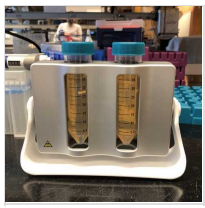

Jan 21, 2022

Manual Nanotrap Concentration and RNA Extraction for SARS-CoV-2 Viral Capture

Matthew Cavallo[1], Stephen P Hilton[1], Jamie VanTassell[1], Julia Raymond[1], Marlene K Wolfe[1], Pengbo Liu[1], Christine Moe[1]

[1]Center for Global Safe WASH, Rollins School of Public Health, Emory University, Atlanta GA USA

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Stephen Hilton

This protocol details a method for SARS-CoV-2 capture and concentration through the use of Nanotrap® Magnetic Virus Particles from 40mL of wastewater sample.

DOI

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Nanotrap, manual Nanotrap, wastewater, SARS-CoV-2, COVID-19, magnetic, magnetic virus particles, Ceres, nano

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Equipment

- pH probe
- Magnetic rack (Dynamag)
- Centrifuge
- QIAamp Viral RNA Mini Kit (Cat. No. 52904 or 52906)
- Conical tube (50mL)
- Eppendorf Research Plus Single Channel Pipette
- LabGard Biological Safety Cabinet - Class 2 A2 Biosafety Cabinet
- Autoclave - Amsco Lab 240 Steam Sterilizer

Materials (per sample)

- 10 µL of BRSV
- 400 µL of

  Nanotrap Magnetic Virus Particles (10) **Ceres Nano Catalog #44202** Step 6

  Molecular Grade
- 1mL of Water **ATCC Catalog #60-2450** Step 10
- Three 1.7mL tubes
- 40 µL of 1X PBS (Phosphate-buffered saline) **Contributed by users**

  Buffer
- 560 µL of AVL **Qiagen Catalog #19073** Step 14

  Carrier RNA **Thermo**
- 5.6 µL of **Fisher Catalog #4382878** Step 14
- 560 µL of 96% ethanol

  Buffer
- 500 µL of AW1 **Qiagen Catalog #19081** Step 24

  Buffer
- 500 µL of AW2 **Qiagen Catalog #19072** Step 25

  Buffer
- 60 µL of AVE **Qiagen Catalog #1020953** Step 28

Concentration Procedure

1 Place a 50 mL conical tube in a tube rack.

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2 Vigorously shake the sample to re-suspend solids. Immediately move on to Step 3.

3 Add **50 mL** of wastewater to the conical tube inside the biosafety cabinet.

4 Transfer **40 mL** of the top supernatant into a new conical tube.

5 Add **10 µL** of BRSV to the conical tube.

6 Add **400 µL** of Nanotrap Magnetic Virus Particles (10) **Ceres Nano Catalog #44202** to the sample and invert 2-3 times to mix together and to create a 10:1 sample volume to particle ratio.

7 Invert samples 3-4 times every 5 minutes at **Room temperature** for **00:20:00** . 20m

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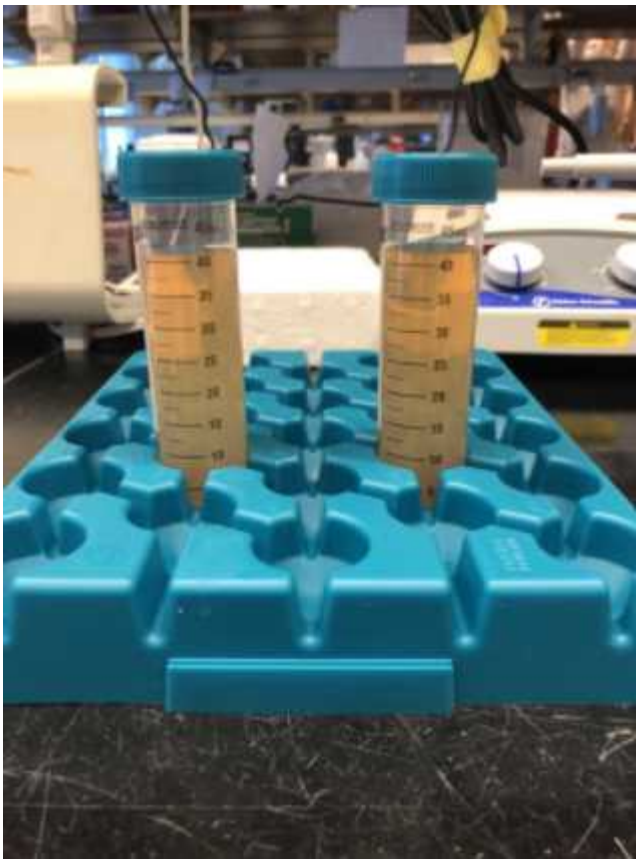

8 Use a magnetic rack to separate the magnetic Nanotrap particles from the sample. Allow the sample to sit in the rack for at least **00:10:00** . 10m

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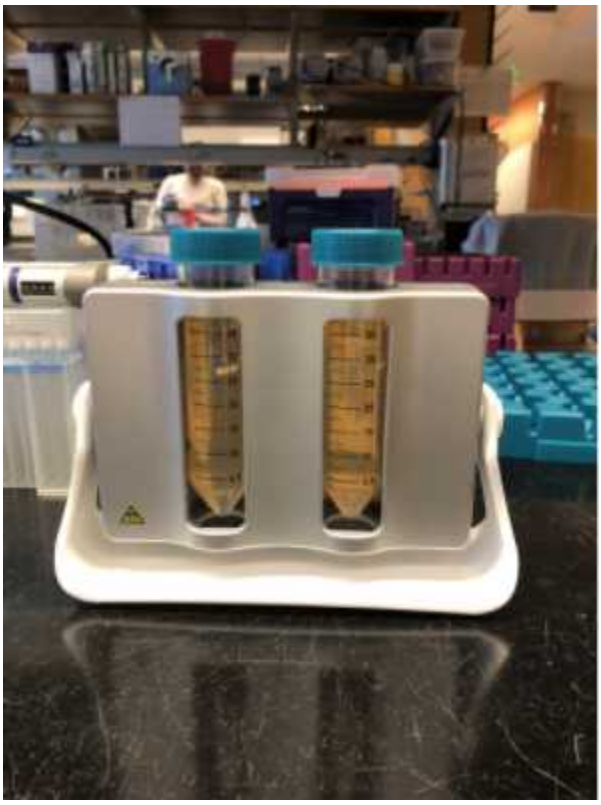

Samples must sit in a magnetic rack for at least 10 minutes to allow for particle separation.

9 Use a pipette to discard the supernatant. Be careful not to disturb the red pellet of Nanotrap particles.

10 Molecular Grade

Add **1 mL** of Water **ATCC Catalog #60-2450** to the conical tube.

Vortex the tube to re-suspend the pellet.

11 Transfer liquid to a 1.7mL tube. Place tubes on magnetic rack and allow to sit for 2m **00:02:00**

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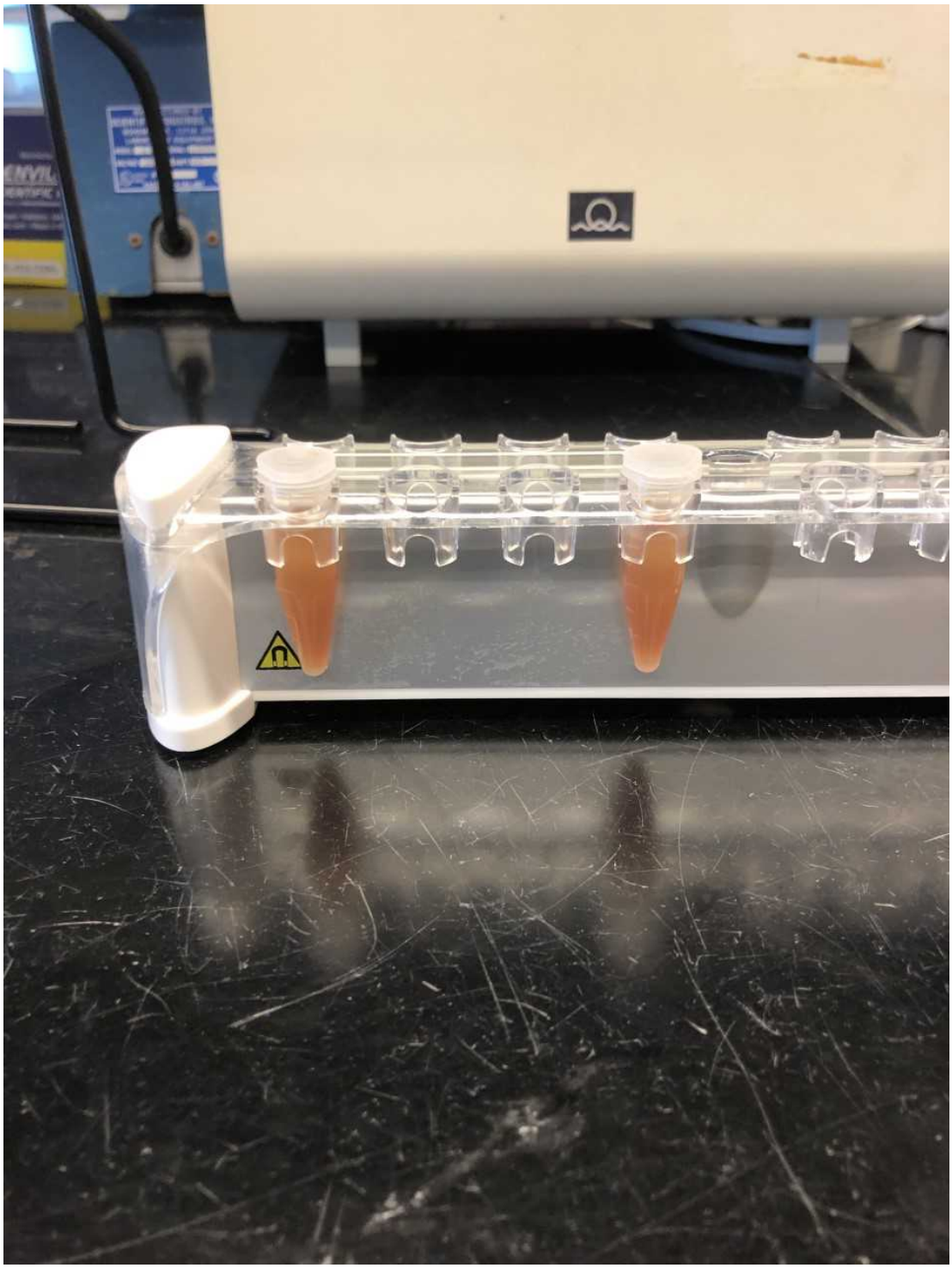

Sample in 1.7 mL tube sits in a magnetic rack for 2 minutes.

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A red pellet of Nanotrap particles congregates to the magnetic side of the 1.7 mL tube.

12 Remove and discard the supernatant. Do not disturb the pellet.

13 Add **140 µL** of 1X PBS to the particle pellet.

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See "Appendix I: Preparing Buffer Solution (1X PBS)" for instructions on how to prepare 1X PBS.

14 In a separate 1.7 mL tube, add **560 µL** of Buffer AVL **Qiagen Catalog #19073** and Carrier RNA **Thermo** **5.6 µL** of **Fisher Catalog #4382878** . Mix well by pipetting up and down several times.

15 In a regular, non-magnetic rack, add the Buffer AVL and carrier RNA to the particle pellet. Suspend the pellet by using the pipette to wash the sides of the conical tube with the lysis buffer, carrier RNA, and 1X PBS mixture.

16 Incubate the sample at **Room temperature** for **00:10:00** . 10m

17 Use a magnetic rack to separate the magnetic Nanotrap particles from the sample. Allow sample to sit on the rack for **00:02:00** . 2m

18 Transfer the supernatant to a 1.7 mL tube and discard the pellet.

Extraction Step (Qiagen Viral RNA Minikit) 11m

19 Add **560 µL** of 96% Ethanol **P212121 Catalog #BE-BDH1156** to sample. Mix well by pipetting up and down several times.

20 Add **630 µL** of the sample mix onto a QIAamp Mini column.

21 Centrifuge sample at full speed for **00:01:00** . Once complete, discard the filtrate. 1m

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22 Repeat Step 26 until all of the sample has gone through the column.

23 Transfer the QIAamp Mini Column into a new collection tube, and discard the tube containing the filtrate.

24 Open the QIAamp Mini Column and add **500 µL** of Buffer AW1 **Qiagen Catalog #19081** . Centrifuge the sample at full speed for **00:01:00** . Once complete, discard the filtrate. 1m

25 Open the QIAamp Mini Column and add **500 µL** of Buffer AW2 **Qiagen Catalog #19072** . Centrifuge the sample at full speed for **00:03:00** . Once complete, discard the filtrate. 3m

26 Place the QIAamp Mini Column into a new collection tube and discard the old one containing filtrate. Centrifuge the sample at full speed for **00:01:00** to remove any buffer AW2 carryover. 1m

27 Place the QIAamp Mini Column into a final, labeled tube and discard the old one containing the filtrate

28 Open the QIAamp Mini Column and add **60 µL** of Buffer AVE **Qiagen Catalog #1020953** and incubate at **Room temperature** for **00:03:00** . 3m

29 Centrifuge the sample at **16000 rpm** for **00:02:00** . 2m

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30 Separate sample into at least two aliquots. Store in **-80 °C** freezer until it is ready for PCR.

Appendix I: Preparing Buffer Solution (1X PBS)

31 Begin making the 10X PBS Solution by mixing the following:

- **80 g** NaCl
- **2 g** KCl
- **14.4 g** Na_2HPO_4
- **2.4 g** KH_2PO_4
- **800 mL** Ddwater

32 Adjust the solution to pH **7.4** by adding 5% NaOH and/or 5% HCl.

- Swirl the mixture after adding either NaOH or HCl.
- Use a pH probe in between adding NaOH or HCl to see if the pH has reached 7.4 yet.
- Wash off tip of probe with DI water in between uses.

33 Add more double distilled water (ddH2O) **Contributed by users** until the volume is **1000 mL** .

34 Dilute the PBS solution by adding **100 mL** of 10X PBS to **900 mL** distilled water to create 1X PBS.

35 Dispense mixture into aliquots and autoclave for **00:20:00** . Store at room temperature.

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