Comparison of Poliovirus Recovery from Water using Three Candidate Filters

Ming-Fong Yang[1], Christa Fagnant[2], Nicola Beck[2], John Scott Meschke[2]
[1]Department of Public Health, National Taiwan University
[2]Department of Environmental & Occupational Health Sciences, University of Washington

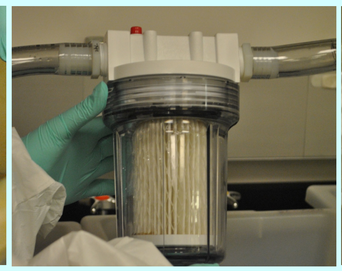
Introduction

- Poliovirus is one of the species of Human enterovirus C. It has three serotypes, PV-1, PV-2 and PV-3. Virus is excreted in feces of infectious individual and shed to the environment.
- Poliovirus is stable in aqueous environment. Infection occurs through fecal-oral route. Flaccid paralysis occurs in 1% of polio infections.
- Acute flaccid paralysis (AFP) surveillance is the gold standard of surveillance in Global Polio Eradication Initiative, launched by World Health Assembly in 1988.
- Though the eradication initiative has reduced new polio cases by 99% and no new cases of PV-2 have been reported since 1999. However, re-emergence of polio epidemic still occurs in some of the previously polio-free areas. The high asymptomatic rate of polio infection limits the reliability of AFP surveillance in some situations.

- Environmental poliovirus surveillance (ENV) is to monitor poliovirus transmission among populations through analyzing environmental specimens contaminated by human feces.
- Environmental surveillance can detect one individual excreting poliovirus in a community of 10,000 people. Its sensitivity in silent transmission can provide valuable information supplementing AFP surveillance.

- Glass wool has been applied in monitoring virus in aqueous environment. The fibrous surface and its electropositive sites catches negatively charged virus when water flows through. It performs best at near-neutral pHs.
- Glass wool filters are inexpensive, reusable, and able to process large volume samples, which make them promising for environmental surveillance.
- In this study, we compare the virus recovery of three filters, glass wool filters, NanoCeram cartridge filters and ViroCap capsule filters. Virus recovery rates were calculated as the ratio of eluate titer to seeded titer.


Glass wool filter


NanoCeram filter


ViroCap filter

Objective

The purpose of this study was to develop enhanced poliovirus environmental surveillance methods using water filtration techniques. Virus recovery rates were determined in parallel for three candidate filter configurations: glass wool filters, NanoCeram cartridge filters and ViroCap capsule filters.

Methods

1. Glass Wool Processing

- Fill the cylinder with dry glass wool. Soak with DI water for 15 minutes.
- Drain water. Fill the cylinder with 1M HCl and let stand for 15 minutes.
- Rinse out HCl with DI water until the effluent is pH 7.
- Fill the cylinder with 1M NaOH and let stand for 15 minutes.
- Rinse out NaOH with DI water until the effluent is pH 7 .
- Store treated glass wool in PBS (1X, pH7)

2. Make Glass Wool Filters

- Pack 150g glass wool into the filter cartridge, then cap the ends. Inject 60ml sterile 1X PBS into the filter. Seal sides with parafilm.


▲Tap water with poliovirus →pump →filter →waste water bucket

•Seed 10^5 or 10^6 PFU of poliovirus into 10 L volumes of sterile tap water.
•Filter the water volumes through one of three different kinds of filter.

ViroCap Filters

Elute
- Pump 175 mL 1.5% Beef extract, 0.05 M glycine buffer (pH 9.5) into inlet end of filter.
- Let stand 30 minutes at room temperature.
- Pump to drain out all eluate from filter.
- Pump the pumped out eluate back into the capsule.
- Pump all eluate into a polypropylene jar

Glass Wool Filters

Elute
- Inject 80ml beef extract (3% beef extract, 0.05M Glycine, pH 9.5) into the filter and let stand for 15 minutes.
- Inject 80ml more beef extract. Continue injecting air into the filter until volume of 175ml is reached or until no more volumes come out.

NanoCeram Filters

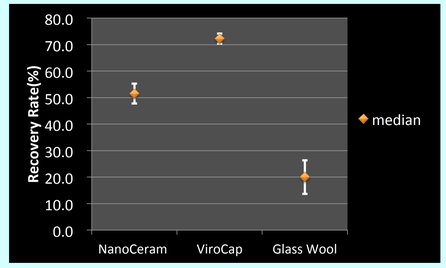
Elute
- Pump 500 mL 1.5% Beef extract, 0.05 M glycine buffer (pH 9.5) into inlet end of filter.
- Let stand 1 minute
- Pump out beef extract to drain out all eluate from filter and store in beaker.
- Pump in the beef extract that was just eluted back into the filter.
- Let stand 15 minutes.
- Pump out beef extract.

Adjust the eluate pH to between 7-7.5

Plaque Assay
- BGMK cell culture in 6-well plates
- Add 200ml eluate or its dilutions to each well. Plate duplicates for each filter. Put into incubator at 37°C and 5% CO_2. Swirl every 15 minutes for even distribution of virus in wells and to prevent cells from drying out.
- After an hour add 2.5 ml avicel/overlay mixture to each well. Incubate at 37°C and 5% CO_2 for 48 hours.

Staining the Cells
- Add 3ml crystal violet solution into each well. Swirl.
- Wait for 3-4 minutes.
- Pour out crystal violet. Wash the cells with 2ml PBS. Pour out PBS.
- Turn the plates upside down to drain the liquids.
- Count Plaques and calculate the recovery rate.

Results



c. The recovery rate of NanoCeram were nearly significantly better than glass wool filter(p=0.057).

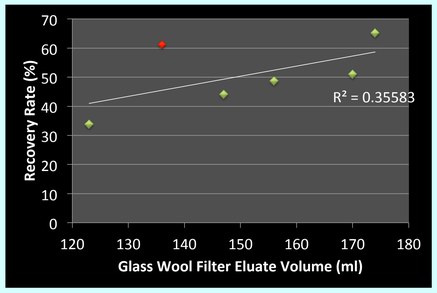


Figure 1.
- The comparison of virus recovery rate is shown by median ± median absolute deviation (MAD).
- Wilcoxon rank sum test:
  a. ViroCap have significantly better recovery rate than glass wool filter ($p<0.05$).
  b. The recovery rate differences between ViroCap and NanoCeram were not significant ($p>0.05$).

Figure 2.
- Correlation between recovery rate and eluate volume wasn't significant (Spearman correlation : 0.657, Kendal's tau correlation: 0.6).
- The outlier(136ml, recovery rate 61.2%, marked red on the figure) affected the correlation.

- Poliovirus was also seeded into 10 L volumes (lake water from Portage Bay, secondary waste water effluent from Westpoint treatment plant, and a 50/50 mixture) and filtered with glass wool filters. Recovery rates dropped significantly to 0.8%-2.2%. Additional trials pending.

Conclusion

- The average recovery rates were 64.1% (37.5%-74.4%) for the ViroCap filters, 47.3% (29.0%-57.2%) for NanoCeram filters, and 19.3% (7.8%-29.4%) for glass wool filters, respectfully. Conclusion: The highest recovery rate was demonstrated for the ViroCap filters.
- Nonparametric test showed no significant correlation between eluate volume and recovery rate.
- Filter based methods offer good recovery of poliovirus from water.
- However, additional information on matrix effect is needed. Development of filtration technique require further comparison with current WHO polio surveillance methods

Reference

- T. HOVI, L. M. SHULMAN, H. VAN DER AVOORT, J. DESHPANDE, M. ROIVAINEN and E. M. DE GOURVILLE (2012). Role of environmental poliovirus surveillance in global polio eradication and beyond. Epidemiology and Infection,140, pp 113 doi:10.1017/S095026881000316X
- World Health Organization. (2003). Guidelines for Environmental Surveillance of Poliovirus Circulation. Geneva , Switzerland : World Health Organization.
- W. J. Lodder, A. M. Buisman, S. A. Rutjes, J. C. Heijne, P. F. Teunis and A. M. de Roda Husman. Feasibility of Quantitative Environmental Surveillance in Poliovirus Eradication Strategies. Applied and Environmental Microbiology, June 2012 vol. 78 no. 11 3800-3805
- Frank J. Loge, Burney A. Kieke and Mark A. Borchardt Elisabetta Lambertini, Susan K. Spencer, Phillip D. Bertz. Drinking Water by Use of Glass Wool Filters Adenoviruses, and Noroviruses from Concentration of Enteroviruses. Applied and Environmental Microbiology, . 2008, 74(10):2990. DOI: 10.1128/AEM.02246-07.

Acknowledgement: Special thanks to: National Taiwan University School of Public Health and Department of Environmental & Occupational Health Sciences, University of Washington for offering this wonderful opportunity; Dr. John Scott Meschke for his instruction and poster revision; Christa Fagnant and Nicola Beck for teaching me the techniques; Dr. Chang-Fu Wu for helping with various procedures and being my adviser of the internship. This poster was supported by Award Number R25ES16150 from the National Institute of Environmental Health Sciences.