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ORIGINAL ARTICLE

Efficacy of an alcohol-based surface disinfectant formulation against human norovirus

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Abstract

Aim: To evaluate the anti-noroviral efficacy of PURELL® surface sanitizer and disinfectant spray (PSS, an alcohol-based formulation) using human norovirus GII.4 Sydney [hNoV, by RT-qPCR and human intestinal enteroid (HIE) infectivity assay] and its cultivable surrogate, Tulane virus (TuV, infectivity assay), compared to sodium hypochlorite (NaOCl) solutions.

Methods and Results: PSS efficacy was evaluated in suspension and on surfaces [stainless steel (SS)] using ASTM methods. Results were expressed as $\log_{10}$ reduction (LR) of genome equivalent copy number (GEC, for hNoV, assayed by RT-qPCR) and plaque forming units (PFU, for TuV, per infectivity assay). In suspension, PSS achieved a 2.9 ± 0.04 LR hNoV GEC irrespective of contact time (30 or 60 s) and soil load (2.5% or 5%). Under all treatment conditions, infectious TuV could not be recovered following exposure to PSS, corresponding to the assay limit of detection (3.1–5.2 $\log_{10}$ PFU). Infectious hNoV could not be detected in the HIE model after exposure to PSS. On SS and 2.5% soil, PSS produced a 3.1 ± 0.1 LR hNoV GEC, comparable to 500 ppm NaOCl for 60 s. With 5.0% soil, PSS produced a 2.5 ± 0.2 LR hNoV GEC, which was similar to 1000–5000 ppm NaOCl for 60 s.

Conclusions: PSS showed high anti-hNoV efficacy by RT-qPCR and in in vitro (TuV) and ex vivo (HIE) infectivity assays and performed similar to 1000–5000 ppm NaOCl for a 60-s contact time on SS with added soil.

Significance and Impact of Study: hNoV remains a significant cause of morbidity globally, partly due to its resistance to numerous surface disinfectants. RT-qPCR results from this study indicate PSS efficacy against hNoV is comparable to NaOCl efficacy. Infectivity assays leveraging TuV and the HIE model for hNoV support and confirm loss of virus infectivity. Collectively, these results indicate the product's ability to inactivate hNoV quickly, which could be beneficial in settings having elevated risk for hNoV transmission.

KEYWORDS

food safety, food virology, human intestinal enteroids, human norovirus, surface disinfection, surface sanitizing, Tulane virus

INTRODUCTION

Human noroviruses (hNoV) are the leading cause of acute gastroenteritis worldwide, responsible for approximately 20 million infections in the United States annually, about 5 million of which are foodborne (Hall et al., 2013; Scallan et al., 2011). Contaminated environmental and food contact surfaces can serve a role in transmission (Lopman et al., 2012; (Barclay et al., 2014) and provide the justification for effective cleaning and disinfection of inanimate surfaces. The efficacy of a surface sanitizer and disinfectant relies not only on the active ingredient(s), but also on the entirety of the product formulation (Macinga et al., 2008). Additionally, product efficacy is affected by several other factors, most notably pre-cleaning of the surface, organic load or soil, type and level of contamination, concentration of the active ingredient(s) and contact time, surface characteristics, temperature and humidity (Rutala & Weber, 1997).

The US Environmental Protection Agency, which regulates antimicrobial surface products, places these products into two broad categories: sanitizers and disinfectants. Sanitizers are restricted to making efficacy claims against bacteria only, while disinfectants are able to make efficacy claims against viruses and bacteria (United States Environmental Protection Agency, 2021). It is well accepted that hNoV are resistant to many common antimicrobial surface products used at manufacturer and/or regulated concentrations and contact times (Girard et al., 2010; Manuel et al., 2017; Moorman et al., 2017; Tung et al., 2016). For example, one study investigating the efficacy of a commonly used quaternary ammonium compound (QAC)-based product found less than one $\log_{10}$ reduction in hNoV genome equivalent copy (GEC) following a 30-s exposure time in suspension (Tung et al., 2016). Similarly, another study tested the efficacy of a variety of household disinfectants against hNoV on stainless steel, including sodium hypochlorite, QAC and alcohol-based products. While the sodium hypochlorite disinfectant resulted in approximately 2 and 3.5 $\log_{10}$ reduction in hNoV GEC after 5 and 10 min contact times, respectively, no reduction was seen for the QAC and alcohol-based disinfectants (Girard et al., 2010). There is a need for surface disinfecting products exhibiting better anti-hNoV activity while being safe to use, compatible with multiple surfaces and environmentally friendly.

Historically, it has been difficult to screen surface disinfectants for anti-hNoV activity due to the absence of a cell culture infectivity model. In its place, scientists have relied on molecular amplification (RT-qPCR) for detection, or else have used cultivable surrogate viruses like feline calicivirus (FCV), murine norovirus (MNV-1), and more recently, Tulane virus (TuV). Recently, cultivation of hNoV was achieved in stem cell-derived human intestinal enteroids (HIE) (Ettayebi et al., 2016), and the suitability of this model for characterizing the inactivation of hNoV was demonstrated for ionizing irradiation and heat (Ettayebi et al., 2016); later, for chemical treatments including alcohols, chlorine (Costantini et al., 2018), hand sanitizers (Escudero-Abarca et al., 2020) and natural compounds found in green tea (Randazzo et al., 2020). However, the HIE model remains time-consuming, expensive and cannot yet be used to quantitatively evaluate disinfectant efficacy.

The purpose of this work was to evaluate the anti-noroviral efficacy of a surface disinfecting product having an alcohol-based formulation using hNoV (as evaluated by RT-qPCR) and its cultivable surrogate, TuV (as evaluated by infectivity assay). Assays were performed in suspension and on stainless steel coupons for contact times of 30 and 60 s, with and without added soil load. Similar experiments were done with hNoV exposed to sodium hypochlorite at concentrations ranging from 100 to 5000 ppm for a 60-s contact time. The anti-hNoV efficacy of the surface disinfecting product was further confirmed using the HIE infectivity model.

MATERIALS AND METHODS

Products

PURELL® Surface Sanitizer and Disinfectant Spray (PSS), an alcohol-based antimicrobial surface chemistry containing 29.4% ethanol as an active ingredient, and other inactive ingredients (i.e. water, isopropanol, lauric acid and potassium hydroxide) with a pH of 12.6–12.9, was provided by GOJO Industries, Inc. (Akron, OH) and was used as per manufacturer's instructions. Sodium hypochlorite solutions (100, 500, 750, 1000, 2000, 3000, 4000 and 5000 ppm) were prepared by diluting commercial bleach in sodium phosphate buffer, pH 7.3 (Life Technologies). Total

chlorine concentration was confirmed using a digital titrator (model 16,900, Hach Co.).

Viruses

Three deidentified human stool specimens (designated as NV14-017, NV14-103 and NV14-117) obtained from a single outbreak of the hNoV GII.4 Sydney epidemic strain (courtesy of Dr. Shermalyn Greene, North Carolina State Laboratory of Public Health, Raleigh, NC) were used as inocula in the experiments. Stool samples were suspended 20% in PBS with clarification by centrifugation (3100 *g* for 5 min at 4°C) and had initial titres of 6–7 $\log_{10}$ genome equivalent copies (GEC) per ml. Residual soil (organic) load in the working suspensions was approximately 2.5% as previously reported (Moorman et al., 2017). Because of limited amounts of each stool sample, NV14-017 and NV14-103 were used without further preparation for in vitro suspension and surface assays with PSS and sodium hypochlorite solutions, respectively. Sample NV14-117, previously confirmed as permissive in the HIE model (Escudero-Abarca et al., 2020), was used in hNoV infectivity assays. To facilitate infection and reduce cytotoxicity in the latter studies, it was necessary to partially purify this stock by serial filtration through 0.8-, 0.45- and 0.22-μm filters (Millipore Sigma) prior to treatment with PSS or 60% ethanol, as previously reported (Ettayebi et al., 2016). Tulane virus cell culture lysate prepared after three consecutive rounds of virus propagation in rhesus monkey kidney (LLC-MK2) cells (Farkas et al., 2008) was used as inoculum for in vitro suspension and surface assays (~7 $\log_{10}$ PFU/ml). All virus stocks were stored at −80°C until use.

In vitro suspension assays

Virucidal suspension assays were performed in accordance with ASTM standard E1052-11 (ASTM International, 2011a), with minor modifications to accommodate smaller inoculum volumes due to limited availability of virus suspensions. Briefly, a 25 μL volume of the 20% virus-containing faecal suspension (~4–5 $\log_{10}$ GEC/assay) or the TuV cell culture lysate (~5 $\log_{10}$ PFU/assay) and the same volume of the faecal suspension or cell culture lysate supplemented with an additional 2.5% soil (tripartite; prepared according to ASTM standard E1053-11 [ASTM International, 2011b], for an approximate total soil load of 5%) was mixed with 225 μl of PSS or appropriately diluted sodium hypochlorite (treatment) or PBS (no treatment control). Contact times were 30 and 60 s for PSS experiments, and 60 s for sodium hypochlorite studies. After the designated contact times, neutralization was accomplished by adding 20 μl of this solution to 180 μl of 10% D/E neutralization broth (Sigma-Aldrich) for hNoV-PSS experiments; M199 cell culture media (Corning Life Sciences) supplemented with 10% FBS (Life Technologies) for TuV-PSS experiments; or 5% sodium thiosulphate for hNoV-sodium hypochlorite experiments. Consistent with recommendations of ASTM E1052-11 (ASTM International, 2011a), neutralized product controls were included. Neutralized suspensions were held frozen at −80°C until assayed by a RNase-RT-qPCR or plaque assay for hNoV and TuV, respectively, as described below.

In vitro surface assays

Virucidal surface assays were done as per ASTM E1053-11 (ASTM International, 2011b), with minor modifications for inoculum volume, using stainless steel coupons. Briefly, 20 μl of 20% virus-containing faecal suspension (~4–5 $\log_{10}$ GEC/assay) or the TuV cell culture lysate (~5 $\log_{10}$ PFU/assay) and the same volume of the faecal or cell culture lysate suspension supplemented with an additional 2.5% soil (tripartite; prepared according to ASTM standard E1053-11 (ASTM International, 2011b), for an approximate total soil load of 5%) was placed onto sterile 5 × 1.5 cm stainless steel coupons (DYMO Corporation) and allowed to dry for approximately 2 h in a biosafety cabinet. A volume of 180 μl of the appropriately prepared product was placed onto inoculated strips for 30 s (PSS only) or 60 s (PSS and diluted sodium hypochlorite) contact times. PBS in place of disinfectant (no treatment controls) were included in all studies. After the designated contact times, neutralization was accomplished by transferring each strip into a 15-ml conical tube containing 1.8 ml of 10% D/E neutralizing broth for hNoV PSS experiments; M199 cell culture media supplemented with 10% FBS for TuV PSS experiments; or 5% sodium thiosulphate for sodium hypochlorite studies, followed by vortexing for 60 s to elute the virus. Consistent with recommendations of ASTM E1053-11 (ASTM International, 2011b), neutralized product controls were included. Eluates were held frozen at −80°C until assayed by a RNase-RT-qPCR or plaque assay for hNoV and TuV, respectively, as described below.

Virus quantification following in vitro suspension and surface assays

Enumeration of TuV was done by plaque assay using LLC-MK2 cells as previously described (Farkas

et al., 2008). Results were presented as $\log_{10}$ reduction (calculated as the difference between the neutralized product control and the treatment) in virus infectivity as PFU. RNase-RT-qPCR was used for quantification of hNoV GEC. Briefly, prior to RNA extraction, 200 µl of the post-neutralization samples were supplemented with 2 µl RNase One (Promega) and 22 µl reaction buffer followed by incubation at 37°C for 15 min. Samples were placed on ice for 5 min to abolish RNase activity followed by RNA extraction using the automated NucliSENS® EasyMag® system (bioMerieux) as per manufacturer's instructions. RNA was eluted in a 25 µl volume of proprietary NucliSENS® elution buffer and quantified by RT-qPCR targeting the conserved OFR1-ORF2 junction of GII hNoV (Jothikumar et al., 2005) using primers: JJV2F, COG2R, and probe RING2-TP. The *TaqMan* RT-qPCR assay was performed in 25 µl volumes (2.5 µl RNA extract) using the Invitrogen Superscript III Platinum One-Step Quantitative RT-qPCR system (Carlsbad, California) and a real-time PCR thermocycler (CFX Biorad, Hercules, CA). Reverse transcription was done at 50°C for 15 min, followed by 45 cycles of amplification at 94°C (10 s), 54°C (20 s), and 72°C (15 s). The resulting Ct values were compared to a standard curve produced by serial dilutions of previously-extracted viral RNA. Results were presented as $\log_{10}$ reduction (calculated as the difference between the neutralized product control and the treatment) in GEC.

Ex vivo suspension assays for hNoV infectivity

Virucidal suspension assays of GII.4 Sydney sample NV14-117 were done using the HIE model to evaluate the effect of PSS on the infectivity of hNoV as previously described (Escudero-Abarca et al., 2020). For these studies, a 10 µl volume of the partially purified 20% virus-containing faecal suspension was mixed with 90 µl of PSS or 60% ethanol. After a 60 s contact time, 10 µl of this solution was added to 90 µl of complete growth media with omitted growth factors (CMGF-) but supplemented with 10% fetal bovine serum for neutralization. Consistent with previous studies, a no-treatment control (untreated virus stock) and a neutralization control (virus exposed to the neutralizer) were used (Costantini et al., 2018; Escudero-Abarca et al., 2020; Randazzo et al., 2020). Prior to infection of the HIEs, neutralized samples were purified using detergent removal spin columns (Thermofisher) to reduce residual cytotoxicity, as per ASTM E1482-12 (ASTM International, 2017).

Human intestinal enteroid monolayer production and infection

Human intestinal enteroids (jejunal), provided by Dr. Mary Estes (Baylor College of Medicine, Houston, TX) were grown as multilobular three-dimensional cultures in Matrigel (Corning Life Sciences) using commercial media (Intesticult, STEMCELL, Technologies) as per manufacturer's recommendations and previously described (Escudero-Abarca et al., 2020; Ettayebi et al., 2016). The 3D cultures were dissociated into single-cell suspensions in CMGF+ media supplemented with 10 µM Y-27632 (Sigma Aldrich) and plated into 96-well plates coated with collagen IV (Corning Life Sciences) to produce undifferentiated monolayers. After 48 h, the media was replaced with differentiation medium and held (4 days) until a confluent monolayer of differentiated enteroids was obtained (Ettayebi et al., 2016).

Monolayer cultures were infected with 100 µl of (i) untreated partially purified NV 14–117 virus stock suspended in PBS (no treatment positive control); (ii) virus stock exposed to CMGF- supplemented with 10% FBS (neutralizer control); and (iii) virus stock exposed to PSS or 60% ethanol followed by neutralization (treatment). These were then incubated for 1 h to facilitate virus binding, followed by washing with CMGF- and overlay with 100 µl of differentiation media containing 500 µM sodium glycochenodeoxycholate (Sigma Aldrich). For each set of infections, duplicate plates were prepared; one plate was removed and stored at −80°C immediately post-infection [corresponding to 1 h post-infection (hpi)] and another was incubated for 72 h prior to freezing (constituting 72 hpi). Frozen plates were subjected to RNA extraction using the Directzol RNA kit (Zymo Research) according to manufacturer's instructions, with RNA elution in a 25 µl volume of DNase/RNase-free water. Viral RNA was quantified by RT-qPCR as described above. Results from the HIE infectivity assay, when virus replication occurred, were reported as $\log_{10}$ increase in hNoV GEC.

Statistical analysis

Suspension and surface assay experiments for hNoV (enumeration by RT-qPCR) and TuV (enumeration by plaque assay) were done in independent triplicates. Suspension assays followed by HIE infectivity were completed in two to three wells for three independent runs, yielding six to nine replicates. Results were expressed as mean ± standard deviation of $\log_{10}$ reduction in hNoV GEC when quantitative assays were done, and as $\log_{10}$ hNoV GEC increase after 72 hpi when the HIE model was used. Statistical

comparisons were made using ANOVA and the Tukey–Kramer test (Minitab) at a $p < 0.05$ level of significance.

RESULTS

Antiviral efficacy of PSS and diluted sodium hypochlorite in suspension

Virucidal suspension assays were performed for PSS using hNoV and TuV at contact times of 30 and 60 s; and for diluted sodium hypochlorite (100, 500, 750, 1000, 2000, 3000, 4000 and 5000 ppm) using hNoV with the single contact time of 60 s. Treatment with PSS resulted in 2.8–2.9 $\log_{10}$ reduction in hNoV GEC [assay limit of detection (LOD) of 5.1 $\log_{10}$ hNoV GEC] at both contact times and with or without additional soil, with no statistically significant impact for exposure time ($p > 0.05$, Figure 1). Regardless of contact time or soil load status, TuV could not be detected by cell culture infectivity following exposure to PSS, corresponding to a 5.2 $\log_{10}$ reduction in infectious virus, which was the assay LOD (data not shown). Comparatively, all sodium hypochlorite concentrations (100–5000 ppm) were effective against hNoV after a 60-s contact time, in the absence of excess soil, producing 4.5 $\log_{10}$ reduction in hNoV GEC, the assay LOD (Figure 1a). When additional soil load was added, sodium hypochlorite was fully effective against hNoV at concentrations ≥500 ppm ($p < 0.05$, Figure 1b).

Antiviral efficacy of PSS and diluted sodium hypochlorite on stainless steel

The same combinations of product, virus, contact time and soil load as described above were repeated in virucidal surface assays. On stainless steel without added soil, PSS produced a $\log_{10}$ reduction in hNoV GEC of 2.2 for a 30-s exposure and 3.1 for 60 s; these were statistically significantly different from one another ($p < 0.05$; Figure 2). Following exposure to PSS on stainless steel surfaces, TuV could no longer be detected by cell culture infectivity, with $\log_{10}$ reductions corresponding to the assay LOD (3.1 $\log_{10}$ with soil and 3.6 $\log_{10}$ without soil) of the assays under all conditions tested (data not

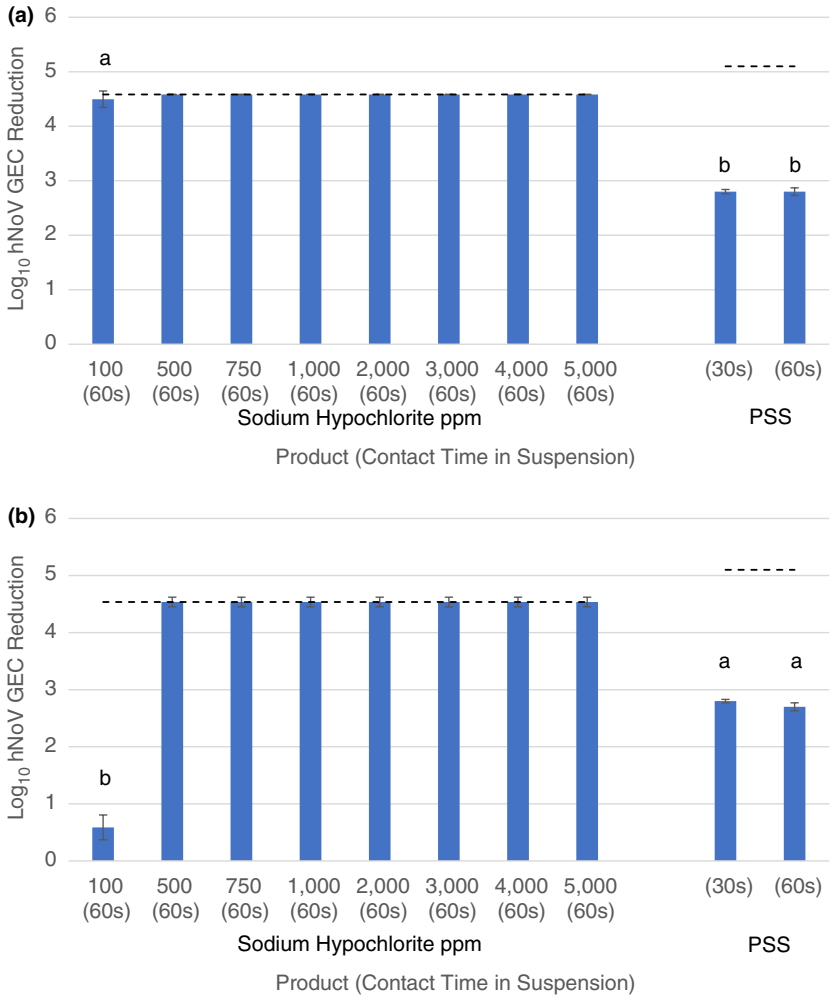

FIGURE 1 Efficacy of various sodium hypochlorite solutions (60 s contact time) and a commercially available alcohol-based surface sanitizer and disinfectant (PSS; 30 and 60 s contact times) against hNoV ($\log_{10}$ hNoV GEC reduction ± standard deviation as evaluated by RNase-RT-qPCR) in suspension (ASTM E1052-11) without additional soil added to the inoculum (Panel A; native soil load ~2.5%), and with additional soil added to the inoculum (Panel B; total soil load of ~5%). The dotted lines represent the limit of detection (LOD) of the assays (LOD 4.5 and 5.1 $\log_{10}$ hNoV GEC for sodium hypochlorite and PSS assays, respectively). Different letters indicate statistically significant differences between treatment types (treatments reaching assay LOD were not included in the statistical analysis)

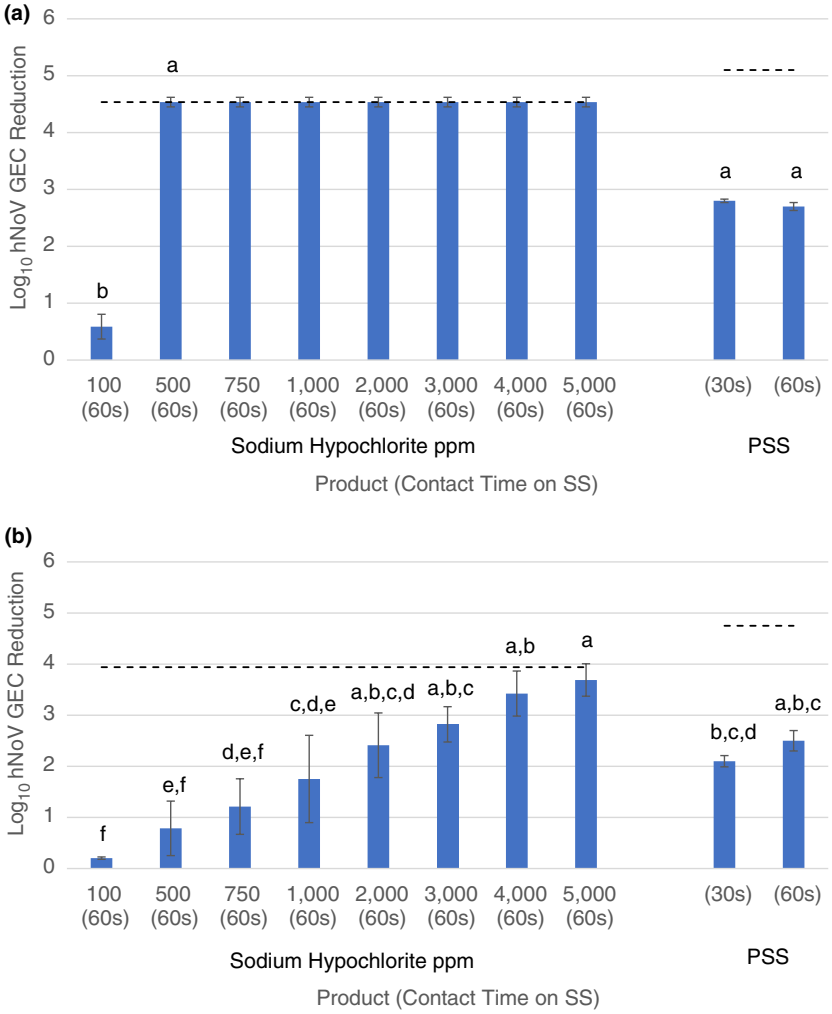

FIGURE 2 Efficacy of various sodium hypochlorite (60 s contact time) solutions and a commercially available alcohol-based surface sanitizer (PSS; 30 and 60 s contact times) against hNoV ($\log_{10}$ hNoV GEC reduction ± standard deviation as evaluated by RNase-RT-qPCR) on stainless steel (SS) surfaces (ASTM E1053-11) without additional soil added to the inoculum (Panel A; native soil load of ~2.5%) and with additional soil added to the inoculum (Panel B; total soil load of ~5%). The dotted lines represent the limit of detection (LOD) of the assays (LOD 3.9 and 4.7 $\log_{10}$ hNoV GEC for sodium hypochlorite and PSS assays, respectively). Different letters indicate statistically significant differences between treatment types (treatments reaching assay LOD were not included in the statistical analysis)

shown). In the absence of additional soil, exposure of hNoV-inoculated surfaces to sodium hypochlorite for 60 s resulted in complete loss of RT-qPCR signal at concentrations ≥750 ppm (assay LOD of 3.9 $\log_{10}$ reduction in hNoV GEC; Figure 2a). When the same set of experiments were done in the presence of added soil, $\log_{10}$ reduction ranging from 0.2–3.7 hNoV GEC were observed for sodium hypochlorite, with a steady and significant increase in efficacy as a function of concentration ($p < 0.05$), but never reaching the assay LOD of 3.9 $\log_{10}$ reduction hNoV GEC (Figure 2b).

Statistical analysis to compare surface study data for PSS directly to that for sodium hypochlorite (Figure 2) revealed no statistically significant differences ($p > 0.05$) in the performance of PSS as a function of contact time. Comparative statistical analysis between PSS and sodium hypochlorite data in surface studies showed that, under low soil load conditions (2.5%), PSS produced a $\log_{10}$ reduction in hNoV GEC that were similar to those produced by 500 ppm sodium hypochlorite for 60 s ($p < 0.05$, Figure 2a). Under high soil load (5%), PSS treatment for 30 s performed similarly to sodium hypochlorite exposure for 60 s at concentrations ranging from 750–4000 ppm, while PSS treatment for 60 s was statistically equivalent to sodium hypochlorite treatment at 1000–5000 ppm for 60 s ($p < 0.05$).

HIE infectivity assays

A 2.8 ± 0.5 $\log_{10}$ hNoV GEC increase at 72 hpi was observed for the no-treatment positive control in the HIE model, while the neutralizer control produced a 2.0 ± 0.1 $\log_{10}$ hNoV GEC increase at 72 hpi; all (6/6) replicates showed evidence of infectious virus. After exposure to PSS for 60 s, hNoV could not be detected at 1 hpi (data not shown) and no replication was observed in the HIE model 72 hpi (0/6 replicates showing evidence of infectious virus; Figure 3). For the 60% ethanol treatment, a 2.1 ± 0.2 $\log_{10}$ hNoV GEC increase at 72 hpi was observed for the neutralization control. There was evidence of bound virus at 1 hpi (data not shown) for

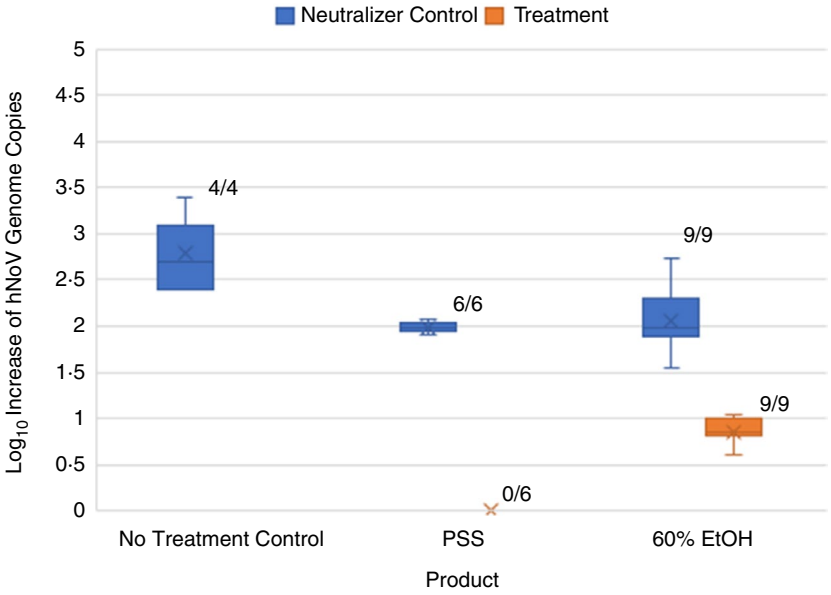

FIGURE 3 Inactivation of hNoV by PSS and 60% ethanol after a 60-s contact time in suspension assay (ASTM method E1052-11), tested in HIE model. Each product test was accompanied by a neutralizer control (NC). A no treatment control was also run independently to ensure consistent replication of the hNoV strain. X denotes the mean; the middle line of the box denotes the median; the top line of the box denotes the 75th percentile; the bottom line of the box denotes the 25th percentile; and whiskers indicate upper and lower values. Fractions listed above the box and whisker plots represent the number of replicates that showed an increase in viral RNA 72 hpi (evidence of viral replication)/total number of replicates

the ethanol treatment, and a 0.9 ± 0.2 $\log_{10}$ hNoV GEC increase 72 hpi; in both cases, 9/9 replicates showed evidence of virus replication.

DISCUSSION

The purpose of this work was to evaluate the anti-noroviral efficacy of an alcohol-based surface disinfecting product. Disinfection assays were performed in suspension and on stainless steel surfaces using hNoV as evaluated by RNase-RT-qPCR, at two different contact times (30 s and 60 s) and under two different soil loading conditions (low, 2.5%; high, 5.0%). The impact of PSS on virus infectivity was confirmed using the HIE infectivity assay and the cultivable surrogate TuV. Parallel studies were done with various dilutions of sodium hypochlorite (corresponding to 100–5000 ppm sodium hypochlorite), a relevant benchmark given US Centers for Disease Control and Prevention (CDC) guidelines recommending 1000–5000 ppm sodium hypochlorite for inactivation of hNoV (Hall et al., 2011). The highest product efficacy was observed in surface assays using an inoculum without additional soil (soil load approx. 2.5%), at a 3.1 ± 0.1 $\log_{10}$ reduction in hNoV GEC after a 60-s exposure. TuV infectivity was eliminated by any treatment with PSS, and similarly, infectious hNoV could not be detected in the HIE model. Novel features of this work include the following: (i) evaluation of a new surface sanitizer and disinfectant formulation specifically designed for use on food contact surfaces; (ii) validation of product efficacy using several different laboratory techniques; (iii) inclusion of infectivity data for hNoV and in parallel, a cultivable surrogate; and (iv) the ability to benchmark product performance to sodium hypochlorite, a widely used solution for disinfection of hNoV.

Because each assay type has advantages and limitations, the use of multiple methods to confirm anti-noroviral efficacy of sanitizers and disinfectants provides cumulative assurance that products effectively abolish virus infectivity. For instance, RT-qPCR produces quantitative results, but the relationship between amplification signals and virus infectivity is not always clear; however, use of an RNase pretreatment provides more reliable results (Escudero-Abarca et al., 2014; Escudero-Abarca et al., 2020; Manuel et al., 2015; Montazeri et al., 2017; Moorman et al., 2017). The HIE model allows for the confirmation of hNoV infectivity but not all human strains can be cultured ex vivo and it does not provide quantitative data on degree of virus inactivation (Costantini et al., 2018; Escudero-Abarca et al., 2020; Ettayebi et al., 2016). Emerging evidence suggests that TuV is superior to the other cultivable surrogates (i.e., FCV and MNV-1) in that it is less sensitive to extremes of pH and certain active ingredients, particularly alcohols (Cromeans et al., 2014). Others have also used multiple, complimentary methods to confirm the efficacy of antivirals against hNoV. For example, one study

used a combination of RNase-RT-qPCR, transmission electron microscopy (TEM), sodium dodecyl sulphate–polyacrylamide gel electrophoreses (SDS–PAGE), Western blot analysis and receptor binding assays to characterize the antiviral efficacy of copper alloys against hNoV (Manuel et al., 2015). Similarly, when investigating the efficacy of high pressure on hNoV, another study utilized a combination of TEM, SDS–PAGE, Western blot analysis and receptor binding assays (Lou et al., 2012). Based on the combined data produced in our study, it is appropriate to conclude that PSS shows promise as an effective anti-hNoV surface disinfectant.

Two commonly used disinfectant active ingredients are sodium hypochlorite and alcohol, both of which have very different modes of action and their own advantages and disadvantages. For example, sodium hypochlorite is well known for its broad antimicrobial spectrum, solubility and persistence in water, rapid bactericidal action, ease of use, low cost, colourless, non-flammable and non-staining characteristics (Rutala & Weber, 1997). Its disadvantages include the potential to irritate mucus membranes, rapid loss of efficacy in the presence of organic materials and poor compatibility with materials like stainless steel, especially at higher concentrations (Rutala & Weber, 1997). Also, at higher concentrations, sodium hypochlorite solutions can react with some chemicals, resulting in the production of toxic gases and odours, including the formation of trihalomethanes which are of public health concern. While sodium hypochlorite is often used as a routine disinfectant to inactivate hNoV (Hall et al., 2011), its efficacy decreases significantly in the presence of excess soil and organic materials (Barker et al., 2004).

Alcohol has been used as an active ingredient for many years (Boyce, 2018), and disinfectant formulations based on alcohol typically include ethyl or isopropyl alcohol solutions sometimes combined with other active ingredients such as quaternary ammonium or phenolic compounds. While alcohols usually have broad bactericidal action, they can also be virucidal, particularly for lipophilic viruses like influenza and coronaviruses, as well as hydrophilic viruses such as enterovirus and rotavirus (Kurtz et al., 1980; Rabenau et al., 2014). Although a 50% ethyl alcohol solution was shown to eliminate the cultivable hNoV surrogate, MNV-1, from stainless steel carrier discs (Magulski et al., 2009), alcohols in general have not been shown to be effective against hNoV (Costantini et al., 2018; Cromeans et al., 2014; Tung et al., 2016), or other cultivable surrogates such as FCV (Tung et al., 2016).

Recent data support that 70% ethanol or isopropanol was incapable of completely inactivating three GII.4 hNoV stains (GII.4 Den Haag, GII.4 New Orleans, and GII.4 Sydney) as evaluated using the HIE model (Costantini et al., 2018). Interestingly, the alcohol content of PSS is less than 30% ethanol, yet exposure of GII.4 Sydney to this product resulted in complete loss of infectivity in the HIE model (Figure 3). Similarly, infectious TuV could no longer be detected after similar exposures to PSS. Combined, these infectivity results suggest that disinfectant formulation in its entirety, rather than alcohol content alone, is crucial for inactivation of hNoV. This hypothesis is strongly supported by previous studies of alcohol-based hand sanitizer formulations (Edmonds et al., 2012). For example, in an in vitro study investigating the efficacy of a 70% ethanol-based hand sanitizer against a variety of non-enveloped enteric viruses, it was reported that antiviral efficacy could be improved by thoughtful formulation (Macinga et al., 2008). Future studies might attempt to delineate the contribution of various ingredients (active and inactive) to product efficacy, but such studies are difficult to perform and will not take into account synergistic or complementary effects when the individual components are combined.

The alcohol-based surface disinfectant tested in our study has an alkaline pH (12.6–12.9) and includes water, a surfactant, and a pH adjuster. It is likely that the surfactant chemical structure and elevated pH of the product plays an important role in its antiviral efficacy against hNoV, even in the presence of excess soil (5%). The isoelectric point (IP) of hNoV is between a pH of 5 and 6 (da Silva et al., 2011; Goodridge et al., 2004) which is physiologically relevant as hNoV is believed to replicate in the duodenum and the jejunum, which each have a similar pH range (Ettayebi et al., 2016). When exposing GI.1 and GII.4 hNoV VLPs to an alkaline solution with a pH of 8.0 and above, rapid loss of attachment efficiency and even viral capsid disintegration has been observed (da Silva et al., 2011). Other studies have shown that, while GII hNoV virus-like particles (VLPs) are generally more stable than GI VLPs in alkaline conditions, mild capsid disassembly, deformation and swelling can be observed when VLPs are stored in alkaline solutions at a pH of 9.0 and higher (Cuellar et al., 2010; Pogan et al., 2018). Deformation of the capsid could facilitate exposure of amino acid residues responsible for receptor binding to external stressors, including alcohols. It is possible that morphological changes to the capsid occur during exposure to surfactants at a high pH, rendering amino acid residues important for receptor binding susceptible to damage via ethanol. This is a plausible mechanism of action for the PSS disinfectant against noroviruses.

An additional strength of our work was that we were able to produce similar data for sodium hypochlorite at various concentrations and a 60-s contact time. While our comparisons involved both suspension and surface assays (Figures 1 and 2), data from surface assays with elevated soil (Figure 2b; 5% soil) are most relevant and

predictive of real-world product efficacy. Comparative analysis of surface assays in the presence of 5% soil revealed that PSS (30 s exposure) had anti-hNoV efficacy similar to that of sodium hypochlorite (60 s exposure) at concentrations ranging from 750 to 4000 ppm (Figure 2b; $p < 0.05$). When PSS exposure was extended to 60 s, this anti-hNoV efficacy resembled that associated with 1000–5000 ppm chlorine (Figure 2b; $p < 0.05$). This efficacy is unique given that PSS is formulated for use on food contact surfaces without a rinse step; any sodium hypochlorite solution above 200 ppm is not approved for use on food contact surfaces per US Food and Drug Administration regulations (Code of Federal Regulations, 2021), without a post-application rinse step with potable water.

The superior performance of PSS compared to food contact surface disinfectant levels of sodium hypochlorite (i.e. 200 ppm or less) can largely be attributed to PSS' enhanced resistance to soils relative to sodium hypochlorite. While sodium hypochlorite solutions demonstrated a $\log_{10}$ reduction in hNoV GEC near the assay LOD in suspension and on stainless steel in the presence of low soil (2.5%, Figures 1 and 2), when additional soil was added (bringing total to 5% organic load) in a surface assay the efficacy was significantly diminished (Figure 2b). This highlights the sensitivity of sodium hypochlorite to organic material, a widely known limitation of many chlorine-based chemistries. Given that sodium hypochlorite is so widely used, this reduction in efficacy in the presence of soil further demonstrates the need for the development of products that are less susceptible to environmental filth during use. PSS demonstrated relatively consistent $\log_{10}$ reductions in hNoV GEC across the suspension and stainless-steel surface assays, indicating maintenance of product efficacy in the presence of soil. This increased soil resilience is attributed to the total formulation of the product, in particular the addition of a surfactant, and the fact that alcohol by itself is relatively more resistant to soils than oxidizing agents such as chlorine (Lambert & Johnston, 2001).

To our knowledge, this research is the first to incorporate the use of TuV as a hNoV surrogate alongside the HIE model for purposes of disinfectant evaluation. The infectivity of TuV was completely lost in all experiments (data not shown; LOD of 3.1–5.2 $\log_{10}$ PFU), a finding which supports and complements the HIE infectivity data, further confirming anti-hNoV efficacy of the product. Tulane virus is a more recently discovered cultivable hNoV surrogate (Farkas et al., 2008) and is considered one of the more appropriate for purposes of approximating efficacy and performance of disinfectants against hNoV (Cromeans et al., 2014). Currently, commercially available surface disinfectants wishing to make anti-hNoV claims must demonstrate efficacy against the FCV surrogate (United States Environmental Protection Agency, 2000); this requirement has the potential to underestimate product efficacy against hNoV given FCV's sensitivity to pH extremes (Cromeans et al., 2014). Data from this study further confirm the utility of TuV as a hNoV surrogate, especially since the TuV infectivity results mirror those of the HIE model.

It is important to note that these experiments were performed using an hNoV GII.4 Sydney strain, the initial inocula sourced from two unique faecal suspensions acquired from two different individuals who were part of the same outbreak. While the 20% faecal suspension used in the RNase-RT-qPCR experiments required no further processing prior to disinfectant efficacy studies, it was necessary to serially filter the hNoV faecal inoculum used in the HIE studies to ameliorate residual cytotoxicity. This may have resulted in a decrease in the organic matter in the inoculum, as well as disaggregation of the viral particles, making them more susceptible to disinfection. Unfortunately, these sample preparation manipulations have been shown to be necessary to effectively use the HIE model for this application (Costantini et al., 2018; Ettayebi et al., 2016). Similar manipulations of the hNoV and TuV inocula could have been done for RNase-RT-qPCR and plaque assays, allowing for a more direct comparison to the HIE model. This was not done in favour of using faecally associated inoculum for those studies, which was deemed a more relevant model for real-world scenarios of surface disinfection in food service settings. However, the fact that the PSS-treated hNoV suspensions failed to bind to the enteroids upon challenge, and in the absence of evidence of virus replication 72 h later (unlike the 60% ethanol benchmark which produced both binding and replication), provides compelling evidence that this alcohol-based surface disinfectant is indeed inactivating infectious hNoV.

One limitation of this study is that its laboratory design by nature may not fully approximate product efficacy in field use. While the surface assay design with the higher soil loads (Figure 2b) is arguably the experiment with most relevance to real world settings, it cannot account for additional variables that may impact efficacy, such as surface type, temperature and humidity conditions, or drying method (e.g. use of a paper towel wipe vs. air drying). Work exploring some of these variables is in progress. An additional limitation is that this study only compares efficacy of two active ingredient types in surface disinfectants used in food settings: sodium hypochlorite and alcohol. Future testing is planned to draw product comparisons for additional active ingredients, such as quaternary ammonium compounds and acid-anionic surfactant blends.

Taken together, the data presented here provide compelling evidence supporting the efficacy of PSS against

hNoV, which is particularly important since this product is designed for use on food contact surfaces which are at-risk for contamination. The results of this study also highlight the need for thoughtful formulation of surface disinfectants, as alcohol content alone does not necessarily predict inactivation of hNoV. Organic load of the environmental surface clearly plays a significant role in the performance of surface disinfectants, and this should be carefully considered in the development of new products and chemistries. The product evaluated in this study shows promise for its anti-hNoV activity, even in the presence of organic soil. It appears to be a good candidate for all settings, especially retail and food service industries, that seek to improve the control of hNoV contamination of food contact surfaces and on high touch locations. Future studies investigating the mechanism of action of this product against hNoV are warranted.

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CONFLICT OF INTEREST

BIEA, RMG, JB, JF and LAJ declare no conflicts of interest. RAL, CSM and JWA are full-time employees of GOJO Industries as scientists.

AUTHOR CONTRIBUTION

All authors contributed to project conception and design of experiments. BIEA, JB and JF performed laboratory-based experiments and data collection. RMG, LAJ and CSM performed data analysis. BIEA prepared the first draft of the manuscript, while RMG, LAJ, RAL, CSM and JWA reviewed and revised prior to submission.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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