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Concentration and total RNA extraction of viruses from nasal swabs V.1

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We use this protocol and it's working

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Abstract

This protocol describes the workflow for concentrating and extracting total RNA from a pool of nasal swab samples, aimed at untargeted RNA sequencing to identify viruses present in nasal secretions. It is based on the NAO protocol **Concentration and nucleic acid extraction of viruses from wastewater influent V.2**. To summarize, nasal secretions are dissociated from swabs (n ≥ 25) into solution, bacteria and human cells are removed, and viral particles are concentrated to a final volume of ~600 µL. Total RNA is extracted from the concentrated product using the QIAamp Viral RNA Mini Kit according to the manufacturer's instructions. We estimate that this protocol yields an average of 84 ng (SE = 15 ng) of total RNA per pooled swab.

Attachments

PDF

Estimate of total RN...

47KB

Guidelines

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

Materials

Materials:
(for one pool of nasal swabs and one negative control)

- Nasal swabs in **Falcon 50 mL Conical Centrifuge Tubes** (VWR No. 21008-178): ≤ 20 swabs per tube
- Clean centrifuge tubes equal to number of tubes containing nasal swabs + one negative control
- 2 x **InnovaPrep Ultra CPT (Unirradiated**) tips for the InnovaPrep Concentrating Pipette Select
- 2 x **0.45 µm PES 75 mm vacuum filtration tops** (VWR No. 10040-470)
- 2 × 100 mL pyrex bottles
- 4 × 5 mL centrifuge tubes
- 1 × 50 mL serological pipette (VWR No. 170358N)
- 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:

- Ice
- **HBSS (Hank's Balanced Salt Solution)**
- **CP Select Elution buffer (Tris)**
- **CP Select Storage Fluid**
- All buffers in **QIAamp Viral RNA Mini Kit (50)**
- 100% ETOH
- 80% ETOH
- Nuclease-free water (NFW)

Equipment:

- **InnovaPrep Concentrating Pipette Select**
- **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)

Safety warnings

Biosafety precautions: Nasal swab samples will be received and stored using both primary and secondary containment. The primary container should remain inside the secondary container until processing begins. Samples must only be handled within a designated biosafety cabinet or fume hood. All laboratory personnel handling these samples are required to wear safety glasses, gloves, lab coats, and N95 face masks to minimize biological aerosol risk. When handling samples in the During transportation between processing stations, samples will be placed in a secondary container that has been disinfected with PreEmpt. In addition, all surfaces—including the outside of the fume hood/sash, centrifuge lid, and rotor—will be wiped down with PreEmpt. Any containers that come into contact with nasal swab samples will be disinfected by soaking with a 10% bleach solution for at least 20 minutes.

Before start

Read the 'Warnings' section for biosafety precautions necessary for handling nasal swab samples. Prepare the biosafety cabinet and fume hood for nasal swab handling. Gather materials and reagents. Ensure proper PPE.

Stage 1: Sample preparation

7m

1 In a biosafety cabinet, remove the nasal swabs (n ≥ 25) from their secondary containers. Ensure that the nasal swabs are evenly distributed among Falcon 50 mL Conical Centrifuge Tubes, with no tube containing more than 20 swabs.

5m

> Note
>
> Falcon 50 mL Conical Centrifuge Tubes should be used as primary containers for collecting nasal swabs to streamline sample processing.

2 Using a 50 mL serological pipette, add the equivalent of 1 mL of HBSS per swab to each tube, taking care not to come into direct contact with the tube. For a negative control, add an equal volume of HBSS to a clean centrifuge tube. Cap and parafilm each tube.

2m

Stage 2: Dissociation of viral particles and removal of large solids

25m

3 Place all tubes on a vortexer using a 50 mL tube adapter. Shake for 1000 rpm, 00:00:30.

1m

4 Excluding the negative control, transfer the liquid contents into clean centrifuge tubes. Leave the swabs in their original tube, cap, and discard. Cap and parafilm each new tube and place in a secondary container.

2m

> Note
>
> For efficient centrifugation and pelleting in 50 mL centrifuge tubes, aim for a volume between 10-20 mL.

5 Transfer all tubes, including the negative control, from the biosafety cabinet to a centrifuge equipped with the appropriate rotor and/or adapters (e.g., Beckman Coulter Avanti J series with JA-14.50 rotor). Centrifuge the samples at 1200 x g, 4°C, 00:10:00. After centrifugation is complete, wait 00:10:00 (as recommended by EHS) to allow aerosols to settle before opening the centrifuge. Remove tubes from the centrifuge, place them in a secondary container, and transfer them to the fume hood.

22m

Stage 3. Removal of suspended solids

22m

6 In the fume hood, prepare two separate 0.45 µm vacuum filtration apparatuses by attaching each filtration unit to a clean 100 mL pyrex bottle. 2m

7 Decant the supernatant from all tubes containing nasal swab samples directly into a 0.45 µm vacuum filtration top, taking care not to dislodge the pellets. 2m

8 Begin vacuum filtration by capping the vacuum filtration top and opening the vacuum line. When complete, cap the pyrex bottle and set aside. 15m

Note

The duration of this step will vary depending on the consistency of the supernatant.

9 For the negative control, decant directly into a 0.45 µm vacuum filtration top. There should not be a pellet. Perform vacuum filtration as was done for the nasal swab samples, cap the pyrex bottle, and set aside. 3m

Stage 4: Concentration of viral particles

25m

10 Perform the "Start-up" protocol for the InnovaPrep Concentrating Pipette Select. 5m

- 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 "ULTRA".

11 Run the concentration protocol for the filtered nasal swab sample. 10m

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

Note

Ensure that the CPT 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 HBSS can be used as the weighted object.

- Press "Start Run", allow the Concentrating Pipette to aspirate the waste, and wait until it beeps to signal the end of the run.
- While holding a 5 mL Eppendorf tube under the Tip, press "Elute" and catch the foam that is dispensed. The eluate should be ~ 600 µL ; this can vary slightly depending on the volume of unconcentrated sample.
- About 1 mL of unconcentrated filtrate should remain in the pyrex bottle. Pipette 600 µL and transfer to a 5 mL Eppendorf tube.

12 Run the concentration protocol for the filtered negative control. Perform in the same manner as the nasal swab sample, including removal of a 600 µL unconcentrated aliquot. Make sure to use a fresh tip. 5m

13 Perform the "Shut Down" protocol for the InnovaPrep Concentrating Pipette Select. 5m
- 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.

Stage 5: Total RNA extraction - QIAamp® Viral RNA Mini Kit 56m

14 Gather the materials and reagents for the QIAamp® Viral RNA Mini Kit in a biosafety cabinet. Equilibrate samples to room temperature. 5m

- Add 25 mL of 100% ETOH to Buffer AW1.
- Add 30 mL of 100% ETOH to Buffer AW2.
- Check Buffer AVL for precipitate, and if necessary incubate at 80 °C until the precipitate is dissolved. **It is not necessary to add carrier RNA.**

Note

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

15 Add 2.4 mL (4x volume) of Buffer AVL to each of the four 5 mL tubes containing 600 µL of concentrated sample and control, and unconcentrated sample and 13m

control. Mix by pulse-vortexing for 00:00:15. Incubate at Room temperature for 00:10:00; spin down.

> Note
>
> The volume of Buffer AVL is scaled according to the manufacturer's recommendation.

16 Add 2.4 mL 100% ETOH to each tube. Mix by pulse-vortexing for 00:00:15; spin down. 3m

> Note
>
> The volume of 100% ETOH should be equal to the volume of Buffer AVL from step 15.

17 For each sample and control, transfer 700 µL to a designated spin-column tube. Centrifuge at 6000 x g, 00:02:00. Discard flow-through. Repeat 8x, or until entire volume has passed through spin-column. Take care to transfer to the correct tube each time. Once finished, transfer the spin-columns to clean collection tubes. 20m

18 Add 500 µL Buffer AW1 to each tube. Centrifuge at 6000 x g, 00:01:00. Discard flow-through. 3m

19 Add 500 µL Buffer AW2. Centrifuge at 6000 x g, 00:03:00. Transfer spin-columns to clean collection tubes. Centrifuge at max rpm, 00:01:00 to remove residual buffer. Transfer spin-columns to designated 1.5 mL Eppendorf tube. 7m

20 Add 50 µL NFW to spin-columns and incubate at Room temperature for 00:02:00. Centrifuge at 6000 x g, 00:01:00 to collect eluate. Discard spin-columns. Store eluates at -80 °C. 5m

> Note
>
> Although the QIAamp® Viral RNA Mini Kit is designed for RNA isolation, some DNA co-precipitation is unavoidable. To eliminate residual DNA, DNase treatment of the eluate is necessary.

Expected result

We estimate that this protocol yields an average of 84 ng (SE = 15 ng) of total RNA per swab from the concentrated products (see Attachment for details). Total RNA per swab from the unconcentrated aliquots is not estimated because these yields are typically below the limit of detection for the Qubit HS RNA assay. Yields are expected to vary depending on the amount of the raw material present on each swab in the pool.

Protocol references

Concentration and nucleic acid extraction of viruses from wastewater influent V.2

Innovaprep FluidPrep Concentrating Pipette Select

QIAamp® Viral RNA Mini Kit