Comparison of Three Filtration Methods to Capture Pathogenic Viruses and Bacteria from Brackish Storm Water

Joshua A. Steele 1*, Meredith R. Raith1, Blythe A. Layton1, A. Denene Blackwood2, Rachel T. Noble2, John F. Griffith1
1Southern California Coastal Water Research Project, Costa Mesa, CA USA; 2UNC Institute of Marine Science, Morehead City, NC USA *joshuas@sccwrp.org

Introduction
Successful molecular analyses of pathogenic bacteria and viruses (or indicators such as bacteriophage) from storm water in urbanized coastal areas are confounded by salinity, silt, sand, and high molecular weight chemical compounds. Direct measurement of important viral and bacterial pathogens at low concentrations is an obvious goal of water quality analyses, but success is hampered by sample concentration and nucleic acid purification necessary for most quantitative molecular techniques. Recent advances in molecular techniques (e.g. digital droplet PCR) have increased the sensitivity of pathogen assays and increased robustness to inhibitory compounds. Simultaneous capture of bacteria and viruses from a complex environmental matrix would enable cost-effective detection and monitoring of pathogens in impaired waters. We tested three filtration methods to capture ssRNA and dsDNA viruses, phage surrogates, and pathogenic bacteria known to occur in recreational waters. The methods would be used on storm water from the San Diego River which drains to a heavily used beach in Southern California which was chosen as a sentinel beach in a wet weather epidemiology study conducted in 2014-2015

Experimental Design Water Matrix
San Diego River Water was collected in San Diego, CA during the February 27 - March 2 2014 storm which had 1.9 in (48.3 mm) total precipitation. The river water had a pH 7.0-7.2 and salinity 5.8-8.1 ppt, and temperature of 16°C. The river water was aged at ambient temperature in the dark for four months prior to the experiment in order to reduce any pathogens present in the sample.

Virus and Bacteria Additions
Triplicate water samples were spiked with two concentrations (high or low; table 1) of bacteria- phage P22 dsDNA, bacteriophage M5 ( ssRNA phage), recombinant Human Adenovirus (dsDNA virus), murine norovirus ( ssRNA Virus), Salmonella enterica enterica Typhimurium, and Campylobacter jejuni and Campylobacter coli.

Virus and Bacteria Capture and Elution Procedures
Spiked water samples were mixed thoroughly and filtered using each of the 3 filtration methods (table 2). HA filters were placed in a sterile microporous tube and flash frozen with liquid N₂. NanoCeram filters were eluted with a 1% NaCl, 0.5M Glycine PBS at pH 9.3. This concentration was aliquoted and flash frozen with liquid N₂. Innovaprev LVC filters were eluted with 0.1% NaCl, 0.5M Glycine PBS, pH 9.3. This concentration was aliquoted and flash frozen with liquid N₂. All buffers and solutions were purchased sterile, nuclease, and virus-free or 0.02 µm filtered.

Extraction and quantification
All filters and elution concentrates were extracted with Mobio Environmental Virus kit with 2-mercaptoethanol added to the lysis buffer and a 2 minute mechanical lysis with glass beads. Extracted DNA or RNA was thawed and quantified by qPCR and digital PCR (ddPCR) assays using published protocols (table 1).

Table 1. Virus and Bacteria Used as assay reference

Table 1. Quantity of Virus and Bacteria after capture and elution or extraction. Numbers are average gene copies recovered from triplicate experiments (standard deviation).

Table 2. Filtration Method Details and Performance Specifications

Conclusions
- There is no single perfect method of capture for viruses in environmental water samples.
- NanoCeram filters were highly variable, but efficiently captured and eluted Murine Norovirus at the lower concentration.
- Innovaprev LVC was comparatively efficient at capturing the RNA viruses.
- HA filters were highly variable, but attained the highest viral recovery efficiency with dsDNA viruses and bacteria.
- Although the Innovaprev LVC was less efficient at capturing viruses in many cases, the large volumes and order of magnitude higher virus recovery should allow for detection of less abundant viruses.

Acknowledgements
Thank you to A. Meunier for providing MNV-1, E. Huang, S. Jiang U.C.Rivine for providing and propagating mAd5 and propagating MNV-1. Thanks also to D. Diehl, J. Brown, L. Mao, M. Griffith, A. Orr. This work was funded by the City and County of San Diego, CA.

References