VIRUS ENTÈRICS
UNIVERSITAT DE BARCELONA

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SAVCA
Servicio de Análisis de Virus en la Cadena Alimentaria y Ciclo del Agua
Services for the Analysis of Viruses in the Food Chain and Water Cycle

Secció Microbiologia, Virologia i Biotecnologia
Departament de Genètica, Microbiologia i Estadística
Facultat de Biologia,
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Protocol for Reexed-25A hollow fiber ultrafilters to concentrate virus, bacteria and protozoa

Background

The method is based on Rexeed-25A-filter. This is a hollow fiber ultrafilter with an area of 2.5 m^2, a priming volume of 137 ml, an inner diameter of the fibers of 185 µm and a pore size of 30 kDa. Thus bacteria, parasites, viruses and everything else in the water with a size exceeding 30 kDa is retained in the filter. The filter can be connected to a tap for on-site filtration or with pump for filtration of grab samples. The filter tolerates a **maximum pressure of 66 kPa**. If oxygen is pumped into the filter, the pressure increase which may damage the pores why a pressure gauge always should be used during filtration.

Supplies

Rexeed- 25A filter (Asahi Kasei Medical America Inc)→ Ref: 525REXEED-25A
Pipettes, disposable 10 ml
Tips for micropipettes
Glass bottles, 1L (sterile)

Reagents

Sodium Polyphosphate (NaPP, 10%)
Antifoam A (1%) → Ref: A5758-100ML Sigma
Tween 80 (10%)
Sterile reagent water
0.01 M Phosphate buffered saline (PBS) pH 7.4
Bovine serum

PRETREATMENT OF THE FILTER

When removing the port caps, put these aside for future use.

1. Prepare the blocking solution by adding 25 ml calf serum to 375 ml sterile reagent grade water.
2. Assemble the filter setup as shown in the next figure using a ring stand and clamp to hold the filter. Marked connection should be secured with hose clamps. Both side ports should be closed with corks.

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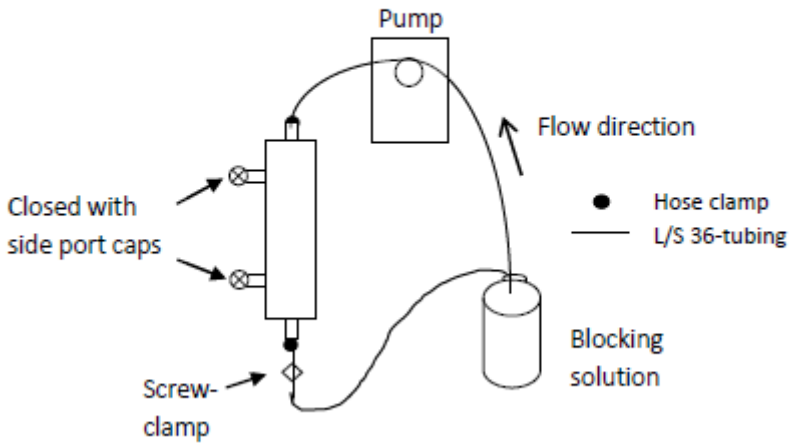

3. Remove the cotton and tapered end of a 10 ml plastic pipette still in the packing and place it in the bottle containing 1L sterile reagent grade water and attach the tubing at the top of the filter to the pipette. Place the tubing from the lower end in a sterile container to collect the rinse water.
4. Start the pump and allow the water to flow through the filter.
5. Transfer the pipette to the bottle containing the blocking solution.
6. Remove the cotton and tapered end of a second 10 ml plastic pipette still in the packing and place it the bottle containing the blocking solution. Attach the tubing from the lower end to the pipette.

If the filter is to be used on site:

7. Circulate the blocking solution through the filter at a speed of 2900 ml/min for at least 5 minutes.
8. Turn off the pump and close the tubing connected to the bottom with the cork. Replace the tubing at the top with a port clamp. Invert the filter and do the same with the tubing at the bottom.
9. Place the filter on a rotary shaker for at least 2h.

If the filter is to be send elsewhere for filtration:

7. Circulate the blocking solution through the filter at a speed of 2900 ml/min for at least 5 minutes.
8. Turn off the pump and close the tubing connected to the bottom with the cork. Remove the filter from ring stand, invert the filter and replace the tubing at the bottom with a port cap.
9. Assemble the filter set up as shown in the figure.

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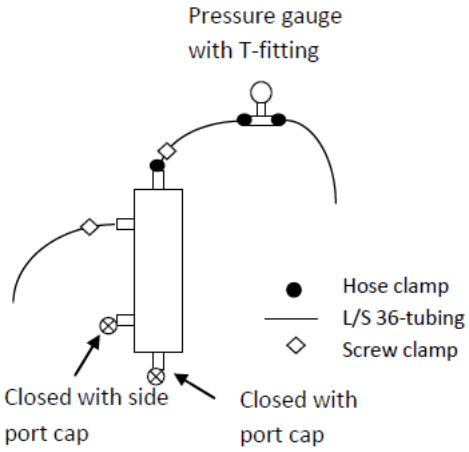
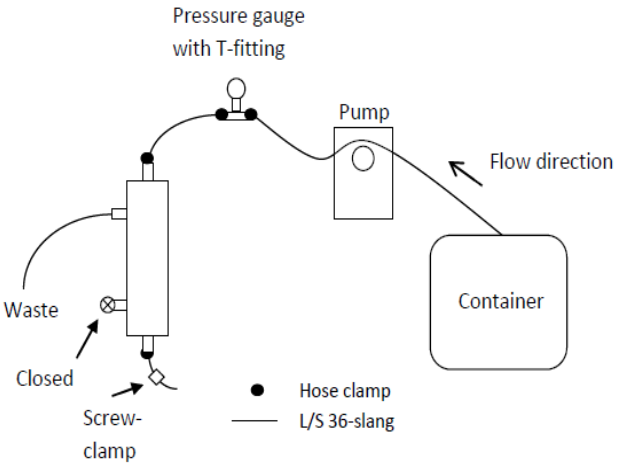


10. Send the filter on ice.

DEAD-END FILTRATION OF A GRAB SAMPLE USING A PUMP

Filtration

1. Assemble the filter according to the next figure except for the tubing connected to the upper side port which should be mounted after that the filter has been flushed with water. Marked connection should be secured with hose clamps and all fittings connected to the Tee-fitting sealed with thread sealing tape.



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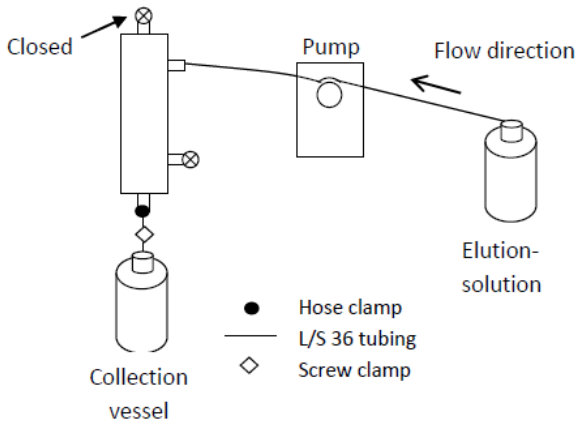
2. Remove the tubing from the sample container and connect it to a 10 ml pipet without a tip and with the cotton removed. Place the pipet in a bottle containing 1L sterile reagent grade water.
3. Remove the port cap at the bottom.
4. Flush the filter at 2900 l/min until the 1L flask is almost empty.
5. Switch the pump off and put the port cap back.
6. Connect the tubing to the upper side port (waste).
7. Begin pumping the water sample through the filter. Switch the pump off before moving the inlet pipet from one container to the next to avoid pumping air into the system. Keep an eye on the pressure during filtration. If it approaches 0,6 Bars, terminate the filtration.
8. When the last container is empty, keep pumping until the pressures starts to increase and then switch the pump off.
9. Carefully release the pressure on the system by letting air into the system by removing the tubing from the peristaltic wheel.

Back-Flush Elution

1. Invert the filter and replace the port cap with a short piece of tubing (appr 1 dm) closed with a screw cap. Take care to avoid any leakage of the concentrate.
2. Assemble the filter setup as shown in the next figure.



3. Make a 0.001% Antifoam, 0.01% NaPP and 0.01% Tween80 solution by adding 0.5 ml 1% Antifoam/10% Tween 80 and 0,5 ml 10% NaPP to 500 ml sterile PBS.
4. Remove the port cap at the bottom and pump the solution through the filter at a speed of 650 ml/min, collecting the eluate in a sterile container, until the system is empty.

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5. Record the volume of the eluate.

FILTRATION FROM A FAUCET

The ultrafilter concentrates all microorganisms, i.e. bacteria, viruses and parasites. The water flows into the inlet (red end) when the outlet (blue end) is close, is forced through the filter pores and exits through the upper side port. After filtration, microorganisms can exit through both end port (red and blue) but not the side ports. Therefore, it is necessary to handle the filter in a manner that prevents leakage from the end ports as the concentration of microorganisms can be high if the water is contaminated.

Procedure

On arrival, the filter contains a blocking solution that has to be rinsed out before filtration. Meanwhile, the filtration rate can be adjusted to approximately 2 l/minute.

1. Flush the lines for a few minutes and close the tap.
2. Connect the tubing at the red end of the filter to the tap. The pressure gauge will then be placed between the tap and the filter.
3. When this has been done, remove the screw clamp from the inlet (red) tubing and the port cap at the bottom. The cap should be put back later!
4. Open the tap slowly and adjust the flow rate to approximately 2 l/minute using a liter measure or a bottle of know volume.
5. Without closing the tap, remove the screw clamp from the side port tubing.
6. Put the bottom port cap back. The water is now forced through the pores and out through the side port. The flow will be somewhat lower than measured above.
7. Filter the desired volume of water. Keep an eye on the pressure gauge. If the pressure increases, the flow rate should be decreased. An increase in pressure may be due to clogging of the pores. If the pressure approaches **0.6 bars**, the filtration has to be terminated.
8. When the filtration is finished, close the tap.
9. Carefully unscrew the upper plastic screw (red end) to which the tubing is attached and replace it a port cap.
10. Replace the tubing at the side port with a side cap. This should be screwed onto the side port.
11. Place the filter in a plastic bag and return to the lab on ice.

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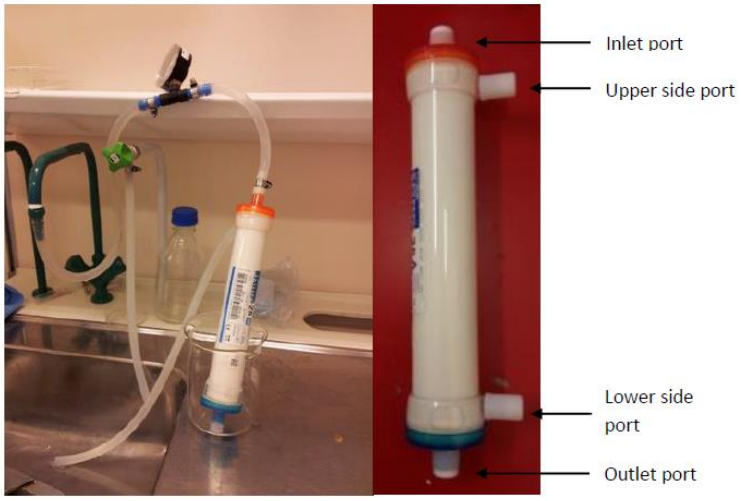

SECONDARY CONCENTRATION

Secondary concentration of tap and grand water using Centricon 70-plus

This method is not suitable for turbid waters as the filter will clog.

Equipment

Centrifuge with swinging bucket rotor that fits 250 ml flat bottom centrifuge flasks and can run at 3500x*g*

Micropipettes with variable settings from 10-1000 µl

Reagents

PBS with 0.001% Antifoam A and 0.01% Tween80

dH2O

Procedure

1. Read the protocol provided by Millipore before starting the concentration.
2. Prerinse the Centricon Plus-70 device as recommended in the protocol.
3. Add 70 ml of the UF-eluate and spin at 3500x*g* for 10 minutes.
4. Empty the filtrate collection cup, add another 70 ml eluate and repeat the centrifugation. Repeat a third time. Note the volume that has been concentrated.
5. If the amount of UF-eluate remaining in the sample filter cup is above the top of the filter cores, decant to a suitable container before proceeding. If not, add 1 ml PBS with 0.001% Antifoam A and 0.01% Tween80 (see above) to the two filter cores and incubate at room temperature for 5 minutes.
6. Turn the concentrate cup upside down and place on top of the sample filter cup. Then carefully invert the device and place in the centrifuge.
7. Spin at 1000xg for 2 minutes to collect the concentrate.
8. Add any UF-eluate from step 5 to the concentrate and determine the total volume.