RNA Extraction of SARS-CoV-2 from Wastewater

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

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ABSTRACT

Modified protocol from RNA-extraction of SARS-CoV-2 from wastewater using the Qiagen QIAmp Viral RNA extraction kit.

PROTOCOL CITATION

Neha Mittal, Vbarua , Nick Stark, Cynthia Gibas, jschluet , Mariya Munir 2021. RNA Extraction of SARS-CoV-2 from Wastewater. **protocols.io** https://protocols.io/view/rna-extraction-of-sars-cov-2-from-wastewater-bqxcmxiw

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SAFETY WARNINGS

Take care to wear appropriate PPE at all times. Extraction protocols are done in a Class IIB Biosafety cabinet.

BEFORE STARTING

Prepare you area with all necessary materials and safety equipment.

1 The biosafety cabinet should be properly sterilized (UV light for 20 minutes and the surface should be cleaned with 70% ethanol). Clean the area with RNA Zap. All extraction work will be done inside the biosafety cabinet.

Clean the equipment/instruments to be used including the centrifuges and pipettes with 70% alcohol and RNA Zap

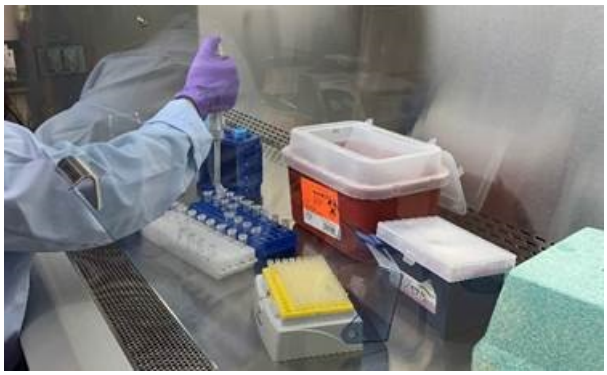
Put on proper PPE (lab coat, N95 fitted masks, gloves, face shield) before handling the wastewater filter.

2 Preparing and labeling all tubes:

- Place sample concentrated vials on a tray in chronological order
- Label 2mL tubes and place in separate tray chronologically in the same positions as the sample vials
- Label QIAamp Mini Spin Column Tubes and place in 3rd separate tray chronologically in the same positions as the sample vials
- Label 1.5 mL tubes in (label same way as sample tubes) and place in separate tray chronologically in the same positions as the sample vials (Label these same exact way as the sample tubes (top and side))
- Label PCR tubes and put them into storage box, same number as sample vials, can label with short abbreviations

3 Transfer 800 uL of AVL Buffer (with carrier RNA already added) into 2mL tubes (the first set of labeled tubes)

4 Transfer 200 uL of corresponding sample solution (1 mL of AVL buffer with the concentrated filter) from the sample vials into the corresponding 800 uL of AVL buffer.



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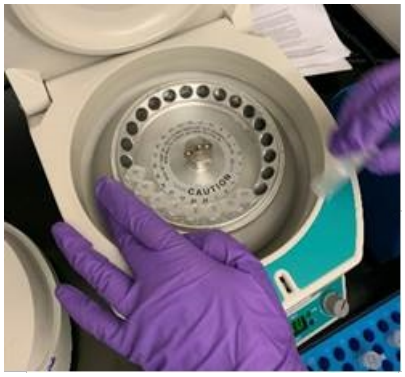
5 Vortex each sample for 15 seconds followed by a quick spin.

6 Incubate samples for 10 minutes at room temperature.

7 Add 800 uL of 100% pure ethanol to each sample and vortex for 15 seconds followed by a quick spin.

8 Add 630 uL of the sample solution to the QIAamp Mini column placed in a 2 mL collection tube.

9 Centrifuge samples for 1 minute at 8(x1000) RPM



10 If you have extra sample, once you've centrifuged, discard the column flow through from the collection tube on the bottom and load another 630 uL onto the column.

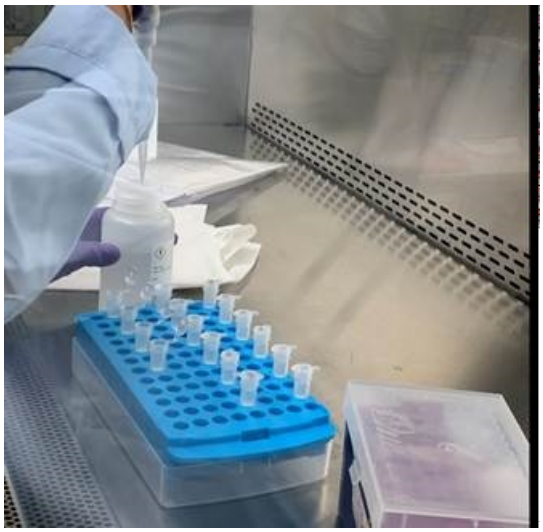
Centrifuge the samples for 1 minute at 8(x1000) RPM

11 Once all sample is in the column, transfer the column to a new 2 mL collection tube.

12 Add 500 uL of AW1 Buffer to each column and centrifuge for 1 minute at 8(x1000) RPM

13 Transfer the columns to a new 2 mL collection tube and discard the filtrate.

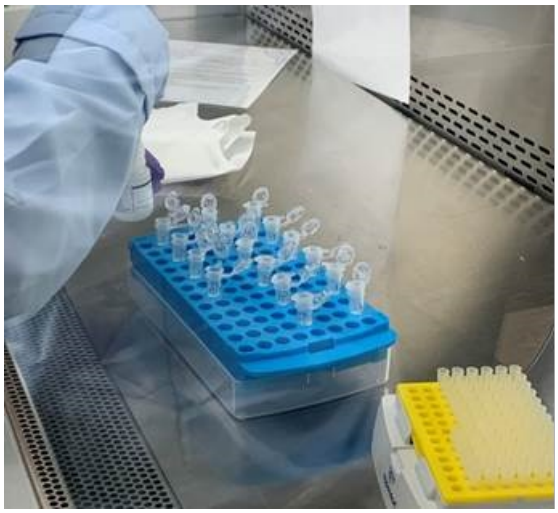
14 Add 500 uL of AW2 Buffer to each column.



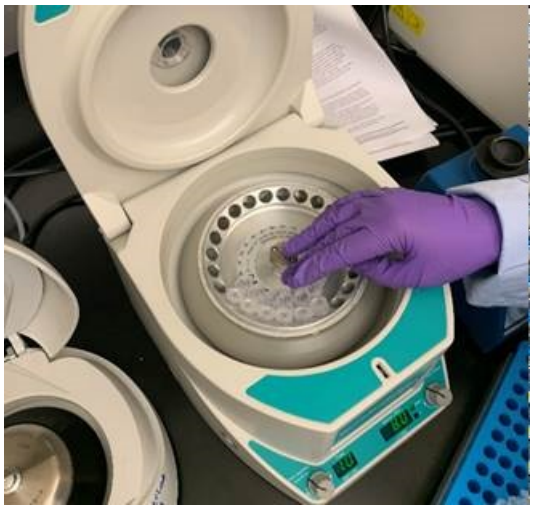
15 Centrifuge for 3 minutes at 13.3(x1000) RPM

16 Transfer the column to a clean 1.5 mL tube and discard the collection tube with filtrate.

17 Add 60 uL of AVE elution buffer and incubate at room temperature for 1 minute.



18 Centrifuge for 1 minute at 8(x1000) RPM



19 Viral RNA is collected at the bottom of the 1.5 mL tube and can be stored at -20 degrees C.

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