**AMERICAN SOCIETY FOR MICROBIOLOGY – May 30 - June 2, 2015**
http://gm.asm.org/

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

J.A. Steele[1], M.R. Raith[1], B. Layton[1], A. D. Blackwood[2], R.T. Noble[2], J.F. Griffith[1]

[1]*Southern California Coastal Water Research Project, Costa Mesa, CA USA*
[2] *UNC Institute of Marine Science, Morehead City, NC USA*

## Abstract

Successful molecular analyses of pathogenic bacteria and viruses (including 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. We compared three filtration approaches varying in capture strategy, mode of concentration, and filtration capacity to concentrate viruses and bacteria from complex storm water samples. Storm water collected March 2, 2014 from the San Diego River (San Diego, CA) was aged in the dark at ambient temperature for 6 months to reduce background native microbial and phytoplankton abundance. We inoculated storm water subsamples with high ($10^8$ per L) or low ($10^5$ per L) concentrations of RNA viruses (coliphage MS2, Murine norovirus), DNA viruses (bacteriophage P22, human Adenovirus), and high ($10^7$ per L) or low ($10^4$ per L) concentrations of pathogenic bacteria (*Salmonella enterica* serovar *Typhimurium*, *Campylobacter jejuni*). Inoculated storm water samples were concentrated using three different methods: 500 ml filtered through electronegative, 0.45µm Millipore-type HA filters (HA); 1L filtered through electropositive, 0.8µm Nanoceram filters (NC); and 20L concentrated via hollow fiber, 30kD InnovaPrep filters (HF). Recovery of the inoculated viruses and bacteria was quantified by qPCR or droplet digital PCR (ddQPCR). HA filters had the highest, most consistent recovery (70-110% recovery of viruses and bacteria), but captured fewer total viruses and bacteria due to the small volumes filtered. NC filters also had high recovery (30-70% recovery of viruses and bacteria), but also were limited by volume. The HF filter had a lower, more variable recovery (7-80% recovery of viruses; 50-80% recovery of bacteria), but captured >10X more viruses and bacteria. Our results suggest that in systems where broad assessment of pathogens and a low limit of detection is desired, it may be necessary to utilize both high recovery HA and larger volume HF filters to quantify pathogenic bacteria and viruses over a wide range of concentrations.