

## Evaluation of electropositive filtration for recovering norovirus in water

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### ABSTRACT

The virus adsorption-elution (VIRADEL) technique has been widely used in the recovery of various enteric viruses in water, and an electropositive filter such as 1 MDS has been commonly applied. However, effective methods of monitoring waterborne norovirus (NoV) have not yet been well characterized and optimized. Hence, in this study, the VIRADEL technique was evaluated and optimized for effectively detecting NoV in water by two commonly used electropositive filters (1MDS and NanoCeram filter). Various elution and concentration methods were evaluated by using both murine norovirus (MNV) and human NoV. Among the tested elution buffers, the most effective was 1.5% beef extract plus 0.01% Tween 80 for both 1MDS (67.5%) and NanoCeram (85.7%) microfilters. The recovery rate of GII-4 human NoV was higher by organic flocculation (86.6%) than by polyethylene glycol (PEG) precipitations (11.6–73.6%). When both 1MDS and NanoCeram filters were tested to detect NoV in surface and groundwater, the sensitivity of NoV recovered by these filters appeared to depend on the types and conditions of environmental water. The results of this study will help to set a standard of detection method for NoV in water.

**Key words** | electropositive microfilter, norovirus (NoV), virus adsorption-elution (VIRADEL) technique, water

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### INTRODUCTION

Norovirus (NoV) is one of the most important waterborne pathogens and is listed on the United States Environmental Protection Agency Contaminant Candidate List (CCL) (US EPA 2008a). It has been identified as the most frequent aetiological viral agent of acute gastroenteritis worldwide (Mead *et al.* 1999; Kim *et al.* 2005; Godoy *et al.* 2006). Based on the nucleic acid sequences of the capsid gene, NoV can be classified into five distinct genogroups (GI–GV). Among them, GI and GII are commonly identified in humans and can be further subdivided into at least 31 different genotypes (Green

*et al.* 2000; Fankhauser *et al.* 2002). Currently, GII-4 is the most prevalent genotype throughout the world and is responsible for 14–61% of all NoV-associated outbreaks (Fankhauser *et al.* 2002; Blanton *et al.* 2006). Recently, NoV incidence has increased markedly in many countries (Kroneman *et al.* 2006; Sakon *et al.* 2007). In South Korea, various genotypes of NoV causing waterborne outbreaks were identified in tap and surface water (KCDC 2007; Lee & Kim 2008).

Because it is impossible to cultivate human NoV using conventional methods, RT-PCR assays have been widely used

to detect NoV in clinical and environmental samples (Ando *et al.* 1995; Doultree *et al.* 1999; Atmar & Estes 2001; Dreier *et al.* 2006). However, molecular methods such as RT-PCR cannot differentiate infectious viruses from non-infectious ones. Recently, murine norovirus (MNV) was found to be culturable by conventional cultivation methods, and was identified as the most suitable surrogate for human NoV (Doultree *et al.* 1999; Duizer *et al.* 2004). However, the suitability of MNV as a surrogate organism for human NoV has not been specifically studied with regard to viral detection in water. Thus, it is necessary to evaluate whether MNV can be used as a surrogate for testing human NoV in water.

The US EPA has established the virus adsorption-elution (VIRADEL) technique as the standard method for recovering enteric human viruses in water (US EPA 2001). In this method, the viruses present in water are first adsorbed to the surface of a filter while water passes through the filter during sampling. Then, the adsorbed virus is eluted from the filter and is further concentrated by either polyethylene glycol (PEG) or organic flocculation. The electropositive 1MDS filter has been widely used for virus detection in water (Sobsey & Glass 1980; Sobsey *et al.* 2004; Wang *et al.* 2005; Hsu *et al.* 2007; Locas *et al.* 2007, 2008). Owing to the predominantly negative charge present on the surface of viruses, use of electropositive filters (e.g. 1MDS) does not require the pre-conditioning of sample waters to adjust parameters including ionic strength and pH (Gerba 1984). Previous studies reported that electronegative filters such as cellulose and nitrocellulose filters are more efficient than electropositive filters in recovering viruses in acidic water (Lukasik *et al.* 2000; Katayama *et al.* 2002; Hsu *et al.* 2007; Victoria *et al.* 2009). Thus, additional steps, such as the addition of an acidifying agent (e.g.  $\text{MgCl}_2$ ) should be taken prior to using electronegative filters.

However, electropositive filters do not require an acidifying process, which gives them an advantage over electronegative filters. These methods have been well described for use with many bacterial and viral agents (Cashdollar & Dahling 2006; Polaczyk *et al.* 2007). Recently, the electropositive NanoCeram filter (Argonide, Sanford, FL) was applied in the sampling of NoV in water (Karim *et al.* 2009). In comparison with the 1MDS filter, which is made of a cellulose medium, the NanoCeram filter is composed of alumina fibres (Karim *et al.* 2009). Regardless of the applied

filter material, the elution conditions (e.g. pH, ionic strength, concentration of detergent) are critical for detecting waterborne viruses using VIRADEL techniques. The different surface characteristics of the 1MDS and NanoCeram filters may require different elution optimization procedures.

The US EPA recommends 1.5% beef extract with 0.05 M glycine (pH = 9.5) as the elution buffer for the VIRADEL method (US EPA 2001). A few studies have reported that the addition of dispersant or surfactant would improve the recovery of microorganisms by reducing the electrostatic attraction between viruses and the membrane (Mendez *et al.* 2004; Hill *et al.* 2005; Polaczyk *et al.* 2007). For the concentration of eluants, both acid and PEG precipitation methods are typically applied. The types of elution buffer and the molecular weight of PEG are important factors in determining the virus recovery efficiency (Lewis & Metcalf 1988; Polaczyk *et al.* 2007). Despite the importance of NoV to public health, VIRADEL methods have not been evaluated and optimized for NoV. The objectives of this study were: (1) to optimize the elution buffer for detecting NoV using the 1MDS and NanoCeram electropositive filters; (2) to compare acid and PEG precipitation in the recovery of NoV; and (3) to evaluate electropositive filters in the detection of NoV in surface and groundwater.

## MATERIALS AND METHODS

### Preparation of murine and human norovirus

Murine norovirus 1 (MNV1) was provided by Dr Herbert W. Virgin IV of the Washington University School of Medicine (St Louis, MO), and was cultured in RAW 264.7 cells cultured in Dulbecco's modified Eagle's medium (DMEM) for three to four days (Wobus *et al.* 2004). The RAW 264.7 cell line (KCLB No. 40071) was provided by the Korea Cell Line Bank (KCLB). The medium for culture was based on DMEM (11965, Gibco, USA), to which 10% fetal bovine serum (16000, Gibco, USA), 10 mM HEPES, 10 mM sodium bicarbonate, gentamycin ( $50 \mu\text{g} \mu\text{l}^{-1}$ ) and 10 mM non-essential amino acid were added. A cell line cultured within a six to eight passage number was used. Infected cells were harvested by centrifuging at  $2,000 \times g$  at  $4^\circ\text{C}$  for 10 min. Further concentration of MNV1 was

obtained by ultrafiltration using the Amicon® Ultra-15 (Millipore, USA) at  $5,000 \times g$  at  $4^\circ\text{C}$  for 10 min. After ultrafiltration, the supernatant was recovered and stored at  $-80^\circ\text{C}$  until recovery testing. The titre of MNV was estimated at  $10^9$  PFU  $\text{ml}^{-1}$ . The Korea Centers for Disease Control and Prevention (KCDC) provided a NoV-positive (GII-4 genotype) stool sample. The titre of NoV was measured using RT-PCR and was estimated to be  $10^7$  RT-PCR units  $\text{ml}^{-1}$ . Human NoV samples also were aliquoted and stored at  $-80^\circ\text{C}$  until further analysis.

### Adsorption and elution

Figure 1 shows a schematic flow of the evaluated elution and concentration procedures used in this study. Either a 142-mm flat 1MDS filter (3 M Cuno, USA) or a 142-mm flat NanoCeram (Argonide, USA) DISC microfilter was fixed onto the filter holder (KS-142-ST, Advantec, Japan). A 1-ml sample of virus (MNV1:  $10^7$  PFU  $\text{ml}^{-1}$ ; Human NoV:  $10^5$  RT-PCR units  $\text{ml}^{-1}$ ) was spiked into 1 l of distilled water; 150  $\mu\text{l}$  of the sample was stored in order to determine the percentage recovery as 100% after RNA extraction process.

The virus sample was passed through a DISC filter using a silicon tube and peristaltic pump (Masterflex L/S, Cole-Parmer Instrument Co., USA) at a flow rate of  $2 \text{ l min}^{-1}$ . After removing the residual water, the elution buffer was poured into the inlet of the filter holder. The elution buffer

was composed of 1.5% beef extract (211520, BD, USA) and 0.05 M glycine (Yakuri Pure Chemicals Co., Japan), and was adjusted to pH 9.5 with 1 M NaOH. Four kinds of elution buffer of different compositions were used for elution, and each included either 0.01% Tween 80 surfactant (63161, Riedel de Haën, Italy), 0.1% sodium polyphosphate dispersant (305553, Aldrich, USA), or both. In addition, a 0.001% antifoaming agent (Antifoam Y-30 emulsion: A5758, Sigma, USA) was added to each of the elution buffers. The elution buffer in the filter holder was allowed to have contact with the microfilter for 30 min at room temperature. The stainless pressure vessel (XX6700P10, Millipore, USA) was filled with elution buffer and was connected to a pressurized nitrogen gas source by a silicon rubber tube. The pressured nitrogen gas was applied to push the elution buffer through the filter for elution. The average volume of elution buffer for elution was 500 ml. All equipment and instrumentation was autoclaved prior to the experiment.

### Secondary concentration

#### Organic flocculation

A 1-ml sample of virus (MNV1:  $10^6$  PFU  $\text{ml}^{-1}$ , human NoV:  $10^5$  RT-PCR units  $\text{ml}^{-1}$ ) was spiked into 500 ml of elution buffer (1.5% beef extract with 0.05 M glycine, pH 9.5). After stirring with a magnetic bar, the pH of the buffer was adjusted to pH  $3.5 (\pm 0.01)$  with 1 M HCl. This state was maintained for 30 min at room temperature. The precipitant was collected with centrifugation at  $2,500 \times g$  at  $4^\circ\text{C}$  for 15–30 min. After carefully disposing of the supernatant, the precipitant was completely dissolved in 10 ml of 0.15 M sodium phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , pH 9.0–9.5). The final concentrated sample volume (FCSV) was measured and adjusted to estimate the recovery rate.

#### PEG precipitation

The PEG re-concentration method for viruses has been previously described (Lewis & Metcalf 1988; Schwab *et al.* 1995, 1996). Here, both PEG 6000 (81253, Fluka, Germany) and PEG 8000 (V3011, Promega, USA) were applied for evaluating the concentration of NoV. A 1-ml sample of virus (MNV1;  $10^7$  RT-PCR units  $\text{ml}^{-1}$ ; human NoV;  $10^5$

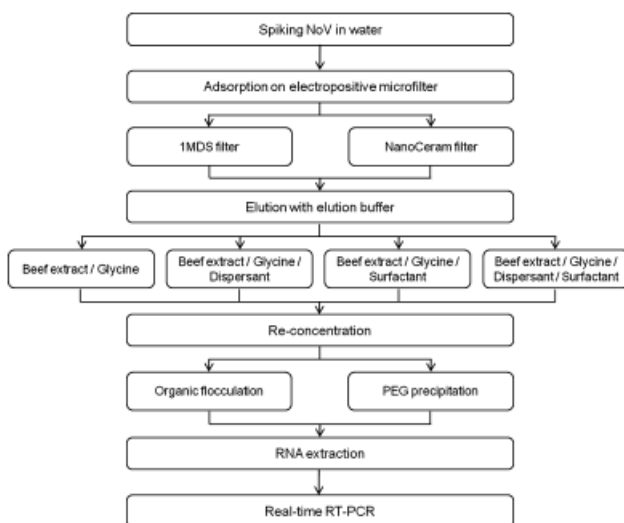


Figure 1 | Schematic flow diagram based on the US EPA virus adsorption-elution (VIRADEL) technique.

RT-PCR units  $\text{ml}^{-1}$ ) was spiked into 500 ml of elution buffer (1.5% beef extract with 0.05 M glycine, pH 9.5). Either PEG 6000 or PEG 8000 was added for a final concentration of 5% and 10% (wt/vol) in buffer with 0.2 M NaCl. This suspension was incubated at 4°C for 2 h. The precipitant was then collected by centrifuging at  $7,000 \times g$  at 4°C for 30 min. After centrifugation, the precipitant was dissolved in about 20 ml of 0.15 M sodium phosphate, and the FCSV was measured.

### Nucleic acid extraction and real-time RT-PCR

Viral RNA of NoV was extracted from 150  $\mu\text{l}$  of each concentrated sample using a QIAmp<sup>®</sup> Viral RNA Mini Kit (52906, Qiagen, USA) according to the manufacturer's instructions. The volume of final purified RNA was 50  $\mu\text{l}$ . Table 1 describes the oligonucleotide primers and probes for MNV and human NoV used in this study. The reaction mixture (25  $\mu\text{l}$ ) for real-time RT-PCR comprised an AgPath-ID<sup>™</sup> One-Step RT-PCR Kit (AM1005, Ambion, USA), which was composed of  $2 \times$  RT-PCR buffer (12.5  $\mu\text{l}$ ), primer (MNV1: 50 pmol 0.2  $\mu\text{l}$ , GII-4 NoV: 10 pmol 0.5  $\mu\text{l}$ ), 100 pmol of probe (MNV1: 0.05  $\mu\text{l}$ , GII-4 NoV: 0.25  $\mu\text{l}$ ),  $25 \times$  RT-PCR enzyme mix (1  $\mu\text{l}$ ), template of viral RNA (2.5  $\mu\text{l}$ ), and nuclease-free water (MNV1: 8.55  $\mu\text{l}$ , GII-4 NoV: 7.8  $\mu\text{l}$ ). Real-time RT-PCR was performed using a 7300 Real-Time PCR System (Applied Biosystems, USA) under the following conditions: MNV1: reverse transcription at 42°C for 10 min, initial denaturation at 95°C for 10 min, and 50 amplification cycles of 15 s at 95°C and 45 s at 65°C; GII-4, NoV: reverse transcription at 42°C for 30 min, initial denaturation at 95°C for 10 min, and 50 amplification cycles of 15 s at 95°C, 30 s at 50°C and 30 s at 72°C.

### Field samples of surface water and groundwater

A total of 10 sampling sites (five surface water and five groundwater sites) were chosen to apply the VIRADEL technique using two different electropositive filters (1MDS and NanoCeram filters). Surface water samples were taken at five different sites on the Han River in metropolitan Seoul, Korea. Groundwater samples were collected in areas of metropolitan Seoul that were suspected to have faecal contamination. The environmental parameters for

the field samples are discussed further below. All samples were collected during November 2008. Both 1MDS and NanoCeram cartridge filters were used to process water sample volumes that measured approximately 200 l and 500 l for surface water and groundwater, respectively. Collected filters were immediately stored at 4°C, and the analysis was performed within 24 h. The elution and re-concentration procedures were performed as previously described. To elute NoV from the filter, 1 litre of elution buffer (1.5% beef extract with 0.05 M glycine, pH 9.5) was used. Initially, the filter housing containing cartridge filter was filled with elution buffer and allowed to have contact with the filter for 30 min at room temperature. And then, the elution buffer was pushed with the remaining secondary elution buffer in a vessel pump using nitrogen gas. Eluate was re-concentrated using the organic flocculation method, and the concentrates were analysed by real-time RT-PCR as described in Table 1 (Kageyama *et al.* 2003; Jothikumar *et al.* 2005).

### Statistical analysis

The efficiency of the concentration methods and elution buffers to recover NoV (MNV1 and GII-4 human NoV) using electropositive disk microfilters (1MDS and NanoCeram) was expressed as a quantitative percentage (%). The recovery of NoV was measured by quantitative RT-PCR, and the percentage was calculated by copy number using the initial spiking NoV as 100%. Every experiment was replicated three times, and the mean and standard deviation are reported. The percentage of recovery from each method was evaluated by applying the Kruskal-Wallis test, and the difference of efficiencies between the two methods was estimated using the Mann-Whitney U-test. All statistical analyses were performed using SPSS software 12.0. *P*-values  $\leq 0.05$  were deemed statistically significant.

## RESULTS AND DISCUSSION

### Evaluation of various elution buffers for 1MDS and NanoCeram filters

The recovery efficiencies of the electropositive filters using various elution buffers are summarized in Table 2.

**Table 1** | Primers and probes for RT-PCR of MNV and human NoV

Genogroup	Oligonucleotide	Orientation	Sequence (5' → 3')	Location*	Reference
MNV1	MNV1 F	+	ACG CCA CTC CGC ACA AA	5614–5630	<i>Lee et al. (2008)</i>
	MNV1 R	–	GCG GCC AGA GAC CAC AAA	5650–5667	
	MNV1 P	+	VIC-AGC CCG GGT GAT GAG-MGB	5632–5646	
GI NoV	COG1F	+	CGY TGG ATG CGN TTY CAT GA	5291–5310	<i>Kageyama et al. (2003)</i>
	COG1R	–	CTT AGA CGC CAT CAT CAT TYA C	5375–5396	
	RING(a)-TP	–	FAM-AGA TYG CGA TCY CCT GTC CA-TAMRA	5340–5359	
	RING(b)-TP	–	FAM-AGA TCG CGG TCT CCT GTC CA-TAMRA	5340–5359	
GII NoV	JJV2F	+	CAA GAG TCA ATG TTT AGG TGG ATG AG	5003–5028	<i>Jothikumar et al. (2005)</i>
	COG2R	–	TCC ACG CCA TCT TCA TTC ACA	5100–5080	
	RING2-TP	+	FAM-TGG GAG GGC GAT CGC AAT CT-TAMRA	5048–5067	

Note : MNV1 = murine norovirus genogroup 1

\*Nucleotide positions based on MNV (accession no. DQ285629), GI NoV (accession no. M87661) and GII NoV (accession no. X86557)

The recovery efficiency of MNV using the 1MDS filter (21.7–54.4%) was higher than that for the NanoCeram filters (3.1–23.4%). The recovery efficiencies of MNV using 1.5% beef extract with 0.05 M glycine were 38.9% and 18.3% from the 1MDS and NanoCeram filters, respectively. The addition of surfactant (0.01% Tween 80) to the elution buffers increased the recovery efficiencies from the 1MDS filter (54.4%) and the NanoCeram filter (23.4%), but the difference was not statistically significant (Kruskal-Wallis test:  $P$ -value > 0.050). In contrast, the addition of dispersant to the elution buffer decreased the viral recovery for both the 1MDS (21.7%) and the NanoCeram (3.1%) filters.

This study indicated that the addition of 0.01% Tween 80 increases the elution efficiencies for NoV. Surfactants have typically been added to eluting solutions to minimize viral adhesion to filter surfaces (*Mendez et al. 2004*). The addition of 0.01% Tween 80 increased the elution efficiencies for NoV in the present study. Another previous study reported that the addition of NaPP dispersant increased the recovery rate of coliphages from the 1MDS filter to 89% (*Polaczyk et al. 2007*). However, the addition of NaPP did not increase the virus recovery efficiencies from the 1MDS and NanoCeram filters employed in the current study. Further, virus recovery from ultrafilters was reported to

have higher recovery with dispersants and surfactants (*Hill et al. 2005*).

Although both 1MDS and NanoCeram filters are electropositive, as mentioned above, the surface characteristics of these two filters are quite different. Unlike 1MDS filter, the NanoCeram filter is made up of nano alumina. That means the NanoCeram filter can be operated in alkaline water without neutralization (*US EPA 2008b*). Zeta potential on the surface of filters is closely associated with higher efficiency of virus capture. The various factors including pH affect the zeta potential on the surface of the filter. In a recovery test with distilled water (pH = 6.1–6.2), different surface characteristics resulted in different viral recovery efficiencies when either a surfactant or a dispersant was present in the elution buffer (Mann-Whitney U-test:  $P$ -value = 0.050; *Table 2*). Surfactant and dispersant in the elution buffer basically minimize the free surface energy, therefore they are more effective for elution (*Polaczyk et al. 2007*). But, the ability of surfactant and dispersant for elution was different between NanoCeram and 1MDS filter. As indicated in this study, the recovery efficiency greatly depends on the conditions of the elution and precipitation. These conditions should be optimized prior to field application.

**Table 2** | Influence of various elution buffers on NoV recovery using electropositive filters

Elution buffer	Electropositive microfilter		P-value**
	1MDS	NanoCeram	
<b>MNV1</b>			
1.5% beef extract + 0.05 M glycine	38.9 (± 17.6)*	18.3 (± 3.7)	0.275
1.5% beef extract + 0.05 M glycine + 0.01% Tween 80	54.4 (± 8.9)	23.4 (± 3.8)	0.050
1.5% beef extract + 0.05 M glycine + 0.1% NaPP	21.7 (± 8.9)	3.1 (± 1.5)	0.050
1.5% beef extract + 0.05 M glycine + 0.01% Tween 80 + 0.1% NaPP	23.8 (± 15.2)	13.5 (± 15.0)	0.275
<b>GII-4 NoV</b>			
1.5% beef extract + 0.05 M glycine	61.1 (± 11.1)	26.5 (± 13.3)	0.275
1.5% beef extract + 0.05 M glycine + 0.01% Tween 80	67.5 (± 40.3)	85.7 (± 25.8)	0.050

NaPP: sodium polyphosphate

\*Recovery efficiency (%) (± standard deviation): The percentage of recovered NoV was calculated using the initial spiking of NoV as 100%. Tests were conducted in triplicate

\*\*P-value was estimated by a Mann-Whitney U-test between 1MDS and NanoCeram filters

### Recovery efficiencies of human NoV

Several elution buffers (1.5% beef extract + 0.05 M glycine, both with and without 0.01% Tween 80) were evaluated to determine the recovery efficiencies of human NoV using the GII.4 strain, which is the most commonly identified NoV strain. The recovery efficiencies of human NoV (26.5–85.7%) were generally higher than those of MNV (3.1–54.4%). Without 0.01% Tween 80, the recovery efficiencies of human NoV using 1.5% beef extract plus 0.05 M glycine were higher from 1MDS than from NanoCeram. On the other hand, when 0.01% Tween 80 was added to the elution buffer, the recovery efficiencies from the NanoCeram filter were significantly higher than those from the 1MDS filter (Mann-Whitney U-test:  $P$ -value = 0.050; Table 2). Based on these results, the best elution buffer for NoV was determined to be 1.5% beef extract + 0.05 M glycine with 0.01% Tween 80.

MNV should be a suitable surrogate for evaluating human NoV in water. Traditionally, feline calicivirus (FCV) was used as a surrogate for NoV (Kadoi & Kadoi 2001). A recent study indicated that MNV would be the most appropriate surrogate because it has similar biological characteristics to human NoV (Karst et al. 2003). Since MNV is typically transmitted by a faecal-oral route, it is more resistant to acid than FCV (Cannon et al. 2006). This resistance could make MNV more attractive for use as a surrogate for human NoV, because concentration methods such as organic flocculation could be applicable for both molecular and infectivity assays. This

study demonstrated that the recovery efficiencies of MNV are not significantly different from those of human NoV under most conditions. However, when NanoCeram filters were used and surfactants were present in the buffer, there was a significant difference between the recovery rates of human NoV and MNV (Table 2).

### Comparison between PEG and organic flocculation for re-concentrating NoV eluates

Table 3 summarizes the recovery efficiency of elution buffers by either acid or PEG precipitation. The average recovery efficiencies by organic flocculation were 42.0% and 86.6% for MNV and GII-4 NoV, respectively. The recovery efficiencies by PEG precipitation were 9.6–18.6% for MNV and 11.1–73.6% for GII-4 NoV. Overall, the recovery efficiencies of organic flocculation were higher than those of PEG precipitation. However, this difference was not statistically significant (Kruskal-Wallis test:  $P$ -value > 0.050). Overall, PEG 8000 had a higher recovery efficiency than PEG 6000. The recovery efficiencies of human NoV were significantly higher than those of MNV when the virus was concentrated by PEG 8000 (Mann-Whitney U-test:  $P$ -value = 0.025). Based on these results, organic flocculation was the better procedure for re-concentrating NoV in the elution buffer.

Organic flocculation is another commonly applied method for re-concentrating viruses in water. If the tested virus was sensitive to acid and subject to cultivation, PEG

precipitation would be a more appropriate concentration method. NoV is resistant to low pH (Koopmans & Duizer 2004; Cannon *et al.* 2006), and organic flocculation resulted in a higher recovery rate, as indicated in Table 3.

### Detection of human NoVs in environmental water samples using the optimized procedure

Table 4 shows the results of the monitoring of NoV in both surface water and groundwater. Human NoV was detected in four out of five surface water samples. Among the four positive samples of surface water, two samples were positive using both the 1MDS and NanoCeram filters. The other two samples of surface water were only positive with the 1MDS filter. For groundwater, two and three out of five samples were positive using the 1MDS and NanoCeram filter, respectively. The results using either the 1MDS or NanoCeram filters were similar, in part. Sample site five was positive with both the 1MDS and NanoCeram filters. In this sample, both GI and GII NoV were positive. The other samples had different results depending on the types of filter used. Both GI and GII NoVs were detected in the groundwater.

A high concentration ( $10^5$  RT-PCR units  $\text{ml}^{-1}$ ) of NoV was spiked in water to compare the recovery efficiencies of NoV in this study. Since the viral concentrations in environmental samples are typically low, a large volume of water should be concentrated into a small volume of elution buffer. However, the initial concentration of the virus in water could be a factor in determining the recovery efficiency. In a

previous filter test with MS2 coliphage (Polaczyk *et al.* 2007), water that was spiked with a low concentration of MS2 resulted in a less efficient recovery compared with experiments using high concentrations. The effect of inoculated viral concentration on the recovery should be further investigated.

Another recent study suggested that the NanoCeram filter could be a useful alternative to the 1MDS filter for viral monitoring of tap water and river water (Karim *et al.* 2009). This study indicated that the sensitivity of filtration depends on the condition and type of environmental samples. The electrostatic interaction between the virus and the membrane surface of the filter depends on various factors, including viral isoelectric points (pHis), water pH and salt concentration (Farrah 1982; Hsu *et al.* 2007; Polaczyk *et al.* 2007; Victoria *et al.* 2009). The isoelectric pH of NoVs ranges from 5.9 to 6.9 (Goodridge *et al.* 2004). Karim *et al.* (2009) reported that the NanoCeram filter efficiently recovered NoV within a pH range of 6.0 to 9.5. The NanoCeram filter is capable of adsorbing virus in wide range of turbidity and salinity conditions as well as pH (Tepper & Kaledin 2007). But, the results of the NanoCeram and 1MDS filter were different in 5 out of 10 samples. Unlike the spiked experiment in the laboratory, various chemicals such as heavy metals and humic acids may be present in environmental field samples. Therefore, we can consider that these chemicals may affect not only the PCR amplification but also the electrostatic interaction. The samples assayed in this study were obtained from different water sources with various environmental

**Table 3** | Efficiency of NoV concentrations from various precipitation methods

Precipitation method		Recovery efficiency (%)* ( $\pm$ standard deviation)		P-value**
		MNV1	GII-4 NoV	
Organic flocculation		42.0 ( $\pm$ 30.5)	86.6 ( $\pm$ 20.7)	0.077
PEG precipitation	PEG concentration			
	PEG 6000			
	5%	12.5 ( $\pm$ 0.4)	11.1 ( $\pm$ 5.3)	0.881
	8%			
	10%	9.6 ( $\pm$ 1.3)		
PEG 8000	5%	18.6 ( $\pm$ 1.3)	73.6 ( $\pm$ 37.7)	0.025
	8%			
	10%	11.8 ( $\pm$ 5.2)		

\*Recovery efficiency (%) ( $\pm$  standard deviation): The percentage of recovered NoV was calculated using the initial spiking of NoV at 100%. Tests were conducted in triplicate.

\*\*P-value was estimated by a Mann-Whitney U-test comparing MNV1 and GII-4 NoV

**Table 4** | Experimental results of NoV in 10 environmental field samples

Source	No.***	Volume (litre)	Turbidity (NTU)	Temp. (°C)	pH	Nitrate nitrogen (NO <sub>3</sub> -N, ppm)	1MDS filter	NanoCeram filter
Surface water	S-1	200	4.98	14.4	7.33	3.57	–	–
	S-2	200	4.03	16.2	8.56	1.95	+* (GI)	–**
	S-3	200	2.36	12.9	7.49	1.66	+ (GI)	–
	S-4	150	1.60	15.3	8.61	5.34	+ (GI)	+ (GI)
	S-5	180	1.37	19.2	7.30	7.38	+ (GI)	+ (GI)
Groundwater	G-1	504	0.78	15.0	6.75	2.37	–	–
	G-2	500	0.51	16.1	5.80	4.55	–	+ (GII)
	G-3	500	0.66	17.6	7.11	13.62	+ (GI)	–
	G-4	500	0.16	20.2	7.01	10.89	–	+ (GI)
	G-5	314	0.37	15.4	6.38	4.25	+ (GI/GII)	+ (GI/GII)

NTU: nephelometric turbidity units

\*+: Positive by RT-PCR with primers and probes described in Table 1

\*\*–: Negative by RT-PCR with primers and probes described in Table 1

\*\*\*Every environmental water sample was taken in the metropolitan Seoul area from different water sources

conditions, and these factors could lead to different experimental results.

## CONCLUSIONS

This study determined the best elution and concentration methods for waterborne NoV. NoV was most effectively recovered by the VIRADEL technique with elution buffer (1.5% beef extract plus 0.01% Tween 80) using both NanoCeram and 1MDS filters and organic flocculation. The sensitivities of two electropositive filters to the recovery of NoV in water varied in different types of water. In conclusion, both the 1MDS and NanoCeram filters could be applied to detect NoV in environmental field samples. Each filter appeared to be more appropriate under specific environmental conditions. Further study to investigate the effects of various environmental conditions on the recovery of NoV should be carried out.

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