Rapid concentration of SARS-CoV-2 RNA in wastewater samples by ultracentrifugation for disease surveillance

Introduction

Wastewater-based disease surveillance is an important tool that health authorities increasingly use to track disease emergence and to monitor transmission trends. Recent efforts have focused on testing wastewater for the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA during the global health crisis [1,2]. Although SARS-CoV-2 transmission is primarily through respiratory droplets and aerosols, viral RNA has also been found in excreta of infected individuals [3,4]. SARS-CoV-2 RNA detected in wastewater can serve as a disease indicator that is not affected by healthcare-seeking behaviors and availability of clinical testing.

Wastewater surveillance often incorporates an initial step of concentrating disease agents and their genetic material. In this application note, we present an ultracentrifugation-based method developed by Green and colleagues [5], utilizing the Thermo Scientific™ Sorvall™ WX+ Ultracentrifuge with the Thermo Scientific™ SureSpin™ 630 Swinging-Bucket Rotor (which has been replaced by the higher-performance SureSpin 632 Swinging-Bucket Rotor, Table 1), to concentrate SARS-CoV-2 RNA in wastewater samples.

Sorvall WX+ Ultracentrifuge

SureSpin 632 Swinging-Bucket Rotor with 38.5 mL and 17.0 mL Bucket Sets

Table 1. Specifications of SureSpin 630 and SureSpin 632 rotors.

	SureSpin 630 rotor	SureSpin 632 rotor
Max. speed (rpm)	30,000	32,000
Max. RCF (x *g*)	166,880 (36.0 mL) 166,880 (17.0 mL)	187,295 (38.5 mL) 191,759 (17.0 mL)
Max. capacity	6 x 36.0 mL	6 x 38.5 mL
K-factor	219	194
Rotor surface color	Black	Bare titanium
Max. cycle number	400	1,200

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Methodology

Concentration of viral particles and nucleic acid

1. Blend wastewater samples to resuspend any settled particulates.
2. Transfer 20 mL of each sample into Thermo Scientific™ 36 mL PET thin-walled ultracentrifuge tubes (Cat. No. 75000471), using a disposable serological pipette.
3. Transfer 12 mL of sucrose cushion [50% sucrose in TNE buffer (20 mM Tris-HCl, pH 7.0, 100 mM NaCl, 2 mM EDTA)] with a serological pipette to below the sample to form distinct layers as shown in Figure 1A.
4. Balance the tubes containing the wastewater samples in batches of six by adding distilled water (<500 µL).
5. Centrifuge the samples using the SureSpin 630 Swinging-Bucket Rotor in the Sorvall WX+ Ultracentrifuge, at 150,000 x *g* for 45 min at 4°C.
6. Discard the supernatants carefully with fresh serological pipettes, and resuspend the pellets, which contain viral particles and nucleic acids (Figure 1B), in 200 µL of 1X PBS.
7. Transfer the suspensions to fresh microcentrifuge tubes and store at –20°C for no more than 24 hours until nucleic acid extraction.
8. Continue with nucleic acid extraction and subsequent qPCR for analysis.

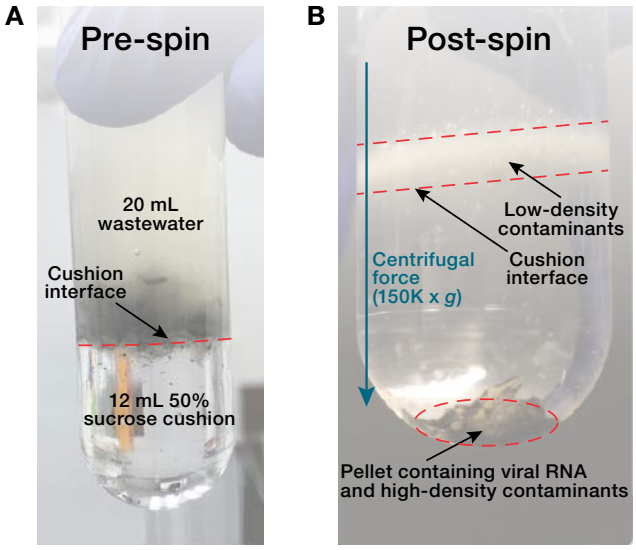

Figure 1. Concentration of viral particles and nucleic acids by ultracentrifugation. (A) Before ultracentrifugation, a wastewater sample and 50% sucrose cushion underneath it form distinct layers. **(B)** After ultracentrifugation, SARS-CoV-2 viral particles and nucleic acids are present in the pellet, and low-density contaminants sit on top of the sucrose cushion. Figure from Wilder ML et al. [5], used with changes under terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License (http://creativecommons.org/licenses/by/4.0/).

Conclusion

This procedure offers a rapid and scalable method for concentration of SARS-CoV-2 viral particles and nucleic acids in wastewater, using a sucrose cushion and the Sorvall WX+ Ultracentrifuge with the SureSpin 630 Swinging-Bucket Rotor. The SureSpin 632 Swinging-Bucket Rotor is recommended as a substitute rotor with increased performance. Removal of low-density contaminants from wastewater samples and pelleting viral particles and nucleic acids via ultracentrifugation help minimize potential interference issues with subsequent nucleic acid extraction and qPCR steps. This method has been shown to enable detection and/or quantification of SARS-CoV-2 in low-prevalence areas (as low as 1 positive test per 10,000 inhabitants), and as rapidly as 4.5 hours after sample arrival [5]. Quick turnaround time and high sensitivity may help public health authorities manage timely responses at the initial stages of SARS-CoV-2 community spread and future viral infections.

References

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