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WORKS FOR ME 1

Membrane Filtration for SARS-CoV-2 Viral Capture

COMMENTS 5

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ABSTRACT

This protocol describes how to perform membrane filtration vacuum technology with wastewater grab samples to allow for SARS-CoV-2 RNA extraction.

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MATERIALS TEXT

Equipment:

- Sterilized forceps
- Alcohol lamp burner
- Benchtop scale
- Centrifuge (Thermo Scientific. SORVALL RC 6+)
- pH Probe (Fisher Scientific. Fisherbrand accumet AB15 plus. pH meter CAT# 13-620-631)
- 5% HCl solution
- 5% NaOH solution
- Aluminum Foil
- 10% Bleach solution
- 70% Ethanol solution
- 100% Ethanol (96% is what is used in the lab)
- Concentrated bleach
- Benchtop Protector Paper
- Eppendorf Research Plus Single Channel Pipette
- LabGard Biological Safety Cabinet - Class 2 A2 Biosafety Cabinet
- Autoclave
- Benchtop biohazard bag(s)
- Benchtop biohazard bag stand (GENESEE SCIENTIFIC CORPORATION. CAT# 30-164)
- Automatic handheld repeater (EppendorfCAT#13-683-552)

Materials Needed (per Sample):

- (1) Field Sample collected and stored at 4C until processing
- (1) Autoclaved centrifuge bottle and lid with O-ring
- (1) Autoclaved 500mL beaker
- (1) Autoclaved Membrane Filtration Cup contained within a (Fisher Scientific.CAT#01-812-55) Sealed Bag
- (1) Sterile 0.45µm (Millipore. CAT#HAWP04700) membrane filter paper
- (1) Powdered Magnesium Chloride ($MgCl_2$)
- (1) 10^4 10µl Bovine Respiratory Syncytial Virus (BRSV) processing control aliquot
- (1) 25mL graduated pipette
- (1) Qiagen RNeasy Mini Kit (Cat. No. 74106)

BEFORE STARTING

Biosafety Level 2 (BSL-2) certification is required due to the nature of the target pathogen.

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Sterilize Biological Hood

1 Turn on the biological hood vent and wait for airflow to start circulating.

2 Carefully remove the vacuum flask and hoses that are attached to the vacuum vent within the biological hood and dispose of any liquid present in the correct biological hazard trash container.

3 Wash and rinse the vacuum hoses using a mixture of hot water and concentrated bleach at a 2:1 ratio.

4 Pour concentrated bleach solution into the vacuum flask to cover the entire bottom surface.

5 Spray 10% bleach solution inside the biological hood and wipe all surfaces with paper towels.

6 After spraying the 10% bleach solution, spray 70% ethanol solution inside the biological hood and wipe all surfaces with paper towels.

7 Carefully reattach the vacuum flask and vacuum hoses to the vacuum vent within the biological hood.

8 Place two pieces of benchtop surface paper underneath the membrane filtration vacuum apparatus that is inside of the sterilized biological hood.

Sterilize Forceps

9 Pour 100% ethanol into a smaller autoclaved beaker to a level that allows for the entire end of forceps to be

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submerged.

10 Carefully light a alcohol lamp burner.

11 Place the forceps that have been submerged in the 100% ethanol and burn the ethanol off to create a visible flame.

12 Place the now sterilized forceps into the biological hood in a position so that the sterilized end is not touching a physical surface to remain aseptic.

13 When forceps are used or come into contact with any surface or filter paper that is contaminated with a sample, they must be re-sterilized using the method given above.

Preparation

14 Prepare and collect all supplies necessary for the number of samples that will be concentrated using membrane filtration.

Note

Sample Processing Procedure

5m

15 Remove the grab sample from the 4°C refrigerator and place under the sterilized biological hood.

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16 Aseptically pour 300 mL of the grab sample into an autoclaved 500mL beaker that is labeled with a sample identifier using labeling tape.

17 After 300mL of the grab sample has been measured, aseptically pour the aliquoted sample into an autoclaved centrifuge bottle that is labeled with the sample identifier using labeling tape.

Note

18 Using aseptic technique, secure the centrifuged bottle lid onto the centrifuge bottle containing the sample and secure the lid by turning a quarter of the way past tight.

19 Balance the sample centrifuge bottles using a benchtop scale that has been sterilized using 10% bleach solution followed by 70% ethanol solution.

Note

5m

20 Centrifuge the sample(s) for 5000 rpm, 00:05:00 .

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Equipment	
Sorvall™ RC 6 Plus	NAME
Centrifuge	TYPE
Thermo Scientific™	BRAND
12121680	SKU
https://www.fishersci.no/shop/products/sorvall-rc-6-plus-centrifuge/12121680	LINK

Note

21 Remove the centrifuged sample(s) from the centrifuge and carefully place them back under the biological hood.

Note

22 Measure out 0.71 g +/- 0.02g of $MgCl_2$ using benchtop scale paper and an autoclaved chemical spatula.

15m

23 Pour the measured $MgCl_2$ into the 500mL beaker that was initially used to measure the sample volume. Put a magnetic stir bar into the beaker and stir the sample on the stirrer for 00:15:00 .

24 Aseptically pour the centrifuged sample into the identically labeled 500mL beaker that now has the $MgCl_2$ to create a final concentration of 25mM.

Note

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25 Place a benchtop calibrated pH probe underneath the biological hood and sterilize the probe, excluding the glass measurement part, and workspace with 10% bleach solution followed by 70% ethanol solution.

26 Adjust the pH to pH 3.5 +/- 0.1 using 5% HCl and 5% NaOH solutions.

Note

27 Use a Eppendorf Research Plus Single Channel Pipette to add 10 µL of BRSV aliquot into the sample.

Note

28 Cover the opening of the sample beakers with aluminum foil and secure the foil by either wrapping around the edge of the opening or with autoclave tape.

29 Gently and carefully swirl the sample to allow for homogeneous mixing of the BRSV throughout the sample beaker.

30m

30 Place the sample beaker into a 4 °C refrigerator for 00:30:00 .

31 While the sample sits in the refrigerator for 30 minutes, place an autoclaved membrane filtration cup into each of the vacuum manifold openings and secure with a quarter turn to create a seal.

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32 Without disturbing the lower half portion of the membrane filtration cup, remove the top cup portion and place topside down onto the benchtop paper that was placed at the start of the procedure preparation.

33 Using the sterilized forceps, aseptically place a 0.45µM membrane filter paper onto the opening of the membrane filtration cup that is still attached to the vacuum manifold.

33.1 When initially setting up the vacuum membrane filtration manifold with filter papers, sterilization of forceps can be done a singular time when all membrane filter cups are autoclaved and placed into the biological hood on the vacuum manifold with no sample(s) present.

33.2 After all membrane filtration cups have a 0.45µM membrane filter paper placed onto their respective opening, reattach the top of the membrane filtration cups to their base.

33.3 Re-sterilize the forceps and place into an aseptic position for later use after sample processing.

34 Label the membrane filtration cups with their respective sample identifier.

Note

35 After the 30-minute refrigeration period is over, pour 150 mL of the sample into a membrane filtration cup that has the membrane filter paper and is sealed tightly on the vacuum manifold.

Note

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36 Check the seals of the vacuum hoses and vacuum flask to make sure that the seals are secure to prevent leakage of any sample liquid.

37 Turn the vacuum vent that is attached to the biological hood on to begin the vacuum process, and subsequently turn the valve on the vacuum manifold for each sample that is being processed.

38 Check the seal on the vacuum hoses and flask once again after the vacuum process has begun.

39 Throughout the filtration process, frequently check the flow of sample liquid in the membrane filtration cups until the whole sample volume has filtered through the membrane. **After 30-45 minutes, if the sample is draining very slowly and a large amount of sample volume still remains to be filtered, follow sub-steps 39.1 - 39.12.**

Note

39.1 Retrieve the sample beaker from the 4°C and place under the biological hood.

39.2 Retrieve a 25mL graduate pipette and an automatic repeater pipette.

39.3 Turn the vacuum valve off for the respective sample.

39.4 Pipette the sample that is remaining within the membrane filtration cup back into the sample beaker and measure the volume amount that is removed.

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39.5 After the sample is removed from the membrane filtration cup, remove the top half of the membrane filtration cup and place top down onto the benchtop paper.

39.6 Using sterilized forceps, roll the membrane filter paper that contains the filtered sample and place into a labeled DNA LoBind tube for RNA extraction.

Note

39.7 Using an Eppendorf Research Plus Single Channel Pipette, place 400 µL of Buffer RLT **Qiagen Catalog #79216** from the Qiagen RNeasy Mini Kit (Cat. No. 74106) into the labeled sample DNA LoBind tube that contains the first sample membrane filter paper.

Note

39.8 Place the sample DNA LoBind tube into a 4°C refrigerator while the remaining sample volume is filtered through a secondary membrane filter paper.

39.9 Re-sterilize the forceps and aseptically place a new 0.45 µM membrane filter paper onto the membrane filtration cup opening and reseal using the top half of the membrane filtration cup.

39.10 Using the same 25mL graduated pipette, replace the measured sample volume into the cup and turn the vacuum valve back to the open position.

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39.11 Once the remaining sample has filtered through the membrane, remove the top half of the cup, roll the membrane filter paper that contains the filtered sample, and place into the labeled DNA LoBind tube that contains the first membrane.

Note

39.12 Add the remaining 400 µL of Buffer RLT **Qiagen Catalog #79216** to the DNA LoBind tube to create a total volume of 800 µL.

40 Once the full sample volume (150 mL +/- 15 mL) has filtered through the membrane, remove the top half of the membrane filtration cup and place top down onto the benchtop paper.

41 Using sterilized forceps, roll the membrane filter paper that contains the filtered sample and place into a labeled DNA LoBind tube for RNA extraction.

Note

42
- Using an Eppendorf Research Plus Single Channel Pipette, place 800 µL of Buffer RLT **Qiagen Catalog #79216** from the Qiagen RNeasy Mini Kit (Cat. No. 74106) into the labeled sample DNA LoBind tube that contains the membrane filter paper.

43 Store DNA LoBind tubes with filters and Buffer RLT in a 4°C refrigerator until all samples are processed and ready for RNA extraction.

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RNA Extraction

10m

44 Insert DNA LoBind tubes that contain the sample filters into a vortex adapter and vortex for 00:10:00 .

3m

45 Centrifuge tube for 00:03:00 at maximal speed.

46 Pipette the available liquid into a new, labeled tube. You may discard the tube with the filter(s) afterwards.

Note

47 Aliquot an equal volume of 70% molecular ethanol to the sample. Mix well by pipetting the mixture up and down several times. Do not vortex after this step.

Note

30s

48 Transfer 700 µL of the sample mixture into a labeled RNeasy spin column. Centrifuge for 00:00:30 at full speed. Discard filtrate. Repeat until all of the sample is filtered through the spin column.

30s

49 Aliquot 700 µL of Buffer RW1 **Qiagen Catalog #1053394** to the RNeasy spin column. Centrifuge for 00:00:30 at full speed. Discard filtrate.

30s

50 Aliquot 500 µL of Buffer RPE **Qiagen Catalog #1018013** to the RNeasy spin column. Centrifuge

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for 00:00:30 at full speed. Discard filtrate.

51 Aliquot 500 µL of Buffer RPE **Qiagen Catalog #1018013** to the RNeasy spin column. Centrifuge for 00:02:00 at full speed. Discard filtrate. 2m

52 Transfer spin column to new 2 mL collection tube and centrifuge for 00:01:00 at full speed. Discard collection tube and filtrate. 1m

53 Place the RNeasy spin column into a labeled 1.7 mL microcentrifuge tube. Add 50 µL of RNA-free water to the center of the spin column. Incubate for approximately 00:01:00 at room temperature. 1m

Note

54 Centrifuge for 00:01:00 at full speed. 1m

55 Add another 50 µL of RNA-free water to the center of the spin column. Incubate for approximately 00:01:00 at room temperature. 1m

56 Centrifuge for 00:01:00 at full speed. 1m

57 Close the cap of the 1.7 mL tube containing the final RNA (you will have a final volume of 100 µL).

58 Store RNA at -20 °C until it is ready for PCR.

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Post-Sample Processing Clean-Up Procedure

59 Place all benchtop paper and all disposable materials into the benchtop biohazard bag.

60 Place the benchtop biohazard bag into the correct biohazard floor trash can for future autoclaving.

61 Sterilize forceps that were used and place into their respective areas for future use.

62 Place all contaminated membrane filtration cups, sample beakers, centrifuge bottles, and any other glassware into a sink for cleaning prior to autoclaving.

63 Using concentrated bleach, adequately wash all glassware, centrifuge bottles, and membrane filtration cups and scrub any solids or residue off.

64 Using lab-grade soap and hot water, adequately wash all glassware, centrifuge bottles, and membrane filtration cups before placing on a drying rack.

65 Follow current lab procedures for autoclaving and store autoclaved materials for future membrane filtration processing of samples.

66 Spray 10% bleach solution throughout the biological hood and wipe all surfaces, manifold, and potentially contaminated surfaces with paper towels.

67 Spray 70% ethanol solution throughout the biological hood and wipe all surfaces, manifold, and potentially contaminated surfaces with paper towels.

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68 Close the biological hood and turn off all vents and lights.

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