Rapid Concentration of SARS-CoV-2 in Wastewater for COVID-19 Monitoring and Public Health Outbreak Mitigation

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Abstract: Researchers around the globe have already begun to use wastewater as a means detect the emergence or spread of COVID-19 outbreaks and it could prove to be a significant epidemiological method to help Public Health officials mobilize resources and help determine the degree and duration of intervention needed in a community by community real-time approach. A concentration method, capable of bridging the gap between relevant sample volumes and the volumetric capacity of rapid molecular methods is needed in order to adequately monitor SARS-CoV-2 in wastewater. The needs of this industry require that target organisms are detectable at very low concentrations from about 10 copies per 100 milliliters of influent to as little as 120 copies per 100 liters of effluent. Rapid methods are only capable of analyzing very small volumes; generally 10 microliters or less. InnovaPrep concentration technologies that can bridge this gap faster, easier, and better than common methods allow.

Introduction

InnovaPrep® was founded in 2009 with the express goal of making common preanalytical microbiology processes faster, easier, and better through mechanical enrichment. Our flagship instrument, The Concentrating Pipette (CP) Select™, is a small bench top instrument that uses dead-end filtration on high-flow single-use pipette tips to capture target particles. The CP Select uses an automated recovery process, termed, *Wet Foam Elution™* to enable efficient extraction of the particles from the filter membrane in seconds and can administer very precise and controlled amounts of foam that produce filter elution volumes as low as 200 microliters. The process maximizes concentration of the target thereby exponentially improving the limit of detection for trace pathogens.

InnovaPrep technologies have been used to concentrate microorganisms including viruses suspended in liquid with the CP Select for several years from a variety of environmental sources such as marine recreational and aquaculture waters, surface waters, storm run-off, cave and spring waters, irrigation waters, as well as wastewater influent and effluent.

COVID-19 and the SARS-CoV-2 Virus

Recently, our customers at wastewater treatment facilities have confirmed that The CP Select can rapidly and efficiently concentrate SARS-CoV-2 from raw influent wastewater samples. Detecting SARS-CoV-2 in wastewater is difficult because it is so dilute, making rapid and easy concentration key to rapid detection. InnovaPrep tools can provide the exponential concentration factors required, can be easily integrated with routine methods used for all waterborne pathogens normally surveilled, while saving labor costs.

Unpublished user data show that the CP Select outperformed HA, PEG, and Celite based methods for concentration of six viruses from 100 mL of raw influent. And the speed of concentration and the ease of use easily beat the traditional methods. For secondary clarifier effluent, similar results were reported, and the InnovaPrep Large Volume Concentrator was used to process 100 liters, for secondary concentration using the CP Select. This powerful combination of techniques tremendously improved the detection limit for those viruses.

Public Health

Monitoring SARS-CoV-2 in a community wastewater could be important for several reasons:

- Routine wastewater surveillance could be used as a non-invasive early-warning tool to alert communities to new COVID-19 infections. Studies have shown that SARS-CoV-2 can appear in feces within three days of infection, which is much sooner than the time taken for people to develop symptoms severe enough for them to seek hospital care — up to two weeks — and get an official diagnosis.
- It could give public-health officials a head-start on deciding whether to introduce measures such as lockdowns and for measuring the success of imposed social interventions.
- Once a vaccine for COVID-19 is widely available, wastewater surveillance can continue to be a tool for assessing the success of the vaccinations.

Monitoring the infectivity level of the virus in the wastewater could be important for several other reasons, from human contact concerns by through aerosols generation by the agitation of processes as well as monitoring the effluent wastewater to ensure cleanliness for aquifer replenishment or surface discharge.

Enveloped viruses, such as the SARS-CoV-2, have been shown to be (T90) infective for up to 36 hours at 10°C in wastewater[1]. It is feasible that sewage and fecal-contaminated water could serve as vectors for certain enveloped viruses especially at lower temperatures.

Suppression of the novel coronavirus will require a full systems approach, including physical biosurveillance to warn operators and prevent exposure through engineering changes and personal protective equipment (PPE) approaches.

Analysis Methods Compatibility for SARS-CoV-2

Reverse transcriptase PCR (rt-PCR), one of the more common detection methods for SARS-Cov-2, can detect whether the virus is present but does not distinguish whether the virus is infectious or non-infectious. Other test methods such as TCID-50 Assays will provide for the detection of a 50% infectious dose. The TCID-50 Assay is more complex and more expensive than rt-PCR and is only needed if infectivity is deemed sufficiently important.

InnovaPrep's Wet Foam Elution technology was developed as a front-end to virtually any analysis method, whether by modern molecular methods or classical culture. There is a caveat however regarding enveloped viruses like SARS-CoV-2. The foaming agent, Tween 20, a component in InnovaPrep's commercial elution fluid, has a different effect depending on whether the virus is enveloped or non-enveloped. Tween 20 will disrupt the lipid layer of enveloped viruses, thus preventing the virus from attaching to and entering into host cells. As a result, only molecular assays are compatible for enveloped viruses.

A modified Dulbecco's Eagle Medium (DMEM) fluid buffer can be used with the CP Select to preserve the infectivity of enveloped viruses during the CP Select elution process. While this DMEM fluid is still in a product development stage it can be made available by special request.

Please contact us if you would like more information on the specific method that has been used for concentrating SARS-CoV-2 from raw wastewater or would like to request a quotation.

Reference

(1) Survivability, Partitioning, and Recovery of Enveloped Viruses in Untreated Municipal Wastewater

Yinyin Ye, Robert M. Ellenberg, Katherine E. Graham, and Krista R. Wigginton*

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WET FOAM ELUTION

How it works

Particles are captured onto a membrane by dead-end filtration and are then recovered into a significantly smaller sample volume. A novel process, termed Wet Foam Elution, was developed by InnovaPrep and is the critical component in InnovaPrep products. The Wet Foam Elution process is similar to how liquid would be used to tangentially rinse particles off of a membrane filter; however, it is much more efficient than liquid rinsing for the following reasons:

- **Volume Expansion**: When rinsing a filter with liquid, most of the liquid volume is used to fill the dead space inside the filter housing; only a small portion of the fluid is actually in contact with the filter surface. This can be minimized to an extent by reducing the cross-sectional area of the fluid path across the filter, but a large portion of the liquid is still underutilized. Foam, however, is 80-90% gas, which fills the empty space without contributing to the final sample volume.
- **Increased Viscosity** - Liquid has a tendency toward "channeling" when flowing across a surface, that is, there is an area of high flow in the center of the fluid path while the portion of flow in contact with the filter surface is much slower. The higher viscosity of foam prevents channeling and creates a more uniform flow across the filter surface.
- **Bubble Dynamics** - The microbubbles in the foam behave as deformable solids. As they travel across the surface of the filter they move as a rigid body with a narrow lubricating layer; effectively squeegeeing the particles off of the surface.
- **Exfoliating Action** - As the microbubbles in the foam impact against each other and burst, the turbulence and energy produced helps to lift particles that are adhering to the membrane.

The Wet Foam Elution process requires very specific high-quality foam in order to be effective. The elution fluid is composed of water, a low concentration surfactant (less than 0.1%), and a pH buffer. This solution is packaged with carbon dioxide gas (CO_2), a significant amount of CO_2 also dissolves into the fluid. During the extraction process, the fluid passes through a valve, to a low-pressure environment causing the dissolved CO_2 to expand and come out of solution to form microbubbles. These microbubbles increase the volume of the fluid sevenfold.

An additional benefit of Wet Foam Elution is the clean buffer exchange. In many situations the starting sample matrix is not the most desirable one for the chosen analysis method. Wet Foam Elution allows the user to select the fluid that the particles will be suspended in after concentration, which maximizes the chances of detection.