Novel lab technique

Concentration and detection of hepatitis A virus and its indicator from artificial seawater using zeolite

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ABSTRACT

Hepatitis A virus (HAV) infection is the leading worldwide cause of acute viral hepatitis, and outbreaks caused by this virus often occur in fecal polluted waters. Rapid concentration and detection of viral contamination in water environments can prevent economic loss and can identify the source of contamination within a short time. However, conventional methods for virus concentration are often laborious, time consuming, and subject to clogging. Furthermore, most methods require a secondary concentration step to reduce the final volume of samples. We developed a method to concentrate HAV from seawater using zeolite in aid of rapid detection. In this method, artificial seawater was inoculated with HAV (7~8 log TCID₅₀) and filtered with zeolite. The viruses were then eluted from zeolite with sodium dodecyl sulfate and detected via real-time PCR (qPCR). Zeolite was able to concentrate HAV from artificial seawater with ~99% efficiency in less than 5 min and was more efficient in seawater than in fresh water. The entire concentration and detection can be done in approximately 2 h. Compared to existing methods, this method eliminated the need for a secondary concentration step as well as the necessity to modify the pH or salinity of the seawater during concentration, and was simple and inexpensive.

Keywords: Hepatitis A virus, seawater, zeolite, concentration

1. Introduction

Hepatitis A virus (HAV) is responsible for a large proportion of food and water-borne illnesses and common source outbreaks have been attributed to fecal contamination of food and drinking water (Rao and Melnick, 1984). Outbreaks of HAV caused by the consumption of raw shellfish have been reported regularly since 1962, and raw or undercooked clams or oysters were implicated as the most frequent vehicle of infection (Conaty et al., 2000; Desenclos et al., 1991; Guillois-Becel et al., 2009; Mason and McLean, 1962; Shieh et al., 2007). HAV appears to be extremely stable in the environment, with only a 100-fold decline in infectivity over 4 weeks at room temperature, and 3~10 months in water (Hollinger and Ticehurst, 1996; Koopmans et al., 2002). HAV appears to be relatively resistant to free chlorine, especially when the virus is associated with organic matter.

Viral contaminated shellfish can be a public health concern, since shellfish are filter feeders that can actively bio-concentrate HAV as well as other microorganisms from contaminated waters as much as 100 fold and retain the infectious virus particles for up to one month (Le Guyader et al., 2012; McLeod et al., 2009; Nappier et al., 2008; Wang et al., 2008). Numbers of viruses in contaminated food or water are usually too low for detection; however, even one infectious HAV is sufficient to cause illness (Fong and Lipp, 2005; Schiff et al., 1984). Therefore, large volumes of water (10-20 L) need to be concentrated in order to test the presence of pathogens. Different types of filters and filtration methods have been used to collect and concentrate viral particles from water samples (Gantzer et al., 1999; Griffin et al., 2003; Lipp et al., 2001; Pallin et al., 1997). However, electropositive filters are easily clogged and have low recovery rates for viruses in marine water; while the recovery of viruses from an electronegative filter requires acidification of the water matrix (Fong and Lipp, 2005; Lukasik et al., 2000; Sobsey et al., 1973; Sobsey et al., 1995). Ultrafiltration methods require equipment of high cost, and their processing volumes are usually limited. Concentrated or eluted water samples usually are further concentrated and purified to reduce the final volume of samples to 1 or 2 ml for processing (Fong and Lipp, 2005; Jiang et al., 2001; Katayama et al., 2002; Lipp et al., 2001; Noble and Fuhrman, 2001). Procedures of secondary

concentration have often been reported to cause inhibitory effects in PCR assays (Arnal et al., 1999; Fong and Lipp, 2005).

In an effort to solve many of the disadvantages associated with those methods we developed a method using zeolite that would reduce the time and cost of virus concentration. Zeolite is known as a molecular sieve, and has been widely used in industry for water purification due to low cost, strong ion-exchange abilities and large adsorption capacity (Widiastuti et al., 2008). Zeolite is a hydrated porous crystalline tectoaluminosilicate with uniform pore size, and can recognize and organize molecules similar to their pore size (Davis and Lobo, 1992). The building units of natural zeolite are Si–O and Al–O bond tetrahedrons. Because the Si^{4+} could be replaced by Al^{3+} , the tetrahedron structure is negatively charged and cations could be adsorbed into the tetrahedron (Niu and Feng, 2005). As a result, zeolite not only possesses great surface area and water adsorption ability but also exhibits strong ion-exchange properties and thus is very suitable for virus adsorption. The zeolite used in this study was subject to a patent-pending process by its manufacturer to alter the surface charge from negative to positive, allowing the zeolite particles to become a dual charged material and adsorb both negative and positive charged particles that are suspended in water. Due to the dual charged property of this zeolite material, HAV could be concentrated from seawater without acidification of the water.

Various studies suggested the ability of zeolite to adsorb viruses and remove contaminants from water. Zeolite was documented to remove 99% of viruses and 100% of *E. coli* from the water, and adsorb up to 5 logs of viruses in less than 1 minute (Bright et al., 2009; Imai et al., 2012; Schulze-Makuch et al., 2002; Schulze-Makuch et al., 2003). Due to the high adsorption capacity of zeolite, it can be used to concentrate suspended virus particles from seawater, and a small volume (1 ml) of eluent can be used to elute adsorbed virus. As no previous studies have conducted their experiment in marine water, this report investigates the efficiency of zeolite to concentrate HAV and its indicator MS2 from artificial seawater.

2. Materials and methods

2.1. Virus and bacteriophage

Hepatitis A virus (HAV) cytopathic strain HM175/18f (ATCC VR-1402) was grown in FRhK-4 cells (ATCC CRL-1688) for 7 days. Viruses were harvested from infected cell lysates by freeze-thawing. Bacteriophage MS2 (ATCC 15597-B1) was propagated in the exponentially growing *Escherichia coli* (ATCC 15597) culture for 16 h at 37°C. The overnight culture of MS2 was clarified by centrifugation at $2300 \times g$ for 10 min at room temperature and filtration through a 0.45 µm filter.

2.2. Plaque assay

The titer of the MS2 culture was determined with plaque assay. Briefly, 10 ml of bottom agar (1.2%) was poured in 100×15 mm Petri dishes and 8 ml agar (0.6%) containing *E. coli* 6 h culture was poured on top. Supplements of glucose, CaCl₂ and thiamine were added to both layers at final concentrations of 0.1%, 2 mM and 10 µg/ml. Thirty microliters of MS2 dilutions were then spread on top of the agar with a hockey stick (bent glass rod). Plates were incubated at 37°C overnight before plaques were counted (Cormier and Janes, 2014).

2.3. TCID₅₀ assay

The tissue culture infectious dose 50% (TCID₅₀) method was used to determine the titer of HAV stock and dilutions. Healthy FRhK-4 cells were seeded in 6-well tissue culture dishes (Sarstedt Inc., Newton, NC) at 90% confluency and incubated at 37°C. After 24 h of incubation at 37°C, 70 µl of serial diluted viruses were added to each well, with 10 replicates per dilution. The dishes were incubated for 7 days at 37°C and checked daily for characteristic cytopathic effect (CPE). The TCID₅₀ was calculated using a modified Reed-Muench equation according to Saganuwan (Saganuwan, 2011).

2.4. Extraction of viral RNA

One hundred forty microliters of virus suspension was incubated with 560 µl of lysis buffer (QIAamp Viral RNA mini kit) for 10 min at room temperature. Absolute ethyl alcohol (560 µl) was added to the lysate and mixed by vortexing. The mixture was transferred to a spin column in 630 µl aliquots. The column was washed twice, and the RNA was eluted with 30 µl of RNase-free water containing 0.04% sodium azide and used in PCR immediately.

2.5. Real-time PCR (qPCR)

PCR was performed with a SmartCycler (Cepheid, Sunnyvale, CA). For MS2, 10.8 µl of RNA was added to 14.2 µl of mixture containing 0.2 µl 125×RT Enzyme Mix (ArrayScript™ UP Reverse Transcriptase, RNase inhibitor), 12.5 µl 2×RT-PCR Mix (SYBR® Green I dye, AmpliTaq Gold® DNA Polymerase, Ultra-pure, dNTPs, ROX™ passive reference, Optimized buffer components), 0.2 mM of each primer, 10 U RNase inhibitor (Life Technologies, Carlsbad, CA). The thermocycling conditions were (O'Connell et al., 2006): 48°C for 30 min, 95°C for 10 min, 40 cycles of (95°C for 15 s, 60°C for 1 min), then 95°C for 1 min, and 65°C for 2 min. The primers were as follows: forward: 5' GTC GCG GTA ATT GGC GC 3'; reverse: 5' GGC CAC GTG TTT TGA TCG A 3'.

For HAV, 9.3 µl of RNA was added to 10.7 µl of mixture containing 5 µl of TaqMan® Fast Virus 1-Step Master Mix (Life Technologies Co. Carlsbad, CA), 900 µM of each primer, and 150 µM of probe. The thermocycling conditions followed recommendations from the manufacturer: reverse transcription at 50°C for 5 min, initial denaturation at 95°C for 20 s, and 40 cycles of amplification (95°C for 3 s, 60°C for 30 s). The primers and probe sequences were as follows (Costa-Mattioli et al., 2002): Forward: 5' TTT CCG GAG CCC CTC TTG 3'; reverse 1: 5' AAA GGG AAA TTT AGC CTA TAG CC 3'; reverse 2: 5' AAA GGG AAA ATT TAG CCT ATA GCC 3'; probe: Fam-ACT TGA TAC CTC ACC GCC GTT TGC CT-Tamra.

2.6. Standard curve

Standard curve was performed by testing 10-fold viral dilutions. The RNA was extracted from 10^{-1} to 10^{-8} dilutions of viral stock using QIAamp Viral RNA mini Kit (Qiagen Inc., Valencia, CA). The slope of the standard curve was generated by plotting the log of viral load versus the cycle threshold (CT) value. For MS2, the viral load was expressed as plaque forming units (PFU) (Bae and Schwab, 2008; Blaise-Boisseau et al., 2010; Furiga et al., 2011; Jothikumar et al., 1998; Langleta et al., 2009; Liu et al., 2011; Ogorzaly and Gantzer, 2006; Shin and Sobsey, 2003). For HAV, the viral load was expressed as TCID₅₀ (Butot et al., 2007; Di Pasquale et al., 2011; Houde et al., 2007). The amplification efficiency (E) was calculated using the following equation: $E = (10^{-\text{slope}}) - 1$ (Klein et al., 1999).

2.7. Artificial seawater

Artificial seawater was made with SeaChem Marine Salt (SeaChem Laboratories Inc. Madison, GA) and distilled water. As research shows that salinity in estuaries can range from 5 to 35 parts per thousand (ppt) (Le Saux et al, 2009; Marsden, 2004; Motes et al, 1998), the salinity of the artificial seawater was adjusted to 0, 10, 20, 30, and 40 ppt with a salinity meter (YSI Inc., Yellow Springs, OH).

2.8. Zeolite

Granular zeolite (Zeobrite® Xtreme) (kindly provided by Mr. Charles Admire and Mr. Stephen Peterson from Zeotech Co., Fortworth, TX) was used in this study. The zeolite granules had a size range of 14 × 30 mesh (0.3-1.4 mm dia). Zeobrite® Xtreme was modified by its manufacturer through a chemically bonded carbon chain that made the zeolite “dual-charged”. The modified zeolite was reported to have a total surface area of 45-60 m²/g and can attract positive particles to its interior and negatively charged particles to its surface.

2.9. Zeolite vs. granular activated carbon

Previous study has demonstrated the efficiency of granular activated carbon (Matrix Carbon™) in concentrating bacteriophage MS2 from seawater (Cormier et al., 2014). In this study, granular activated

carbon was compared to zeolite in their efficiencies to concentrate MS2 from artificial seawater. In a filtration protocol, 500 ml of artificial seawater (10 ppt) was spiked with 6~7 log PFU of MS2. The seawater was then filtered with 3 g of zeolite. Thirty microliters of sample was drawn from pre-filtered and post-filtered water and plated in the plaque assay. The same procedure was repeated with granular activated carbon. In addition, 500 ml of seawater was spiked with 6~7 log PFU of MS2 and filtered first with 3 g of granular activated carbon and subsequently with 3 g of zeolite. Thirty microliters of water sample was drawn each time after filtration and plated in the plaque assay. Alternatively, 500 ml of spiked seawater was first filtered with 3 g of zeolite and subsequently with 3 g of granular activated carbon. The quantity of MS2 adsorbed to the adsorbent would be reflected in the difference between the pre-filtered and the post-filtered water samples.

In a suspension protocol, 500 ml of seawater was spiked with 6~7 log PFU of MS2, after which 3 g of activated carbon was suspended in the seawater for 15 min with the help of a magnetic stir bar. The water was then separated from the activated carbon and filtered twice with 3 g of zeolite. Alternatively, 3 g of zeolite was suspended in 500 ml of spiked seawater for 15 min. Thirty microliters of samples were drawn from all pre-filtered and post-filtered water samples and plated in plaque assay.

2.10. Concentration and elution of MS2 with zeolite

Five hundred milliliters of seawater (10 ppt) was spiked with 6~7 log PFU of MS2 and filtered with 3 g of zeolite. One hundred forty microliters of seawater was taken before and after zeolite filtration and used for RNA extraction. One point five milliliters of each of the following eluents: 0.25% trypsin-EDTA solution (Sigma-Aldrich Co. St. Louis, MO), 0.6 M NaCl solution (pH adjusted to 2.6), 0.1% sodium dodecyl sulfate (SDS) and viral RNA lysis buffer (QIAamp Viral RNA mini kit, Qiagen Inc., Valencia, CA) was incubated with the zeolite for 30 min with mild agitation for elution (Cooney et al., 1999a; Cooney et al., 1999b; Zhao et al., 2010).

2.11. Concentration and elution of HAV with zeolite

Fifty milliliters or 500 ml of seawater (10 ppt) were spiked with 6~8 log TCID₅₀ of HAV and filtered with 2 g of zeolite. One hundred forty microliters of seawater was taken before and after zeolite filtration and used for RNA extraction. One milliliter of eluent was incubated with the zeolite for 30 min with mild agitation for elution. The eluent was separated from the zeolite after 30 min of incubation and briefly centrifuged. RNA was extracted from the supernatant with QIAamp Viral RNA mini kit as previously described and used in PCR immediately. RNA was extracted from 140 µl pre-filtered water sample and 140 µl post-filtered water sample and qPCR was performed. The quantity of HAV adsorbed to the adsorbent would be reflected in the difference between the pre-filtered and the post-filtered water samples.

2.12. Selection of optimal eluent

Sodium chloride (0.6 M, 1 M, 1.4 M, 1.8 M, 2.2 M, 2.6 M, 3 M, 3.4 M, 4.2 M, 4.6 M, 5 M, 5.4 M and 5.8 M), phosphate buffer (0.5 M; pH 1-13), glycine (0.1 M, pH 2; 0.1 M, pH 9; 0.1 M glycine with 0.5 M NaCl and 0.05% Tween 80, pH 8), beef extract (3%, pH 9; 3%, with 10% FBS; 3%, in pH 8 McIlvaine buffer; 10%, with 10% FBS; 10%, in pH 8 McIlvaine buffer), pepsin (0.25% and 10%), proteinase K (200 µg/ml), trypsin-EDTA (0.25%), urea (8 M), EDTA disodium (0.1%, pH 13; 0.1 M, pH 10; 1%, pH 13; 10%, pH 10), Triton X-100 (5% and 10%), Tween 80 (5% and 10%), and sodium dodecyl sulfate (SDS) (1%, 2%, 5%, and 10%; pH 1-13 (maintained with 0.5 M phosphate buffer)) were tested for HAV elution from zeolite and the optimal eluent was selected based on the quantity of eluted HAV.

2.13. Extraction of viral RNA from eluate

The eluate was separated from the zeolite after 30 min of incubation and briefly centrifuged. The supernatant was used for RNA extraction with QIAamp Viral RNA mini kit. Every 140 µl of the supernatant was incubated with 560 µl of lysis buffer (QIAamp Viral RNA mini kit) for 10 min at room temperature. Absolute ethyl alcohol (560 µl) was added to the lysate and mixed by vortexing. The mixture was transferred to a spin column in 630 µl aliquots. The column was washed twice, and the RNA

was eluted with 30 µl of RNase-free water containing 0.04% sodium azide and used for PCR immediately. Since EDTA can inhibit the activity of Mg^{2+} which plays a critical role in PCR, excess Mg^{2+} was added to the RNA elution buffer for EDTA eluates. NaCl was added to the SDS eluates to a 0.2 M final concentration during RNA extraction. NaCl keeps SDS soluble in 70% ethanol and minimizes precipitation with the RNA (Oswald, 2007).

2.14. Optimization of elution temperature and time span

The temperature and time span of virus elution were optimized for maximum elution efficiency. Zeolite was incubated with 1 ml of the optimal eluent for 30 min at 30°C, 42°C and 100°C, and the optimal temperature was selected based on the quantity of eluted HAV. Virus elution was then performed at 15 min, 30 min, 60 min and 120 min, for selection of the optimal time span.

2.15. Effect of salinity on concentration efficiency

The impact of salinity on the efficiency of the concentration method was tested by inoculating the same level of HAV (7.7 log units per 50 ml water) into artificial seawater of different salinities (0, 10, 20, 30 and 40 ppt) and comparing the quantity of eluted HAV under each salinity.

2.16. Large scale concentration of HAV

A total of 7.8 ± 0.2 log units of HAV were inoculated into 10 L of artificial seawater (10 ppt). Two grams of zeolite was suspended in the artificial seawater by manual stirring and 140 µl of water sample was taken after the viruses have been adsorbed to the zeolite. The adsorbed HAV was eluted from zeolite with the optimal eluent under the optimal elution temperature and time span. RNA was extracted from both the water sample and the eluate, and quantified with qPCR. The quantity of HAV in the water sample was compared to that in the eluate, and this comparison served as an indication of the efficiencies of direct extraction vs. zeolite extraction.

2.17. Statistical analysis

The effects of salinity, elution temperature and time span on the concentration/elution efficiency were analyzed statistically by comparing the means of HAV detected under each condition. Pairwise comparisons were analyzed using Tukey's HSD with JMP software(SAS Institute Inc. Cary, NC).

3. Results

3.1. Zeolite vs. granular activated carbon

The level of MS2 in the artificial seawater filtered with zeolite dropped by 90%. In contrast, the level of MS2 in the water filtered with activated carbon remained the same. Furthermore, seawater filtered first with activated carbon and then with zeolite did not have significantly less MS2 than the water filtered with only zeolite. Interestingly, water filtered first with zeolite and then with activated carbon had even more MS2 remained.

Suspending granular activated carbon in the artificial seawater reduced the level of MS2 by 60%, and further filtration with zeolite dropped the MS2 level by an additional 15~20%. Suspending zeolite in the seawater reduced the level of MS2 by 90%.

3.2. Concentration and elution of MS2 with zeolite

Interestingly, PCR amplification of the spiked seawater sample filtered with zeolite revealed that only ~60% MS2 was removed from the water, unlike the results from the plaque assay which showed a 90% reduction of MS2 after filtration. Furthermore, trypsin-EDTA, SDS and viral lysis buffer all failed in eluting MS2 from zeolite, since no positive PCR amplification was observed from eluates. Only 0.6 M NaCl had moderate success; 1.5 ml of 0.6 M NaCl solution eluted 3.7 ± 0.2 out of 7.3 ± 0.2 log PFU of MS2 from zeolite.

3.3. Concentration and elution of HAV

PCR detection of spiked water sample before and after zeolite filtration revealed a concentration efficiency of 99.8 ± 0.01 % in 50 ml seawater, and 97 ± 0.1 % in 500 ml seawater (data not shown), regardless of inoculation level ($5.5 \sim 7.8$ log TCID₅₀). PCR detected 4.0 ± 0.07 out of 7.8 ± 0.2 log units of HAV from zeolite concentration in 10 L of spiked seawater. In comparison, direct extraction of viral RNA from seawater samples detected only 2.3 ± 0.1 log units of HAV. In addition, the entire concentration and detection only took 2 h to complete.

Surprisingly, the majority of eluents tested showed inability to elute HAV from zeolite, as indicated by negative PCR amplification of the eluates. Ten percent beef extract (maintained in pH 8 Mcllvaine buffer) and SDS (5%, 10%) were the only eluents able to elute HAV from zeolite, but SDS far exceeded beef extract regarding elution efficiency. Beef extract (10% (v/v) in pH 8 Mcllvaine buffer) was only able to elute at most 3.7 out of 7.8 ± 0.2 log units of adsorbed HAV from zeolite, while 5% SDS was able to elute 5.5 ± 0.2 log units of HAV. SDS was completely unable to elute HAV at 1% and 2%, and was only effective at 5% and 10% (data not shown). In addition, 5% SDS was also far less foamy than 10% SDS and was thus far easier to handle. Furthermore, SDS (5%) dissolved in basic phosphate buffer (pH 8-12) eluted more HAV than SDS dissolved in acidic buffer (pH 1-7) (Fig 1). SDS had the highest elution efficiency in phosphate buffer of pH 11.2 (Fig 2).

3.4. Optimal elution time and temperature

Incubating zeolite with 5% SDS (pH 11.2) at 42°C eluted 0.4 ~ 0.5 log units more HAV than at 30°C, and 3.0 log units more HAV than at 100°C. Not only did the elution efficiency drop significantly at 100°C, the extreme temperature also accelerated the evaporation of eluates. Adjustment of elution time revealed optimal elution efficiency at 60 min (Fig 3).

3.5. Effect of salinity on concentration efficiency

The concentration efficiency was much higher in seawater than in fresh water. The efficiency was highest in seawater of 10 ppt, and 5.5 ± 0.2 out of 7.7 ± 0.05 log units of HAV was detected (Fig 4). Even

though the efficiency varied in water of different salinities, zeolite concentration was overall effective, being able to detect at least 4.8 log units of HAV.

4. Discussion

Consistent with the previous study (Cormier and Janes, 2014), granular activated carbon was able to remove a significant amount of MS2 from spiked seawater through 15 min of suspension. However, simply filtering the seawater through activated carbon did not result in any removal of MS2. On the other hand, zeolite was able to remove 90% of MS2 from the water through filtration, which was 30% more than what granular activated carbon removed through suspension. In addition, pre-treating seawater with activated carbon through either filtration or suspension before filtering with zeolite was slightly less effective than filtering the water with only zeolite.

Cell infectivity assay was not used in the concentration/elution of HAV due to the time-consuming nature of HAV propagation *in vitro*. Instead, qPCR was used as an indication of how efficient zeolite was in virus concentration. Results indicated that zeolite was able to adsorb up to 99% of HAV from the seawater. It appeared that zeolite was more efficient at concentrating HAV than MS2.

While NaCl solution had moderate success in eluting MS2, it failed to elute HAV from zeolite. A variety of other commonly used eluents were also unsuccessful at the task. It was theorized that MS2 was adsorbed to zeolite via electrostatic attraction, but it was unclear which type of interaction was involved in the adsorption of HAV. Anions in the seawater such as chloride have much higher ionic strength than HAV and should theoretically prevent the adsorption of HAV. The observation that zeolite concentration was even more efficient in seawater than in fresh water suggested that electrostatic attraction was not the only force involved in virus concentration. This hypothesis was further supported by the fact that sodium chloride

failed to elute HAV off zeolite. The protein capsid of virus typically contains ionizable amino acids such as glutamic acid, aspartic acid, histidine, and tyrosine (Gerba, 1984). Like many organic chemicals, these individual carboxyl and amino groups, depending on the pH of the surrounding environment, can gain or lose a proton giving the capsid a net electrical charge. Under ambient conditions, most of the enteric viruses are negatively charged (Fong and Lipp, 2005; Sobsey et al., 1973; Sobsey et al., 1995). Since the zeolite used in this study was modified to have “dual charge” ion exchange property, the ionic strength of the eluent was gradually increased to find the optimal salt concentration at which point the viruses would be eluted. Increasing ionic strength has long been recognized as a way to weaken the bond between virus particles and the adsorbent (Pierce, 1974; Zhuang and Jing, 2003). This was attributed to the effect of ion shielding, which at higher ionic strength decreased the electrostatic attraction between the viral particles and the surface of the adsorbent and consequently decreased virus sorption. Since NaCl was the only solute in the eluent, the ionic strength of the eluent depended only on the concentration of the NaCl solution. Theoretically by increasing the salt concentration (salt gradient) in the eluent, the molecules bond to the adsorbent should start to elute. However, it was observed that by increasing the concentration of sodium chloride to its saturation (5.8 M), HAV was still not detected in the eluate.

Phosphate buffer has also been documented to be used as an eluent, due to its ability to enhance ionic strength of the solution (Chu et al., 2000; Storkus et al., 1993; Zhuang and Jing, 2003). Chu and others (2000) discovered that MS2 was removed from sand columns in high ionic strength phosphate buffer. The most commonly used phosphate buffers consist of a mixture of monobasic dihydrogen phosphate and dibasic monohydrogen phosphate. By varying the amount of each salt, a range of buffers can be prepared. Phosphates have a very high buffering capacity and are highly soluble in water (DeAngelis, 2007). Phosphate buffer was also unable to elute HAV; however, the presence of phosphate buffer significantly enhanced the eluting ability of SDS, suggesting a synergistic effect.

Glycine was recommended as the standard eluent for the recovery of enteric viruses from water, wastewater, and coastal sediments (Gerba et al., 1977a; Gerba et al., 1977b; Gerba et al., 1978; Gerba,

1980; LaBelle and LaBelle et al., 1980; LaBelle and Gerba, 1982; Tsai et al., 1983). As an alkaline proteinaceous substance, beef extract has been used for recovery of enteric viruses from water and was documented to achieve high recovery rate of virus elution from glass cartridge fiber (Berg et al., 1971; Katzenelson et al., 1976; Landry et al., 1978; Wellings et al., 1975; Wellings et al., 1976). While beef extract had limited ability to elute HAV, glycine was completely incapable, indicating that the bonding force between HAV particles and the zeolite was particularly strong.

The attempt at using chelation to elute HAV was also unsuccessful. EDTA (ethylenediaminetetraacetic acid) is an organic chelating agent and used widely to sequester metal ions such as Ca^{2+} and Al^{3+} . Once bound to EDTA, these metal centers tend not to form precipitates or to interfere with the action of other substrates in the environment (Bartlett et al., 1972; Bisque, 1961). EDTA has a high affinity for aluminum and was documented to remove aluminum atoms from zeolite (Beaumont and Barthomeuf, 1972). Since aluminum is responsible for the ion-exchange property of zeolite, it was expected that EDTA would sequester aluminum and reverse any electrostatic attraction formed by zeolite. Due to the incapability of EDTA to elute HAV, it was further concluded that electrostatic attraction was not the main contribution to the adsorption of HAV.

Nonionic surfactants Triton X-100 and Tween 80 and ionic surfactant SDS are commonly used in virus adsorption/elution to control the surface tension of the fluid and to prevent virus adsorption. While Triton X-100 would be expected to affect nonionic adsorption only, the anionic SDS would be expected to affect both ionic and nonionic adsorption and specifically counteract the adsorption forces (American Society for Testing and Materials, 1995; Fujito, B.T., Lytle, C.D. 1996; Lytle et al., 1992; Retta et al., 1991). Unlike Triton X-100 and Tween 80, SDS denatures protein in addition to solubilizing them. The success of SDS in eluting HAV and the incapability of Triton X-100 and Tween 80 suggested that protein denaturation was necessary to breaking off the bond between HAV and zeolite.

The basic building blocks of zeolite form a network of pores in the crystal, and the pores have dimensions that are comparable with the size of the molecules that can be adsorbed (Smit and Maesen, 2008). The zeolite used in this study have modified surface and can be used as an amphoteric ion-exchanger. The interaction between zeolite and HAV is likely to be surface-based adhesion, and could be either physisorption or chemisorption. The fundamental interacting force of physisorption is caused by van der Waals force, thus the adsorption is non-specific and reversible and can occur at low temperature. Chemisorption on the other hand involves a chemical reaction between the surface and the adsorbate, and is thus highly specific and relatively irreversible. Ion-exchange is a type of chemisorption (Oura et al., 2003). Zeolite easily removed 99% of the suspended HAV from the seawater at room temperature, thus the attraction between zeolite and HAV is more likely to be a form of chemisorption. Due to the ion-exchange property of zeolite and weak negative charge of HAV, we propose to consider the concentration via a protein ion-exchange model. Established protein ion-exchange models suggest that the adsorption of protein onto an adsorbent is primarily electrostatic attraction, but also involve interplay of colloidal van der Waals, short-range repulsion forces, short-range attraction, surface charge density, and size dependent interaction (Roth et al., 1994). We theorized that the pore size of the zeolite used in this study was very close to the size of HAV capsid protein, thus the virus particles that were adsorbed onto zeolite remained in the pores and since adsorption occurs via short-distance interaction, common eluents could not access the pores that were occupied by the viruses. The sole success of SDS suggested that a protein denaturant was necessary in releasing HAV from the pores, possibly by unfolding the capsid protein and releasing the viral RNA. The failure of protease to release adsorbed HAV may be due to the size of the protease too large to access the pores.

Since zeolite was able to concentrate 99% of the virus from the seawater, once the seawater volume is scaled up, the concentration/elution method will be very effective and the sensitivity will be even more profound compared to direct detection.

In conclusion, zeolite can effectively concentrate HAV from seawater without the need to modify the acidity or salinity of the seawater, and only a small volume of eluent (1 ml) is needed to elute virus particles from zeolite, with no secondary concentration procedure necessary. Zeolite could potentially be used for virus concentration from shellfish growing waters at low cost, and be conveniently transported to the lab for analysis. Compared to current methods, this method does not involve complex machinery, and can be done in approximately 2 h.

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References

- Abbaszadegan, M., Monteiro, P., Ouwens, R.N., Ryu, H., Alum, A., 2006. Removal and inactivation of *Cryptosporidium* and microbial indicators by a quaternary ammonium chloride (QAC)-treated zeolite in pilot filters. J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng. 41(6), 1201-1210.
- American Society for Testing and Materials. 1995. Standard test method for resistance of materials used in protective clothing to penetration by bloodborne pathogens using viral penetration as a test system. Standard F16 71-95.
- Arnal, C., Ferre-Aubineau, V., Besse, B., Mignotte, B., Schwartzbrod, L., Billaudel, S., 1999. Comparison of seven RNA extraction methods on stool and shellfish samples prior to hepatitis A virus amplification. J. Virol. Methods. 77, 17-26.
- Bae, J., Schwab, K.J., 2008. Evaluation of Murine norovirus, Feline calicivirus, Poliovirus, and MS2 as surrogates for Human norovirus in a model of viral persistence in surface water and groundwater. Appl. Environ. Microbiol. 74, 477-484.

392 Bartlett, R. J., Riego, D. C., 1972. Effect of chelation on the toxicity of aluminum. *Plant. Soil.* 37(2),
393 419-423.

394 Beaumont, R., Barthomeuf, D., 1972. X, Y, aluminum-deficient and ultrastable faujasite-type zeolites: I.
395 Acidic and structural properties. *J. Catal.* 26(2), 218-225.

396 Berg, G., Dahling, D. R., Berman, D., 1971. Recovery of small quantities of viruses from clean waters
397 on cellulose nitrate membrane filters. *Appl. Microbiol.* 22, 608-614.

398 Bisque, R.E., 1961. Analysis of carbonate rocks for calcium, magnesium, iron, and aluminum with
399 EDTA. *J. Sediment. Res.* 31(1), 113-122.

400 Blaise-Boisseau, S., Hennechart-Collette, C., Guillier, L., Perelle, S., 2010. Duplex real-time qRT-PCR
401 for the detection of hepatitis A virus in water and raspberries using the MS2 bacteriophage as a process
402 control. *J. Virol. Methods.* 166 (1–2), 48–53.

403 Butot, S., Putallaz T., Sánchez G., 2007. Procedure for Rapid Concentration and Detection of Enteric
404 Viruses from Berries and Vegetables. *Appl. Environ. Microbiol.* 73(1), 186–192.

405 Bright, K. R., Sicaire-Ruelas, E. E., Gundy, P. M., Gerba, C. P., 2009. Assessment of the Antiviral
406 Properties of Zeolites Containing Metal Ions. *Food. Environ. Virol.* 1(1), 37-41.

407 Chu, Y., Jin, Y., Yates, M.V., 2000. Virus transport through saturated sand columns as affected by
408 different buffer solutions. *J. Environ. Qual.* 29, 1103-1110.

409 Conaty, S., Bird, P., Bell, G., Kraa, E., Grohmann, G., McAnulty, J. M., 2000. Hepatitis A in New South
410 Wales, Australia, from consumption of oysters: the first reported outbreak. *Epidemiol. Infect.* 124, 121-
411 130.

412 Cooney, E.L., Booker, N.A., Shallcross, D.C., Stevens, G.W., 1999. Ammonia Removal from
413 Wastewaters Using Natural Australian Zeolite. I. Characterization of the Zeolite. *Separ. Sci. Technol.*
414 34(12), 2307-2327.

415 Cooney, E.L., Booker, N.A., Shallcross, D.C., Stevens, G.W., 1999. Ammonia Removal from
416 Wastewaters Using Natural Australian Zeolite. II. Pilot Scale Study Using Continuous Packed Column
417 Process. *Separ. Sci. Technol.* 34(14), 2741-2760.

418 Costa-Mattioli, M., Monpoeho, S., Nicand, E., Aleman, M.H., Billaude, S., Ferre', V., 2002.
 419 Quantification and duration of viraemia during hepatitis A infection as determined by real-time RT-PCR.
 420 J. Viral. Hepat. 9, 101-106

421 Cormier, J., Janes, M., 2014. A double layer plaque assay using spread plate technique for enumeration
 422 of bacteriophage MS2. J. Virol. Methods. 196, 86–92.

423 Cormier, J., Gutierrez M., Goodridge, L., Janes, M. 2014. Concentration of enteric virus indicator from
 424 seawater using granular activated carbon. J. Virol. Methods. 196, 212–218.

425 Davis, M.E., Lobo, R. F. 1992. Zeolite and molecular sieve synthesis. Chem. Mater. 4 (4), 756–768.

426 DeAngelis, K.M. 2007. Phosphate buffer. Available at:
 427 <http://www.cnr.berkeley.edu/soilmicro/methods/phosphate%20buffer.pdf>. Accessed 12 January 2013.

428 Desenclos, J.C., Klontz, K.C., Wolfe, L.E., Hoecherl, S., 1991. The risk of Vibrio illness in the Florida
 429 raw oyster eating population, 1981-1988. Am. J. Epidemiol. 134(3), 290-297

430 Di Pasqualea, S., Paniconia, M., Auricchioa, B., Oreficea, L., Schultzb, A.C., De Medicia, D., 2010.
 431 Comparison of different concentration methods for the detection of hepatitis A virus and calicivirus from
 432 bottled natural mineral waters. J. Virol. Methods. 165(1), 57-63.

433 Fong, T.T., Lipp, E.K., 2005. Enteric Viruses of Humans and Animals in Aquatic Environments: Health
 434 Risks, Detection, and Potential Water Quality Assessment Tools. Microbiol. Mol. Biol. Rev. 69(2), 357.

435 Fujito, B.T., Lytle, C.D., 1996. Elution of viruses by ionic and nonionic surfactants. Appl. Environ.
 436 Microbiol. 62(9), 3470-3473.

437 Furiga, A., Pierre, G., Glories, M., Aimar, P., Roques, C., Causserand, C., Berge, M., 2011. Effects of
 438 ionic strength on bacteriophage MS2 behavior and their implications for the assessment of virus
 439 retention by Ultrafiltration membranes. Appl. Environ. Microbiol. 77(1), 229–236.

440 Gantzer, C., Senouci, S., Maul, A., Levi, Y., Schwartzbrod, L., 1999. Enterovirus detection from
 441 wastewater by RT-PCR and cell culture. Water Sci. Technol. 40, 105-109.

442 Gerba, C. P., Goyal, S. M., Snith, E. M., Melnick, J. L., 1977. Distribution of viral and bacterial
 443 pathogens in a coastal canal community. Mar. Pollut. Bull. 8, 279-282.

444 Gerba, C. P., Smith, E. M., Melnick, J. L., 1977. Development of a quantitative method for detecting
 445 enteroviruses in estuarine sediments. Appl. Environ. Microbiol. 34, 158-163.

446 Gerba, C. P., Smith, E. M., Schaiberger, G. E., Edmond, T. D., 1978. Field evaluation of methods for the
 447 detection of enteric viruses in marine sediments, p. 64-74. In C. D. Litchfield and P. L. Seyfried (ed.),
 448 Methodology for biomass determinations and microbial activities in sediments. ASTM Special Technical
 449 Publication 673. American Society for Testing and Materials, Philadelphia, PA.

450 Gerba, C.P., 1984. Applied and theoretical aspects of virus adsorption to surfaces. *Adv. Appl. Microbiol.*
 451 30,133-168.

452 Griffin, D.W., Donaldson, K.A., Paul, J.H., Rose, J.B., 2003. Pathogenic Human Viruses in Coastal
 453 Waters. *Clin. Microbiol. Rev.* 16(1), 129-143.

454 Guillois-Becel, Y., Couturier E., Le Saux, J.C., Roque-Afonso, A. M., Le Guyader, F.S., Le Goas, A.,
 455 Pernes, J., Le Behec, S., Briand, A., Robert, C., Dussaix, E., Pommepuy, M., Vaillant, V., 2009. An
 456 oyster-associated hepatitis A outbreak in France in 2007. *Euro. Surveill.* 14(10), 1-6.

457 Hollinger, F., Ticehurst, J., 1996. Hepatitis A virus. In: *Fields Virology* (Fields, B.N., Knipe, D.M.,
 458 Howley, P. et al., Eds.), pp. 735-782. Lippincott-Raven, Philadelphia, PA.

459 Houde, A., Guevremont, E., Poitras, E., Leblanc, D., Ward, P., Simard, C., and Trottier, Y.L., 2007.
 460 Comparative evaluation of new TaqMan real-time assays for the detection of hepatitis A virus. *J. Virol*
 461 *Methods.* 140, 80–89.

462 Imai, K., Ogawa, H., Bui, V. N., Inoue, H., Fukuda, J., Ohba, M., Yamamoto, Y., Nakamura, K., 2012.
 463 Inactivation of high and low pathogenic avian influenza virus H5 subtypes by copper ions incorporated
 464 in zeolite-textile materials. *Antivir. Res.* 93(2), 225–233.

465 Jiang, S., Noble, R., Chui, W. P., 2001. Human adenoviruses and coliphages in urban runoff-impacted
 466 coastal waters of Southern California. *Appl. Environ. Microbiol.* 67,179–184.

467 Jothikumar, N., Cliver, D.O., Mariam, T.W., 1998. Immunomagnetic capture PCR for rapid
 468 concentration and detection of hepatitis A virus from environmental Sam-ples. *Appl. Environ.*
 469 *Microbiol.* 64 (2), 504–508.

470 Katayama, H., Shimasaki, A., Ohgaki, S., 2002. Development of a virus concentration method and its
 471 application to detection of enterovirus and Norwalk virus from coastal seawater. *Appl. Environ.*
 472 *Microbiol.* 68, 133–139.

473 Katzenelson, E., Fattal, B., Hostovesky, T., 1976. Organic flocculation: an efficient second-step
 474 concentration method for the detection of viruses in tap water. *Appl. Environ. Microbiol.* 32, 638-639.

475 Klein, D., Janda, P., Steinborn, R., Müller, M., Salmons, B., Günzburg, W. H., 1999. Proviral load
476 determination of different feline immunodeficiency virus isolates using real-time polymerase chain
477 reaction: influence of mismatches on quantification. *Electrophoresis*. 20, 291-299.

478 Koopmans, M., von Bonsdorff, C. H., Vinjé, J., de Medici, D., Monroe, S., 2002. Foodborne viruses.
479 *FEMS. Microbiol. Lett.* 26, 187-205.

480 LaBelle, R., Gerba, C. P., 1980. Influence of estuarine sediment on virus survival under field conditions.
481 *Appl. Environ. Microbiol.* 39, 749-755.

482 LaBelle, R., Gerba, C. P., Goyal, S. M., Melnick, J. L., Cech, I., Bodgan, G. F., 1980. Relationships
483 between environmental factors, bacterial indicators, and the occurrence of enteric viruses in estuarine
484 sediments. *Appl. Environ. Microbiol.* 39, 588-596.

485 LaBelle, R., Gerba, C. P., 1982. Investigations into the protective effect of estuarine sediment on virus
486 survival. *Water. Res.* 16, 469-478.

487 Landry, E. F., Vaughn, J. M., Thomas, M. Z., Vicale, T. J., 1978. Efficiency of beef extract for the
488 recovery of poliovirus from wastewater effluents. *Appl. Environ. Microbiol.* 36, 544-548.

489 Langleta, J., Ogorzalya, L., Schrotterb, J.C., Machinalb, C., Gaboriaudc, F., Duvald, J.F.L., Gantzer, C.,
490 2009. Efficiency of MS2 phage and Q phage removal by membrane fil-tration in water treatment:
491 applicability of real-time RT-PCR method. *J. Membr. Sci.* 326, 111–116.

492 Le Guyader, F.S., Atmar, R.L., Le Pendu, J., 2012. Transmission of viruses through shellfish: when
493 specific ligands come into play. *Curr. Opin. Virol.* 2(1), 103-110.

494 Le Saux, J.C., Le Vaillant, G., Guillermou, G., Vilbas, J.V., Deter, J., Delyon, B., Le Guyader, S.,
495 Pommeupuy, M., 2009. Prediction of faecal contamination in shellfish production areas: Interest and
496 limits of the salinity parameter. *ICMSS09 – Nantes, France – June 2009*.

497 Lipp, E. K., Lukasik, J., Rose, J. B., 2001. Human enteric viruses and parasites in the marine
498 environment. *Methods. Microbiol.* 30, 559-588.

499 Liu, J., Kibiki, G., Maro, V., Maro, A., Kumburu, H., Swai, N., Taniuchi, M., Gratz, J., Toney, D., Kang,
500 G., Houpt, E., 2011. Multiplex reverse transcription PCR luminex assay for detection and quantitation of
501 viral agents of gastroenteritis. *J. Clin. Virol.* 50 (4), 308–313.

502 Lukasik, J., Scott, T. M., Andryshak, D., Farrah, S. R., 2000. Influence of salts on virus adsorption to
503 microporous filters. Appl. Environ. Microbiol. 66, 2914-2920.

504 Lytle, C. D., Routson, L. B., Cyr, W. H., 1992. A simple method to test condoms for penetration by
505 viruses. Appl. Environ. Microbiol. 58, 3180-3182.

506 Marsden, I.D., 2004. Effects of reduced salinity and seston availability on growth of the New Zealand
507 little-neck clam *Austrovenus stutchburyi*. M.E.P.S. 266, 157–171.

508 Mason, J.O., McLean, W.R., 1962. Infectious hepatitis traced to the consumption of raw oysters. Am. J.
509 Epidemiol. 75(1), 90-111.

510 McLeod, C., Hay, B., Grant, C., Greening, G., Day, D., 2009. Inactivation and elimination of human
511 enteric viruses by Pacific oysters. J. Appl. Microbiol. 107(6), 1809-1818.

512 Motes, M. L., DePaola, A., Cook, D. W., Veazey, J. E., Hunsucker, J. C., Garthright, W. E., Blodgett,
513 R. J., Chirtel, S. J., 1998. Influence of Water Temperature and Salinity on *Vibrio vulnificus* in Northern
514 Gulf and Atlantic Coast Oysters (*Crassostrea virginica*). Appl. Environ. Microbiol. 64(4): 1459-1465.

515 Nappier S.P., Graczyk, T.K., Schwab, K.J., 2008. Bioaccumulation, retention, and depuration of enteric
516 viruses by *Crassostrea virginica* and *Crassostrea ariakensis* oysters. Appl. Environ. Microbiol. 74(22),
517 6825-6831.

518 Niu, Q., Feng, N., 2005. Effect of modified zeolite on the expansion of alkaline silica reaction. Cement.
519 Concrete. Res. 35, 1784-1788.

520 Noble, R. T., Fuhrman, J. A., 2001. Enteroviruses detected by reverse transcriptase polymerase chain
521 reaction from the coastal waters of Santa Monica Bay, California: low correlation to bacterial indicator
522 levels. Hydrobiologia. 460, 175–184.

523 O’Connell, K.P., Bucher, J.R., Anderson, P.E., Cao, C.J., Khan, A.S., Gostomski, M.V., Valdes, J.J.,
524 2006. Real time fluorogenic reverse transcription PCR assays for detecting bacteriophage MS2. Appl.
525 Environ. Microbiol. 72 (1), 478–483.

526 Ogorzaly, L., Gantzer, C., 2006. Development of real-time RT-PCR methods for specific detection of F-
527 specific RNA bacteriophage genogroups: application to urban raw wastewater. J. Virol. Methods. 138,
528 131–139.

529 Oswald, N., 2007. Ethanol Precipitation of DNA and RNA: How it works. In: Cloning and Expression.
 530 Available at: <http://bitesizebio.com/articles/the-basics-how-ethanol-precipitation-of-dna-and-rna-works/>.
 531 Accessed 12 August 2012.

532 Oura, K., Lifshits, V.G., Saranin, A., Zotov, A.V., Katayama, M., 2003. Surface Science, An
 533 Introduction. Berlin: Springer.

534 Pallin, R., Wyn-Jones, A. P., Place, B. M., Lightfoot, N. F., 1997. The detection of enteroviruses in large
 535 volume concentrates of recreational waters by the polymerase chain reaction. J. Virol. Methods. 67, 57-
 536 67.

537 Pierce, S., Strauss, E.G., Strauss, J.H., 1974. Effect of Ionic Strength on the Binding of Sindbis Virus to
 538 Chick Cells. J. Virol. 13(5), 1030-1036.

539 Protocol: TaqMan® Fast Virus 1-Step Master Mix. Available at:
 540 http://tools.lifetechnologies.com/content/sfs/manuals/cms_084608.pdf. Accessed 12 January 2011.

541 Rao, V. C., Melnick, J.L., 1986. Environmental Virology. In J.A. Cole, C.J. Knowles, and D.
 542 Schlessinger (eds), Aspects of Microbiology. American Society for Microbiology, Washington, DC, vol,
 543 13.

544 Retta, S. M., Herman, W. A., Rinaldi, J. E., Carey, R. F., Herman, B. A., Athey, T. W., 1991. Test
 545 method for evaluating the permeability of intact prophylactics to viral-size microspheres under simulated
 546 physiologic conditions. Sex. Transm. Dis. 18, 111-118.

547 Roth, C.M., Ungerb, K.K., Lenhoff, A.M., 1996. Mechanistic model of retention in protein ion-exchange
 548 chromatography. J. Chromatogr. A. 726, 45-56
 549

550 Saganuwan, S.A., 2011. A modified arithmetical method of Reed and Muench for determination of a
 551 relatively ideal median lethal dose (LD50) Afr. J. Pharm. Pharmacol. 5(12), 1543-1546.

552 Schiff, G. M., Stefanovi, G. M., Young, B., Pennekamp, J. K., Minimum human infectious dose of
 553 enteric viruses (echovirus-12) in drinking water, 1984, p. 222–228. In J. L. Melnick (ed.), Enteric viruses
 554 in water, vol.15. Karger, Basel, Switzerland.

555 Schulze-Makuch, D., Pillai, S. D., Guan, H., Bowman, R., Couroux, E., Hielscher, F., Totten, J.,
 556 Espinosa, I. Y., Kretzschmar, T., 2002. Surfactant-modified zeolite can protect drinking water wells
 557 from viruses and bacteria. Eos. Trans. 83(18), 193–201.

558 Schulze-Makuch, D., Bowman, R. S., Pillai, S. D., Guan, H., 2003. Field Evaluation of the Effectiveness
559 of Surfactant Modified Zeolite and Iron-Oxide-Coated Sand for Removing Viruses and Bacteria from
560 Ground Water. *Ground. Water. Monit. R.* 23, 68–74.

561 Shieh, Y.C., Khudyakov, Y.E., Xia, G., Ganova-Raeva, L.M., Khambaty, F.M., Woods, J.W., Veazey,
562 J.E., Motes, M.L., Glatzer, M.B., Bialek, S.R., Fiore, A.E., 2007. Molecular Confirmation of Oysters as
563 the Vector for Hepatitis A in a 2005 Multistate Outbreak. *J. Food. Prot.* 70(1), 145-150.

564 Shin, G. A., Sobsey, M. D., 2003. Reduction of Norwalk Virus, Poliovirus 1, and Bacteriophage MS2 by
565 Ozone Disinfection of Water. *Appl. Environ. Microbiol.* 69(7), 3975-3978.

566 Smit, B., Maesen, T.L.M., 2008. Molecular Simulations of Zeolites: Adsorption, Diffusion, and Shape
567 Selectivity. *Chem. Rev.* 108, 4125–4184.

568 Sobsey, M. D., Wallis, C., Hendersen, M., Melnick, J. L., 1973. Concentration of enteroviruses from
569 large volumes of water. *Appl. Microbiol.* 26, 529-534.

570 Sobsey, M., Battigelli, D., Handzel, T., Schwab, K., 1995. Male-specific coliphages as indicators of viral
571 contamination of drinking water. AWWA Research Foundation, Denver, CO.

572 Storkus, W.J., Zen, H.J. III, Salter, R.D., Lotze, M.T., 1993. Identification of T-Cell Epitopes: Rapid
573 Isolation of Class I-Presented Peptides from Viable Cells by Mild Acid Elution. *J. Immunother.* 14, 94-
574 103.

575 Tsai, S.C., Ellender, R.D., Johnson, R.A., Howell, F.G., 1983. Elution of viruses from coastal sediments.
576 *Appl. Environ. Microbiol.* 46(4), 797-804.

577 Wang, D., Wu, Q., Yao, L., Wei, M., Kou, X., Zhang, J., 2008. New target tissue for food-borne virus
578 detection in oysters. *Lett. Appl. Microbiol.* 47(5), 405-409.

579 Wellings, F. M., Lewis, A. L., Mountain, C. W., Pierce, L.V., 1975. Demonstration of virus in
580 groundwater after effluent discharge onto soil. *Appl. Microbiol.* 29, 751-757.

581 Wellings, F.M., Lewis, A. L., Mountain, C. W., 1976. Demonstration of solids-associated virus in
582 wastewater and sludge. *Appl. Environ. Microbiol.* 31, 354-358.

583 Widiastuti, N., Wu, H., Ang, M., Zhang, D. K., 2008. The potential application of natural zeolite for
584 greywater treatment. *Desalination.* 218 (1–3), 271–280.

Zhao, M., Sun, L., Fu, X., Gong, X., 2010. Influence of ionic strength, pH, and SDS concentration on subunit analysis of phycoerythrins by SDS-PAGE. *Appl. Biochem. Biotechnol.* 162(4), 1065-1079.

Zhuang, J., Jin, Y., 2003. Virus retention and transport in Al-oxide coated sand columns: effects of ionic strength and composition. *J. Contam. Hydrol.* 60, 193-209.

Figure legends

Figure 1. Effect of eluent pH on the efficiency of the concentration/elution process. Fifty milliliters of 10 ppt artificial seawater was spiked with 5.91 log units of HAV and filtered with 2 g of zeolite. Five percent SDS solutions of pH 1 to 13 were used as eluents and the amount of eluted HAV under each condition was displayed.

Figure 2. Further optimization of eluent pH. Fifty milliliters of 10 ppt artificial seawater was spiked with 7.35 ± 0.05 log units of HAV and filtered with 2 g of zeolite. Five percent SDS solutions of pH 11 to 12 were used as eluents and the amount of eluted HAV under each condition was analyzed using Tukey's HSD with JMP software. Means that are denoted by the same letter are not statistically different.

Fig. 3. Effect of elution time on the efficiency of the concentration/elution process. Fifty milliliters of 20 ppt artificial seawater was spiked with 7.7 ± 0.05 log units of HAV and filtered with 2 g of zeolite. The zeolite was subsequently incubated with 5% SDS (pH 11.2) for 15 to 120 min at 42 °C and the amount of eluted HAV under each condition was analyzed using Tukey's HSD with JMP software. Means that are denoted by the same letter are not statistically different.

Fig. 4. Effect of salinity on the efficiency of the concentration/elution process. Fifty milliliters of artificial seawater (0 to 40 ppt) was spiked with 7.7 ± 0.05 log units of HAV and filtered with 2 g of zeolite. The zeolite was subsequently incubated with 5% SDS (pH 11.2) for 60 min at 42 °C and the amount of eluted HAV under each condition was analyzed using Tukey's HSD with JMP software. Means that are denoted by the same letter are not statistically different.

Figure 1

Figure 2

Figure 3

Figure 4