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🌐 Concentration and nucleic acid extraction of viruses from aggregated airplane waste

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We use this protocol and it's working

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Abstract

This protocol details our workflow for performing concentration and total nucleic acid extraction from aggregated airplane waste for the purposes of untargeted RNA and DNA sequencing of viruses present in wastewater.

Protocol overview: In this protocol, 40 mL of aggregated airplane waste is concentrated to a final volume of 400 uL using the InnovaPrep Concentrating Pipette Select. Prior to concentration, the waste sample is treated with Tween 20 and sonicated to dissociate viral particles from solids in the wastewater matrix. The waste sample is filtered using a 0.45 um PES 75 mm filtration unit, with an added pre-filter (2um glass fiber) to remove solids and bacteria. This filtrate is then concentrated with the Concentrating Pipette, using Ultra CPT tips and recommended device settings for the InnovaPrep modified wastewater processing protocol. Nucleic acids are then extracted from the concentrated product using the Zymo quick-DNA/RNA Viral kit using the manufacturer protocol with a few modifications that we have found to be helpful.

Guidelines

RNA processing and handling: Please review **Protocol Note: Working with RNA Samples** before handling RNA samples.

Materials

Green-Z Spill Control Solidifier

Materials:

(for one sample replicate and one negative control)

- 2 x InnovaPrep Ultra CPT (Unirradiated) tips for the InnovaPrep Concentrating Pipette Select
- 3 x 0.45 um PES 75 mm vacuum filtration tops (VWR No. 10040-470)
- 3 x 0.22 um PES vacuum filtration top (VWR No. 10040-444)
- 3 x 2 um Cytiva Whatman Glass Microfiber Prefilter (Fisher Scientific No. 09-874-86)
- 2 x 100 mL pyrex bottles
- 4 x 50 mL falcon tubes (VWR No. 21008-178)
- Green-Z Spill Control Solidifier (Safetec SKU 42010)
- 2 x 5 mL centrifuge tubes
- 3 x 25 mL serological pipettes (Thermo Fisher No. 170357N)
- 1.5 mL microcentrifuge tubes (VWR No. 1420-2600)
- PREempt wipes (VWR No. 10822-456)
- Parafilm (Millipore Sigma No. HS234526C)
- RNaseZap
- Kimwipes (VWR No. 34120)
- Paper towels
- Filtered micropipette tips

Reagents:

- Tween 20
- 1x Phosphate Buffered Saline (w/o Ca & Mg)
- Ice
- CP Select Elution buffer (Tris)
- CP Select Storage Fluid
- Zymo quick-DNA/RNA Viral Kit and buffers

Equipment:

- InnovaPrep Concentrating Pipette Select
- Branson M 1800 Sonicator (filled $\frac{3}{4}$ with tap water)
- Attachment for vortexer (Cole-Parmer No. UX-04724-89)
- Scientific Industries Vortex Genie 2
- Vacuum Line
- Micropipettes (1000 uL, 200 uL, 10 uL, 2 uL, 1 uL) and holder
- Timer
- Floor Centrifuge (Ex: Beckman Coulter Avanti J series)
- Rotor compatible with 50 mL tubes (Ex: Beckman Coulter JA-14.50)



Troubleshooting

Safety warnings

! Biosafety precautions: All raw wastewater samples will be received and stored with primary and secondary containment. The primary container (the bottle or falcon tube) should remain in the secondary container (a Ziploc bag containing paper towels to absorb spills) until processed. All raw samples must be handled within a dedicated fume hood or biosafety cabinet. All laboratory personnel handling these samples must use safety glasses, gloves, and lab coats. Samples will be transported between processing stations within a secondary container that has been cleaned with PreEmpt. All surfaces (outside of the fume hood/sash, centrifuge lid and rotor, etc.) will also be wiped down with PreEmpt.

Do not mix bleach with aggregated airplane waste. Aggregated airplane waste contains strong detergents which may react with bleach to create toxic gas. Liquid airplane waste should be disposed of by mixing with Green-Z Spill Control Solidifier in a ziploc bag. All autoclave-able bottles that are in contact with waste samples will be cleaned by rinsing with water (which is disposed of in Green-Z), and then filling with 10% bleach for at least 20 minutes before disposal.

Before start

Read the 'Safety Warnings' section for biosafety precautions necessary for handling raw airplane waste samples. Prepare the fume hood for wastewater handling, gather materials and reagents (centrifuge tubes, serological pipettes, pipette tips, micropipettes, a marker, strips of parafilm, PBS). Label centrifuge tubes (two for each influent sample, and two for a negative control sample). Ensure proper PPE.



Reagent Preparation

7m

- 1 Prepare a [M] 10 % volume Tween 20 stock solution. For a 40 mL stock, combine 4 mL of Tween 20 with 36 mL of 1X PBS. Filter sterilize with a 0.22 um vacuum filtration unit.

7m

Expected result

This recipe produces enough 10% Tween 20/PBS solution for approximately 20 dissociation treatments.

Part 1: Waste Handling, Dissociation, Filtration

49m

2

5m

Safety information

Refer to the 'Safety Warnings' section for biosafety precautions necessary for handling raw wastewater samples.

Transfer the waste sample (within secondary container) from the refrigerator to the fume hood. Remove the sample bottle from the secondary container and unseal the bottle by removing the affixed Parafilm.

- 3 Prepare aliquots of aggregated airplane waste. Invert the bottle of waste several times to re-suspend contents, then carefully open it. Using a fresh 50 mL serological pipette, aspirate and dispense 20 mL of waste into two 50 mL centrifuge tubes each. Repeat for desired number of sample replicates. For a negative control, add 20 mL of 1X PBS to two centrifuge tubes each using a fresh 50 mL serological pipette, for a total volume of 40 mL .


10m

Note

The 40 mL sample is separated into two 50 mL tubes, each containing 20 mL of airplane waste and 20 mL of dilution and dissociation reagent (step 4).

Additionally, EHS recommended limiting sample volume to 40 mL in each tube to reduce the risk of leaks.



4 Add  18 mL of 1X PBS to each centrifuge tube. 2 mL of **[M] 10 % volume** Tween 20 stock solution to each centrifuge tube for a final concentration of **[M] 5 % (v/v)** Tween 20.


3m

5 Cap and parafilm all bottles and centrifuge tubes.


5m

Safety information

Return the waste bottle to secondary containment, wipe exposed surfaces with pre-empt wipes, and return the remaining waste to refrigeration.

6 Place all centrifuge tubes on a vortexer using a 50 mL tube adapter. Shake at maximum speed for  00:02:00 .

3m

7 Transfer all centrifuge tubes from the vortexer to a sonication bath, and sonicate for  00:01:00 at 40 kHz. Use a paper towel to dry the tubes when finished.

1m

8 Prepare vacuum filtration units.

5m

Note

One pre-filter/filter unit will be used for each waste sample centrifuge tube, so for one replicate of waste (two waste sample centrifuge tubes), prepare two pre-filter/filter units. One pre-filter/filter unit will be used for each negative control sample, so for one negative control replicate (two centrifuge tubes), prepare one pre-filter/filter units.

8.1 Place a 2 um pre-filter in a 0.45 um vacuum filtration cup. Repeat for all pre-filter/filter units needed.

1m

Note

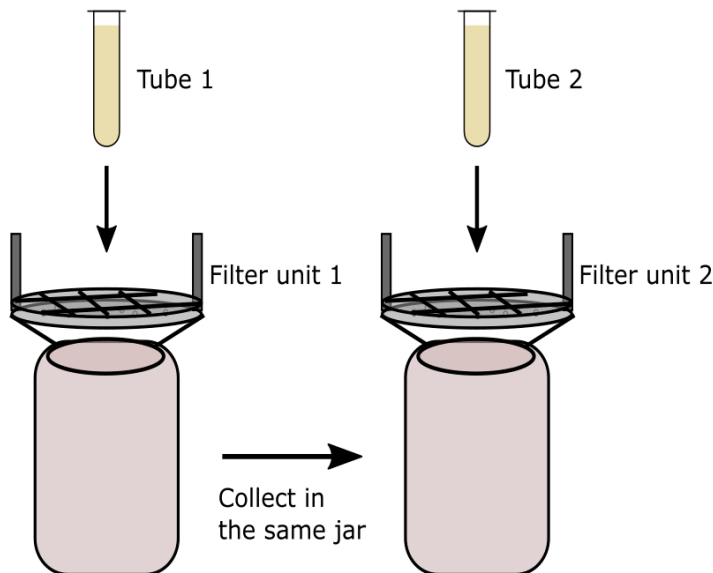
Cytiva Whatman Glass Microfiber Prefilters are oriented in the box as they should be placed in the filtration cup. Ensure that the pre-filter is flush with the surface of the filtration cup, and also centered such that sample liquid cannot seep past the pre-filter's edges. Tweezers are useful for proper placement.

9 Perform filtration for the waste sample.

14m

Note

Waste sample tubes are filtered separately because they easily clog the pre-filter/filter units. Filtrate is combined into one bottle prior to concentration in order to increase the concentration of viral particles in the concentrated eluate.



Centrifuge tubes contain a total of 80 mL of sample (40 mL x 2 tubes). Each tube is filtered with a new filter and pre-filter, and the total volume is collected into one collection bottle.

- 9.1 Attach one pre-filter/filter unit to a clean 100 mL pyrex bottle. Decant one waste sample tube into the filtration cup. Begin vacuum filtration by capping the vacuum filtration top and opening the vacuum line.

7m

Expected result

The filtrate volume will vary depending on the amount of solid material in the sample. Filtration may occur slowly such filtrate drips at a low yet consistent rate.

- 9.2 When filtration is complete, turn off the vacuum line and replace the used pre-filter/filter unit with a fresh one by attaching it to the same pyrex bottle with the waste sample filtrate. Decant the second waste sample tube into the fresh filtration cup. Begin vacuum filtration by capping the vacuum filtration top and opening the vacuum line. When complete, cap the pyrex bottle and set aside.

7m

**Note**

A filtration cup and pre-filter which are saturated in waste may smell unpleasant. To mitigate this, seal the filtration cup in a bag prior to discarding into a larger biowaste bin.

10 Perform filtration for the negative control sample.

2m

10.1 Attach one pre-filter/filter unit to a clean 100 mL pyrex bottle. Decant both centrifuge tubes into the filtration cup. Begin vacuum filtration by capping the vacuum filtration top and opening the vacuum line. When complete, cap the pyrex bottle and set aside.

2m

Part 2: Concentration via InnovaPrep Concentrating Pipette Select

1h

11 Perform the "Start-up" protocol for the InnovaPrep Concentrating Pipette Select.

- Turn on the Concentrating Pipette, and navigate to "Maintenance" and then "Start-up". Follow the prompts.
- Check that the maintenance tip is in place.
- Place the waste line in the proper position.
- Remove the storage fluid line and insert the foam elution canister.
- Ensure that the screen reads "WWULTRA".

5m

12 Run the concentration protocol for the filtered waste sample.

15m

12.1 Remove the maintenance tip and place a fresh Ultra CPT into the tip port. Lower the tip into the sample.

1m

Note

Ensure that the Tip is as close to the bottom of the sample bottle as possible. If necessary, the bottle can be balanced on its edge while a weighted object holds down the top of the Concentrating pipette. A bottle of PBS can be used as the weighted object.

12.2 Press "Start Run", allow the Concentrating Pipette to aspirate the waste, and wait until it beeps to signal the end of the run.

10m

**Note**

A timer will run on the display during the concentration. The Concentrating Pipette will stop aspirating and begin to beep when the Tip detects air instead of liquid sample. Concentration should take roughly 5 min, but can vary based on the consistency of the sample.

12.3 While holding a 5 mL centrifuge tube under the Tip, press "Elute" and catch the foam that is dispensed.

2m

12.4 After the foam degasses, add  400 μ L of the Zymo DNA/RNA shield reagent.

2m

Expected result



The eluate should be roughly 400 μ L. The Zymo DNA/RNA shield reagent is added at a 1:1 ratio of sample:reagent.

13 Run the concentration protocol for the negative control. Perform as is done for the waste sample.

5m

Note

Make sure to use a fresh Tip for each sample.

14 Incubate samples with added Zymo DNA/RNA shield reagent at room temperature for  00:30:00 . If samples are sitting for longer then store at  4 $^{\circ}$ C

30m

15 Perform the "Shut Down" protocol for the InnovaPrep Concentrating Pipette Select.

- Navigate to "Maintenance" and then "Shut Down".
- Place the maintenance tip into the tip port.
- Remove the elution canister.
- Check to ensure that there is adequate storage fluid and insert the storage fluid line.
- Turn off the device and remove the waste line.

5m

Part 3: Nucleic Acid Extraction - Zymo quick-DNA/RNA Viral Kit


6m

16 Gather the materials and reagents for the Zymo quick-DNA/RNA Viral Kit in the Biosafety Cabinet. Equilibrate samples to room temperature.

5m



**Note**

Refer to the "Guidelines" section for instructions on processing and handling RNA samples.



- 17 Add  1600 μL of Viral DNA/RNA buffer to the sample (a 2:1 ratio) and vortex briefly to mix. 3m

Note



The volume is scaled according to the manufacturers recommendation.


- 18 Transfer up to  700 μL of lysate into a Zymo-Spin™ IIC-XLR Column in a collection Tube and centrifuge for  12.000 x g, 00:02:00 . Discard the flow-through in the miniprep waste container. 8m

Repeat until full lysate volume is processed.

- 19 Add  500 μL of Viral Wash Buffer to the column, centrifuge for  12.000 x g, 00:00:30 and discard the flow-through. 30s

- 20 Repeat the previous step. 2m

- 21 Add  500 μL ethanol (95-100%) to the column and centrifuge for  12000 x g, 00:01:00 to ensure complete removal of the wash buffer. 1m





- 22 Transfer the column to a clean collection tube, and centrifuge at  12000 x g, 00:01:00 to remove any remaining ETOH. 1m

Note

This step is not included in the Zymo manual, but reduces the chance of ETOH carryover and subsequent inhibition of downstream enzymatic steps.

- 23 Carefully, transfer the column into a 1.5 mL nuclease-free tube. 1m



- 24 Add  50 μ L DNase/RNase-Free Water directly to the column matrix and incubate at RT for  00:01:00 . Centrifuge for  12000 x g, 00:00:30 to collect the eluate.
- 25 Place all extracted nucleic acids in a freezer set to  -80 °C

1m 30s

3m

Note

Optional: Perform nucleic acid quantification prior to freezing. Perform RNA or DNA quantification using the Qubit HS RNA assay kit or the Qubit 1X dsDNA Assay kit.

Protocol references

[Innovaprep FluidPrep Concentrating Pipette Select](#)

[Zymo Research Quick-DNA/RNA Viral Kit](#)