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Mini Meta Study: Improved Pathogen Detection of Air, Surface and Liquid Samples through Rapid Concentration

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Introduction:

Contamination outbreak investigations are an important role of Public Health officials and First Responders for ensuring that the source of contamination is found quickly to reduce additional incidences of harm to the public. Sample sources come from food, liquid, surface, and air samples containing pathogens that are often dilute.

Workflow in these cases need streamlining as much as possible which may include using rapid molecular analysis methods rather than traditional culture methods, pooling samples (and removing an aliquot for follow-up analysis if needed), and using alternative sample preparation approaches.

New sample preparation approaches are of interest to biosurveillance teams to save time to detection, as common methods take hours or days in order to enrich the threat organisms to a detectable level - or centrifugation steps that require tedious transfer steps which often incur efficiency losses and also limits the sample volume amount that can be used.

Background:

This need for rapid enrichment has been addressed by InnovaPrep through development of a rapid concentration platform called the Concentrating Pipette. To use, a filtered Concentrating Pipette Tip (CPT) is snapped into the device and lowered into the liquid sample. With a button press, the sample is pumped through the CPT. Fluid from the sample goes to waste while the pathogens are trapped on the internal membrane of the CPT. Once the entire sample has been processed, the pathogens are rapidly recovered from the membrane surface with an automated tangential flush using carbonated **Wet Foam Elution™**. The final sample volume is user selectable from from 150 µL to 1 mL.

During the elution the wet foam expands to six times its original liquid volume and becomes highly viscous, allowing it to act at the membrane surface and recover the particles. It then collapses into small concentrated volumes that more closely match the input volume of molecular analysis methods. A clean buffer exchange is simultaneously achieved removing potential inhibitors.

Use Cases for Outbreak Investigations:

The InnovaPrep Wet Foam Elution™ technology was first developed for integration into advanced biodefense and biodetection systems, but it has application with any analysis method where increased sensitivity is needed. Fields of application include, but are not limited to water, food, and drug safety, environmental monitoring, outbreak monitoring, and biodefense.

Three sampling scenarios performed by university and government agencies illustrate the advantage of large volume sampling + rapid sample concentration for **liquid, surfaces**, and **air samples**. Each of the below cases have been separately reported by the users in recent peer reviewed publications.

Concentrated Surface Samples

WISCONSIN UNIVERSITY OF WISCONSIN-MADISON

NASA Jet Propulsion Laboratory California Institute of Technology

Characterization of Aspergillus fumigatus Isolates from Air and Surfaces of the International Space Station Knox, et al.

Surface Sampled: International Space Station cupola window pane
Surface area sampled: 1m^2
Surface wipe: polyester wipe (9 by 9 in.; ITW Texwipe, Mahwah, NJ) was folded two times and soaked in 15 ml of sterile molecular-grade water for 30 min, followed by transfer to a sterile zip lock bag.

Method:

- Each wipe was aseptically taken out from the zip lock bag and transferred to a 500-ml sterile bottle containing 200 ml of sterile PBS.
- The bottle with the wipe was shaken for 2 min followed by concentration with an InnovaPrep Concentrating Pipette using 0.45µm hollow fiber polysulfone pipette tips and PBS elution fluid.
- The environmental control and each sample were concentrated to 4 mL.
- A 200-µL aliquot was serially diluted in PBS to estimate the cultivable population.
- Concentrated samples were diluted in PBS (up to 10^6 of each original sample), plated on the media (100 µl; in duplicates) Reasoner's 2A agar (R2A) for environmental bacteria and PDA for fungi, and incubated at 25°C for 7 days; CFU were then counted.

Air, Surface, and Liquid Sample Matrix Reduction for Improved Detection

	Pooled Air Samples Collected in Liquid	1 m^2 Surface Wipes	Environmental Liquids
Starting volume	20 mL	200 mL	100 mL
Final volume	0.6 mL	4 mL	0.25 mL
Theoretical Concentration Factor	33X	50X	400X

• Actual Concentration Factor = Theoretical Concentration Factor x Efficiency

Concentrated Air Samples

Detection of an Avian Lineage Influenza A (H7N2) Virus in Air and Surface Samples at a New York City Feline Quarantine Facility Blachere et al.

"To establish the presence of infectious virus on airborne particles, SKC BioSampler samples were concentrated using the InnovaPrep Concentrating (IPC) Pipette. A SKC aerosol sample (15 ml) was drawn through a single disposable ultrafiltration concentrating pipette tip and extracted to a final volume of 0.7 ml, using the manufacturer supplied DMEM/N_2O elution fluid. To increase the likelihood of detecting infectious virus, three samples were first pooled together (Final volume ~20 ml) and similarly run through an ultrafiltration concentrating pipette tip. The final extracted sample volume was 0.6 ml. A 0.2 ml volume of each IPC sample was injected into individual 10-day old embryonated chicken eggs. Quantitative PCR detection of the M1 gene was used to quantify viral loads from the resultant allantoic fluid.

Increasing the A(H7N2) egg inoculum from pooled samples with the InnovaPrep Concentrating Pipette by approximately 33-fold led to a 47-fold increase in M1 copies. Likewise, concentrating sample K03 approximately 22-fold resulted in a dramatic 2 x 10$^{7-8}$ - fold increase in M1 copies and enabled hemagglutination detection with a final HA titer of 512 HA units (HAU)/50 µL allantoic fluid."

Concentrated Liquid Samples

UNIVERSITY OF MARYLAND USDA

Comparison of methods to determine the microbial quality of alternative irrigation waters Hsin-Bai Yina , et al

For the concentrator method, each water sample with appropriate dilution (total volume 100 mL) was concentrated using a bio-concentrator and a 0.45 µm Concentrating Pipette Tip (InnovaPrep) to a ~250 µL concentrate, and the entire ~250 µL concentrate was spread plated onto agar with the appropriate incubation conditions. "The concentrator method is comparable to membrane filtration method for analyzing microbiological quality of creek water and roof-harvested water. It is superior in detecting virulence genes associated with *E. coli* O157:H7 in creek water. The concentrator method is simple, highly effective, and rapid in detecting bacterial populations in irrigation water and can be used as an alternative to traditional tedious membrane filtration method."

Conclusions:

The Concentrating Pipette bio-concentrator is a fast, easy to use tool for concentrating microorganisms from an aqueous sample matrix for improved detection. 97%-99.75% matrix reduction and the final elution was achieved in under 5 minutes. When paired with a rapid molecular method, sample to answer can be achieved within hours.