash ($p = 0.77$). Gender did not have a significant impact on wash time for the baseline ($p = 0.89$) or the post Glo Germ™ handwash ($p = 0.53$). Table 2 summarizes rinse time data. No significant difference between foaming and liquid handsoap occurred in terms of rinse time for both the baseline ($p = 0.056$) and the post Glo Germ™ handwash ($p = 0.43$). Gender did not have a significant impact on wash time for the baseline rinse ($p = 0.48$) or the post Glo Germ™ hand rinse ($p = 0.34$).

The minimum number of pumps dispensed for the baseline handwash for both foaming and liquid handsoap was 1 pump, while the maximum was 2 pumps. The minimum number of pumps dispensed for the post Glo Germ™ handwash for foaming and liquid handsoap was 1 pump, while the maximum was 3 and 2 pumps, respectively. No significant difference between foaming and liquid handsoap occurred with respect to the number of pumps of soap for either the baseline ($p = 0.35$) and the post Glo Germ™ handwash ($p = 0.29$). Gender did not have a significant impact on the amount of soap dispensed in the baseline ($p = 0.072$) or in the post Glo Germ™ handwash ($p = 0.29$).

Prior to conducting this study, another study with 12 different participants (4 men and 8 women) was conducted. Here, no baseline handwash was completed. The average wash time for foaming and liquid handsoap was 13.58 ± 6.14 s and 15.33 ± 6.56 s, respectively. Average rinse time for foaming and liquid handsoap was 9.08 ± 4.08 s and 10.92 ± 4.80 s, respectively. No significant difference between foaming and liquid handsoap occurred in terms of wash time ($p = 0.26$), rinse time ($p = 0.22$), and total concentration Glo Germ™ remaining ($p = 0.92$). Gender did not have a significant effect on total concentration of Glo Germ™ remaining on hands ($p = 0.29$) or on wash time ($p = 0.74$) or rinse time ($p = 0.88$). No participants used more than 1 pump of the liquid handsoap; however, five and six participants used 1 and 2 pumps of foaming handsoap, respectively, while one participant used 4 pumps of foaming handsoap. No significant difference was found in the concentration of Glo Germ™ remaining when number of pumps of soap used changed ($p = 0.68$).

4. Discussion

Comparison of foaming and liquid handsoap, especially concerning behavior in response to soap type, is extremely limited in the published literature. To our knowledge, no studies investigating the effects of soap type on HW behavior are available in the published literature. Overall, the results of the present study indicate that no significant difference exists in behavior in terms of HW time, rinsing time, and amount of soap dispensed.

Despite the finding that no significant difference appears to exist in HW time and rinsing time between foaming and liquid handsoap, on average participants consistently washed and rinsed hands for a longer amount of time with liquid handsoap as compared to foaming

handsoap. However, it is important to note that while no statistically significant difference was found in wash times or rinse times between the two soaps at an alpha level of 0.05, the p-value for the baseline hand rinse at 0.056 is very close to alpha (0.05), indicating that it is nearly significant. Having a p-value so close to alpha calls into question the potential for the finding of a significant result, particularly if a larger sample size were used, resulting in increased statistical power. While the lathering of soap in hands is a large component of the removal of microorganisms from hands, rinsing adds additional physical removal of bacteria through the water flow, primarily for transient microorganisms, (Price 1938, Todd et al. 2010). Miller et al. (2011) conducted a study on reduction of *Escherichia coli* on skin with three different methods of HW: 1) running water; 2) running water and friction; and 3) running water with friction and soap. The authors found that simply holding contaminated hands passively under running water was ineffective, but the friction from energetically rubbing hands together while holding hands under water improved the decontamination of hands (Miller et al. 2011). Although the additional reduction in microorganisms due to rinsing of hands is unclear in the published literature, a significant increase in rinsing time between foaming and liquid handsoap could present an opportunity for increased removal of microorganisms from hands; therefore, additional investigation into the impact of soap type on rinse time may be warranted.

One inherent limitation with the present study is that it is an observational study. While participants were not informed as to what researchers would be observing and recording prior to their participation, it is entirely possible that participants inferred what was being studied and then altered their HW behavior accordingly. It is known that people are responsive to the presence of an outside observer (Ram 2013). Ram et al. (2010) conducted an observational

study in Bangladesh, and the researchers embedded acceleration sensors within the soap to assess the reactivity of participants to structured observation. Results of the study indicated that on days participants were observed, the use of the sensored soap increased by approximately 35%. This led the authors to conclude that individuals substantially alter HW behavior in the presence of an observer, particularly in participants who are aware of the social expectations of hand hygiene (Ram et al. 2010). However, structured observation is still a commonly used method in HW research, though it does require caution when interpreting data (Ram 2013). Even taking this possible observation bias into consideration, the mean HW times for both foaming and liquid handsoap in the present study are still well below the recommended 20 s HW time, indicating that this time is more than likely not being met on a daily basis by the general population. Additionally, although this cannot be determined, the possibility exists that if behavior was changed because of the presence of an observer, that behavior would be equally changed for both soap types, negating the effect.

As mentioned previously, an additional limitation of the present study is that of sample size. Our study utilized the observations of 12 individuals (6 males and 6 females) over two weeks. Although a significant difference in HW time was not found, a slight increase in HW time consistently occurred for liquid handsoap, and it is possible that an increased sample size would result in decreased variance and a corresponding finding of a significant difference in HW behavior in response to soap type. Throughout the published literature, numerous studies have low statistical power because of inadequate sample size or issues in the experimental design (Eng 2003, Freiman et al. 1978). Freiman et al. (1978) conducted a review of published clinical literature (n = 71) with negative results and found that the alpha and beta remained almost

completely unmentioned in the studies reviewed; thus, concluding that many of the "negative" trials had the potential to be false negatives. For many of these trials, an inadequate sample size was chosen leading to a low power, and an increased possibility that a false negative conclusion was observed. In my study, after consulting with a statistician, it was determined that the sample size (n=12), though somewhat low, would be chosen because of allotment of available resources.

In originally setting up this experiment, one of the primary goals was to use Glo Germ™ as an indicator to determine if HW behavior was impacted by soap type (foaming versus liquid). Glo Germ™, a lotion with a fluorescent compound, is more commonly used to qualitatively observe HW behavior (Benoit et al. 2015, Biran, et al. 2009 Ling et al. 2012). Typically, participants rub the fluorescent lotion onto their hands and then wash their hands. After washing their hands, participants view their hands under a blacklight in order to visualize where the fluorescent compound is remaining, thus indicating that these are the areas missed during HW. Through this experiment, my goal was to take this qualitative tool and apply it in a quantitative manner to determine if amount of the fluorescent compound remaining on hands would correlate with a behavioral difference in participants between the two soap types. Establishment of a standard curve allowed us to determine concentration of the fluorescent compound remaining on participants' hands. However, the remaining fluorescent compound on hands proved to be a difficult tool to use as a measure of HW behavior since concentration of fluorescent compound remaining on participant's hands was rather low, and the concentration remaining varied greatly from person to person.

Gibson et al. (2013) used Glo Germ™ powder fluorescent compound in a mock retail deli environment to understand the impact of workers on cross contamination as well as to identify critical areas of contamination. Similar to the present study, the results found by Gibson et al. (2013) were rather variable for each participant, but the researchers were able to determine the areas within the deli environment that were more likely for cross-contamination. Shaw et al. (2015) used Glo Germ™ lotion to understand the spread of cross contamination by field workers in a strawberry field not only on workers (from head to toe), but also onto the strawberries and in the strawberry field. In this particular study, once nightfall was reached, pictures were taken of field workers and the field to visualize where contamination took place. While this study was not quantitative in terms of the amount of Glo Germ™ transferred to surfaces, it provided insight into the quantity and spread of cross contamination that can occur during harvest (Shaw et al. 2015). Benoit et al. (2015) also used Glo Germ™ to quantitatively determine the spread of *Listeria monocytogenes* between deli meats and product contact surfaces. In this study, Glo Germ™ was spread on deli meats, and the transfer to product contact surfaces was measured by photographing surfaces under UV-light and using image processing software to quantitatively analyze the photos. These data were then compared to *L. monocytogenes* transfer data collected under equivalent conditions. The authors found that Glo Germ™ was a reasonable surrogate to rapidly quantify simulated *L. monocytogenes* cross-contamination (Benoit et al. 2015). While Glo Germ™ can serve as a beneficial tool in HW training, based on my experiment, it appears to be much more useful as a qualitative tool rather than as a quantitative tool, especially considering the large amount of variance that can occur between participants. Time spent HW and rinsing proved to be more reliable measurements of HW behavior in this experiment.

5. Conclusions

Known as one of the most effective methods in preventing disease transmission, the importance of HW is especially critical in the food service industry as food handlers have a multitude of contact points in the food preparation chain in which contaminated hands can contaminate the food supply (Miller et al. 1994, Paulson 2000). Despite recommendations by the WHO and CDC that HW should occur for a minimum of 20 s and strict guidelines established by the USFDA in the Food Code, researchers have shown repeatedly that these guidelines are often not being met, and people in daily life as well as in the healthcare industry and the food industry are often washing hands for less time than the recommended 20 s handwash (Burton et al. 2011, Meengs et al. 1994, Soap and Detergent Association 2007, Strohbehn et al. 2008). Foaming handsoap is relatively new in the world of HW, and to my knowledge, limited research in the published literature comparing the effectiveness of foaming to liquid handsoap exists. Additionally, no studies have been conducted on the impact of soap type (foaming or liquid) on HW behavior. It is essential to understand if HW behavior changes in response to the type of soap used, as a significant change in HW behavior could result in a significant change in effectiveness of soap in removing microorganisms during HW. The results of my study indicate that no significant difference exists in HW time or rinsing time between foaming and liquid handsoap.

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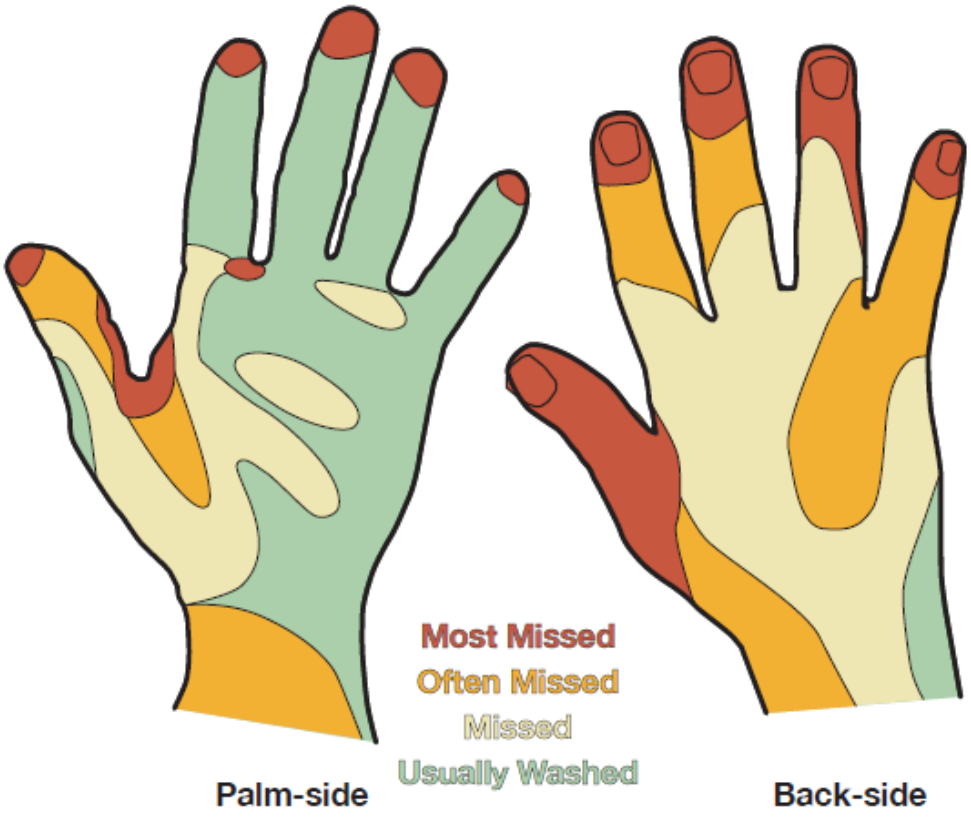
Table 1: Time spent washing hands

Time spent washing hands (s)			
		Foam	**Liquid**
Baseline Hand Wash	Min	5	6
	Max	18	24
	Mean	11.17	13.83
	Std Dev	3.93	7.30
	p value	0.29	
Post Glo Germ™ Hand Wash	Min	7	6
	Max	30	24
	Mean	13.33	14.25
	Std Dev	6.23	7.70
	p value	0.77	

Table 2: Time spent rinsing hands

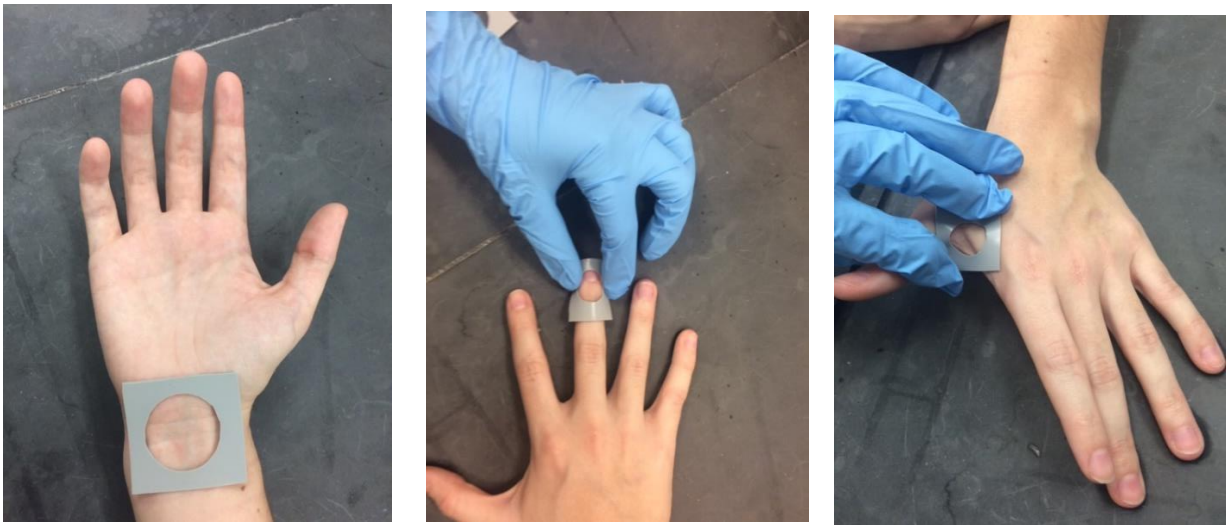
Time spent rinsing hands (s)			
		Foam	**Liquid**
Baseline Hand Rinse	Min	3	6
	Max	15	19
	Mean	7.42	10.75
	Std Dev	3.32	4.33
	p value	0.056	
Post Glo Germ™ Hand Rinse	Min	5	4
	Max	20	24
	Mean	9.58	11.33
	Std Dev	4.10	5.84
	p value	0.48	

Figure 1: Areas of the hands most frequently missed during hand washing.



Source: *HandwashingforLife® 2004*

Figure 2: Areas of the hand swabbed with standard templates



(a) **(b)** **(c)**

Figure 2: Locations of the hand swabbed with standard templates. (a) depicts the palmar side of the wrist. (b) depicts the lower half of the nail/upper portion of the middle finger. (c) depicts the skin between the thumb and index finger on the ventral side of the hand.

Figure 3: Glo Germ™ Standard Curve

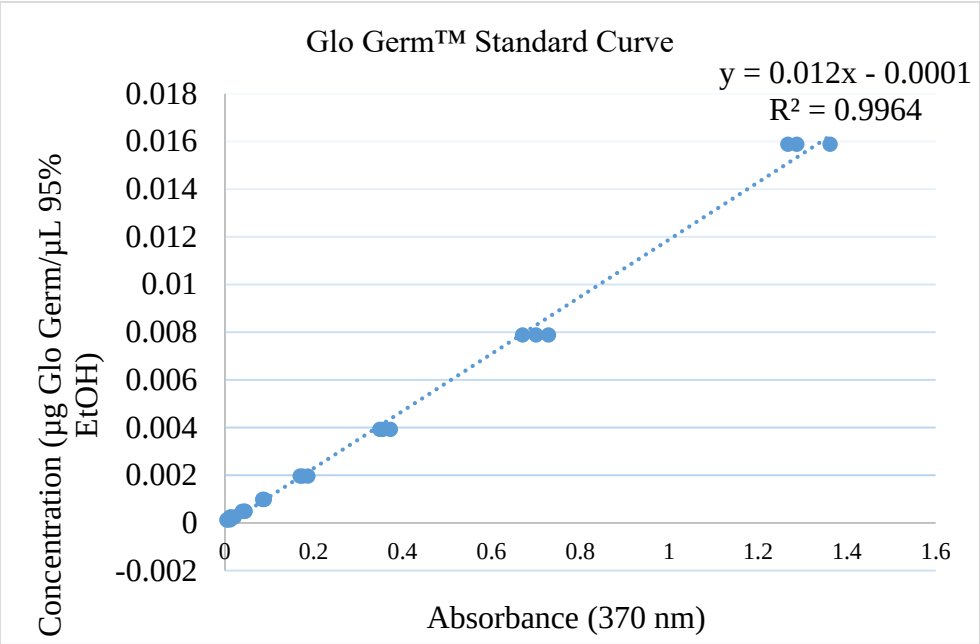


Figure 3: Standard curve made by creating a two-fold dilution of Glo Germ™ lotion in 95% ethanol (EtOH) and measuring the absorbance at 370nm. Slope equation of standard curve was used to solve for concentration (y) of Glo Germ™ fluorescent compound remaining on hands after absorbance (x) was measured for each participant.

Chapter 5: Comparison of two plain soap types for removal of bacteria and viruses from hands with specific focus on food service environments

Abstract

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

1. Introduction

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

The general population uses HW as an important step in disease prevention, and this is especially critical within a food service environment (Miller, James-Davis, & Milaneis, 1994).

The hands of food service employees may become contaminated with foodborne pathogens during critical stages in food service including after using the restroom, handling raw materials (e.g., meats, vegetables, eggs, etc.) and after touching contaminated surfaces (Miller, James-Davis, & Milaneis, 1994). Therefore, studies on the efficacy of HW agents are essential to ensure that HW procedures are optimized for removal of pathogenic microorganisms from hands during food service.

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

readily as liquid handsoap. Meanwhile liquid handsoaps typically have increased surfactant levels as well as additional salts that allow for the formation of micelles (personal communication provided by M. Caetta, VCI Formulation Specialist at GOJO Industries, Inc.) that aid in the removal of dirt and oils as well as microorganisms.

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

2. Materials and Methods

2.1. Study design

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

participants or any carryover effects that could potentially be present throughout the four weeks of the study).

2.2 Participant recruitment and training

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

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

2.3 Preparation of inocula

2.3.1. Preparation of MS2 bacteriophage

A stock of MS2 bacteriophage (ATCC 15597-B1; American Type Culture Collection, Manassas, VA) was prepared through propagation in *E. coli* C3000 followed by chloroform extraction of the infected cell lysate as described previously by Gibson, Crandall, & Ricke (2012). The stock concentration of MS2 bacteriophage was determined to be approximately 10^{11} PFU/mL by the double agar layer (DAL) method. One milliliter aliquots of MS2 were stored at -80ºC. The phage stock was diluted with 0.1% peptone (Becton Dickinson and Company, Sparks, Maryland) to approximately 6.78×10^8 PFU/mL.

2.3.2. Preparation of E. coli C3000

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

2.4. Hand decontamination prior to inoculation

To eliminate resident microorganisms on the hands of participants prior to inoculation with test organisms, hands were treated with a conditioning wash as described by Fuls et al. (2008) with modifications. Modifications included using 1 pump of antibacterial handsoap (The Dial Corporation, Scottsdale, Arizona) with subjects scrubbing hands for 30 s and rinsing hands

for 10 s. Hands were also twice soaked in 70% ethyl alcohol and dried thoroughly before inoculation with microorganisms.

2.5. Inoculation of hands

Hand inoculation of *E. coli* C3000 and MS2 was performed by the palmar surface method (PSM) as described in the ASTM Standard Test Method E2870-13 with modifications. One-hundred microliters of prepared *E. coli* or MS2 inoculum were pipetted onto the palm of each hand (200 µl total) for an average of 1.25×10^8 CFU total (hands combined) or 1.36×10^8 PFU total (hand combined), respectively. The participants were asked to rub the palms and fingers of each hand against each other for 10 ± 1 s in order to spread the inoculum on the palms and fingers of each hand. Following inoculation, the hands were air-dried for 20 ± 5 s.

2.6. Selection of soaps

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

2.7. Handwashing and drying

After allowing the inocula to dry, the participants were immediately asked to wash their hands with one of the test soaps using the method described in Section 2.2. Following the 10 s

handwash, hands were rinsed for 10 s. Participants then dried hands for 10 s with three paper towels, and hands were immediately sampled for recovery of microorganisms using the glove juice method (GJM; Section 2.8.).

2.8. Recovery of microorganisms by GJM

Remaining microorganisms were recovered using the GJM described in ASTM E1174-13 with modifications (ASTM 2013b). Modifications included using 35 mL of 0.1% peptone (Becton Dickinson and Company) sampling solution in each glove. As the soaps used did not contain antimicrobial agents, no neutralizers were added to the sampling solution. An additional modification included recovering all of the remaining liquid from each glove and transferring to a sterile, glass sample jar (VWR) and using a portion of the remaining solution to dilute and plate. Volumetric adjustments were made based on total volume recovered (Equation 1).

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

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

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

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

Equation 4: $LR = \log10 (I_{RE}/R_M)$

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

2.9. Detection of microorganisms in recovered sampling solution

For *E. coli* C3000, 1 mL aliquots of each dilution were plated onto 3M Petrifilm™ *E. coli*/coliform count plates (3M) in duplicate and incubated at 37°C for 24 h. After 24 h, CFU were counted. A negative control of 1mL of 0.1% peptone was plated as well as a positive control of 1 mL of *E. coli* C3000 at a concentration of 10^1 to 10^2 CFU/mL. For MS2, the DAL method was used as described previously (Gibson, Crandall, & Ricke, 2012). Following incubation for 16-24 h at 37ºC PFU were counted, and the PFU/mL were determined. A negative control with 100 µL of log phase *E. coli* C3000 plus 100 µL of 0.1% peptone was analyzed in each set of experiments. A positive control of 100 µL of 1×10^2 PFU/mL MS2 and 100 µL of log phase *E. coli* C3000 was also performed.

2.10. Statistical Analysis

JMP® Pro 11 (SAS, Cary, NC) statistical analysis software was used for all data analysis. As the study was primarily based on a Latin Square design, with elements of a split plot design, a mixed model was used to test the significance of differences that occurred in reduction of

microorganisms for each soap type (foaming or liquid) as well as for each microorganism type (*E. coli* or MS2). Sequence and week were incorporated into the model to account for any variance that may have occurred due to learning by either the participants or researchers throughout the study as well as for any potential cross-over effect that may have occurred between weeks of the study. Gender was also added as an effect in the model to determine any significance. Multiple comparison of the interaction means was performed with Tukey's honest significant difference (HSD) test. Results were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Efficacy of foaming and liquid handsoaps

All 24 participants completed all 4 weeks of the study. The average age of the participants was 31 years of age. As stated in Section 2.5, the initial inoculum on hands was on average 1.25×10^8 CFU *E. coli* C3000 and 1.36×10^8 PFU MS2. Table 1 reports the average total log reductions for *E. coli* C3000 and MS2 on hands for foaming handsoap, liquid handsoap, and the baseline wash with water. However, this baseline water only wash was not considered a treatment in the mixed model used for statistical analyses (Section 2.10).

3.2. Factors influencing HW efficacy

Statistical analysis of log reductions indicates no significant difference in efficacy of foaming and liquid handsoap for overall microbial removal ($p = 0.56$). However, the reduction of microorganisms on hands was significantly different depending on the type of microorganism with increased reductions occurring for hands inoculated with *E. coli* C3000 as compared to hands inoculated with MS2. Based on Tukey's HSD test, MS2 reduction was significantly less

with foaming soap when compared to *E. coli* reductions with foaming soap ($p = 0.0008$). Table 1 shows the comparison of log reduction for each microorganism by treatment. Gender did not have a significant impact on the reduction of microorganisms on hands ($p = 0.44$).

4. Discussion

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

Although the exact reasons behind these differences in reduction are not clear, it is known that MS2 particle size (24-27 nm)—and human enteric viruses in general—is much smaller than that of bacteria such as *E. coli* (500 nm in diameter) (Strauss & Sinsheimer, 1963; Abbsazadegan, Mayer, Ryu, & Nwachuku, 2007). In addition, MS2 bacteriophage is strongly hydrophobic and this property impacts its attachment to particulates (Bales, Li, Maguire, Yahya, & Gerba, 1993; Shields & Farrah, 2002). With respect to lower reductions in MS2 when using foaming soap when compared to *E. coli*, a possible theory is that the increased surfactant levels of liquid soap create conditions in which the smaller, hydrophobic MS2 particles are surrounded by micelles and are more readily removed from the hands. Conversely, the decreased surfactant concentrations of the foaming handsoap creates conditions that do not allow for the same micelle formation (personal communication provided by M. Caetta, VCI Formulation Specialist at GOJO

Industries, Inc.); therefore, limiting the ability to surround the particles with the micelle structure and making it difficult to remove the smaller, hydrophobic MS2 particles present on hands.

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

With respect to MS2 removal reported in previously published studies, Sickbert-Bennett et al. (2005) observed that tap water was actually the most effective for removal of MS2 from hands with 1.89 to 2.56 log PFU reduction followed by plain, liquid soap with 1.54 to 2.03 log PFU reduction. A baseline wash with tap water was completed in the present study, and although this was not technically a 'treatment', the results reported here for tap water (1.20 log PFU reduction) do not corroborate the findings by Sickbert-Bennett et al. (2005) while similar results for plain, liquid handsoap are reported (2.23 log PFU reduction). Soap volume is one difference between the present study and that of Sickbert-Bennett et al. (2005). The authors of that study used 3 mL of liquid handsoap with a 10 s handwash, while the present study used half this amount of soap (1.5 mL) and a 10 s handwash. Although both studies used a 10 s handwash time, the difference in soap volumes may have an effect on the differences in reduction of MS2 by plain, liquid handsoap occurring between the two studies.

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

Compared to studies on HW efficacy against bacteria, very few studies have been reported on the reduction of human enteric viruses or viral surrogates achieved through HW with either liquid or foaming handsoaps (Ansari, Sattar, Springthorpe, Wells, & Tostowaryk, 1989; Lin et al., 2003; Liu, Yuen, Hsiao, Jaykus,, & Moe, 2010; Mbithi, Springthorpe, & Sattar, 1993; Sickbert-Bennett et al. 2005; Stowell et al., 2014). Liu, Yuen, Hsiao, Jaykus, & Moe (2010) investigated the efficacy of liquid soap for removal norovirus, using two different finger pad protocols—the standard ASTM method (E 1838-2) and a modifications of this method. The average log reductions achieved with a liquid handsoap containing 0.5% triclosan were 0.67 and 1.10 log for the standard and modified ASTM methods, respectively. Even though the described study used a liquid handsoap containing an antimicrobial agent, the reported log reductions were

lower than those reported in the present study and more similar to the baseline water only wash. Numerous differences occurred between the present study and that of Liu, Yuen, Hsiao, Jaykus, & Moe (2010) including microorganisms selected, method of detection (molecular detection versus plaque assay for infectivity), and the HW treatment method.

Lin et al. (2003) compared HW techniques to remove *E. coli* and caliciviruses under natural fingernails. For nails inoculated with *E. coli*, reductions of 1.18 log CFU were reported for both tap water and plain, liquid soap, respectively; meanwhile, reductions of feline calicivirus (FCV) were 1.97 and 1.82 log PFU for tap water and plain, liquid soap, respectively. Reductions reported by Lin et al. (2003) for FCV are comparable to the reductions reported for MS2 in the present study, while the reductions achieved for *E. coli* are quite a bit lower than the reductions reported in the present study. While the palmar surface method of inoculation was applied in the present study, Lin et al. (2003) utilized contact of the underside of the nail with inoculated ground beef to simulate real-life contamination of fingernails which may explain the difference in log reduction values. Additional studies evaluating the efficacy of various HW agents including plain, liquid soap in removal of enteric viruses (i.e. hepatitis A virus, poliovirus type 1, human rotavirus) from hands have reported log reduction values ranging from 1 to 1.2 logs (Ansari, Sattar, Springthorpe, Wells, & Tostowaryk, 1989; Mbithi, Springthorpe, & Sattar, 1993). Compared to the present study, these log reduction values are nearly 1-log lower than those reported for MS2 in the present study and are more similar to the baseline water wash removal. Again, many of these differences can be explained by variations in methodology even though several standard methods for the evaluation of HW agents exist both in the U.S. and the European Union (E.U.).

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

Here, it is important to acknowledge some of the limitations of the present study. In food service facilities, food handlers' hands are potentially covered with numerous organic materials including oils and food particles as well as both resident and transient microorganisms. However, to eliminate unnecessary variables for the initial evaluation of foaming versus liquid handsoap participant hands were decontaminated at the beginning of each study day and then inoculated with only one test microorganism at a time. Based on this, one limitation of the present study is that the experimental design does not replicate the conditions that would be present in a food service facility. To address this, future research on the effect of additional organic material on hands on the reduction of both viruses and bacteria on hands when washed with foaming and liquid handsoap would be beneficial. An additional limitation is the use of a short HW time in our study (10 s wash and 10 s rinse). This HW time was selected to be more representative of what has been observed and reported in the peer-reviewed literature as well as based on the authors' experience (Graham 1990; Meengs, Giles, Chisholm, Cordell, & Nelson, 1994; Munger

& Harris, 1989; Sickbert-Bennett et al. 2005; Strohbehn, Sneed, & Meyer, 2008). However, it should be noted that the FDA *Food Code 2013* recommends a 20 s HW time with vigorous scrubbing for 10 to 15 s therefore the 20 s may include the rinse time as well. A final, potential limitation is the low recovery efficiency of the GJM. In order to keep the recovery solution in the glove, a volume of 35 mL was chosen for each glove. This lower volume may have had an impact on the recovery efficiency. However, as discussed in section 2.8, recovery efficiency was incorporated into the overall log reduction that occurred on hands to account for this. Moreover, previously published studies that utilized a GJM rarely report recovery efficiency, and because of this we were unable to accurately compare our recovery efficiency with that of other studies.

5. Conclusions

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

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Table 1: Comparison of log reduction by treatment

Treatment	Total Log_{10} CFU or PFU Reduction (± SD)		
	Microorganism		
	E. coli	**MS2**	**p-value**
Foaming	2.76 (0.70)	2.10 (0.57)	0.0008
Liquid	2.52 (0.58)	2.23 (0.51)	0.079
Water	2.45 (0.93)	1.20 (0.49)	<0.0001

Chapter 6: Overall Conclusions

For quite some time now, handwashing (HW) has been accepted as a method to prevent disease transmission not only in the general population, but especially in select industries including the health care field and the food service industry. The food service industry has established strict guidelines for employees to follow to prevent the transmission of harmful pathogens to consumers. One such guideline is the United States Food and Drug Administration's (FDA) Food Code which details the steps needed for completion of a thorough and appropriate handwash, as well as specific instances in which food service workers will need to wash their hands (after using the restroom, switching between raw and ready-to-eat food, etc.) (USFDA 2013).

Regardless of strict guidelines on proper HW, food service workers often fail to comply with the established policies, and numerous foodborne illness outbreaks occur yearly because of inadequate HW (Green et al. 2006). One important step in reducing foodborne illness caused by food handlers is to understand and correct this lack of compliance. An additional method to reduce foodborne illness is to understand and optimize the various factors that impact the HW process (Conover and Gibson 2016). Soap type (foaming (F) versus liquid (L)) is once such variable that may impact the HW process. To my knowledge no research studies have been conducted comparing the effectiveness of foaming and liquid handsoap. Foaming handsoap is relatively new in the realm of HW with the first generic system introduced in 1999 by Deb Group Limited.

The primary objectives of my research were to I) conduct a survey of soap type and soap volume in food service establishments in Washington County, Arkansas II) to determine if handsoap type (foaming versus liquid) affects HW behavior and III) to determine if a difference exists in the effectiveness of non-antimicrobial F and L handsoap by measuring the reduction of microorganisms on hands inoculated with non-pathogenic E. coli and MS2 bacteriophage – a surrogate for the study of human enteric viruses such as norovirus.

In order to begin this research, a list of 75 food service locations in Washington County, Arkansas was generated based on a set of exclusion criteria as well as random number generations. Soap samples were collected from these food service locations to determine average soap volume as well as prevalence of soap type. Soap samples were collected from each location in triplicate (three "pumps" from the same soap dispenser on the same day), and the volume was measured. Sixty-eight of the 75 locations were sampled. I determined that 54.4% (n = 37) and 47.06% (n = 32) had F and L soap types, respectively. The average volume of F and L handsoap was 0.64 ± 0.21 mL and 1.19 ± 0.46 mL, respectively. This information determined the selection of representative F and L handsoap volumes used in chapters 4 and 5.

In order to understand HW behavior, 12 volunteers completed a series of handwashes in which no training in proper HW was given. A baseline handwash was completed as the participant entered the lab, while the second handwash involved the application of a known amount of Glo Germ™ (GG) fluorescent lotion to hands prior to the handwash. Following both the baseline and GG HW, hands were swabbed in three locations to recover remaining GG. Swabs were eluted and absorbance was measured at OD_{370nm} and remaining GG was quantified

using a standard curve. No significant difference in behavior was determined in terms of GG remaining, HW time in the baseline handwash and post GG handwash, and baseline handrinsing time and post GG handrinse. Average HW time for the baseline handwash was (F) 11.17 ± 3.93 s and (L) 13.83 ± 7.30 s, and for the post GG handwash (F) 13.33 ± 6.22 s and (L) 14.25 ± 7.70 s. While no statistically significant difference in behavior occurred between F and L handsoap, a consistent increase in both wash time and rinse time for L handsoap did occur, indicating that there may be a possible benefit to using liquid handsoap in food service establishments. Additional research in this area may be beneficial to determine if this difference in wash time has an impact on microbial reductions.

Finally, in order to compare the effectiveness of foaming and liquid handsoap, hands of 24 participants were inoculated by the palmar surface method with *Escherichia coli* C3000 or MS2 bacteriophage. Participants washed their hands following a standard protocol with a standardized soap volume and a 10 s HW time. Following this, remaining microorganisms were recovered from hands and remaining microorganisms were quantified by standard spread plate and plaque assays for *E. coli* and MS2, respectively. Hands inoculated with *E. coli* had an average log reduction of 2.79 ± 0.71 and 2.52 ± 0.58 log CFU for foaming and liquid handsoap, respectively. The mean log reduction for hands inoculated with MS2 was 1.98 ± 0.60 and 2.26 ± 0.49 log PFU for foaming and liquid handsoap, respectively. These data indicate that there is no significant difference in the effectiveness between foaming and liquid handsoap in overall microbial removal. However, regardless of soap type, the type of microorganism impacted overall log reduction, with a greater log reduction occurring for hands inoculated with *E. coli* as

compared to hands inoculated with MS2. Future research into impact of soap type will be beneficial to understand the potential decrease in reduction occurring for viruses with foaming handsoap.

Overall, this research has provided insight into the impact different types of soap (F versus L) have on handwashing behavior, as well as the difference in effectiveness between F and L handsoap on microbial reduction on hands, particularly when focusing on the removal of bacteria and viruses from hands. Additional research will be needed to better understand the potential impact the difference in wash times between F and L handsoap has on microbial reduction as well as the effect of F and L handsoap on bacteria and virus removal on hands and the potential impact this may have on the food service industry.

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Appendix

Figure 1: Schematic of experimental design (Chapter 5):

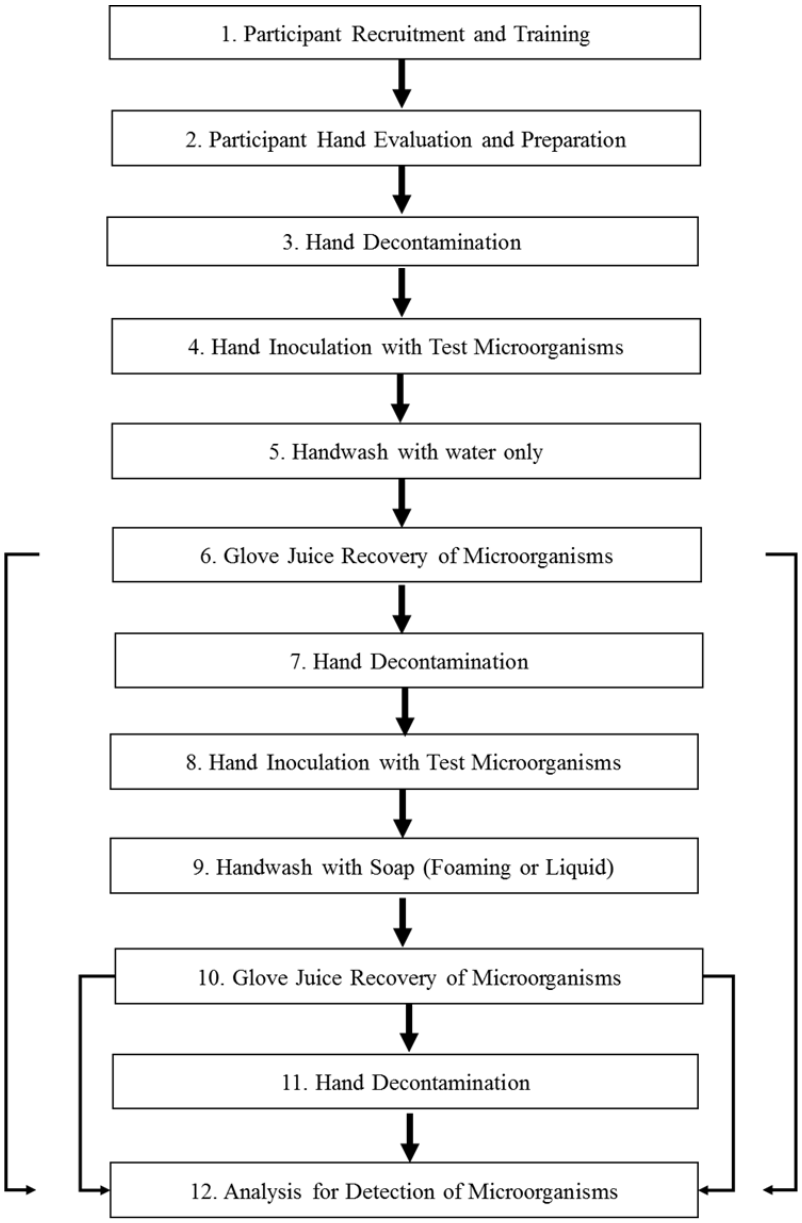


Figure 1: Schematic of experimental design of Chapter 5. Step 1 was completed before participation in the study began. Steps 2-12 were completed each time (4 times total) a participant completed the experiment.

IBC Approval Letter:

UNIVERSITY OF ARKANSAS

Office of Research Compliance

April 13, 2015

MEMORANDUM

TO: Dr. Kristen Gibson

FROM: W. Roy Penney
Institutional BioSafety Committee

RE: IBC Protocol Approval

IBC Protocol #: 15013

Protocol Title: "Comparative Efficacy of Foaming and Non-Foaming Handsoap in Reduction of Microorganisms in Handwashing "

Approved Project Period: Start Date: April 9, 2015
Expiration Date: April 8, 2018

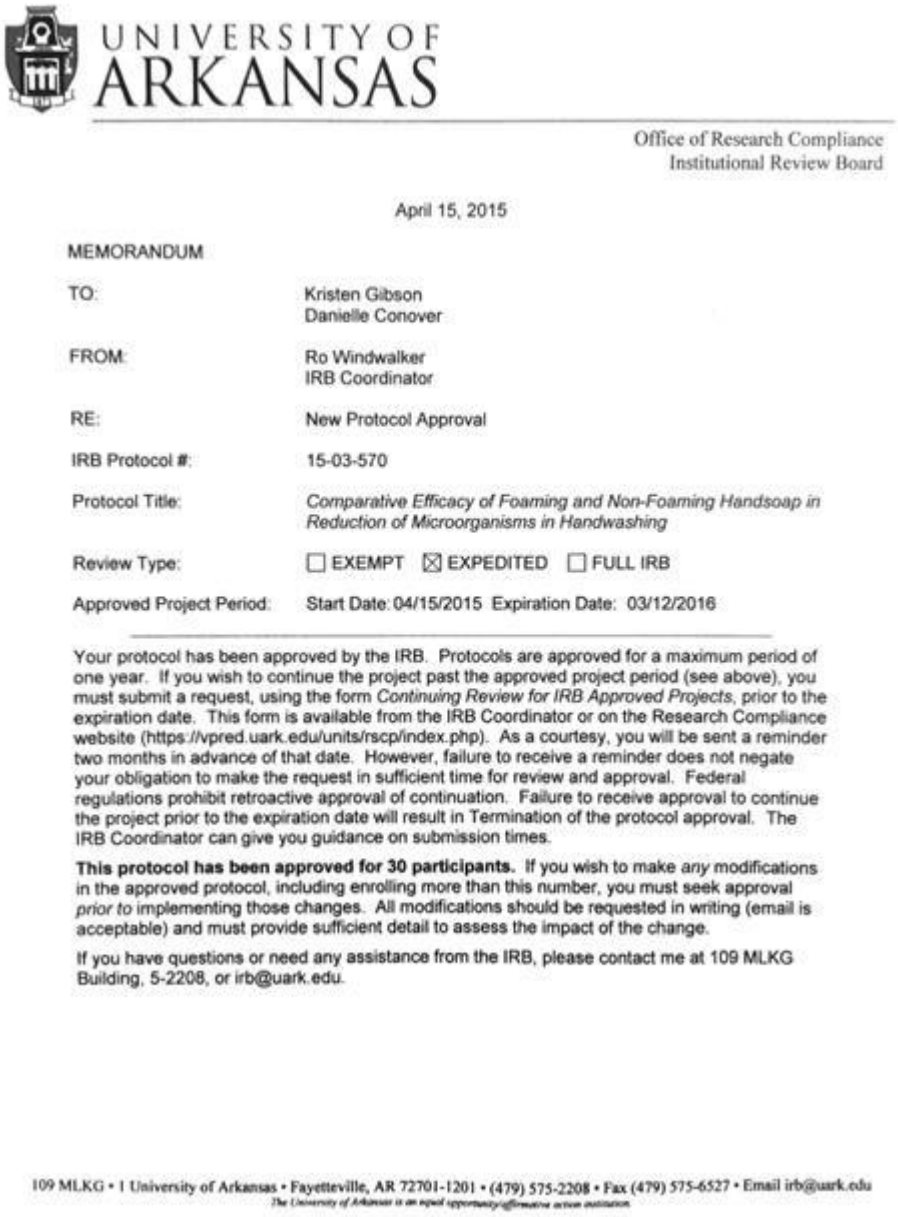
The Institutional Biosafety Committee (IBC) has approved Protocol 15013, "Comparative Efficacy of Foaming and Non-Foaming Handsoap in Reduction of Microorganisms in Handwashing" You may begin your study.

If further modifications are made to the protocol during the study, please submit a written request to the IBC for review and approval before initiating any changes.

The IBC appreciates your assistance and cooperation in complying with University and Federal guidelines for research involving hazardous biological materials.

109 MLKG • 1 University of Arkansas • Fayetteville, AR 72701-1201 • 479-575-4572 • Fax: 479-575-6527 • http://rscp.uark.edu

IRB Approval Letter:



UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

April 15, 2015

MEMORANDUM

TO:	Kristen Gibson Danielle Conover
FROM:	Ro Windwalker IRB Coordinator
RE:	New Protocol Approval
IRB Protocol #:	15-03-570
Protocol Title:	*Comparative Efficacy of Foaming and Non-Foaming Handsoap in Reduction of Microorganisms in Handwashing*
Review Type:	☐ EXEMPT ☒ EXPEDITED ☐ FULL IRB
Approved Project Period:	Start Date: 04/15/2015 Expiration Date: 03/12/2016

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (https://vpred.uark.edu/units/rscp/index.php). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 30 participants. If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 109 MLKG Building, 5-2208, or irb@uark.edu.

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