Standard Operating Procedure for Performing Backflush of Ultrafilters for Bacterial Pathogens

Issue Date: February 1, 2021
Revision 1 - Date: August 16, 2019
Revision 2 – Date: January 9, 2020
Revision 3 – Date July 26, 2020
Revision 4 – November 2020
Revision 5 – February 1, 2021
Revision 6 – January 5, 2022

Prepared

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Table of Contents

REVISIONS

August 16, 2019

1. Section 4.1 - Added language: “Filtrate port tubing: Silicone Tubing size 36 (Geotech, Item # 77050011)”.
2. Section 5.5.5 – Added language: “Be sure to push the tubing on the side port all the way to where the port meets the ultrafilter.”
3. Added “Section 5.5.5.1 If using the Cole Parmer L/S 36 tubing, a clamp should be used to secure the hosing to the ultrafilter side port. This tubing can slip off the side port when under pressure and must be secured.”
4. Added “Section 5.5.5.2. If using the Geotech size 36 tubing, no clamp is needed.
5. Under Photograph 1. Added language: “****NOTE 3:* If using Cole Parmer L/S 36 tubing, you must secure the tubing to the side port with a hose clamp. If using Geotech size 36 tubing, no hose clamp is needed. Be sure to push the tubing on the side port all the way to where the port meets the ultrafilter.”

January 9, 2020

6. Section 4.1 – Added language - “15) Zip-ties”
7. Section 5.5.5.1. – Added language - “Alternatively, a zip-tie may be used in the place of a hose clamp.”

July 1, 2020

8. Minor edits to text and added additional options to supply list.

July 26, 2020

9. Minor edits to text based upon CFSAN and ORA review.

November 2020

10. Minor edits – added exact number of clamps and rods needed for setup of pump.

February 1, 2021

11. Minor edits as part of periodic review of text.

January 5, 2022

12. Edited to add an alternate to the Y-30 antifoam solution (Note in Equipment and Supplies Section) due to manufacturer discontinuing the product.
13. Added note about pump platform construction (also in Equipment and Supplies Section)

SCOPE AND APPLICABILITY

The procedure will be used to backflush an ultrafilter intended for bacterial pathogen analysis.

SUMMARY OF METHOD

The ultrafilter is backflushed with a 500 mL solution of 0.5% Tween 80, 0.01% sodium polyphosphate, and 0.001% Antifoam Y-30 Emulsion.

HEALTH AND SAFETY WARNINGS

Sterile disposable gloves should be worn during all steps of this procedure to prevent possible exposure to waterborne pathogens and prevent cross-contamination of sample. Since the system is under pressure, a face shield is recommended to protect eyes and mouth. Please consult safety protocols developed for your specific laboratory for exact requirements in terms of precautions and PPE.

EQUIPMENT AND SUPPLIES

Equipment

1. Peristaltic Pump (Geotech; http://www.geotechenv.com/ Geopump™ Peristaltic Pump Series II Package (includes easy-load II pump head and batteries), Geotech, Cat No. 91352123 or equivalent)

Disclaimer: Names of vendors or manufacturers are provided here as examples of suitable product sources; inclusion does not imply endorsement by the US Food and Drug Administration or the Department of Health and Human Services. Alternative products may be used if they provide equivalent performance to the items cited in this SOP.

Supplies

1) Ultrafilter-related supplies
   a) Filtrate port tubing:
      i) Silicone Tubing L/S 36 (Masterflex Cole-Parmer # 96410-36)
      ii) Silicone Tubing size 36 (Geotech, http://www.geotechenv.com/ Item # 77050011)
      iii) Plastic hose clamp (Cole-Parmer # 6832-04; Min ID: 0.402”, Max ID: 0.449”); (Another option: Grainger Item# 1ENP7; Max 0.452) See photo in Appendix A. Zip ties, *optional*, in place of hose clamp (example: Cole Parmer 40 Pound Solid Nylon Cable, Zip Ties, 5.5" L, White; 1000/PK; Fisher Scientific Cat. NC9269761)
2) Paper towels
3) Scissors
4) Bench protector paper, to catch water from leaks (Fisher Scientific, Cat. 14-206-65, or similar)

5) Face shield, *optional* (Fisher Scientific, Cat. 17-310, or similar) *Note*: Please consult safety protocols of individual laboratories
6) Nitrile or latex sterile, disposable gloves
7) 10% bleach
8) 70% ethanol
9) Tween® 80
10) Sodium polyphosphate (NaPP)
11) Antifoam Y-30 Emulsion – *Note:* Due to potential unavailability of the Y-30 solution from Sigma, and no other known manufacturers, it is currently recommended to make your own Y-30 solution by mixing the antifoam A concentrate in propylene glycol to make a 30% solution. The recommended Sigma product numbers are: 398039 for the propylene glycol (available in 25 ml, 500 ml, and 2 L sizes) and A6852-100G for the Antifoam A (100 g size) concentrate.
12) Sterile stir bar
13) 500 mL bottle, sterile, one per sample
14) 1 Liter bottle, sterile, one per sample
15) Sterile distilled water
16) Rods and clamps for assembly to hold up pump (*NOTE:* this is just one example of how to construct an elevated platform for the pump, other configurations and set-ups are fine to assist with raising the pump for ease in backflushing filters).
    1. Eisco Aluminum Rod 1/2 in. Diameter x 12 in. Length - Round Bar (0.50 in. x 12 in.); Fisher Scientific, Cat. S24256 (need four per set up)
    2. Troemner™ Stainless Steel Support Rod, Length: 22.76 inches, Diameter: 0.51 inches; Fisher Scientific, Cat. 02-300-253 (need four per set up)
    3. Troemner™ Two-Prong Single Adjust Swivel Clamp; Fisher Scientific, Cat. 02-216-212 (need eight per set up)
    4. Troemner™ Talboys™ Labjaws™ Regular Clamp Holder; Fisher Scientific, Cat. 02-300-201 (need two per set up)
    5. Bel-Art™ SP Scienceware™ Compact Support Stand, 21 x 16cm L x W footprint; Fisher Scientific, Cat. 12-947-976 (need one per set up)
    6. Eisco™Aluminum Rods, Dia. x L: 12mm x 60cm (this would be the rod that screws into the stand); Fisher Scientific, Cat. 04-999-113 (need one per set up)

PROCEDURE

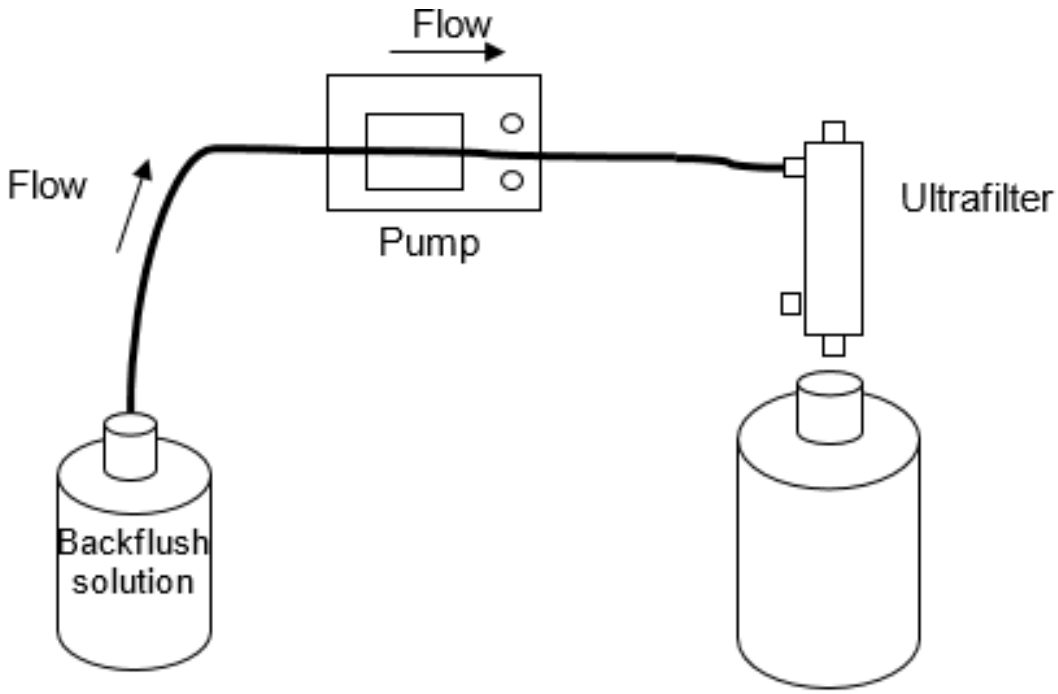
14. Put on gloves.
15. Create backflush solution. A 500 mL volume is required per ultrafilter to be backflushed.
    Note: The backflush solution (0.5% Tween® 80/0.01% NaPP/0.001% Antifoam Y-30) can be made ahead of time but s*hould be discarded after 48 hours*.
    a) Make a 10% NaPP/1% Antifoam Y-30 stock solution in a 10 mL conical tube. It is fine to prepare this stock solution ahead of time and store it for no longer than a week at 4°C. After this time or if before this time if bacterial growth is observed, it should be discarded.
    b) Add 1 g NaPP to 10 mL DI water. Shake vigorously to dissolve the NaPP.

c) Add 100 µL Antifoam Y-30 to the solution and gently mix by inverting repeatedly.
d) Add 500 mL sterile distilled water to a 500-mL bottle (glass or plastic).
e) Add 2.5 mL of Tween® 80 to the 500 mL sterile distilled water. Swirl vigorously (don't shake) to dissolve the Tween® 80. *Note: Be patient, the Tween® 80 can take a while to dissolve*. Attempt to limit the amount of foam produced, but some foam is inevitable.
f) Add 500 µL of the 10%NaPP/1% Antifoam Y-30 stock solution to the 500mL sterile distilled water and Tween® 80 and swirl gently to mix. Alternatively, stir with a sterile stir bar for at least 5 min to dissolve the Tween 80". Again, attempt to limit the amount of foam produced. This is your backflush solution. Set aside for later use or store at 4°C for no more than 48 hours.

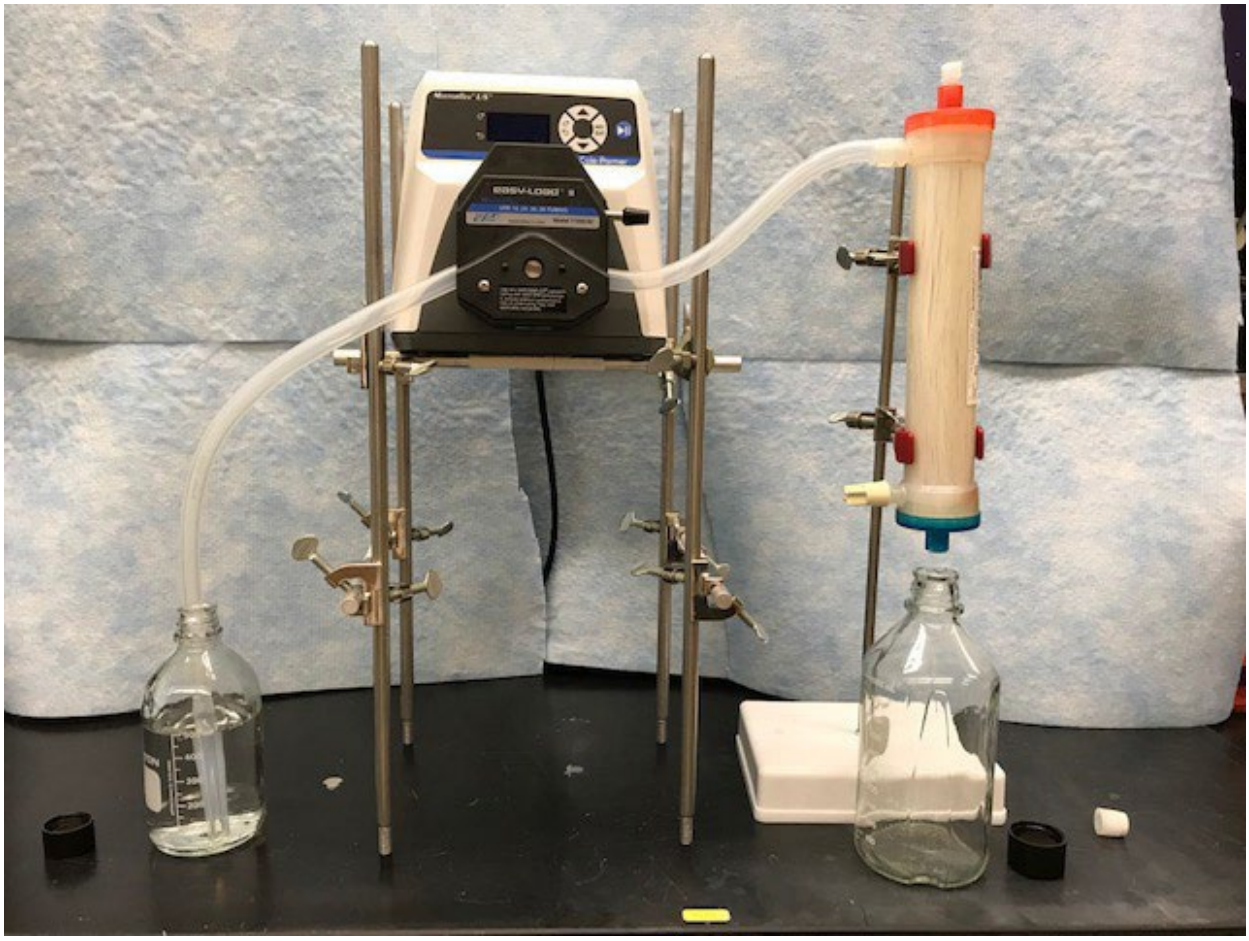
16. *If filters have been held at 4°C or on ice packs from shipping*, allow the filters to warm to room temperature on bench for approximately 1 hour. *Since there exists limited experimental information in terms of viability of bacterial cells following long-term storage of the ultrafilter, it is recommended that filters be processed upon receipt in the laboratory.*
17. Record all pertinent details of the ultrafilter sample on your 1-Liter sample collection bottle.
18. Place clean bench protectors on the lab bench where backflushing will be performed.
19. Assemble the filtration system as shown in Figure 1 and Photographs 1 and 2.
    a) Set up the pump on the assembly of rods and clamps.
    b) Prepare the ultrafilter for backflushing.
        i. Unscrew and remove the cap from one end port of the ultrafilter (blue end). Backflush solution will exit this port and fill the 1 Liter bottle upon backflushing.
        ii. Remove the cap from the top side port (located farthest away from the now-open end port). This is where the L/S 36 tubing will be attached. See Photograph 1.
        iii. The remaining bottom side port should already be secured for shipping by either a rubber storage cap or the original side port cap. If the side port cap was used, check to ensure it is finger tightened. If a rubber storage cap was used, a zip tie must be applied to the base of the cap and pulled as tight as possible to ensure the rubber cap does not falls off or leak. Appendix A contains photos of these items for reference.
        iv. Secure the ultrafilter vertically to the stand using clamps. Place the ultrafilter/stand to the right of the pump.
20. Position a 1-L bottle directly under the end port, making sure the ultrafilter doesn't touch the lip of the bottle.
21. Push size 36 tubing onto the open side port. A 3-foot section of tubing is typically sufficient. Be sure to push the tubing on the side port all the way to where the port meets the ultrafilter and secure with either a hose clamp or zip-tie. *Note: If using the Cole Parmer L/S 36 tubing, a clamp should be used to secure the hosing to the ultrafilter side port. This tubing can slip off the side port when under pressure and must be secured. Alternatively, a zip tie may be used in the place of a hose clamp.* If using the Geotech size 36 tubing, no clamp is needed.

22. Feed the tubing through a peristaltic pump and place the end of the tubing into the backflush solution.
23. Ensure the two (2) remaining caps on the ultrafilter are secured tightly to prevent leaks.
24. Put on face shield.
25. Ensure pump is set to clockwise flow direction. Pump backflush solution through the ultrafilter at 650 mL/min, capturing backflush solution into the bottle. If the pump doesn't have a digital display set the dial to a slow pumping rate. *BE VERY CAREFUL - THE SYSTEM IS UNDER PRESSURE.*
26. Continue pumping until no backflush solution remains in the 500 mL bottle and the flow out of the ultrafilter has slowed to a trickle. It will be necessary to briefly pump air after the backflush solution is used up in order to get all the liquid out of the filter. *Do not pump air into the filter for more than approximately 10 seconds.*
27. Turn off the pump.
28. Replace the lid on the 1-Liter bottle and ensure the bottle is properly labeled. *This is your sample.* Refrigerate until analysis. *Since there exists limited experimental information in terms of viability of bacterial cells following long-term storage in the backflush solution, it is recommended that backflush be enriched for pathogens within 24 hours.* Measure and record sample volume, if necessary. Sample volume is typically between 500-700 mL.
29. Equipment disassembly and cleanup
    7. Wearing a face shield, release the L/S 36 tubing by *slowly* pushing the lever on the pump and removing from ultrafilter port. Discard tubing. *BE VERY CAREFUL - THE SYSTEM IS UNDER PRESSURE*. The pump head lever should be gradually raised to allow the air to be released slowly.
    8. After backflushing, the ultrafilter may be stored if required by laboratory-specific protocol with regards to sample retains. However, backflushing the filter a second time is not likely to result in the additional recovery of bacterial pathogens. Be sure to cap all open ports (side and end) to prevent leaks.
    9. If discarding the ultrafilter, the rubber storage cap, side port cap(s) and/or blood port cap (Appendix A contains photos of these items for reference) can be removed and disinfected for future use as spare parts by soaking in 10% bleach solution for 30 minutes, rinsing with sterile water, and allowing to air dry.
    10. Discard bench protector. Disinfect area with 10% bleach followed by 70% ethanol.
30. Proceed to pathogen-specific detection method or desired secondary concentration method.

Figure 1. Ultrafilter Backflush Setup



Photograph 1. Backflush Pump and Ultrafilter Setup – Front view



*NOTE: Bench protector paper is missing from the lab bench in Photograph 1. Bench protector paper should be placed under the entire assembly, including the 500 mL bottle and the 1 Liter bottle.
**NOTE 2: A zip-tie (not pictured) must be attached to the rubber storage cap on the bottom side port to ensure the rubber storage cap remains in place.
***NOTE 3: If using Cole Parmer L/S 36 tubing, you must secure the tubing to the side port with a hose clamp. If using Geotech size 36 tubing, no hose clamp is needed. Be sure to push the tubing on the side port all the way to where the port meets the ultrafilter.

Photograph 2. Backflush Pump and Ultrafilter Setup – Side View

*See Notes under Photograph 1.

Appendix A

Photographs of Hose Clamp, Blood Port Cap, and Rubber Storage Cap

Photo 1. Hose clamp
(Cole-Parmer # 6832-04; Min ID: 0.402”, Max ID: 0.449”)

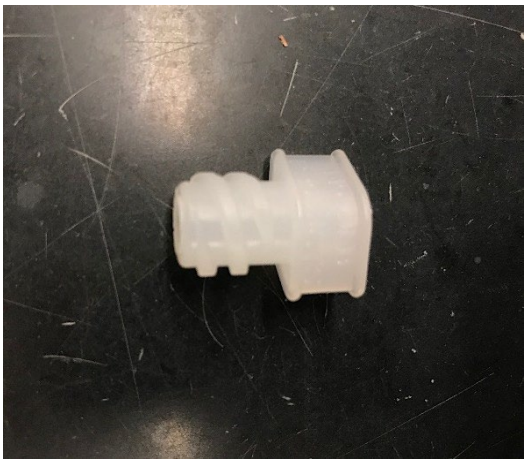


Photo 2. Blood Port Cap
(Molded Products # MPC-40)

Photo 3. Rubber Storage Cap
Dialysate Port Storage Cap (Molded Products #MPC-60D)