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Short communication

Concentration of enteric viruses from tap water using an anion exchange resin-based method

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A B S T R A C T

Detecting low concentrations of enteric viruses in water is needed for public health-related monitoring and control purposes. Thus, there is a need for sensitive, rapid and cost effective enteric viral concentration methods compatible with downstream molecular detection. Here, a virus concentration method based on adsorption of the virus to an anion exchange resin and direct isolation of nucleic acids is presented. Ten liter samples of tap water spiked with different concentrations (10–10,000 $TCID_{50}$/10 L) of human adenovirus 40 (HAdV-40), hepatitis A virus (HAV) or rotavirus (RV) were concentrated and detected by real time PCR or real time RT-PCR. This method improved viral detection compared to direct testing of spiked water samples where the ΔCt was 12.1 for AdV-40 and 4.3 for HAV. Direct detection of RV in water was only possible for one of the three replicates tested (Ct of 37), but RV detection was improved using the resin method (all replicates positive with an average Ct of 30, $n = 3$). The limit of detection of the method was 10 $TCID_{50}$/10 L for HAdV-40 and HAV, and 100 $TCID_{50}$/10 L of water for RV. These results compare favorably with detection limits reported for more expensive and laborious methods.

It is estimated that consumption of contaminated drinking water causes more than 19 million illnesses every year in the United States (Reynolds et al., 2008). Enteric viruses are a major cause of diverse waterborne diseases, from mild gastroenteritis to life threatening conditions such as hepatitis and meningitis. These viruses infect the intestinal tract of humans and are shed in very high numbers into the stools (Bosch, 1998; Koopmans and Duizer, 2004; Schultz et al., 2011). Enteric viruses have very low infectious doses, ranging from 1 to 100 viral particles (Appleton, 2000; Bresee et al., 2002; Koopmans and Duizer, 2004; Ikner et al., 2011). Thus, water contaminated with very low concentrations of viral particles represents a significant health risk. Given these factors, sensitive methods to detect viruses at low concentrations in water samples are needed (Wu et al., 2011), which make procedures for virus concentration especially critical (Jones et al., 2009).

Although several methods for concentration of enteric viruses from water have been developed, limitations such as need for expensive equipment, difficulty in processing large volumes, low efficiency, excessive processing time, requirement for sample conditioning and incompatibility with downstream detection techniques have prompted continuous searches for new and improved concentration methods. At present, the most widely used concentration methods are based on adsorption of viral particles through surface charges. However, this technique requires elution of virus from the filters, which is often inefficient and necessitates the need for large volumes of elution buffer that has to be reprocessed using a secondary concentration technique. This in turn lowers the overall efficiency and increases method processing time and cost (Wyn-Jones and Sellwood, 2001; Ikner et al., 2011).

Among enteric viruses, rotavirus (RV) is the most important causative agent of infantile diarrhea; it is estimated that more than 600,000 RV related deaths occur worldwide each year (Parashar et al., 2006). This virus belongs to the family *Reoviridae*, genus *Rotavirus*, and has been linked to outbreaks through consumption of contaminated drinking water (Gratacap-Cavallier et al., 2000). Human adenoviruses (HAdV) belong to the family *Adenoviridae*, genus *Mastadenovirus* and comprise 54 serotypes classified into seven species. The two members of the species *Human adenovirus* F, also known as enteric adenoviruses (HAdV-40 and HAdV-41), are present in high amounts in the feces of young children with acute gastroenteritis, and are second only to RV as a major cause of

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Table 1
Characteristics of the viruses used in this study.

Pathogen	pI[a]	Nucleic acid[b]	Capsid characteristics[b]	Size[b]
Human adenovirus 40	4.5	dsDNA	Icosahedral with protruding fibers	70–90 nm
Hepatitis A virus	2.8	ssRNA	Icosahedral	22–30 nm
Rotavirus	8.0	dsRNA	Icosahedral with trimeric spikes	80–100 nm

Q4 [a] Isoelectric points. *Source*: Michen and Graule (2010).
[b] *Source*: King (2010).

infantile viral diarrhea (King, 2012). As HAdV are very persistent in water, they are considered to be a conservative indicator of human viral fecal contamination (Jiang, 2006; Mena and Gerba, 2009). Hepatitis A virus (HAV) is the only species of the genus *Hepatovirus*, family *Picornaviridae* (King, 2012). HAV causes acute hepatitis in humans, replicates in the hepatocytes and is transmitted by the fecal–oral route through contaminated food and water. Worldwide, clean drinking water is an inverse predictor of HAV infection rates (Jacobsen and Koopman, 2005).

The objective of this study was to test a novel and simple method to concentrate different enteric viruses from tap water. This method is based on adsorption of the viruses to an anionic exchange resin dispersed into the water sample, followed by direct isolation of nucleic acids from the resin, thus eliminating the need for elution and secondary concentration steps. In small volumes of water (50 ml) this method was previously shown to allow for effective concentration and detection of enteric virus indicator F-RNA coliphages (Pérez-Méndez et al., 2014). In this study, HAdV-40, HAV and RV were selected for evaluation with the resin-based method due to their public health relevance and diversity of surface structure, size, nucleic acid content and isoelectric points (Table 1).

Viruses and cell lines were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA) and propagated in accordance with ATCC protocols. Strain Dugan (ATCC® VR-931™) was propagated in HEK-293 cells (ATCC® CRL-1573™). HAV virus strain HM175/18f (ATCC® VR-1402™) was propagated in FRhK4 cells (ATCC® CRL-1688™) and human RV strain Wa (TC adapted) (ATCC® VR-2018™) was propagated in MA-104 clone 1 cells (ATCC® CRL-2378.1™). The titers of the viral stocks were determined by assaying at least six replicates of 10-fold serial dilutions of the virus sample, and tissue culture infectious doses 50% ($TCID_{50}$/ml) were calculated using the Reed–Muench method (Reed and Muench, 1938).

Samples (10 L) of dechlorinated tap water (pH 8.2) from the Fort Collins, CO municipal water supply were spiked with each virus to obtain final viral concentrations of 10, 100 and 10,000 $TCID_{50}$/10 L. After thorough mixing, total nucleic acid was extracted from a 140 μl sample using the QIAmp Viral RNA kit© (Qiagen, Valencia, CA, USA). For virus concentration 0.5 g of the anion exchange resin, Amberlite IRA-900 (Polysciences, Warrington, PA, USA), was added to the 10 L spiked water sample and mixed continuously at room temperature for 90 min, using a stirring bar. At the end of the mixing period, stirring was stopped, the resin was allowed to settle for 1 min, and collected using a wide bore tip serological pipette (orifice size of 2–3 mm). The resin was then transferred to a 50 ml conical tube, and any remaining liquid was removed using a pipet tip. Nucleic acid isolation from the resin-adsorbed viruses was accomplished by adding 560 μl of AVL buffer (QIAmp Viral RNA kit) to the resin and incubating for 10 min at room temperature with occasional agitation. The nucleic acid-containing supernatant was transferred to a 1.5 ml Eppendorf tube and then processed according to manufacturer's instructions. For both water and resin samples, the nucleic acid (DNA or RNA) was eluted in 60 μl of AVE buffer (QIAmp Viral RNA kit).

All viruses were detected with commercially available real time PCR (for adenoviruses) or real time RT-PCR (for HAV virus and RV) kits (Ceeram, La Chapelle-sur-Erdre, France) using a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Five microliters of nucleic acid extract from the water sample or from the resin was tested in each reaction. Positive, negative and internal amplification controls provided in each kit were tested. In addition, nucleic acid isolated from resin incubated in 10 L of sterile water served as a second negative control, and produced the expected negative result. For each virus and each viral concentration tested, three independent biological replicates were performed.

HAdV-40 was not detected in water samples containing 10 $TCID_{50}$, but when the resin-based method was performed, the virus was detected in all three replicates with an average threshold cycle (Ct) of 31.7 (Table 2). Water samples spiked with 100 or 10,000 $TCID_{50}$ of HAdV-40 produced positive results by real time PCR (average Ct of 36.8 and 30.3, respectively); however, use of the resin-based method improved target detection considerably (average Cts of 28.3 and 18.3, respectively). Ct results of the water samples were compared to Ct results of the resin samples using a *t*-test, and statistically significant differences were found for both HAdV-40 concentrations tested (p-value < 0.001). Similarly, HAV was not detected in the water when 10 $TCID_{50}$ were spiked in 10 L samples, and it was detected in only one of three replicates when spiked with 100 $TCID_{50}$. However, this virus was detected in all the replicates at both 10 and 100 $TCID_{50}$ when the resin-based method was used (average Cts of 36.3 and 33.7, respectively). When 10,000 $TCID_{50}$ of HAV were spiked, direct detection was possible (Ct = 35.1) but the use of the resin (Ct = 30.8) improved HAV detection significantly (p-value > 0.001). The resin-based concentration method demonstrated similar performance when evaluated with RV. In these samples direct detection from water was not possible at 10 or 100 $TCID_{50}$, and was only detected in one of three replicates at 10,000 $TCID_{50}$. When resin was used, RV was detected at all concentrations tested, producing a Ct of 38.9 for 10 $TCID_{50}$ (only one replicate was positive), an average Ct of 36.4 for 100 $TCID_{50}$ and 30.0 for 10,000 $TCID_{50}$ samples. The limit of detection of the concentration method for each virus was deemed to be the lowest concentration of the virus detected as positive in all three replicates. Therefore, the limit of detection of the method was 10 $TCID_{50}$/10 L (or 10^{-3} $TCID_{50}$/ml) for HAdV-40 and HAV, and 100 $TCID_{50}$/10 L (or 10^{-2} $TCID_{50}$/ml) for RV.

For all the enteric viruses tested in this study, the use of the resin-based method improved detection (measured by ΔCt). Provided a 100% efficient RT-PCR reaction, a gain of one Ct corresponds to a 2 fold increase in target concentration. Therefore, the increased concentration of the target due to the use of the resin can be estimated as $2^{\Delta Ct}$ and was calculated to be 4300× for HAdV-40, 128× for RV and 20× for HAV. This improvement was dependent on the viral load in the sample, where ΔCt was larger when viruses were at higher concentrations. Such behavior may be explained by the fact that viral adsorption to surfaces is influenced by the frequency of impacts of the virus with the adsorbent surface, and the probability of impacts increases with the concentration of the virus (Gerba, 1984).

Viral adsorption to the anionic resin is expected to occur through electrostatic interactions. However, although RV is expected to

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