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Rapid Concentration to Support Improved Detection of Indicator Bacteria in Recreational Waters

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Rapid detection of fecal indicator bacteria in recreational waters is needed to ensure public safety. Molecular and other rapid microbiological detection technologies have progressed significantly in the last several decades and provide the greatest potential for overcoming these challenges. However; their development has significantly outpaced development of sample concentration techniques, which are necessary for rapid detection of low concentrations of bacteria. InnovaPrep has developed a suite of systems for concentration of bacteria and other biological particles from liquid samples. One automated system, the Concentrating Pipette, was tested using water samples taken from three recreational sources including one freshwater beach location and two saltwater beach locations. The samples were rapidly concentrated and analyzed by both qPCR and classical culture plating.

Rapid detection of fecal indicator bacteria in recreational waters is needed to ensure public safety. Molecular and other rapid microbiological detection technologies have progressed significantly in the last several decades and provide the greatest potential for overcoming these challenges. However; their development has significantly outpaced development of sample concentration techniques, which are necessary for rapid detection of low concentrations of bacteria.

InnovaPrep has developed a suite of systems for concentration of bacteria and other biological particles from liquid samples. Volumes of water from a few milliliters to tens of liters of water are processed through flat membrane filters or hollow fiber membrane filters to capture any biological particles that are present. The biological particles are then efficiently recovered from the membrane surface with an automated tangential flush using carbonated "wet foam". The wet foam is expanded up to six times the original liquid volume and becomes highly viscous, allowing it to act at the membrane surface and recover the particles into volumes significantly smaller than can be attained with traditional liquid elutions. The foam quickly collapses into a flat liquid, ready for analysis. The process is scalable, efficient, and typically results in concentration factors of approximately 1000X per concentration stage.

CONCENTRATING PIPETTE

The device for concentration that was used in this testing was the InnovaPrep Concentrating Pipette an automated micro-particle concentrator which quickly and efficiently concentrates waterborne bacteria, viruses, DNA, protozoa, or other particles of interest from large liquid sample volumes into liquid volumes of clean buffer as small as 200 microliters.

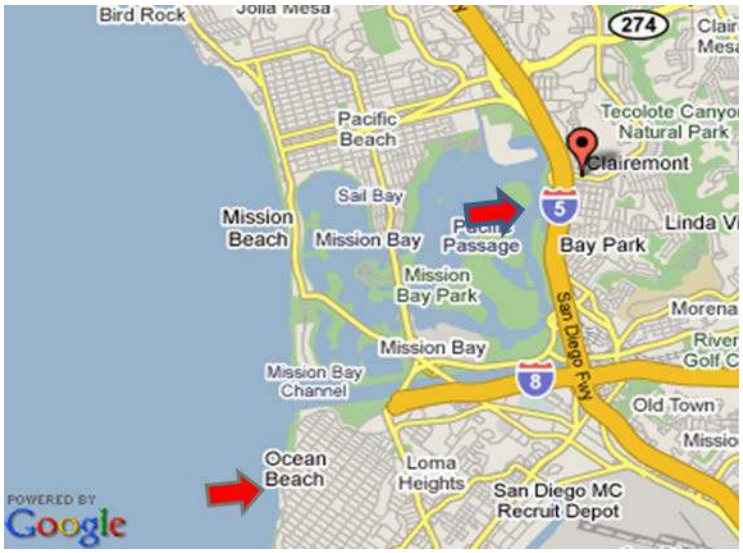
Test series were performed with water samples taken from three recreational sources including one freshwater beach location and two saltwater beach locations.

Ocean Water Samples: Ocean Beach and Mission Bay in San Diego, CA

The saltwater samples were collected from two locations in Southern California:

1.) Mission Bay is a saltwater lagoon located south of Pacific Beach in San Diego. The bay is part of the recreational Mission Bay Park, the largest man-made aquatic park in the country.

2.) Ocean Beach, a popular swimming and surfing beach located on the Pacific Ocean at



the estuary of the San Diego River.

MISSION BAY TESTING

Three 100 mL samples were autoclaved for 20 minutes to kill background flora. The samples were then spiked (0.9 CFU/mL) with *Enterococcus faecalis* using BioMerieux BioBalls (NCTC 12697).

The three samples, along with a control sample (of PBS spike 1.2 CFU/mL) were concentrated using the InnovaPrep Concentrating Pipette (CP). The single-use concentrating pipette tip chosen for this testing was a flat Polycarbonate Track Etch membrane with a pore size of 0.4 µm and a surface area of 8.5 cm^2 shown at right.

	Mission Bay	Ocean Beach	PBS
Time	1:11	1:10	1:04
Average final concentrate volume	295 µL	282 µL	189 µL
Average % Efficiency	58.1%	63.8%	54.9%
Concentration factor	203X	221X	287X
Extract #2			
Average % Efficiency	6.3%	7.9%	10%
Average total efficiency Extract 1&2	64.4%	71.7%	65%

The Mission Bay samples took an average 1:11 minutes to filter and elute the 100 mL volume into an average final concentrate of 295 µL. Analysis was performed by plating, incubation, and enumeration. The recovery efficiency of the 1st elution was an average 58.1%. Each pipette tip was eluted twice to bring the final recovery average to 64.4% and a concentration factor of 203X.

OCEAN BEACH TESTING

Sample type	Mission Bay	Ocean Beach
Sample volume	100 mL each	
Enterococcus faecalis BioBall™ spike	0.9 CFU/mL	1.05 CFU/mL
Pretreatment	Autoclave 20 min	none
Pipette tip pre-filter	none	10 µm syringe filter
Filter tip type	0.4 Polycarbonate Track Etch (PCTE)	
Control	PBS spike 1.2 CFU/mL	
# Runs	3 each	
Analysis by plating, incubation, and enumeration		

Three 100 mL samples were filtered using a 10 µm syringe filter to remove the sand from the sample. The samples were then spiked (1.05 CFU/mL) with *Enterococcus faecalis* using BioMerieux BioBalls.

The three samples, along with a control sample (of PBS spike 1.2 CFU/mL) were concentrated using the InnovaPrep Concentrating Pipette (CP). The single-use concentrating pipette tip chosen for this testing was a flat Polycarbonate Track Etch membrane with a pore size of 0.4 µm and a surface area of 8.5 cm.

The Ocean Beach samples took an average 1:17 minutes to filter and elute the 100 mL volume into an average final concentrate of 282 µL. Analysis was performed by plating, incubation, and enumeration. The recovery efficiency of the 1st elution was an average 63.8%. Each pipette tip was eluted twice to bring the final recovery average to 71.7% and a concentration factor of 221X.

The PBS control sample gave, on average, a smaller final concentrate of 189 µL which resulted in a better concentration factor of 287%. The recovery efficiency of the first elution was 54.9% and improved to 65% total recovery efficiency with a second elution of the pipette tip.

Freshwater Samples Lake of the Ozarks

A Freshwater sample was collected from Lake of the Ozarks. The Missouri freshwater lake has a total of 1,150 miles (1,850 km) of shoreline—mostly privately owned, and two swimming beaches.

FRESH WATER METHOD E. COLI SPIKING/QPCR

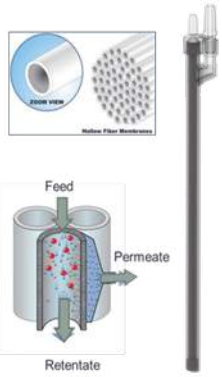
1 Liter samples of lake water were autoclaved (1250 C, 45 min.) to kill background flora. The autoclaved samples were spiked with E. coli (NCTC 12923) at two levels.

- Low level – 0.1 cfu/mL (10 cfu/100 mL) and
- High level – 1 cfu/mL (100 cfu/100 mL)

The single-use concentrating pipette tip chosen for this testing was a Polysulfone hollow fiber membrane pipette tip, shown below , with a pore size of 0.45 µm and a surface area of 80 cm^2 (a 10X surface area increase over the flat membrane tips used in the salt water testing above).

Each of the three 1 Liter samples were concentrated in an average 9:15 minutes .

The DNA extraction was performed using a modified MOBIO Powerwater method, which resulted in a final volume of 50 µL. Quantitative PCR (qPCR) was performed using the Applied Bioscience StepOne thermocycler. For each sample, 5 µL sample vol/triplicate was used for amplification using the conserved flanking regions of the 16s rRNA gene, the ITS region and the 23S rRNA gene (IEC primers) and SYBR Green and allowed to run for 40 cycles.



SINGLE-USE HOLLOW FIBER CONCENTRATING PIPETTE TIPS (HF CPTS)

Low Level: 0.1 CFU/mL *E. coli*

	Run 1	Run 2	Run 3	Blank	Avg.	St. Dev.
Cycle Threshold	32.2	32.4	32.4	32.8	**32.3**	0.07
Quantity	36.0	34.3	34.8	26.6	**35.0**	0.90
Blank Corrected Quantity	9.4	7.6	8.2	-	**8.4**	0.90

High Level: 1 CFU/mL *E. coli*

	Run 1	Run 2	Run 3	Blank	Avg.	St. Dev.
Cycle Threshold	30.7	30.7	30.0	35.2	**30.5**	0.39
Quantity	332	324	523	15	**393**	113
Blank Corrected Quantity	317	309	508	-	**378**	113

FRESH WATER METHOD *E. COLI* SPIKING/PLATING

Each 1 Liter sample was concentrated in an average 9:09 minutes with an average concentration factor of 2,119X with the first elution. A second elution gave an average 9.9% improvement on efficiency for a total recovery efficiency of 83.2%.

- Average number spiked: 116 cfu in 1 L
- Average number recovered: 85 cfu in 376 µL

AUTOMATED FILTER ELUTION

	Run 5	Run 6	Run 7	Run 8	Avg.	St. Dev.
Time	6:44	7:47	13:48	8:17	**9:09**	
Concentrate vol.	376 µL	394 µL	383 µL	352 µL	**376 µL**	**18 µL**
Efficiency	76.7%	86.2%	69.0%	61.2%	**73.3%**	**10.7%**
Concentration factor	2,151X	2,308X	1,898X	1,832X	**2,119X**	**207X**
Extract #2						
Efficiency	1.7%	5.2%	17.2%	15.5%	**9.9%**	**7.6%**
Total Efficiency (Extracts 1&2)	78.5%	91.4%	86.2%	76.7%	**83.2%**	**6.8%**

CONCLUSION:

The InnovaPrep 'wet foam elution' technology has shown to be exceptionally efficient and effective for its ability to deliver concentrated samples of waterborne pathogens from relatively large volumes (up to 3 liters) to rapid detection methods such as real-time polymerase chain reaction systems in a fraction of the time compared to other methods.

For very large liquid volumes, InnovaPrep offers a manual kit called the Large Volume Concentration (LVC) Kit, capable of concentrating bacteria, parasites, and viruses from up to 40 liters.

Future research plans include development of a modification to method 1611 and reducing the volume mismatch between concentration and detection for qPCR and Isothermal detection using lyophilized reagents.