Journal of Virological Methods 162 (2009) 272–275

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Short communication

A novel method for concentrating hepatitis A virus and caliciviruses from bottled water

Katarina Kovač[a,*], Ion Gutiérrez-Aguirre[b], Marko Banjac[c], Matjaž Peterka[c], Mateja Poljšak-Prijatelj[d], Maja Ravnikar[b], Janet Zimšek Mijovski[d], Anna Charlotte Schultz[e], Peter Raspor[a,*]

[a] Chair of Biotechnology, Microbiology and Food Safety, Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
[b] Department of Biotechnology and Systems Biology, National Institute of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia
[c] BIA Separations, d.o.o., Teslova 30, SI-1000 Ljubljana, Slovenia
[d] Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloška 4, SI-1000 Ljubljana, Slovenia
[e] National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark

Article history:
Received 23 February 2009
Received in revised form 13 July 2009
Accepted 21 July 2009
Available online 29 July 2009

Keywords:
Monolithic columns
Virus concentration
HAV
FCV
Water

A B S T R A C T

Human enteric viruses are detected frequently in various types of environmental water samples, such as irrigation water, wastewater, recreational water, ground or subsurface water and even drinking water, constituting a primary source of gastroenteritis or hepatitis outbreaks. Only a few, but still infective number of viral particles are normally present in water samples, therefore an efficient virus concentration procedure is essential prior to molecular detection of the viral nucleic acid. In this study, a novel chromatographic technology, Convective Interaction Media® (CIM) monolithic supports, were optimized and applied to the concentration of hepatitis A virus (HAV) and feline calicivirus (FCV), a surrogate of norovirus (NoV), from water samples. Two-step real-time RT-qPCR was used for quantitation of the virus concentration in the chromatographic fractions. Positively charged CIM QA (quaternary amine) monolithic columns were used for binding of HAV and FCV present in previously inoculated 1.5 l bottled water samples. Column bound viruses were eluted from the monolith using 1 M NaCl to a final volume of 15 ml. Elution volume was concentrated further by ultracentrifugation. When the CIM/ultracentrifugation method was compared with another concentration method employing positively charged membranes and ultrafiltration, the recovery of HAV was improved by approximately 20%.

© 2009 Elsevier B.V. All rights reserved.

Noroviruses (NoVs) and hepatitis A virus (HAV) are among the most important human food-borne viruses and cause serious outbreaks of infection, which are, in some cases, related to potable water supply resources (Bosch et al., 1991; Cliver, 1997; Craun et al., 2002; Hejkal et al., 1982; Kaplan et al., 1982; Koopmans and Duizer, 2004; Kukkula et al., 1999). These viruses, as well as other enteric viruses, are transmitted by the faecal–oral route and infect primarily the gastrointestinal tract of the host. Patients suffering from gastroenteritis or hepatitis A may excrete from 10^5 to 10^{11} virus particles per gram of stool (Farthing, 1989; Koopmans and Duizer, 2004). Food or water can be contaminated directly by faecal matter and vomitus or indirectly by exposure to contaminated surfaces (Fiore, 2004; Frankhauser et al., 1998; Lamhoujeb et al., 2008).

Both viruses are small and non-enveloped with a positive sense, single stranded RNA genome. HAV belongs to the genus *Hepatovirus* of the *Picornaviridae* family and causes acute hepatitis in humans worldwide (Stanway et al., 2005). The incidence of HAV infection varies considerably among and within countries (Mast and Alter, 1993). It is an increasing problem in Western European countries because of the decreased immunity of the population in countries with higher hygiene standards (Koopmans and Duizer, 2004). NoVs are members of *Caliciviridae* family and cause acute gastroenteritis (Green et al., 2001). The incidence is highest among younger children, but illness also occurs in adults and asymptomatic infections are common (Codex Alimentarius, 1999; Koopmans and Duizer, 2004). NoV and HAV are highly infectious and exhibit variable levels of resistance to heat and disinfection agents (Koopmans and Duizer, 2004).

In the water environment, the fate of microbial enteric pathogens may take several potential routes. Exposure to waterborne enteric virus infections originates mainly from shellfish grown in contaminated waters, contaminated drinking water, food crops grown in land irrigated with wastewater and/or fertilized with sewage, and, to a lesser extent, from sewage-polluted recre-

* Corresponding authors. Tel.: +386 1 4231161; fax: +386 1 2574092.
E-mail addresses: katarina.kovac@bf.uni-lj.si (K. Kovač), peter.raspor@bf.uni-lj.si (P. Raspor).

0166-0934/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jviromet.2009.07.013

ational waters (Bosch et al., 2006; Rzezutka and Cook, 2004). Two studies confirming the presence of RNA with nucleotide sequences specific for NoV in different brands of European mineral water indicate that bottled water could also be an important source of viral infection (Beuret et al., 2000, 2002).

A few, usually undetectable, viral particles are typically present in water samples, however, since such low viral concentrations are able to cause disease, efficient concentration methods are needed urgently (Koopmans et al., 2002). A variety of strategies had been used to concentrate viruses from water samples, which are based mostly on adsorption-elution techniques, precipitation, ultrafiltration, lyophilization and ultracentrifugation (Bosch et al., 2006). During last decade, a new chromatographic medium, monolithic supports, was developed and applied successfully to the concentration and detection of several plant and human viruses (Branovic et al., 2003; Gutiérrez-Aguirre et al., 2008a; Kramberger et al., 2004). Convective Interaction Media® (CIM) is a monolithic chromatographic support made from a single block of porous material, with highly interconnected channels that enable very rapid transfer of the sample molecules between the mobile and the stationary phase. This rapid mass transfer is based on convective flow (Štrancar et al., 2002). The diameter of the larger channels is about 1500 nm, and therefore large enough to allow the circulation of molecules such as virus particles. All these properties contribute to enhance the speed of the separation process and to reduce the back pressure, non-specific binding, product degradation and minor changes in the structure of the biomolecule. CIM supports are easy to handle and increase the productivity of chromatographic processes by at least one order of magnitude over traditional chromatographic columns packed with porous particles (Barut et al., 2008; Štrancar et al., 2002; Urthaler et al., 2005).

The aim of this study was to apply CIM monolithic chromatographic supports (BIA Separations, d.o.o., Ljubljana, Slovenia) for the concentration of HAV and feline calicivirus (FCV), a NoV surrogate, from water samples. HAV strain HM 175/18f and FCV strain VR-782 were propagated in cell cultures at the Istituto Superiore di Sanità, Rome, Italy. Viral titers of HAV and FCV were 2.7×10^7 $TCID_{50}$/ml and 2×10^7 $TCID_{50}$/ml, respectively. Stocks of viruses were aliquoted and stored at −80 °C. A known initial concentration of virus samples was used for preparing dilutions in phosphate buffer saline (PBS), which were used in the experiments.

Preliminary experiments were carried out initially in order to optimize virus binding to the CIM monolithic supports and to determine the elution conditions. Since most enteric viruses are negatively charged at ambient pH (Lipp et al., 2001), positively charged CIM QA (quaternary amine) monolithic columns were chosen for the concentration of the two viruses selected. The mobile phase, 50 mM phosphate buffer pH 7, optimized already for the binding of rotaviruses to CIM QA (Gutiérrez-Aguirre et al., 2008a), was used. Different stepwise gradient elutions (0.5 M, 1 M, 1.5 M and 2 M NaCl in 50 mM phosphate buffer, pH 7) were carried out to determine the NaCl concentration needed for elution of the bound viruses from the CIM QA matrix. 1 M NaCl eluted both types of viruses efficiently. Therefore, 50 mM phosphate buffer with 1 M NaCl was used as an elution buffer in subsequent concentration experiments. The method was optimized further in order to reduce the eluate volume, after concentration using CIM QA-8 ml tube, from 15 ml to around 150 μl by the addition of a secondary concentration step consisting of ultracentrifugation.

The optimized method was compared to the modified method of Gilgen et al. (1997) to ascertain its efficiency for concentrating viruses from 1.5 l bottled still water samples (COOP, Bologna, Italy). Prepared dilutions of both viruses were spiked together in water samples reaching different final concentrations. In order to test the ability of the CIM QA monoliths to concentrate minimal amounts of viruses, very low concentrations were included in the experiment. Each of five water samples (2–6) were spiked simultaneously with 10-fold serial dilutions of the viral stocks, using the ranges 10–10^5 $TCID_{50}$ of HAV and 10^5–10 $TCID_{50}$ of FCV, while sample 1 was used as a negative control. All experiments for both methods were carried out in triplicate.

First, viruses were concentrated using a CIM QA – strong anion exchanger in combination with ultracentrifugation (the "in-house" method). The 8 ml CIM QA tube was connected to an HPLC pump (Knauer, Berlin, Germany). Samples were pumped through the tube column at a flow rate of 50 ml/min. Unbound components were washed with at least 10 column volumes (CV) of 50 mM phosphate buffer, pH 7. Afterwards, the bound viruses were eluted from the matrix using 50 mM phosphate buffer, pH 7 with 1 M NaCl at lower flow rate. The elution was monitored spectrophotometrically at 280 nm with a modular UV–vis detector (Knauer) equipped with a preparative flow cell. A 15 ml eluate was usually obtained. The eluted sample was concentrated further by ultracentrifugation (Beckman L8-80M) at 85,750 × *g* at 4 °C. After 1 h, the supernatant was discarded and the pellet was resuspended in 560 μl of AVL buffer containing carrier RNA (QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany)), and proceeded with RNA isolation according to the manufacturer's instructions. The CIM QA tube was regenerated and stored as recommended by the manufacturer.

The second concentration method (the "reference" method) was modified slightly from the originally described by Gilgen et al. (1997). Briefly, samples were concentrated using 0.45 μm pore size Sartolon polyamide membrane filter with a diameter of 47 mm, cat. No. 25006-47-N (Sartorius, Goettingen, Germany). Membrane bound viruses were eluted using 10 ml of tris–glycine beef extract (TGBE) buffer, pH 9.5. After shaking at 500 rpm for 20 min at room temperature in order to elute viruses, the pH of the sample was adjusted to 8 using 1 M HCl. The samples were concentrated further using the Amicon Ultra-15 Centrifugal Filter Units with Ultracel-100 Membrane (Millipore, Billerica, Massachusetts) at 4000 × *g* for 13 min to about 150 μl. PBS was added to each sample to reach the final volume of 500 μl and RNA was extracted from the whole volume using NucliSens MiniMAG System (Biomérieux, Marcy l'Étoile, France).

Independent standard curves were generated to account for the different RNA extraction procedures (QIAamp Viral RNA Mini Kit (Qiagen) and NucliSens MiniMAG System (Biomérieux)) used for each concentration method. Newly constructed specific primers and probes and the protocol for reverse transcription (RT) followed by amplification using the Taqman Universal PCR Master Mix (Applied Biosystems, Foster city, USA) were kindly provided by Di Pasquale et al. (Istituto Superiore Di Sanità, Rome, Italy). A non-template control was included in each run. The RNA was first transcribed into cDNA and applied in duplicates to the FCV and HAV specific qPCR. The equations of the linear regression and the R^2 value were obtained for each prepared curve. Detection limits and quantitation ranges of FCV and HAV were then estimated (Table 1) based on standard curves. The quantitation range was determined taking into consideration the coefficient of variation of real-time RT-qPCR duplicates which was calculated as the ratio of standard deviation to the mean. Values showing a coefficient of variation lower than 30%, were assumed to be within the range of quantitation (Boben et al., 2007; Burns et al., 2004; Gutiérrez-Aguirre et al., 2008b).

With both types of viruses, the RT-qPCR detection limit for the "in-house" method (RNA extraction using QIAamp Viral RNA Mini Kit) was 1 $\log_{10}$ more sensitive comparing to the RT-qPCR detection limit of the reference method (RNA extraction using NucliSens MiniMAG System). The quantitation ranges for FCV were the same for both extraction methods (1–10^5 $TCID_{50}$), while the quantitation range for HAV was 1 $\log_{10}$ broader for the "in-house"

Download English Version:

https://daneshyari.com/en/article/3407248

Download Persian Version:

https://daneshyari.com/article/3407248

Daneshyari.com