Rapid SARS-CoV-2 Viral Isolation from Wastewater

Key Advantages

> Nanotrap® Magnetic Virus Particles concentrate SARS-CoV-2 wastewater sample with no centrifugation or filtration.

Introduction

According to the Centers for Disease Control and Prevention, SARS-CoV-2 can be shed in the feces of individuals with asymptomatic or symptomatic infections, making SARS-CoV-2 RNA detection in sewage a COVID-19 indicator that is independent of healthcare-seeking behaviors and access to clinical testing.[1] Due to low concentrations in water samples such as sewage, SARS-CoV-2 viral particles must be concentrated prior to nucleic acid isolation. The three most common approaches for viral particle concentration are filtration and concentration using electronegative membranes, ultrafiltration, and precipitation. These approaches are time consuming and cumbersome, requiring access to complex laboratory equipment like centrifuges, which limits the daily sample throughput. [2-5]

Here we describe a simple and rapid approach, using Nanotrap® Magnetic Virus Particles to capture and concentrate SARS-CoV-2 from wastewater samples prior to nucleic acid isolation. Because it requires no filtration or centrifugation steps, this approach is amenable to high throughput implementation.

Materials and Methods

Wastewater

Three separate lots of SARS-CoV-2 positive wastewater were obtained from a facility in Minnesota during the summer of 2020. Upon receipt, samples were stored at 4°C for one week.

SARS-CoV-2 Concentration

Each lot of wastewater was split into 2 x 40 mL aliquots and a single 140 µL aliquot. To each 40 mL aliquot, six hundred microliters of Nanotrap® Magnetic Virus Particles (SKU 44202) were added. Each sample was briefly vortexed and left to incubate at room temperature with no additional mixing for 30 minutes. After the incubation, samples were placed on a magnetic separation rack for 2 minutes to allow the Nanotrap® Magnetic Virus Particles and the captured SARS-CoV-2 virions to pellet. The supernatant was removed and discarded. The particle pellets were then re-suspended in 140 µL of PBS.

RNA Extraction

Viral RNA was extracted from each sample using a QIAamp® Viral RNA Mini Kit (QIAGEN). To each 140 µL PBS / Nanotrap® particle sample, 560 µL of QIAGEN Virus Lysis Buffer AVL was added and incubated for 10 minutes at room temperature. Samples were then placed on a magnetic separation rack for 1 minute to allow particles to separate, and the supernatant was added to 560 µL of 100% ethanol.

As described above, as a control, RNA was extracted from 140 µL of each unprocessed wastewater sample (i.e. samples that had not been processed with Nanotrap® particles). Briefly, 560 µL of QIAGEN Virus Lysis Buffer AVL was added to each sample, which were incubated for 10 minutes at room temperature. Five hundred-sixty microliters of 100% ethanol was then added, followed by a brief vortex.

All samples (those concentrated with Nanotrap® particles and not) were then added to the QIAamp Mini columns, centrifuged, and the flow-through was discarded. Two washes, one with Buffer AW1 and a second with Buffer AW2 (500 µL each), were performed with columns being placed in new collection tubes between washes. To ensure residual ethanol was removed, additional centrifugation was performed at maximum speed, and columns were transferred to a clean 1.5 mL collection tube. For elution, 50 µL of Buffer AVE was added to the columns and incubated for 1 minute at room temperature, followed by centrifugation, eluting the RNA into a clean microcentrifuge tube for downstream testing.

Real-Time RT-PCR Analysis

The extracted viral RNA was analyzed using using 2019-nCoV RT-PCR CDC assay probes (IDT). The kit reagents and RT-PCR controls were prepared according to the manufacturer's

instructions. For each reaction, 15 µL of the prepared reaction mix was added to an optical 96-well reaction plate and mixed with 5 µL of RNA template. The plate was sealed with an optical adhesive film, loaded into a Roche LightCycler® 96, and run using the amplification settings specified by IDT. Each lot of wastewater was processed with Nanotrap® particles twice. Each RNA extraction was analyzed by real-time RT-qPCR in triplicate.

Results

Nanotrap® Magnetic Virus Particles offer a simple and rapid approach for concentration of SARS-CoV-2 from large volume wastewater samples. This method is useable without the need to filter the raw wastewater sample. Results for 40 mL of processed samples show that Nanotrap® Magnetic Virus Particles improve the Ct value of a downstream real-time RT-PCR assay by ~5, as compared to unprocessed samples, indicating that this method will work well for low viral load samples moving forward (see **Figure 1**).

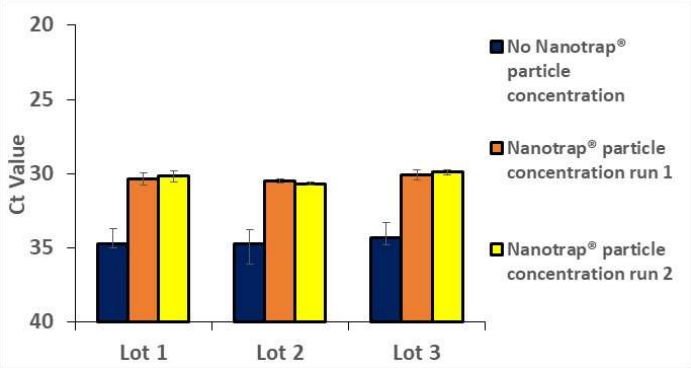

Figure 1: Concentrating wastewater samples using Nanotrap® particles is a simple and rapid approach that shows enhanced viral detection over samples processed without Nanotrap® particles. Each bar is the average of three real-time RT-PCR replicates. Error bars are 1 standard deviation. Blank water samples showed no detectable viral RNA.

Conclusions

Nanotrap® Magnetic Virus Particles enable SARS-CoV-2 detection in large volume raw wastewater samples, allowing them to serve as a good alternative to time consuming and cumbersome viral concentration approaches that rely on centrifugation or filtration. Nanotrap® Magnetic Virus Particles can be used in liquid handling systems to automate sample analysis.

References

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