Handwashing Water Temperature Effects on the Reduction of Resident and Transient (*Serratia marcescens*) Flora when Using Bland Soap

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**ABSTRACT**

For many years, sanitarians have specified that hands be washed using warm or hot water to reduce cross-contamination risks, with various authors indicating temperatures between 38°C and 48.9°C. However, it has been suggested that these temperatures may contribute to skin damage when frequent handwashing is necessitated (in health care and food service). This study evaluates the bacterial reduction efficacy of water temperature during normal handwashing. The hands of two groups of four experimental subjects were soiled with sterile or contaminated substances (tryptic soy broth and hamburger meat). Uninoculated menstruum was used to study the effects of treatment temperatures on resident microflora reduction, while *Serratia marcescens*-inoculated menstruum was used to study treatment effects on transient microorganism reduction. Following contamination with appropriate media, one hand was immediately sampled to obtain baseline (control) data, using the “glove-juice” technique for microorganism recovery. Hands were then moistened with water at the assigned temperature (4.4°C, 12.8°C, 21.1°C, 35°C or 48.9°C), washed 15 s with bland soap, and rinsed 10 seconds at the same temperature as was used before; and the opposing hand was then sampled. Results indicate that water temperature has no effect on transient or resident bacterial reduction during normal handwashing when bland soap is used.

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INTRODUCTION

A critical and thorough evaluation of a simple handwashing reveals numerous variables that must be considered to achieve maximum or appropriate degerming of the hands and fingernail regions. Numerous studies have explored topics such as type of soap (e.g., antibacterial vs. plain, liquid vs. bar), amount of soap and handwashing technique, nailbrush or sanitizer use, drying technique (e.g., cloth vs. paper towels, paper towels vs. air-drying), and application of hand sanitizers (post-wash liquids). Although studies indicate that these variables are crucial in achieving effective removal of transient bacteria from the hands under controlled testing conditions, testing to determine specific guidelines for water temperatures and flow rates is rarely mentioned in the scientific literature. Many of the currently employed handwashing practices may be based on untested traditions that could actually result in compromised skin health. With so many variables involved in such a “simple” procedure, it would make sense to explore and maximize all possible aspects of the process while minimizing negative collateral. This is especially important because many observations of food service workers have revealed what are considered poor habits in handwashing techniques. Studies indicate that handwashing compliance drops considerably without supervision and monitoring, or in situations where skin damage occurs. This further amplifies the need to strengthen knowledge of all variables that might improve or weaken daily handwashing prac-

<table>
<thead>
<tr>
<th>Issue #</th>
<th>Submitter</th>
<th>Requested change from 110°F (43°C) minimum</th>
<th>Reasons given for change requested</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000-I-24</td>
<td>M. Scarborough (GA Dept. of Human Resources, Div. Publ. Health)</td>
<td>37.7°C (100°F)</td>
<td>1. No science (110°F vs. 100°F)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Plumbing code @100°F max. (safety concerns)</td>
</tr>
<tr>
<td>2000-I-25</td>
<td>J. Budd (Healthminder/Sloan Valve Co.)</td>
<td>35°C (95°F)</td>
<td>1. No scientific basis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Max. soap efficacy at 35°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3. Hand comfort</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4. Hot water discourages hand washing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>to 110°F (43°C)</td>
<td>2. Possible scalding</td>
</tr>
<tr>
<td>2000-I-27</td>
<td>B. Adler (MN Dept. of Health)</td>
<td>Impose temp. range 110°F (43°C) to 130°F (54.4°C)</td>
<td>1. Need upper limit or subject to OSHA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Food workers don’t wash 25 s so cannot scald</td>
</tr>
<tr>
<td>2000-I-28</td>
<td>F. Reimers (H.E.B. Grocery Co.)</td>
<td>“Tempered” to warm</td>
<td>1. No science</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Max. soap efficacy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3. 110°F risks injury</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4. Waste water as wait for temp. at 110°F</td>
</tr>
</tbody>
</table>
Two types of flora, transient and resident, exist on the hands. The transient flora are generally removed fairly easily. They do not have adhesion characteristics that hold them to the skin’s surface (8) and are somewhat suppressed by secretions and competitive exclusion by normal resident flora. Resident flora are removed more slowly. Because of co-evolution, resident flora have adapted to conditions on the skin surface that cause rapid die-off of most transients. Invaginations such as the nail fold, hair follicles and sebum-producing sebaceous glands support a rich resident flora. Transient flora may consist of pathogens, spoilage bacteria or harmless environmental species. Under certain conditions transient flora can change status and become permanent residents. Resident flora as a rule are not pathogenic types.

Frequent or prolonged exposure of the skin to microbial contamination in soils, skin damage or fissures provide portals of entry to deeper tissue and may result in the presence of many pathogenic bacteria among the resident species (11,27).

Removal of viable bacteria, dirt and grease from the skin is accomplished by friction and surfactant action, which lowers surface tension. Alkaline detergent solutions remove bacteria from skin more efficiently than acid or neutral so-

### TABLE 2. A comparison of resident flora and transient flora studies

<table>
<thead>
<tr>
<th></th>
<th>Resident flora</th>
<th>Transient flora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Laboratory</td>
<td>BioScience Laboratories</td>
<td>Silliker Research Laboratories</td>
</tr>
<tr>
<td>Location</td>
<td>Bozeman, MT</td>
<td>South Holland, IL</td>
</tr>
<tr>
<td>Study Director</td>
<td>D. Paulson J. Budd</td>
<td>V. Gangar</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M. Arenas</td>
</tr>
<tr>
<td>Test Subjects</td>
<td>Paid Volunteers</td>
<td>Laboratory Workers</td>
</tr>
<tr>
<td>No. Test Subjects</td>
<td>4 (3 Females, 1 Male)</td>
<td>4 (1 Female, 3 Male)</td>
</tr>
<tr>
<td>Test subjects age (range)</td>
<td>26 - 56</td>
<td>24 - 25</td>
</tr>
<tr>
<td>Test temperatures (°C)</td>
<td>4.4, 12.8, 21.1, 35, 48.9</td>
<td>4.4, 12.8, 21.1, 35, 48.9</td>
</tr>
<tr>
<td>Test temperatures (°F)</td>
<td>40, 55, 70, 95, 120</td>
<td>40, 55, 70, 95, 120</td>
</tr>
<tr>
<td>Test soil</td>
<td>Tryptic soy broth (TSB) 1.0 ml (0.5 ml/hand)</td>
<td>1.0 (ml/hand)</td>
</tr>
<tr>
<td>Y- irradiated ground beef (GB)</td>
<td>3.0 grams (1.5 g/)</td>
<td>3.0 grams</td>
</tr>
<tr>
<td>Microbial inoculum</td>
<td>None</td>
<td>S. marcescens</td>
</tr>
<tr>
<td>No. test days/soil/subject</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Temperature/Subject</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total data points/temperature</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Mean baseline count Log10</td>
<td>TSB 6.05</td>
<td>6.91</td>
</tr>
<tr>
<td></td>
<td>GB 6.40</td>
<td>7.21</td>
</tr>
<tr>
<td>Amount of time massaged with TSB and GB</td>
<td>45 seconds</td>
<td>2 minutes</td>
</tr>
<tr>
<td>Amount of time TSB and GB air-dried</td>
<td>2 minutes</td>
<td>1 minute</td>
</tr>
<tr>
<td>Amount of soap used for handwashing</td>
<td>3 ml</td>
<td>3 ml</td>
</tr>
</tbody>
</table>
lutions do (20), forming the basis for skin sampling solutions used in this study (37).

Added to the aforementioned studies are the many references to warm or hot water use for hand-washing from the Internet or popular press. These references are meant to provide information to food workers or consumers. Questions need to be answered regarding water temperature guidelines with respect to handwashing: Do soaps perform better depending on the water temperature for handwashing? Does hot water help cleanse the hands better than cool or plain tap water? What are the physiological changes of the skin when different temperature/soap combinations are used? Does water temperature make a significant difference in reducing the numbers of transient and/or resident bacteria on the hands?

The effective water temperature used for washing and rinsing hands has been under debate recently at the Year 2000 Conference for Food Protection. Six issues were brought before Council I with regard to FDA Food Code hand washing water temperature specifications. The 1999 Food Code (36) requires sinks used for handwashing to be equipped so as to be “capable of providing water of at least 43°C (110°F), accomplished through use of a mixing valve or a combination faucet.” An outline summarizing the issues brought forth by the various submitters at the Year 2000 Conference, including requested changes and reasons given for those changes, is provided in Table 1.

All but one of the issue submissions requested temperature decreases with the intent of improving hand comfort, as the discomfort associated with higher temperatures results in decreases in hand washing frequency or compliance (I-23, I-25). Several submitters note a lack of scientific information on the subject (I-24, I-25, I-28). There is concern that a minimum handwashing temperature of 43°C (110°F) in addition to causing discomfort (I-23, I-26), will result in injury or scalding (I-28, I-24, I-26) and may even be in conflict with local plumbing codes (I-24). Two submitters point out that soaps currently available target maximum effectiveness at around 35°C (95°F) (I-25, I-28). Two submitters requested that the minimum temperature of 43°C (110°F) be changed to warm water (I-23, I-28) or that it be tempered to a range of 29.5°C (85°F) to 43°C (110°F). And finally, one submission (I-27) sought to place an upper temperature limit of 54.4°C (130°F), for fear that these regulations would be subject to OSHA scrutiny and criticism without a limit. Interestingly, it was noted in this submission, through reference to the Consumer Product Safety Commission, that second- or third-degree burns have been shown to occur in the elderly at temperatures not much over 43°C (110°F). Council I and the General assembly of voting delegates passed a recommendation to lower the Food Code water temperature minimum to 29.5°C (85°F).

The universe of food handling situations requiring effective personal hygiene runs from temporary handwash stations set up in produce fields to advanced state-of-the-art kitchens used to produce extended-shelf-life ready-to-eat foods sold at retail. In many of these situations, it is difficult to provide water meeting strict temperature ranges. Further, it is difficult to manage and monitor food handlers to insure that the 43°C (110°F) temperature minimum is maintained during all handwashing activities. When subject to regulatory inspections, violations are given to food industry entities based on Food Code specifications. Therefore, in the interest of possibly increasing handwashing compliance or efficacy and clarifying the importance of this issue to enforcement authorities, handwashing studies were undertaken.

In a literature search for effect of water temperature on hygienic efficiency, only two experimental studies shed light on this issue. Both of these involved hand sampling studies, in which the objective was to remove and enumerate as many bacteria on the hands as possible, either as normal or transient flora. In hand scrubbing experiments, Price (27) found that at temperatures from 24°C (75.2°F) to 56°C (132.8°F) there was no difference in de-germing rate. Because he scrubbed hands with a brush for a specific period of time, each in turn in a series of sterile wash basins, he might have been capable of seeing differences upon counting the flora in each basin. After conducting over 80 experiments in a 9-year period, Price concluded that the largest variable in determining the rate of removal of bacteria from the hands was the vigorousness of scrubbing. Other factors, such as soap used or water temperature, were less important. In later hand sampling experiments implementing the glove juice method for recovery of microorganisms, no differences in isolation rates were seen at either 6°C (42.8°F) or 23°C (73.4°F) (12). Although this information is inconclusive and does not answer questions concerning bacterial loads suspended in a confounding soil, they tend to indicate that there may not be a very great difference in efficacy over a range of temperatures from 6°C (42.8°F) to 56°C (132.8°F).

Various menstruum have been used for handwashing efficacy studies. For studies involving transient flora, the most often used soil is tryptic soy broth (TSB). Microorganisms exhibit good survivability, with even distribution of contaminating microorganisms into skin cracks, creases and invaginations being possible. Ground beef probably represents the most appropriate menstruum because of concern for risks of E. coli O157:H7 infection, but is only occasionally used (30, 31). Numerous cases of foodborne illness have been tied to poor personal hygiene after ground beef preparation.

On the basis of all the information gained from the literature search and analysis, experiments
were performed to determine if there was a superior temperature or range of temperatures for removal of bacterial contamination from hands during handwashing. This involved contaminating hands with marker bacteria and washing hands with soap and water, followed by counting resident and transient (marker) bacteria. Because it was realized that both the use of antimicrobial soap and drying with paper towels would confound and alter the effects of water temperature washing and rinsing, bland soap was used and hands were not dried with paper towels.

MATERIALS AND METHODS

This study was performed at BioScience Laboratories (for resident bacteria) and Silliker Research and Laboratory Services (for transient bacteria). Table 2 provides a comparison of methods used for testing in the two laboratories.

A stable pigmented strain of Serratia marcescens (SLR 1421) was used to simulate transient hand contamination. This organism is used frequently used in hand disinfection studies (5, 22, 23, 24, 28).

Tryptic soy agar (TSA) and tryptone glucose yeast (TGY) agar spread plates, deionized water, sterile stripping fluid, Butterfield’s phosphate buffer solution, phosphate buffer with 0.1% Triton X-100, TSB with 1% Tween and 0.3% lecithin, sterile latex-free surgical gloves, alcohol, and Ivory® liquid soap (non-antimicrobial) were used.

Subjects rinsed both hands under running tap water at the designated temperature, and shook off any excess. Three ml of Liquid Ivory® Soap was dispersed into the subjects’ cupped hands and rubbed over all surfaces, including the lower third of forearms, making sure not to lose any soap. After complete soap dispersal, a small amount of tap water was added, and subjects lathered their hands and forearms vigorously for 15 s. Subjects then rinsed their hands and forearms for 10 s under running tap water maintained at a flow rate of 7.6 liters/min (2 gallons/min) at the designated temperature, after which they shook the hands two times to remove excess moisture. While still wet, the subjects’ hands were gloved for sampling using the Glove Juice technique.

Glove juice sampling procedure

The effectiveness of bacterial reductions from the hands was evaluated using the glove juice recovery method as described in ASTM test methods (4). Following the prescribed wash and rinse procedure, sterile, powder-free latex gloves were donned. Seventy-five ml of Sterile Stripping Fluid (aqueous phosphate buffer with 0.1% Triton) were instilled into the glove, the wrists were secured, and attendants massaged the hands through the gloves in a uniform manner for 60 s. Aliquots of the glove juice were removed and serially diluted in Butterfield’s Phosphate Buffer solution containing 1.0% Tween 80 and 0.3% Lecithin as product neutralizers.

Enumeration

For normal (resident) bacteria, duplicate spiral plates were prepared from appropriate dilutions using TSA with product neutralizers. The plates were incubated at 30°C ± 2°C (86°F ± 2°F) for 48 h. Colonies were counted and the data recorded using the CASBA™ 4 plate-counting system.

For transient (Serratia marcescens) bacteria, Samples were spread on TGY agar following appropriate dilutions, and incubated at 35°C (95°F) for 24 to 48 h. Any pink colonies observed were considered to be S. marcescens, while the others were considered to be normal flora. The number of bacteria were tabulated using the following formula:

\[ B = A \left( \sum x/n \right)^{10D} \]

Where:

- B = estimated number of microorganisms
- A = portion volume = 75 ml
- \( \sum x/n \) = average CFU per plate for each dilution level
- D = dilution level

Subjects for normal (resident) flora experiment

The constant exposure of microbiology laboratory technicians to sanitizers and the necessity of disinfection provides the potential for high variability in the resident or “normal” flora and physiological condition of their hands and forearms. Working daily with various microorganisms that are not considered part of the normal (resident) skin flora (including agents used in their testing and evaluation) increases the susceptibility of these individuals to infection and skin damage. For this reason, volunteers were used to get a more accurate picture of the effects of water washing temperature on resident flora.

Between the ages of twenty-six and fifty-six four healthy subjects were selected, three females and one male. All subjects’ hands and forearms were free from clinically evident dermatosis, injuries, open wounds, hangnails, or any other disorder that could compromise the subject and the study. Participation was restricted to individuals not currently using any topical or systemic antimicrobials, steroids, or other medication known to affect the resident microbial flora of the skin.

The “pre-test period, seven days prior to the testing portion of the study, was designed to generate optimum levels of resident flora for testing purposes. During this period, subjects were instructed to avoid using medicated soaps, lotions, deodorants and shampoos, as well as skin contact with solvents, detergents, acids and bases, or other
products known to affect the microbial population of the skin. Avoidance of UV tanning beds and swimming or bathing in biocide-treated pools or hot tubs was mandatory. During this period, subjects were supplied with a personal hygiene kit, containing non-medicated soap, shampoo, deodorant, lotion, and rubber gloves to be worn when contact with antimicrobials, solvents, detergents, acids, or bases could not be avoided. For subjects’ safety, leaving the lab once the testing began was prohibited.

Testing period of normal (resident) flora

Each subject was utilized for approximately one-half hour every other day of the test period, excluding weekends and holidays (a total of ten test days per subject). Subjects were instructed to avoid washing their hands for two hours prior to testing, and fingernails were trimmed to a free-edge of less than 1 mm if not already done. All jewelry was removed from the hands and arms prior to washing.

Testing of normal (resident) flora with TSB

On each of the five test days, subjects had 1.0 ml (0.5 ml per hand) of TSB placed into their cupped hands in ten aliquots of approximately 0.1 ml. The broth was distributed evenly over both hands, not reaching above the wrists, by gentle continuous massage for 45 s. After a timed two-minute air dry, the non-dominant hand of each subject was sampled for baseline using the Glove Juice Sampling technique. Subjects washed their hands as previously described, and the other hand was then sampled using the Glove-Juice technique. These procedures were repeated each day, with the non-dominant hand being used for baseline sampling for each subject on each test day. The water temperature for the handwashes on each test day was adjusted for subjects to wash at a different temperature. Test days one through five were performed at the following water temperatures, respectively: 4.4°C (40°F), 12.8°C (55°F), 21.1°C (70°F), 35°C (95°F), and 48.9°C (120°F).

Testing of normal (resident) flora with ground beef

On each of five test days, subjects handled and smeared three grams of gamma-irradiated hamburger meat on their hands for two minutes. After a timed two-minute air dry, the non-dominant hand of each subject was sampled for baseline using the glove juice sampling technique. Subjects washed their hands as previously described, and the other hand was then sampled using the glove-juice technique. These procedures were repeated each day, with the non-dominant hand being used for baseline sampling for each subject on each test day. Wash and rinse temperatures were each day identical to those used for the resident flora with TSB testing.

Testing of transient flora with TSB and gamma-irradiated ground beef

Four laboratory workers, one female and three males, twenty-four to twenty-five years of age, were chosen for this experiment. Testing was performed over a four-week period.
period in order to alternate left and right hands for baseline readings for each temperature and inoculum. Testing procedures for the ground beef were identical to testing for normal (resident) flora, with the addition of $1 \times 10^8$ S. marcescens. Testing with TSB was similar to the tests for transient flora, with the following exceptions: the addition of $1 \times 10^8$ S. marcescens, a two-minute massage period of broth into the hands, and a one-minute drying period. Subjects washed their hands as previously described, with the opposing hand being used for baseline on alternate days. Hands were washed as previously described, and the glove juice technique was utilized for recovery.

Methods of analysis of normal (resident) and transient bacteria

The plate count data collected from this study were evaluated using MiniTab® statistical computer software. Prior to performing a statistical analysis, exploratory data analysis was performed. Stem-leaf ordering, letter value displays, and box plots were generated. Geometric mean colony counts were obtained and log or % reductions in transient and normal flora were determined from these values through comparisons to baseline counts. The experiments were analyzed for significance using statistical ANOVA software. A series of two-sample Student t-tests were conducted using the 0.05 significance level for Type 1 ($\alpha$) error and corrected for multiple comparisons on means.

RESULTS AND DISCUSSION

Because a number of submitters at the Conference for Food Protection brought forward the issue of skin injury and possible scalding at temperature above 43°C (110°F), a review of pertinent literature was undertaken to determine if facts support lowering of the temperature for reasons other than efficacy. The Consumer Product Safety Commission has noted that residential water heater thermostat settings should be set at 49°C (120°F) to reduce the risk of the majority of tap water scald injuries. Although the majority of scalding incidents in the home occur in children under the age of five and in the elderly, third-degree burns are known to result from a 2 s exposure to 66°C (150°F), 6 s at 60°C (140°F) and 30 s at 54.4°C (130°F) (35). As we age, our skin becomes thinner, losing suppleness. This fact is important, as many seniors are now actively involved in the food industry. Due to the elder risk particularly, some have recommended that water be delivered from the tap at even lower temperatures, of less than 43°C (110°F) (33).

The activity of soaps, friction, and rinsing become crucial because the temperatures recommended in handwashing water alone would not provide thermal destruction of pathogenic microorganisms. Relevant to the discomfort issue (brought forward as issues 1-23 and 1-26) is a study involving dishwashing soaps. In that study, participants could withstand only water temperatures of 43°C, 45°C, and 49°C (110°F, 113°F, and 120°F), with tolerance levels related to discomfort peaking at one minute (9). Even though this is considerably longer than the 10 to 25 s exposure period that would result from hand-wash-
ing, it is indicative of the fact that temperatures from 43°C to 49°C (110°F to 120°F) are at the discomfort threshold.

Appropriate handwashing duration (15 seconds) for this study was determined through review of various governmental agency recommendations and previous handwashing study observations (1, 3, 10, 36). Suggested lathering times by specific agencies are: the 1999 FDA Food Code (20 seconds) (36), the American Society for Testing and Materials (ASTM) (15 seconds) (3), The Association for Professionals in Infection Control and Epidemiology (APIC) (minimum of 10 seconds) (10), and The American Society for Microbiology (ASM) (a 10 to 15 s vigorous scrub) (1). Several studies support a washing duration of at least 10 seconds, with sufficient transient removal efficiency achieved by 30 seconds. A study by Stiles and Sheena (32) involving workers in a meat processing facility determined that a wash of 8 to 10 s was too short for adequate soil removal from the hands. A study by Ojajarvi (21) compared a 15 s and a two-minute wash, with the latter providing only an additional 3% transient bacterial reduction. Two observational studies were reviewed in the health care and food service industries to determine average durations in the real world. A study of nurses (34) revealed an average wash time of 21 s, while a survey of restaurant employees (4) showed that the average duration was 20 s.

After experiments were completed, log_{10} reductions of each individual handwashing were calculated by subtracting counts obtained after handwashing from baseline data. Statistical analysis using ANOVA, was performed, with no statistical difference seen between any set of handwashing and rinsing temperatures for normal (resident) or transient flora with either of the two contaminating soils. Figures 1 and 2 show log_{10} reduction results for the range of temperatures used in these experiments for normal (resident) flora soiled with TSB and with gamma irradiated ground beef, respectively. Four data points are provided at each temperature and soil. Two log_{10} reduction data points for both TSB and ground beef appear as negative for transient flora. Polynomial regression analysis was performed to display potential trends even though no statistical significance could be shown. In respect to normal (resident) flora, although rising temperature reduction efficacy seemed to increase slightly with TSB inocula, a slight decrease in efficacy was seen with ground beef. Resident TSB and ground beef R^2 values of 0.0135 and 0.1861, respectively, provide evidence of the lack of a relationship between the two variables.

Figures 3 and 4 show log_{10} reduction results for transient flora in TSB and gamma irradiated ground beef, respectively, at temperatures tested. Only one negative log_{10} reduction figure was observed. While polynomial regression showed a slight increase in efficacy with increasing temperature for ground beef inoculum, both high 48.9°C (120°F) and low 4.4°C (40°F) temperatures tended to have higher log_{10} reductions than the mid temperatures tested. Again, TSB and ground beef R^2 values of 0.1065 and 0.1174, respectively, provide evidence of a lack of relationship between the two variables.

The geometric mean log_{10} reduction for all transient flora experiments involving both TSB and ground beef inocula was 1.9,
Figure 4. Handwashing efficacy (log_{10} reduction) for transient flora (S. marcescens) in TSB at selected water washing and rinsing temperatures

whereas the resident flora log_{10} reduction was 0.2 for both menstruum. These log_{10} reduction figures are in agreement with results from other similarly performed studies of both resident (6, 19) and transient flora (2, 7, 26).

A comparison of log_{10} reduction variability (as seen in Fig. 1-4) was reviewed for trends that could indicate increased or decreased variability with certain temperatures under specific inoculum conditions. Coefficient of variation values for each temperature group for both resident and transient flora as well as both menstruum were determined by obtaining the ratio of the standard deviations of each group to the mean log_{10} reductions. Figure 5 shows the coefficient of variation (expressed in percent) for each testing condition. Coefficients of variation are fairly consistent for transient flora, with resident flora data exhibiting a great deal of variation. Overall, there appeared to be a slightly lower variation in log_{10} reduction figures for the 48.9°C (120°F) temperature over the 35°C (95°F) group. Variability data from the 4.4°C (40°F) and 12.8°C (55°F) groups were similarly low, with variability for temperature ranges peaking at 21.1°C (70°F).

Subjects freely commented that the water at a temperature of 4.4°C (40°F) was uncomfortable. In issues brought before the CFP, temperatures at or above 43°C (110°F) were argued to be uncomfortable. Taken together with the variability noted, it suggests that participants more consistently wash their hands when water temperatures are between 35°C (95°F) and 48.9°C (120°F).

Friction has been identified as a key element in removing microbial contaminants from hands (11, 27). Friction applied during the hand drying process is instrumental in finishing the process. Removal of transient flora appears to be even more friction dependent than removal of resident flora. Surfactant and antimicrobial compounds in soap are responsible for lifting soil and killing microorganisms suspended in the soil. When bland soap is used to wash hands, handwashing efficacy appears to be dependent on the effects of surfactant action of the soap along with friction applied during the washing and rinsing process. Rinsing also provides the necessary removal by dilution. To facilitate appropriate rinsing of the hands, some personal hygiene consultants have suggested the practice of using thicker, higher-viscosity soaps in larger doses, which would require a longer, more vigorous rinsing routine.

Price (27), upon noticing that in his scrubbing experiments water temperature had little effect at de-germing of the skin, commented that water applied to the skin at a given temperature quickly reaches equilibrium with normal skin surface temperature unless hands are totally immersed.

Skin oils derived from sebum are liquid in the sebaceous gland and solidify on the skin surface. Beef tallow melts in the range of 35°C to 40°C (95°F to 104°F), while lard or butterfat are liquefied at temperatures around 30°C (86°F) (15). If handwashing efficacy for both resident and transient floras embedded in both natural and artificially applied fats depended on thermal melting, then log_{10} reduction figures should have been greatest at the highest temperature and least at temperatures that cause these fats to congeal.

Fats such as tallow or lard are distinguished from oils in that oils are liquids at room temperature.
Hand soap formulations are designed to lift soil through their foaming action, dispersing and solubilizing organic soils using detergent surfactants. Primary micelles are present, having hydrophilic and hydrophobic groups attached to the ends of the surfactant monomer. Soaps with multiple surfactants form mixed micelles, which increases efficiency with various soil mixtures. In water and organic soil mixtures, these form complex micelle structures around hydrocarbon moieties (encapsulation), resulting in microemulsions. Thus, the soap provides a "bridge" between the oily droplet and water, permitting the soapy water to "wash away" greasy material.

Price (27) described the contradictory aspect of soap, which tends to reduce surface friction. Soaps of his day were not the more developed formulas now available and used in this experiment. In the experiments described here, a 3-ml aliquot of bland soap was used to remove a total of one gram of TSB or three grams of ground beef. Use of lower quantities of soap would obviously provide lower surfactant effectiveness. The quantity of soap used for handwashing has the ability to affect handwashing efficacy, as shown by Larson (14). Several studies (13, 16, 17, 18, 19, 21, 25, 29, 31) have used soap amounts in the range of 2.5 to 5.0 ml in their handwashing protocol. The higher levels are considered excessive, except in hospital infection control. Many food service operations set soap dispensers at 1 ml per pump, and employees often times use multiple pumps. As the experiments described here utilized 1.5 grams ground beef menstruum per hand, 3 ml of soap was chosen to represent an amount found to be significantly effective in an earlier study (14). In that study, it was determined that 3-ml of soap provided greater bacterial reductions than did 1 ml for a liquid, nonantimicrobial soap. Observations of soap usage by health care employees in the hospital setting were also performed, as nine different departments, from labor and delivery to psychology, determined average soap use to be around 2.18 ml per incidence, compared to 3.5 by the general population (14).

Surfactants in soap have surface tension lowering capabilities. The vigorous rubbing action of hands creates a rapid formation of surfaces and changing pressure gradients, which develop and increase micelle formation. The combined action of soap, friction and dilution appears to outweigh any advantage that temperature might have in the liquefying of fats, which would normally occur in the range of 30°C to 40°C (86°F to 104°F).

Many antimicrobials are inactivated by the presence of organic soils or soaps. Several writers have suggested that these antimicrobial ingredients present in soaps are not in contact with microorganisms long enough to provide sufficient antimicrobial action. Of the commonly used antimicrobial ingredients employed in soap products, only iodophors have been shown to exhibit temperature-dependent antimicrobial effects due to temperature-dependent dissociation constants for PVP and iodine present in the formulation. For these reasons, even if antimicrobial agents were present in soap, it is doubtful that water temperature would have a significant effect on overall hygienic efficiency. It should also be noted that under real-life conditions, hands would be dried (usually with paper towels) and that further bacterial reductions in the range of 1 log<sub>10</sub> are seen, reducing any slight difference in efficacy with antimicrobial soaps.
REFERENCES


ACKNOWLEDGMENTS

Funding for this project was provided by a grant from the Georgia-Pacific Health Smart Institute.