Efficiency of Reovirus Concentration from Water with Positively Charged Filters

Walter Q. Betancourt*¹, Sherif Abd-Elmaksoud², Charles P. Gerba¹

The University of Arizona¹

Water & Energy Sustainable Technology (WEST) Center

2959 W Calle Agua Nueva

Tucson, AZ 85745

**Environmental Virology Laboratory², Department of Water Pollution Research, National Research
Centre, Cairo, Egypt.**

Corresponding author*:

Phone: (520)-621-6163

Mobile: (520)-539-4827

wbetancourt@email.arizona.edu

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

Abstract

This study examined the efficacy of reovirus concentration from large volumes of water using two positively charged filters: Zeta Plus 1MDS and NanoCeram. The results indicated that an average of 61% and 81% of input reoviruses were effectively recovered, respectively, from recycled water and tap water using NanoCeram filtration.

Keywords: reovirus, recovery, concentration, water

Acknowledgments

The authors acknowledge financial support provided by the United States Department of Agriculture-National Institute of Food and Agriculture, Grant number 20166800725064, that established CONSERVE: A Center of Excellence at the Nexus of Sustainable Water Reuse, Food and Health. The authors thankfully acknowledge critical review of the manuscript by Kelly Bright.

The potential significance of reovirus in water has generally been overlooked because of uncertainty in the nature of illness caused by this virus in humans. Reovirus appears to occur in greater or equal numbers than all enteric viruses in domestic wastewater and many surface waters; however, evidence of a plausible waterborne transmission has not been documented (Betancourt and Gerba, 2016). Aulicino *et al.* (1996) reported the highest (100%) detection rate of reovirus in primary and secondary treated wastewater using electropositive membranes (Virosorb 1 MDS-AMF Comp., Cuno Division, Meriden, U.S.A.) and cell culture combined with virus identification by polyacrylamide gel electrophoresis (PAGE). In addition, Sedmak *et al.* (2005) reported more frequent detection of reovirus (91%) than enteroviruses (85%) and adenoviruses (65%) in influent and treated effluent (reovirus only was detected in 29% of samples) using organic flocculation and cell culture combined with a direct fluorescent antibody assay. More recent studies conducted by Qiu *et al.* (2015) using NanoCeram filters reported infectious reovirus as the only enteric virus detected by an integrated cell culture – real time polymerase chain reaction assay (ICC-qPCR) after chlorine disinfection and ultrafiltration of the final effluent. These studies and others (Betancourt and Gerba, 2016) suggest the relative resistance of reovirus to wastewater treatment processes compared to other enteric viruses. However, no published literature could be found on the efficiency of reovirus concentration from waters by the use of these positively charged filters. The goal of this study was to determine the efficiency of reovirus concentration by positively charged filters. The most commonly used positively charged filters for the concentration of viruses from large volumes of water are the Zeta Plus 1MDS and NanoCeram. Both filters are components of the United States Environmental Protection Agency's (USEPA) Method 1615 developed with the goal of providing a standardized approach for the measurement of enterovirus and norovirus occurrence by cell culture and reverse transcription real time polymerase chain reaction RT-qPCR (Cashdollar and Wymer 2013; Fout and Cashdollar 2016).

Reovirus Type 3 Dearing strain (T3D) was obtained from the American Type Culture Collection (ATCC® VR824TM; Manassas, VA) and propagated in monolayers of LLC-MK2 cells (ATCC® CCL7.1™; Manassas, VA). Reovirus titration and quantification in recovery efficiency assays were performed using the Reed-Muench method to determine the tissue culture infectious dose that affected 50% of the wells (TCID₅₀) (Payment and Trudel 1993). Similarly, poliovirus 1 (PV-1; strain LSc-2ab) obtained from the

Department of Virology and Epidemiology at the Baylor College of Medicine (Houston, TX) was propagated and titrated in monolayers of Buffalo green monkey kidney (BGM) cells (obtained from Dan Dahling at the United States Environmental Protection Agency, Cincinnati, OH) and used for virus spikes and recovery efficiency assays.

Tucson municipal tap water and tertiary treated wastewater were the water matrices used for the recovery efficiency experiments. Average values of pH, total dissolved solids (TDS), turbidity and conductivity for tap water were 7.65, 335 mg/L (± 25 mg/L), 0.28 NTU (± 0.14 NTU), and 882 $\mu\text{S}/\text{cm}$ (± 44 $\mu\text{S}/\text{cm}$), respectively. Total organic carbon was less than 1 mg/L. Tertiary treated wastewater was produced in a facility utilizing primary sedimentation dissolved air flotation (DAF), four parallel five-stage Bardenpho processes, disc filtration, and chlorination disinfection. The effluent water quality parameters determined at the time of sample collection were pH 7.12 – 7.28, TDS 837 mg/L, biological oxygen demand (BOD) 2-5.6 mg/L, and turbidity 1.70 NTU.

One hundred to 1000 liters of dechlorinated tap water and tertiary treated wastewater seeded with 10^7 TCID₅₀/mL of reovirus or poliovirus 1 were filtered through sterilized electropositive NanoCeram VS2.5-5 (Argonide Corporation, Sanford, FL) and Zeta Plus Virosorb 1MDS (Cuno, Meriden, CT) cartridge filters at an average flow rate of 4.77 ± 1.40 liters per minute. After the primary virus concentration on the NanoCeram filters, viruses were eluted by passing 420 mL of 1.0% (wt/vol) sodium polyphosphate (NaPP; Sigma-Aldrich, St. Louis, MO) solution with 0.05 M glycine (pH 9.0) through the filter under positive pressure (N₂ gas) as previously described (Ikner et al. 2011). Secondary virus concentration was performed using Centricon Plus-70 ultrafilter (100-kDa cutoff; Millipore, Billerica, MA). Briefly, a 70-ml volume of the NanoCeram filter eluate was added to the Centricon filter and concentrated via centrifugation (1,900 X g for 8 min). The viral concentrate was collected via inversion of the filter followed by further centrifugation for 2 min at 800 X g obtaining an average volume of 3.5 ± 0.5 ml for the secondary concentrates. For the 1MDS filters, the elution of viruses involved two separate elution steps with 500 ml each of 1.5% beef extract (BD Bacto™ Beef Extract, catalog no. 211520; pH 9.0, Sparks, MD) containing 0.05 M glycine (Fisher Scientific, NJ). Viruses were further concentrated by organic

flocculation of the beef extract at pH 3.5 as outlined in the 1996 U.S. EPA Information Collection Rule method (ICR, EPA/600/R-95/178, April 1996) (Fout et al. 1996). Organic flocculation as a secondary concentration step for viruses with the NanoCeram filter was also used for comparison purposes. The overall method efficiency for the recovery of both viruses was based on the number of viruses in the secondary concentrate compared with an equivalent volume from the original 100- to 1000-liter water sample (e.g., 70 ml/420 ml total eluate concentrated using Centricon filter $\approx$ 16.67 liters/100 liters total influent). The number of viruses in the secondary concentrate was then divided by the number of viruses from the equivalent volume examined and then multiplied by 100. The cell culture infectivity assay was used in the recovery efficiency experiments to quantitatively evaluate any effect on virus infectivity introduced by the method.

The results of this study indicate that NanoCeram filtration combined with NaPP for virus elution and ultrafiltration for secondary virus concentration provided higher recovery efficiencies of reovirus and poliovirus from recycled water than organic flocculation (Table 1). Reovirus recovery and concentration with the salt-based solution was more effective than the proteinaceous (beef extract) solution. However, larger variability in viral recovery was observed for recycled water than tap water, more likely associated with the presence of unidentified factors in the recycled water affecting the adsorption and/or elution components of the recovery method. The evaluation of such factors affecting the recovery of reovirus from recycled water was beyond the scope of this research. Our study was conducted to evaluate the recovery efficiency of reovirus by two adsorption-elution procedures commonly used for the recovery of enteric viruses from large volumes of water. The latter is critical to estimate actual levels of human viruses released by full-scale wastewater treatment processes that can be affected by technical and management deficiencies leading to human exposure via recycled water (Gerba et al. 2017).

Wastewater involves large concentrations of organic substances such as humic acid and proteins that exhibit colloidal behavior similar to virus particles. The presence of these proteinaceous and other colloidal substances (suspended-mineral colloids or low-density phytoplankton) competing with viruses for adsorption sites may adversely affect the efficiency of virus recovery and concentration from complex

water matrices such as recycled water. Studies have shown that the filter surface charge and surface area can also influence virus adsorption capacity, with larger filter surface areas providing more adsorption sites per unit area (Gibson and Borchardt, 2016). Features of the NanoCeram and 1MDS cartridge filters have been previously described and relatively high recovery efficiencies for enteroviruses (from 30 to 70%) in tap water have been reported (Karim et al. 2009; Ikner et al. 2011). These studies, however, have not included testing for recovery efficiency of reovirus which differs in size, genome type and resistance to chemical and physical inactivation processes with respect to enteroviruses and viral surrogates. Reovirus was efficiently recovered from large volumes of water using the method described by Ikner et al. (2011) which includes the NanoCeram filter followed by virus elution with NaPP and Centricon ultrafilters for secondary concentration. Organic flocculation as prescribed by EPA method 1615 with either NanoCeram or 1 MDS may underestimate the level of this virus in water.

References

- Aulicino, E. A., Mastrantonio, A., Orsini, E., Bellucci, C., Muscillo, M., & LaRosa, G. (1996). Enteric viruses in a wastewater treatment plant in Rome. *Water Air Soil Pollution*, 91, 327–334.
- Betancourt, W.Q., & Gerba, C.P. (2016). Rethinking the Significance of Reovirus in Water and Wastewater. *Food Environmental Virology*. 8(3): 161 – 173.
- Cashdollar, J.L., & Wymer, L. (2013) Methods for primary concentration of viruses from water samples: a review and meta-analysis of recent studies. *Journal of Applied Microbiology* 115:1–11
- Fout, G.S., & Cashdollar, J.L. (2016). EPA Method 1615. Measurement of Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR. II. Total Culturable Virus Assay. *Journal of the Visualized Experiments*. (115), e52437, doi:10.3791/52437.

1 Fout, G. S., Schaefer, F. W. III, Messer, J.W., Dahling, D.R., & Stetler.R.E. (1996). Information collection
2 rule (ICR) microbial laboratory manual. EPA/600/R-95/178. U.S. Environmental Protection Agency,
3 Washington, DC.

4
5 Gerba C., Betancourt W.Q, & Kitajima, M. (2017). How much reduction of virus is needed for recycled
6 water: A continuous changing need for assessment? Water Research 108: 25-31

7
8 Gibson, K.E., & Borchardt, M.A. (2016). Methods for virus recovery in water. In S. M. Goyal & J.L.
9 Cannon (Eds.). Viruses in Food. (2nd ed., pp. 277 – 301). Switzerland; Springer.

10
11 Ikner LA, Soto-Beltran, M., & Bright, K.R. (2011) New method using a positively charged microporous
12 filter and ultrafiltration for concentration of viruses from tap water. Applied and Environmental
13 Microbiology 77:3500–3506

14
15 Karim, M.R., Rhodes, E.R., Brinkman, N., Wymer, L., & Fout. G.S. (2009) New electropositive filter for
16 concentrating enteroviruses and noroviruses from large volumes of water. Applied and Environmental
17 Microbiology 75:2393–2399

18
19 Qiu, Y., Lee, B. E., Neuman, N., Ashbolt, N., Craik, S., Maal-Bared, R., & Pang, X. L. (2015). Assessment
20 of human virus removal during municipal wastewater treatment in Edmonton, Canada. Journal of Applied
21 Microbiology, 119, 1729–1739.

22
23 Sedmak, G., Bina, D., MacDonald, J., & Couillard, L. (2005). 9-year study of the occurrence of culturable
24 viruses in source water for two drinking water treatment plants and the influent and effluent of a
25 wastewater treatment plant in Milwaukee, Wisconsin (August 1994 through July 2003). Applied and
26 Environmental Microbiology, 71, 1042–1050.

- 1 Payment, P., Trudel, M. (1993). Isolation and identification of viruses. In P. Payment & M. Trudel (Eds.),
- 2 Methods and Techniques in Virology. (pp. 19 – 40). Marcel Dekker, New York.
- 3