

# Comparison of the Activity of Alcohol-Based Handrubs Against Human Noroviruses Using the Fingerpad Method and Quantitative Real-Time PCR

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**Abstract** Noroviruses (NoV) are the most common cause of acute nonbacterial gastroenteritis in the United States, and human hands play an important role in their transmission. Little is known about the efficacy of hand hygiene agents against these highly infectious pathogens. We investigated the activity of seven commercially available hand hygiene products against human noroviruses by in vivo fingerpad tests. The in vivo activity of alcohol-based handrubs ranged from 0.10 to 3.74 log reduction and was not solely dependent on alcohol concentration. A handrub (VF481) based on 70% ethanol and a blend of other skin care ingredients reduced Norwalk virus (NV) by 3.74 log in 15 s and provided significantly greater NV reduction than all the other products tested ( $P < 0.001$ ). Furthermore,

VF481 was the most effective product tested against the NoV genogroup II strains Snow Mountain virus (GII.2) and a GII.4 strain. These results demonstrate that alcohol by itself is not effective against NoV, but effective formulation of alcohol-based handrubs can achieve significant reduction of norovirus RNA on fingers.

**Keywords** Norovirus · Quantitative RT-PCR · Handrub · Fingerpad · ASTM

## Introduction

Noroviruses (NoV) are the major cause of acute nonbacterial gastroenteritis in humans worldwide (Widdowson et al. 2005; Blanton et al. 2006; Lopman et al. 2002). In the United States, NoV account for 59% of the estimated 76 million food-related infections (Mead et al. 1999) and have also been implicated in outbreaks in both long-term care and acute care facilities (Wu et al. 2005; Hansen et al. 2007). Human NoV are classified into three genogroups, designated GI, GII, and GIV, and multiple clusters (genotypes) within each genogroup. Norwalk virus (NV) and Snow Mountain virus (SMV) are prototype GI.1 and GII.2 genotypes, respectively. GII.4 is the most commonly detected NoV genotype throughout the world and accounted for 62% of all reported NoV outbreaks from 2001 to 2007 (Siebenga et al. 2009).

Hands are known to be an important vehicle for the transmission of NoV (Bidawid et al. 2004; Todd et al. 2008; Moe et al. 2001), and the use of hand hygiene products that are effective at removing or inactivating NoV is likely to be a critical part of an effective NoV infection control strategy (Bidawid et al. 2004; Lages et al. 2008; Kampf et al. 2005). Despite the important role of hands in

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the transmission of NoV, little is known about the efficacy of hand hygiene practices in reducing the spread of NoV.

Alcohol-based handrubs have some advantages over traditional hand washes, particularly when running water is not accessible. The effectiveness of handrubs against bacteria (Davis et al. 2006; Kampf et al. 2003; Weber et al. 2003), and viruses, including influenza (Kramer et al. 2006), rotavirus (Sattar et al. 2000; Sattar and Ansari 2002), hepatitis A virus (Mbithi et al. 1993), poliovirus (Mbithi et al. 1993), adenovirus (Sattar et al. 2000), and rhinovirus (Sattar et al. 2000) has been widely studied. The efficacy of handrubs against human NoV has been rarely reported primarily due to the lack of an available animal model or routine culture system for studying human NoV infectivity.

Although preliminary results from Straub et al. (2007) demonstrate that it may be possible to culture NoV, routine and replicable culture-based methods to quantify infectious human NoV are not yet available. Most studies on the efficacy of disinfectants and antiseptics against NoV have employed surrogate animal caliciviruses, including feline calicivirus (FCV) (Gehrke et al. 2004; Kampf et al. 2005) and more recently murine norovirus (MNV) (Cannon et al. 2006; Macinga et al. 2008; Park et al. 2010; Okunishi et al. 2010; Magulski et al. 2009), to predict the behavior of human norovirus. These viruses are useful because they can be measured via cell culture assays and possibly by animal challenge studies. However, the appropriateness of FCV and MNV as surrogates for human NoV has been debated because they do not always behave in the same manner in environmental persistence or inactivation studies (Park et al. 2010). Specifically, FCV is quite pH sensitive under acidic conditions, while MNV is sensitive to desiccation (Cannon et al. 2006) and is likely much more susceptible to ethanol inactivation (Belliot et al. 2008; Magulski et al. 2009; Park et al. 2010). These observations are inconsistent with current knowledge of the behavior of the human NoV. Synergistically formulated ethanol-based handrubs have recently been reported to have significantly enhanced antiviral efficacy against several non-enveloped viruses, including FCV and MNV (Macinga et al. 2008; Belliot et al. 2008). Moreover, the results obtained using surrogate viruses may not be a good indication of the actual efficacy of these products against human NoV strains. It is therefore important to evaluate hand hygiene products against human NoV to determine the ability of antiseptics to reduce transmission of NoV by hands.

Quantitative real-time PCR (RT-qPCR) is currently the best available method for the enumeration of human NoV genomic copies in food, clinical, and environmental samples (Topping et al. 2008; Duizer et al. 2004; Rodriguez-Lazaro et al. 2007). Recently, we combined RT-qPCR and

the ASTM fingerpad methodology (Sattar and Ansari 2002; E-1838-02, ASTM International 2002) to test hand hygiene products against Norwalk Virus (NV). Our results indicated that the performance of an antimicrobial handwash was similar to that of a water rinse, and that a handrub based on 62% ethanol was not effective at reducing NV on fingerpads (Liu et al. 2010). The aim of this study was to examine the efficacy of several commercial alcohol-based handrubs against three human NoV strains, NV (GI.1), SMV (GII.2) and a recent GII.4 isolate, using the fingerpad method and RT-qPCR assays for virus quantification. This study expands our previous work with NV by including additional human NoV strains and evaluating the efficacy of additional hand hygiene agents—including those previously shown to be effective against surrogate animal caliciviruses (Macinga et al. 2008).

## Materials and Methods

### Antimicrobial Test Products

A total of seven commercially available hand hygiene products were evaluated (Table 1): seven products against NV (Table 2 and Fig. 1), three against SMV (Table 3), and two against GII.4 (Table 4). The benchmark handrub (PURELL Original Instant Hand Sanitizer, subsequently referred to as “Benchmark”), PURELL Instant Hand Sanitizer VF447 (subsequently referred to as “VF447”), and PURELL Instant Hand Sanitizer VF481 (subsequently referred to as “VF481”) are commercially available products from GOJO Industries Inc., Akron, OH). Other products that are commercially available handrubs were purchased through standard market distributors and were all tested before their expiration dates on the label.

### Virus Inoculum

Norwalk virus and Snow Mountain virus were obtained from the stool samples of previously healthy adult volunteers who became infected with norovirus in previous human challenge studies (Lindesmith et al. 2003, 2005). GII.4 norovirus was kindly provided by Dr. Lee-Ann Jaykus (North Carolina State University), and was confirmed by RT-PCR and sequencing a 172 bp fragment of the capsid region that showed 100% homology with 2006-USA GII.4 Minerva strain. The stool samples were diluted to 20% suspensions with RNase-free water, vortexed briefly, and centrifuged at  $550\times g$  for 30 s. To ensure the safety of the study participants, careful screening and strict exclusion criteria for volunteer enrollment and post decontamination procedures were performed as described below.

**Table 1** Test products and ingredients

Test product	Product format	Active ingredient <sup>a</sup>	Other ingredients <sup>a</sup>
Benchmark PURELL Instant Hand Sanitizer (GOJO Industries)	Gel Sanitizer	62% (v/v) ethanol	Water, glycerin, propylene glycol, sodium hydroxide, tocopheryl acetate, carbomer, fragrance
PURELLVF447 (GOJO Industries)	Gel Sanitizer	70% (v/v) ethanol	Water, isopropyl alcohol, isopropyl myristate, glycerin, diisopropyl sebacate, citric acid, PEG/PPG-20/6 dimethicone, pentaerythrityl tetra-di- <i>t</i> -butyl hydroxyhydro-cinnamate, hydroxypropylcellulose, polyquaternium-37, methylchloroisothiazolinone, methylisothiazolinone
PURRELLVF481 (GOJO Industries)	Gel Sanitizer	70% (v/v) ethanol	Water, isopropyl alcohol, copper gluconate, diisopropyl sebacate, PEG/PPG-20/6 dimethicone, pentaerythrityl tetra-di- <i>t</i> -butyl hydroxyhydrocinnamate, polyquaternium-37
Endure 300 (Ecolab. Inc.)	Gel Sanitizer	70% (v/v) ethanol	Water, isopropyl alcohol, carbomer, propylene glycol, aminomethyl propanol, fragrance
Sterillium Virugard (Bode Chemie)	Sanitizer Rub	95% (w/w) ethanol	Alkane/cyclo alkane mixture, glycerin, myristyl, alcohol, hexane
Germstar Noro (Soaptronic LLC)	Sanitizer Rub	63% (w/w) ethanol	Water, isopropanol, emollient complex, fragrance
Anios Gel 85 NPC (Laboratoires ANIOS)	Gel Sanitizer	85% (v/v) ethanol	Aqua, glycerin, acrylates/C10-30 alkyl acrylate crosspolymer, bisabolol, caprylic/capric triglycerides PEG-4 esthers, PEG-8 caprylic/capric glycerides, aminomethylpropanol, ethylpropanediol

<sup>a</sup> As represented on the product labels

**Table 2** Efficacy of handrubs against Norwalk virus after a 15 s exposure

Treatment	<i>N</i>	Mean log reduction (SD)
VF481	12	3.74 (0.85) <sup>a</sup>
VF447	12	2.04 (0.78) <sup>b</sup>
Endure 300	12	1.49 (0.62) <sup>b</sup>
Sterillium Virugard	12	0.10 (0.17)
Germstar Noro	12	0.11 (0.22)
Anios Gel 85 NPC	6	1.27 (0.22) <sup>b</sup>

<sup>a</sup> Significantly greater reduction compared to all other test products ( $P < 0.001$ )

<sup>b</sup> Significantly greater reduction compared to Sterillium Virugard and Germstar Noro ( $P < 0.01$ )

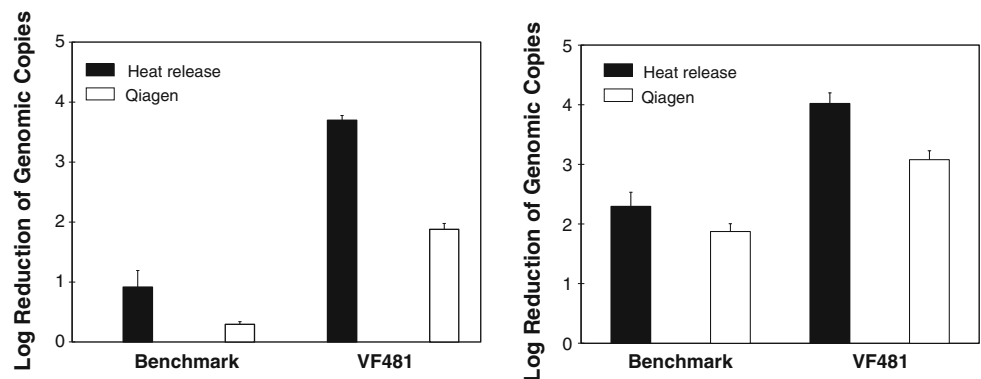
### Human Test Subjects

Study protocols involving human volunteers were reviewed and approved by the Institutional Review Board of Emory University. A total of 30 adult volunteers, between 18 and 50 years of age, were enrolled in the study. After providing informed consent, both hands of each volunteer were carefully inspected prior to each experiment to ensure that they were free of any cuts, abrasions or rashes.

### Fingerpad Method

A modification of the ASTM (American Society for Testing and Materials) standard E-1838-02 fingerpad method,

**Fig. 1** Efficacy of benchmark and VF481 against Norwalk virus (*left*) and GII.4 (*right*) at 15 s exposure time using heat release (HR) and Qiagen (QI) RNA extraction methods ( $N = 11$  for Norwalk trials and  $N = 12$  for GII.4 trials)



**Table 3** Comparison of in vivo efficacy of handrubs against Snow Mountain virus (GII.2) after 15 s exposure

Hand hygiene treatment	N	Log reduction (mean $\pm$ SD)
Benchmark sanitizer	12	1.22 (0.31)
VF447	12	0.30 (0.24) <sup>b</sup>
VF481	12	2.27 (1.70) <sup>a</sup>

<sup>a</sup> Significantly greater log RNA reduction compared to VF447 and benchmark sanitizer ( $P < 0.001$ )

<sup>b</sup> Significantly lower log RNA reduction compared to benchmark sanitizer ( $P < 0.001$ )

**Table 4** Comparison of in vivo efficacy of handrubs against NoV GII.4 strain after 15 s exposure

Hand hygiene treatment	N	Log reduction (mean $\pm$ SD)
Benchmark sanitizer	12	2.30 (0.82)
VF481	12	4.02 (0.61) <sup>a</sup>

<sup>a</sup> Significantly greater log RNA reduction compared to benchmark sanitizer using heat release RNA extraction ( $P < 0.0001$ )

which has been described in detail previously, was used in this study (Sattar and Ansari 2002; Macinga et al. 2008; E-1838-02, ASTM International 2002). The modification involved not scraping the fingers after the handrub use in order to avoid physical removal of the virus and to more accurately model actual use of a handrub by a consumer. Prior to inoculation of the fingerpads, the volunteers were asked to clean their hands completely by washing with a mild soap followed by sanitizing with a solution of 70% ethanol.

#### Inoculation of Fingerpads

Ten microliters of a 20% suspension of NV (approximately  $6.3 \times 10^6$  genome copies), SMV (approximately  $2.0 \times 10^8$  genome copies), or GII.4 NoV (approximately  $6.4 \times 10^6$  genome copies) were placed on the center of each fingerpad of volunteers' hands. For determination of input virus (time 0 control), the seeded inoculum was eluted immediately from the thumbs after fingerpad inoculation (without drying) with 990  $\mu$ l of Hanks Balanced Salt Solution (HBSS). The thumbs were decontaminated as described below, and the virus inocula on other digits were air dried for 20 min.

#### Test Method for Handrub Test Products

For all handrub test products, the fingerpads were exposed to 1 ml of the test substance in an open vial after virus inoculation and drying, respectively, for a contact time of 15 s without inversion. Upon completion of product

testing, the volunteers were instructed to wash their hands with antibacterial liquid soap, and their fingerpads were then decontaminated by pressing onto a paper towel soaked with 10% bleach for 2–3 min.

#### Virus Elution from Fingerpads

To elute the virus remaining on a control fingerpad after drying or on fingerpads after treatment with test products, the volunteers were asked to place the contaminated area of the fingerpad over the mouth of a 1.7 ml plastic vial containing 1 ml of HBSS. The vial was inverted and the eluent in the vial was allowed to remain in contact with the inoculated area for 10 s. The vial was next inverted 20 times with the fingerpad still in place. The soak and inversion steps were repeated once. The vial was then turned upright, and the eluate remaining in contact with the fingerpad was scraped against the inside rim of the vial to recover as much of the fluid as possible.

#### Virological Analysis

##### Recovery of Viruses from Eluates

The viruses in the eluates were precipitated by adding 12 mg polyethylene glycol (PEG) 8000 (Sigma, St. Louis, MO), incubated at 4°C for 1 h, and centrifuged at  $12,000 \times g$  for 15 min. The virus-containing pellets were reconstituted in 50  $\mu$ l of RNase-free water prior to freezing at  $-80^\circ\text{C}$ .

##### RNA Extraction and Real-time RT-PCR

For release of RNA, a heat release RNA extraction was used as described previously (Schwab et al. 1997) for all experiments except for those presented in Fig. 1. An alternative RNA extraction method, the QIAamp Viral RNA kit (QIAGEN, Valencia, Calif.), was used in accordance with the manufacturer's instructions for the experiments in Fig. 1. NV specific RT-qPCR (genogroup I) that targets the RNA-dependent RNA polymerase region of the NV was carried out following methods described previously (Teunis et al. 2008). SMV and GII.4 RNA were quantified using a norovirus GII broadly reactive RT-qPCR assay that the primers and probe span the open reading frame 1 (ORF1) and ORF2 junction region (Kageyama et al. 2003). To generate a standard curve for NV RNA quantification, a full-length NV RNA standard was in vitro transcribed from NV plasmid cDNA with T7 RNA polymerase (Ambion Inc.), serially diluted and quantified by UV absorbance at 260 nm. Similarly, a SMV RNA standard was generated from SMV plasmid with a 2179-bp insert (nt 3,000–5,178) spanning the entire RNA-dependent

RNA polymerase region) and used for the quantification of SMV and GII.4 samples in this study. NoV genome copies in the test samples were estimated by comparing the cycle threshold (Ct) number to that of the RNA standards. All test samples were assayed in duplicate, and the estimated number of genome copies for each sample was an average of replicate test wells.

The log reduction in NoV genome copies associated with exposure to each hand hygiene product was calculated by subtracting the log-transformed NoV titer for each product from the log-transformed baseline control (virus genome copies remaining after 20 min drying). The NoV log reduction for each hand wash product was calculated by averaging the log reductions from all the replicate fingerpads (both hands of all subjects in the experiment). If no viral RNA was detected in a sample, we assumed that the sample had  $\leq 5$  genomic copies (half of the limit detection of the NV specific RT-qPCR assay) and used 5 to calculate log reduction from the baseline control.

To test for PCR inhibition, 10  $\mu$ l of the test product was mixed with 980  $\mu$ l of HBSS and 10  $\mu$ l of 20% NV stool suspension, and then 10- and 100-fold dilutions were amplified by TaqMan real-time RT-PCR. The Ct values from these serially diluted samples were compared with those from serially diluted baseline samples that only contained NV mixed with HBSS. We considered a difference of 1.5 or more between the Ct values of the solutions with and without test product (comparing the same dilutions) as an indication of PCR inhibition in the solution with test product.

### Statistical Analyses

To examine the differences in virus reduction between each hand hygiene product, a one-way analysis of variance (ANOVA) test with Tukey's post hoc analysis was performed at an alpha level of 0.05 using the SAS 9.2 (Statistical Analysis Software) PROC GLM. Paired *t* tests were used to examine the differences between Qiagen vs. heat release RNA extraction and Norwalk virus vs. GII.4 strain. Only data from side-by-side conditions in a single trial were compared in each analysis.

## Results

### NV RNA Reduction by Multiple Hand Hygiene Products

Previous experiments in our lab demonstrated that a handrub based on 62% ethanol (Liu et al. 2010) and a 70% ethanol in water (control test article) were ineffective (Data not shown, mean log reductions = 0.03) at reducing NV RNA. In this study, we examined the efficacy of six

additional hand hygiene products against Norwalk virus (Table 2). There were considerable differences in the viral RNA reductions by the different hand hygiene products, with mean log reductions from 12 replicate fingerpads (6 fingerpads for Anios Gel 85 NPC) ranging from 0.10 to 3.74. Exposure to VF481 gave the greatest mean reduction of NV RNA (3.74 log) that was significantly higher than any other product tested ( $P < 0.001$ ). Sanitizers VF447, Endure 300 and Anios Gel 85 NPC, provided moderate NV RNA reduction (1.27–2.04 log) and were significantly different than the baseline controls ( $P < 0.001$ ). The lowest NV RNA reductions were by Sterillium Virugard and Germstar Noro and were not significantly different from the baseline control ( $P > 0.05$ ).

### NoV Strains Exhibit Unique Sensitivities to Alcohol-Based Handrubs

To investigate potential strain-to-strain differences in NoV reduction by handrubs, we evaluated the efficacy of the two most effective hand hygiene products in our first experiments (VF481 and VF447) and the benchmark handrub product against SMV in a trial with six subjects. VF481 was again the most effective product, reducing SMV RNA by a mean 2.27 log reduction using heat release RNA extraction method (Table 3). However, SMV RNA reduction was less than that observed for VF481 against NV RNA (3.74 log reduction) in the previous trial in Table 2. The SMV RNA reduction by VF481 was significantly different from the baseline control samples ( $P < 0.0001$ ) and also from the RNA reductions associated with the benchmark sanitizer ( $P = 0.0055$ ) and VF447 ( $P < 0.0001$ ).

The performance of the benchmark sanitizer and VF481 were also compared against NoV GII.4 using heat release RNA extraction method (Table 4). VF481 reduced the GII.4 RNA by a mean of 4.02 log in a 15-s exposure in comparison to 2.30 log for the benchmark sanitizer at the same exposure time ( $P < 0.0001$ ).

### Impact of RNA Extraction Methods on Estimates of NoV Reduction

Two RNA extraction methods, heat release and Qiagen, were compared for NV and GII.4 NoV to determine if the RNA reductions we observed were due to PCR inhibition rather than virus inactivation. Two products were examined in this experiment—the 62% ethanol “benchmark” handrub (Liu et al. 2010) and VF481. For both test products, the mean NoV RNA reductions were lower in samples processed by the Qiagen method (1.88 log for VF481 and 0.29 log for benchmark against NV) compared to the reductions measured in aliquots of the same samples processed by the heat release method (3.70 log for VF481 and

0.92 log for the benchmark sanitizer against NV) (Fig. 1, left). This difference was significant ( $P < 0.0001$ ) for VF481 but not significant ( $P = 0.09$ ) for the benchmark sanitizer. Similar trends were observed for the GII.4 virus (Fig. 1, right) when comparing the Qiagen and heat release results ( $P = 0.01$  for VF481, and  $P = 0.25$  for the benchmark). These findings suggest that the NoV RNA reductions observed for some test products (VF481 and possibly others) using the heat release method may be overestimated due to PCR inhibitors even though the samples were tested at a 1:100 dilution. Despite these differences, both extraction methods indicated similar trends in terms of the relative magnitude of RNA reduction by each product for the two different NoV strains. For both extraction methods and for both virus strains, VF481 produced significantly greater mean NoV RNA reductions than the benchmark sanitizer ( $P < 0.0001$ ).

## Discussion

In this study, we examined the efficacy of seven commercial hand hygiene products against multiple NoV strains using the fingerpad method and RT-qPCR. A wide range of efficacy (between 0.10 and 3.74 log reduction) was observed. The most effective product tested, VF481, is a 70% ethanol gel containing additional ingredients that appear to potentiate the virucidal activity of the product. The mean NoV RNA log reductions produced by VF481 ranged from 2.27 for SMV (Table 3) to 3.74 for NV (Table 2) and 4.02 for GII.4 (Table 4) using the heat release method. The efficacy of the other five commercial products (with ethanol concentrations from 62% to 95% [see Table 1]) against NV ranged from 0.10 to 2.04 log reduction (Table 2). These results indicate that formulation plays an important role in product efficacy and that alcohol alone does not dictate NoV reduction. In products like VF447 and VF481, additional ingredients, such as citric acid, polyquaternium-37 or copper gluconate (Table 1), may work with the ethanol to help denature the viral capsid protein. Further studies are needed to specifically examine the effect of these additives on viral RNA and clarify our understanding of the mechanism of action. However, the increased magnitude or broader spectrum of virucidal activity from these synergistic blends has been reported in two previous studies using norovirus animal surrogates as well as poliovirus, rotavirus, adenovirus, hepatitis A virus, and bacteriophage MS2 (Kramer et al. 2006; Macinga et al. 2008). Mean MNV log reduction (measured by plaque assay) was 1.16 for the benchmark sanitizer vs.  $\geq 3.68$  for VF447 in in vitro studies and 0.91 for 75% ethanol vs. 2.48 for VF447 in fingerpad studies (Macinga et al. 2008).

Because NoV strains are highly diverse, handrubs need to be effective against a range of NoV strains including the predominant circulating epidemiological NoV strains. Our results indicate differences in NoV reduction depending on the virus strain and demonstrate the importance of testing more than one strain. The GII.4 NoV was more readily reduced from fingerpads than NV by both VF481 and the benchmark sanitizer (Fig. 1). This finding is consistent with previous research (Butot et al. 2008, 2009) but is somewhat surprising because GII.4 NoV strains have been the predominant NoV outbreaks strains for years (Siebenga et al. 2009), therefore we expected they might be more resistant to inactivation on hands. In contrast, the SMV strain appeared to be more resistant than NV to the handrubs tested but was still significantly reduced by VF481 (Table 3). These findings suggest that VF481 could be helpful in controlling outbreaks due to various NoV strains. Further evaluation of other strains would be of value.

This study extends our previous work and knowledge (Liu et al. 2010) in two ways. First, it demonstrates that some alcohol-based handrubs can be effective against human NoV as measured by RNA reduction using RT-qPCR, and that there are significant differences in the ability of various hand hygiene products to reduce these viruses on fingerpads. These findings highlight the need for evidence-based decision-making about hand hygiene products in settings where NoV transmission and outbreaks can occur. Second, different human NoV strains display different susceptibilities to hand hygiene agents, so it is important to evaluate these products against several NoV strains.

Several questions arise because the methodology in this study uses the presence of RNA genome copies as an indicator of infectious virions. However, it is possible that naked RNA from inactivated virus may be detected by RT-PCR in the fingerpad eluates—thus resulting in an underestimate of the efficacy of the hand hygiene agent. We addressed this concern in our previous study where we compared samples pre-treated with RNase H to duplicate untreated samples and found no significant difference between the results (Liu et al. 2010). However, some investigators have reported differences in the log reductions of surrogate animal caliciviruses measured by RT-PCR and by plaque assay (Belliot et al. 2008; Park et al. 2010). Therefore, reduction in measurable viral RNA should be considered a conservative measure of the efficacy of a hand hygiene product, and side-by-side comparisons with culture methods have confirmed that significant reductions in RNA titer consistently reflect significant reductions in infectivity of MNV (Park et al. 2010).

Another potential concern is that the observed virus reduction may have been overestimated due to PCR inhibitors in the test products. A side-by-side comparison of the RNA heat release method and the Qiagen RNA extraction method, which is designed to remove PCR inhibitors, showed less NoV RNA reduction in samples processed by the Qiagen method than the same samples processed by the heat release method. For the benchmark sanitizer, there was not a significant difference between the measured reductions from the two RNA extraction methods. However, for VF481, the PCR titer of NoV RNA was significantly lower in the samples tested by the heat release method and may be due in part to residual PCR inhibitors in these samples even though they were tested at a 1:100 dilution. These results suggest that the heat release results for all the test products in this study may represent the upper bound of NoV reduction by these products. Future method developments, such as using immunomagnetic separation (IMS) assay to detect RNA from virions with intact capsids and developing an internal RNA control to directly control for the presence of PCR inhibitors is under investigation.

The true measure of efficacy of a hand hygiene product is the impact on NoV outbreak control or prevention in high-risk settings where NoV outbreaks commonly occur, such as food service and food processing, healthcare (acute care hospitals and long-term elder care facilities), cruise ships and military vessels. Is the 2–4 log NoV reduction associated with VF481 sufficient to significantly reduce or prevent NoV transmission via hands? One recent report indicates that a NoV outbreak in an infirmary in Hong Kong was successfully contained by directly observed hand hygiene with WHO formulation of a handrub with 80% vol/vol ethanol (Cheng et al. 2009). Further field studies are needed with products that have demonstrated efficacy against human noroviruses.

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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  $7.3 \pm 0.20$ . 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 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  $1 \times 10^9$  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 non-medicated 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  $5.0 \times 10^8$  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  $\text{KH}_2\text{PO}_4$ , 10.1 g of  $\text{Na}_2\text{HPO}_4$ , and 1.0 g of isoocetylphenoxypolyethoxyethanol 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,  $7.1 \pm 0.2$ ) 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<sup>a</sup>

Step	Food Code-compliant procedure for hand washing products	SaniTwice <sup>b</sup> 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	

<sup>a</sup> All application procedures were initiated within 10 s of completing the 90-s drying step.

<sup>b</sup> 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.

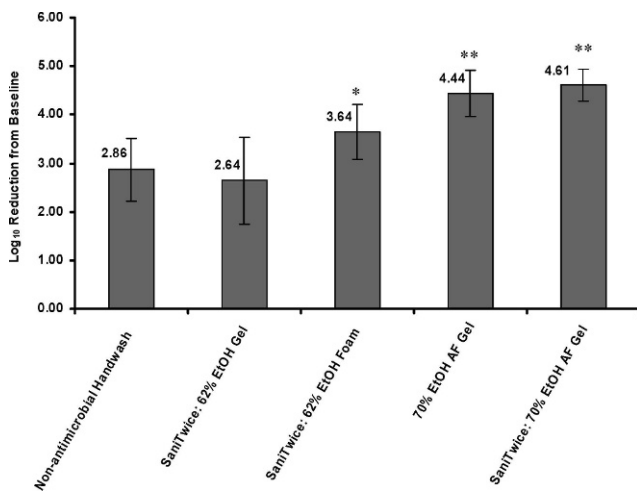


FIGURE 1. Log reduction from baseline for microbial contamination of hands moderately soiled with contaminated beef broth after application of test products. Error bars represent standard deviation. Data are from two separate studies. In study 1 ( $n = 11$ ), nonantimicrobial hand washing product and 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 70% EtOH foam.

All SaniTwice regimens were equivalent to or better than the Food Code hand washing protocol. Reductions from baseline ranged from  $2.64 \pm 0.89$  log CFU/ml for SaniTwice with the 62% EtOH gel to  $4.61 \pm 0.33$  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 ( $3.64 \pm 0.57$ -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 ( $4.44 \pm 0.47$ -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 ( $4.61 \pm 0.33$ -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  $2.69 \pm 0.32$  and  $2.65 \pm 0.33$ , 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).

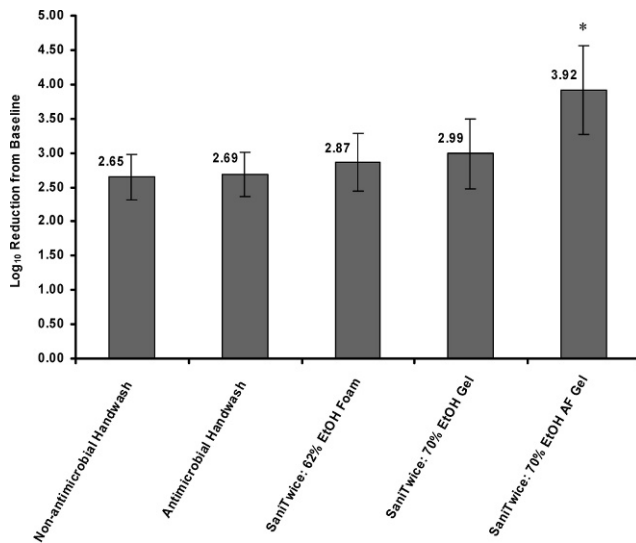


FIGURE 2. Log reduction from baseline for microbial contamination of hands heavily soiled with contaminated uncooked hamburger after application of test products and protocols. Error bars represent standard deviation. Data are from study 3 ( $n = 15$ ), in which five test configurations were evaluated. \*  $P < 0.05$  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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# Comparative Efficacy of Alcohol-based Hand Sanitizers and Antibacterial Foam Handwash against Noroviruses Using The Fingerpad Method

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## ABSTRACT

**Background:** Noroviruses are commonly associated with outbreaks of acute non-bacterial gastroenteritis in food service establishments, and hands are a principal vehicle of this transmission. Alcohol-based hand sanitizers and antibacterial foam handwashes are popular hand hygiene products, but little is known about their effectiveness against noroviruses on contaminated hands.

**Methods:** We examined the efficacy of two commercial alcohol-based hand sanitizers (one based on 62% ethyl alcohol, and one based on 70% isopropyl alcohol), a new formulation (based on 70% ethanol and a synergistic blend of polyquaternium-37 and citric acid), one commercially available antibacterial foam handwash (0.5% chloroxylenol active ingredient), and a hard water rinse control against Norovirus using the ASTM (American Society of Testing and Materials) E1838-02 standard method. Approximately  $6.3 \times 10^7$  Norwalk Virus (NV) or  $8.9 \times 10^7$  Snow Mountain Virus (SMV) particles were inoculated on each fingerpad. NV and SMV RNAs were extracted by a heat-release method and RNA titers were assayed by a one-step TaqMan real-time quantitative RT-PCR.

**Results:** The 70% ethanol-based hand sanitizer, antibacterial foam handwash and water rinse resulted in average of  $1.36 (\pm 0.49)$ ,  $1.53 (\pm 0.82)$  and  $1.40 (\pm 0.34) \log_{10}$  NV RNA reductions, respectively. All three hygiene methods provided a significant reduction of NV compared to a dried virus control ( $P < 0.001$ ), but were not significantly different from each other ( $P > 0.05$ ). The 62% ethanol-based hand sanitizer reduced the NV titers by an average of  $0.57 (\pm 0.31) \log_{10}$  and was significantly different from the control ( $P < 0.001$ ). The 70% isopropanol-based hand sanitizer reduced the NV titers by an average of  $0.00 \log_{10} (\pm 0.31)$  and was not significantly different from the control ( $P > 0.05$ ). A regimen of the antibacterial foam handwash followed by the 70% ethanol hand sanitizer produced the best reduction of NV ( $3.81 \log_{10} (\pm 0.30)$ ). The activity of all products was lower against SMV with the antibacterial foam handwash alone achieving an average  $\log_{10}$  reduction ( $0.94 \pm 0.51$ ) that was significant compared to the dried virus control ( $P < 0.001$ ).

**Significance:** These results demonstrate that handwashing with water and antibacterial foam are effective methods to remove NV from fingers. The results also show it is feasible for an alcohol-based hand sanitizer to give significant NV removal on contaminated fingers. This new synergistically formulated hand sanitizer is therefore a viable option to reduce the spread and risk of NV in food service or other settings. Because SMV is more difficult to remove than NV on human fingerpads, a regimen of handwashing followed by sanitizing may be the most appropriate hand hygiene strategy.

## INTRODUCTION

Outbreaks of human norovirus (NoV) often originate in food service establishments and the hands of food handlers are thought to be a principal vehicle for NoV transmission. Hand washing is therefore considered to be an important method to control NoV transmission. Previous studies indicated that alcohol-based hand sanitizers had a significant effect against feline calicivirus (FCV), a surrogate for human NoV on human hands (1). Recently, mouse norovirus (MNv) has been considered as a more appropriate surrogate for human NoV, but questions continue as to the relevance of these viruses because both FCV and MNv belong to different calicivirus genera than the human viruses. A previous study by our group demonstrated that hand wash with water alone or an antibacterial soap effectively reduced Norwalk virus (NV) from contaminated fingerpads but a 62% ethanol-based hand sanitizer was not effective for NV removal on human hands (2). In this study, we tested the efficacy of three marketed alcohol-based hand sanitizers (PURELL Food Code Compliant [62% Ethanol], Product 1 [60% Ethanol], and Product 2 [70% Isopropanol]), a marketed antimicrobial handwash (MICRELL Antibacterial Foam Handwash [0.5% Chloroxylenol]) and a new synergistically formulated hand sanitizer (PURELL VF447 [70% Ethanol]) foam hand wash against Norwalk virus and/or Snow Mountain Virus using a standard ASTM fingerpad method.

## METHODS

**Virus inocula:** Norwalk Virus and Snow Mountain Virus were obtained from the stool samples of two experimentally infected volunteers in our previous studies. The stool was diluted 20% in RNase free water prior to seeding on volunteers' fingerpads.

**ASTM Standard Method for Testing Handwash Agents using fingerpads:** We collected samples from volunteers following the standard methods (3) of the American Society of Testing and Materials (ASTM E 1838-02) for handwash agents using fingerpads. Figure 1 shows the sample collection procedures. The foam handwash product was exposed to virus for 15 seconds followed by a 10 second hard water rinse. All other test products were exposed to virus for 30 seconds and were not followed by a rinse.

**Hand hygiene products:** Products used in this study were PURELL Food Code Compliant Instant Hand Sanitizer, Product 1, Product 2, PURELL VF447, and MICRELL Antibacterial Foam Handwash. Table 1 shows the active ingredient and concentration of the handwash products that were tested.

**Virus concentration:** The Norwalk virus eluates were precipitated by the addition of 12% polyethylene glycol (PEG) 8000, incubated for 2 h at 4°C and centrifuged at  $12,000 \times g$  for 10 min. The supernatant was discarded and the precipitate was suspended in sterile DNase-RNase free water and stored at -80°C until real-time amplification.

**RNA extraction and real-time RT-PCR:** Norovirus RNA was extracted by a heat-released RNA extraction method (4). NV real-time RT-PCR method has been described before (2). SMV real-time RT-PCR was followed by Kagayama's method (5).

**Statistical analysis:** The viral genomic copies for each sample were  $\log_{10}$  transformed. The  $\log_{10}$  reduction for each handwash agent was calculated by subtracting the  $\log_{10}$  transformed virus for each agent from the  $\log_{10}$  transformed baseline control. We performed a paired t-test to examine the difference in  $\log_{10}$  reduction between the dry control and each individual handwash product.

Table 1. Tested Products Used in This Study

Product Name	Active Ingredient	Concentration
PURELL VF447	Ethanol	70%
PURELL Food Code Compliant	Ethanol	62%
MICRELL Antibacterial Foam Handwash	Chloroxylenol	0.5%
Product 1	Ethanol	60%
Product 2	Isopropanol	70%

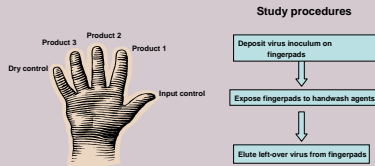


Figure 1. Diagram of American Standard Test Method for *in vivo* evaluation of the activity of handwash agents using the fingerpad method (ASTM E 1838-02).

## RESULTS

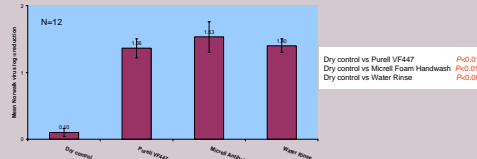


Figure 2 depicts the efficacy of PURELL VF447, MICRELL Antibacterial Foam Handwash, and a hard water rinse alone against NV compared to a dried virus control on two hands of 6 subjects. The graph illustrates the mean  $\log_{10}$  NV reduction compared to the baseline virus levels eluted from fingerpads.

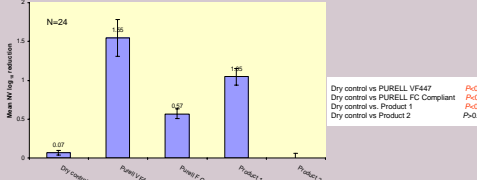


Figure 3 demonstrates the efficacy of PURELL VF447, PURELL Food Code Compliant, Product 1 and Product 2 against NV compared to a dried virus control for 24 subjects. The graph depicts the mean  $\log_{10}$  NV reduction by each product compared to the baseline virus levels.

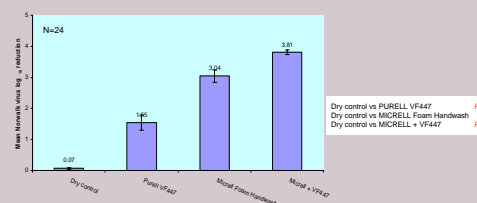


Figure 4 illustrates the efficacy of PURELL VF447, MICRELL Antibacterial Foam Handwash and a regimen of MICRELL followed by VF447 against NV for 24 subjects. The graph shows the mean  $\log_{10}$  NV reduction compared to the baseline virus levels. **Note: a "blot dry" step using a KimWipe was used after all MICRELL washes.**

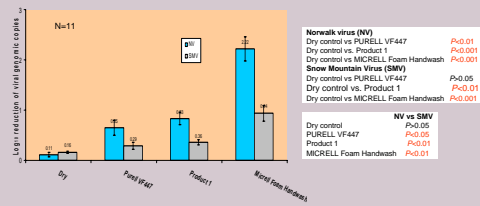


Figure 5. The mean  $\log_{10}$  NV and SMV reduction of PURELL VF447, Product 1 and MICRELL Antibacterial Foam Handwash compared to the baseline virus levels for 11 subjects using the ASTM fingerpad method.

## SUMMARY AND CONCLUSIONS

- PURELL VF447, MICRELL Antibacterial Foam Handwash and a hard water rinse were effective at reducing Norwalk Virus on human hands. MICRELL Foam was also effective for SMV removal.
- PURELL Food Code Compliant had a relatively weak activity against NV compared to PURELL VF447, MICRELL Antibacterial Foam Handwash and a hard water rinse. The effectiveness of all these products was statistically better than the dried virus control.
- Product 2 was not effective for NV removal on human hands in this study. This result is not surprising in that a previous study demonstrated isopropanol to be inferior to ethanol against calicivirus (6).
- The regimen of MICRELL Antibacterial Foam Handwash followed by PURELL VF447 was significantly better than MICRELL or PURELL VF447 foam alone for removing NV on human hands.
- Comparison of test products side-by-side against NV and SMV demonstrated that SMV is significantly harder to remove / kill than NV.
- The reduction of NoV RNA measured in this study may be due to physical removal and/or chemical inactivation. Some products appeared to give better physical removal of the stool suspension inocula as assessed by the color of the eluate. Further studies are needed to elucidate the mechanism of NoV reduction by different handwash agents.

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