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Research Note

SaniTwice: A Novel Approach to Hand Hygiene for Reducing Bacterial Contamination on Hands When Soap and Water Are Unavailable

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ABSTRACT

The risk of inadequate hand hygiene in food handling settings is exacerbated when water is limited or unavailable, thereby making washing with soap and water difficult. The SaniTwice method involves application of excess alcohol-based hand sanitizer (ABHS), hand ''washing'' for 15 s, and thorough cleaning with paper towels while hands are still wet, followed by a standard application of ABHS. This study investigated the effectiveness of the SaniTwice methodology as an alternative to hand washing for cleaning and removal of microorganisms. On hands moderately soiled with beef broth containing *Escherichia coli* (ATCC 11229), washing with a nonantimicrobial hand washing product achieved a 2.86 ($\pm$0.64)-log reduction in microbial contamination compared with the baseline, whereas the SaniTwice method with 62% ethanol (EtOH) gel, 62% EtOH foam, and 70% EtOH advanced formula gel achieved reductions of 2.64 $\pm$ 0.89, 3.64 $\pm$ 0.57, and 4.61 $\pm$ 0.33 log units, respectively. When hands were heavily soiled from handling raw hamburger containing *E. coli,* washing with nonantimicrobial hand washing product and antimicrobial hand washing product achieved reductions of 2.65 $\pm$ 0.33 and 2.69 $\pm$ 0.32 log units, respectively, whereas SaniTwice with 62% EtOH foam, 70% EtOH gel, and 70% EtOH advanced formula gel achieved reductions of 2.87 $\pm$ 0.42, 2.99 $\pm$ 0.51, and 3.92 $\pm$ 0.65 log units, respectively. These results clearly demonstrate that the in vivo antibacterial efficacy of the SaniTwice regimen with various ABHS is equivalent to or exceeds that of the standard hand washing approach as specified in the U.S. Food and Drug Administration Food Code. Implementation of the SaniTwice regimen in food handling settings with limited water availability should significantly reduce the risk of foodborne infections resulting from inadequate hand hygiene.

Foodborne diseases are a serious public health concern *(3, 4, 15),* but despite preventive efforts there has been little recent progress in reducing infections caused by foodborne pathogens *(6).* Faulty food handling practices, particularly improper hand washing, contribute significantly to the risk for foodborne disease *(11–13, 19, 25–27, 29).* Proper hand hygiene reduces the risk of transmission of pathogens from hands to food *(7, 20, 21)* and is associated with a reduction in gastrointestinal illness *(2, 8, 18).* The U.S. Food and Drug Administration (FDA) Food Code for retail establishments requires hand washing as a preventive method and provides specific guidance on proper hand washing procedures *(30).* The five-step hand washing procedure outlined in the FDA Food Code consists of (i) rinsing under warm running water, (ii) applying the manufacturer-recommended amount of cleaning compound, (iii) rubbing the hands vigorously, (iv) rinsing thoroughly under warm running water, and (v) thoroughly drying the hands with individual paper towels, a continuous clean towel system, or a heated or pressurized hand air drying device. According to the Food Code, alcohol-based hand sanitizers (ABHS) may be used in retail and food service only after proper hand washing.

ABHS are recommended as an alternative to traditional hand washing in the health care setting *(5).* Alcohols are highly effective against a range of bacterial pathogens, fungi, enveloped viruses, and certain nonenveloped viruses *(2, 10).* Although considered to be ineffective antimicrobial agents in the presence of visible dirt or proteinaceous material, alcohol-containing products were more effective than those containing triclosan *(2, 14)* or detergents *(17)* for removing microorganisms from hands contaminated with organic material. In health care facilities and other environments, easily accessible ABHS have resulted in greater hand hygiene compliance and reduction in infections *(1, 9, 16, 31).* Although ABHS are approved for use in the health care environment, the FDA does not regard these agents as adequate substitutes for soap and water in the food service setting *(30).*

A reliable hand hygiene method is needed for food service settings in which adequate hand washing facilities are limited or unavailable. These settings include portable bars, buffet lines, outdoor events, and catering functions at which the only available hand hygiene facility often is either ''trickle hand washing'' (i.e., hand washing done from a

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portable container of water over a bucket or other type of basin) or simply the use of a paper towel or damp cloth to rub the hands. These methods may be inadequate for proper hand cleansing.

SaniTwice (a registered trademark with James Mann, Handwashing for Life, Libertyville, IL) is a two-stage hand cleansing protocol that is performed using ABHS when water is not available. In this study, we evaluated the microbiological efficacy of the SaniTwice method on the hands of adult human participants. These studies were designed to assess (i) the antimicrobial efficacy of various ABHS used with the SaniTwice regimen as compared with that of a standard hand washing method with soap and water on soiled hands and (ii) the impact of the active ingredient and/or formulation of a hand sanitizer on antibacterial efficacy when used in a SaniTwice regimen.

MATERIALS AND METHODS

Test products. All test products in this study were manufactured by GOJO Industries (Akron, OH). Two hand washing products were evaluated: a nonantimicrobial product (GOJO Luxury Foam Handwash) and an antimicrobial product (MICRELL Antibacterial Foam Handwash, 0.5% chloroxylenol active). Four ABHS also were evaluated: a 62% ethanol (EtOH) gel (PURELL Instant Hand Sanitizer Food Code Compliant), a 62% EtOH foam (PURELL Instant Hand Sanitizer Foam), a 70% EtOH gel (PURELL 70 Instant Hand Sanitizer), and a 70% EtOH Advanced Formula (AF) gel (PURELL Instant Hand Sanitizer Advanced Formula VF481).

Overall study design. Three studies were conducted by BioScience Laboratories (Bozeman, MT) to determine the in vivo antimicrobial efficacy of various test product configurations under conditions of moderate or heavy soil. The order of use of each product was determined randomly. A two-step testing sequence was used for all products. Each volunteer completed the baseline cycle, where hands were contaminated with moderate or heavy soil (as described below) containing *Escherichia coli* (ATCC 11229), and samples were collected for baseline bacterial counts. Following the baseline sampling, participants completed a 30-s nonmedicated soap wash followed by the product evaluation cycle, which consisted of a contamination procedure, application of the test product, and subsequent hand sampling. Between uses of different test products, participants decontaminated their hands with a 1-min 70% EtOH rinse, air drying, and a 30-s nonmedicated soap wash. A minimum of 20 min elapsed before the next testing sequence began. Baseline and postapplication samples were evaluated for the presence of *E. coli*. Testing was performed according to the FDA health care personnel hand washing product evaluation method *(28)* and modified as described previously *(22)*.

The study was approved by the Gallatin Institutional Review, an independent review board unaffiliated with BioScience Laboratories, and was conducted in compliance with Good Clinical Practice and Good Laboratory Practice regulations. All participants provided written informed consent.

Participants. The study enrolled healthy adults with two hands. All participants were free of dermal allergies or skin disorders on the hands or forearms.

Preparation of inoculum. *E. coli* was used to test the efficacy of the test procedures. A 2-liter flask was filled with 1,000 ml of tryptic soy broth: 30.0 g of dehydrated tryptic soy broth medium (BD, Franklin Lakes, NJ) added to 1 liter of deionized water, heated, and sterilized for a final pH of 7.3 ± 0.20. The broth was inoculated with 1.0 ml of a 24-h culture of *E. coli* grown from a cryogenic stock culture. The flask was incubated for 24 h, and the suspension was used for challenge.

Hand contamination procedures. For the moderate soil study, a 24-h culture of *E. coli* was suspended in beef broth (Swanson low sodium beef broth, Campbell Soup Company, Camden, NJ) at 1×10^9 CFU/ml. Three aliquots of 1.5 ml were transferred into each participant's cupped hands. Each aliquot was distributed over the entire front and back surfaces of the hands up to the wrists during a 20-s period and allowed to air dry for 30 s after the first and second aliquots and for 90 s after the third aliquot. After samples were collected for baseline bacterial counts and hands were decontaminated with a 30-s wash with nonmedicated soap, a second cycle of contamination was initiated. After the 90-s final drying step, participants applied the randomly assigned test product.

For the heavy soil study, 5.0-ml aliquots of the challenge suspension of *E. coli* were transferred to 4-oz (113-g) portions of sterile 90% lean ground beef and distributed evenly with gloved hands to achieve contamination levels of approximately 5.0×10^8 CFU per portion. Each participant then kneaded the inoculated raw hamburger for 2 min. Hands were air dried for 90 s and then sampled for baseline counts. After a 30-s decontamination with nonmedicated soap, the cycle was repeated, and the test product was applied.

Test article or product application and SaniTwice procedure. The hand washing procedure used for the nonantimicrobial and antimicrobial hand washing products was consistent with Food Code specifications. Table 1 shows the stepwise product application procedures for all test configurations.

Bacterial recovery and microbial enumeration. Within 1 min after contamination for baseline evaluation or after product application, powder-free sterile latex gloves were placed on each participant's hands and secured above the wrist, and 75 ml of sterile stripping fluid (0.4 g of KH_2PO_4, 10.1 g of Na_2HPO_4, and 1.0 g of isooctylphenoxypolyethoxyethanol in 1 liter of distilled water, pH adjusted to 7.8) was transferred into each glove. Following a 60-s massage of the hands through the gloves, a 5.0-ml aliquot of the glove rinsate sample was removed and diluted in 5.0 ml of Butterfield's phosphate buffer solution with product neutralizers. Each aliquot was serially diluted in neutralizing solution, and appropriate dilutions were plated in duplicate onto MacConkey agar plates (BD; 50.0 g of dehydrated medium added to 1 liter of deionized water, heated, and sterilized; final pH, 7.1 ± 0.2) and incubated for 24 to 48 h at 30°C. Colonies were counted and data were recorded using the computerized Q-COUNT plate-counting systems (Advanced Instruments, Inc., Norwood, MA).

Data analysis and statistical considerations. The estimated log transformed number of viable microorganisms recovered from each hand (the R value) was determined using the formula $R = \log(75 \times C_i \times 10^D \times 2)$, where 75 is the amount (in milliliters) of stripping solution instilled into each glove, C_i is the arithmetic average colony count of the two plate counts at a particular dilution, D is the dilution factor, and 2 is the neutralization dilution.

Descriptive statistics and confidence intervals were calculated using the 0.05 level of significance for type I (alpha) error. Statistical calculations of means and standard deviations were

TABLE 1. *Test product application procedures*[a]

Step	Food Code–compliant procedure for hand washing products	SaniTwice[b] procedure for ABHS	Procedure for 70% EtOH AF gel
1	Wet hands with water at 40°C	Dispense ~3 ml of product into cupped hands	Dispense ~1.5 ml of product into cupped hands
2	Apply ~1.5 ml of product	Rub vigorously over hands for 15 s to simulate washing	Rub hands together until dry
3	Lather for 15 s	Clean thoroughly with two paper towels	
4	Rinse with water for 10 s	Dispense additional ~1.5 ml of product	
5	Pat dry with two paper towels	Rub hands together until dry	

[a] All application procedures were initiated within 10 s of completing the 90-s drying step.
[b] SaniTwice is a registered trademark with James Mann (Handwashing for Life, Libertyville, IL).

generated for the log recovery data from baseline samples, postproduct application samples, and the log differences between baseline and postapplication samples. Product comparisons were made using a one-way analysis of variance with post hoc analysis (Bonferroni's multiple comparison test) using the 0.05 level of significance for alpha error.

RESULTS

Reduction in microbial contamination of moderately soiled hands. Two studies were conducted to evaluate microbial count reductions on hands that had been contaminated by handling beef broth containing *E. coli*. Reductions from baseline produced by the five test product configurations in these two studies are shown in Figure 1.

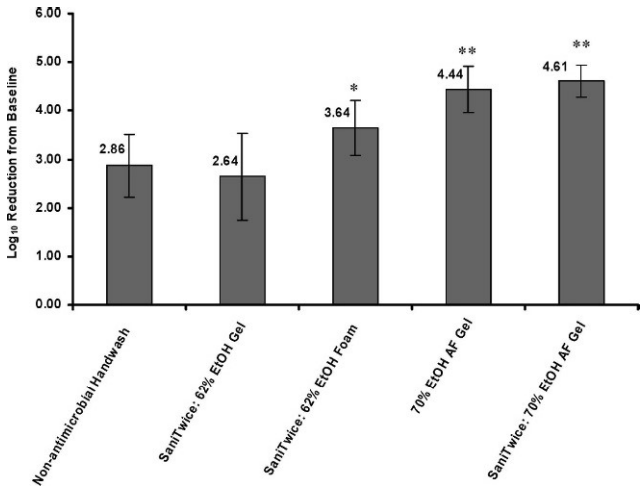

FIGURE 1. *Log reduction from baseline for microbial contamination of hands moderately soiled with contaminated beef broth after application of test products. Error bars represent standard deviation. Data are from two separate studies. In study 1* (n = *11), nonantimicrobial hand washing product and SaniTwice with 62% EtOH gel were compared. In study 2* (n = *12), the conditions evaluated were nonantimicrobial hand washing product, SaniTwice with 62% EtOH foam, 70% EtOH AF gel without SaniTwice, and SaniTwice with 70% EtOH AF gel. Results for nonantimicrobial hand washing product represent pooled data from both studies.* * $P < 0.05$ *for SaniTwice with 62% EtOH foam versus nonantimicrobial hand washing product or SaniTwice with 62% EtOH gel.* ** $P < 0.05$ *for 70% EtOH AF gel or for SaniTwice with 70% AF gel versus nonantimicrobial hand washing product, SaniTwice with 62% EtOH gel, or SaniTwice with 62% EtOH foam.*

All SaniTwice regimens were equivalent to or better than the Food Code hand washing protocol. Reductions from baseline ranged from 2.64 ± 0.89 log CFU/ml for SaniTwice with the 62% EtOH gel to 4.61 ± 0.33 log CFU/ml for SaniTwice with the 70% EtOH AF gel.

SaniTwice using the 62% EtOH gel was equivalent to the nonantimicrobial Food Code hand washing protocol. However, SaniTwice using the 62% EtOH foam (3.64 ± 0.57-log reduction) was more effective than SaniTwice with the 62% EtOH gel and the Food Code hand washing protocol ($P < 0.05$).

The 70% EtOH AF gel was the most effective sanitizing product. When used independently, it was significantly more effective (4.44 ± 0.47-log reduction) than SaniTwice with 62% EtOH foam or 62% EtOH gel or the nonantimicrobial hand washing product ($P < 0.05$ for all comparisons). Although the log reduction data suggest that SaniTwice with 70% EtOH AF gel (4.61 ± 0.33-log reduction) was equivalent to the 70% EtOH AF gel used independently, this lack of differentiation was most likely due to the limitations of the assay. The 4.61-log reduction was at the limit of detection for all participants using 70% EtOH AF gel with SaniTwice but for only half the participants using 70% EtOH AF gel alone. Therefore, the log reductions produced by the 70% EtOH AF gel after either a single sanitization or the SaniTwice regimen are likely underestimated, and the log reductions in both cases would likely be higher if the limits of detection were lower.

Reduction in microbial contamination of heavily soiled hands. Figure 2 shows microbial count reductions produced by test product configurations on hands that had been contaminated by handling ground beef containing *E. coli*. All SaniTwice regimens tested were equivalent to or better than the Food Code hand washing protocol, indicating that under conditions of heavy soil, the SaniTwice procedure is as effective as hand washing. The performance of the antimicrobial hand washing product was equivalent to that of the nonantimicrobial hand washing product in this heavy soil challenge, with log reductions of 2.69 ± 0.32 and 2.65 ± 0.33, respectively. SaniTwice with the 70% EtOH AF gel outperformed all other sanitizer configurations tested and was superior to hand washing for reduction of organisms on heavily soiled hands ($P < 0.05$ for comparisons of SaniTwice with 70% EtOH AF gel versus each of the other procedures).

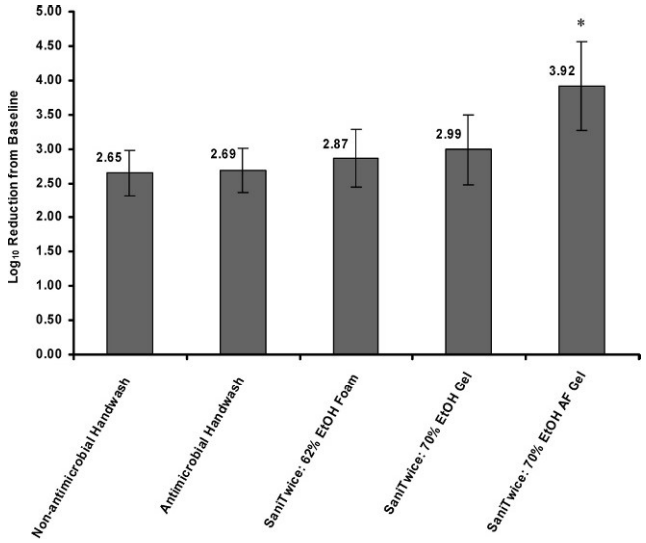

FIGURE 2. *Log reduction from baseline for microbial contamination of hands heavily soiled with contaminated uncooked hamburger after application of test products and protocols. Error bars represent standard deviation. Data are from study 3 (*n = *15), in which five test configurations were evaluated.* * P < *0.05 for SaniTwice with 70% AF gel versus nonantimicrobial hand washing product, antimicrobial hand washing product, SaniTwice with 62% EtOH foam, or SaniTwice with 70% EtOH gel.*

Two ABHS used with SaniTwice under both moderate and heavy soil conditions produced greater log reductions in the moderate soil condition. Mean log reductions using SaniTwice (moderate versus heavy soil) were 3.64 versus 2.87 for 62% EtOH foam and 4.61 versus 3.92 for 70% EtOH AF gel.

DISCUSSION

The SaniTwice method for hand disinfection was equivalent or superior to hand washing with soap and water for reducing viable bacteria on hands in the presence of representative food soils. Although the raw hamburger was a more difficult soil to penetrate, as demonstrated by approximately 1.0-log lower reductions compared with challenge by contaminated beef broth, the SaniTwice method with ABHS was equivalent to hand washing even under this worst-case simulation, underscoring the efficacy of this new method and indicating a potentially greater margin of safety.

The ABHS products used in this study exhibited a range of antimicrobial efficacy, suggesting that product formulation and the concentration of active ingredient may play a role in the observed efficacy. The impact of formulation was indicated by the significantly higher efficacy of the 62% EtOH foam compared with the 62% EtOH gel when challenged with moderate soil. This difference may be due to the additional foaming surfactants in the foam formulation, which may aid in lifting and removing bacteria and soil from the hands during the SaniTwice procedure. In addition, SaniTwice with the 70% EtOH AF gel was superior to SaniTwice with the 70% EtOH gel and 62% EtOH foam under heavy soil conditions. The 70% EtOH AF gel, whether tested as a single application or with the SaniTwice method, was superior to hand washing and to the 62% EtOH gel or foam under moderate soil conditions. The 4.44-log reduction with a single use of the 70% EtOH AF gel demonstrates its high antimicrobial efficacy, which is further enhanced when used with the SaniTwice method. The 70% EtOH AF gel contains a patent-pending blend of ingredients that enhance the activity of the alcohol and likely contribute to the high efficacy observed in this study. The SaniTwice procedure gives the benefit of skin cleansing and soil removal, which is not obtained with single use of a product. The efficacy of ABHS used with SaniTwice against nonenveloped enteric viruses, which are more difficult to eradicate, remains to be determined.

In support of previous findings *(23),* the findings in this study indicate that the decontamination efficacy was similar for the antimicrobial and nonantimicrobial hand washing products under heavy soil conditions, suggesting that the cleansing properties of the surfactants in these soaps and the mechanical action of hand washing may be the primary contributors to efficacy rather than the antimicrobial activity of any constituent of the formulations. It is expected that with heavy hand soiling, the surfactant effect drives efficacy, and typical antibacterial constituents will have little additional effect.

In this study, SaniTwice was an effective hand hygiene regimen at least equivalent to hand washing with soap and water for reducing microbial contamination, even under worst case conditions of high bacterial load and heavy food soils. The current FDA Food Code allows use of ABHS only on hands that have been cleaned according to the recommended hand washing protocol *(30)*. However, other than substitution of an ABHS for soap and water, the SaniTwice protocol mirrors the FDA-specified hand washing sequence. SaniTwice is at least as effective as hand washing when used with standard-efficacy ABHS; when used with a high-efficacy ABHS, the SaniTwice protocol is superior to washing with soap and water. The Food Code provides few specific recommendations for achieving good hand hygiene when water (or other hand washing supplies and equipment) is unavailable or limited. The Food Code (Section 2-301.16) severely restricts hand sanitizers by allowing use only after proper hand washing or in situations in which no direct contact with food occurs *(30)*.

A potential solution to this gap in food safety practices is SaniTwice. The SaniTwice studies described here provide convincing scientific rationale for including the SaniTwice approach in the Food Code as an alternative method of hand hygiene when standard hand washing is impractical. The simplicity and ease of use of the SaniTwice method, which requires only a supply of ABHS and paper towels, should allow this protocol to be applied to various food service settings and other areas in which hand hygiene is needed but safe water is unavailable or in short supply.

The findings in the present study support and extend those from previous studies; ABHS used alone or in combination with hand washing can be effective for decontaminating hands in the presence of organic soils *(17, 23, 24)*. A well-formulated ABHS in conjunction with

the SaniTwice regimen can have high efficacy, even in the presence of high organic load. Therefore, a reevaluation of the longstanding paradigm defining the use of ABHS in the presence of organic soils in both food handling and health care environments is warranted.

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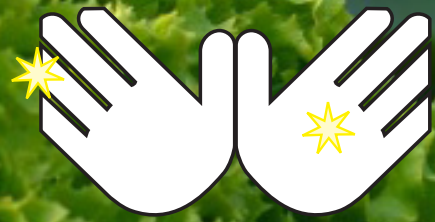

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Hand Hygiene Regimens for the Reduction of Risk in Food Service Environments

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ABSTRACT

Pathogenic strains of *Escherichia coli* and human norovirus are the main etiologic agents of foodborne illness resulting from inadequate hand hygiene practices by food service workers. This study was conducted to evaluate the antibacterial and antiviral efficacy of various hand hygiene product regimens under different soil conditions representative of those in food service settings and assess the impact of product formulation on this efficacy. On hands contaminated with chicken broth containing *E. coli,* representing a moderate soil load, a regimen combining an antimicrobial hand washing product with a 70% ethanol advanced formula (EtOH AF) gel achieved a 5.22-log reduction, whereas a nonantimicrobial hand washing product alone achieved a 3.10-log reduction. When hands were heavily soiled from handling ground beef containing *E. coli,* a wash-sanitize regimen with a 0.5% chloroxylenol antimicrobial hand washing product and the 70% EtOH AF gel achieved a 4.60-log reduction, whereas a wash-sanitize regimen with a 62% EtOH foam achieved a 4.11-log reduction. Sanitizing with the 70% EtOH AF gel alone was more effective than hand washing with a nonantimicrobial product for reducing murine norovirus (MNV), a surrogate for human norovirus, with 2.60- and 1.79-log reductions, respectively. When combined with hand washing, the 70% EtOH AF gel produced a 3.19-log reduction against MNV. A regimen using the SaniTwice protocol with the 70% EtOH AF gel produced a 4.04-log reduction against MNV. These data suggest that although the process of hand washing helped to remove pathogens from the hands, use of a wash-sanitize regimen was even more effective for reducing organisms. Use of a high-efficacy sanitizer as part of a wash-sanitize regimen further increased the efficacy of the regimen. The use of a well-formulated alcohol-based hand rub as part of a wash-sanitize regimen should be considered as a means to reduce risk of infection transmission in food service facilities.

Foodborne diseases are a serious and growing public health concern both in the United States *(8, 19)* and worldwide *(46)*. The Centers for Disease Control and Prevention attributed 9.4 million illnesses, nearly 56,000 hospitalizations, and more than 1,300 deaths to foodborne pathogens annually in the United States *(33)*. Many researchers believe that foodborne diseases are underreported *(27, 39, 43)*.

The ever-changing nature of pathogens, including the emergence of new ones, is contributing to an increase in foodborne diseases *(5)*. Enterotoxigenic *Escherichia coli* has been implicated in one of the largest foodborne outbreaks reported in the United States to date *(3)*. According to the Foodborne Disease Outbreak Surveillance System (1998 to 2002), 31% of foodborne disease outbreaks and 41% of cases of infection with known etiology can be attributed to human norovirus (HNV) *(27),* and HNV is now recognized as the most significant cause of infectious gastrointestinal illnesses, with a growing number of virulent strains circulating *(4, 9, 16, 44)*.

Poor personal hygiene of food service workers, in particular improper hand washing, contributes significantly to the risk of foodborne diseases *(15, 17, 26, 38, 41)*. The majority of HNV infection outbreaks are attributed to contamination of food via unwashed or improperly washed hands of food handlers *(5, 9, 23)*. HNVs have a low infective dose *(37, 44),* persist in the environment, and are resistant to chlorination and freezing *(23, 35, 44)*. These factors contribute to an increased risk of HNV illness transmission. Heavily soiled items are frequently encountered in food service settings when preparing food, and antimicrobial agents are considered to be less effective in the presence of such items *(6)*. The U.S. Food and Drug Administration (FDA) Food Code requires that food service workers wash their hands with a cleaning compound and water before using alcohol-based hand rubs (ABHRs) *(42)*. Although an improvement in compliance among food handlers with personal hygiene risk factors was observed between 1998 and 2008 in retail food facilities, hand washing practices were the most out-of-compliance risk factor for every type of facility evaluated *(40)*. In 2008, hand washing practices were not being followed in 76% of restaurants and approximately 50% of delicatessens *(40)*. In another study, compliance with Food Code recommendations for frequency of washing during production, service, and cleaning phases in restaurants was only 5% *(36)*.

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TABLE 1. *Test products*

Test product	Description	Abbreviation
GOJO Luxury Foam Handwash	Nonantimicrobial hand washing product	Nonantimicrobial hand wash
MICRELL Antibacterial Foam Handwash	0.5% Chloroxylenol hand washing product	PCMX hand wash
GOJO Antibacterial Plum Foam Handwash	0.3% Triclosan hand washing product	Triclosan hand wash
PURELL Instant Hand Sanitizer Foam	62% Ethanol foam ABHR	62% EtOH foam
PURELL Instant Hand Sanitizer Advanced Formula VF481	70% Ethanol gel ABHR	70% EtOH gel

Various hand hygiene regimens reduce the risk of transmission of pathogens from the hands of food service workers to the food they handle and prepare *(10, 29, 30)*. Proper hand hygiene has been associated with reductions of gastrointestinal illness ranging from 42 to 57% *(5, 11, 25)*. However, some interventions are more effective for removing pathogens than are others. Hand washing with soap and water was more effective for reducing contamination on the hands than was rinsing with water or not washing at all *(7, 10)*. Antimicrobial agents are more effective for removing bacteria on hands than is nonantimicrobial soap *(13, 30)*. Even ABHRs used alone decontaminate hands at least as effectively as does washing with soap and water *(12, 34)*. However, the combination of hand washing followed by the use of ABHRs produces even greater reduction of bacteria on hands *(18, 29, 30, 32)*. When water is unavailable, a two-stage hand cleansing protocol using an ABHR known as the SaniTwice method (a registered trademark, James Mann, Handwashing for Life, Libertyville, IL) was at least as effective for removing bacteria from the hands as was only washing with soap and water *(12)*.

A critical need remains for hand hygiene products with increased efficacy against hard-to-kill pathogens. Typical ABHR activity against nonenveloped enteric viruses varies depending on the type and concentration of alcohol *(5, 6, 14, 21)*. Different strains of HNVs may be more resistant to antimicrobial agents than others *(24)*. Several studies have been conducted on newly formulated ABHRs with significantly improved inactivation of nonenveloped viruses *(24, 28)*. A 70% ethanol advanced formula (EtOH AF) gel reduced HNV by 3.74 log units in 15 s, a significantly greater HNV reduction than produced by six other commercially available hand hygiene products *(24)*. This gel was the most effective product tested against two strains of HNV.

Quantitative data are scarce on the relative health impact of different hygiene interventions *(5)*, in particular hand hygiene product performance against organisms commonly found in food service facilities, i.e., in food soils. This series of studies was designed to determine the antimicrobial effectiveness of various hand hygiene product regimens under moderate and heavy food soil conditions and against the murine norovirus (MNV), a surrogate for HNV. The impact of specific product formulation on antimicrobial efficacy also was evaluated.

MATERIALS AND METHODS

Test products. The test products, which were manufactured by GOJO Industries (Akron, OH), are described in Table 1.

Product application. Table 2 shows the stepwise product application procedures for all test methods.

Participants. The study participants were healthy adults with two hands and were free of dermal allergies or any skin disorders on the hands or forearms. These studies were conducted in compliance with good clinical practice and good laboratory practice regulations and approved by local institutional review boards. All participants provided written informed consent.

Overall design for antibacterial efficacy studies. The purpose of the studies was to determine the antibacterial efficacy of various blinded test product configurations versus a relevant foodborne pathogen presented under conditions of moderate or heavy food soil. The order of use of each product configuration was determined randomly. All testing of antibacterial efficacy was performed using a modification of the ASTM International E1174-06 method *(1)*. For both the moderate and heavy soil tests, a two-step testing sequence was used for all products. For the moderate and heavy soil tests 18 and 12 participants, respectively, tested each configuration. Each participant completed a baseline cycle, in which hands were contaminated with *E. coli* (ATCC 11229) in moderate soil (chicken broth) for the first study and in heavy soil (sterile ground beef *(31)*) in the second study. Samples were collected for baseline bacterial counts. After the baseline sampling, participants completed a 30-s nonmedicated soap wash followed by the product evaluation cycle, which consisted of a contamination procedure, application of the test product, and subsequent hand sampling. Baseline and postapplication samples were evaluated for the presence of *E. coli*. Each participant was used for only one test configuration and, on completion of testing, decontaminated their hands with a 1-min 70% EtOH rinse, air drying, and a 30-s nonmedicated soap wash.

Preparation of inoculum. A 2-liter flask was filled with 1,000 ml of tryptic soy broth, i.e., 30.0 g of dehydrated tryptic soy broth medium (BD, Franklin Lakes, NJ) added to 1 liter of deionized water, heated, and sterilized (final pH 7.3 $\pm$ 0.20). The broth was inoculated with 1.0 ml of a 24-h culture of *E. coli* grown from a cryogenic stock culture. The flask was incubated for 24 h, and the suspension was used for the contamination challenge.

Hand contamination procedures. For the moderate soil study, a 24-h culture of *E. coli* was suspended in commercially available chicken broth (Swanson chicken broth, Campbell Soup Company, Camden, NJ) to a final concentration of 1×10^9 CFU/ml. Three aliquots of 1.5, 1.5, and 2 ml were transferred into each participant's cupped hands. Taking care not to drip the suspension, each aliquot was distributed over the front and back surfaces of the hands up to the wrists for 20 s; hands were air dried for 30 s after the first and second aliquots and for 90 s after the third aliquot. After samples were collected from the hands for baseline bacterial counts, the hands were washed for 30 s with a

TABLE 2. *Test product application procedures*[a]

Step	Wash	Sanitize	Wash-sanitize regimen	SaniTwice regimen[b]
1	Wet hands with water at 40°C	Dispense 1.5 ml of product into cupped hands	Wet hands with water at 40°C	Dispense 3 ml of sanitizer into cupped hands
2	Apply 1.5 ml of product	Rub hands together until dry	Apply 1.5 ml of product	Rub vigorously over hands for 15 s to simulate washing
3	Lather for 30 s		Lather for 30 s	Clean thoroughly with two paper towels
4	Rinse with water for 30 s		Rinse with water for 30 s	Dispense additional 1.5 ml of product
5	Pat dry with two paper towels		Pat dry with two paper towels	Rub hands together until dry
6			Apply 1.5 ml of sanitizer to hands	
7			Rub until dry	

[a] All application procedures were initiated within 10 s of completing the 90-s drying step.
[b] SaniTwice is a registered trademark with James Mann (Handwashing for Life, Libertyville, IL).

nonmedicated soap, and a second cycle of contamination was performed. After the 90-s drying step, participants applied the randomly assigned test product.

For the heavy soil study, 5.0-ml aliquots of the challenge suspension of *E. coli* was transferred to 4-oz (113-g) portions of sterile 90% lean ground beef and distributed evenly with gloved hands to achieve contaminant levels of approximately 5.0×10^8 CFU per portion. Each participant then kneaded the inoculated raw hamburger for 2 min. Hands were air dried for 90 s and then sampled for baseline counts. After a 30-s decontamination with nonmedicated soap, the cycle was repeated, and the test product was applied.

Bacterial recovery and microbial enumeration. Within 5 min after contamination for baseline evaluation and after product application, oversized powder-free sterile latex gloves were placed on each participant's hands, and 75 ml of sterile stripping fluid (0.4 g of KH_2PO_4, 10.1 g of Na_2HPO_4, and 1.0 g of isooctylphenoxypolyethoxyethanol in 1 liter of distilled water, pH adjusted to 7.8) was transferred into each glove. After a 60-s massage of the hands through the gloves, a 5.0-ml sample of the rinsate was removed from the glove and diluted in 5.0 ml of Butterfield's phosphate buffer solution with product neutralizers. Each aliquot was serially diluted in neutralizing solution, and appropriate dilutions were plated in duplicate onto MacConkey agar plates (50.0 g of dehydrated medium [BD] added to 1 liter of deionized water, heated, and sterilized; final pH 7.1 $\pm$ 0.2) and incubated for 24 to 48 h at 30°C. Colonies were counted and recorded using the computerized Q-Count plate-counting systems (Advanced Instruments, Inc., Norwood, MA).

Data analysis and statistical considerations. The estimated log-transformed number of viable microorganisms recovered from each hand (the R value) was determined using the formula $R = \log(75 \times C_i \times 10^D \times 2)$, where 75 is the volume (in milliliters) of stripping solution instilled into each glove, C_i is the arithmetic average colony count of the two plate at a particular dilution, D is the dilution factor, and 2 is the neutralization dilution.

Descriptive statistics and confidence intervals were calculated using the 0.05 level of significance for type I (alpha) error. Statistical calculations of means and standard deviations were generated on the log recovery data from baseline samples, post–product application samples, and the log differences between baseline and post–product application samples. Product comparisons were made using a one-way analysis of variance with post hoc analysis (Bonferroni's multiple comparison test) at $\alpha = 0.05$.

Overall design for HNV study. The purpose of the HNV study was to determine the virucidal activity of various hand hygiene regimens against HNV. Because routine culture and infectivity assays of HNV are not possible, HNV surrogates are routinely used to evaluate the virucidal activity of disinfectants and antiseptics. MNV, which is a suitable surrogate for HNV *(45),* was used in this study. A modification of ASTM International E2011-09 method for evaluating hygienic hand wash formulations for virus-eliminating activity using the entire hand *(2)* was utilized in this study. The modification involved the use of the glove rinsate sampling method and a randomized cross-over design. A total of six participants completed testing on all of the products.

Virus inoculum. Strain MNV-G (Yale University, New Haven, CT) was confirmed by direct serial dilution and inoculation onto host cells. Virus stocks were stored in an ultracold freezer (≤ -60°C). Frozen viral stocks were thawed on the day of test. The

TABLE 3. E. coli *recovery and reductions in the presence of moderate food soil load*

Application procedure	Test products	Mean ± SD *E. coli* (log CFU/ml)		Statistical analysis[a]			
		Baseline recovery	Reduction				
Wash	Nonantimicrobial hand wash	8.58 ± 0.46	3.10 ± 0.61	A			
Wash	PCMX hand wash	8.62 ± 0.65	3.56 ± 0.74	A	B		
Wash-sanitize	Nonantimicrobial hand wash + 62% EtOH foam	8.32 ± 0.64	3.81 ± 0.89		B	C	
Wash-sanitize	PCMX hand wash + 62% EtOH foam	8.25 ± 0.45	4.16 ± 0.91			C	
Wash-sanitize	Nonantimicrobial hand wash + 70% EtOH AF gel	8.49 ± 0.42	5.13 ± 0.71				D
Wash-sanitize	PCMX hand wash + 70% EtOH AF gel	8.57 ± 0.53	5.22 ± 0.60				D

[a] Configurations with the same letter are statistically equivalent, and configurations with different letters are statistically different, with each letter increase (B through D) indicating that a configuration had a significantly higher log reduction.

titer of the stock virus was at least 1×10^7 $TCID_{50}$ (median tissue culture infective dose) per ml. The organic soil concentration was adjusted to at least 5% fetal bovine serum of the volume of the viral suspension.

Hand contamination procedures. Before viral contamination, participants washed their hands with nonmedicated soap for 1 min, rinsed their hands, and dried their hands with sterile paper towels. Each participant's hands were then submerged to the wrists in a solution of 70% EtOH for 10 s. The solution was distributed over the entire front and back surfaces of the hands up to the wrists for 90 s and allowed to air dry until evaporation was complete. The alcohol submersion procedure was then repeated. The participants' hands were rinsed with approximately 200 ml of deionized water and dried with an air blower. After their hands were dry, participants waited at least 20 min until the next round of viral contamination and treatment. Each participant's hands were contaminated with 1.5 ml of MNV. The virus was rubbed over the entire surface of both hands for 90 s, not reaching above the wrists. The hands were dried for approximately 90 s. For the baseline control, samples for virus recovery were collected immediately after drying. A decontamination procedure was completed after the baseline sample collection, and a randomly assigned product regimen was applied. The decontamination procedure was repeated after all subsequent treatment rounds. Samples were collected from the participants' hands, and the required controls were evaluated for the amount of MNV capable of replicating in cell culture.

Elution of virus. Within 5 min after each treatment regimen, loose-fitting powder-free sterile latex gloves were placed on each participant's hands, and 40 ml of recovery medium was transferred into each glove. After a 60-s massage of the hands through the gloves, the rinsate was transferred from the glove to a sterile tube, vortexed, and serially diluted in cell culture medium. Appropriate dilutions were inoculated onto the host cell culture (RAW 264.7, ATCC TIB-71) and absorbed for 20 to 30 h at 36 ± 2°C with 5% ± 1% CO_2. The cultures were incubated for another 3 to 6 days at 36 ± 2°C with 5% ± 1% CO_2 to allow for the development of viral infection.

Calculation of virus titer and reduction. The host cells were examined microscopically for the presence of infectious virions. The resulting virus-specific cytopathic effects (CPE) and test agent–specific cytotoxic effects were scored by examining both test samples and controls. The presence of residual infectious virions was scored based on virus-induced CPE. The $TCID_{50}$ per milliliter was determined using the Spearman-Karber method *(22)*. When a sample contained no detectable virus, a statistical analysis was performed based on the Poisson distribution *(20)* to determine the theoretical maximum possible titer for that sample. The log viral reduction value was calculated by subtracting the log virus units of the treatment regimen samples from the log baseline units. Descriptive statistics and confidence intervals were calculated ($\alpha = 0.05$). Statistical calculations of means and standard deviations were generated on the log recovery data from baseline samples, post–product application samples, and the log differences between baseline and post–product application samples. Test configuration comparisons were made using a one-way analysis of variance with post hoc analysis (Bonferroni's multiple comparison test) at $\alpha = 0.05$.

RESULTS

Reduction in microbial contamination of moderately soiled hands. Reductions of *E. coli* on moderately soiled hands (chicken broth) ranged from 3.10 log CFU/ml for the nonantimicrobial hand wash to 5.22 log CFU/ml for the wash-sanitize regimen with the 0.5% chloroxylenol (PCMX) hand wash and the 70% EtOH AF gel (Table 3). Although the differences were not significant, the PCMX hand wash achieved higher log reductions than did the nonantimicrobial hand wash for all regimens tested. Regimens including the 70% EtOH AF gel were superior to all other configurations ($P < 0.001$). The reductions for the majority of subjects were at the limit of detection (complete kill) for both regimens that included the 70% EtOH AF gel; therefore, these reductions may actually be underestimated. Overall, the wash-sanitize regimen was significantly superior to hand washing alone with one exception. The PCMX hand wash alone was equivalent in efficacy to the nonantimicrobial hand wash followed by the 62% EtOH foam.

Reduction in microbial contamination of heavily soiled hands. The four product configurations tested under conditions of heavy soil load produced *E. coli* log reductions ranging from 3.97 to 4.60 log CFU/ml (Table 4). The antimicrobial agent in the hand washing product did not impact efficacy of the regimen; the reductions produced by the same sanitizer used in combination with the 0.3% triclosan hand wash or the PCMX hand wash were equivalent. However, the choice of sanitizer did have a significant impact on efficacy. All configurations that included the 70% EtOH AF gel were superior in

TABLE 4. E. coli *recovery and reductions in the presence of heavy food soil load*

Application procedure	Test products	Mean ± SD *E. coli* (log CFU/ml)		Statistical analysis[a]
		Baseline recovery	Reduction	
Wash-sanitize	PCMX hand wash + 62% EtOH foam	7.50 ± 0.19	4.11 ± 0.48	A
Wash-sanitize	Triclosan hand wash + 62% EtOH foam	7.54 ± 0.18	3.97 ± 0.45	A
Wash-sanitize	PCMX hand wash + 70% EtOH AF gel	7.53 ± 0.19	4.60 ± 0.52	B
Wash-sanitize	Triclosan hand wash + 70% EtOH AF gel	7.46 ± 0.19	4.51 ± 0.43	B

[a] Configurations with the same letter are statistically equivalent, and configurations with different letters are statistically different, with a letter increase (B) indicating that a configuration had a significantly higher log reduction.

performance to configurations that included the 62% EtOH foam ($P < 0.05$).

Inactivation of MNV on soiled hands. A third study was conducted to evaluate four hand hygiene configurations against MNV, a surrogate for HNV. Hand washing with the nonantimicrobial hand wash was minimally effective against MNV, producing a <2-log reduction (Table 5). Sanitizing with the 70% EtOH AF gel was significantly more effective than hand washing for reducing MNV ($P < 0.01$). Using a wash-sanitize regimen was more effective than either hand washing or sanitizing alone ($P < 0.05$). The SaniTwice method with the 70% EtOH AF gel was the most effective regimen, achieving a >4-log reduction of MNV ($P < 0.01$).

DISCUSSION

Previous findings suggest that hand hygiene regimens reduce the risk of transmission of pathogens from the contaminated hands of food service workers to food *(10, 29, 30)*. The findings from our studies support and extend those from previous studies by demonstrating that hand hygiene regimens can be effective even in the presence of high organic loads and against nonenveloped viruses such as HNV.

These studies further demonstrate the improved effectiveness of wash-sanitize regimens over hand washing or sanitizing alone. In the presence of moderate food soil, the combination of the 70% EtOH AF gel with either a nonantimicrobial hand wash or an antimicrobial hand washing product each achieved >5-log reductions of *E. coli*. In contrast, hand washing achieved only a <3.6-log reduction. In the presence of heavy food soil, the use of 70% EtOH AF gel after the antimicrobial foam hand washing product in two different configurations achieved a 4.51-log reduction and a 4.60-log reduction, respectively. In the HNV study, hand washing alone produced a <2-log reduction. When used as part of a wash-sanitize regimen that included the 70% EtOH AF gel a 3.19-log reduction was achieved. These findings demonstrate that the addition of a high-efficacy sanitizer to a hand washing regimen results in a greater reduction of microorganisms. This finding is consistent with those of others, who reported that the primary factor influencing final microorganism levels on the hands is sanitizer use *(30)*.

The current FDA Food Code *(42)* allows use of ABHRs only on hands that have been cleaned according to the recommended hand washing protocol. The Food Code (section 2-301.16) also severely restricts hand sanitizers by allowing their use only after a proper hand washing or where no direct contact with food occurs. The SaniTwice regimen has previously been shown to be an effective means for the reduction of bacteria on the hands when soap and water are unavailable. In the MNV study, use of the SaniTwice protocol with the 70% EtOH AF gel achieved a >4-log (>99.99%) reduction of MNV and was the most effective regimen tested. This combination is significantly more effective than hand washing or sanitizing alone and more effective than a wash-sanitize regimen. Therefore, these data indicate that the SaniTwice regimen is an effective method for significantly reducing bacteria and nonenveloped viruses.

In the studies presented here, the configurations that included the 70% EtOH AF gel consistently provided superior performance. These findings are consistent with previous findings that the in vivo activity of ABHRs is not solely dependent upon alcohol concentration *(12, 24, 28)*. In a previous study, the 70% EtOH AF gel provided significantly greater HNV reduction than did other hand hygiene products that contained >85% ethanol *(24)*.

TABLE 5. *MNV recovery and reductions*

Application procedure	Test products	Mean ± SD MNV (log $TCID_{50}$/ml)		Statistical analysis[a]
		Baseline recovery	Reduction	
Wash	Nonantimicrobial hand wash	6.98 ± 0.20	1.79 ± 0.29	A
Sanitize	70% EtOH AF gel		2.60 ± 0.41	B
Wash-sanitize	Nonantimicrobial hand wash + 70% EtOH AF gel		3.19 ± 0.31	C
SaniTwice	70% EtOH AF gel		4.04 ± 0.33	D

[a] Configurations with the same letter are statistically equivalent, and configurations with different letters are statistically different, with each letter increase (B through D) indicating that a configuration had a significantly higher log reduction.

Similarly, an earlier version of the 70% EtOH AF gel was more effective than hand hygiene products containing 95% ethanol and 75% isopropanol *(28)*. Liu et al. *(24)* suggested that the additional ingredients in these novel ABHRs (a synergistic blend of polyquaternium polymer and organic acid) may work with the ethanol to denature the viral capsid protein. These comparisons demonstrate the importance of formulation in product efficacy.

As illustrated in the *E. coli* study with heavy food soil, the lower log reductions produced by the regimen including the PCMX hand wash with the 70% EtOH AF gel reflects the fact that the raw hamburger was a greater challenge than was the moderate soil (chicken broth). Despite this challenge, use of the 70% EtOH AF gel as part of the hand hygiene regimen probably would provide increased protection against the transmission of foodborne illness because it produced at least 0.5-log greater reductions than did washes paired with a typical hand sanitizer. A wash-sanitize regimen including a high-efficacy formulation should be used in high-risk environments in which uncooked meat is handled in the same vicinity as ready-to-eat foods.

A limitation of our study was that a surrogate virus, MNV, was utilized. Although MNV has been extensively studied and is considered an acceptable surrogate for HNV, the results obtained with this virus may not be an exact reflection of the actual efficacy of these products against various HNV strains. Future efforts should focus on developing routine and repeatable culture-based methods to quantify infectious HNV. Currently, clinical studies should focus on improving hand hygiene compliance by food handlers and on determining the effectiveness of hand hygiene regimens in food service settings.

This series of studies reveals that wash-sanitize regimens, particularly those including a well-formulated ABHR, can be highly efficacious, even in the presence of high organic loads and against HNV. Consequently, the inclusion of such formulations as part of a hand hygiene regimen could be a primary intervention for reducing the risk of infection transmission in food service facilities.

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Ability of Hand Hygiene Interventions Using Alcohol-Based Hand Sanitizers and Soap To Reduce Microbial Load on Farmworker Hands Soiled during Harvest

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ABSTRACT

Effective hand hygiene is essential to prevent the spread of pathogens on produce farms and reduce foodborne illness. The U.S. Food and Drug Administration Food Safety Modernization Act Proposed Rule for Produce Safety recommends the use of soap and running water for hand hygiene of produce handlers. The use of alcohol-based hand sanitizer (ABHS) may be an effective alternative hygiene intervention where access to water is limited. There are no published data on the efficacy of either soap or ABHS-based interventions to reduce microbial contamination in agricultural settings. The goal of this study was to assess the ability of two soap-based (traditional or pumice) and two ABHS-based (label-use or two-step) hygiene interventions to reduce microbes (coliforms, *Escherichia coli,* and *Enterococcus* spp.) and soil (absorbance of hand rinsate at 600 nm [A_{600}]) on farmworker hands after harvesting produce, compared with the results for a no-hand-hygiene control. With no hand hygiene, farmworker hands were soiled (median A_{600}, 0.48) and had high concentrations of coliforms (geometric mean, 3.4 log CFU per hand) and *Enterococcus* spp. (geometric mean, 5.3 log CFU per hand) after 1 to 2 h of harvesting tomatoes. Differences in microbial loads in comparison to the loads in the control group varied by indicator organism and hygiene intervention (0 to 2.3 log CFU per hand). All interventions yielded lower concentrations of *Enterococcus* spp. and *E. coli* ($P < 0.05$), but not of coliforms, than were found in the control group. The two-step ABHS intervention led to significantly lower concentrations of coliforms and *Enterococcus* spp. than the pumice soap and label-use ABHS interventions ($P < 0.05$) and was the only intervention to yield significantly fewer samples with *E. coli* than were found in the control group ($P < 0.05$). All interventions removed soil from hands ($P < 0.05$), soap-based interventions more so than ABHS-based interventions ($P < 0.05$). ABHS-based interventions were equally as effective as hand washing with soap at reducing indicator organisms on farmworker hands. Based on these results, ABHS is an efficacious hand hygiene solution for produce handlers, even on soiled hands.

Increases in produce-associated outbreaks highlight the need for effective microbial risk management on produce farms and in packing sheds. In the United States, from 1999 to 2008, contaminated produce was responsible for at least 23% of all reported foodborne illnesses *(33)*. Produce contamination may occur at various points in the farm-to-fork continuum *(19, 31)*. Some produce-associated outbreaks have been thought to be caused by infected farmworker and, possibly, inadequate hand hygiene *(14, 16, 42)*.

Farmworker hands may be vehicles for microbial contamination of produce *(23, 29)*. Harvest and packing, often done by hand, have been associated with increases in microbial contamination *(2, 18, 22)*. A 2010 study found that of seven major fruit and vegetable crops, all were either exclusively or partially harvested by hand *(7)*. Because "workers often touch produce with their bare hands" the U.S. Food and Drug Administration Food Safety Modernization Act (FSMA) Proposed Rule for Produce Safety states that hand washing is a "key control measure in preventing contamination" of produce *(39)*.

Effective hand hygiene reduces microbial risks and disease in health care and community settings *(1, 6, 43)*, but there are few data on its efficacy in food handling settings *(4)*, and it has just begun to be studied in the agricultural environment. The FSMA Proposed Rule for Produce Safety defines hand hygiene as "washing hands thoroughly, including scrubbing with soap and running water … and drying hands thoroughly using single-service towels, clean cloth towels, sanitary towel service or other adequate hand drying devices" *(39)*. However, soil on farmworker hands may limit the ability of hand washing to remove or inactivate microbes. Thus, it is important to assess the hypothesis that hand washing with soap is the most efficacious hygiene intervention for the agricultural envi-

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ronment. In addition, hand washing with soap may be difficult to achieve on every occasion specified in the rule due to barriers such as limited access to potable water near all work areas. Alcohol-based hand sanitizers (ABHS) are a logical alternative because they do not require potable water, and a large body of evidence exists to show that their antimicrobial efficacy results in reduced spread of infection in health care environments *(6, 43)*. The FSMA Proposed Rule for Produce Safety prohibits the sole use of ABHS because "the effectiveness of hand sanitizers has been shown to be highly dependent upon the removal of organic material from the hands prior to their use" *(39)*. However, a large body of research suggests that the efficacy of ABHS is not impacted when hands are soiled *(10, 12, 25, 26, 28, 30, 35)*. One limitation of ABHS is that hands may still appear dirty, even if microbes have been inactivated. One method that may address this limitation is SaniTwice, a two-step technique where an excess of ABHS is applied to hands and removed with paper towels, followed by a second ABHS application *(11)*. This technique has been shown to reduce *Escherichia coli* on hands soiled with beef broth and raw hamburgers *(11)* and to reduce bacteria and soil on agricultural workers' hands *(13)*.

The goal of this study was to assess the ability of two soap-based and two ABHS-based hygiene interventions to reduce microbes and soil on farmworker hands after harvesting produce, compared with a no-hygiene control. Traditional (nonantibacterial and nonabrasive) soap was included as the current "gold standard" *(38)*. Pumice soap was chosen because it may be able to remove particles and organic compounds from hands that traditional soaps do not. ABHS interventions were included as waterless hygiene options as alternatives to traditional soap. The two-step ABHS intervention was included because of its previously demonstrated efficacy on soiled hands *(10)*.

MATERIALS AND METHODS

Setting and population. This study took place over a 4-week period in August and September 2014 on a farm that produces tomatoes in the state of Nuevo León, Mexico. The farm exported its produce to the United States and sold it to Mexican retailers and had established food safety protocols in place, as well as a dedicated food safety specialist on site. Approval for research on human subjects was conferred after ethics review by Emory University (institutional review board no. 00035460).

The study population consisted of 181 farmworkers who were employed by this farm to harvest tomatoes. Participants routinely used gloves for tomato harvest but removed them when participating in our study in order that the interventions be tested on the most highly soiled and microbially contaminated hands possible. During each of the five nonconsecutive days of the study prior to study enrollment, the farm food safety specialist introduced the study staff, who described the study and solicited volunteers. Inclusion criteria included that the participant was an employee of the farm assigned to harvest tomatoes and provided oral informed consent to participate in the study according to the institutional review board–approved protocol. There were no exclusion criteria. Oral consent was documented by study staff for each participant.

Farm activities and intervention groups. After consent was received, the farmworkers were randomly assigned to one of five groups (described below), and each was given a name tag to indicate his or her group and unique sample identifier. To standardize the microbial load on farmworker hands, all farmworkers were asked to wash their hands with traditional (nonantibacterial and nonabrasive) soap (~3.5 ml of Pearl Lotion Hand Soap; Noble Chemical, Inc., Lancaster, PA) and potable water at a nearby hand washing station stocked with paper towels for drying (Servitoalla double-ply, 28 by 22.8 cm; Pétalo, Kimberly-Clark, Mexico City, Mexico). All potable water used in the study was provided by the Universidad Autónoma de Nuevo León (UANL) laboratory and assured to have no coliforms, *E. coli,* or *Enterococcus* spp. in a 100-ml aliquot (see "Absorbance and microbial analyses" for general description of microbial assays). The farmworkers were then asked to harvest tomatoes for 1 to 2 h (collecting approximately 30 bins per person), using their standard procedure but without gloves. After harvesting, each farmworker completed activities described below based on their assigned group, following the instructions and demonstration of study staff (Fig. 1). A convenience sample of at least 10 participants per study group also had their hands photographed before and after the activities described below.

After harvesting, individuals in the control group did not perform any hand hygiene. Individuals in the label-use ABHS group used ABHS according to the product label instructions, with minor modifications. Individuals in this group received one pump of sanitizer gel (~3.5-ml of GOJO Purell Advanced Instant Hand Sanitizer, active ingredient 70% ethanol; GOJO Industries, Akron, OH) in the palm of one hand. They were then asked to rub their hands in the following manner used in all interventions: rub hands palm-to-palm, rub each palm on the dorsal surface of the opposite hand, and interlace fingers to distribute product over the fingers. They were asked to continue rubbing their hands until dry.

Individuals in the two-step ABHS group performed SaniTwice hand hygiene as described previously, with minor modifications *(11)*. Briefly, they received three pumps of sanitizer gel (~10.5 ml, enough to keep hands wet for 20 s) in the palm of one hand. They were then asked to rub their hands as described above for about 20 s. After ~20 s of rubbing, they were given a paper towel to remove all remaining sanitizer on their hands. They then followed the steps described above for the label-use ABHS group.

Individuals in the traditional soap group received two pumps of potable water (approximately 220 ml) to wet their hands. They then received one pump (~3.5 ml) of the same traditional soap used by all participants prior to harvesting. They were asked to rub their hands as described above for about 20 s. After rubbing, they rinsed their hands with three pumps of the potable water provided (approximately 330 ml). A paper towel was provided, and they were asked to dry their hands as they normally would.

Individuals in the pumice soap group received two pumps of pumice soap (~6 ml of GOJO Natural Orange Pumice Hand Cleaner, a gel-based surfactant formula with pumice particles; GOJO Industries) in the palm of one hand. They were then asked to rub their hands as described above for about 20 s. During this rubbing, they also received a splash of potable water (approximately 2 ml). After rubbing, they rinsed their hands with three pumps of the potable water provided (approximately 330 ml). A paper towel was provided, and they were asked to dry their hands as they normally would.

Immediately after the activities described above were completed, the farmworkers were asked to provide a hand rinsate sample by inserting one hand in a Whirl-Pak bag (Nasco, Fort Atkinson, WI) containing 750 ml of sterile 0.1% peptone water (Thermo Fisher Scientific, Waltham, MA) while study staff massaged their fingers through the bag for 20 to 30 s. This process

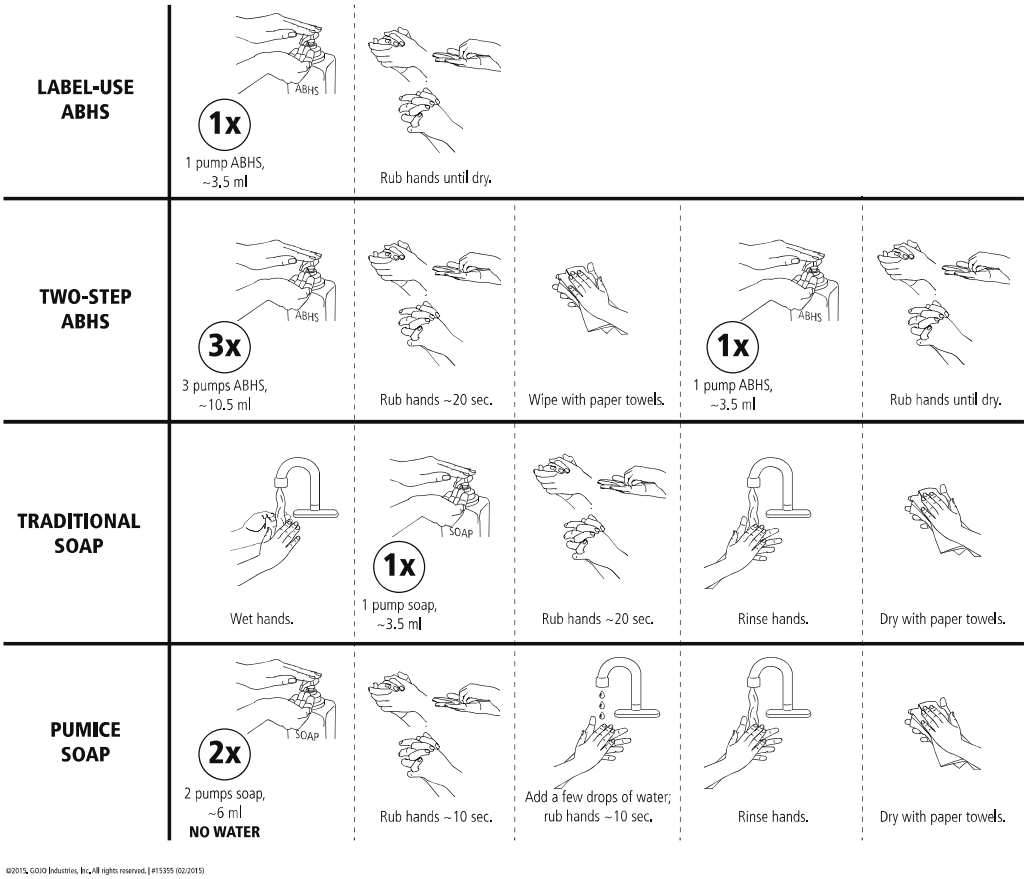

FIGURE 1. *Visual description of the two ABHS-based and two soap-based hand hygiene interventions. Illustrations in this figure are courtesy of GOJO Industries, Inc.*

was repeated for the second hand. The worker was provided a paper towel and small token of thanks for participation (e.g., bottled water, a cap, a bandana, or similar item). The labeled hand rinsate sample was stored on ice packs in a cooler. For each study staff member collecting samples, at the end of the day, an additional unopened Whirl-Pak bag containing 750 ml of peptone water was retained as a negative collection control. All samples were transported to the Laboratory of Microbial Biochemistry and Genetics at UANL, where they were stored at 4°C until analysis. Analysis was performed within 48 h of field collection. If the microbial analysis results were outside the quantifiable range and a repeat analysis was necessary, the repeat analysis was conducted within 72 h of field collection.

Absorbance and microbial analyses. Absorbance readings of hand rinsate at 600 nm (A_{600}) were taken to objectively measure the matter removed from hands during sampling, used as a proxy for "dirtiness of hands," referred to as "soil" herein. Absorbance reading is an objective approach to assessing dirt on hands that is comparable to assessing the turbidity of hand rinse samples *(27)* and may be preferable to other, subjective methods, such as visual inspection of hands *(25)*. Rinsate samples were inverted several times to resuspend any particulate matter, and then an aliquot was taken for measurement of absorbance at 600 nm (A_{600}) using a spectrophotometer (Sequoia Turner, Mountain View, CA).

Samples were analyzed in random order (without regard to study group) to detect and enumerate coliforms, *E. coli,* and *Enterococcus* spp., three common, nonpathogenic types of bacteria used to indicate microbial load, hereinafter called indicator bacteria. Serial volumes of each hand rinse sample (100 μl, 1 ml, and 10 ml) were filtered through separate 0.45-μm-pore-size cellulose filters (EMD Millipore Corporation, Billerica, MA) using a vacuum manifold filtration system (Pall Corporation, Port Washington, NY). When filtering volumes of less than 10 ml, the funnel (with the vacuum closed) was prefilled with 10 ml of peptone water before the sample was added to allow even sample dispersion across the membrane prior to opening the vacuum. Following filtration through duplicate membranes for each serial volume of rinsate, each membrane was placed on a separate petri dish containing solidified agar for bacterial enumeration. To enumerate *E. coli* and coliform bacteria, membranes were placed on chromogenic Bio-Rad Rapid'*E. coli* 2 agar (Bio-Rad, Hercules, CA) and incubated at 44°C for 24 h for enumeration of typical colonies (pink to purple for *E. coli* and both blue to green and pink to purple for coliforms). To enumerate *Enterococcus* bacteria, membranes were placed on Kenner Fecal *Streptococcus* agar (BD, Franklin Lake, NJ) plates and incubated at 37°C for 48 h before enumeration of red-centered colonies. For all three organisms, the limit of detection was 37 CFU per hand and the upper limit of quantification was 8.3 log CFU per hand.

The remaining sample rinsate was stored at 4°C for no more than 72 h postcollection and reprocessed, as described above, for cases in which colony counts were inconsistent or larger than assay detection limits (e.g., more than 250 colonies per plate). For each day of sample collection, study staff processed a negative sample collection control (described above), a negative water control (sampled from the municipal water used for hand rinsing in the field), and a positive control (mixture of *Enterococcus faecalis* [ATCC 19433], *Salmonella enterica* serovar Typhimurium [ATCC 19428] as a surrogate for coliforms *(15),* and *E. coli* [ATCC

TABLE 1. *Proportions of hand rinsate samples positive for indicator bacteria from the control group and four intervention groups of workers harvesting tomatoes on a farm in Mexico*

Group[a]	No. of positive samples/total no. of samples (%) tested for[b]:		
	Coliforms	*Enterococcus* spp.	*E. coli*
Control	30/42 (71)	41/42 (98)	10/42 (24)
Label-use ABHS	28/34 (82)	31/34 (91)	2/34 (6)
Two-step ABHS	21/35 (60)[c]	28/35 (80)	0/35 (0)[d]
Traditional soap	28/35 (80)	31/35 (89)	2/35 (6)
Pumice soap	35/35 (100)[d]	35/35 (100)	1/35 (3)

[a] The control group samples were collected after farmworkers harvested tomatoes for 1 to 2 h. Hand rinsate samples were collected from the four intervention groups immediately after performing hand hygiene.

[b] Values are for hand rinsate samples tested for the given indicator bacteria within each study group.

[c] Result is significantly different from the result for the pumice soap group ($\alpha = 0.05$)

[d] Result is significantly different from the result for the control group ($\alpha = 0.05$)

25922]; American Type Culture Collection, Manassas, VA). The positive control was created by growing each strain overnight on tryptic soy broth (Difco, BD) and then seeding 1 ml of each strain into 11 ml of sterile 0.85% NaCl (Sigma Aldrich, St. Louis, MO), pH 7.0.

Data entry and statistical analyses. All data were entered independently by two trained individuals into separate Microsoft Excel databases (Microsoft, Redmond, WA), compared, and reconciled by review of the original laboratory forms. An additional check showed no discrepancies when 5% of the original laboratory forms were randomly selected and compared against the final database. Statistical analyses were performed using Stata 10 (STATA Corp., College Station, TX), JMP Pro 10, and SAS 9.3 (SAS Institute Inc., Cary, NC). The Shapiro-Wilk test *(32)* indicated that all data (e.g., absorbance values of hand rinsates and log-transformed indicator organism concentrations) were not normally distributed (data not shown). Therefore, all statistical tests used were nonparametric. When calculating the concentrations of indicator bacteria, any sample without detectable bacteria was assigned a value of 18.5 CFU per hand, half the limit of detection *(37)*. Geometric means and standard deviations are used to describe bacterial concentrations as a convenience to the reader *(40)*, and medians and standard deviations are used to describe absorbance data. To compare differences in percentages of samples positive for microbial indicators across study groups, a Pearson χ^2 test *(9)* and Bonferroni correction *(17)* were used. To compare A_{600} and microbial concentration values across study groups, the Kruskal-Wallis test *(20)* followed by the Steel-Dwass multiple comparison procedure *(8)* were used.

RESULTS

In general, farmworkers' hands became contaminated with indicator bacteria (Table 1 and Fig. 2, control) and soiled while they harvested produce, prior to hand hygiene (Fig. 3, control). The percentages of samples positive for coliforms (71%) and *Enterococcus* bacteria (98%) in the control group were high (Table 1) relative to the percentage of samples positive for *E. coli* (24%) (Table 1). The concentrations of bacteria on control group hands ranged widely: coliform concentrations in positive samples ranged from the lower limit of detection to the upper limit of quantification (37 CFU per hand to 8.3 log CFU per hand) (Fig. 2), *Enterococcus* concentrations in positive samples ranged from 93 CFU per hand to the upper limit of quantification (8.3 log CFU per hand) (Fig. 2), and *E. coli* concentrations in positive samples ranged from the lower limit of detection (37 CFU per hand) to 3.3 log CFU per hand. The geometric mean concentrations of coliforms (3.4 log CFU per hand) and *Enterococcus* bacteria (5.3 log CFU per hand) in control group samples were relatively high (Fig. 2) compared with the geometric mean concentration of *E. coli* bacteria (1.7 log or 50 CFU per hand) (Fig. 2). For microbial assays, all negative and positive controls consistently yielded the expected results. The median absorbance of control hand rinsate samples was 0.48, and the values varied greatly across the control group, ranging from A_{600} 0.05 to 1.36. The visual appearance of hands postharvest and preintervention is shown in the "before intervention" photographs of hands in Figure 4. It appears that in just a few hours of harvesting produce, the farmworkers' hands accumulated high concentrations of some indicator bacteria and soil.

While hygiene interventions did not completely eliminate indicator bacteria from hands, in general, all hand hygiene interventions effectively reduced the concentrations of some bacteria. However, there were differences in the performance of the four interventions tested.

Compared with the results for the control group, none of the hand hygiene interventions yielded a significantly lower coliform concentration or percentage of samples positive for coliforms (Table 1 and Fig. 2). However, the two-step ABHS group had lower concentrations of coliforms than the label-use ABHS and pumice soap groups ($P < 0.05$) (Fig. 2). Compared with the control group, all four intervention groups had lower concentrations of *Enterococcus* spp. ($P < 0.05$) (Fig. 2), although similar to the result for coliforms, none of the hand hygiene interventions yielded significantly lower percentages of samples positive for *Enterococcus* than in the control group (Table 1). The two-step ABHS group had lower concentrations of *Enterococcus* than the label-use ABHS and pumice soap groups ($P < 0.05$) (Fig. 2). For *E. coli*, all four hand hygiene interventions yielded significantly lower concentrations on hands than were found in the control group ($P < 0.05$, Fig. 2). However, two-step ABHS was the only intervention to have significantly fewer samples with detectable *E. coli* than the control group, and this group had no samples positive for *E. coli* ($P < 0.05$) (Table 1). The other three interventions had only 1 or 2 samples positive for *E. coli* (3 to 6%), compared with 10 samples positive for *E. coli* (24%) in the control group (Table 1), but these differences did not reach statistical significance.

Using absorbance measurements of hand rinsate samples as a proxy for soil, all four interventions yielded significantly less soil on hands than in the control group (range, A_{600} 0.05 to 1.36); soap-based interventions (range, A_{600} 0.00 to 0.15) yielded significantly less soil remaining

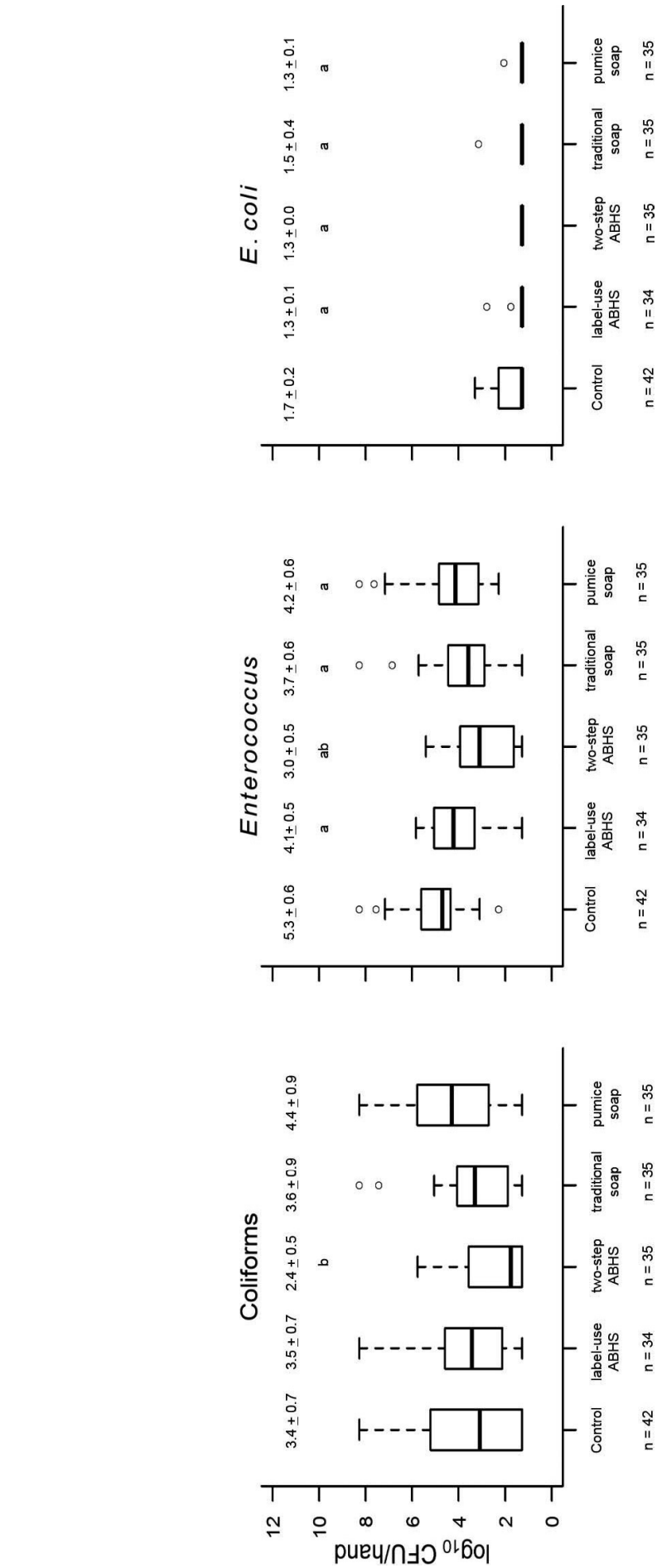

FIGURE 2. *Concentrations of coliform,* Enterococcus, *and* E. coli *bacteria in hand rinsate samples from the control group and four hand hygiene intervention groups of workers harvesting tomatoes. For each study group, the boxes display the quartiles (25th, 50th, and 75th) and whiskers extend to 1.5 times the interquartile range. Any data points outside the whiskers are displayed individually as dots. The values above each study group box plot indicate the geometric mean bacterial concentration and standard deviation (log CFU per hand). The control group samples were collected after farmworkers harvested tomatoes for 1 to 2 h. The four intervention groups had hand rinsates collected immediately after performing hand hygiene. a, significantly different from the control group ($\alpha = 0.05$); b, significantly different from the label-use ABHS and pumice soap groups ($\alpha = 0.05$)*

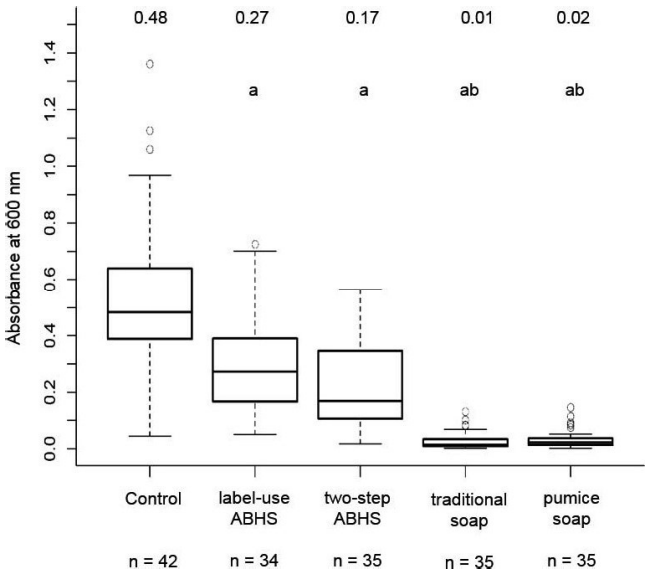

FIGURE 3. *Absorbance (at 600 nm) in hand rinsate samples from the control group and four intervention groups of workers harvesting tomatoes. For each study group, the boxes display the quartiles (25th, 50th, and 75th) and whiskers extend to 1.5 times the interquartile range. Any data points outside the whiskers are displayed individually as dots. The value above each study group box plot indicates the median absorbance (A_{600}). The control group samples were collected after farmworkers harvested tomatoes for 1 to 2 h. The four intervention groups had hand rinsates collected immediately after performing hand hygiene. a, significantly different from the control group ($\alpha = 0.05$); b, significantly different from the label-use ABHS and two-step ABHS groups ($\alpha = 0.05$)*

on hands than ABHS-based interventions (range, A_{600} 0.02 to 0.73) ($P < 0.05$) (Fig. 3). These absorbance results confirm the trends seen in the "after intervention" photographs taken of hands (Fig. 4).

DISCUSSION

The goal of this study was to assess the ability of two soap-based (traditional or pumice) and two ABHS-based (label-use or two-step) hygiene interventions, compared with a no-hand-hygiene control, to reduce microbes (coliforms, *E. coli,* and *Enterococcus*) and soil (A_{600} of hand rinsate) on farmworker hands after harvesting produce. Without intervention, farmworkers' hands were contaminated with high concentrations of indicator bacteria and were heavily soiled after 1 to 2 h of harvesting tomatoes. All four hygiene intervention groups had lower concentrations of *Enterococcus* and *E. coli* on their hands than the control group. Furthermore, all four interventions yielded significantly less soil remaining on hands, soap-based interventions more so than ABHS-based interventions. Based on these results, ABHS can be viewed as a promising hand hygiene solution for produce handlers, even on soiled hands. To build on these findings, future studies could investigate the efficacy of ABHS for pathogen inactivation on soiled hands in a controlled setting (e.g., an experimental greenhouse).

Farmworkers' hands were heavily soiled and contaminated with high concentrations of indicator bacteria after 1 to 2 h of harvesting tomatoes. The control group results are supported by our previous field observational study of microbial contamination of produce, environmental samples, and farmworkers' hands *(23),* where we found that 16 to 41% of farmworkers' hands had detectable *E. coli,* 92 to 100% had detectable coliforms, and 70 to 99% had detectable *Enterococcus* bacteria, depending on the type of produce harvested. The lower percentage of samples positive for *E. coli* than of samples positive for coliforms and *Enterococcus* is expected, as *E. coli* is a gram-negative species of bacteria indicative of fecal contamination from a warm-blooded animal, whereas *Enterococcus* spp. (a genus of gram-positive bacteria) and coliforms (a general group of bacteria) are larger, more general categories of indicator bacteria. It is unlikely that the presence of these indicator bacteria is simply a result of poor sanitation and hygiene practices among the farmworkers given that they washed their hands with soap and water before beginning harvest and their sole activity was harvesting produce. It is more likely that farmworkers' hands are accumulating organic matter and indicator bacteria present in the agricultural environment (e.g., on plants, soil, or produce bins). Both coliforms and *Enterococcus* are naturally present in the guts of animals *(5, 36),* but they are also present in the environment *(36)* and could be introduced into the agricultural environment through various pathways (e.g., irrigation water, soil amendments, or contaminated tools or equipment). Similarly, the *E. coli* seen on some farmworker hands after harvest may indicate recent fecal contamination from a warm-blooded animal *(36)* or may indicate past environmental contamination, as *E. coli* is known to be persistent in the environment *(41).*

Farmworkers in all four intervention groups had lower concentrations of *Enterococcus* and *E. coli* on their hands than those in the control group. These results indicated that all four interventions were efficacious at reducing the concentrations of viable microbes on hands. The soap-based interventions likely reduced bacterial concentrations because soap is, by definition, an emulsifier, meaning it suspends hydrophobic compounds and, with them, any particles and microbes. These particles and microbes are then removed when hands are rinsed. These traditional soap and pumice soap intervention results are consistent with the results from a pilot study of a hand hygiene intervention using foam soap on soiled farmworker hands *(13).* The ABHS-based interventions likely reduced bacterial concentrations because ethanol, the active ingredient in the ABHS, is an effective antimicrobial agent *(3, 24).* These results suggest that ABHS can be an efficacious hand hygiene method, even on soiled hands. Although the soap-based and ABHS-based interventions work by different mechanisms, they were both efficacious at reducing microbes on soiled hands.

No intervention resulted in lower concentrations of coliforms than in the control group. Given the high variability of coliform concentrations in the control and all intervention groups and the generally small reductions (0 to 2 log) in coliforms previously reported with hand washing with foam soap and ABHS in the field *(13),* a larger sample size would likely have been needed for these interventions to demonstrate a statistically significant difference in coliform

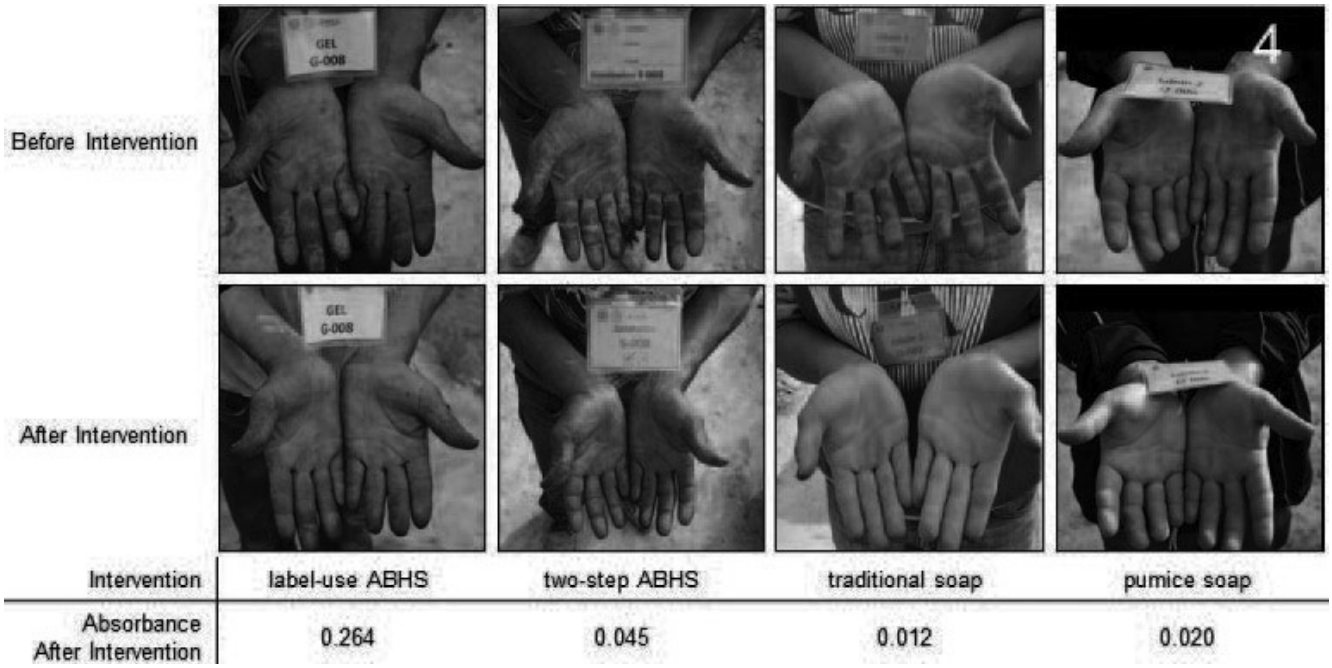

FIGURE 4. *Photographs of hands and corresponding individual hand rinsate absorbance readings from samples collected after intervention from study participants—workers harvesting tomatoes on a farm in Mexico. Photographs were taken immediately before and after each worker performed hand hygiene.*

concentration compared with the control group. In a previous study comparing two-step ABHS and foam soap to a control group, only two-step ABHS had significantly lower levels of coliforms (~2 log *(13)*) than the control group. These results suggest that coliforms may be more persistent on hands than *E. coli* and *Enterococcus* spp. after hand washing or ABHS use. Given that total coliforms are poor indicators of fecal contamination in an environmental setting *(36),* it is unclear whether this result has a practical application in hand hygiene techniques.

All four interventions significantly removed soil from hands, soap-based interventions more so than ABHS-based interventions. It was expected that soap-based interventions would be the most efficacious at soil removal, given soap's emulsion properties described above. The removal of soil from hands with label-use of ABHS was a somewhat unexpected result, as the intervention does not involve wiping or removing anything from the hands. This result contradicts previous research on alcohol-based gels *(21, 34)*. However, study participants' hands were quite heavily soiled, and particles may have been solubilized in the ABHS and then dropped to the ground as the liquid portion evaporated. The two-step ABHS intervention uses paper towels to remove excess ABHS *(11)*; it is likely that additional soil particles were also removed by the paper towel when wiping dry.

The label-use ABHS and pumice soap interventions were similar to the traditional soap intervention in their effectiveness at reducing the microbial load on farmworker hands. However, the two-step ABHS intervention was more efficacious than the label-use ABHS and pumice soap interventions and was at least as efficacious as traditional soap at reducing microbes on soiled farmworker hands. The two-step ABHS intervention resulted in significantly lower percentages of positive samples and lower geometric mean concentrations of all indicators than did the label-use ABHS intervention (concentrations of coliforms and *Enterococcus* bacteria) (Fig. 2) and pumice soap intervention (prevalence and concentrations of coliforms and concentrations of *Enterococcus* bacteria) (Table 1 and Fig. 2). These results confirmed the results in a previous study of hand hygiene interventions with farmworkers harvesting jalapeños, where the same two-step ABHS intervention resulted in 1 to 2 log CFU fewer bacteria per hand than were found for the control group and performed better at eliminating indicator bacteria than hand washing with foam soap *(13)*. The results suggest that the most efficacious hand hygiene intervention in the agricultural environment may be a dual-mechanism intervention, such as the two-step ABHS, that combines physical removal from hands (e.g., with paper towels) with inactivation of indicator bacteria (e.g., by ethanol, the active ingredient in the ABHS and an effective antimicrobial agent *(3, 24)*).

This study has several strengths and limitations. It addresses a gap in the hand hygiene literature by evaluating the efficacy of hygiene interventions in an agricultural environment under real-use conditions. The study also compares an array of hygiene interventions, both soap based and ABHS based. Although the study was conducted on only one farm with participants harvesting only one type of produce, the similarity of the results to those of a previous pilot study evaluating foam soap and two-step ABHS on a different farm with different produce *(13)* suggests that these results may be broadly applicable to the agricultural field environment during produce harvest.

The results of this field evaluation of hand hygiene techniques have several implications. Hands may be a source of produce contamination if a farmworker is ill, and

hands may also contribute to produce contamination by transferring indicator bacteria from the environment (e.g., soil, water, or produce bins) to the produce during harvest. These results show that the performance of hand hygiene interventions can vary with the hygiene product and technique, and hand hygiene recommendations may need to be tailored to meet the environment and availability of hygiene resources. Hand hygiene performed incorrectly or with an ineffective product may not improve the microbial quality of hands even if they appear cleaner after hygiene. Although they did not remove soil as well as soap-based interventions, the ABHS-based interventions reduced the concentrations of indicator bacteria similarly to the soap-based interventions and can be viewed as efficacious hand hygiene solutions even on soiled hands.

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