HA Filtration of Wastewater for SARS-CoV-2 detection

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1 *Works for me* This protocol is published without a DOI.

UNC Charlotte COVID WBE

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ABSTRACT

This protocol provides the information on how to concentrate SARS-CoV-2 virus in wastewater sample using a vacuum filtration in conjunction with electronegative filter (type HA) method. The volume of wastewater sample that could be filtered at once depends on the turbidity of the sample.

PROTOCOL CITATION

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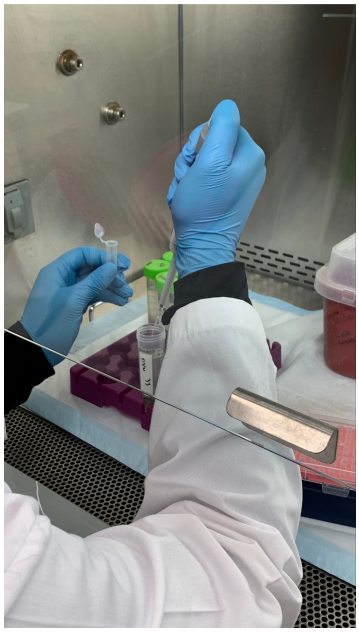
GUIDELINES

Working with wastewater samples requires appropriate IBC approvals and PPE requirements for your institutions.

1 Put on proper PPE (lab coat, N95 fitted masks, gloves, face shield) before handling the wastewater sample.

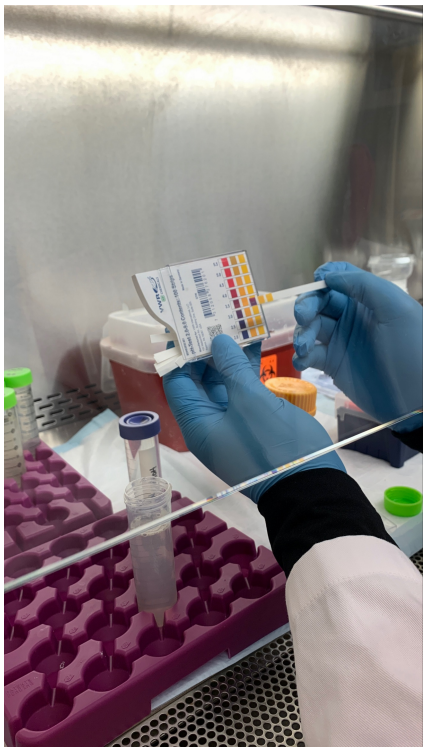
2 The biosafety cabinet should be properly sterilized (UV light for 20 minutes and the surface should be cleaned with 70% ethanol). Gloves should be sprayed with 70% ethanol prior to starting.

3 Place a 40 mL aliquot of wastewater into a 50 mL conical tube.
Add 4 µL of Bovine Coronavirus modified live vaccine (BCoV) to 40 mL of wastewater.



4 Adjust the pH of the wastewater sample to 3.5-4 using 10 M HCl either with pH paper or pH meter. While adding the HCl the sample should be properly mixed.

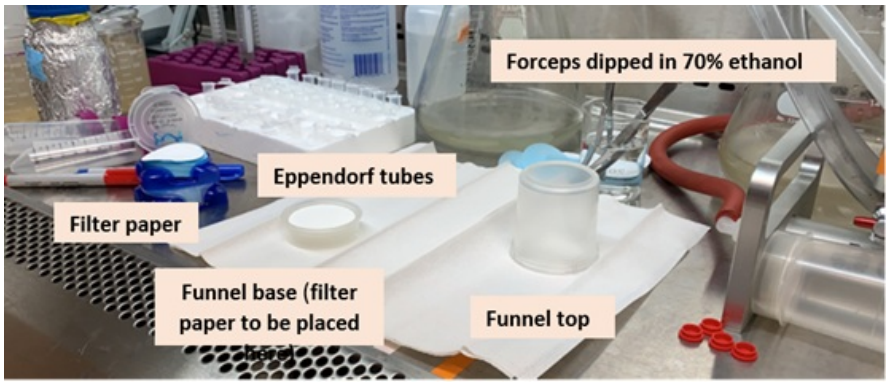
N.B. If there are multiple samples pH paper is more favorable to avoid cross-contamination by using the same pH electrode for every sample. If pH meter is used electrode should be cleaned with 10% bleach, followed by 2% sodium thiosulfate and distilled water.



5 Add 400 µL of 2.5M $MgCl_2$ x 6 H_2O per 40 mL of sample, mix the sample properly.

6 Pass the entire volume of sample through a disposable filter funnel fitted with electronegative (type HA) filter of 0.45 µm pore size and 90 mm diameter with a vacuum filtration manifold .

7 Following filtration, fold the filter paper using sterilized forceps dipped in 70% ethanol and place in the labeled Eppendorf tube containing 1 mL of lysis buffer (AVL with carrier RNA, Qiagen).



8 Repeat steps 1- 7 for each replicate.

N.B. For the same sample, the filter funnel can be the same, but the filter paper should be new for every replicate. But for different sample the filter funnel and the filter paper should be changed every time.

9 For each set of wastewater samples collected and processed, a method blank was prepared.

N.B. Add 4 µL BCoV to 40 mL phosphate buffer (1X PBS) and repeat step 5-7.

10 After completing the filtration work, clean the biosafety cabinet with 10% bleach and 70% ethanol and turn on the UV lights for 20 minutes.

11 Add 10% bleach to any liquid waste generated during the process and let it sit for 30 minutes before disposing.