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STANDARDS RESEARCH

Testing the Effect of Faucet Flowrate on Handwashing Efficacy

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Authors

Sadru Walji, Graduate Student, University of Waterloo

Shazia Tanvir, Research Associate, University of Waterloo

Marc Aucoin, Professor, University of Waterloo

William A. Anderson, Professor, University of Waterloo

Advisory Panel

Jessica Fullerton, University Health Network and The Ottawa Hospital

Marianne Lee, H.H. Angus & Associates Limited Consulting Engineers

Nick Stark, H.H. Angus & Associates Limited Consulting Engineers

Andrew Cutz, Health+Safety Matters & Associates

Kay Penn, CSA Group

Sayward Fetterley (Project Manager)

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Executive Summary

Handwashing is an important part of infection control, safety, and hygiene in a wide variety of circumstances, including health care and food services. There are many factors that may determine the efficacy of handwashing, including the type of soap, the type of faucet (touchless or otherwise), the length of handwashing time, and the specific handwashing procedure. One factor that has not been examined in much detail is the potential effect of the water flowrate coming out of the faucet. One small study published in 2009 concluded that low flowrate may result in poorer handwashing results, but no further research has been replicated and reported. Therefore, the objective of this study was to examine the effect on handwashing of water flowrate from touchless faucets under laboratory-controlled conditions.

Over 21 participants were recruited to wash their hands using an established public health protocol and a non-antimicrobial soap. In this protocol, the hands were initially wetted, then a 15-second lathering with soap was used with attention paid to contacting all the skin surfaces, followed by a rinse under the faucet and drying with paper towels. After washing, the participants' hands were sampled to quantify the numbers of naturally occurring bacteria (resident flora) remaining on the skin. Four laminar flow touchless faucets were used, pre-set at flowrates of 0.5, 1, 1.5, and 2.2 gallons per minute (gpm). Each participant used every faucet twice over the course of a three-week study so that up to 42 handwashing samples were measured for each flowrate. A subset of six participants was subsequently used to test the effect of the highest and lowest flowrates on hands that were initially heavily contaminated with a non-pathogenic strain of *Escherichia coli,* to examine the potential effects of water flowrate on non-resident flora.

The results showed that the flowrate had no significant effect on the quantities of naturally occurring bacteria remaining after washing. On average, approximately 300,000 microbes (5.5 log CFU) could be recovered from a participant's hand after washing at any flowrate. Of those participants whose hands were initially contaminated with *E. coli,* handwashing removed 99.3% of the *E. coli,* confirming the importance of handwashing in contaminant removal. However, there was no statistically significant difference between the lowest (0.5 gpm) and highest (2.2 gpm) water flowrates used in the removal of *E. coli.*

These results are consistent with other literature reports on various aspects of handwashing that quantified the bacteria remaining on hands using similar microbiological sampling techniques as those used in this study. It is concluded that faucet flowrate has no significant effect on handwashing efficacy, under the specific conditions used in this testing.

Comparison of these results with the 2009 published study (the only other available study) that did find a flowrate effect indicates that the prior study used much lower flowrates, ranging from 0.3 to 1 gpm, and that the study's conclusions showed a relatively weak effect of flowrate (Thornley et al., 2009). That study also used a different handwashing protocol whereby the lathering and washing took place under running water, in contrast to the protocol in this work where a 15-second lathering with soap was then followed by rinsing with the running water for an uncontrolled period of time.

There may be scenarios not examined in this current study where faucet flowrate might have a more significant effect, such as when hands are more heavily soiled with additional organic matter before washing. However, for normal day-to-day handwashing scenarios using the given protocol, the effect of flowrate appears to be insignificant within the 0.5 to 2.2 gpm range tested.



"The importance of hand hygiene for prevention of infection transmission is well established. "

1 Introduction

1.1 Background

The importance of hand hygiene for prevention of infection transmission is well established (Allegranzi & Pittet, 2009). Designs of faucets and sinks have been developed that assist with hand hygiene practices in health care facilities by minimizing splashing and cross-contamination of hands (CSA Z317.1-16). However, other factors in handwashing can play a significant role in the efficacy, or degree of cleanliness, achieved by handwashing. Some of these factors include the length of time, and the type and amount of soap. It is commonly found that, even with intense scrubbing and using antibacterial soaps, many thousands of bacteria remain on the hands as part of the natural and normal "resident flora".

A number of past research studies have examined some of these various aspects of handwashing and its effects on the residual microbial burden remaining on the hands. For example, the use of two different microbiocidal foaming soaps has been studied and compared to a non-biocidal soap (Arbogast, Bowersock, Parker, & Macinga, 2019). Handwashing technique and timespan has also been examined in some detail (e.g., Larson et al., 2001; Montville, Chen, & Schaffner, 2002; Pereira, Lee, & Wade, 1997; Siddiqui et al., 2017). Conover and Gibson (2016) provided an extensive review of the wide range of study methods that have been used for evaluating the efficacy of handwashing. Antibacterial soaps tend to be a bit more effective than other types, but the length of time spent handwashing tends to have a more limited impact (Conover & Gibson, 2016; Montville & Schaffner, 2011).

Drying of the hands after washing is known to be important for minimizing subsequent transfer of microbes to other surfaces (Patrick et al., 1997). Some reviews suggest that the method used for drying hands seems to have no conclusive effect on hand hygiene (Conover and Gibson, 2016). Others, such as Huang et al. (2012), point out that there is a lot of variability among studies and research techniques used to examine this factor, leading to some difficulties in drawing conclusions. As examples of this variability, Gustafson et al. (2000) found no statistically significant difference between cloth towels, paper towels, warm forced air and spontaneous room air evaporation. Suen et al. (2019) found that a jet air dryer was marginally better than warm air or paper towels, but the difference was very small. However, Pitt et al. (2018) found that paper towels were more effective at microbe removal from hands than jet air or warm air dryers. Concerns have been noted with the potential spread of microbes during air dryer use through aerosolization and dispersal (Huang et al., 2012). Kimmitt and Redway (2015) measured much higher levels and transmission distances of viruses (MS2 bacteriophage) with jet air dryer usage versus warm air and paper towel drying. Pitt et al. (2018) also measured more extensive microbial contamination on

the walls near jet and warm air dryers in contrast to paper towel dispensers. Overall, there is a reasonable basis for preferring paper towels as a drying method to achieve good microbial reduction while minimizing further spread of microbes into the local environment.

In spite of all these previous studies, one aspect of the handwashing process has apparently never been extensively examined: the effect of water flowrate onto the hands during parts of the handwashing procedure. New health care facilities tend to use touchless (sensor-activated) faucets to minimize cross-contamination between users, and these faucets have pre-set flowrates. For economic and environmental reasons, it may be desirable to use as low a flowrate as practical. However, the impact of using a low flowrate on the handwashing process and efficacy is uncertain.

There appears to be only one report where flowrates ranging from 1 to 3.8 litres per minute (Lpm) (0.3 to 1.0 U.S. gallons per minute) were tested with 12 subjects for residual *E. coli* contamination (Thornley et al., 2009). That small study concluded that lower flowrates did lead to slightly higher levels of residual contamination on the hands. The authors concluded that lowering flowrates down to 1 Lpm is likely to expose people to an increased risk of communicable disease transmission, but a specific recommendation about a minimum flowrate was not determined. Similar work has not been reproduced and reported by others, leading to uncertainty about this one conclusion and resulting in a lack of information on what minimum flowrate might be required to achieve good hand hygiene.

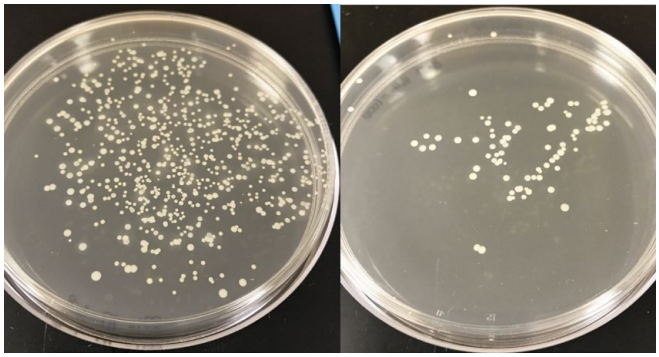
Therefore, it was the objective of this study to determine if water flowrate had an effect on the efficacy of handwashing, all other factors being held constant, and to identify if a minimum recommended water flowrate needs to be established.

1.2 Microbiological Aspects

Microbes, and more specifically bacteria, are ubiquitous in the environment and can be found on almost all surfaces and in the air. This includes human skin and hands, where hundreds to thousands of microbes typically reside on every square centimetre. This natural "resident flora" is generally harmless, but pathogenic bacteria can be picked up on the hands when touching various contaminated surfaces in some circumstances.

The number of bacteria on the hands cannot be easily determined directly. It typically requires that the hands be sampled in some way to remove bacteria from the surface of the skin into a sterile liquid. A measured portion of this liquid is then spread onto agar plates (Petri dishes) that provide nutrients for the bacteria to grow. Once they have reproduced sufficiently to be visible to the eye as a colony (see Figure 1), the colonies can be counted. This count of "colony forming units" (CFU) is an indication of the number of bacteria that were initially in the sample collected from the hands. There is substantial inherent variability in this method, due to variability in the success of removing the bacteria into the liquid and variability in how well different bacteria will grow on the agar nutrients. Therefore, it is typical in this type of microbiological work to use multiple replicated samples and statistical analysis to take the variability into account before drawing conclusions.

Figure 1: *Typical set of agar plates showing microbial colonies growing on the surface after a 24-hour incubation.*



The numbers of bacteria tend to be large and therefore it is common to express the counts in terms of logarithms. As an example, CFU counts of 1,000, 10,000, and 100,000 would be quantified as "3 log, 4 log, and 5 log", respectively, and a 3.5 log CFU would correspond to 3,162 CFU counts (the anti-log of 3.5). Likewise, the efficacy of handwashing is often described in terms of "log reduction". For example, a decrease in CFU counts from

10,000 to 1,000 would be quantified as a 1 log reduction (logarithm of 10,000 divided by 1,000). Log reductions are also related to percent reductions as in the following example: 1, 2, and 3 log reductions are equivalent to 90%, 99%, and 99.9% reductions, respectively.

2 Methodology

2.1 Equipment

Four handwashing stations were set up, each consisting of a vitreous china American Standard sink (ICS Scrub Sink Model 9118.111.020) and a touchless controlled flow faucet. The faucets were selected to cover a range of flowrates, specifically 0.5, 1.0, 1.5, and 2.2 gallons per minute (gpm). The 0.5 gpm faucet was from Sloan (Optima Plus Model EBF750-4-BDT with 0.5 gpm multi-laminar flow spray head), and the 1.0, 1.5, and 2.2 gpm laminar flow faucets were from American Standard (Selectronic IC Proximity Faucet Model 6055193.002). All flows listed here are nominal, and measured values of flow under actual operating conditions, which are given in the Results and Discussion section, were very close to these nominal values. The handwashing stations were set up in a temporary laboratory location at the University of Waterloo as shown in Figure 2. The building's municipal water supply was used as the water source for all handwashing with a mixture of hot and cold water to supply a lukewarm mixture at the sinks.

2.2 Study Design

2.2.1 Naturally Occurring Bacteria on Hands

A group of 21 study participants was recruited from among the faculty, staff, and graduate students at the University of Waterloo. Approval for this study and methods was granted by the University of Waterloo Research Ethics Committee (ORE #40339). The participants spanned a range of ages and the group was approximately gender-balanced. The participants' occupations ranged from office to laboratory work within the University.

A randomized and duplicated statistical design was employed to test the hypothesis that the faucet flowrate has an effect on handwashing efficacy. Using this design, each participant would be sampled for microbial load remaining on their hands after handwashing with an assigned faucet and following a prescribed handwashing protocol (Public Health Ontario, 2009). With this design, the effects of handwashing under each of the four faucets was sampled up to 42 times. For a negative control, each participant was also sampled once with no handwashing, providing a baseline set of data with up to 21 replicates.

Figure 2: *Left: Handwashing stations, with faucets nominally set at 2.2, 1.5, 1.0, and 0.5 gpm, from left to right. Equivalent nominal flowrates in litres per minute are 8.3, 5.7, 3.8, and 1.9 Lpm, respectively. Right: Close-up of one handwashing station showing interior layout.*



The study was carried out over a three-week period, during which most participants were sampled only once per day. For a few participants who were sampled twice in one day, a minimum four-hour delay between sampling was used to ensure there was ample opportunity for hand recontamination. Participants were asked to avoid antimicrobial soaps and materials but were not required to avoid handwashing at other times, and they were expected to carry on with their normal routine. Participants selected the dates and times of the nine sampling events over the three-week period, according to their availability and daily work schedule.

2.2.2 Contaminated Hands

A subset of six volunteers from the participants were recruited, and each volunteer's right and left hands were sampled three times after inoculation (contamination) with a standard culture of a non-pathogenic *Escherichia coli DH5α (E. coli)*. The three sampling events were (1) without washing to assess the level of contamination due to the inoculation procedure; (2) after washing and drying with a faucet set at 0.5 gpm; and (3) after washing and drying with a faucet set at 2.2 gpm. According to the study design, the order of these washing and sample processes was randomized to eliminate potential order bias. For each volunteer, all three sampling events were performed on the same day, and the six volunteers were sampled on different days over a period of several weeks.

2.3 Hand Sampling

For each sampling event, each participant followed a Public Health Ontario (2009) handwashing protocol, namely wet hands with water, apply soap, lather hands for 15 seconds (ensuring all hand surfaces are contacted), rinse thoroughly under running water, and pat hands dry with paper towel. Handwashing was performed using a commercially available non-antibacterial, fragrance-free, natural ingredient liquid hand soap (Nature Clean[1], Walmart Canada). To reduce variability between participants, the researchers specified and monitored the application of the liquid soap using a single pump of the bottle. The volume of soap dispensed with a single pump was measured to be on average 1.6 ± 0.1 mL. Participants washed their hands at one of the four stations, one person at a time to minimize possible cross-contamination between stations.

For the first part of the study, participants' hands were not inoculated or contaminated before washing, relying instead on the natural resident flora developed on their hands during the course of a normal working day. Participants were asked to refrain from using antibacterial soaps and other such products during the course of the study.

For the latter part of the study examining the effect of microbial contamination, a measured amount of an *E. coli* culture was added to the participants' hands and they thoroughly spread it all over their hand surfaces, including the palms, back of the hands, and between the fingers. Participants then waited one to two minutes for their hands to dry and absorb the culture liquid before proceeding to the handwashing and sampling steps. Preliminary testing with this method demonstrated that approximately 10^6 cfu/mL of *E. coli* could be recovered from the participants' hands using the sampling method described in the next paragraphs.

A variant of the "glove juice" method was used for sampling the bacterial load remaining on each of the participants' two hands after each handwashing trial. This common method was adapted from a combination of ASTM methods and other research literature (ASTM E2755; ASTM E2870; Pereira et al., 1997; Mahl, 1989; Kampf, Ostermeyer, Heeg, & Paulson, 2006; Conover & Gibson, 2016). In this method, an extra-large nitrile glove was placed on each hand and 20 mL of sterile sampling medium was added (tryptic soy broth with 0.5% lecithin and 1% Polysorbate 80, pH 7.1 to 7.5). The addition of lecithin and Polysorbate 80 is reputed to ameliorate residual disinfectant effects and promote the release of bacteria from the skin surface, respectively. Each hand (palm, back, fingers) was massaged by the researcher for one minute in a consistent pattern to recover bacteria from the skin surface, and then the glove was removed. Participants were asked to remove rings before sampling but were not required to remove rings before washing.

[1] Containing water, potassium oleate, glycerin, and sodium chloride according to the manufacturer's website.



"Microbial concentrations in the municipal water supply were measured ... to monitor for possible variations in the water quality."

For the examination of the naturally occurring bacteria on participants' hands, shortly after sampling the liquid was diluted with sterile fresh medium (1:10 ratio) and 0.1 or 0.01 mL was then spread onto agar plates containing the same tryptic soy medium, with lecithin and Polysorbate 80. The plates were incubated for 24 hours at 37°C, and the number of colonies on each plate was counted and recorded (CFU). Colony counts of much greater than 300 on an individual plate were usually discarded as being unreliable. The CFU counts were multiplied by the dilution factors to calculate the total number of bacteria recovered from the hand. The counts from several dilutions were combined using a geometric mean.

For the participants whose hands were pre-contaminated with *E. coli,* the same sampling approach was used except that MacConkey agar was used with 24- to 48-hour incubation at 35°C. This methodology is more specific and selective for *E. coli* bacteria and it eliminated the background interference from resident flora on participants' hands.

2.3.1 Study Controls

Faucet flowrates were measured and verified at the start of the study and throughout the sampling period. Water temperatures were measured and controlled to be as consistent as possible throughout the study period, with an average temperature of 29.8°C (±4.1°C). Samples of the sterile sampling medium were tested by agar plating as a negative control, to ensure that sterility was maintained. Preliminary testing confirmed that the sterile nitrile gloves had no significant antibacterial effects, consistent with the findings of Landers and Dent (2014). Likewise, the paper towels were tested by sampling and agar plating and had no significant contribution to microbial counts.

The use of a randomized and duplicated assignment of faucets for participants helped to ensure that day-to-day variations in conditions were not likely to bias the results in any consistent way. For example, a specific single faucet was not repeatedly used on a specific day. Instead, participants were assigned to use a different faucet on each sampling day, and different participants used different faucets on each day.

Likewise, for participants with hands intentionally contaminated with *E. coli,* the order of handwashing flowrates and sampling was randomized and completed on different days for each volunteer to spread any bias effects across samples.

Microbial concentrations in the municipal water supply were measured frequently using the same agar plate counting technique to monitor for possible variations in the water quality. Of the 54 water samples analyzed during the study, 48 showed zero microbial counts, indicating that the water supply was not a likely source of resident flora microbial contamination on the hands during and after washing.

3 Results and Discussion

3.1 Faucet Flowrate Confirmation

The nominal faucet flowrates were 2.2, 1.5, 1.0, and 0.5 gpm, according to the manufacturers' information. Actual flowrates from each faucet were measured eight times over the course of the three-week study to quantify the flowrates rather than using nominal values, since these might be affected by fluctuations in supply pressure or other factors.

The average actual (vs. nominal) flowrates were as follows: 2.18 (2.2), 1.48 (1.5), 1.02 (1.0), 0.50 (0.5) gpm, or in litres per minute as 8.3, 5.6, 3.9, and 1.9 Lpm, respectively. The relative standard deviation of these flow measurements was ±3.5%, indicating that the variability in flowrate was consistently very small over the study period. Therefore, the manufacturers' nominal faucet flowrates are a very good representation of the actual flowrate achieved in this study, and the nominal flowrates are used throughout this report.

3.2 Data Overview

Each sampling event resulted in a colony count for the left and right hand of each participant. Using the known dilution factors and total volume of sampling liquid (described in Section 2.3, Hand Sampling), these were converted into a total colony count per hand (colony forming units, or CFUs). In this report, the base 10 logarithm will be cited (log CFU) in accordance with common practice in the literature and for ease of presentation. For example, log CFU results of 3, 4, or 5 represent actual colony counts of 1,000, 10,000, or 100,000, respectively (or 10^3, 10^4, 10^5).

3.2.1 Handwashing Sampling Results – Resident Flora

The effects of handwashing on the microbial load remaining on hands has been reported several times in the literature, with a variety of handwashing scenarios and soaps or disinfectants. For example, Arbogast et al. (2019) reported a mean reduction in log CFU of 2.04 for non-biocidal soap, and between 3.09 and 3.70 for two different antimicrobial soaps after one handwashing using a challenge microbe (*Serratia marcescens*). Montville and Schaffner (2011) reported that log CFU reduction after handwashing tends to be much smaller when the resident flora of the hands are used rather than an applied "challenge" microbe. They report a mean log reduction of 0.31 for resident flora based on a meta-analysis of other studies.

In this study, for naturally occurring bacteria ("resident flora") on the participants' hands, the overall mean of colony counts (log CFU) with and without handwashing were 5.52 (n = 159) and 5.56 (n = 20), respectively, while the standard deviations were 0.52 and 0.48, respectively. Therefore, the log reduction caused by handwashing in this study was found to be 0.04. The effect of handwashing was relatively small in this study, but this is consistent with the analysis by Montville and Schaffner (2011) who found a 0.31 log reduction (±0.34 standard deviation) for resident flora on hands. The small log reduction of 0.04 (1.1% removal) in this study therefore falls within one standard deviation of the Montville and Schaffner analysis.

Since the left and right hands were sampled separately, the overall data were analyzed to determine if there was a consistent difference for resident flora. Without washing (n = 20), the left and right log CFU counts were 5.61 (±0.59) and 5.51 (±0.41), respectively, with the standard deviations of the counts shown in parentheses. The difference between counts on the left and right hands would not be considered statistically significant. Likewise, with washing (all flows combined, n = 159) the left- and right-hand counts (±standard deviations) were 5.57 (±0.57) and 5.48 (±0.54), respectively. This difference between hands would again not be considered statistically significant. Therefore, it was concluded that the effect of handedness was not likely a biasing factor in analyzing and interpreting the data.

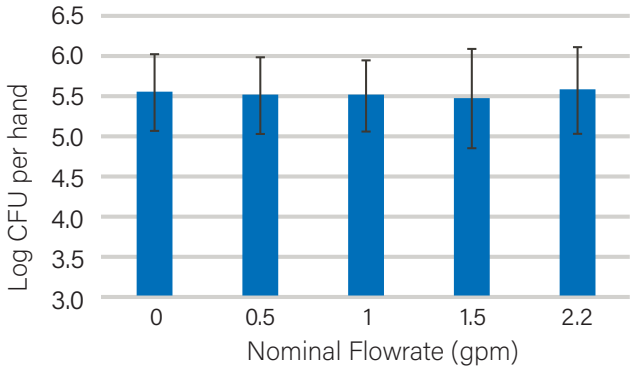
Based on the 21 participants and the repeated sampling in this study design, the "power" of the testing can be estimated from statistical theory, assuming a 95% confidence level for concluding that there are differences. The overall mean colony count for all data in this study (excluding no-washing controls) is 5.52 log CFU, with a standard deviation of 0.52 log CFU. For a sample size of up to 42 tests for each faucet, a 0.32 log CFU difference between test conditions can be detected with a power of 0.8.[2]

[2] That is, the probability of missing a real difference of this magnitude (0.32) between faucets is 20%, and of incorrectly detecting a difference is 5%. These are commonly used criteria in experimental studies. Calculated using https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html

An overview of the entirety of the resident flora study data is provided in Figure 3, showing the geometric mean and standard deviations of the colony counts for all participants and each nominal faucet flowrate.

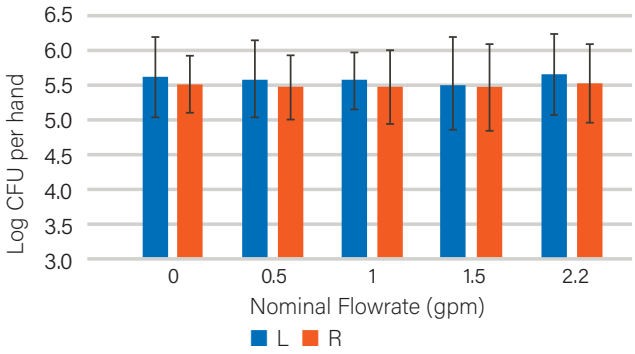
Examination of Figure 3 suggests that there is no statistically detectable difference in the resident flora remaining on the hands after washing at various flowrates. Furthermore, using the handwashing materials and protocols in this study there was no significant effect on the resident flora bacterial counts that could be recovered from the hands, whether the hands were washed or not. Although these results seem counterintuitive to some extent, they are consistent with other findings and several factors that can influence these measurements, as discussed further in Section 3.3, Discussion of Results.

Figure 3: *Mean logarithm of colony counts (log CFU) from resident flora hand sampling for each different faucet, including a control with no washing (labelled "0"). Left- and right-hand samples are combined into the averages. Error bars show plus/minus one standard deviation estimated from the sampling data.*



Overall, the data did not show any significant effect of left- or right-hand sampling, but the averages for each faucet are shown in Figure 4 to illustrate. Although there is potentially a slightly smaller log CFU result for the right hands, the error bars on the data indicate that it is not possible to conclude the differences are experimentally real.

Figure 4: *Mean logarithm of resident flora colony counts (log CFU) from left (L) and right (R) hand sampling for each different faucet, including a control with no washing (labelled "0"). Error bars show plus/minus one standard deviation estimated from the sampling data.*



3.2.2 Handwashing Sampling Results – Contaminated Hands

The raw data for *E. coli* colony counts in the samples recovered from each of the volunteers' hands are shown in Figure 5.

Figure 5: *E. coli microbial counts for samples recovered from the left (L) and right (R) hands of six volunteers before washing (control), as well as after washing under water flowrates of 0.5 and 2.2 gpm, after contamination with a non-pathogenic strain of E. coli. Hands were also dried with paper towels prior to the hand sampling. Each bar within a grouping on the graph represents a result from one volunteer.*

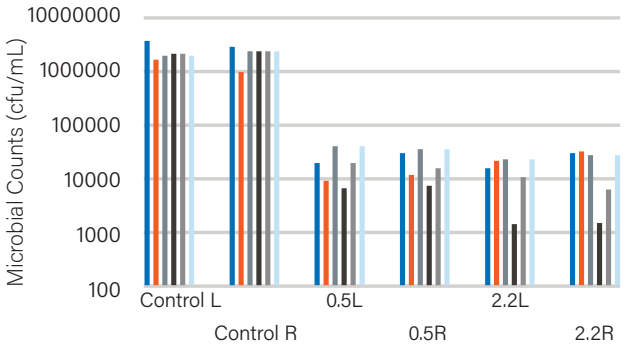
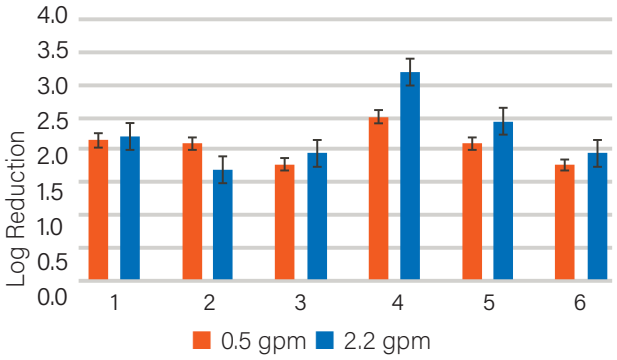


Figure 5 illustrates several important observations. First, from the left- and right-hand controls it is confirmed that the methodology used in the study was able to consistently apply and recover the *E. coli* microbial inoculation to both hands of all the volunteers. Secondly, although there is some variability between volunteers, there is no statistically significant bias towards right- or left-hand microbial application and recoveries under any conditions (95% confidence level).

It is also visually apparent from Figure 5 that there is potentially a small effect of faucet flowrate on the microbial loads that were recoverable from the volunteers' hands. To compare effects more directly and quantitatively, "log reductions" were calculated for the geometric means of left- and right-hand microbial counts for each volunteer. These results are shown in Figure 6.

Figure 6: *Log reductions of E. coli microbial counts from washing (versus controls), for results from right and left hands combined (as geometric means), for six volunteers washing at 0.5 and 2.2 gpm. Error bars show the standard error of the mean reduction for each flowrate.*



Based on the overall combined left- and right-hand results for all six volunteers, there was a 2.06 log reduction (±0.27 standard deviation) after washing at 0.5 gpm, and a 2.23 log reduction (±0.53 standard deviation) after washing at 2.2 gpm. Although the 2.2 gpm flowrate appears to be slightly more efficacious, a Student's *t*-test (probability 0.49) indicates that there are no statistically significant differences between the two flowrates. Therefore, any apparent differences in flowrate effects could readily be due to random variability in the data. Since these two flowrates represent the extreme low and high levels tested in the experiments with resident flora, it can be surmised that the other flowrates in-between these would also show no significant difference.

As noted in the previous section for resident flora measurements, the flowrate appears to have no direct relationship with handwashing efficacy. This may be because of the protocol used in these studies, whereby the faucet flow only plays a direct role in the rinsing of hands to remove residual soap. The removal of bacteria may be more directly affected by the nature of the scrubbing action, which in this study was carefully controlled to be a 15-second period with attention paid to scrubbing all surfaces of the hands, according to the protocols provided by Public Health Ontario (2009).

3.3 Discussion of Results

3.3.1 Data Validation and Comparison – Resident Flora

The bacterial counts obtained in this study can and should be compared to other literature where a similar glove juice method was applied, using resident flora (as opposed to purposely applied bacteria). There are several relevant studies published in the health care and food preparation literature. For example, Larson et al. (2001) reported mean log counts (±standard deviations) of 4.80 (±0.92) and 5.15 (±1.11) for a pre-scrub sampling of hands that had been initially washed for 10 seconds with a non-antimicrobial soap. Log counts after an alcohol treatment or traditional 6+ minute surgical scrub fell to 3.86 (±0.84) and 4.34 (±1.24), respectively. The fact that approximately 10,000 or more bacteria can still be recovered by the glove juice method, even after an extensive scrubbing protocol, is instructive for interpretation of results.

Likewise, Pereira et al. (1997) measured similar counts on the order of 5.2 log CFU before a scrubbing protocol, using a glove juice method. After scrubbing, the log counts decreased to between 4.2 and 4.9, but in that research, a variety of strong antimicrobial compounds were used (chlorhexidine gluconate, povidone iodine, isopropanol, ethanol). Waterman et al. (2006) also used a glove juice method and measured counts equivalent to approximately 5.8 log CFU for pre-scrub veterinary students.

The "resident flora" results from the present study fall within a similar range of log CFU counts per hand as in these literature reports, thus confirming that the methods and results are reasonably consistent with known data where the glove juice method was used to measure the resident flora on participants' hands.

In addition to comparing the log CFU counts, the standard deviations of count data should be examined and compared to literature reports. Standard deviation is a measure of the "spread" and variability of data and is also an indication of how consistent a technique might be when applied to sampling. A smaller value of standard deviation is considered better, as it indicates less variability in the data and makes it easier to draw statistical conclusions.

As noted above, the standard deviations in the data from Larson et al. (2001) were in the range of ±0.84 to 1.24. The work cited above by Pereira et al. (1997) and Waterman et al. (2006) did not explicitly report standard deviations. However, other literature on hand sampling (using a variety of techniques and some challenge organisms) can also provide some insight into typical standard deviations. Thornley et al. (2009) cited standard deviations ranging from 0.62 to 0.86. Standard deviations cited by Montville and Schaffner (2011) range from 0.34 to 1.13 for log reductions, which is not quite the same measure but related to log counts.

The standard deviations in this current study ranged from approximately 0.41 to 0.59. In comparison with other literature, these standard deviations are consistent and somewhat smaller than some reports. This indicates that the trials were well controlled and subject only to the natural variability that is found in other bacterial sampling work with handwashing.

3.3.2 Data Validation and Comparison – Contaminated Hands

The overall efficacy and importance of handwashing to remove contaminants was confirmed in these tests. For all washing tests combined, the log reduction in microbial counts averaged 2.14, or in other words 99.3% of the *E. coli* were removed by handwashing using the provided protocol.

This is a similar result to those reported elsewhere in literature for handwashing with non-antimicrobial soaps. For example, Fuls et al. (2008) measured a 1.72 log reduction (98.1%) in *Serratia marcescens* inoculated on hands after a 15-second wash with non-antimicrobial soap. Similarly, Arbogast et al. (2019) measured a mean 2.04 log reduction (99.1%) after one wash with inoculated *Serratia marcescens.* Using *E. coli,* Mahl (1989) also determined a mean 1.72 log reduction after sampling from three fingers of each volunteer washing with a non-antimicrobial Ivory liquid soap. Fischler et al. (2007) applied approximately 6.46 log CFU per hand of *E. coli,* and after a 15-second wash with non-antimicrobial soap measured a log reduction of approximately 2 log CFU (or 99% reduction).

Therefore, it is clear that the results with *E. coli*–contaminated hands obtained in this study are consistent with similar types of inoculated-hand washing studies reported previously in peer-reviewed literature where water flowrate was not a studied factor.

3.3.3 Other Potential Factors

In this study, factors such as soap volume and washing time were controlled to minimize their influence on the measured results. This was meant to help ensure that the faucet flowrate was the primary variable that would potentially impact the measured bacterial loads remaining on each hand. The literature provides some insights into these and other factors that may play a role in handwashing efficacy, and these are described in the following subsections.

3.3.3.1 Wash Time, Temperature, and Rings

In this study, a 15-second lathering time was employed for all participants. Montville and Schaffner (2011) pointed out that there is a relatively limited amount of data on the effects of washing/lathering times. Their data suggested that a wash time of 120 seconds may be better than 30 seconds, but within the range of 15 to 30 seconds there was no obvious effect of time. Likewise, Fuls et al. (2008) noted no significant improvement for non-antimicrobial soaps when wash time was increased from 15 to 30 seconds and when hands were purposefully contaminated with *Shigella flexneri.*



"A 15-second lathering time was employed for all participants."

Conover and Gibson (2016) cited several studies where wash time was observed for people in the medical and food services industries. The average wash time ranged from 9.5 to 15.3 seconds when not strictly controlled. This suggests that the wash time used in this study was fixed at a reasonable length, which may be achieved on average in a population where proper hand hygiene is encouraged.

One aspect of the washing process was not controlled in this study, namely the pre-lather wetting of hands and the post-lather rinsing. Participants were permitted to use their own judgement with respect to these times, which likely varies depending on the feeling of the hands and personal preference. For example, Burton et al. (2011) noted that participants washed on average for 12 seconds when water alone was used, but 14 seconds when water and soap were used together, presumably to allow for removal of the feeling of soap residues on the hands. In the present study, the rinsing times were not strictly measured but for many participants lasted on the order of a few seconds (by observation). It is possible that some participants used slightly longer rinsing times for the lower flowrate faucets to compensate for the lower water volume, but there is no concrete observational evidence for a consistent difference. Anecdotally, participants seemed satisfied with the lower flowrates in terms of the user experience, and the variation between rinse times for participants appeared to be consistent from day to day.

The water temperature during this study averaged 29.8°C (standard deviation ±4.1°C, range 20 to 35°C), with some fluctuations due to daily variations in water supply temperature and pressure. Michaels et al. (2002) studied the effect of water temperature on resident flora removal using non-antimicrobial soaps with temperatures in the range of 4.4 to 48.9°C. They found no effect on the extent of resident bacterial flora removal over this temperature range, with sampled log reductions ranging from -0.6 (i.e., an increase) to 0.9, with an average of approximately 0.2. It is not expected that the smaller temperature range in this study would have a significant effect either, and the results from Michaels et al. were consistent in magnitude with those from this study.

Some participants wore rings during washing, and some removed them. Most participants removed rings before sampling by the glove juice method. The presence or absence of rings is not expected to have a significant biasing effect on the results of this study. A study by Waterman et al. (2006) on surgeons after handwashing with and without rings (smooth band) failed to detect any significant difference between the two groups, even after three hours of surgical glove wear. This suggests that such rings and the skin underneath are not a significant reservoir of microbes after handwashing.

3.3.3.2 Amount of Soap Applied

In this study, participants used one pump of the hand soap bottle to apply the liquid soap, in a volume of approximately 1.6 mL. It is possible that there were

variations in soap volume, depending on the accuracy and consistency in pushing down the soap dispenser pump, and therefore this possible effect is considered from the literature data.

Montville and Schaffner (2011) found no strong correlation between soap volume and efficacy, for a range of less than 1 mL to greater than 5 mL. Conover and Gibson (2016) cited some works that show a larger volume (3 to 5 mL) was more effective for antimicrobial soaps, but that a smaller volume was less effective for non-antimicrobial soaps. Fuls et al. (2008) concluded that once a minimum level of surfactancy (or detergency) is achieved, further increases in soap volume do not have any significant effect when non-antimicrobial soaps are used. However, there is an effect for antimicrobial soaps since increased volumes result in higher concentrations of the antimicrobial agent available on the hands.

In the context of this study, the applied amount of soap was within the typical and expected range based on previous reports. It is also not expected from these literature reports that small variations in the soap volume from person to person would negatively affect or bias the results of this study.

3.3.3.3 Microbial Load on Hands

In the literature reports, there are two main approaches to experimentally investigating handwashing efficacy, and both were employed in this study. One approach relies on the resident flora on the participants' hands, developed through growth of bacteria on the skin and picked up from contact with soiled surfaces. The other approach uses the application of "challenge microbes" such as *E. coli* or other bacteria. The efficacy of handwashing is known to depend on which of the two approaches is used, since resident flora are more difficult to remove during handwashing (Montville & Schaffner, 2011). The application of challenge organisms to hands raises additional biosafety and ethical issues for a research study, which were addressed in this study by using a non-pathogenic strain of *E. coli* and following procedures developed in consultation with the University's Office of Research Ethics and Biosafety Officer.

Although the resident flora on participants' hands were variable, they tended to centre on 5.56 log CFU for unwashed hands, with a relatively narrow standard deviation of 0.48, or a relative standard deviation of 8.6%. Therefore, there did not appear to be a wide range of initial bacterial loads on participants' hands. This may be due to several factors, including the time of year (late February to mid-March) where lower temperatures and relative humidity could suppress microbial growth on the skin surface. Likewise, the study participants worked in relatively clean environments, unlike some in the medical and food service industries where heavy soiling loads may be picked up on the hands over the course of a day.

Montville and Schaffner (2011) concluded that initial bacterial levels on the hands had a significant effect on the measured efficacy of soaps. A higher initial CFU count (>6 log) tended to show more differences in log reduction when comparing soaps and handwashing protocols. The initial loads in this study were close to 6 log (5.56 log), suggesting that this should be close to a range where differences might become more pronounced. However, as noted above, resident flora also tend to be more difficult to remove during handwashing, potentially reducing the measurable differences.

For the participants with hands inoculated by *E. coli,* the average initial microbial load per hand was 7.64 log CFU, excluding any resident flora which were also present as discussed above. The effects of these resident flora were excluded from the counts by using an agar which was selective for the applied *E. coli.* This microbial load falls within the range of other studies reported by Montville and Schaffner (2011) for inoculated hands, i.e., 2.24–8.95 log CFU, with a variety of microbes including *E. coli* strains. This initial load is well above the 6 log level that Montville and Schaffner suggested would show more pronounced differences in log reduction when comparing handwashing protocols. In this study, there was a significant difference between washed and unwashed hands (2.14 log, or 99.3%), although no significant difference in washing as the two extremes of flowrate could be detected even with these relatively high initial microbial loads.

3.3.4 Comparison with Thornley et al. Results

As noted above, the only literature report dealing with flowrate effect on handwashing efficacy is from Thornley et al. (2009). Therefore, it is important to compare and contrast this study with those results, and a summary of the testing conditions is given in Table 1.

For comparison to this study and the results shown previously in Figures 3 and 6, the results from Thornley et al. (2009) were extracted from their paper and plotted in Figure 7. In the original study, Thornley et al. used a statistical technique (linear mixed effects regression of log CFU versus log flowrate) to determine that the flowrate was a statistically significant factor in reducing log CFU counts. From Figure 7, however, it is not immediately obvious that the flowrate effect is that strong. There is a clear difference between the unwashed hands loaded with the *E. coli* challenge bacteria, and the hands that are washed. This difference is approximately log 2 CFU/mL (99%), which is similar in magnitude to the difference measured in this study. However, the differences between washed hands at different flowrates are not obvious in Figure 7. It is possible that the statistical technique Thornley et al. employed was overly biased by the large counts for unwashed hands, and there are statistical methodological reasons why a regression analysis is not necessarily the best approach for analyzing this data and drawing conclusions.[3]

Figure 7: *Results taken from Thornley et al. (2009), showing mean log CFU/mL and reported standard deviations. A flow of "0" represents E. coli-contaminated hand samples before washing.*

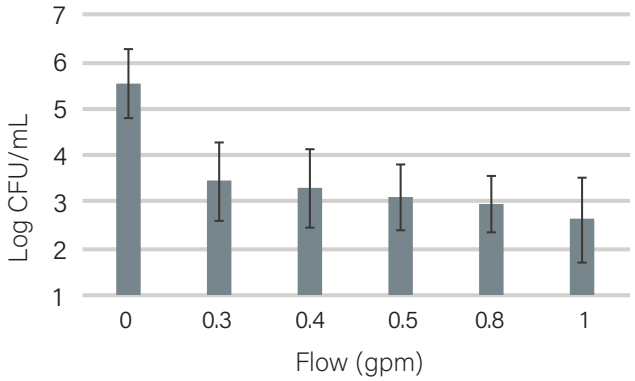


Table 1: *Comparison of study parameters and methodology used in Thornley et al. (2009) and this study.*

Parameter	Thornley et al. (2009)	This Study	This Study
Faucet flowrates	1, 1.5, 2, 3, 3.8 Lpm 0.3, 0.4, 0.5, 0.8, 1 gpm	1.9, 3.8, 5.7, 8.3 Lpm 0.5, 1.0, 1.5, 2.2 gpm	1.9, 8.3 Lpm 0.5, 2.2 gpm
Microbes	*E. coli* from fecal sample	Resident flora	*E. coli DH5-alpha*
Sampling method	Sterile chamois contact sample	Glove juice recovery	Glove juice recovery
Initial loads	5.54 log CFU/mL*	5.56 log CFU	7.64 log CFU
Soap	Non-antimicrobial	Non-antimicrobial	Non-antimicrobial
Soap volume	0.25 mL	1.6 mL	1.6 mL
Wash protocol	5 seconds lathering, 15 seconds washing with friction and selected water flowrate	Pre-wet, 15 seconds lathering, rinse with selected flowrate	Pre-wet, 15 seconds lathering, rinse with selected flowrate
Sample size	12 participants, one measurement at each flow	21 participants, two measurements at each flow	6 participants, one measurement at each flow

** The quantification method for CFU/mL is not clear from the Thornley et al. (2009) study and may not be directly comparable to the initial load measured in this study.*

[3] Arguably, using data from zero flow (i.e., unwashed) introduces a discontinuity into the regression analysis. The unwashed sample is a different condition that should not be included in the continuous progression of flowrate data.

Other differences between the Thornley et al. (2009) study and this study include the nature of the handwashing protocol, as summarized in Table 1. It appears that the Thornley et al. study used running water for 15 seconds with hand friction to accomplish the washing, whereas in this study the Public Health Ontario (2009) protocol was used, where the water was only used after 15 seconds of lathering. This difference in protocol makes it difficult to compare and extrapolate from the Thornley et al. (2009) results. Another key difference is that the Thornley et al. study used much lower flowrates than those in this study, which ranged from 0.5 to 2.2 gpm.

In principle, the handwashing protocol used in this study is less dependent on water flowrate to achieve handwashing efficacy than that used by Thornley et al. (2009). Lathering for 15 seconds with good contact and friction provides the removal mechanism for bacteria from the hand surfaces, and the water flow removes the residual soap and bacteria lifted off the surfaces. Therefore, the effect of friction and detergency are decoupled somewhat from the water flowrate in the protocol used in this study. The Thornley et al. washing protocol had simultaneous water flow with friction and detergency action, and direct comparison with the results of this study and other literature are difficult because of this difference.

4 Conclusions

The resident flora microbial loads on the participants' hands were measured and fell within expected ranges as mentioned in a variety of related literature studies, both before and after washing using the provided handwashing protocol. Handwashing had a non-significant, minor effect on the resident flora, removing only approximately 1% of the recoverable microbes. However, tests with participants whose hands were initially purposefully contaminated with *E. coli* confirmed that handwashing resulted in a removal of approximately 99.3% of the added *E. coli* microbes, and these results were likewise comparable with similar studies reported in the literature.

In both cases, there was no discernable effect of water flowrate on handwashing efficacy when all other relevant factors were held constant. It can be concluded that flowrates ranging from 0.5 to 2.2 gpm are satisfactory for handwashing efficacy when the Public Health Ontario (2009) handwashing protocol is followed.

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