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The evaluation of hollow-fiber ultrafiltration and celite concentration of enteroviruses, adenoviruses and bacteriophage from different water matrices

Eric R. Rhodes*, Emma M. Huff, Douglas W. Hamilton, Jenifer L. Jones

Biohazard Assessment Research Branch, Microbiological and Chemical Exposure, Assessment Research Division, National Exposure Research Laboratory, Office of Research and Development, United States Environmental Protection Agency, 26 Martin Luther King Dr., MS-587, Cincinnati, OH 45268, USA

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A B S T R A C T

The collection of waterborne pathogen occurrence data often requires the concentration of microbes from large volumes of water due to the low number of microorganisms that are typically present in environmental and drinking waters. Hollow-fiber ultrafiltration (HFUF) has shown promise in the recovery of various microorganisms. This study has demonstrated that the HFUF primary concentration method is effective at recovering bacteriophage φX174, poliovirus, enterovirus 70, echovirus 7, coxsackievirus B4 and adenovirus 41 from large volumes of tap and river water with an average recovery of all viruses of 73.4% and 81.0%, respectively. This study also evaluated an effective secondary concentration method using celite for the recovery of bacteriophage and enteric viruses tested from HFUF concentrates of both matrices. Overall, the complete concentration method (HFUF primary concentration plus celite secondary concentration) resulted in a concentration factor of 3333 and average recoveries for all viruses from tap and river waters of 60.6% and 60.0%, respectively.

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1. Introduction

Different types of enteric viruses can be excreted in the feces of infected humans. These viruses can be pathogenic to humans who come into contact with contaminated matrices and can be spread through food and/or water (Fong and Lipp, 2005; Sinclair et al., 2009). Illnesses caused by the enteric viruses can include gastroenteritis, conjunctivitis, hepatitis, respiratory infections, encephalitis, paralysis and myocarditis (Fong and Lipp, 2005; Sinclair et al., 2009), suggesting that these waterborne microbes can have a significant impact on public health. Enteric viruses have been detected in numerous water matrices including recreational waters (Centers for Disease, C. and Prevention, 2004; Heerden et al., 2005; Sinclair et al., 2009), drinking waters (Anderson et al., 2003; Parshionikar et al., 2003; Riera-Montes et al., 2011) and wastewaters (Belguith et al., 2007; Brinkman et al., 2013; Fong et al., 2010).

One of the most promising techniques for concentrating viruses from water is hollow-fiber ultrafiltration (HFUF), which captures and concentrates the viruses by size exclusion. Any virus that is larger than the cutoff are trapped in the filter (Hill et al., 2005, 2007; Polaczyk et al., 2007; Rhodes et al., 2011), then eluted and the concentrated sample is collected. Hollow-fiber ultrafiltration that is setup in a tangential flow pattern has been shown to be very efficient at recovering multiple types of microorganisms, including viruses (Francy et al., 2009; Gibson and Schwab, 2011; Hill et al., 2005, 2007; Holowecky et al., 2009; Kuhn and Oshima, 2002; Leskinen et al., 2010; Lindquist et al., 2007; Polaczyk et al., 2008; Rajal et al., 2007; Rhodes et al., 2011, 2012; Simmons et al., 2001; Winona et al., 2001). Moreover, with a 2015 cost of approximately 20 U.S. dollars it is less expensive than the other capsule filter options (e.g. 1MDS costs approximately 200 U.S. dollars) and can collect multiple microorganisms simultaneously. Hollow-fiber ultrafilters also show promise in use with source water as demonstrated in a few studies which showed that hollow-fiber ultrafilters can recover multiple microorganisms from both ground and surface water (Gibson and Schwab, 2011; Leskinen et al., 2010; Olszewski et al., 2005). However, if hollow-fiber ultrafiltration using inexpensive disposable filters is going to be an option for concentration as part of a method, then their use with different types of water and different virus types needs to be evaluated.

After HFUF concentration a secondary concentration method usually needs to be performed that can further reduce the

* Corresponding author. Tel.: +1 513 569 7308; fax: +1 513 569 7117.
E-mail address: rhodes.eric@epa.gov (E.R. Rhodes).

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sample volume. These secondary concentration methods need to be evaluated for different viruses and for different matrices as well. Organic flocculation or polyethylene glycol (PEG) precipitation are two common procedures for secondary concentration (Fout et al., 1996; Katzenelson et al., 1976; Lewis and Metcalf, 1988; Schwab et al., 1995). An alternative procedure using celite has been developed (Dahling and Wright, 1986b) and this procedure has been optimized for the concentration of enteroviruses and adenoviruses (McMinn, 2013; McMinn et al., 2012; Rhodes et al., 2011).

This study evaluates the use of hollow-fiber ultrafiltration primary concentration and celite secondary concentration to recover different enteroviruses, φX174 bacteriophage and adenovirus 41 from large volumes of tap water and river water samples. The enteroviruses that were chosen for this study are poliovirus, enterovirus 70, echovirus 7, coxsackievirus B4. These viruses cover the majority of the 4 different groups of enteroviruses. Poliovirus belongs to group C, coxsackievirus B4 and echovirus 7 belong to group B and enterovirus 70 is in group D. This study did not include an enterovirus from group A. Also a bacteriophage, which are viruses that infect bacteria, was evaluated because it could potentially be used as a surrogate for viruses. Since they are routinely found in higher numbers than pathogenic viruses, may behave in a similar manner through different treatment processes and transport through the natural environment, and are easier to detect, we wanted to determine how well these organisms were recovered by HFUF relative to pathogenic viruses.

2. Materials and methods

2.1. Viruses

Bacteriophage φX174 (ATCC#87210), poliovirus 1 Chat (ATCC#VR-1562), echovirus 7 (ATCC#VR-1047), enterovirus 70 (ATCC#VR-836), coxsackievirus B4 (ATCC#VR-184) and adenovirus 41 (ATCC#VR-930) were used in this study. The virus stocks were tittered according to Sections 2.8 and 2.9 then were diluted to a target concentrations of either 1×10^3, 1×10^4 or 1×10^5 PFU/mL or genomic copies/mL (Table 1) in 1× phosphate buffered saline (PBS) pH 7 (P3744, Sigma-Aldrich, St. Louis, MO) prior to addition to the water samples.

2.2. Tap water sample collection

Tap water lines at Environmental Protection Agency laboratories, Cincinnati, OH were purged for at least 5 min prior to sample collection. 100 L tap water samples were collected in a 30 gallon polypropylene tank (EW-06317-73, Cole Parmer, Vernon Hills, IL), dechlorinated with 7.5 g sodium thiosulfate pentahydrate (72050, Sigma-Aldrich) and amended with 10.0 g sodium hexametaphosphate (68915-31-1, Fisher Scientific, Pittsburgh, PA) as the tank filled (Rhodes et al., 2011).

2.3. River water sample collection

River water samples were collected in 20 L polypropylene carboys from the Ohio River near Cincinnati, OH. The samples were transferred to the laboratory within 1 h of collection. Turbidity was measured using a LaMotte Turbidity Meter 2020e (LaMotte, Chesterton, MD) and results expressed as Nephelometric Turbidity Units (NTU). The carboys were emptied into a 30 gal polypropylene tank to the 50 L mark and amended with 5.0 g sodium hexametaphosphate (Fisher Scientific).

2.4. Hollow-fiber ultrafiltration primary concentration method

The hollow-fiber ultrafilter assembly was set up to run in a tangential flow similar to those published previously (Francy et al., 2009; Hill et al., 2007; Holowecky et al., 2009; Lindquist et al., 2007; Polaczyk et al., 2008; Rhodes et al., 2011, 2012). The concentration procedure and setup was followed precisely as previously described (Rhodes et al., 2011, 2012). The filtration was performed with Asahi Kasei Rexeed 25S ultrafilters (Dial Medical Supply, Chester Springs, PA), and I/P precision brushless drive with an easy load pump head (Cole Parmer, Vernon Hills, IL). The Asahi Kasei ultrafilters have a molecular cutoff of 30 kDa, an inner fiber diameter of 185 μm and a total surface area of 2.5 m^2. The final elution step was performed using an elution solution (0.01% Tween 80 (P1754, Sigma-Aldrich), 0.01% Sodium Hexametaphosphate (68915-31-1, Fisher Scientific), 0.001% Antifoam A (A5758, Sigma-Aldrich). New Masterflex® I/P-73 tubing and ultrafilters were used for each virus and run. All other tubing and connectors and were sterilized with a 10% bleach solution and autoclaved prior to reuse. Pressure gauges were sterilized with 10% bleach.

2.5. Celite secondary concentration method

The concentrate obtained from the primary concentration method was amended with BBL beef extract powder (212303, BD Biosciences, Sparks, MD) at a concentration of 1.5 g per 100 mL of the sample concentrate with mixing. After the beef extract dissolved, Celite 577 (577, Fluka Chemical Co., Ronkonkoma, NY) was added to the mixture at a concentration of 0.1 g per 100 mL of the sample concentrate (McMinn, 2013; McMinn et al., 2012; Rhodes et al., 2011). The concentrated river water samples were highly turbid and thus were centrifuged prior to celite addition in order to remove particulate matter. Otherwise, the sample would clog the AP20 filter. Only the river water samples were subjected to a centrifugation step prior to celite addition to remove the particles that were concentrated during the ultrafiltration process. The collected material was not assayed for viral loss. Specifically, after the addition of the beef extract and after it was completely dissolved the sample was stirred for 15 min, then centrifuged at 3300 × *g* for 15 min. The supernatant was decanted into a sterile beaker and then celite was added. The pH of the mixture was brought to 4.0 using 1 N HCl and the mixture was stirred slowly for 10 min. The celite was collected on an AP20 filter (75 mm diameter, 2.0 μm pore size, glass fiber filters with binder resin, AP207500, Millipore Corp, Bedford, MA) using a Buchner funnel, a side-arm flask and suction. The AP20 filter and its Buchner funnel housing were placed on a sterile 250 mL side-arm flask and 35 mL of 1X PBS pH 9.0 (P3744, Sigma-Aldrich) was added drop wise to elute the virus from the celite, resulting in a secondary concentrate. The pH of the secondary concentrate was adjusted to 7.0–7.5 if needed.

2.6. Tap and river water sample processing

2.6.1. Primary concentration

Water samples were seeded with a single virus type and concentrated as described in Section 2.4, resulting in a final sample volume of 300–350 mL. To prepare the samples for cell culture, approximately 50 mL was filter sterilized through a 0.2 μm Acrodisc® syringe filter (4525, Pall, Ann Arbor, MI), which was pretreated with a 1.5% beef extract solution. The filtered sterilized sample was stored at −80 °C until analysis (φX174 samples were stored at 5 °C and analyzed within 24 h).

2.6.2. Secondary concentration

Unspiked water samples were concentrated as described in Section 2.4. The primary concentrate was seeded with a single virus