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Applicability of integrated cell culture reverse transcriptase quantitative PCR (ICC-RTqPCR) for the simultaneous detection of the four human enteric enterovirus species in disinfection studies

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Abstract

A newly developed integrated cell culture reverse transcriptase quantitative PCR (ICC-RTqPCR) method and its applicability in UV disinfection studies is described. This method utilizes a singular cell culture system coupled with four RTqPCR assays to detect infectious serotypes that represent the four human-related species of enterovirus (e.g., *Enterovirus A*, *Enterovirus B*, *Enterovirus C* and *Enterovirus D*). Evaluation of the RTqPCR assays was conducted with coxsackievirus A10, echovirus 30, poliovirus 1 and enterovirus 70 and resulted in 100% specificity for the tested assays. A comparison of ICC-RTqPCR between the individual enterovirus serotypes and a mixture of all four viruses resulted in an approximate 1:1 correlation, demonstrating a lack of competition during incubation in cell culture and RTqPCR. The simultaneous detection of multiple human enterovirus species within mixed cultures is relevant to many applications, including virus disinfection studies. This high-throughput, multiplex approach delivers the practical benefits of time- and cost-efficient, and consequently will help the collection of more data leading to more statistically sound data sets for direct comparison of four enterovirus inactivation rates.

Keywords

multiplex integrated cell culture quantitative PCR; enteroviruses; UV disinfection

Introduction

Enteroviruses are non-enveloped icosahedral animal viruses that contain a single-stranded, 7.5 kb RNA genome. Enteroviruses can utilize a variety of host animals including bovine, porcine, simian, and human. Enteroviruses are classified as members of the *Picornaviridae* family and the *Enterovirus* genus, which was recently reclassified into 13 species (e.g.,

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Enterovirus A-J and *Rhinovirus* A-C) (ICTV, 2017). The species that have been documented to cause human infections are *Enterovirus A*, *Enterovirus B*, *Enterovirus C*, *Enterovirus D*, *Rhinovirus A*, *Rhinovirus B* and *Rhinovirus C*. The most widely recognized enterovirus, poliovirus, is a member of *Enterovirus C*. Due to their occurrence in source water and limited information available regarding their inactivation by drinking water treatment practices, enteroviruses, which includes (sero)types of polioviruses, coxsackieviruses, echoviruses and the numbered enteroviruses (e.g., enterovirus 71), have been listed on all iterations of the U.S. Environmental Protection Agency's Contaminant Candidate Lists (CCL) 1-4 (USEPA, 2017).

The tissue culture based method has been the gold standard for the detection of infectious viruses. Specifically, the plaque assay and a cytopathic effect (CPE) coupled with most probable number (MPN) or median tissue culture infective dose (TCID₅₀) have been used for the quantification of infectious enteric viruses (Abbaszadegan et al., 2007; USEPA, 1996). However, the tissue culture assay is time-consuming, expensive, and labor intensive. As a result, water disinfection studies relying on the culture assay often calculate inactivation rate constants of target human enteric viruses with a limited number of data points. Furthermore, the CPE produced after infection from multiple types of viruses has similar visual effects and therefore cannot determine the identity of the infectious virus. These disadvantages, inherent to the method, present critical barriers in assessing the efficacy of disinfection applications against human enteric viruses. To overcome some of these hurdles, tissue/cell culture based methods have been integrated with rapid molecular-based detection methods and have been successfully applied in disinfection studies of human adenoviruses and enteroviruses (Ding et al., 2017; Gerrity et al., 2008; Ko et al., 2005; Rodriguez et al., 2013; Ryu et al., 2015). Of particular interest, Mayer et al. (2010) reported the simultaneous quantification of two serotypes of *Enterovirus B* (coxsackievirus B6 and echovirus 12) and one serotype of *Enterovirus C* (poliovirus 1) using a singular tissue culture line with three serotype specific reverse transcriptase quantitative PCR (RTqPCR) assays. Although Mayer et al. (2010) could simultaneously detect three serotypes of enteroviruses, only two of the four species relevant to human health are represented. This study builds upon previous work to develop an integrated cell culture-RTqPCR (ICC-RTqPCR) method for the simultaneous detection of a broader range of enteroviruses relevant to human health. Furthermore, sample throughput was increased by optimizing the procedures of ICC-RTqPCR with 24-well cell culture trays followed by 96-well DNA extraction format to provide more data points, and we investigated the ICC-RTqPCR applicability in disinfection studies.

Materials and methods

Target enteroviruses preparation and cell line

Serotype representatives of the four human enteric species (*Enterovirus A*, *Enterovirus B*, *Enterovirus C* and *Enterovirus D*) were selected and prepared for the present study (Table 1). The selected enteroviruses were obtained from the American Type Culture Collection (Manassas, VA) and consisted of coxsackievirus A10 (*Enterovirus A*, ATCC#VR-168), echovirus 30 (*Enterovirus B*, ATCC#VR-322), and enterovirus 70 (*Enterovirus D*,

ATCC#VR-836). In addition, poliovirus 1 was used to represent *Enterovirus C*. All enterovirus serotypes were propagated in buffalo green monkey kidney (BGMK) cells as described previously (Dahling and Wright, 1986). To obtain high titer stock, BGMK cells were infected with each virus and incubated until cytopathic effect (CPE) was observed. After three freeze-thaw cycles, cell lysate containing viruses was centrifuged at $2500 \times g$ for 30 minutes to remove cell debris. The pellet was discarded, and the supernatant containing virus was centrifuged again, at $10,000 \times g$ for 10 minutes to remove any remaining small cellular debris. The concentrations of virus stocks were titered by the plaque forming unit (PFU) cell culture method, and the stocks were stored at -80°C .

One-step reverse transcriptase quantitative PCR (RTqPCR)

For each of the four enterovirus species representatives, specific RTqPCR assays with a hydrolysis probe with minor groove binder (MGB) moiety (Kutyavin et al., 2000) were developed. Briefly, the complete genome sequences for the selected serotypes of enteroviruses were obtained from the GenBank (NR) database. Several sets of primers and probes were designed using Primer Express (Applied Biosystems, Foster City, CA, USA), followed by *in-silico* evaluation of the RTqPCR assays using the National Center for Biotechnology Information (NCBI) BLASTn (<http://www.ncbi.nlm.nih.gov/BLAST>) (Altschul et al., 1997). One assay per virus was selected (Table 1) and used for further experimental tests for assay specificity and sensitivity.

The one-step RTqPCR assay was performed in 25 μL reaction mixtures containing 1X TaqMan RT-PCR and RT enzyme mix (Applied Biosystems, Foster City, CA), 0.2 μM (final concentration) of each primer and FAM-labeled MGB probe, and 2 μL of viral RNA template. The amplification conditions involved reverse transcription at 48°C for 15 min, RT inactivation and initial denaturation at 95°C for 10 min, and then 40 cycles of 95°C for 15 s and 60°C for 1 min. The RTqPCR assays were performed using a 7900 HT Fast Real-Time Sequence Detector (Applied Biosystems). All assays were performed in triplicate in MicroAmp Optical 96-well reaction plates with MicroAmp Optical Caps (Applied Biosystems). In addition, mixed virus stocks with all four tested enteroviruses were analyzed for demonstrating the effect of RTqPCR performance by virus mixture.

PCR data were analyzed using ABI's Sequence Detector software (version 2.2.2). Two independent standard curves for each RTqPCR run were generated by plotting threshold cycle (Ct) values against RNA standards prepared using five 10-fold serial dilutions of each purified enterovirus, and the relative quantification was performed by assigning 10 viral genome copies to the lowest dilution within the range of quantification (ROQ). The percent amplification efficiencies were calculated by the instrument manufacturer's instructions (Applied Biosystems). The detection limits (DL) of RTqPCR assays in a unit of PFU/reaction were calculated by the following equation.

$$\text{DL(PFU / reaction)} = \text{SC(PFU / mL)} \times \text{LDF} \times \text{TV(mL)}$$

where SC (PFU/mL) is the virus concentration of each stock, LDF is a dilution factor from the lowest quantifiable dilution of the tested twelve 10-fold serial dilutions, TV is the template volume of RTqPCR used (mL).

Integrated cell culture RTqPCR (ICC-RTqPCR)

Cultures for ICC-qPCR were grown in 24-well cell culture trays (Thermo Fisher Scientific Inc.) in Dulbecco's Minimum Essential Medium (DMEM) (Thermo Fisher Scientific Inc., Waltham, MA) supplemented with 10% fetal calf serum (Thermo Fisher Scientific Inc.) and maintained in 5% (vol/vol) CO₂. Virus inoculation procedures for cell culture analysis were conducted as described by Mayer et al. (2010) with the exception of adding 100 µL of inoculum to each of four wells per sample. The trays were then incubated for 24 hours at 37°C in a 5% CO₂ incubator. After incubation, the cell monolayers were washed five times with 1 mL of PBS to remove extracellular viruses and then harvested by scraping after a freeze-thaw cycle. These washing steps would not completely remove all the non-infectious virus attached to cells, which could cause false positive signals. Four rinse control replicates with inactivated enteroviruses by a UV dose of 100 mJ/cm² were processed along with each set of ICC-RTqPCR standards (Mayer et al., 2010). Each mean copy number was subtracted from the respective sample. Viral RNA from cell harvest with infectious viruses was extracted and purified with the RNeasy 96 kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. RNA extracts were stored at -80°C until the RTqPCR assay was performed.

ICC-RTqPCR standard curves were constructed to quantify infectious viruses using four 10-fold serial dilutions from each virus stock (i.e., 10⁴ to 10¹ PFU for CVA10 and 10⁵ to 10² PFU for the other three viruses) as described by Ryu et al. (2015). Briefly, the data points were fitted by the following equation:

$$Y = aX + b$$

where X (genome copy/rxn) is the quantity resulting from the ICC-RTqPCR assay, Y (PFU/rxn) is the original number of infectious viruses spiked into each dilution, a and b are unknown parameters in the standard curve. The concentrations of infectious viruses in pre-inoculation samples (i.e., the dependent variable, Y=the original virus concentration prior to replication) were estimated using the regression equation with ICC-RTqPCR quantities (i.e., the independent variable, X). The estimated Y values were used to calculate the log inactivation of each enterovirus. Besides the effect of RTqPCR performance by virus mixture, ICC-RTqPCR performance was evaluated by parallel comparison of individual and mixed virus stocks.

UV-exposure experimental operation

UV-exposure experiments were performed as previously described (Gerrity et al., 2008; Ryu et al., 2015). Briefly, a bench-scale collimated beam apparatus containing two 15-watt, low-pressure, mercury arc bulbs which emitted monochromatic, germicidal light at a peak wavelength of 254 nm was used. The water samples were prepared by adding four enteroviruses to 200 mL of Butterfield's buffer (i.e., to a final concentration of 10⁵ PFU/

mL). Separate samples (i.e., 10 mL in 60×15 mm Petri dishes) were used for each exposure time to prevent fluctuations in intensity due to varying water depths. UV disinfection experiments with three different exposure times ranged from 10 to 30 mJ/cm² were performed. The samples were continuously mixed using a low-speed stir plate and 8×1.5-mm Teflon-coated stir bars to minimize surface disruptions and to prevent vortexing while providing a homogenous distribution of viruses.

Statistical analysis

Log inactivation of enteroviruses (I) is defined by the following equation, values for inactivation kinetics were calculated using multiple UV doses, and the data points were fitted by regression analysis:

$$I = -\log_{10}\left(\frac{N_d}{N_0}\right) = k(U_d)$$

where N_0 and N_d (PFU/mL) are the initial concentration and the concentration of infectious enterovirus after a specified UV dose, k (cm²/mJ) is the inactivation rate constant, U_d (mJ/cm²) is the calculated UV dose. The intercept of the first-order kinetics was set to 0 at UV dose = 0 mJ/cm².

As described above, the effect of virus mixture for RTqPCR and ICC-RTqPCR assays was examined. The Pearson correlation coefficient (Pearson's r) was calculated for the parallel comparison of individual and mixed virus stocks. SPSS version 12.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. P values of <0.05 were considered statistically significant. Also, two quantities from individual and mixed virus stocks were fitted by the following regression equation:

$$\text{Log}_{10}(\text{mixed virus stock}) = a \times \text{Log}_{10}(\text{individual virus stock}) + b$$

where a and b are unknown parameters in linear regression.

Results

Validation of serotype-specific one-step RTqPCR assays

Twelve 10-fold serial dilutions of each target virus stock and six concentrations of three non-target viruses (i.e., a total of 18 controls per virus) were analyzed for assay sensitivity (i.e., detection limit) and assay specificity (i.e., cross-amplification rate), respectively, for each of the four RTqPCR. In addition, six no-template controls were included for each RTqPCR run which none of the controls showed a positive result, indicating the absence of contamination in the RTqPCR experiments. Each RTqPCR assay was tested for specificity using six 10-fold dilutions of the three non-target viruses, ranging from 10³ to 10⁸ viral genome copies in each reaction (GC/rxn). All reactions were negative and resulted in 100% specificity for the four serotype-specific assays. The ROQ and detection limit were determined using serial dilutions of the target virus stocks. The optimum ROQ for all the RTqPCR assays were between 10¹ and 10⁵ (GC/rxn), while the unit of the detection limit was converted to PFU

per reaction as described in Table 2. The detection limits of all the tested RTqPCR assays were 2-3 orders of magnitudes lower than the theoretical detection limit of a cell culture method, which is 1 PFU. In addition to evaluating the RTqPCR assays with individual virus stock, mixed virus stocks were analyzed. RTqPCR quantities for each virus alone and in a mixture showed a high cross-correlation (Pearson's r of 0.9218), and, as shown in Figure 1, the slope of the regression equation was near one ($a=0.9673$, $b=0.1226$, $R^2=0.9503$). Altogether, this result suggested further development of one cell culture system coupled with multiple RTqPCR assays which are required for identifying and differentiating the four enteroviruses present in a same cell culture mixture.

Validation of simultaneous detection of infectious enteroviruses from BGMK cell line

The optimum spiking levels of each enterovirus serotype into the same cell culture well was determined with different concentrations ranging between 10^1 and 10^5 PFU/mL and 24-hour incubation, as described by Mayer et al. (2010). A spiking concentration of 10^5 PFU/mL for all the tested viruses except for CVA10 was optimal for the simultaneous quantification of individual viruses. CVA10 quantities leveling off at 10^5 PFU/mL indicated lack of cell capacity of each well in a 24-well tray for virus infection and replication. Spiking concentrations of 10^4 PFU/mL for CVA10 and 10^5 PFU/mL for the other viruses were identified as optimal for the simultaneous quantification of mixed viruses (Fig. 2). Using ICC-RTqPCR standard curves, ICC-RTqPCR quantity was converted to initial virus concentrations for the quantification of infectious viruses prior to their replication in host cell culture. The converted infectious virus quantities were used to calculate log inactivation of viruses in disinfection studies. While the slopes of the regression equations for PV1 was near one ($a=1.02$), those for the other viruses ranged from 0.83 to 0.90 (Fig. 3). ICC-RTqPCR performance was also evaluated with a mixed virus stock and compared to the individual results. There was an approximate 1:1 correlation between ICC-RTqPCR quantities for each virus alone and in a mixture. The linear regression equations for four enteroviruses are as follows:

$$\text{CVA10: Log (mixed virus stock)} = 0.965 \times \text{Log (individual virus stock)} - 0.13 \quad R^2 = 0.994$$

$$\text{Echo30: Log (mixed virus stock)} = 1.020 \times \text{Log (individual virus stock)} - 0.11 \quad R^2 = 0.999$$

$$\text{PV1: Log (mixed virus stock)} = 0.961 \times \text{Log (individual virus stock)} + 0.05 \quad R^2 = 0.998$$

$$\text{EV70: Log (mixed virus stock)} = 0.950 \times \text{Log (individual virus stock)} - 0.20 \quad R^2 = 0.999$$

Disinfection application

Bench scale low-pressure UV collimated beam tests were performed using a mixed stock of the four viruses for constructing UV dose-response curves with a UV dose range of 10-30

mJ/cm². The extent of log inactivation of the enteroviruses for the triplicate UV experiments is illustrated in Figure 4. The inactivation rate constants, k , from linear regression curves ranged from 0.1727 cm²/mJ to 0.09 cm²/mJ (R^2 ranging from 0.88 to 0.99) and were used to estimate the UV dose requirements for virus inactivation (Table 3). For a 2-log inactivation credit, UV doses averaged approximately 12, 22, 14, and 19 mJ/cm² for CVA10, Echo30, PV1, and EV70, respectively.

Discussion

The newly developed one-step RTqPCR assays targeting four representatives of each human species of enteroviruses showed relatively high sensitivity to the targeted enterovirus and no cross-contamination with the other non-targeted enteroviruses examined (i.e., 100% assay specificity). In addition, an approximate 1:1 relation of RTqPCR quantities with individual and mixed virus stocks and their high cross-correlation (i.e., Pearson's r of 0.9218) suggested negligible RTqPCR inhibition or cross-amplification from the other non-target viruses. In terms of assay sensitivity, all the RT-qPCR assays detected 10 genome copies which correspond to several orders of magnitudes lower than 1 PFU. Indeed, the RTqPCR is able to detect the entire population of targeted viruses including non-infectious or infectious but not culturable (IBNC) ones. One possible explanation is that non-infectious and IBNC virions exist in the virus stocks used. It should be noted that the ratio of infectious virus to physical particles can vary by orders of magnitude among different batches of preparations due to uncertainties of virus propagation procedures (e.g., cell type, cell passage number and incubation periods) and stock storage conditions (e.g., freezing/thawing cycles).

BGMK cell line is commonly used for the detection of infectious enteroviruses (Dahling and Wright, 1984; USEPA, 1996; Fout et al., 2010). However, a conventional tissue culture method, including BGMK cells, cannot differentiate infection from a mixed virus suspension. The integration of a cell culture method capable of detecting infectious enteroviruses and RTqPCR that can differentiate individual viruses in a mixed culture can be used in a variety of applications, including virus disinfection studies. This is a significant development, which allows simultaneous detection of infectious enteroviruses from four different species in a single disinfection experiment. This multiplex approach would provide not only practical benefits (e.g., time- and cost-saving) but facilitate the collection of more data, leading to more statistically sound data sets for direct comparison of inactivation rates.

Similar replication rates of spiking viruses were considered to select an optimum spiking level of mixed viruses. However, the addition of four different enteroviruses onto the same cell culture monolayer could have resulted in a virus serotype being more efficient in infection and replication than other serotypes. The effect of ICC-RTqPCR performance by virus mixture was demonstrated by parallel comparison of quantification with individual and mixed virus stocks. The regression slopes of ICC-RTqPCR for all the viruses were close to one, suggesting a negligible viral replication from competition. In order to avoid limiting cell host capacity in disinfection experiments, it was suggested that CVA10 stock be diluted to a spiking concentration of 10⁴ PFU/mL. Our results for optimum virus spiking levels are comparable to the previous research. Mayer et al. (2010) reported an optimum spiking

concentration of 10^4 TCID₅₀/mL for three tested enteroviruses (coxsackievirus B6, echovirus 12 and poliovirus 1) in 24-well tray format and 24-hour post-incubation period.

Successful optimization of ICC-RTqPCR for simultaneous quantification of four enteroviruses can enable its application in disinfection studies. However, with slopes of ICC-RTqPCR standard curves not equal to one (Fig. 3), significant discrepancies between inactivations calculated using PFU and ICC-RTqPCR quantities may exist. In this study, the slopes of all the tested viruses except for PV1 were below one, resulting in overestimation of each inactivation calculated using ICC-RTqPCR quantities directly rather than PFU conversion by a standard curve. Mayer et al. (2010) reported that regression slopes of TCID₅₀ and ICC-RTqPCR quantities for coxsackievirus B6, echovirus 12, and PV1 were 0.97, 0.72 and 1.27, respectively. These results illustrated the need to construct a ICC-RTqPCR curve for quantifying inactivation in disinfection studies.

The UV inactivation of enteroviruses exhibited linear inactivation kinetics (Fig. 4). CVA10 has the greatest inactivation rate constant (k), followed by PV1, EV70, and Echo30. The range of k values are comparable to other studies using conventional tissue culture methods (Ma et al., 1994; Meng and Gerba, 1996; Gerba et al., 2002) and ICC-RTqPCR (Mayer et al., 2010). Four-log₁₀ inactivation was achieved at a UV dose of 44 mJ/cm² for Echo30, whereas UV doses for the other enteroviruses were below 40 mJ/cm². Overall, UV dose requirements of all four enteroviruses determined in this study were much lower than a LP monochromatic 254 nm UV dose of 186 mJ/cm² for achieving 4-log₁₀ inactivation of enteric viruses suggested by USEPA (2006) for the treatment of drinking water supplied from surface water sources. The UV doses for the enteroviruses yielded much greater inactivation rate constants than MS2 coliphage and the most UV resistant adenoviruses (Beck et al., 2017). Mayer et al. (2010) reported that ICC-RTqPCR showed relatively low estimates of log₁₀ inactivation (i.e., higher UV dose requirements) compared to the conventional tissue culture assays, suggesting that an ICC-RTqPCR approach could be used as a conservative estimate of UV disinfection efficacy.

In conclusion, an ICC-RTqPCR assay for the simultaneous detection of four human-relevant species of infectious enteroviruses was developed and successfully applied for use in disinfection studies. These optimization procedures mainly focused on a high-throughput format employing a 24-well cell culture tray and 96-well DNA extraction kit. Furthermore, the multiplexed, high-throughput strategy allows collection of more data, which can provide more statistically sound data sets for the direct comparison of inactivation rates. The ICC-RTqPCR assay can facilitate the construction of a database of UV inactivation efficacy for enteroviruses in water, which can be used by officials for potential regulatory determinations of these USEPA CCL microorganisms.

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Fig. 1.
Comparison of one-step RTqPCR for individual and mixed virus stocks.

Fig. 2. Optimization of spiking levels of each virus in the cell culture well. Each column represents the mean value of four replicate samples of the indicated virus PFUs inoculated. The error bars represent ± 1 standard deviation.

Fig. 3. ICC-RTqPCR standard curves relating ICC-RTqPCR quantities to PFU (i.e., initial infectious virus concentrations) for quantifying inactivation.

Fig. 4. Log₁₀ inactivation of infectious enteroviruses after exposure to low-pressure UV at 254 nm. The error bars represent ± 1 standard deviation from triplicate samples per UV dose.

Table 1

Summary of oligonucleotide primers and probes for TaqMan RTqPCR

Species	Serotype (Abbreviation, Strain)	Primer or probe sequence (5' to 3') ^a	T _a (°C) ^b	Size (bp) ^c
<i>Enterovirus A</i> (EV-A)	Coxsackievirus A10 (CVA10, Kowalik)	Forward: CCCTTCCCGGTTGAACAT Reverse: GCCGCGCTGTACTG Probe: 6FAM-AGAAATAACGCACCTCTGA-MGBNFQ	60	55
<i>Enterovirus B</i> (EV-B)	Echovirus 30 (Echo 30, Bastianini)	Fwd: GGAGCGCAAGTGTCAACACA Rev: TTCCCGCTAGCACTTAAACCA Probe: 6FAM-AAAACTGGAGCACATGAGA-MGBNFQ	58	62
<i>Enterovirus C</i> (EV-C)	Poliovirus 1 (PV1, Mahoney)	Fwd: AACGCGGCTTCGAAACAG Rev: TTGATGGGCTCGGTGAACCT Probe: 6FAM-ACTTCTCTCAAGACCC-MGBNFQ	59	59
<i>Enterovirus D</i> (EV-D)	Enterovirus 70 (EV70, J670/71)	Fwd: CCCCGGTGAAGTTGCATAGA Rev: CTAAAGCGGTAAACGGATTTCATC Probe: 6FAM-TGTACCCACGGTTGAAA-MGBNFQ	60	62

^a A 5' reporter dye of 6-Carboxyfluorescein (6-FAM) and a 3' nonfluorescent quencher (NFQ) with minor groove binder (MGB) moiety

^b Optimum annealing temperature

^c Approximate product size determined from *in silico* analysis

Table 2

One-step RTqPCR performance

Assays	ROQ ^a (genome copy/reaction)	DL ^b (PFU/reaction)	Amplification Efficiency (%)	R ²
CVA10 (EV-A)	10 – 10 ⁵	1.4×10 ⁻³	100	0.998
Echo 30 (EV-B)	10 – 10 ⁵	5.0×10 ⁻³	101	0.999
PV1 (EV-C)	10 – 10 ⁵	5.0×10 ⁻²	101	0.999
EV70 (EV-D)	10 – 10 ⁵	5.6×10 ⁻³	101	0.999

^aRange of quantification^bDetection limit of RTqPCR assays in a converted unit

Table 3

Calculated UV dose requirements for inactivation of human enteroviruses

Virus	UV dose (mJ/cm ²) for specified level of inactivation			
	1-log ₁₀ (90.0%)	2-log ₁₀ (99.0%)	3-log ₁₀ (99.9%)	4-log ₁₀ (99.99%)
CA10 (EV-A)	6	12	17	23
Echo 30 (EV-B)	11	22	33	44
PV1 (EV-C)	7	14	21	27
EV70 (EV-D)	9	19	28	37