Scientific Brief

COVID-19 Detection Through Concentration of SARS-CoV-2 Virus in Wastewater

Introduction

Tracking the spread of COVID-19 is one of the most critical means available to scientists for containing and ultimately eliminating the disease. Unfortunately, tracking efforts are significantly complicated by the fact that many individuals do not report mild symptoms, or are asymptomatic. Detection of SARS-CoV-2 viral particles in human wastewater provides a sensitive early-warning system for the spread of COVID-19 in communities[1,2] without needing to rely on symptom-based case reporting. In this paper, state-of-the-art of COVID-19 detection via examination of viral RNA in human wastewater is discussed.

As the COVID-19 pandemic continues to devastate communities, epidemiologists conduct systematic public health surveillance to calculate case counts and estimate the prevalence of the disease. Health care workers record case numbers, hospitalizations, and deaths so that they can judge whether the disease is rising or falling in a particular area, and nail down the precise location of local outbreaks. The more closely a disease outbreak can be tracked, the greater the odds of containing and stopping it.

Water based epidemiology (WBE) is a technique that has been used for the last 50 years to track toxic chemicals, illicit drugs, and human pathogens present in the wastewater catchment of communities or populations[3]. WBE is an extremely helpful tool for epidemiologists; while hospitals and clinics can track self-reported cases and cases identified through contact tracing, WBE can potentially identify previously undetected disease present in the population. There is an increasing body of evidence that SARS-CoV-2 viral shedding in fecal matter takes place in infected individuals, regardless of whether that individual is symptomatic.[4] Consequently, screening for the SARS-CoV-2 virus in wastewater is gaining ground as an early detection system for COVID-19 outbreaks.

Viral Concentration

Wastewater samples are highly diluted samples, and thus detection of SARS-CoV-2 relies on a primary concentration of viral RNA particles, followed by amplification of the virus's genetic signature using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR), and the subsequent detection of the amplified signal. The first step in this sequence, the viral concentration step, is crucial to the validated detection of SARS-CoV-2 viral RNA.[5] Figure 1 lays out the workflow of how wastewater samples are collected, concentrated, and processed so that SARS-CoV-2 viral RNA can be accurately detected.

Due to the rapid emergence of COVID-19 as a global public health threat, wastewater detection methodologies for SARS-CoV-2 are still in the process of being refined.[6] As with any concentration method, the effectiveness of viral recovery is impacted by the efficiency of adsorption and extraction of the target molecule. Loss of virus particles to sample processing lowers recovery rates, decreasing the sensitivity of the detection method. However, avoiding concentration altogether risks the possibility of obtaining a false negative; a grave risk in the case of a disease as dangerous and highly contagious as COVID-19.

There are several different viral concentration methods available to SARS-CoV-2 researchers, each with varying efficiency in the recovery of enveloped virus[7]. Among the various approaches that have been evaluated for viral detection, ultrafiltration is the most thoroughly studied and has been well documented to successfully concentrate viruses from wastewater[8,9]. It is also a very convenient method for the concentration of viral particles. It is gentle and highly efficient, typically resulting in greater than 90% recovery.

SARS-CoV-2 Detection Methodology

Several recently published studies have mentioned the use of centrifugal filters, including the Jumbosep™ centrifugal device from Pall, as a convenient and effective spin device for use in the primary concentration of SARS-CoV-2 particles[1,6]. In these studies, ultrafiltration via large volume centrifugal devices is determined to be one of the most efficient concentration methods currently available for SARS-CoV-2 detection from human wastewater.

Figure 1
SARS-CoV-2 detection in wastewater samples, workflow using the Jumbosep centrifugal device in viral concentration step

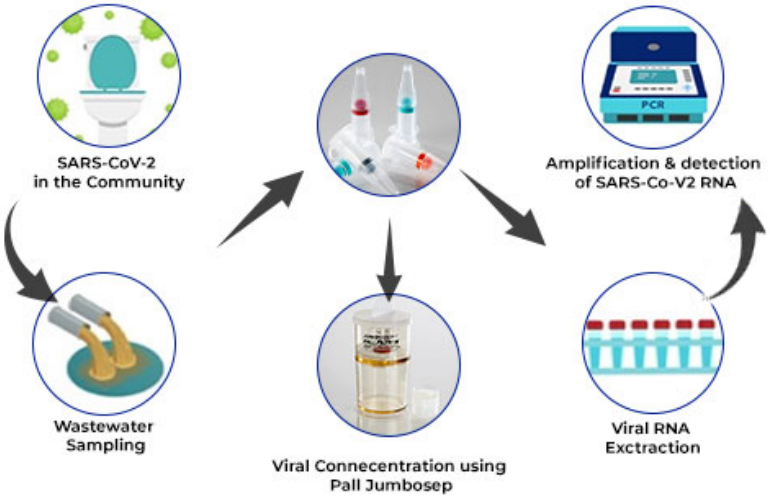


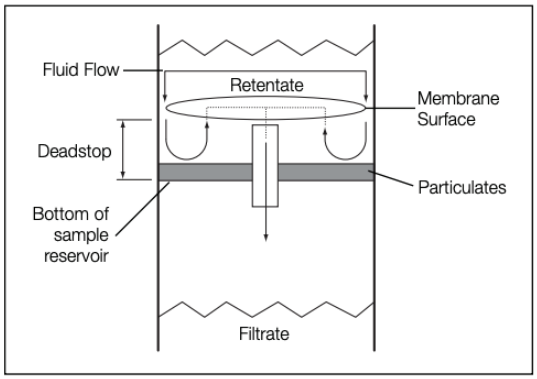
Figure 1 above shows the workflow process of wastewater samples from potentially infected individuals with COVID-19 are collected, with viral concentration being an essential step prior to viral RNA extraction, amplification and detection of SARS-CoV-2 RNA

Jumbosep centrifugal devices provide the rapid and convenient concentration, diafiltration, and fractionation of biological samples. This uniquely designed, large volume, spin filtration product can accommodate samples up to 60 mL and concentrate a 60 mL sample to 5 mL in typically 30 minutes. Pall also supplies the Microsep™, centrifugal device which can handle smaller volumes of 0.5 mL – 5 mL if desired.

Jumbosep and Microsep centrifugal devices are being used to collect and concentrate organic particles in wastewater that are then tested for SARS-CoV-2. Using either device for the detection of SARS-CoV-2 in wastewater catchment samples is easy and straightforward (Figure 2). High-speed centrifugation results in the retention of molecules above the MWCO of the low-protein-binding Omega filtration membrane in the sample reservoir. Target molecules are collected into the filtrate receiver.

Pall Centrifugal Ultrafiltration devices can accommodate starting volumes of between <50 µL to 60 mL and are compatible with several different RNA extraction kits. Following extraction, the SARS-CoV-2 RNA is amplified via RT-PCR. Jumbosep centrifugal filters typically concentrate 20 – 60 mL samples to 5 mL and achieve 50X concentration and >90% recovery in just minutes. Microsep centrifugal devices can rapidly concentrate 0.5 – 5 mL sample volumes to 65 – 100 uL and achieve 50X concentration and >90% recovery in just minutes.

Figure 2
Principal of the Jumbosep centrifugal device.



Decontamination and Re-use

Cost and environmental impact constitute a formidable hurdle to scale-up of wastewater-based COVID-19 detection. Jumbosep's re-usable format is economical and environmentally friendly. The Jumbosep product features a sample reservoir and filtrate receiver, both manufactured from low protein binding and chemically resistant polysulfone material. The sample reservoir and filtrate receiver can be sanitized and reused.

Repeated use of Jumbosep centrifugal devices for concentration requires a simple and effective decontamination procedure to prevent carry-over contamination in subsequent samples. In a recent study, Pall evaluated the Jumbosep device and found zero carryover of viral protein between successive uses

The decontamination process used in this study consisted of a one-hour immersion in a solution of a neutral detergent and 0.05 % sodium hypochlorite followed by three washes with molecular grade water. Jumbosep centrifugal devices which had first been used for concentrating a known SARS-CoV-2 positive wastewater sample were then treated with the above described decontamination process (Condition A) or a mock treatment (Condition B) which consisted solely of three washes with molecular grade water and omitted the detergent/ sodium hypochlorite immersion step.

As shown in Table 1, these results indicate that the decontamination process is effective in preventing carry-over of viral nucleic acids to successive samples without impacting the detection assay.

Table 1

SARS-CoV-2 Real-Time RT-PCR test results of water sample concentrates from Jumbosep centrifugal device

		Real-Time RT-PCR	
Sample	**Decontamination Procedure**	**Average Ct**	**Score**
Wastewater concentrate positive for SARS-CoV-2	NA	33.11	Positive
Molecular biology grade water	Condition A	>38	Negative
Molecular biology grade water	Condition B	36.68	Positive
Molecular biology grade water with plasmid E	Condition A	22.72	Positive
NTC control	NA	—	Negative

Table 1 presents the results of a SARS-CoV-2 Real-Time RT-PCR assay carried out on the resulting sample concentrates. Samples obtained from devices that were decontaminated (Condition A) tested negative in the SARS-CoV-2 Real-Time RT-PCR test, whereas samples obtained from mock-treated devices tested positive. The decontamination process did not affect the downstream detection as shown by the fact that the Real-Time RT-PCR assay was able to detect SARS-CoV-2 E gene sequences in a sample consisting of molecular biology grade water spiked with plasmid E after concentrating it in a decontaminated device.

Conclusions

The ability to accurately track the presence of COVID-19 in communities plays a critical role in the ongoing effort to eliminate the disease. Nearly 80 % of U.S. households are served by municipal wastewater collection systems,[10] making wastewater based COVID-19 detection a potentially powerful surveillance tool, particularly in those communities where COVID-19 clinical testing is currently unavailable.

WBE for the SARS-CoV-2 virus is a rapidly developing field with the prospect of detecting outbreaks of COVID-19 infection before they are identified through patient testing. SARS-CoV-2 detection in wastewater samples is critically dependent on efficient concentration and subsequent extraction of viral RNA particles. Centrifugal ultrafiltration has been identified as one of the most efficient means of concentrating SARS-CoV-2 virus in wastewater, and well-documented as being compatible with several common RNA extraction methodologies.

The Jumbosep centrifugal device provides for rapid and efficient detection of SARS-CoV-2 in human wastewater samples. Jumbosep is simple to use, and available in a large volume format commonly favored for wastewater sampling. In addition, the device is re-usable, with zero carryover contamination or risk of false positives from sample to the next. In conclusion, the Pall Jumbosep device and its related counterpart products provide a proven economical solution to the urgent need for WBE COVID-19 detection.

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