P4-10

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 X 10^6 Norwalk Virus (NV) or 8.9 X 10^6 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$0.35)]. 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 4ºC and centrifuged at 12,000 × g for 10 min. The supernatant was discarded and the precipitate was suspended in sterile DNase-RNase free water and stored at -80ºC until molecular 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 Kageyama'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%

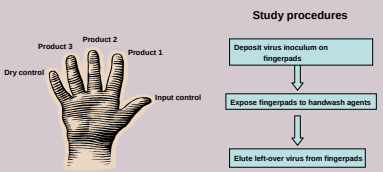

Figure 1. Diagram of American Standard Test Method for *in vivo* evaluation of the activity of handwash agents using the fingerpad method (ASTM E 1838-02).

RESULTS

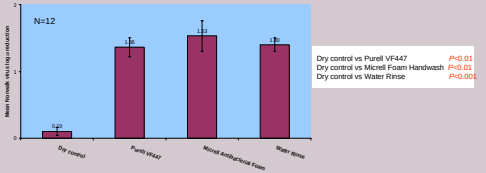

Figure 2 depicts the efficacy of PURELL VF447, MICRELL Antibacterial Foam Handwash, and a hard water rinse alone against NV compared to a dried virus control on two hands of 6 subjects. The graph illustrates the mean $\log_{10}$ NV reduction compared to the baseline virus levels eluted from fingerpads.

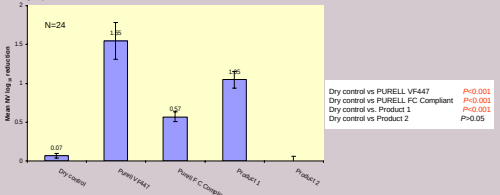

Figure 3 demonstrates the efficacy of PURELL VF447, PURELL Food Code Compliant, Product 1 and Product 2 against NV compared to a dried virus control for 24 subjects. The graph depicts the mean $\log_{10}$ NV reduction by each product compared to the baseline virus levels.

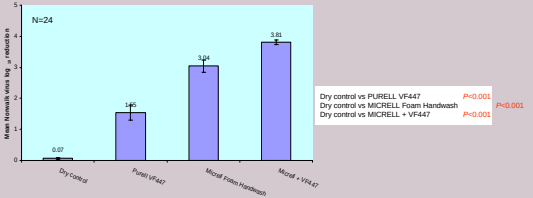

Figure 4 illustrates the efficacy of PURELL VF447, MICRELL Antibacterial Foam Handwash and a regimen of MICRELL **followed by** VF447 against NV for 24 subjects. The graph shows the mean $\log_{10}$ NV reduction compared to the baseline virus levels. Note: a "blot dry" step using a KimWipe was used after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

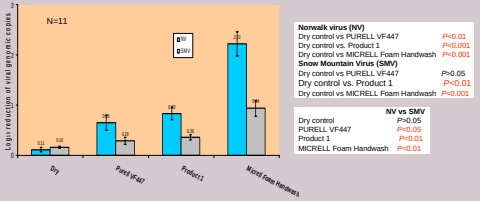

Figure 5. The mean $\log_{10}$ NV and SMV reduction of PURELL VF447, Product 1 and MICRELL Antibacterial Foam Handwash compared to the baseline virus levels for 11 subjects using the ASTM fingerpad method.

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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