



SARS-CoV-2 Wastewater RNA Concentration and Extraction (Nanotrap® and NucleoMag® RNA Water)

Brett Rasile¹, Kendra Maas¹

Jan 20, 2021

¹University of Connecticut

 Works for me dx.doi.org/10.17504/protocols.io.bn58mg9w

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Kendra Maas

ABSTRACT

Process

DOI

dx.doi.org/10.17504/protocols.io.bn58mg9w

PROTOCOL CITATION

Brett Rasile, Kendra Maas 2021. SARS-CoV-2 Wastewater RNA Concentration and Extraction (Nanotrap® and NucleoMag® RNA Water). **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bn58mg9w>



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CREATED

Oct 28, 2020

LAST MODIFIED

Jan 20, 2021

OWNERSHIP HISTORY

Oct 28, 2020 brett.rasile

Jan 18, 2021 Kendra Maas

PROTOCOL INTEGER ID

43936

Virus Capture & Concentration

10m

1 Create a 1:100 dilution of **Zoetis Bovine Rhinotracheitis-Parainfluenza-Respiratory Syncytial Virus (BRSV) Vaccine** in H₂O.

2 Collect **500 mL** of wastewater in a 500mL or 1L plastic sample bottle.



500mL of wastewater collected in 500mL plastic bottles.

- 3 Spike the **500 mL** wastewater sample with **500 µl** of **1:100 BSRV** to achieve a 1µL/mL concentration of **1:100 BSRV**.

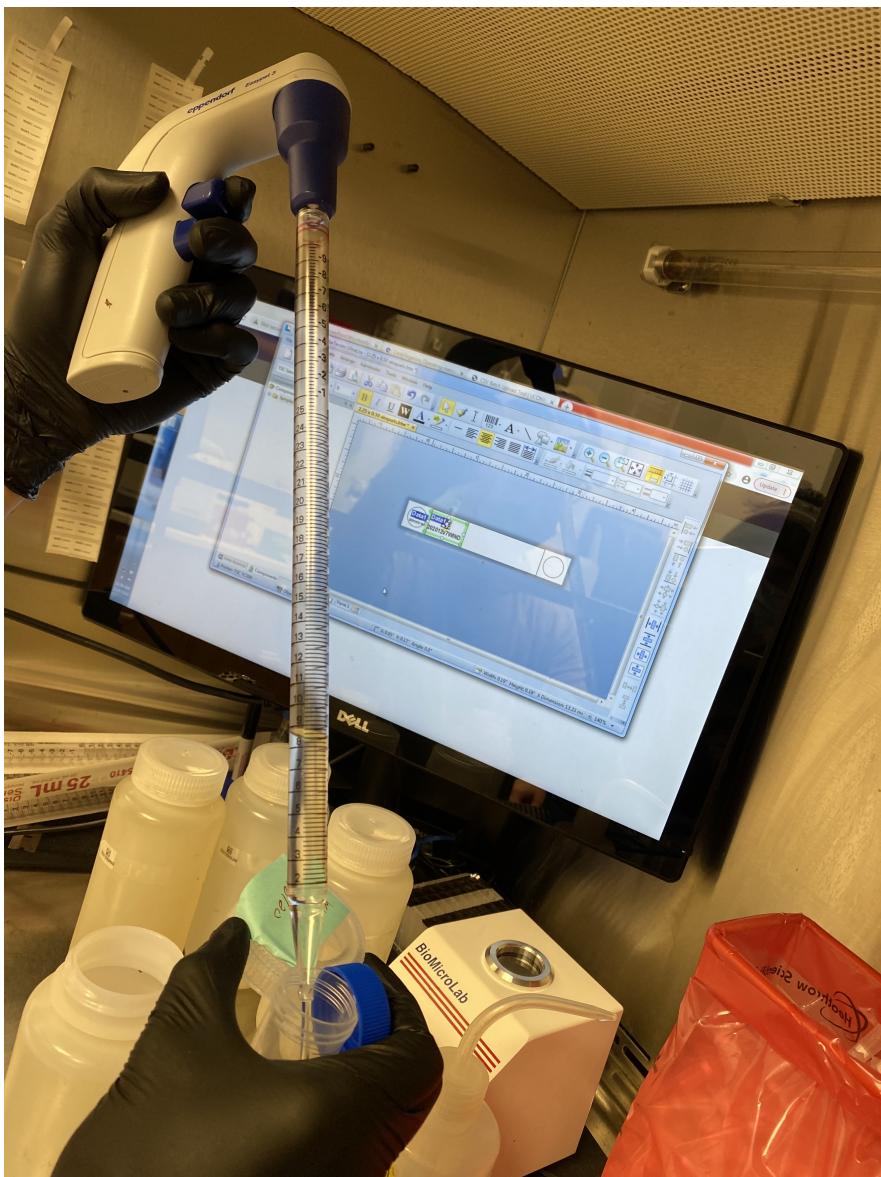
This can be applied to wastewater samples greater or less than 500mL, as long as a 1µL/mL concentration is achieved.

- 4 Mix the added 1:100 BRSV into the wastewater sample by aggressively inverting the bottle several times or shaking.

- 5 Incubate spiked wastewater samples for a minimum of **00:10:00** at room temperature (RT) to allow large aggregates to sediment at the bottom of the sample bottle. 10m

- 6 Use a 25mL serological pipette to transfer top **40 mL** of spiked wastewater to a 50mL conical tube.

Make sure to **gently** transfer from the **top** of the wastewater sample as to not disturb the sedimented particles at the bottom of the bottle.



Transferring 40mL of spiked and settled wastewater samples from 500mL bottles to 50mL conical tubes.

7 Add **600 μ L** of Ceres Magnetic Nanotrap® particles to the **40 mL** spiked wastewater aliquot.

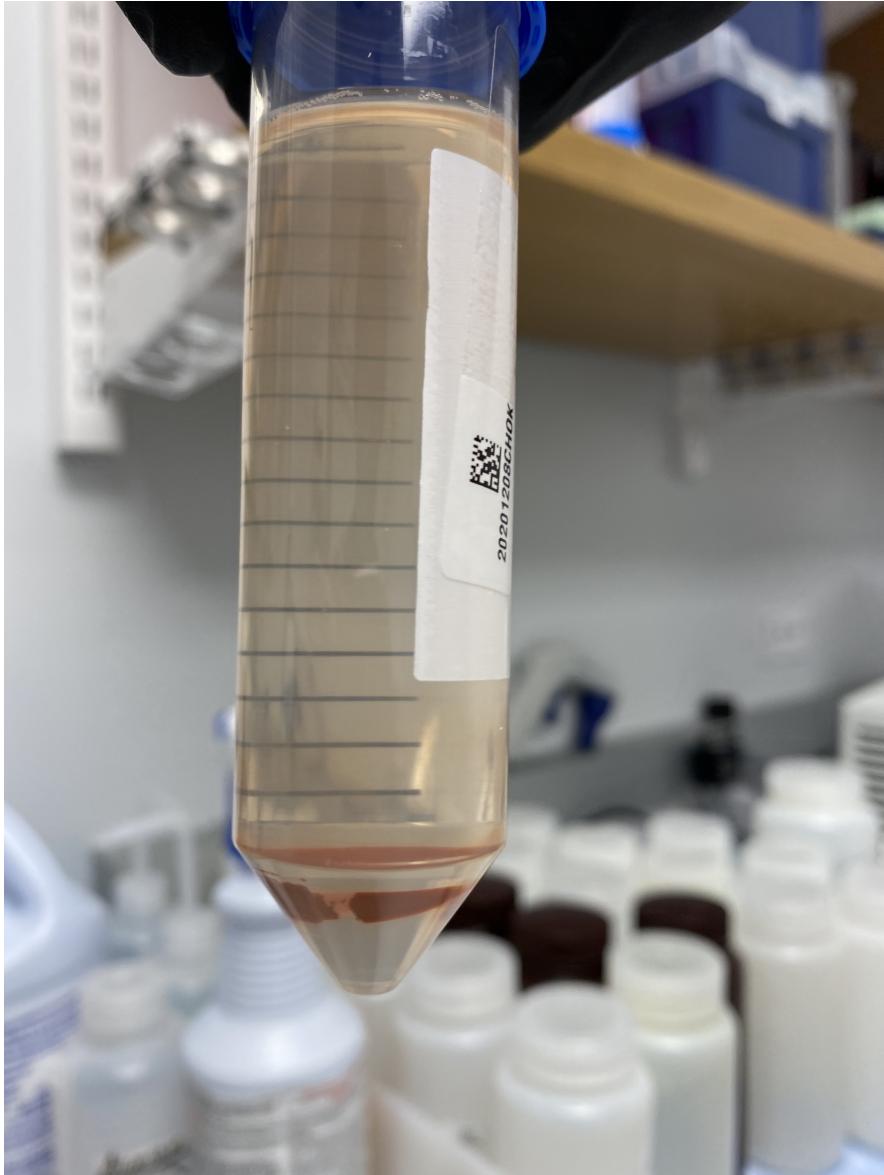
7.1 Invert the wastewater samples several times to incorporate the Magnetic Nanotrap® particles and incubate for 20 minutes at RT.

8 Place conical tubes into custom magnet racks and allow magnets to attract Magnetic Nanotrap® particles for a minimum of 20 minutes at RT.

The samples should be allowed to sit on the magnets long enough so that the supernatant is relatively clear. Some wastewater samples may be too turbid to reach this point, however.



Conical tubes with wastewater samples after incubation on magnets. Notice the rust-red Magnetic Nanotrap particles that have precipitated at the bottom of the tubes near the magnets.



Wastewater sample with Magnetic Nanotrap particles completely out of solution following magnet incubation.

- 9 Keeping the conical tubes fixed to the magnets, pour off the supernatant carefully as to not disturb the pellet of Magnetic Nanotrap® particles at the bottom of the conical tubes.

Viral RNA Extraction (Nucleomag® RNA Water)

39m

- 10 Add **500 µl** of **Buffer MWA1** to the falcon tubes.

- 10.1 Vortex to resuspend the Magnetic Nanotrap® pellets in the Buffer MWA1.

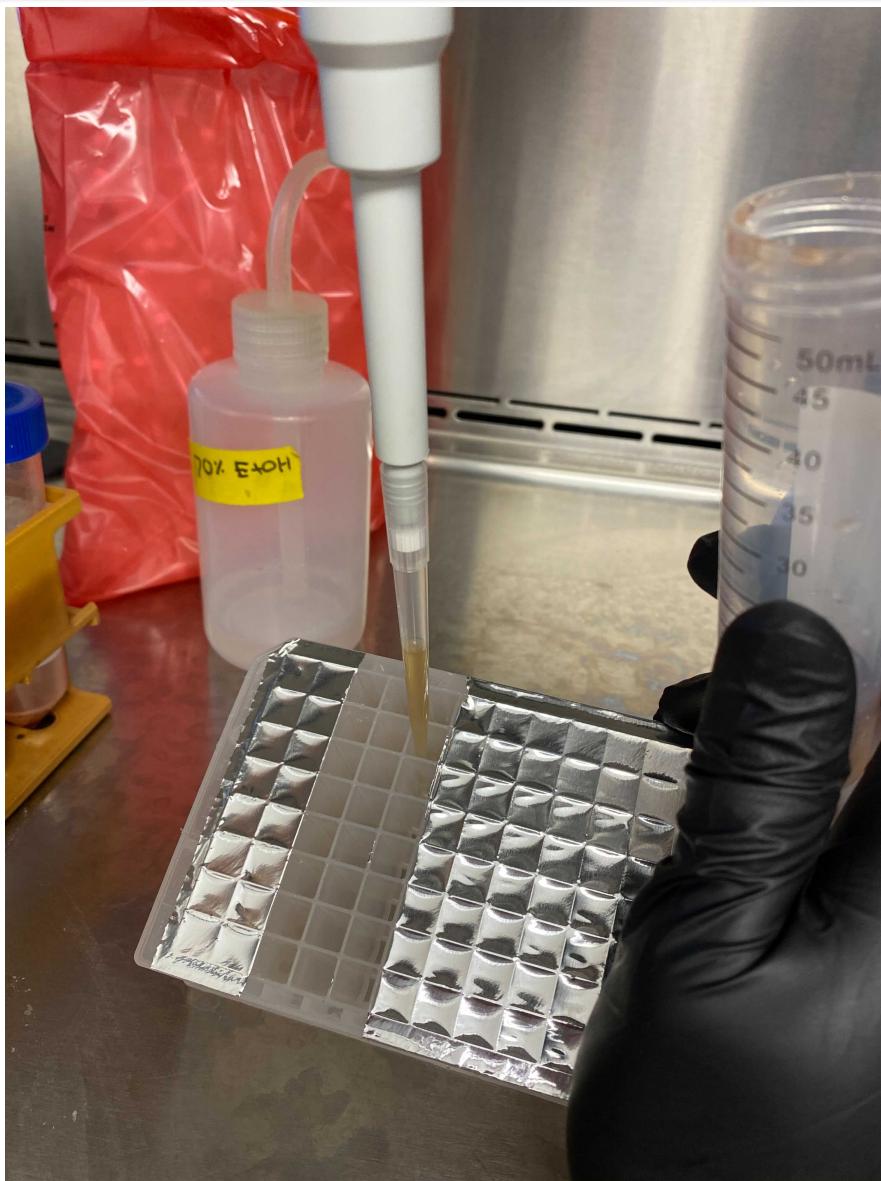
- 10.2 Incubate samples for **00:10:00** at RT.

10m

11 Place conical tubes on the custom magnet racks to separate the Magnetic Nanotrap® particles.

11.1 With the tubes on the magnets, transfer \square 450 μ L of lysate to a 2mL deep well plate.

Use barcode labels on the falcon tubes to transfer sample ID's to a LIMS program when transferring lysate to the deep well plate.



Transferring lysate to a 2mL deep well plate for RNA extraction. Columns not being used in the current extraction run are sealed using non-pierceable foil.

12 Add \square 475 μ L of Buffer MWA2 and \square 25 μ L of NucleoMag® B-Beads to the lysate in the deep well plate.

12.1 Shake the sample plate for **00:05:00** at **1400 rpm**, **56 °C**. 5m

12.2 Place the sample plate on a plate magnet for at least **00:05:00** to separate NucleoMag® B-beads. 5m

At each magnet step, allow as much time as needed for the NucleoMag® B-beads to completely precipitate near the magnets.

12.3 Use a multichannel pipette to remove the supernatant from each well.

Ensure that NucleoMag® B-beads are not removed with the supernatant. If beads are drawn up with the supernatant, dispense the liquid back into the well and incubate on a magnet for a few minutes to allow the NucleoMag® B-beads to settle at the magnet.

13 Add **850 µl** of **Buffer MWA3** to each well.

13.1 Shake the sample plate for **00:02:00** at **1400 rpm**, **56 °C**. 2m

13.2 Place the sample plate on a plate magnet for at least **00:02:00** to separate NucleoMag® B-beads. 2m

13.3 Use a multichannel pipette to remove the supernatant from each well.

14 Add **850 µl** of **Buffer MWA3** to each well.

14.1 Shake the sample plate for **00:02:00** at **1400 rpm**, **56 °C**. 2m

14.2 Place the sample plate on a plate magnet for at least **00:02:00** to separate NucleoMag® B-beads. 2m

14.3 Use a multichannel pipette to remove the supernatant from each well.

15 Add **850 µl** of **Buffer MWA4** to each well.

15.1 Shake the sample plate for **00:02:00** at **1400 rpm**, **56 °C**.

2m

15.2 Place the sample plate on a plate magnet for **00:02:00** to separate NucleoMag® B-beads.

2m

15.3 Use a multichannel pipette to remove the supernatant from each well.

Take extra care to remove as much supernatant as possible at this step. Carryover of the wash buffer can cause PCR/qPCR/library prep to fail.

16 Place plate on shaker at **56 °C** for approximately 30 minutes to allow beads to air dry.

Ensure beads are thoroughly dry before proceeding, carry over EtOH from **Buffer MWA4** can ruin downstream experiments.

17 Add **60 µl** of **RNase-free H₂O** to each well.

17.1 Shake the sample plate for **00:05:00** at **500 rpm**, **56 °C**.

5m

17.2 Place the sample plate on a plate magnet for **00:02:00** to separate NucleoMag® B-beads.

2m

18 Transfer eluted RNA to a 96-well elution plate for further processing.