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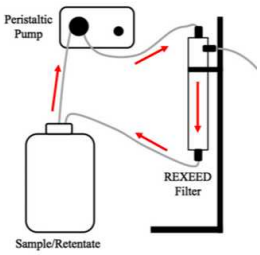
Hollow Fiber Ultrafiltration for Concentrating Large Volumes of Liquid Samples



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We use this protocol and it's working

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Abstract

This Standard Operating Procedure (SOP) was created for the Southern Nevada Water Authority Research and Development (R&D) Research Microbiology group. It describes the concentration of water samples (e.g., Las Vegas Wash water, wastewater, surface water, etc.) for downstream molecular analyses. Hollow fiber ultrafilters, such as the Rexeed #25S filters or alternative ELISIO-25H filters, paired with a peristaltic pump, can concentrate large volumes of water samples (here, specifically 10L wastewater samples), through 30 kDA membranes. This procedure can be modified to provide higher equivalent sample volumes (ESVs) by increasing initial sample size or continuing concentration and reducing eluate volumes.

Image Attribution

Katerina Papp

Materials

EQUIPMENT AND REAGENTS

- Centrifuge with swinging bucket rotor and 50mL tube adapter (e.g., AVANTI J-HC, Beckman Coulter)
- Peristaltic pump (e.g., Masterflex L/S Easy Load II)
- Sterile silicone tubing (L/S 24)
- Tubing connectors if needed
- Sterile 0.5 L glass beakers
- Sterile 0.1 L glass beakers
- Graduated cylinder (1 L capacity or larger)
- 1-2L autoclavable glass bottles
- Sample carboy or container (e.g., 10 L Nalgene carboy)
- Access to sink or container for permeate discharge
- Hollow fiber ultrafilter of choice (e.g., previously REXEED 25S Dialysis filter but no longer available, currently use ELISIO-25H from NIPRO Corporation)
- Support stand with large clamp
- Pipettes (P-1000, P-200 or P-100)
- Pipette tips (1000 µL, 200 µL)
- 50 mL conical falcon tubes
- 1.5 mL microcentrifuge tubes
- Biohazard waste container
- Scale
- Weigh boats
- Parafilm
- Paper towels, kimwipes
- Notebook
- Sodium Polyphosphate (NaPP, Sigma-Aldrich)
- Tween-80 (Sigma-Aldrich)
- Antifoam Y-30 (Sigma-Aldrich)
- Reference material for spiking (e.g.,Bovine rota-coronavirus, Calf Guard, Zoetis)
- 10% bleach bath
- 10% bleach
- 70% ethanol

Safety warnings

It is each employee's responsibility to follow applicable District Environmental, Health, Safety, and Corporate Security (EHS&CS) procedures and to identify, evaluate, and follow prescribed controls for the hazards posed by their work. All required training should be current and a safe performance self-assessment should be performed prior to beginning each task. This should include the following steps:
Assess the risk
Analyze how to reduce the risk
Act to ensure safe operations
Record assessment and analysis results on a blank Job Hazard Analysis Form (SNWA only)
If you cannot eliminate or manage the risk, do not continue and inform management.

Before start

DEFINITIONS

- HFUF – Hollow-Fiber Ultrafiltration
- NaPP – Sodium Polyphosphate
- PPE – Personal Protective Equipment
- SDS – Safety Data Sheet

PRINCIPLES OF HFUF

- *Drain* the storage solution
- *Block* the pores
- *Concentrate* the sample
- *Elute* any remaining sample

Solution Preparation

1 **Preparation**

1.1 Don the appropriate PPE.

1.2 Prepare and clean bench space.

1.3 Prepare enough glass bottles based on volume of each solution needed. See equations below to calculate volume of each solution.

1.4 Prepare 1-L (or larger) graduated cylinder.

1.5 Prepare scale and weigh boats.

1.6 Prepare pipette (P-100) and tips (100-µL).

1.7 Prepare waste container.

1.8 Prepare 70% ethanol

2 **Make Blocking solution (0.01% sodium polyphosphate (NaPP))**

2.1 Calculate the volume of blocking solution using the equation below:

Blocking solution (L) needed = number of samples × 0.3 L

Note

Note: Volume of blocking solution can be increased (e.g., to 0.5 L), if desired.

2.2 Measure out the required volume of DI water using the graduated cylinder and transfer into glass bottles.

Note

Note: If preparing 1 L of solution in a 1-L glass bottle, you MUST transfer a minimum of 0.3 L into another bottle such that each 1-L bottle only contains 0.5 - 0.7 L of solution prior to autoclaving. Failure to do so will result in solution overflowing (loss) during autoclaving.

2.3 Weigh out 0.1 g NaPP for each liter of solution.

2.4 Add NaPP to DI water (e.g., 0.1 g NaPP to 1 L of DI water in a 2-L glass bottle).

2.5 Dissolve NaPP by thoroughly shaking the bottle by hand or use a magnetic stirrer and plate.

2.6 Label bottle with solution name, chemical composition, date prepared, and initials.

2.7 Place autoclave tape on bottle.

2.8 Loosen cap and sterilize solution by autoclaving at 121 °C for 20 minutes.

2.9 Remove bottles from autoclave ONLY once cooled. Use heat resistance glove to remove bottles.

2.10 Tighten cap and store solution at room temperature.

3 Make Elution Solution (0.01% NaPP, 0.01% Tween-80, 0.001% Antifoam Y-30)

3.1 Calculate the volume of elution solution using the equation below:

Elution solution (L) needed = number of samples × 0.06 L

> Note
>
> **Note:** Volume of blocking solution can be increased (e.g., to 0.3 L), if desired.

3.2 Measure out the required volume of DI water using the graduated cylinder and transfer into glass bottles.

> Note
>
> **Note:** If preparing 1 L of solution in a 1-L glass bottle, you MUST transfer a minimum of 0.3 L into another bottle such that each 1-L bottle only contains 0.5 - 0.7 L of solution prior to autoclaving. Failure to do so will result in solution overflowing (loss) during autoclaving.

3.3 Weigh out 0.1 g NaPP for each liter of solution and add to bottle.

3.4 Using a P-1000, pipette 0.1 mL (100 µL) of Tween-80 into each liter of solution.

3.5 Using a P-100, pipette 0.01 mL (10 µL) of Antifoam Y-30 into each liter of solution.

Note

Note: Tween-80 is very viscous, aspire and dispense Tween-80 very slowly to avoid air bubbles in pipette tip and minimize adhesion to pipette tip walls. Antifoam Y-10 is white, the solution will clear out after autoclaving.

3.6 Dissolve components by thoroughly shaking the bottle by hand or use a magnetic stirrer and plate.

3.7 Label bottle with solution name, chemical composition, date prepared, and initials.

3.8 Place autoclave tape on bottle.

3.9 Loosen cap and sterilize solution by autoclaving at 121 °C for 00:20:00 .

3.10 Remove bottles from autoclave ONLY once cooled. Use heat resistance glove to remove bottles.

3.11 Tighten cap and store solution at room temperature.

Sample Preparation

4 Don the appropriate PPE, including face shield if desired

Example of optional face shield

5 Inspect the 10 L sample and make sure that container does not leak.

6 **Optional**: Perform pre-filtration for difficult samples using a filtration apparatus and Whatman filters

Example pre-filtration setup

7 Weigh out 1 g of NaPP using a small weigh boat

8 Carefully remove sample container cap and add the NaPP to the sample. This results in a 0.01% NaPP amendment.

9 Add 1 mL of spike reference material of choice .

Note

Note: The volume of reference material to add depends on its starting concentration. Determine the starting concentration of your reference material stock and spike in the appropriate volume based on desired final concentration in the sample. The R&D Microbiology group frequently uses an attenuated vaccine-strain of bovine rota-coronavirus as reference material (Calf-Guard, Zoetis). This material is rehydrated into 50 mL of 1X TE buffer, quantified, and subsequently used as process and recovery control. Since it is in liquid form, it is easily pipetted directly into the sample prior to sample processing. Usually we quantify the stock at the same time we quantify the sample for most accurate results.

10 Close sample container tightly and mix sample and spiked constituents throuroughly by shaking/rolling the carboy for approximately 1 minute.

11 Set carboy aside at room temperature in an upright position.

Ultrafiltration Apparatus Setup

12 Securely fasten the clamp onto the metal rod stand about two-thirds of the way up.

13 Tighten the REXEED 25S filter (or other) in the clamp stand

Note

Note: Check direction of flow on filter cartridge and orient filter such that the flow direction is vertical from top to bottom (e.g., blue end at the bottom, red end at the top for the REXEED 25S filter).

14 Remove caps from top and bottom ports of the filter.

15 Allow the filter to drain into a small (e.g., 0.2-L) beaker placed in a large secondary leakproof container (e.g., large rectangular plastic basket).

Note

Note: Alternative filters do not have a liquid storage solution that needs to be drained. This will result in a lower volume of recovered blocking solution. (Step 24)

16 Connect a long piece of LS tubing to the top port and a shorter piece of tubing to the bottom port by twisting the tubing onto the port back and forth until it is snug. Make sure the tubing is tight on both ports but especially the top one. Use small hose clamps to secure the tubing if needed.

17 Leave permeate (side) port caps on for now (i.e. ports are closed).

18 Pass the top tubing end through a peristaltic pump.

19 Make sure the pump will run in the correct direction (clockwise).

20 Place both ends of the tubing into a 1-L beaker placed inside a large secondary container (e.g., large rectangular plastic basket).

Blocking solution recirculation

Note

Note: It is recommended to set up the apparatus on a solid, stable, low-profile platform on the floor close to a sink such that tubing can reach into the sink. The pump should be placed above the filter (e.g., on a stable shelf or bench).

Ultrafilter Blocking

21 Pour 0.3-0.5 L of blocking solution into the 1-L beaker, make sure both ends of the tubing are fully submerged.

22 Turn on the pump and check that the system is running correctly.

23 Adjust the pump speed to a reasonable flow rate

Note

Note: As the pump runs, the blocking solution is pulled from the beaker through the peristaltic pump into the ultrafilter. It runs through the filter and comes out via the bottom port and is directed back into the beaker through the tubing. The system forms a closed loop with blocking solution being recirculated through the filter multiple times.

24 Recirculate the blocking solution through the filter for 3-5 minutes.

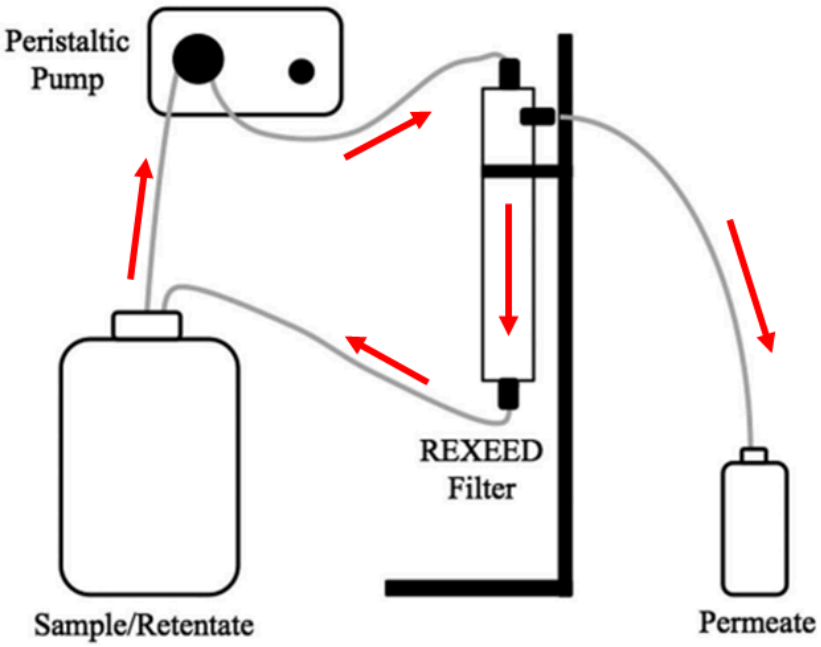
25 Lift both tubing ends out of the solution and push air through the system to drain excess blocking solution from the filter.

Note

Note: If not using a filter with a liquid storage solution, there may need to be more blocking solution used and there may not be any excess blocking solution in the filter

Sample Concentration

26 Swap the blocking solution containing beaker for the amended sample carboy. The carboy should also be placed inside a large secondary container in case of leaks or spills.



27 Place the tubing inside the carboy. Make sure all tubing connections are tight – especially at top and bottom of the ultrafilter.

Note

Note: For non-REXEED filters- clamping/parafilm or adapters should be used to ensure a tight fit between tubing and ultrafilter.

28 Remove the top side permeate port cap.

29 Connect a piece of tubing to this port for permeate discharge.

30 Place a waste container underneath the permeate tubing (placed inside a secondary container) or discharge into a sink

31 Check tubing connections one more time.

32 Turn on the pump to desired speed (600 – max speed – should be displayed on the dial).

33 Watch the sample being pulled through the tubing into the ultrafilter, then back into the sample carboy.

34 Check the permeate tubing. Permeate should be coming out shortly after the concentration process has started.

35 **Optional:** Collect 200 mL of permeate after ~ 3 min of concentration into 250-mL Nalgene bottle if permeate analysis is desired.

36 Never leave the concentration process unattended.

37 **IF A LEAK OCCURS:**

38 Tighten or change the affected tubing.

39 Clean any spills with 10% bleach followed by 70% ethanol.

40 Restart the pump.

41 **If the ultrafilter becomes completely clogged** (i.e., sample is leaking through the top port and the leak cannot be fixed), elute (elution process described in SAMPLE ELUTION) and use a new ultrafilter to concentrate the remaining sample. Drain then block the new ultrafilter the same way as the first one. Then continue sample concentration. For new ultrafilter elution, use same elution solution as before

42 When sample volume in carboy gets low (pump is pulling in air), tilt the carboy and stabilize in tilted position by placing an object below and on the side.

43 When the system pulls in air again, pause the pump.

44 Lift tubing slightly and allow remaining sample to drain back into the carboy.

45 Once sample had stopped dripping out from the tubing, place tubing into a clean 1-L graduated beaker, placed inside a secondary container.

46 Carefully pour the remaining sample from the 10-L carboy into the glass beaker.

47 Parafilm the top of the beaker and around the tubing to prevent aerosolization.

48 Continue concentration until desired volume is achieved (~ 50 mL). It is relatively easy to monitor the level of the remaining sample using the beaker's graduation marks.

49 Keep the retentate (= concentrated sample) in the 1-L beaker for now.

Sample Elution

35m

50 Leave the system, including tubing and retentate, as is for now.

51 Take off the permeate discharge tubing from the permeate port.

52 Place it in a 10% bleach bath.

53 Cap the permeate port.

54 Pour 60-100 mL of elution solution into a new 150-200mL beaker.

55 Place inside the secondary container close to the 1-L beaker containing the retentate.

56 Transfer the tubing from the 1-L beaker containing the retentate into the 200-mL beaker containing the elution solution.

57 Parafilm the 1-L beaker containing the retentate. Carefully set aside now.

58 Parafilm the 200-mL beaker and around the tubing.

59 Check that tubing on filter ports is snug and tight.

60 Turn on the pump.

61 Watch the elution solution being pulled up into the tubing, pushed through the filter, and recirculated back into the beaker. No permeate is discharged since the port is capped. The apparatus forms a loop, so the elution solution is recirculating through the system in the same way as for ultrafilter blocking.

62 Allow the elution solution to circulate through the system for 00:05:00 . The systems is placed in a secondary container in case minor spills occur. 5m

63 Pull the tubing out of the elution solution and push air through the system to recover as much solution as possible from the ultrafilter and tubing.

64 Shake the ends of the tubing gently against the wall of the beaker to remove drops.

65 Place the ends of the tubing into a stable waste container (e.g., another 1-L beaker) in case sample is still drips.

66 Combine eluate and retentate in the same beaker.

67 Mix gently by swirling, estimate total concentrate volume.

68 Split the concentrate into equal parts by transferring into 50-mL conical tubes.

69 Record concentrate volume using graduation marks of the conical tubes (The tubes are graduated, and the marks are relatively accurate in terms of volume measurement).

	A	B	C	D	E	F	G
	Sample name/ID	**Starting Volume**	**[Spike] and vol. added**	**Concentrate volume (retentate + eluate)**	**Pellet volume**	**Supernatant Volume transferred**	**Note**
	Example 1	10 L	1 mL BCoV	100 mL	10 mL	250 uL	filter clogged

Example of data to record for HFUF process

70 Carefully close the conical tubes to avoid overflow (they may be full).

71 Label conical tubes with sample name, sample type, date, and initials.

72 Place the conical tubes in a swinging bucket rotor. Make sure the rotor is balanced. Balance the rotor with conical tubes filled with the appropriate amount of water if needed.

73 Centrifuge at 3200 x g for 00:30:00 to pellet solids. 30m

74 Record the volume of pellet formed at the bottom of each conical tube.

75 Transfer 1 mL of supernatant into a clean 1.5-mL tube for liquid phase analysis.

76 Label the tube with sample name, sample type, date and initials.

77 Freeze immediately.

78 Store the remaining 50-mL conical tubes containing the centrifuged sample concentrate in the fridge for optional further analysis. (e.g., solids phase analysis)

Cleanup

20m

79 Carefully disconnect the tubing from the filter and remove while holding the open ends upwards.

80 If tubing is to be reused, submerge all parts in a 10% bleach bath overnight. Make sure that the bleach gets inside all tubing parts.

81 Close the ultrafilter ports and dispose of the filter in biohazard waste bag.

82 Place all disposable items (e.g., used parafilm, paper towels etc.) in a biohazard waste bag.

83 Submerge all used labware (e.g., beakers, bottles) in 10% bleach overnight.

84 Fill the sample carboy with 10% bleach, allow to sit overnight.

85 Wipe the peristaltic pump, filter support stand and clamp with 70% ethanol.

86 Store all cleaned equipment and consumables away.

87 Clean area with 10% bleach followed by 70% ethanol.

88 Next day, wash all labware with soap and water. Rinse thoroughly.

89 Cover all openings with aluminum sheets.

90 Put a piece of autoclave tape on each item.

91 Sterilize by autoclaving at 121 °C for 00:20:00 . 20m

Acknowledgements

Katerina Papp created this internal document- Katherine Crank converted it to protocols.io format only.