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Immunomagnetic separation combined with RT-qPCR for determining the efficacy of disinfectants against human noroviruses

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Summary Little is known about the effectiveness of disinfectants against human noroviruses (NoV) partially because human NoV cannot be routinely cultured in laboratory. The objective of this study was to develop a NoV monoclonal antibody-conjugated immunomagnetic separation (IMS) procedure combined with real-time reverse transcription polymerase chain reaction (RT-qPCR) assays to study the *in vitro* efficacy of disinfectants against human NoV. Monoclonal antibodies against Norwalk virus (NV, GI.1) and NoV GII.4 were produced using unique NoV capsid proteins, and the antibodies were conjugated to magnetic Dynalbeads. The immunomagnetic beads were used to simultaneously capture intact NoV in samples and effectively remove PCR inhibitors. We examined the efficacy of ethanol, sodium hypochlorite, nine commercially available disinfectants, and one prototype disinfectant using the IMS/RT-qPCR. The sensitivity of this procedure was approximately 100 virus particles for both the NV and GII.4 viruses. The average log reductions in *in vitro* activities varied between disinfectants. The prototype disinfectant produced

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an average 3.19-log reduction in NV and a 1.38-log reduction in GII.4. The prototype disinfectant is promising of inactivating NoV. This method can be used to evaluate *in vitro* activity of disinfectants against human NoV. The IMS/RT-qPCR method is promising as an effective method to remove PCR inhibitors in disinfectants and enable the evaluation of the efficacy of disinfectants.

Introduction

Noroviruses (NoV) are a leading cause of acute nonbacterial gastroenteritis outbreaks in adults [1] and the second most important viral pathogen of acute nonbacterial gastroenteritis in young children [2]. These viruses are classified into five genogroups (GI to GV), and each genogroup is further divided into multiple clusters [3]. Norwalk virus (NV) is the prototype of the GI group, and GII.4 is the predominant epidemic strain that accounted for 62% of all reported NoV outbreaks from 2001 to 2007 in the US [4]. GII.4 is also the most commonly reported genotype in pediatric NoV infections [5,6].

NoV are readily transmitted in human populations by the consumption of contaminated food, water, contact between individuals, and contaminated inanimate surfaces. Because these viruses are highly contagious among humans [7], persistent in environments [8], and resistant to disinfectants [9], prevention and control of outbreaks and sporadic diarrhea is challenging. Among the possible effective measures, the disinfection of contaminated surfaces is an effective strategy for interrupting NoV transmission [10]; however, little is known about the effectiveness of disinfectants against human NoV [11,12]. One reason for this lack of knowledge is that human NoV cannot be routinely cultured *in vitro*, which complicates the evaluation of the efficacies of disinfection strategies. As there is no *in vitro* culture system for human NoV (HuNoV), nucleic acid-based diagnostic methods have been the dominant tools. However, there are two major obstacles to the use of these nucleic acid-based detection tools in the evaluation of disinfectants: (1) the presence of PCR inhibitors in disinfectants and virus stocks that usually apply human fecal specimens, and (2) the inability to obtain intact virions. One approach to removing PCR inhibitors and allowing for the detection of intact virions is the use specific virus antibody-mediated immunomagnetic separation (IMS) assays. IMS assays can potentially effectively and simultaneously remove PCR-inhibiting substances and capture intact virions to allow for subsequent quantitative real-time RT-PCR (RT-qPCR) that only quantifies the intact virions (Fig. 1). IMS assays have been reported to successfully isolate human noroviruses from contaminated food [13], environmental water [14], and stool samples [15], but such assays have not been used to study the efficacy of disinfectants against HuNoV. The objective of this study was to develop a NoV monoclonal antibody-conjugated IMS procedure combined with NoV RT-qPCR assays to study the *in vitro* efficacy of disinfectants against GI.1 and GII.4. We examined the efficacies of ethanol, sodium hypochlorite, nine commercially available disinfectants, and one prototype disinfectant developed by Kim Laboratories Inc. (Kim Laboratories Inc, Rantoul, IL, USA).

Materials and methods

Test disinfectants and controls

A total of 10 disinfectants (Table 1), including nine commercial disinfectants and one prototype disinfectant provided by Kim Laboratories Inc., were evaluated. An experimental negative control (DNase- and RNase-free deionized water) served to represent the input virus. A product negative control (70% ethanol) with previous evidence [16] of ineffectiveness against HuNoV and a product positive control (sodium hypochlorite) that has

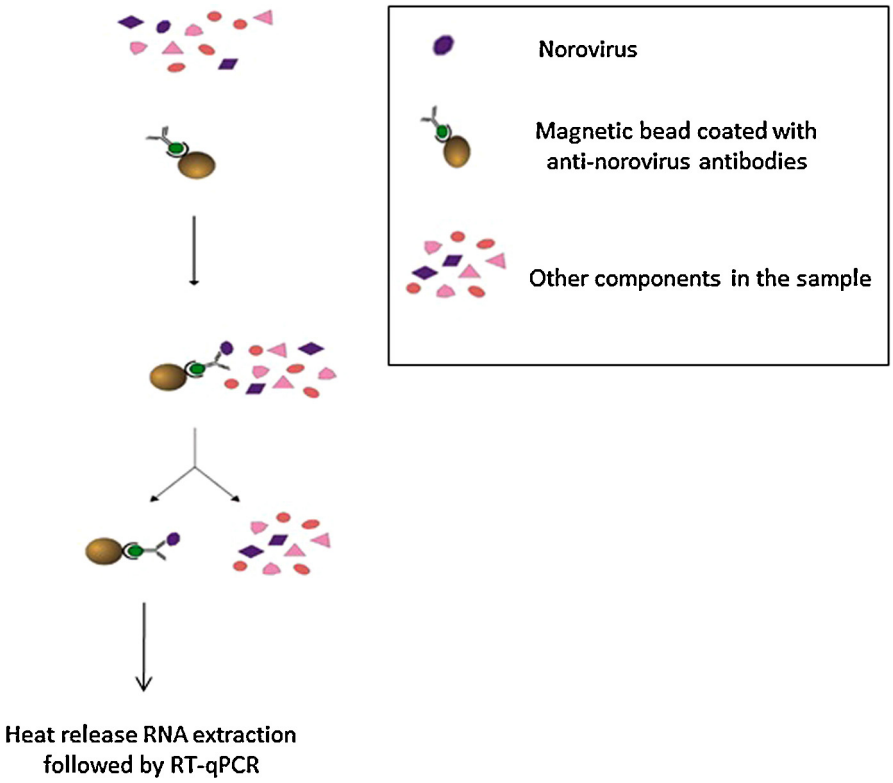

Figure 1 Diagram illustrating the procedures for the isolation of norovirus from the samples using magnetic beads coated with anti-norovirus antibodies.

shown complete inactivation of HuNoV [16] were processed under ambient temperature (70—74 °F [21—23 °C]) using the same laboratory procedures that were employed for the test products.

Human norovirus inoculum

Norwalk virus was obtained from the stool sample of an infected volunteer in a previous human challenge study [17]. GII.4 norovirus was obtained from an outbreak and was confirmed by RT-PCR and sequencing. The stool samples were diluted to 20% suspensions in RNase-free water, vortexed briefly, and centrifuged at 550 × *g* for 30 s. Aliquots of the supernatants were used as the sources of the viruses.

Monoclonal antibodies against NV and GII.4

The monoclonal antibodies (mAbs) to NV (Catalog number MABG12) and GII.4 (Catalog number MABG22) were produced by Kim Laboratories Inc. These mAbs were generated by immunizing mice using unique NoV capsid antigens identified by Kim Laboratories. According to the general guidelines of monoclonal antibody production, the antigens were conjugated to the carrier immunogen keyhole limpet hemocyanin to ensure high-yield antibody production and injected into mice (i.p.) twice over a 6-week time period. Next, the sera were collected from immunized mice and assayed for NoV antibody production against the immunized antigens using an enzyme immunoassay (EIA). The mice with positive antibody reactions were given a final boost immunization prior to fusion of the spleen cells with the Sp2/O myeloma cell line. The resultant fusion hybridomas were screened for reactivity against the antigens and NoV VLPs (Virus-like Particles) using EIA.

Conjugation of antibodies to IMS beads

Commercially available M-280 Dynalbeads (Dynal Biotech, Oslo, Norway) were used for conjugation to the NoV antibodies. Fifty milligrams of the beads were conjugated with 2 mg of the antibody according to the manufacturer's instructions. The final concentration of IMS bead was 20 mg/mL in the provided buffer.

In vitro suspension procedure

Suspension tests for the virucidal activities of the disinfectants were performed as described

Table 1 Test products and ingredients.

Test product (vendor)	Active ingredient and concentration (%)[a]	Other ingredients[a]	Affected microorganisms[a]	Application recommendation[a]
Daesung (Daesung C&C, Korea)	Hydrogen peroxide (22%), acetate (6%), dehydroacetic acid (4%)	95.26% other ingredients	Kills a wide variety of organisms including a list of respiratory and enteric bacteria and viruses	Dilute 1:500 with water for ready to use solution. 4 min exposure of the disinfectant to target pathogens
Ajax D-125 formula (Microgen Inc., NJ USA)	2.37% Alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}) dimethyl benzyl ammonium chloride; 2.37% alkyl (68% C_{12}, 32% C_{14}) dimethyl ethylbenzyl ammonium chloride	95.26% inert ingredients	Kills 141 microorganisms including bacteria, antibiotic resistant bacteria and viruses	Dilute 1:64 with water for ready to use solution. 4 min exposure of the disinfectant to target pathogens
Clorox Disinfectant Wipes (Clorox, USA)	0.145% alkyl (C_{12}—C_{16}) benzyl ammonium chloride; 0.145% alkyl (C_{12}—C_{18}) ethylbenzyl ammonium chloride	1.0—5.0% isopropanol 94.71—98.71% other ingredients	Kills 99% bacteria in 15 s; kill flu virus, HIV, MRSA/STAPH	Wipe disinfecting areas
Vital Oxide (Vital Oxide, USA)	0.2% Chlorine dioxide; 0.125% alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}) dimethyl benzyl ammonium chloride; 0.125% alkyl (68% C_{12}, 32% C_{14}) dimethyl ethylbenzyl ammonium chloride	99.55% other ingredients	Kills a wide variety of organisms including a list of respiratory and enteric bacteria and viruses and hepatitis B and C viruses.	Use as is. Allow surfaces to remain wet for 5 min for virus inactivation and 10 min for bacteria disinfection
Aseptic-HB™ (Ecolab, USA)	0.07% Alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}) dimethyl benzyl ammonium chloride; 0.07% alkyl (68% C_{12} and 32% C_{14}) dimethyl ethylbenzyl ammonium chloride	99.86% inert ingredients	Against hepatitis B virus, virucidal, bactericidal, fungicidal and HIV-1	Use as is. Use 10 min contact time for other viruses, bacteria and fungi
SaniClean Quat (Namkang, Korea)	17.5% Alkyl (C_{12}—C_{18}) dimethyl ammonium chlorides; 5% ethylenediaminetetraacetic acid	77.5% inert ingredients	Both Gram positive and negative bacteria, fungi, and viruses	Dilute 1:500 with water. Allow surface to remain wet for 5 min for disinfection
Lysol® 4 in 1 (Reckitt Benckiser, UK)	0.086% alkyl (67% C_{12}, 25% C_{14}, 7% C_{16}, 1% C_8—C_{10}—C_{18}) dimethyl benzyl ammonium chloride; 0.0216% alkyl (68% C_{12} and 32% C_{14}) dimethyl ethylbenzyl ammonium chloride 3.2% lactic acid	96.7% other ingredients including dipropylene glycol monobutyl ether, alcohols, ethoxylated, sulfonic acids	Eliminates odor-causing bacteria and kill the flu virus; kills 99.9% viruses and bacteria	Leave surfaces wet for 10 min
Oxivir-TB (JohnsonDiversey Inc, USA)	0.5% Hydrogen Peroxide	1—5% benzyl alcohol and 94.5—98.5% other ingredients	Kills a wide variety of organisms including bacteria, antibiotic resistant bacteria, viruses and fungi	Allow surface to remain wet 1 min to kill HIV-1, HBV and HCV, 5 min to kill Tb and 10 min to kill fungi
Pure Green 24 (Pure Green LLC, USA)	0.003% Silver, 4.84% citric acid	95.151% other ingredients	Kills a wide variety of organisms including bacteria, antibiotic resistant bacteria, viruses and fungi	Contact time varies by microorganisms; remain 10 min to kill norovirus
Beta formula (Kim Laboratories, Rantoul, IL)	0.07% Alkyl dimethyl benzyl ammonium chloride; 0.07% alkyl dimethyl ethylbenzyl ammonium chloride	Fortified with proprietary extracts and other surfactants, 97% inert ingredients	Kills a wide variety of organisms including bacteria, viruses and fungi	Use as is. Spray and wet surface for 1 min and wipe out remains

[a] As represented on the product labels.

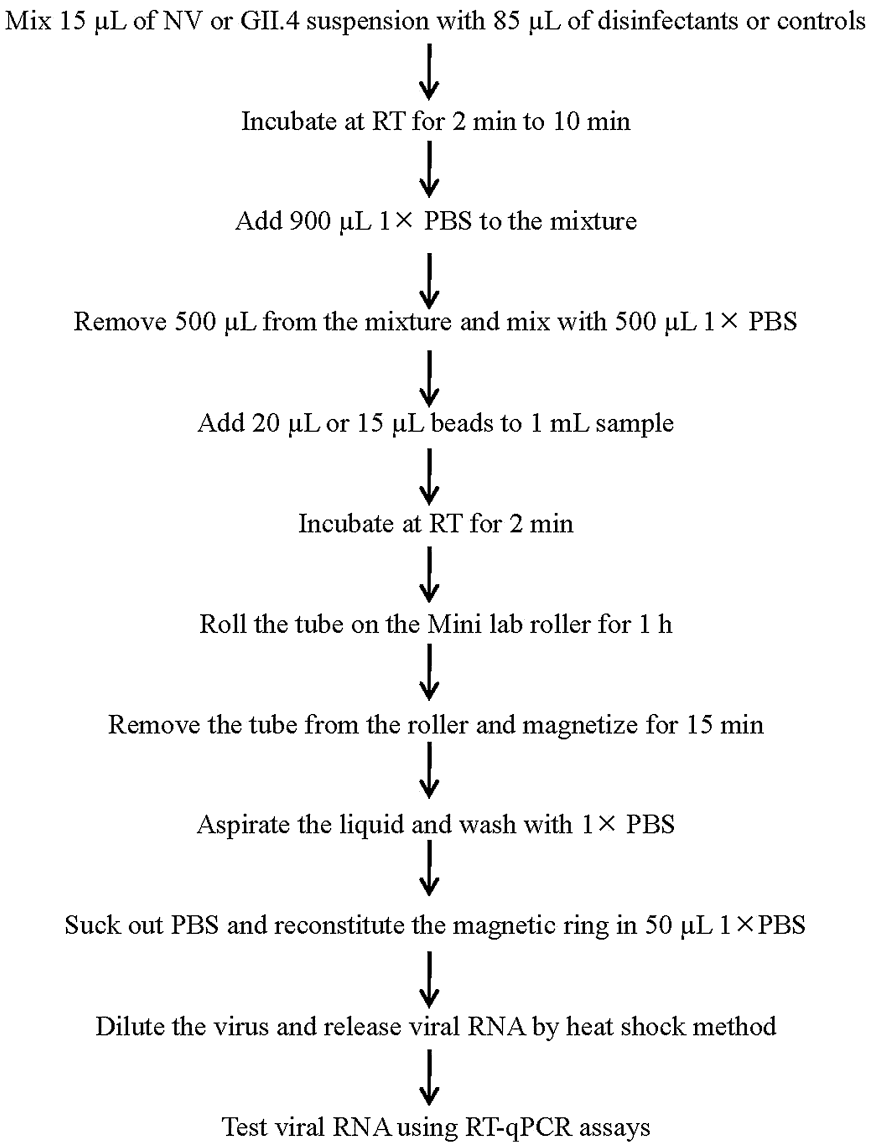

Figure 2 Flow diagram of the *in vitro* test of the disinfectants against human noroviruses using IMS followed by real-time RT-PCR.

previously [16]. Fig. 2 shows a flow diagram of the sample collection and the IMS procedure. Several concentrated test products were diluted using 1 × phosphate-buffered saline (PBS, 0.01 M, pH 7.4, Dulbecco's modification) according to the instructions of the commercial disinfectants. Briefly, 15 μL of the 20% NV stool suspension was mixed with 85 μL of each test disinfectant solution, water, 70% ethanol, or 1600 ppm sodium hypochlorite. The virus–disinfectant (or control) mixture was quickly vortexed and incubated for 2, 4, or 10 min as indicated by the instructions on the product labels. Immediately following the exposure period, 900 μL PBS was added to the mixture, and a half volume (500 μL) was removed from this tube and added to a tube containing 500 μL of PBS for further neutralization of the reaction.

IMS procedure

One milliliter of the sample collected from the aforementioned *in vitro* suspension procedure was mixed with 20 μL (approximately 3×10^7 beads) of the NV antibody-conjugated beads or 15 μL (approximately 2×10^7 beads) of the GII.4 antibody-conjugated magnetic beads. The sample–bead mixture was incubated at room temperature for 2 min, rolled on a Minilab rotator (Lab International Inc., Pittsburg, PA) at room temperature for 1 h, and removed from the roller. The

beads with captured viruses were collected with a PolyATtract® System 1000 (Promega, Madison, WI) and washed with PBS. The beads were then suspended in 50 μL of 1 × PBS and transferred to a 1.7-mL sterile tube.

Heat-release RNA extraction and RT-qPCR

Heat-release RNA extraction was used to release the RNA as described previously [18]. NV-specific RT-qPCR was performed following previously described methods [19]. GII.4 RNA was detected using a NoV GII broadly reactive RT-qPCR assay [20]. To generate a standard curve for the NV RNA quantification, a full-length NV RNA standard was transcribed *in vitro* from a NV plasmid cDNA. Similarly, a Snow Mountain virus (SMV, GII.2 cluster) RNA standard was generated from a SMV plasmid and used for the quantification of the GII.4 RNA. The log reduction associated with the exposure to each disinfectant was calculated by subtracting the log-transformed NoV titer for each product from the log-transformed baseline control. The average log reduction for each disinfectant was calculated from the replicate trials and the duplicate reactions per sample. If no viral RNA was detected in a reaction, we used half of the detection limit of the NoV RT-qPCR to calculate the log reduction.

Results

Sensitivity of the IMS/RT-qPCR

A series of tenfold dilutions of known NV titers (3.5×10^6 — 3.5 genomic copies) in 1 mL PBS were prepared and processed by IMS. Following IMS and heat release RNA extraction, RT-qPCR was used to determine the limit of detection of the NV IMS/RT-qPCR. The NV IMS/RT-qPCR exhibited an endpoint detection in the 10^{-4} dilution, which corresponded to approximately 100 of the spiked viruses. Similarly, the detection limit for GII.4 IMS/RT-qPCR was determined to be 6.9×10^2 (Table 2).

Optimal quantity of IMS beads for the concentration of NoV

To determine the optimal quantity of IMS beads for the separation of NVs, different volumes (2 μL, 5 μL, 10 μL, and 20 μL) of NV mAbs conjugated IMS beads were added to 1 mL of PBS containing approximately 10^6 NVs. The four different volumes exhibited no significant binding activity to the NVs as detected by NV-specific RT-qPCR (data not shown), but 20 μL of the IMS beads bound with the highest number of NVs, and increasing the amount of IMS beads did not increase the binding signal between the IMS beads and the NVs. Therefore, 20 μL of NV IMS beads in a 1-mL sample was used in the subsequent experiments. The optimal volume of GII.4 beads was determined using the same procedure that was used for NV, and this procedure revealed that 15 μL of the GII.4 IMS beads produced the best results.

In vitro efficacies of the disinfectants

Previous *in vitro* experiments in our laboratory have demonstrated that 70% ethanol is ineffective at reducing NV RNA and that 1600-ppm sodium hypochlorite exhibits a clear NV inactivation effect [16]. In the present study, sodium hypochlorite at a concentration of 1600 ppm produced complete inactivation of NV with an average of 4.84-log reduction and also completely inactivated GII.4 with an average 3.74-log reduction in a 2-min contact time. In contrast, 70% ethanol exhibited mean log reductions of 0.81 and 0.14 for NV and GII.4, respectively. When we examined the efficacies of nine commercial disinfectant products and one prototype disinfectant, considerable variations in the viral RNA log reductions produced by the different disinfectants against NV and GII.4 were observed. The average log reductions of the disinfectants against NV and GII.4 ranged from −0.98 to 3.19 and −0.95 to 1.38, respectively. Ammonium chloride-based disinfectants exhibited no effect against either NV or GII.4. The disinfectant Oxivir-TB (JohnsonDiversey, Sturtevant, Wisconsin) produced small log reductions of NoV; 1.11 and 0.94 log-reductions against NV and GII.4, respectively were

Table 2 Detection limits of IMS beads combined with RT-qPCR.

	Initial genomic copies	Dilution					
		−1	−2	−3	−4	−5	−6
NV	3.5×10^6	+	+	+	+	−	−
GII.4	6.9×10^6	+	+	+	+	−	−

Table 3 *In vitro* efficacy of disinfectants against Norwalk virus (GI.1) and NoV GII.4 strain.

Disinfectant name	Mean log reduction (SD[a])	
	NV	GII.4
Ethanol Control	0.81 (0.57)	0.14 (0.13)
Bleach Control	4.84 (0.03)	3.74 (0.05)
Daesung	−0.20 (0.07)	−0.04 (0.06)
Ajax	0.06 (0.21)	−0.02 (0.04)
Clorox Disinfectant Wipes	−0.01 (0.22)	−0.95 (0.10)
Vital Oxide	−0.98 (0.47)	−0.8 (0.65)
Aseptic-HB	−0.44 (0.17)	−0.87 (0.08)
Namkang	−0.13 (0.22)	−0.12 (0.17)
Lysol	2.29 (0.37)	0.21 (0.10)
Oxivir-TB	1.11 (0.08)	0.94 (0.13)
Pure Green 24	−0.67 (0.07)	−0.73 (0.13)
Beta formula	3.19 (0.67)	1.38 (0.40)

[a] Standard deviation.

observed. Lysol (Reckitt Benckiser, UK), with the active ingredient of lactic acid, produced an average log-reduction in NV of 2.29 and an average log-reduction in GII.4 of 0.21. Exposure to the prototype disinfectant developed by Kim Laboratories Inc. resulted in the greatest mean reductions of NV RNA (3.19) and GII.4 RNA (1.38). Pure Green 24 containing citric acid and silver iron was ineffective in reducing the NV and GII.4 RNA titers (Table 3).

Discussion

Inanimate surfaces such as food processing surfaces, door handles, and toilet handles are known to be important vehicles for NoV transmission [21], and the use of effective disinfectants is a critical NoV infection control strategy [10]. Despite the important role of disinfectants in the control of NoV transmission, little is known about the efficacies of disinfectants in reducing the spread of NoV partially because of the lack of routine and replicable culture-based methods and small animal models to determine intact human NoV. Given these circumstances, most studies seeking to determine the efficacy of disinfectants and antiseptics against NoV have employed surrogate caliciviruses, including feline calicivirus (FCV) [22] and murine norovirus (MNV) [23], to estimate the effectiveness of disinfectants against HuNoV. However, the results obtained using surrogate viruses might not represent actual efficacy against HuNoV strains.

Immunomagnetic separation in which monoclonal or polyclonal antibodies are conjugated to magnetic beads and used to isolate and concentrate specific virus particles for subsequent PCR amplification has been used for NoV detection in clinical and environmental samples [13—15]. IMS is able to effectively remove interfering substances from clinical or environmental samples and separate them from the specific virus particles. This process ensures that the nucleic acids detected during subsequent PCR amplifications are derived from intact virions and reduces the possibility of detecting disrupted virus particles and naked RNA. This technique has been used for several viruses [24—26] and is particularly appropriate for non-cultivable viruses such as NoV. Previous studies have successfully employed IMS and RT-PCR to detect NoV particles in fecal specimens [15], NoV-contaminated water [14], and food samples [13] and indicate that sensitivity and efficiency of this assay are more promising than those of available methods for the detection of NoV in stool samples [26]. For example, Gilpatrick et al. used an IMS/RT-PCR assay to detect Norwalk virus in stool samples from experimental human infections and compared this assay to heat release/RT-PCR and an antigen ELISA. The detection limit of the IMS/RT-PCR was 250—750 genomic equivalents/ml of 10% stool suspension, and the sensitivity of the IMS/RT-PCR was significantly better than those of the heat release/RT-PCR and the antigen ELISA [26]. In the present study, we applied IMS followed by RT-qPCR with the objective of detecting intact NoV particles from samples for testing the efficacy of disinfectants against NV and GII.4 strains. The monoclonal antibody-coated immunomagnetic beads successfully detected NV and GII.4 in stool suspension dilutions containing approximately 100 of the respective virus particles and found a sensitivity that was similar to that of the previous study [26]. Nonspecific binding by magnetic beads to heterologous viruses has been reported by other

investigators [26,27], but this was not an issue in the present study because the samples in this study contained only the specific NoV genotype. Additionally, two levels of specificity were provided: (1) the monoclonal antibodies used in this study exhibited more specific binding than the polyclonal antibodies [15], and (2) the RT-qPCR used specific primers and probes for NoV amplification.

The traditional methods that have been used to evaluate the virucidal activities of disinfectants are based on cell cultures and are considered to be the gold standard. However, culturing methods are not always available for some pathogens such as human noroviruses. In such situations, virucidal activity is normally tested using surrogate viruses that are capable of growing, but the test results from surrogates do not represent the effects of disinfectants against the non-cultivable virus. To test the effectiveness of disinfectants against non-cultivable viruses, molecular assays such as PCR are typically used; however, one major concern regarding PCR is that presence of nucleic acid does not indicate the presence of intact virions because it is possible for naked nucleic acid to be detected by PCR. Some investigators [28] have sought to address this issue by pretreating samples with RNase H under the assumption that the RNase will degrade the naked nucleic acid prior to the application of PCR. In this study, we addressed this issue in an alternative manner; we used immunomagnetic beads conjugated to NV monoclonal antibodies to isolate the virions and subsequently used real-time PCR to detect the viruses.

A previous study [16] from our group indicated that ethanol has no effect against human Norwalk virus; however, in the present study, ethanol produced a 0.81 log-reduction in Norwalk virus. The discrepancy between these two studies is the result of the different methodologies used to determine the efficacies. In the previous study, viral RNA was extracted from NV samples that were treated with ethanol; thus, the detection of viral RNA should have been indicative of the presence of intact virions, disrupted virions, and naked RNA. In contrast, the ethanol-treated NV samples in this study were processed by IMS first, and the detection of viral RNA thus indicated only the presence of intact virions; hence, the viral titers were lower in the ethanol-treated samples, and the log reduction was higher.

Several commercial disinfectants based on different concentrations of alkyl dimethyl benzyl ammonium chloride and/or alkyl dimethyl ethylbenzyl ammonium chloride failed to inactivate NV and GII.4 in the 4-min or 10-min exposure periods. These results are consistent with those of a previous study that reported the ineffectiveness of a similar disinfectant formulation against FCV, a surrogate for HuNoV [22]. However, that study reported that alkyl dimethyl ethylbenzyl ammonium chloride formulated with other additives is effective against FCV [29], which suggests that a synergistic effect might have contributed to the virucidal activity. Interestingly, nearly all of the disinfectants that use ammonium chlorite as the active ingredient in this study produced negative log reductions compared to the baseline control. This phenomenon is likely associated with two factors: (1) ammonium might have positive effects on norovirus RNA yields as reflected in a previous study in which optimal concentrations of ammonium were found to promote the RNA expression of some endometrial genes [30], and (2) ammonium likely helps the removal of PCR inhibitors from the samples [31]. Either or both of these factors could have resulted in higher viral RNA titers from the RT-qPCR detections of the treated samples and consequently led to negative log reductions compared to the baseline control.

Hydrogen peroxide has been widely used for microbial inactivation. Previous studies have reported the effectiveness of hydrogen peroxide compounds against bacteria [32], parasites [33], and viruses [34]. In the present study, Oxivir-TB with 0.5% hydrogen peroxide exhibited moderate virucidal activities against both NV and GII.4, which suggests that hydrogen peroxide has broad spectrum antimicrobial and antiviral activities.

Unsurprisingly, Pure Green 24 containing citric acid and silver iron was ineffective against NV and GII.4 because citric acid has shown mixed virucidal activities previous studies. For example, 2% citric acid was found effective in the inactivation of both foot-and-mouth disease virus and African swine fever virus dried on a wood surface for 30 min at 22 °C [35], but a suspension test indicated that a citric acid compound was ineffective against avian influenza viruses at low temperature [36], and a controlled clinical trial of hand disinfection with an antiviral hand treatment containing 2% citric acid in 62% ethanol did not significantly reduce rhinovirus (RV) infection or RV-related common cold illnesses in young adult volunteers [37]. As norovirus is one of the pathogens that is most resistant to disinfectants, it is less likely to be inactivated by disinfectants with citric acid as the active ingredient than the aforementioned viruses.

The prototype disinfectant developed by Kim Laboratories Inc., produced a 3.19 log-reduction in NV and a 1.38 log-reduction in GII.4, and these results indicate the promising activity of this disinfectant against HuNoV. Together with the results

from other effective disinfectants used in this study, it is obvious that GII.4 is more resistant to disinfectants than NV. These observation coincides with those of previous epidemiological studies in which GII.4 strains were found to have caused greater numbers of outbreaks than GI viruses worldwide since the 1990 s [38—40]. The persistence of GII.4 in the environments has been reported previously [8], and the evidence for the resistance of GII.4 to disinfectants from the present study might help to elucidate the epidemiological disparity between the GII.4 and GI viruses.

Conclusion

The IMS/RT-qPCR assay developed in this study appears to be an effective method for the removal of PCR-inhibiting substances from samples and the subsequent detection of intact virions using RT-qPCR. To our knowledge, this is the first breakthrough address the issues of PCR inhibitors while evaluating disinfectant products using molecular assays. The current prototype disinfectant developed by Kim Laboratories was the most efficacious among the tested disinfectants in the inactivation of HuNoV, and the disinfectants with active ingredients of alkyl dimethyl benzyl ammonium chloride and/or alkyl dimethyl ethylbenzyl ammonium exhibited the worst efficacies against human noroviruses.

Conflict of interest statement

Dr. Myung Kim and Dr. James Slauch are shareholders of Kim Laboratories, Inc.

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Ethical approval

Not required.

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