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

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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 were tested using 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 5.8-8.1ppt, and temperature of 16°C. The river water was aged at ambient temperature in the dark for 6 months prior to the experiment in order to reduce any pathogens present in the sample.

Figure 1 Satellite view of the San Diego River (A) near the beach discharge and a photograph of the sampling site under the Ingraham Street Bridge (B).

Virus and Bacteria Additions

Triplicate water samples were spiked with two concentrations (high or low; table 1) of bacteriophage P22 (dsDNA phage), bacteriophage MS2 ((+)ssRNA phage), recombinant Human Adenovirus (dsDNA virus), murine norovirus ((+)ssRNA Virus), *Salmonella enterica enterica* Typhimurium , and *Campylobacter jejuni* and *Campylobacter coli*.

Table 1. Virus and Bacteria Used for Capture and PCR assay reference

Virus / Bacteria	Strain	High Spike Concentration	Low Spike Concentration	Molecular Assay	Assay Reference
Human Adenovirus	rAdV-5	10^8 copies per L	10^5 copies per L	qPCR, ddPCR	Jothikumar et al. 2005
Bacteriophage P22	ATCC 19585-B1	10^9 copies per L	10^6 copies per L	qPCR	Masago et al. 2008
Bacteriophage MS2	ATCC 15597-B1	10^8 copies per L	10^5 copies per L	ddRT-PCR	Conn et al. 2012
Murine Norovirus	MNV-1	10^9 copies per L	10^6 copies per L	ddRT-PCR	Leen Baert et al. 2008
Salmonella enterica Typhimurium	ATCC 19585	10^8 copies per L	10^5 copies per L	ttr gene ddPCR invA gene ddPCR	Malorny et al. 2004 González-Escalona et al. 2009
Campylobacter jejuni & coli	ATCC 33560 ATCC 33559	10^7 copies per L	10^4 copies per L	ddPCR	Lund et al. 2004

Virus and Bacteria Capture and Elution Procedures

Spiked water samples were mixed thouroughly and filtered using each of the 3 filtration methods (table 2). HA filters were placed in a sterile microcentrifuge tube and flash frozen with liquid N_2. NanoCeram filters were eluted with a 1% NaPP, .05M Glycine PBS at pH 9.3. This concentrate was aliquoted and flash frozen with liquid N_2. Innovaprep large volume concentrator filters were eluted with foam containing 0.075% Tween-20 and 25mM Tris buffer. This concentrate was aliquoted and flash frozen with liquid N_2. 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).

dsDNA Virus Capture

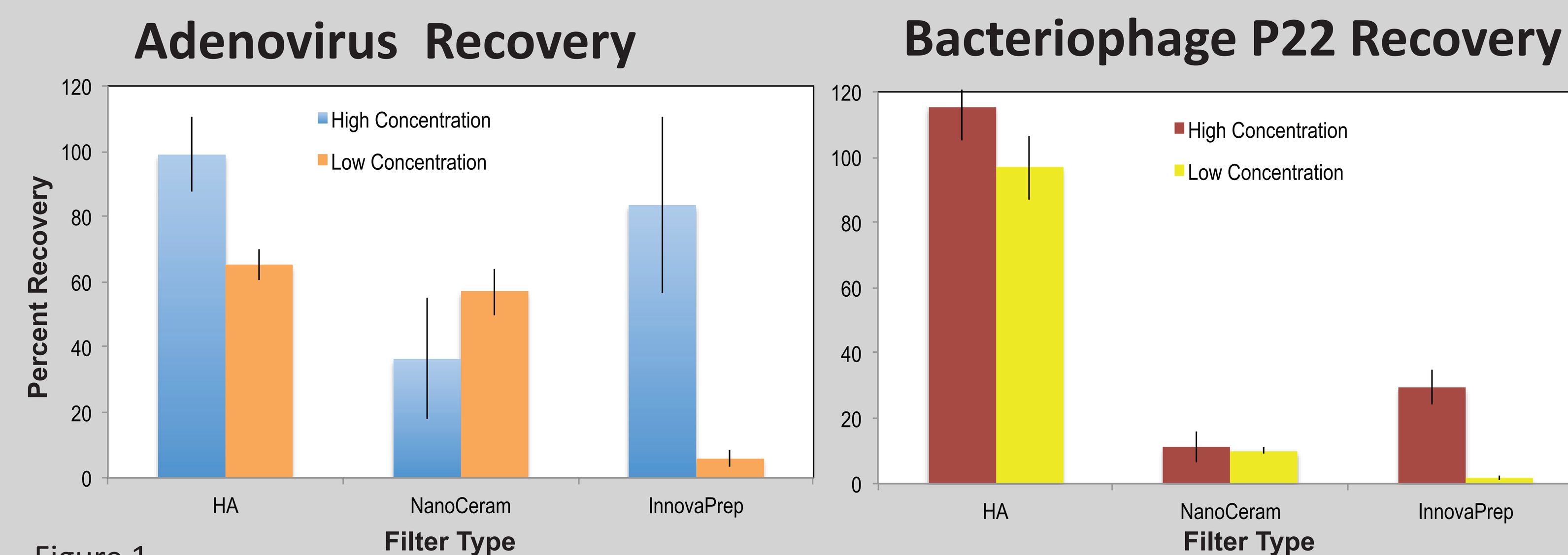

Figure 1
Percent recovery of dsDNA viruses measured as gene copies post filtration and extraction/gene copies spiked. HA filters performed the best capturing the dsDNA viruses. The greater than 100% recovery likely due to different lysis and extraction efficiencies between the spiked virus and the virus captured on the filters. The low percent recovery in the low concentrations for the InnovaPrep LVC for Adenovirus and for the NanoCeram disc and the InnovaPrep LVC in the P22 recovery is likely due to inhibition. The P22 assay is a traditional qPCR and is less robust to inhibition

(+) ssRNA Virus Capture

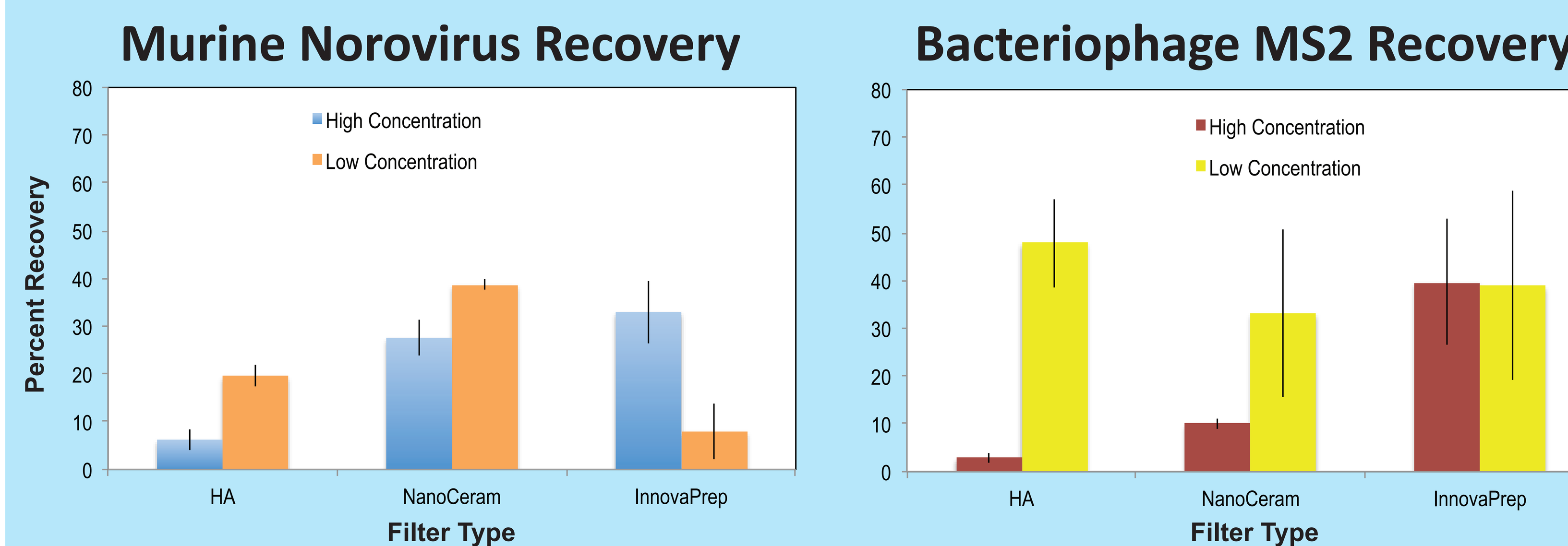

Figure 2.
Percent recovery of (+)ssRNA viruses measured as gene copies post filtration and extraction/gene copies spiked. The NanoCeram disc and HA filter had high recovery of viruses in the low concentrations. Inhibiton is a particularly difficult problem for RNA viruses since the reverse transcriptase and the *Taq* polymerase can be inhibited by large macromolecules, humic acids, metals, and phenolic compounds in the storm water. Many of the high concentration samples had to be diluted in order to be in range of the ddPCR, and some showed inhibition, which may account, in part, for the high variation in bacteriophage P22 measurements.

Table 2. Filtration Method Details and Performance Specifications

Filter Name	Filter Type	Filter Size	Nominal Pore Size	Sample Additions (pre-filtration)	Volume Filtered	Elution Buffer	Filtration + Elution Time (minutes)	Final Concentrate volume
Millipore type HA	mixed cellulose ester	47 mm	0.45 µm	25 mM $MgCl_2$, pH 3.5	0.5 L	N/A	45-60	N/A
Argonide NanoCeram Disc	nano-alumina coated glass fiber	47 mm	0.8 µm	N/A	1 L	1.0% NaPP, 0.05 M Glycine, PBS pH 9.3	30-60	20 mL
Innovaprep Large Volume Concentrator	Fresenius polysulfone hollow fiber dialysis filter	200 µm inner diameter; 2.0 m^2 surface area	30 kD	N/A	20 L	.075% Tween-20, 25 mM Tris-HCl foam	25-40	50-80mL

HA method adapted from Katayama et al. 2002
NanoCeram method adapted from Li et al. 2010 & Ikner et al. 2011

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Campylobacter and *Salmonella* Capture

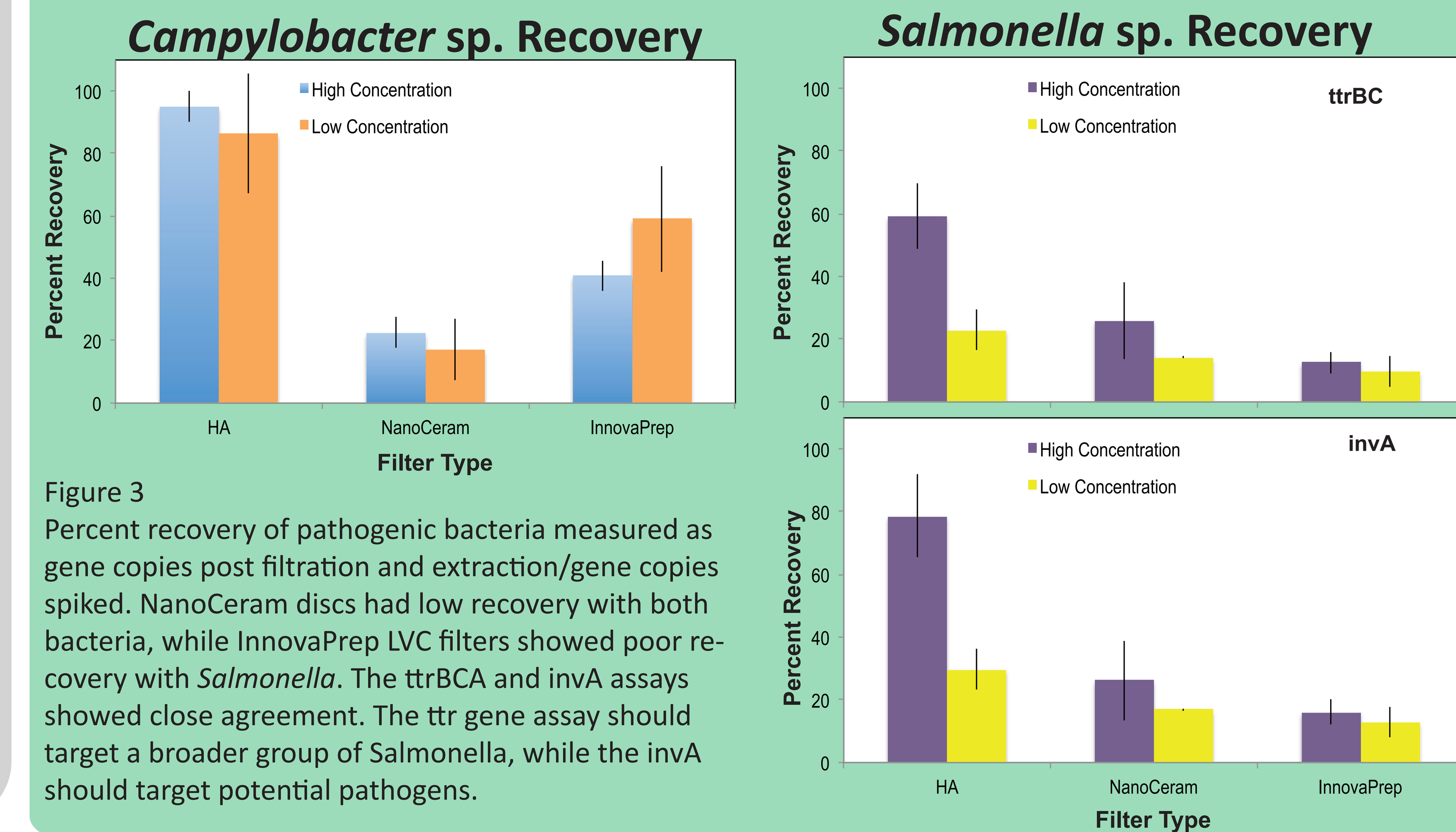

Figure 3
Percent recovery of pathogenic bacteria measured as gene copies post filtration and extraction/gene copies spiked. NanoCeram discs had low recovery with both bacteria, while InnovaPrep LVC filters showed poor recovery with *Salmonella*. The ttrBCA and invA assays showed close agreement. The ttr gene assay should target a broader group of Salmonella, while the invA should target potential pathogens.

Table 3. Quantity of Virus and Bacteria after capture and elution or extraction. Numbers are avaerage gene copies recovered from triplicate expermients (standard deviation).

Virus / Bacteria	HA High	NanoCeram High	InnovaPrep High	HA Low	NanoCeram Low	InnovaPrep Low
Human Adenovirus	$1.1x10^8$ ($7.9x10^6$)	$7.2x10^7$ ($3.7x10^7$)	$4.1x10^9$ ($1.3x10^9$)	$2.0x10^4$ ($1.4x10^3$)	$3.4x10^4$ ($4.4x10^3$)	$1.3x10^6$ ($5.5x10^5$)
Bacteriophage P22	$2.5x10^8$ ($4.6x10^7$)	$4.6x10^7$ ($1.6x10^7$)	$2.5x10^9$ ($5.7x10^8$)	$2.74x10^5$ ($2.9x10^4$)	$5.7x10^4$ ($4.7x10^3$)	$2.0x10^5$ ($9.7x10^4$)
Bacteriophage MS2	$2.8x10^6$ ($7.9x10^5$)	$2.0x10^7$ ($6.2x10^5$)	$1.5x10^9$ ($4.4x10^8$)	$2.7x10^3$ ($5.3x10^2$)	$3.7x10^3$ ($2.0x10^3$)	$8.9x10^4$ ($4.9x10^4$)
Murine Norovirus	$3.8x10^7$ ($1.3x10^7$)	$3.0x10^8$ ($4.0x10^7$)	$7.7x10^9$ ($9.9x10^8$)	$1.5x10^5$ ($1.6x10^4$)	$2.9x10^5$ ($8.2x10^3$)	$8.5x10^5$ ($5.0x10^5$)
Salmonella enterica Typhimurium ttrBCA	$1.3x10^7$ ($2.2x10^6$)	$1.3x10^7$ ($5.1x10^6$)	$2.2x10^9$ ($6.3x10^8$)	$1.0x10^4$ ($2.9x10^3$)	$1.2x10^4$ ($4.0x10^2$)	$1.7x10^5$ ($8.7x10^4$)
Salmonella enterica Typhimurium invA	$1.7x10^7$ ($2.7x10^6$)	$1.1x10^7$ ($4.0x10^6$)	$2.9x10^9$ ($7.2x10^8$)	$1.3x10^4$ ($2.65x10^3$)	$1.5x10^4$ ($2.5x10^3$)	$2.3x10^5$ ($8.7x10^4$)
Campylobacter jejuni & coli	$9.8x10^6$ ($1.9x10^6$)	$4.7x10^6$ ($1.7x10^6$)	$1.7x10^8$ ($2.1x10^7$)	$1.0x10^4$ ($2.3x10^3$)	$3.0x10^4$ ($1.7x10^4$)	$2.8x10^5$ ($8.2x10^4$)

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.
- InnovaPrep 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 InnovaPrep 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.
- HA and NanoCeram discs remain limited by the volume of stormwater they allow through before clogging.
- PCR inhibition and assay sensitivity is always an issue, although ddPCR can help alleviate both.

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