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Sampling and viral concentration for SARS-CoV-2 detection in wastewater

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GUIDELINES

This protocol has been effective at recovering enveloped viruses genes for qPCR detection, but not appropriate when infective viruses are desired.

STEPS MATERIALS

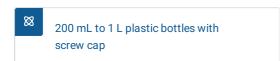
NAME	CATALOG #	VENDOR	
200 mL to 1 L plastic bottles with screw cap			
0.25 N glycine buffer (pH 9.5)			
2× phosphate-buffered saline (PBS, pH 7.2)			

EOUIPMENT

NAME	CATALOG #	VENDOR
Ultracentrifuge	XPN-90	
Centrifuge	5424	

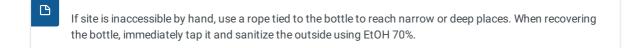
Sampling wastewater

Autoclave plastic containers and expose to UV for 10 min.



2 Fill bottles with wastewater at sampling points using sterile gloves.





3 Place bottles in a refrigerated container (i.e. with ice flakes or gel packs) until returning to the lab. Our sampling days usually take 4-6 hours since first sample is collected.

 2× phosphate-buffered saline (PBS, pH 7.2)

5 Place 42 mL of sewage water in an appropriate ultracentrifugation tube and ultracentrifugate as follows:

1h

3100000 x g

84°C

© 01:00:00

6 Discard supernatant resuspend pelleted viral particles as follows:

6.1 Discard supernatant.

6.2 Resuspend viral particles in $\square 3.5$ ml glycine buffer.

5m

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30m 6.3 Incubate on ice for \bigcirc **00:30:00** 5m Add 3.5 ml PBS and gently mix. 15m Clarify supernatant by centrifugation as follows: **◎12000 x g © 00:15:00** 1h Finally recover viruses by ultracentrifugation as follows: **③100000 x g** 84°C **© 01:00:00** 10 Resuspend viruses in 200 µl PBS . Process immediately for nucleic acid extraction or store at 8-80 °C.