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Optimization and evaluation of a method to detect adenoviruses in river water

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Adenoviruses are often implicated in recreational water disease outbreaks but existing methods for their detection perform poorly within these matrices. In this study, small volume (100 mL) concentration was used to identify processes that promoted recovery of adenovirus from river water. Several alternative secondary concentration techniques were investigated and compared to the baseline method consisting of primary concentration via filtration, followed by celite mediated secondary concentration. The alternative secondary concentrations included multiple filter elutions, soaking the filter for 15 min prior to elution and concentration using pre-treated celite (river water, 1.5% and 3% milk) instead of a filter. Modifications of the viral nucleic acid extraction technique were also evaluated. Concentration using pre-treated celite and a modified extraction technique (10 min boil and a 1 h ProK incubation at 37 °C) recovered significantly higher levels of adenovirus ($P = 0.001$) than other methods tested. This optimized method increased recovery of spiked adenovirus (57 ± 27%) compared to baseline method performance (4 ± 3%) indicating that use of pre-treated celite as opposed to filtration significantly improves recovery. Application of the optimized concentration method to larger volume (1 L) of river water resulted in similar recoveries (42 ± 19%) underlying the utility of this method to detect adenovirus from environmental samples.

1. Introduction

Viral pathogens are important etiological agents in recreational waterborne disease outbreaks. Specifically adenoviruses are commonly detected in human waste (Pina et al., 1998; Levidiotou et al., 2009; Nakanishi et al., 2009; Svraka et al., 2007; Kitajima et al., 2014) and are believed to be responsible for a number of reported waterborne illnesses (Sinclair et al., 2009; Mena and Gerba, 2009; Leclerc et al., 2002). Adenoviruses are non-enveloped, medium sized (90–100 nm) double-stranded DNA viruses with a complicated structure consisting of icosahedral capsid interspersed with protein fibers of varying lengths and complexities (Russell, 2009; Mangel and San Martin, 2014). The 51 known human serotypes are subdivided into six subgenera (A–F) and can infect wide variety of tissues (Russell, 2009; Mangel and San Martin, 2014). Adenoviruses are known to be fairly resistant to treatments commonly used to disinfect wastewater (Thompson et al., 2003; Calgua et al., 2014) and this inherent stability could result in adenoviruses remaining infective for prolonged periods, increasing the chances of human exposure if released into recreational water bodies.

Typically human viruses are found in low concentrations in drinking and ground waters requiring large volumes of water (100–1600 L) to be concentrated in order to attain detectable levels. This is usually achieved by primary concentration using some form of filtration (electropositive, electronegative, size exclusion, glass wool, etc.) to capture viruses present, followed by secondary concentration where viruses are released from filter surfaces using an elution buffer (e.g., beef extract, milk) and then concentrated via flocculation. Depending on the method, samples can undergo additional concentration (e.g., nucleic acid extraction) further reducing final sample volumes (Cashdollar et al., 2013; Karim et al., 2009; Aslan et al., 2011).

Processing large volumes of environmental water presents unique challenges including the need for field deployable filtration, while addressing common issues of filter fouling due to buildup of suspended organics and inorganics on filter surfaces. Additionally, the multiple steps (filtration, elution, secondary concentration, tertiary concentration and extraction) required to concentrate viruses from large volumes, introduce opportunities for viral loss (Ahmed et al., 2015a). Large volume sampling could be avoided if more targeted virus concentration techniques existed. Depending on the contamination level present, viruses can be detected in smaller volumes (1–10 L) of water (Victoria et al., 2014; Rigotto et al., 2010; Lee et al., 2014). Working with reduced sample volumes

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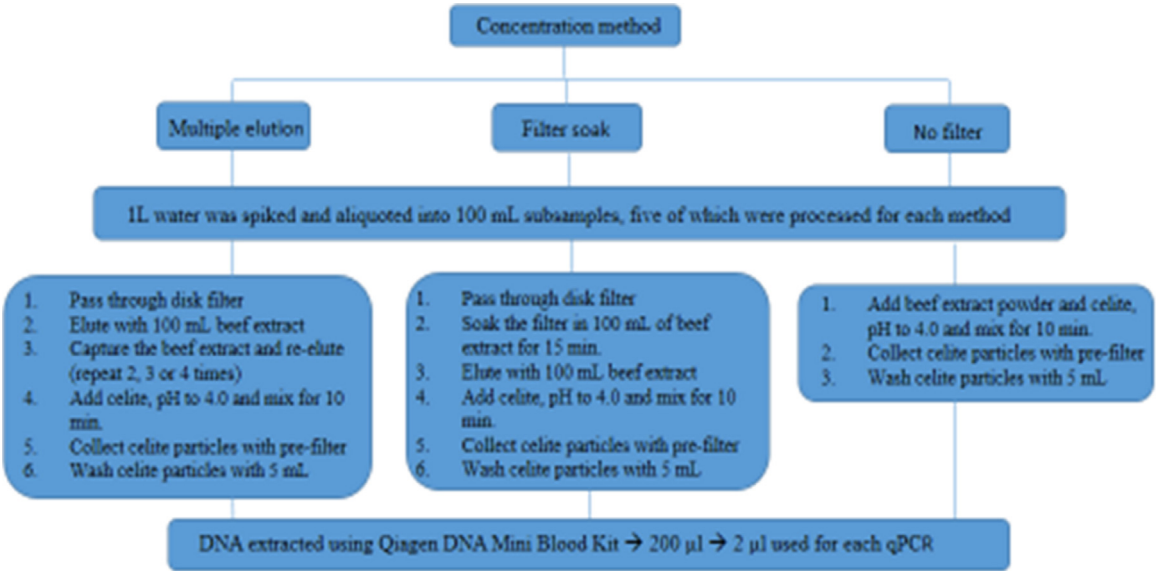

Fig. 1. Description of the primary concentration methods used.

has numerous advantages including easier manipulation of samples and fewer concentration steps resulting in quicker processing times and lower amounts of inhibitory substances co-concentrated (Hata et al., 2011).

Concentration of adenoviruses via standard techniques is challenging likely due to the highly complex structure of the icosahedral capsid which allows the initial attachment to the surface of the filter but at the same time complicates the subsequent steps. Earlier studies have shown effective capture of adenovirus on filter surfaces (~99%), but low recoveries (2–35%) compared to other viruses (60–80%) using similar concentration methods (Gibbons et al., 2010; Sobsey and Glass, 1984; Ikner et al., 2011; Zhang et al., 2013; Pang et al., 2012). This data suggests that a majority of viral losses occur during filter elution and that avoiding filters could alleviate much of the initial virus losses. In addition, latter concentration steps could also be modified to further promote higher viral recovery. Our earlier work indicated that adenoviruses can be efficiently recovered when spiked during secondary concentration without the use of a concentrating filter (McMinn et al., 2012) and that certain combinations of primary and secondary concentration techniques can have a negative effect on virus recovery (McMinn, 2013). In addition, losses of viral nucleic acids occur during chemical extraction when using binding silica membrane columns (Hata et al., 2011) common to most commercial nucleic acid extraction/purification kits which may lead to further underestimation of the viral concentrations.

The main objective of this study was to develop simple and effective techniques for concentrating adenovirus from small volumes of river water by (i) evaluating sample matrix effect on recovery of adenovirus, i.e., tap water, primary effluent and river water, (ii) evaluating alternatives to traditional primary virus concentration procedures, (iii) testing various secondary elution techniques with modifications and (iv) optimizing extraction of adenovirus nucleic acids prior to molecular detection.

2. Materials and methods

2.1. Virus stock

Wild-type adenovirus 5 (#AD104-S, O.D. 260, Boise, ID) was used for all spiking experimentation. Stock virus was quantified via real-time, quantitative PCR and diluted to working concentrations (~10^4–10^5 genomic copies per mL) in sterile 1X PBS (171 mM NaCl, 3.3 mM KCl, 10 mM Na_2PO_4) at pH 7.0. Working stocks were aliquoted into 1.5 mL cryogenic vials and stored at −80 °C. For each experiment, 1 mL of thawed AdV5 working stock was seeded into either 1 L of dechlorinated tap water, 1 L of river water or 1 L of primary sewage effluent. Spiked water was mixed for 10 min to assure even distribution of virus and 100 mL portions were aliquoted and used as replicates ($n = 5$).

2.2. Sampling

Three different sample matrices were evaluated in this study (tap water, primary effluent and river water) to determine their effect on the recovery of spiked adenovirus using traditional concentration techniques. Tap water was collected in the laboratory and dechlorinated prior to experimentation using 0.07 g/L sodium thiosulfate (Sigma, St. Louis). Primary effluent was collected from a local waste water treatment plant in sterile 1 L polypropylene bottles and transferred to the laboratory on ice. River water was collected from the Ohio River approximately 12 m from shore in sterile 1 L polypropylene bottles and transferred to the laboratory on ice. All samples were allowed to warm to room temperature prior to experimentation.

2.3. Alternative secondary concentration

Several alternative secondary concentration procedures were compared to the baseline method described in (Zhang et al., 2013). Briefly, the baseline method consisted of passing 100 mL of spiked sample through a 47 mm 1MDS (Bedford, MD) electropositive filter disk, eluting the filter with 100 mL of 1.5% desiccated beef extract (Becton, Dickson and Company, Sparks, MD) and using the celite secondary concentration technique to concentrate to a final volume of 5 mL (Ikner et al., 2011). In order to enhance the release of AdV5 from filter surfaces following primary concentration, several virus elution alternatives were tested including soaking the filter ("filter soak" treatment) and multiple filter elutions (Fig. 1). For the filter soak protocol, following filtration 1MDS disk filters were removed from the filter housing using sterile forceps, and soaked in 100 mL of 1.5% desiccated beef extract with 0.05 M glycine at pH 10 for 15 min. Filters were removed from the beef extract, placed back in the filter housing and the 100 mL of beef extract used to soak