IRAOLA LAB

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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)		

EQUIPMENT

NAME	CATALOG #	VENDOR
Ultracentrifuge	XPN-90	
Centrifuge	5424	

Sampling wastewater

1 Autoclave plastic containers and expose to UV for 10 min.

200 mL to 1 L plastic bottles with screw cap

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

Sampling points may be diverse and accessibility to water sources will be sometimes difficult depending on site infrastructure. Consider these aspects before starting sampling.

If site is inaccessible by hand, use a rope tied to the bottle to reach narrow or deep places. When recovering the bottle, immediately tap it and sanitize the outside using EtOH 70%.

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.

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Concentration by ultracentrifugation 1h

4 Prepare your reagents and materials:

Ultracentrifuge

Beckman Coulter XPN-90

Your ultracentrifuge must reach 100,000 x g and cool down to 4° C

Centrifuge

Bench centrifuge

Eppendorf 5424

0.25 N glycine buffer (pH 9.5)

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

5 Place 42 mL of sewage water in an appropiate ultracentrifugation tube and ultracentrifugate as follows: 1h

100000 x g

4 °C

01:00:00

6 Discard supernatant resuspend pelleted viral particles as follows:

6.1 Discard supernatant.

6.2 Resuspend viral particles in **3.5 ml glycine buffer** . 5m

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6.3 Incubate on ice for **00:30:00** 30m

7 Add **3.5 ml PBS** and gently mix. 5m

8 Clarify supernatant by centrifugation as follows: 15m

12000 x g

00:15:00

9 Finally recover viruses by ultracentrifugation as follows: 1h

100000 x g

4 °C

01:00:00

10 Resuspend viruses in **200 µl PBS** .

11 Process immediately for nucleic acid extraction or store at **-80 °C** .

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