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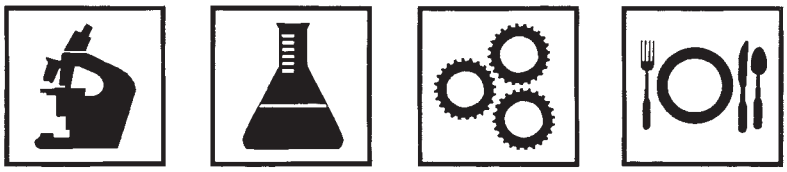

Hand Hygiene Regimens for the Reduction of Risk in Food Service Environments

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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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