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# Improved Inactivation of Nonenveloped Enteric Viruses and Their Surrogates by a Novel Alcohol-Based Hand Sanitizer[▽]

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**Norovirus is the leading cause of food-related illness in the United States, and contamination of ready-to-eat items by food handlers poses a high risk for disease. This study reports the in vitro (suspension test) and in vivo (fingerpad protocol) assessments of a new ethanol-based hand sanitizer containing a synergistic blend of polyquaternium polymer and organic acid, which is active against viruses of public health importance, including norovirus. When tested in suspension, the test product reduced the infectivity of the nonenveloped viruses human rotavirus (HRV), poliovirus type 1 (PV-1), and the human norovirus (HNV) surrogates feline calicivirus (FCV) F-9 and murine norovirus type 1 (MNV-1) by greater than 3 $\log_{10}$ after a 30-s exposure. In contrast, a benchmark alcohol-based hand sanitizer reduced only HRV by greater than 3 $\log_{10}$ and none of the additional viruses by greater than 1.2 $\log_{10}$ after the same exposure. In fingerpad experiments, the test product produced a 2.48 $\log_{10}$ reduction of MNV-1 after a 30-s exposure, whereas a 75% ethanol control produced a 0.91 $\log_{10}$ reduction. Additionally, the test product reduced the infectivity titers of adenovirus type 5 (ADV-5) and HRV by ≥3.16 $\log_{10}$ and ≥4.32 $\log_{10}$, respectively, by the fingerpad assay within 15 s; and PV-1 was reduced by 2.98 $\log_{10}$ in 30 s by the same method. Based on these results, we conclude that this new ethanol-based hand sanitizer is a promising option for reducing the transmission of enteric viruses, including norovirus, by food handlers and care providers.**

Proper and frequent hand hygiene is crucial for infection control (10, 25). Whereas hand washing with soap and water remains common and relevant, influential organizations such as the U.S. Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) now recommend alcohol-based hand sanitizers (ABHS) for hand hygiene when hands are not visibly soiled (10, 42). Among the advantages of ABHS over traditional soap-and-water washing are (i) a faster microbial kill, (ii) a greater reduction in microbial load, (iii) a broader spectrum of microbicidal activity, (iv) relative ease of use and time savings, (v) better skin tolerance in spite of frequent use, (vi) convenience and freedom from dependence on sinks and running water, and (vii) water conservation (7–10, 38, 42). These factors, together with evidence for higher levels of compliance with hand hygiene and reduced rates of certain types of hospital-associated infections, have promoted wide acceptance of ABHS in health care and moderate acceptance in food industries (7, 10, 19, 37).

While improving compliance with hand hygiene in the food service and food processing industries remains a challenge, the presence of nonenveloped viruses, which are more difficult to inactivate by commonly used hand sanitizers, adds another layer of concern. Statistics from the Foodborne-Disease Outbreak Surveillance System (1998 to 2002) indicate that viral pathogens (predominantly norovirus) accounted for 33% of outbreaks and 41% of cases of infection with known etiology (27). The annual number of food-related infections in the United States is estimated at 76 million, with viruses accounting for 79% and human norovirus (HNV) alone accounting for 59% of such cases (30). The 2005 Food Code, which regulates hand hygiene in the food service sector, prescribes hand washing as the primary means for hand decontamination, with the use of ABHS only as an adjunct to hand washing (37). It is well documented that ethanol alone can rapidly inactivate vegetative bacteria, fungi, and enveloped viruses, but its activity against nonenveloped viruses varies more widely (10). There remains a need for hand sanitizers with demonstrated activity against a broad spectrum of nonenveloped viruses, including 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 (6, 13). Feline calicivirus (FCV), one such surrogate, survives well in the environment and is relatively resistant to chemical challenges (13, 14, 16, 28). However, since FCV is primarily a respiratory pathogen and appears to be less acid tolerant than HNV, its relevance as a surrogate for HNV has been questioned by some investigators (11). The recent in vitro cultivation of murine norovirus type 1 (MNV-1) provides an alternative and perhaps a more suitable surrogate than FCV (11, 40, 41).

This study presents findings of the in vitro and in vivo activities of a new synergistically formulated ABHS against several nonenveloped viruses, including surrogates for HNV.

## MATERIALS AND METHODS

**Antimicrobial test articles.** Purell VF447 (GOJO Industries Inc., Akron, OH), the test product used in this study, contains 70% ethanol (vol/vol) as the active ingredient and is formulated with a synergistic blend of polyquaternium-37 (PQ-37) and citric acid, as well as gelling and skin conditioning ingredients (M. Snyder, D. R. Macinga, and J. W. Arbogast, U.S. patent application 11/499,227.).

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TABLE 1. Mammalian viruses and corresponding cell lines used in this study

| Virus (ATCC strain) | Host cell (ATCC strain) |
|---|---|
| Adenovirus type 2 (VR-846) | A-549 (CCL-185) |
| Adenovirus type 5 (VR-1516) | 293 (CRL-1573); Vero (CCL-81) |
| Feline calicivirus, F-9 (VR-782) | CrFK (CCL-94) |
| Hepatitis A virus, HM-175 (VR-2093) | FRhK-4 (CRL-1688) |
| Murine norovirus type 1, P3[a] | RAW 264.7 (TIB-71) |
| Poliovirus type 1 Sabin (VR-1000) | Vero (CCL-81) |
| Human rotavirus, Wa (VR-2018) | MA-104 (CRL-2378.1) |

[a] A gift from H. W. Virgin, Washington University, St. Louis, MO.

The benchmark hand sanitizer, Purell Instant Hand Sanitizer (GOJO), contains 62% ethanol (vol/vol) as the active ingredient. The 75% ethanol (vol/vol) reference solution was prepared in distilled water.

**Bacteriophage MS2 suspension assays.** MS2 was used as the surrogate for nonenveloped mammalian viruses to screen for compounds with virucidal activity. A 0.1-ml aliquot of MS2 (ATCC 15597-B1) suspension was added to 9.9 ml of the test substance. After a 60-s contact time at room temperature with mixing, a 0.1-ml volume was removed and neutralized by dilution in 9.9 ml of Dey-Engley neutralizing broth. Further 10-fold serial dilutions were prepared in Dey-Engley broth. The viable phage remaining were quantified by infecting *Escherichia coli* (ATCC 15597) by using the soft agar technique (20).

**Viruses, cell lines, and viral propagation.** The mammalian viruses used and their respective host cell lines are listed in Table 1. Human adenovirus type 5 (ADV-5; ATCC VR-1516) was grown in 293 cells (ATCC CRL-1573) and enumerated by plaque assay in Vero cells (ATCC CCL-81) because 293 cells yielded higher titers of the virus and Vero cell monolayers proved more stable for the 7 to 8 days of incubation required for the plaque assay. All other viruses were grown and plaque assayed using the appropriate cell line as indicated in Table 1. The procedures for growing cell monolayers and for preparing virus pools have been described previously (6, 32).

**Infectivity assays.** To determine the infectious titer of MNV-1 in virucidal suspension assays, conventional plaque assay techniques were employed as previously reported (11). Briefly, RAW 264.7 cells were dispensed in 60-mm-diameter cell culture plates at a density of $2 \times 10^6$ cells per plate and grown to 80 to 90% confluence in 5 ml of complete minimum essential medium at 37°C. Cell monolayers were infected with 200 μl of a 10-fold dilution series of the virus for 1 h at 37°C and, after the inoculum was removed, the cells were overlaid with 5 ml of medium containing 0.5% agarose and incubated for 48 h. A second agarose overlay, which included 0.75% neutral red solution (Sigma-Aldrich), was then added (3 ml), and plaques were counted at 5 to 8 h thereafter. Plates with 5 to 50 plaques were used to determine the virus titer in PFU. Infectivity assays for all other viruses in suspension assays were done by measuring the residual infectious virus detected by the virus-induced cytopathic effect (26). Viral titers were expressed as the $-\log_{10}$ of the 50% titration endpoint for infectivity ($TCID_{50}$) per 0.1 ml as calculated by the method of Spearman-Kärber (21).

Infectivity assays for fingerpad experiments were done by plaque assay using a slightly different method from that described above. Host cell monolayers were prepared in 12-well cell culture plates (Corning, Ithaca, NY). Each well received 2 ml of cell suspension in growth medium and was incubated at 37°C with 5% $CO_2$ until cells were confluent. Samples for assay were serially diluted in Earle's balanced salt solution (EBSS; Invitrogen, Burlington, ON, Canada). Cell monolayers were infected with 100 μl of each serial dilution and incubated at 37°C in 5% $CO_2$ for 90 min with gentle rocking every 30 min. Two milliliters of warm (45°C) sterile agar overlay was placed in each well, and the plates were incubated at 37°C in 5% $CO_2$ until plaque formation. Thereafter, monolayers were fixed in 2 ml of 3.7% formaldehyde (Anachemia; Lachine, QC, Canada) in normal saline for at least 4 h. Formaldehyde was discarded, and agar plugs were removed by holding the plates under running warm tap water. The fixed cells were then stained with 0.1% (wt/vol) aqueous crystal violet solution for 20 min (6). Plaques were then counted, and their numbers were recorded.

Each plaque assay plate included at least two wells which served as negative controls to make sure that the cell monolayers were not contaminated. One well was used as a positive control to ensure the presence of infectious virus and its ability to infect the host cells used in the assay. An assay was regarded as valid only if the negative controls remained intact and there was detectable degeneration of the cells in the positive control (33).

**Suspension assays.** Suspension tests for virucidal activity were performed using a modification of ASTM standard E-1052 (3). A 4.5-ml sample of the test substance was dispensed into a sterile 15-ml conical tube and mixed with 0.5 ml of the virus suspension. The mixture was vortexed for 10 s and held for the remainder of the 30-s exposure time. Immediately following the exposure period, a 0.1-ml aliquot was removed from the tube, neutralized by 10-fold serial dilution into minimum essential medium containing 10% fetal bovine serum and titrated for the presence of viable virus by infectivity assay. For MNV-1, infectivity was determined by plaque assay. In instances where residual cytotoxicity was noted after exposure to a test substance, the sample was removed in accordance with the column chromatography method recommended by ASTM standard E-1482, prior to plaque assay (4). For all other viruses, infectivity was determined by virus-induced cytopathic effect, and $\log_{10}$ reductions were calculated by subtracting the $TCID_{50}$ of the test treatment from the $TCID_{50}$ of the virus control (21, 26). Consistent with recommendations of ASTM standard E-1052 (3), virus controls, neutralization controls, and cytotoxicity controls were also performed.

**Fingerpad method.** The fingerpad assay used in this study was based on ASTM standard E-1838-02, which has been described in detail previously (2, 32, 33). Prior to the initiation of the study, we obtained formal approval from the Ottawa Hospital Research Ethics Board. Briefly, each potential adult panelist was screened, using a set of inclusion/exclusion criteria, briefed on the objective and procedures for the study, and requested to review and sign an informed consent form. Just before their fingerpads were contaminated, the panelist thoroughly washed and dried his/her hands, using a nonmedicated liquid soap to remove any dirt and oil from the skin. To reduce any transient microorganisms on the panelist's hands, about 3.0 ml of 75% (vol/vol) ethanol was then dispensed onto the panelist's cupped palms, and he/she rubbed the alcohol over the entire surface of both hands until the liquid had evaporated completely.

Each virus to be tested was suspended in a soil load to simulate the presence of body secretions or excretions. The two types of soil loads, as permitted by ASTM standard E-1838-02 (2), were 5% bovine serum (final concentration) and a mixture of three components (tryptone, bovine serum albumin, and bovine mucin), with a total protein content roughly equal to that in 5% bovine serum (33).

To determine the number of PFU of the test virus placed onto each fingerpad, we placed 10 μl of the inoculum on each of the panelist's thumb pads and immediately eluted the pads ("time-zero control") as described below; the thumb pads were immediately decontaminated by pressing them for 2 to 3 min against a paper towel soaked in either 75% ethanol (for AD and HRV) or a 1:10 dilution of domestic bleach (for MNV-1, poliovirus [PV], and hepatitis A virus [HAV]). Each fingerpad was then contaminated with 10 μl of the test virus suspension, and the inocula were allowed to become visibly dry under ambient conditions, which required 20 to 25 min. To assess the loss in virus infectivity due to drying, one randomly selected fingerpad was eluted immediately upon drying, and another was eluted at the end of the experiment; the average level of infectious virus on these two fingerpads constituted the "baseline control."

The dried inocula on at least two randomly selected fingerpads (one on each hand) were then each exposed simultaneously to 1 ml of the test formulation contained in a 2-ml cryovial (Sarstedt Inc., Montreal, QC, Canada) for the desired contact time. The material remaining on each treated fingerpad was eluted with 1 ml of EBSS containing 0.1% (vol/vol) Tween-80 (BioShop, Burlington, ON, Canada). The degree of virus inactivation was determined by subjecting the eluates, from the control and the treated digits, to a plaque assay. Additional controls were included to determine (i) the level of infectious virus in the suspensions for the contamination of hands, (ii) any interference to virus infectivity that subcytotoxic levels of the test formulations might cause, and (iii) the absence of extraneous viral or bacterial contamination in the host cells (33). These controls ensured that a decreased (or lack of) recovery of virus after exposure to the test substance was due to inactivation by the test formulation.

**Statistical considerations.** For comparing the $\log_{10}$ reductions of a minimum of six replicates for various test articles against MS2, a one-way analysis of variance (ANOVA) with a Bonferroni post hoc analysis for multiple comparisons was performed at an alpha level of 0.05. Statistical comparisons of VF447 and the 75% ethanol control activities against MNV-1 were evaluated with Student's *t* test ($\alpha = 0.05$). Statistical analyses were conducted with GraphPad InStat version 3.06 (GraphPad Software, Inc., San Diego, CA).

## RESULTS

**Identification of ethanol potentiators.** Previous reports, as well as work in our laboratories, have demonstrated that ethanol possesses relatively weak activity against certain nonen-

TABLE 2. In vitro inactivation of MS2 after 60-s exposure to various alcohol mixtures

| Active ingredient | Potentiator (% wt/wt) | MS2 $\log_{10}$ reduction |
|---|---|---|
| NaOCl (ppm) | | |
| 100 | None | ≥5.85 |
| Ethanol (%) | | |
| 62 | None | −0.10 |
| 70 | None | −0.02 |
| 78 | None | 0.16 |
| 78 | Benzethonium chloride (0.5%) | 0.34 |
| 78 | Chlorhexidine gluconate (2%) | 0.48 |
| 78 | Copper gluconate (0.04%)[a] | 0.18 |
| 78 | Silver zeolite (0.24%)[b] | 0.58 |

[a] 60 ppm copper.
[b] 60 ppm silver.

veloped viruses, including PV and the HNV surrogate FCV (5, 13, 29). A suspension assay with bacteriophage MS2 was used to identify compounds capable of potentiating the activity of ethanol against nonenveloped viruses. MS2 was chosen because it has been demonstrated to be a good model for evaluating hand hygiene products which target nonenveloped viruses and because it is safe and relatively simple to handle (20).

Table 2 shows that MS2 is highly resistant to ethanol, which exhibited minimal activity in concentrations of up to 78%. In contrast, viable MS2 became undetectable after a 60-s exposure to 100 ppm of sodium hypochlorite (≥5.85 $\log_{10}$ reduction). Various compounds with known antimicrobial activity (including the topical antimicrobials chlorhexidine gluconate and benzethonium chloride and the transition metals copper and silver) produced small but measurable increases in the activity of ethanol. A large number of additional compounds did not improve the activity of ethanol against MS2 (data not shown). Interestingly, the addition of either citric acid or the cationic polymer PQ-37 each increased the activity of 78% ethanol against MS2 (Fig. 1). Statistical analysis using one-way ANOVA revealed that the increase in activity against MS2 resulting from the addition of PQ-37 to ethanol was significant ($P < 0.001$), whereas the increase from the addition of citric acid to ethanol was not statistically significant. Surprisingly, the combination of ethanol, PQ-37, and citric acid was synergistic against MS2, resulting in a reduction (4.36 $\log_{10}$) that was significantly higher than that of ethanol in combination with either citric acid (0.60 $\log_{10}$) or with PQ-37 (1.06 $\log_{10}$) alone ($P < 0.001$). Similar results were observed at lower ethanol concentrations, although the overall $\log_{10}$ reductions were lower (data not shown).

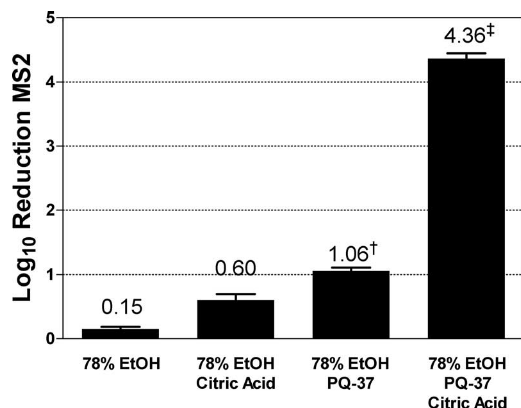


FIG. 1. Virucidal activity of various alcoholic mixtures as measured by a suspension test against MS2, with a 60-s exposure at room temperature. When they were included, citric acid was at 0.25% (wt/wt), and PQ-37 was at 0.4% (wt/wt). The bar heights indicate the mean values from a minimum of six replicates, and the error bars indicate standard errors of the means. † indicates significantly greater activity than 78% ethanol (EtOH) by one way ANOVA ($P < 0.001$). ‡ indicates significantly greater activity than all other alcoholic mixtures ($P < 0.001$).

TABLE 3. In vitro inactivation of MS2 after 60-s exposure to products with different active ingredients

| Product | Active ingredient(s) | MS2 $\log_{10}$ reduction |
|---|---|---|
| Benchmark hand sanitizer | 62% ethanol | 0 |
| VF447 | 70% ethanol | 1.91 |
| A | 71% ethanol | 0 |
| B | 95% ethanol | 0.90 |
| C | 54.1% ethanol, 10% 1-propanol, 5.9% 1,2-propanediol, 5.7% 1,3-butanediol | 1.79 |
| D | 70% ethanol, 0.1% 1,3-butanediol | 0 |
| E | 70% isopropanol | 0.05 |
| F | 75% isopropanol | 0.18 |
| G | 45% isopropanol, 30% 1-propanol, 0.2% mecetronium etilsulfate | 0 |

Based on the above results, a hand sanitizer was formulated for optimized virucidal performance, skin compatibility, and aesthetics. The formulation (termed Purell VF447 and hereafter referred to as VF447) contains 70% ethanol, PQ-37, citric acid, hydroxypropyl cellulose, and multiple skin-conditioning ingredients. Table 3 compares the in vitro virucidal activity of VF447 to that of several commercially available hand sanitizers with differing active ingredients against the MS2 surrogate after a 60-s exposure. Products containing up to 95% ethanol, up to 75% isopropanol, or mixtures of alcohols exhibited minimal virucidal activity against MS2. Only VF447 and the product based on a blend of alcohols and diols (23) inactivated MS2 by >1 $\log_{10}$ (1.91 $\log_{10}$ and 1.79 $\log_{10}$, respectively).

TABLE 4. In vitro comparison of benchmark sanitizer activity compared to that of VF447

| Virus | Virus $\log_{10}$ reduction[a] | |
|---|---|---|
| | Benchmark hand sanitizer | VF447 |
| Feline calicivirus | 1 | ≥4.75 |
| Murine norovirus | 1.16 | ≥3.68 |
| Adenovirus type 2 | 0.5 | 2.75 |
| Hepatitis A virus | 0 | 1.75 |
| Poliovirus type 1 | 0 | 3.5 |
| Rotavirus | ≥5.75 | ≥4.75 |

[a] Values indicate reduction after a 30-s exposure. ≥ indicates inactivation values below that of the detection limit of the assay.

TABLE 5. In vivo efficacy of 75% ethanol against murine norovirus compared to that of VF447

| Treatment | Exposure time (s) | No. of fingerpads examined | Mean $\log_{10}$ reduction | SD[b] | $P$ value |
|---|---|---|---|---|---|
| VF447[a] | 30 | 16 | 2.48 | 0.45 | <0.0001 |
| 75% ethanol | 30 | 8 | 0.91 | 0.57 | |

[a] Values shown are the combined results of two individual lots.
[b] SD, standard deviation.

TABLE 6. In vivo efficacy of VF447 against five nonenveloped mammalian viruses

| Virus | Exposure time (s) | No. of fingerpads examined | Mean $\log_{10}$ reduction[a] | SD[b] |
|---|---|---|---|---|
| Adenovirus type 5[c] | 15 | 8 | ≥3.16 | 0.12 |
| | 30 | 12 | ≥3.12 | 0.43 |
| Rotavirus[c] | 15 | 12 | ≥4.32 | 0.06 |
| | 30 | 12 | ≥3.84 | 0.35 |
| Poliovirus type 1 | 30 | 12 | 2.98 | 0.50 |
| Hepatitis A virus | 30 | 2 | 1.32 | NA |

[a] ≥ indicates that no viruses were recovered from the treated fingerpads, values were calculated from baseline controls.
[b] SD, standard deviation; NA, not applicable.
[c] Values represent combined results of two individual lots.

**Virucidal activity of the test product against nonenveloped enteric viruses by suspension assays.** Table 4 shows a comparison of the virucidal activity of VF447 with that of a benchmark hand sanitizer (Purell Instant Hand Sanitizer; 62% ethanol) against several nonenveloped enteric viruses and their surrogates. The activity of the benchmark sanitizer varied considerably depending on the virus, and ranged from no reduction of HAV to >5 $\log_{10}$ reduction for human rotavirus (HRV) after a 30-s contact. The activity of VF447 was substantially higher for the ethanol-resistant nonenveloped viruses, reducing the infectivity of HAV, PV, and ADV-2 by 1.75 $\log_{10}$, 3.5 $\log_{10}$, and 2.75 $\log_{10}$, respectively. Of note was the improvement in virucidal activity against the HNV surrogates FCV (≥4.7 $\log_{10}$ reduction) and MNV-1 (≥3.68 $\log_{10}$ reduction). In each case, no infectious virus was detectable after a 30-s exposure to VF447, whereas the benchmark product produced only marginal reductions of the HNV surrogates (approximately 1 $\log_{10}$) after the same exposure time.

**Inactivation of MNV-1 on fingerpads.** Table 5 shows a comparison of the activity of VF447 with that of a 75% ethanol control against MNV-1 on the fingerpads of adults, using ASTM standard E-1838-02. The test product achieved an average reduction of 2.48 $\log_{10}$ in 30 s, which was significantly greater than that achieved by the 75% ethanol control (0.91 $\log_{10}$) at the same exposure time ($P < 0.0001$).

**Inactivation of additional nonenveloped viruses on fingerpads.** VF447 was further evaluated against additional nonenveloped enteric viruses, utilizing the fingerpad method (Table 6). Experiments using a 30-s contact resulted in no detectable recovery of ADV-5 and HRV from the majority of the fingerpads exposed to VF447. Baseline, input, and plaque interference controls (described in Materials and Methods) demonstrated that this was due to the inactivation of virus to a level below that of the detection limit of the assay (33). The average reduction values were ≥3.12 $\log_{10}$ and ≥3.84 $\log_{10}$ for ADV-5 and HRV, respectively. The test product was slightly less effective against PV (2.98 $\log_{10}$ reduction) and demonstrated lower activity against HAV (1.32 $\log_{10}$ reduction) after a 30-s exposure.

Because the test product reduced ADV-5 and HRV levels to below those of the detection limits after 30 s, additional experiments were conducted using a 15-s contact. Again, no virus was recovered from the majority of the fingerpads exposed to the test product, resulting in average estimated reduction values of ≥3.16 $\log_{10}$ and ≥4.32 $\log_{10}$ for ADV-5 and HRV, respectively.

## DISCUSSION

This broad, multilaboratory study describes the antiviral performance of a new ethanol-based hand sanitizer optimized for activity against nonenveloped enteric viruses. The antiviral activity of the test product, VF447, was evaluated by both in vitro and in vivo methodologies at multiple time points against a broad set of nonenveloped enteric viruses and their surrogates. Overall, VF447 demonstrated in vitro virucidal activity that was superior to that of the benchmark sanitizer (Table 4) and those of a variety of commercial ABHS (Table 3) and performed statistically better than a 75% ethanol control solution, using in vivo fingerpad assays (Table 5).

The test product, VF447, was formulated with ethanol, which is recognized by the U.S. Food and Drug Administration as a safe and efficacious active ingredient for health care antiseptic drug products (15). An activity level of 70% was chosen since this is the maximum level of compliance with international food code regulations. PQ-37 and citric acid were included in VF447 because the combination of these two ingredients with ethanol was found to be synergistic, providing the greatest overall enhancement of virucidal action (Fig. 1). Additional experiments demonstrated that PQ-37 could be substituted with alternative polyquaterniums to produce similar results (Snyder et al., U.S. patent application 11/499,227.). Polyquaterniums are typically poor microbicides, and in fact, PQ-37 was found to have little microbicidal activity by itself. While the mechanism by which organic acid and polyquaterniums potentiate the activity of ethanol remains poorly understood, preliminary studies suggest that charge density plays a role (D. R. Macinga and J. W. Arbogast, unpublished data).

The sensitivity of FCV to low pH has been recently reported (11), and the acid-labile nature of rhinoviruses is also well known (18, 24, 36). Our findings show that the potentiation of the virucidal activity of ethanol by the addition of organic acids is virus specific. For example, when comparing similar formulations, we typically observed greater activity against HAV when acid was absent (D. R. Macinga and J. W. Arbogast, unpublished data). Overall, it appears that the addition of PQ-37 and citric acid uniquely maximizes the activity against mammalian nonenveloped viruses while still meeting the critical skin care performance and aesthetic requirements accepted in actual use.

The enteric viruses and their surrogates selected for this

study represent the majority of food-borne viral pathogens. As stated previously, HNV is the most epidemiologically important virus in food settings, followed closely by HAV (17). Rotaviruses have also been occasionally incriminated in food-borne outbreaks (1). The PV used here is prototypical of human enteroviruses and is often used in assessing antiseptics and disinfectants for their activities against viruses (e.g., European virucidal test norm, EN 14476 [14a]). While ADV-40 and -41 are incriminated in cases of gastroenteritis, they remain relatively difficult to work with in the laboratory. In view of this, ADV-2 and ADV-5 were used as surrogates for them in this study. It should be noted that the transmission of these enteric viruses is not limited to food settings. For example, HNV has plagued the cruise line industry for years, and its growing importance in long-term-care facilities has been documented recently (12, 39).

To our knowledge, this is the first published study that uses MNV-1 as a surrogate for HNV for the fingerpad method. The literature suggests that MNV-1 provides a more relevant surrogate because it belongs to the same genus as that of HNV and is also transmitted by the fecal-oral route in its natural host (22, 41). Nevertheless, because of the high relevance of HNV and the inability to assay the virus by standard means, both FCV and MNV-1 were used as surrogates for HNV in this study. The two surrogates were found to exhibit similar sensitivities to the products tested (Table 4). Whereas the benchmark sanitizer reduced each surrogate by approximately 1 $\log_{10}$ in 30 s, VF447 reduced both surrogates to a level below the detection limit in 30 s. These results confirm previous studies demonstrating the ethanol resistance of FCV and the suitability of MNV as an HNV surrogate when ethanol-based products are evaluated. Furthermore, the results demonstrate that antiviral activity is not dictated entirely by ethanol level and illustrates the benefits of the VF447 formulation.

Methodology challenges, limitations, and choices with hand hygiene product efficacy testing are typically a source of debate and discussion. The 15- and 30-s contact times evaluated in this study are highly relevant, as ABHS typically take about 15 to 30 s to dry, depending primarily on the amount applied to the hands. In previous studies that evaluated ABHS by the fingerpad method, contact times ranged from 20 to 60 s (23, 32). It can be argued that a 60-s contact time is not relevant to the end user; however, a 60-s contact time may have some scientific value (e.g., when comparing soil types and load effects). In addition to more testing at 15 s or less, future studies using actual HNV and intervention epidemiology studies to assess real-world infection rate outcomes would be highly valuable.

In this study, the initial screening with bacteriophage MS2 proved to be a useful tool for predicting the virucidal activity of test formulations against mammalian nonenveloped viruses. The relevance of in vitro suspension assays can be argued and should not be the sole means for evaluating the efficacy of hand hygiene products. They are, however, a critical tool in screening and ranking test articles because they afford major cost and time savings compared to that of in vivo testing. The fingerpad protocol is currently the best method to predict the real-world efficacy of hand hygiene products against viruses and has been shown to be comparable with whole-hand methods (35). In this set of experiments, the virucidal activity observed for VF447 in the suspension assays was a reliable predictor of in vivo activity measured by fingerpad experiments (e.g., the high activity of VF447 against the HNV surrogates by both methods). In conclusion, the use of MS2 for quick screening of technologies, in vitro suspension assays for comparing multiple products at multiple time points, and the fingerpad method for final confirmation were approaches that were both efficient and effective.

The results of this study demonstrate that VF447 is a promising adjunct to hand washing for use in multiple settings, including the food service and food processing industries. Previous work has demonstrated that the combination of hand washing and hand sanitizing produces greater reductions in bacterial levels than either practice alone (31). Future studies to understand the benefits of a washing/sanitizing regimen using VF447 or comparable formulations would be highly desirable. VF447 may also be appropriate for reducing the risk of viral infections in areas with light soiling, such as the intermediate spaces of restaurants (e.g., around cash registers, order pick-up areas, drive-through windows, or sinks receiving minimal or restricted use). Beyond its use by the food industry, VF447 could be a good infection control tool for the cruise line and the hospitality industries, where norovirus outbreaks often occur and patrons do not always have ready access to sinks nor the discipline to perform hand washing.

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