A Method of Assessing the Efficacy of Hand Sanitizers: Use of Real Soil Encountered in the Food Service Industry

D. L. CHARBONNEAU,* J. M. PONTE, AND B. A. KOCHANOWSKI

The Procter & Gamble Co., Cincinnati, Ohio, USA

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ABSTRACT

In many outbreaks of foodborne illness, the food worker has been implicated as the source of the infection. To decrease the likelihood of cross-contamination, food workers must clean and disinfect their hands frequently. To ensure their effectiveness, hand disinfectants should be tested using rigorous conditions that mimic normal use. Currently, several different methods are used to assess the efficacy of hand disinfectants. However, most of these methods were designed with the health care worker in mind and do not model the specific contamination situations encountered by the food worker. To fill this void, we developed a model that uses soil from fresh meat and a means of quantifying bacteria that is encountered and transferred during food preparation activities. Results of studies using various doses of para-chloro-meta-xylenol and triclosan confirm that the method is reproducible and predictable in measuring the efficacy of sanitizers. Consistent, dose-dependent results were obtained with relatively few subjects. Other studies showed that washing hands with a mild soap and water for 20 s was more effective than applying a 70% alcohol hand sanitizer.

Despite the wealth of information available on the mechanisms of transmission of foodborne illness, recent epidemiologic data indicate that the incidence of foodborne illness is increasing (1). It is speculated that the rise in foodborne illness is associated with several factors, including increased importation of food that is not produced under optimal hygiene conditions and consumption of meals away from home (4). In a number of outbreaks of foodborne illness, the food worker has been implicated as the source of contamination. For several reasons, including hand contamination and improper handling and storage of ready-to-eat foods, the food worker has a great potential for becoming the source of foodborne disease (5, 6, 13−15).

A useful weapon in the prevention of foodborne illness is an effective sanitation program that includes a comprehensive hand hygiene component (6). The most practical hand hygiene program is centered around the use of disinfectant products to eliminate transient bacteria from the food worker’s hands. In the past few years, much interest has been placed on the use of hand cleaning products with robust antimicrobial efficacy. However, many of these products are harsh to hands, which negatively affects hand washing compliance. The fact that hand washing compliance is as important as product efficacy has been largely ignored. For hand hygiene to be optimal, the hand cleaner must be mild enough so the worker will not resist using the product often in an effective regimen.

To ensure their effectiveness, hand disinfectants must be tested using rigorous test conditions that mimic normal use conditions. Normally, these products are used in the hand washing process not for cleaning and disinfecting hard surfaces. Thus, the use of in vitro methods with artificial surfaces (e.g., carrier tests and bacterial suspension tests) seems inappropriate for the evaluation of hand washing products. In addition, since these products are intended to be used for the prevention of cross-contamination, the methodology should take into account this aspect of bacterial transfer. Currently, several different methods are used to assess the efficacy of hand disinfectants (2, 3, 7−12, 16−18). Most of these methods are designed to evaluate hand disinfectants that are used in the hospital setting and do not emphasize the specific needs of the food worker. In most of these published methods, the fingertips of the hands are artificially inoculated with a pure culture of bacteria. Samples are then collected either by placing a fluid-filled bag or glove on the hand, massaging it, and plating its contents (“glove juice method”) (10) or by pressing fingers onto the surface of an agar plate (2, 3, 7−9). Standard plate counting techniques are used to enumerate bacterial coverage in most of these methods. More recently, image analysis has been used to quantify results (7, 12).

A major concern with the use of the “glove juice method” is that it samples areas of the hands that may not be involved with the transmission of pathogens (e.g., back of the hand). Use of the finger press method alone does not provide a true quantitative measure of efficacy. In addition, both methods require manual counting of bacterial colonies, which is time-consuming and prone to error. Finally, in both these methods, hands are artificially soiled with pure bacterial suspensions of a single organism. In practice, the worker typically comes into contact with mixtures of bacteria in the presence of fat or other soil. Clearly, since none of these methods were designed with the specific needs of the food worker in mind, new models must be developed that are appropriate for products to be used in food service.

* Author for correspondence. Tel: 513-622-2896; Fax: 513-622-2626; E-mail: Charbonneau.dl@pg.com.
In this article, we describe a new method for the evaluation of hand washing soaps and hand disinfectants. In this model, we use fresh, raw meat (either chicken or ground beef) as the source of soil, because it incorporates bacteria in the presence of a soil load that is consistent with the situation encountered in the food service environment. As described previously (7, 12), imprints of the whole palm were taken to encompass the major areas of the hand that are responsible for the transmission of pathogens. Image analysis of the plates was used for the purpose of quantitative assessments. The data indicate that our method can be used to assess the efficacy of hand washing agents in removing bacteria from the hands of meat handlers. The results also indicate that washing hands with a mild soap was more effective than 70% ethanol in eliminating viable bacteria from meat-soiled hands.

**MATERIALS AND METHODS**

**Subjects.** People who had open cuts on their hands or were taking antibiotics were not allowed to participate in these studies.

**General test procedure.** For each experiment (see studies A through E below), the procedure depicted in Figure 1 was followed. On entry into each study, subjects washed their hands with 1.9 g of nonmedicated liquid soap to remove transient organisms. The soap was dispensed from a commercial dispenser onto the palm of the left hand of each subject. Subjects briefly wet both hands with tap water (40 ± 5°C, at a flow rate of 4 liter/min), then rubbed the soap all over their hands and the lower one third of each forearm (taking care not to lose or dilute the formulation). Hands were then lathered and washed vigorously for 20 s, rinsed under tap water (40 ± 5°C, at a flow rate of 4 liter/min) for 30 s, and dried with one to two paper towels.

After washing their hands with the nonmedicated liquid soap, subjects contaminated both hands by handling either a whole, fresh raw chicken or 2 lb of hamburger for 45 s. Chickens were massaged both inside and out, and raw hamburger was kneaded with the hands. After contamination, hands were air dried for approximately 1 min. Both hands were then sampled for bacteria (baseline) using the palm imprint method (7, 12).

After baseline evaluations were completed, the antibacterial efficacy of various soaps and sanitizers was tested. In each experiment, subjects again washed their hands and lower forearms for 20 s with a nonmedicated liquid soap (see above) and recontaminated their hands (see above) with the same chicken and/or hamburger they had used previously. Results of preliminary studies indicated that bacterial levels on the chicken or hamburger samples were not altered by repeated handling (unpublished data). Subjects then washed their hands with a nonmedicated liquid soap or test soap (see studies A through D below) and repeated the palm imprint procedure. All test soaps were administered in a single-blind fashion. On completion of their respective experiment, participants sanitized their hands with Hibistat (Zeneca, Wilmington, Del.) for 30 s.

**Study A: effect of nonmedicated liquid soap on contamination by ground beef or chicken.** The purpose of this study was twofold: to determine the antibacterial effect of a nonmedicated liquid soap and to determine whether ground beef or chicken should be used to soil the hands in subsequent experiments. Five volunteers (three women, two men) participated in the study. After
washing their hands for 20 s with nonmedicated liquid soap (see general test procedure above), three subjects handled chicken and two subjects massaged hamburger. After baseline imprints were obtained, subjects washed their hands with nonmedicated soap and repeated the imprint procedure. After washing the agar off with nonmedicated soap, they recontaminated their hands with the alternate soil (i.e., those who initially handled chicken switched to hamburger and vice versa) and repeated the imprint procedure (before and after washing again with nonmedicated soap). Thus, in this experiment, measurements for both meat products (before and after washing with nonmedicated soap) were obtained from each subject. Based on the results of this study, chickens were used to soil the hands in subsequent experiments.

Study B: dose response of PCMX. The purpose of this study was to determine the efficacy of different doses of para-
FIGURE 3. Graphic representation of image analysis data obtained from subjects sanitizing hands by washing with nonmedicated soap or applying various concentrations of (A) PCMX or (B) triclosan. Data are presented as mean ± standard error of pixel area covered by both hands of all subjects (for PCMX, n = 4; for triclosan, n = 6). Palms were contaminated by handling a fresh, raw chicken. Treatments are compared with baseline untreated controls (BL).

chloro-meta-xylenol (PCMX) in eliminating bacteria from hands. A total of four subjects (three women and 1 man) participated. The effect of washing with 1.9 g of a nonmedicated liquid soap for 15 s (positive control) was assessed first. After the nonmedicated liquid soap imprint was obtained, the participants washed their hands with the same nonmedicated liquid soap for 20 s. After handling the chicken again for 45 s, 1 ml of a formulation containing 0.12, 0.24, 0.48, 0.95, 1.9, or 3.75% PCMX, 48% isopropanol, 5% propylene glycol, and the remainder of the DC200 silicone was placed in the subjects’ palms. The subjects then rubbed their hands together for 5 s and were instructed to distribute the solution over the entire surface of the hands. Five minutes later, hand imprints were made to enumerate the bacteria remaining on the hands. After the imprint procedure was completed, 100 ml of an inactivator solution containing 2% Tween 80, 0.1% lecithin, and 0.1% sodium thiosulfate was placed onto the subjects’ hands, and they were instructed to wash for 20 s with the nonmedicated liquid soap. This cycle was repeated until all participants had used all the treatments (in order of dose). Results of preliminary experiments indicated that the outcome was not influenced by order of treatment (data on file).

Study C: dose response of triclosan. The purpose of this study was to determine the efficacy of different doses of triclosan
on eliminating bacteria from hands. As with study B, a nonmedicated liquid soap was used as a positive control. A total of six subjects (three of each sex) participated. The protocol followed was identical to that of study B (PCMX), with the exception that the subjects’ hands were treated with formulations containing 0.1, 0.25, 0.5, 1.0, 2.5, or 5.0% triclosan instead of PCMX.

**Study D: variability in efficacy of nonmedicated liquid soap.** Data from each participant in studies B and C were analyzed and plotted separately to determine if efficacy of the nonmedicated liquid soap varied from participant to participant.

**Study E: effect of soap and water compared with alcohol gel sanitizer.** The purpose of this experiment was to compare the effect of a nonmedicated liquid soap with that of a sanitizer containing 70% ethanol in eliminating viable bacteria from soiled hands. In addition, the effect of using both products in sequence was assessed. Four subjects (two women and two men) were used. After baseline imprints were obtained, subjects washed the soil off with the nonmedicated liquid soap, resoiled their hands with a raw, fresh chicken, washed with the nonmedicated liquid soap again for 20 s, and repeated the imprint procedure. After subjects washed the soil off with the nonmedicated liquid soap and recontaminated their hands, alcohol gel sanitizer (1.5 ml) was dispensed onto each hand. Subjects then washed their hands until dry and repeated the imprint procedure. The subjects then washed and recontaminated their hands one more time. Afterward, they washed their hands with the nonmedicated liquid soap and then with the alcohol gel sanitizer (1.5 ml per hand). Final imprints of both hands were then obtained.

**Quantification of bacteria.** Bioassay plates (Nunc, 240 by 25 mm) containing hand medium (as described by Leyden and coworkers) were used for all imprints performed in this study, except cycloheximide was omitted (7). Plates were placed into a NUaire Class II Biological Safety Cabinet for 1 h before use. After hands were contaminated and dried, subjects touched the palms of their hands onto individual plates. Plates were then incubated at 48 ± 4 h at a temperature of 33.0 ± 2°C and a relative humidity of 60 ± 4%. Photographic images of the plates were then taken and stored on compact disks. Images displayed in this article were chosen at random.

The images from the palm imprint plates were visualized using a MTI CCD72 black-and-white video camera. Plates were illuminated from underneath using a Fostec Ace 1 fiber optic illuminator with an iris diaphragm and the light control adjusted to an optimal setting. The images were captured using a COMPAQ DeskPro XE 560 computer equipped with Image Pro Plus software Version 1.3 (Media Cybernetics). The bacterial growth was converted into dark pixels by the software, and the area of dark pixel coverage was calculated using Image Pro Plus Software. All data gathered using the system were immediately downloaded to an Excel (Microsoft) version 4.0a spreadsheet for analysis.

**Data analysis.** The hand imprint data from the video image (converted to dark pixels) was used directly from the spreadsheet. Data from studies B and C were analyzed using a single-factor analysis of variance. Between-group comparisons were made using a least significant difference test. The level of significance was $P \leq 0.05$. The percent reduction in area coverage was calculated using the following formula:
Effect of Soap and Water and Alcohol Gel Sanitizer
Used Alone and in Combination

FIGURE 5. Bacterial coverage of palms before and after washing with nonmedicated soap, treatment with alcohol gel sanitizer, or using both products sequentially. Results shown are from both hands of one representative subject. Palms were contaminated by handling a fresh, raw chicken.

Percent Reduction
= (Baseline Measurement − Treatment Measurement × 100)/Baseline Measurement

RESULTS AND DISCUSSION

Effect of nonmedicated liquid soap on contamination by ground beef or chicken. Images from subjects washing with the nonmedicated liquid soap after handling ground beef or chicken (study A) are shown in Figure 2. The baseline area of dark pixel coverage for both hands ranged from 29,046 to 59,517 after massaging ground beef and from 37,882 to 68,999 after handling fresh, raw chicken. After washing with the nonmedicated liquid soap, pixel area coverage was reduced to 1,566 to 38,017 for subjects handling fresh, ground beef and to 5,789 to 34,794 for subjects handling fresh, raw chicken. Because bacterial coverage on the hands of subjects handling chicken was greater and variability was lower compared with beef, chicken was chosen as the inoculum for all further experiments.

Dose responses of PCMX and triclosan. Under the conditions used in this study (study B), a 5-s application of 0.12 to 3.75% PCMX significantly reduced the amount of viable transient bacteria on the palms of participants (Fig. 3A). The effect was dose dependent from 0.48 to 3.75%. A concentration of at least 0.95% PCMX was required to reduce bacterial levels by 80% or more. In this experiment, a dose-response relationship was observed when using data from as few as four participants. This indicates that the chicken test model is a good screening tool for small-scale preliminary studies.

Results of the experiment with triclosan (study C) are depicted in Figure 3B. All concentrations used in the study significantly reduced bacterial levels. Use of triclosan concentrations greater than 1.0% did not increase efficacy dramatically.

Nonmedicated liquid soap variability. The individual values for pixel area coverage after contamination and washing with a nonmedicated liquid soap (study D) for the 10 subjects who participated in studies B and C are shown in Figure 4. As noted, the amount of bacteria present on the hands after contamination and washing with the nonmedicated liquid soap varied from participant to participant. In these subjects, the amount of bacterial reduction induced by washing with the nonmedicated liquid soap ranged from 82.96 to 100%. The degree of reduction achieved did not correlate with the initial amount of bacteria present. Taken together, these data suggest that in a certain number of people a significant amount of bacteria remains on their hands after washing with soap and water for 15 s. There are several possible reasons for this variability in hand washing efficacy among the participants. Poor-quality washes may be associated with the technique used in the hand washing procedure. Some individuals do not achieve optimal coverage during the lathering process or do not apply an appropriate amount of force when scrubbing. Efficacy of washing may also depend on the condition of the skin at the time of the washing procedure. If the skin is dry, it may be more difficult to remove the adherent bacteria from the surface. It is possible that longer washing times may reduce the person-to-person variability. Therefore, to reduce the potential for bacterial transfer, food workers may need to wash their hands for longer than 15 s or wash more often.

Effect of soap and water compared with alcohol gel sanitizer. As shown in Figure 5, a 20-s wash with the nonmedicated liquid soap and water was more effective at removing bacteria from palms than application of a gel sanitizer containing 70% alcohol (study E). Interestingly, use of both products in sequence resulted in more bacteria being transferred versus soap and water alone. This suggests that use of alcohol on the hands after washing them may increase transfer of bacteria that are normally sequestered. The finding that the combination of soap and water plus alcohol enhances bacterial transfer has been reported in the
literature previously by two independent investigators (8, 11). The mechanism by which the combination of soap and water plus alcohol increases the numbers of bacteria that transfer from the hands is yet to be determined. It has been speculated that this combination raises bacteria from deeper layers of the skin (8, 11).

Altogether, results of these studies show that our method, which involves use of real soil and image analysis for quantification, is an accurate and easy means to evaluate the antimicrobial efficacy of hand washing soaps and sanitizers in relatively few subjects. We believe that this method is a much more realistic procedure for food workers than existing ones and has many applications in the evaluation of hand washing agents designed for use in the food service industry.

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REFERENCES