



APR 05, 2024

OPEN  ACCESS**DOI:**

[dx.doi.org/10.17504/protocols.io.
dm6gpz8p5lzp/v1](https://dx.doi.org/10.17504/protocols.io.dm6gpz8p5lzp/v1)

Protocol Citation: Brett Rasile, Kendra Maas, Archana Anand, Michael Hajkowski 2024. Microbe/Phage Wastewater DNA/RNA Concentration and Extraction (Nanotrap® and NucleoMag® RNA Water).

protocols.io

<https://dx.doi.org/10.17504/protocols.io.dm6gpz8p5lzp/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Microbe/Phage Wastewater DNA/RNA Concentration and Extraction (Nanotrap® and NucleoMag® RNA Water)

 Forked from [SARS-CoV-2 Wastewater RNA Concentration and Extraction \(Nanotrap® and NucleoMag® RNA Water\)](#)

Brett Rasile¹, Kendra Maas¹, Archana Anand², Michael Hajkowski²

¹University of Connecticut; ²San Francisco State University

Coronavirus Method Development Community

2019-nCoV Wastewater Epidemiology

[1 more workspace](#) ↓



Archana Anand

SFSU

Protocol status: Working

We use this protocol and it's working

Created: Apr 05, 2024

Last Modified: Apr 05, 2024

PROTOCOL integer ID: 97861

ABSTRACT

Wastewater epidemiology is a method used to study the diseases affecting a population. Conventional methods of wastewater collection for composite samples have involved the usage of autosamplers that require installation in sewers with appropriate expertise and certification. Tampon-based wastewater sampling is a cost-effective and simple way to collect wastewater to study microbial pathogens. It is also a safer alternative as tampons can be deployed and retrieved without opening manhole covers. In this protocol we detail a wastewater sampling strategy using tampons to obtain composite samples.

Next, wastewater-based epidemiology has predominantly been targeted, where scientists have looked for specific pathogens and use biomarkers for the same. Notably, traditional wastewater filtration methods (i.e. skim milk filtrations and membrane filtration) require large sample sizes with varying nucleic acid yields (Ahmed 2022). Recently, Nanotrap[©] technology has been shown to have a higher nucleic acid yield using a smaller sample size when compared to traditional membrane filtration techniques, making it a better tool for scientists to concentrate microbial nucleic acids in wastewater for wastewater epidemiology. Specifically, the Ceres Nanotrap[©] technology was developed to trace severe acute respiratory syndrome (SARS-CoV-2) in public wastewater and Nanotrap[©] particles have been used specifically to track viral pathogenic shedding in wastewater of: Endogenous pepper mottle virus (PMMoV), Influenza A, CrAssphage, and monkey pox. However, there is little known about the success rates of different downstream analysis techniques to concentrate microbes in wastewater in an untargeted fashion. Therefore, it remains unclear whether one method will work better than the other if one were to be interested in characterizing the microbial community composition and abundance in a wastewater sample. To this end, in this protocol we detail a wastewater processing method involving Ceres Nanotrap[©] technology and NucleoMag[®] RNA Water.

Virus Capture & Concentration

10m

- 1 Create a 1:100 dilution of **Zoetis Bovine Rhinotracheitis-Parainfluzena-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 \ddagger 500 mL wastewater sample with \ddagger 500 μL of **1:100 BSRV** to achieve a 1 $\mu\text{L}/\text{mL}$ concentration of **1:100 BSRV**.

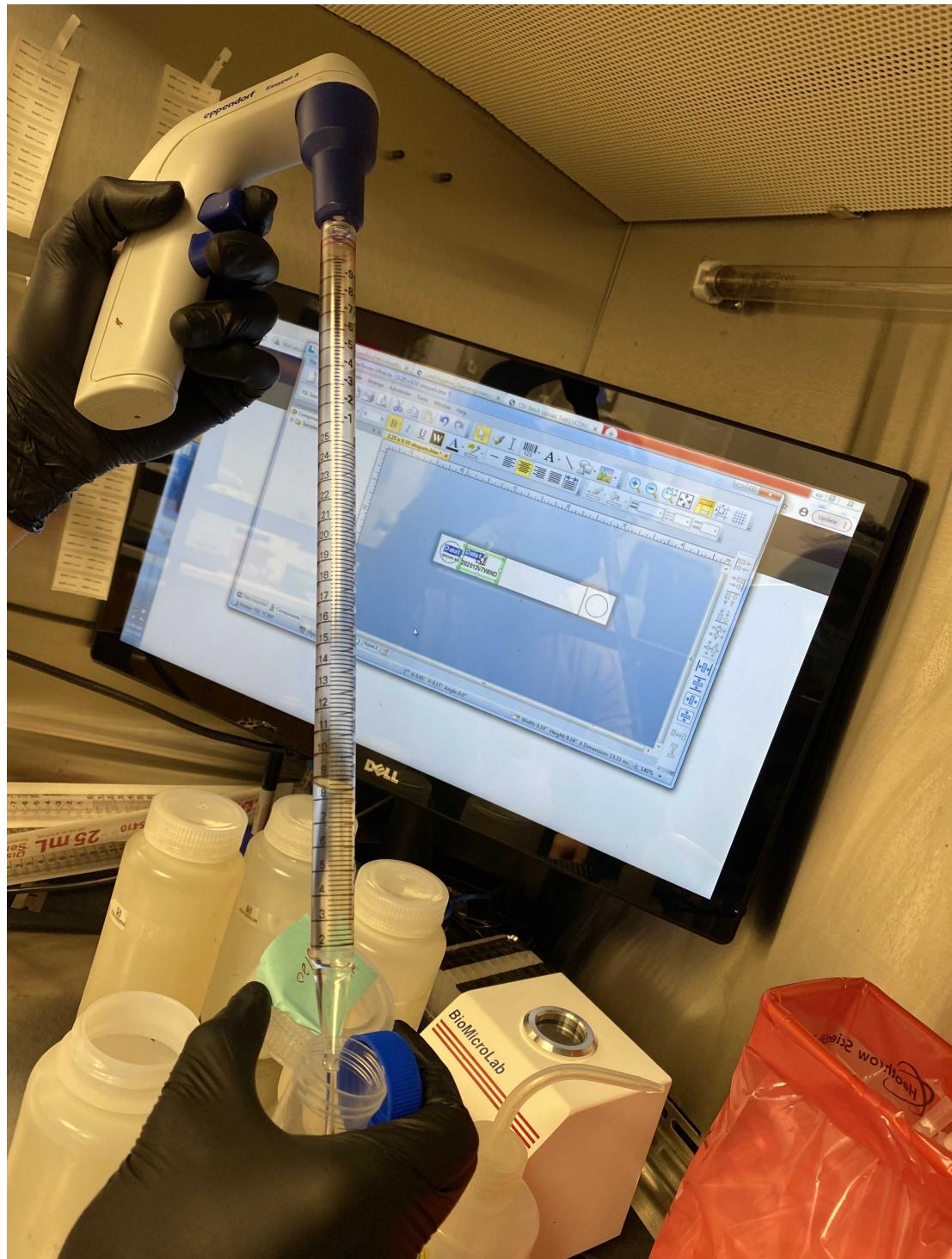
Note

This can be applied to wastewater samples greater or less than 500mL, as long as a 1 $\mu\text{L}/\text{mL}$ 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  10m large aggregates to sediment at the bottom of the sample bottle.
- 6 Use a 25mL serological pipette to transfer top  40 mL of spiked wastewater to a 50mL conical tube.

Note

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 $\text{a } 600 \mu\text{L}$ of **Ceres Magnetic Nanotrap® particles** to the $\text{a } 40 \text{ mL}$ spiked wastewater aliquot.

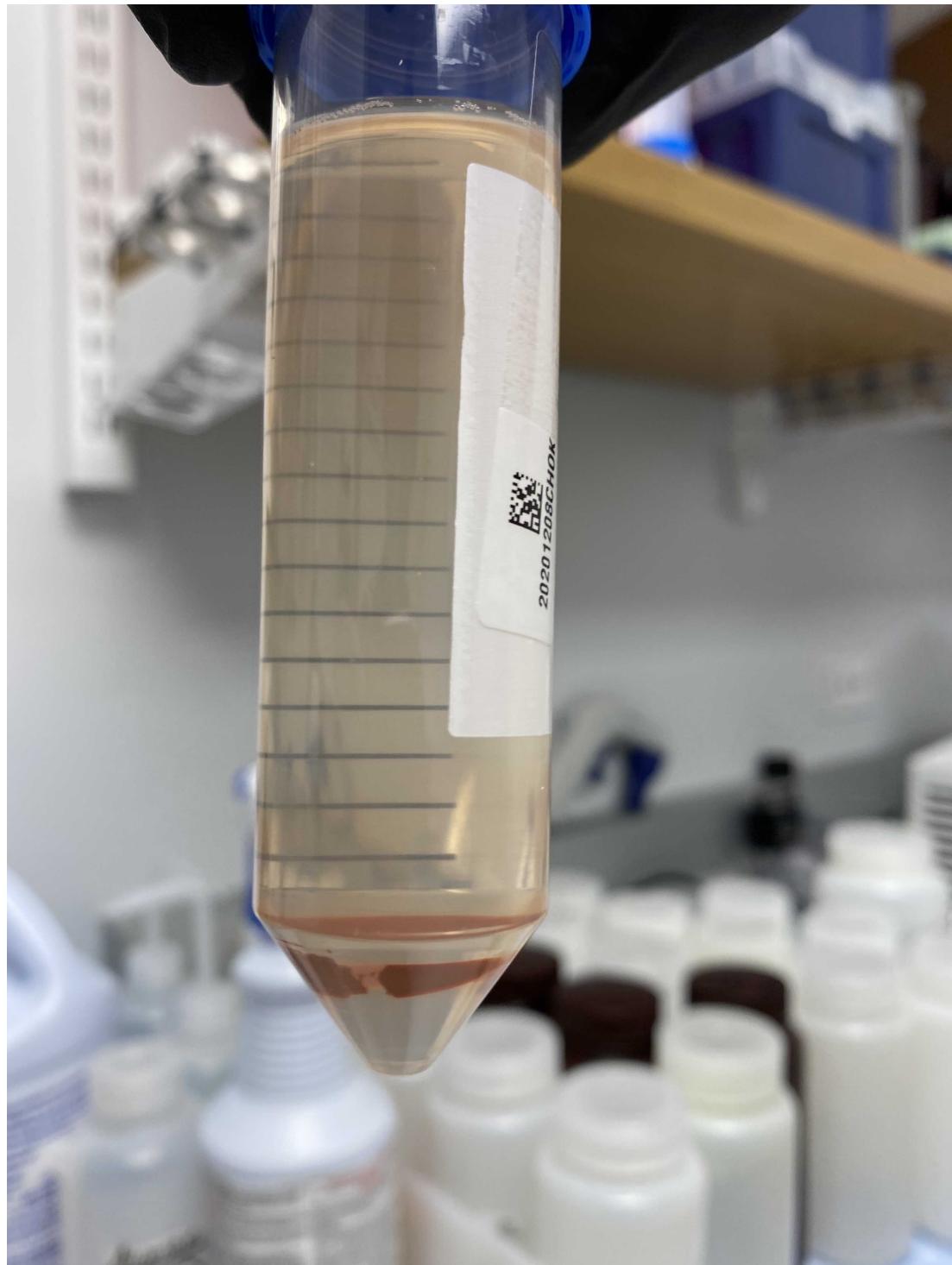
- 7.1** Invert the wastewater samples several times to incorporate the Magnetic Nanotrap® particles and incubate for 20 minutes at RT.
- 8** Place conical tubes into custom magnet racks and allow magnets to attract Magnetic Nanotrap® particles for a minimum of 20 minutes at RT.

Note

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)

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.

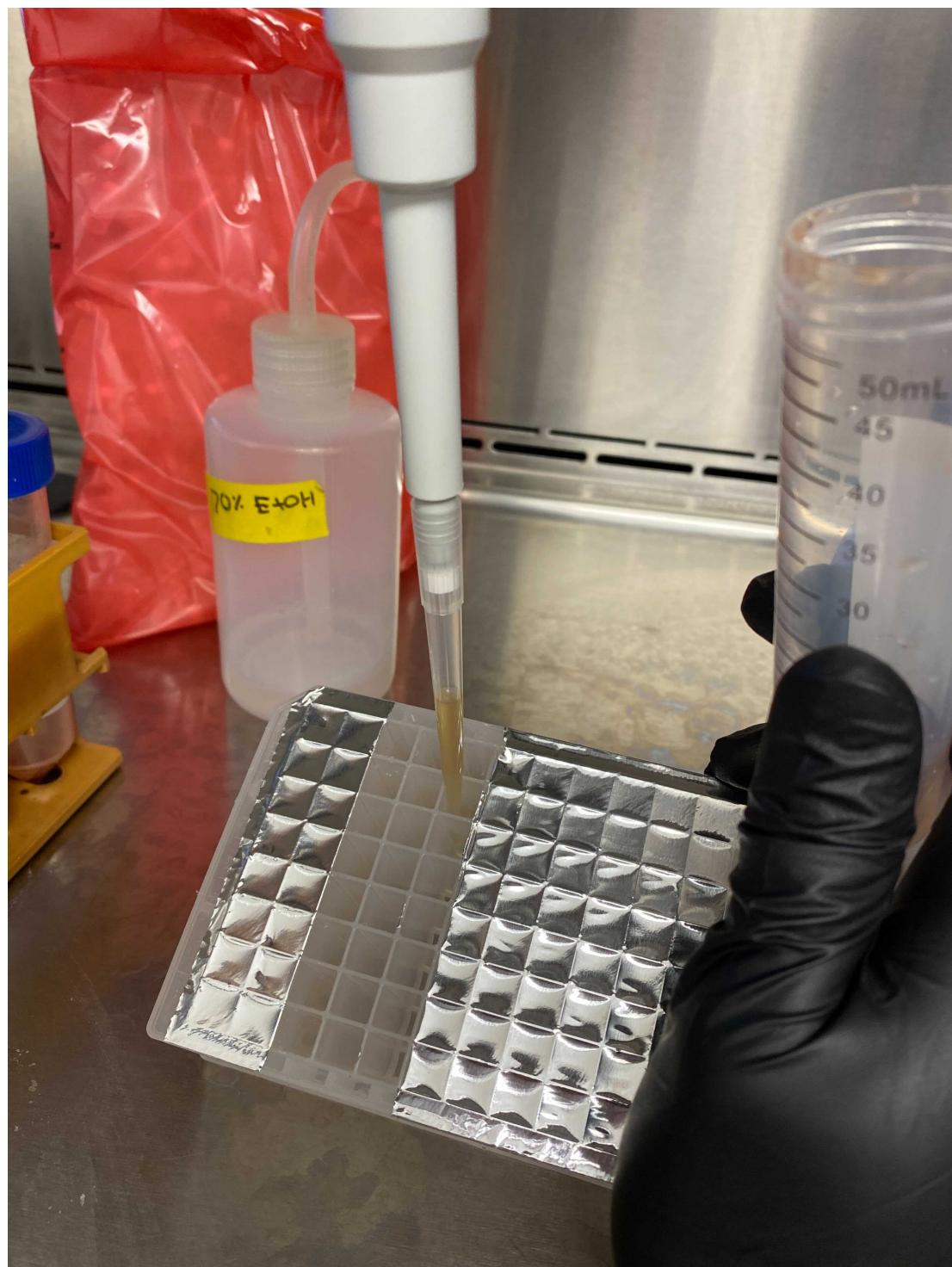
10m

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

11.1 With the tubes on the magnets, transfer  450 µL of lysate to a 2mL deep well plate.

Note

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 $\text{a } 475 \mu\text{L}$ of **Buffer MWA2** and $\text{a } 25 \mu\text{L}$ 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® beads.

5m

Note

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.

Note

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® 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® 2m beads.

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.

Note

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.

Note

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.