Standard Operating Procedure for Dead-End Ultrafiltration Water Sampling in the Field for Bacterial Pathogens Using REXEED 25S Replacement Filters

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Prepared By:

Ai Kataoka
Research Microbiologist
Center for Food Safety and Applied Nutrition
US Food and Drug Administration

Roberto Guzman
Microbiologist
Center for Food Safety and Applied Nutrition
US Food and Drug Administration

Julie Ann Kase, PhD
Microbiologist
Center for Food Safety and Applied Nutrition
US Food and Drug Administration
Contact info: Julie.Kase@fda.hhs.gov

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1. SCOPE AND APPLICABILITY

This procedure will be used to filter up to 100 L of water using an ultrafilter at a field sampling site to collect bacteria for pathogen analyses in the laboratory. Included are instructions on how to properly backflush the filters in a laboratory setting to retrieve any bacteria captured.

2. SUMMARY OF METHOD

Using a field-portable peristaltic pump, volumes up to 100 L of water will be passed continuously through a Clearum H22, Xevonta Hi23, Elisio 25H or equivalent dialysis ultrafilter having a pore size small enough to capture any bacteria and other microorganisms present in the site water. Parameters such as time, date, and Sample ID will be recorded on the filter using permanent marker. The ultrafilter will be kept at 4ºC (refrigerated or on ice packs) after sample collection. Following sampling, a backflush procedure is performed in the laboratory with a 500 mL solution of 0.5% Tween 80, 0.01% sodium polyphosphate, and 0.001% Antifoam Y-30 Emulsion.

3. HEALTH AND SAFETY WARNINGS

Disposable gloves are recommended to be worn during all steps of this sample collection procedure to prevent possible exposure to waterborne pathogens and contamination of the filtration components. Gloves are mandatory when removing caps from the ultrafilter, attaching tubing to the filter ports or otherwise touching exposed parts of the ultrafilter. For backflush procedure, disposable gloves should be worn and changed appropriately during all steps to prevent possible exposure to waterborne pathogens and to prevent cross-contamination of samples. Since the filtration 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 personal protection equipment (PPE).

4. EQUIPMENT AND SUPPLIES FOR DEAD-END ULTRAFITRATION

Disclaimer: Names of vendors or manufacturers are provided 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.

NOTE: Ultrafilter sampling kits can be made ahead of time to facilitate field activities. One kit is prepared per ultrafilter. See Appendix C for details.

1. Filtrate port tubing (Note this tubing is not sold pre-sterilized, although the Masterflex Silicone Tubing L/S 36 (sold in 25-foot rolls; VWR, Item #MFLX96410-36) **Note**: this tubing is not sold pre-sterilized, although the likelihood of pathogen presence is infinitesimal. If concern over sterility exists, contact manufacturer for autoclaving instructions or consider using an approximately 3-foot piece of tubing from same lot as a control sample that could be flushed with sterile water that is subsequently tested accordingly.

a. Alternative tubing (less expensive but doesn't work as well) – Silicone Tubing, Size 36 (sold in 25-foot rolls; Geotech Environmental Equipment, Item # 77050011, http://www.geotechenv.com/)
b. Alternative tubing (needs to be used with the smaller DIN adapter and clamp) L/S 24 tubing Masterflex, (Cole-Parmer, Item # 96410- 24); Small Plastic hose clamp - (Grainger, Item # 1ENJ3, Max 0.406")

2. Kidney Dialysis Ultrafilters: Clearum H22 (Item #1428580) and Elisio 25H (Item # 9120016) can be purchased from Henry Schein Corporation (1-800-472-4346); Xevonta Hi23 (Item # 7204405) can be purchased from Optimal Golden Max LLC (1-800-778-4718)

3. SNP-8 plastic hose clamps (for DIN adapter), (Cole-Parmer, Item # EW06832-08). See Fig. 4, Appendix A

4. SNP-12 plastic hose clamps (for side port), (Cole-Parmer, Item # EW06832-12). See Fig. 7, Appendix A

5. Zip ties (Fisher Scientific, Item # NC9269761 or equivalent)

6. SNP-4 Plastic hose clamp, (Cole-Parmer, Item # EW06832-04 or Grainger, Item # 1ENP7 for DIN adapter MPC-855). See Fig. 1, Appendix A

7. DIN Adapter (Molded Products, Item # MPC-855, sold by the set, sterile, 2 pieces per set; https://moldedproducts.com/); See Fig. 2, Appendix A. Alternative DIN Adapter – larger version, not pre-sterilized, only used with the L/S 36 tubing (Molded Products, Item # MPC- 855NS.375). **Note**: can use zip ties instead of clamps and thus no pliers needed. See Fig. 3, Appendix A.

8. Blood Port Caps (Molded Products, Item # MA-40); See Fig. 5 in Appendix A

9. Dialysate Port Storage Cap (Molded Products, Item # MPC-60D) See Fig. 6 in Appendix A

10. Two 20L-cubitainers or other container with demarcations for measuring total volume (liters) passed through filter if not using a flow meter (having two containers will allow for water measurements to continue while one container is being emptied).

11. Mosquito/insect fiberglass mesh (sterile) for sampling waters with substantial, visible debris

12. Permanent Marker

13. Sealable, watertight plastic bags (e.g., Ziploc® 2.5 gallon) for storing filter after sampling.

14. Spray bottle of 70% ethanol (to field-disinfect scissors, pliers, and gloved hands)

15. Needle nose pliers (to close and remove the hose clamps)

16. Cutting pliers to cut the zip ties, if used

17. Cooler or other suitable container for keeping filters chilled after collection and during transport to laboratory.

18. Heavy duty tape (Gorilla Tape or equivalent since will be used to seal cooler for shipment and will need to withstand rough treatment)

19. Ice / freezer packs

20. Bench protector paper (Fisher Scientific, Item # 14-206-65, or similar)

21. UPS or FedEx Shipping label(s) for Next Day Delivery, if shipping

22. Trash bag or biohazard bag depending on individual laboratory guidelines.

23. Nitrile or latex disposable gloves

24. Scissors (capable of cutting tubing)

25. 50 mL conical tubes or other clean, sterile container for storing clamps and caps.

26. Flat head and #1 Phillips head screwdrivers (if the easy-load II pump head on Geopump needs to be moved)

27. Umbrella (golf-type or other large size, if bench protector paper not available) if sampling in sunny conditions to protect ultrafilter during collection and prevent pump from overheating.

28. Sodium thiosulfate, (Fisher Scientific, Item # S446) (Only needed for chlorinated water)

29. Peristaltic Pump (Geotech Environmental Equipment, Item # 91352123, http://www.geotechenv.com/peristaltic_geopump.html or equivalent) with one battery (recommend purchasing extra batteries)

30. Extra batteries for field sampling pump (Geotech Environmental Equipment, Item # 77250000 or equivalent)

31. DC to AC converter for pump operation (Geotech Environmental Equipment,

Item # 81400127 or equivalent) – this piece of equipment has alligator clips (to attach to a car battery) and a place to plug in the pump. It provides a way to power the pump in the field if the battery is depleted.

32. Turbidity Meter (optional) with a resolution of 0.01 NTU, a range up to at least 200 NTU, and compliant with EPA 180.1 method or ISO 7027 standard.

33. Flow meter (optional and can be used instead of containers for measuring pump output). Recommend – Item # 113900-9511 Rust Automation & Controls Inc. rustco.com

34. Sterile DI water for priming filter (1L per filter)

5. EQUIPMENT AND SUPPLIES FOR BACKFLUSH PROCEDURE

1. Silicone Tubing L/S 36 Masterflex (Cole-Parmer, Item # 96410-36) or Silicone Tubing size 36 (Geotech Environmental Equipment, http://www.geotechenv.com/ Item # 77050011)

2. Plastic hose clamp, SNP-12 (Cole-Parmer, Item # EW06832-12); See Fig. 7 in Appendix A.

3. Zip ties, optional, in place of hose clamp (Fisher Scientific, Item # NC9269761 or similar). See Fig. 7 in Appendix A.

4. Paper towels

5. Scissors

6. Needle nose pliers (to close the hose clamps)

7. Bench protector paper (Fisher Scientific, Item # 14-206-65, or similar)

8. Face shield, optional (Fisher Scientific, Item # 17-310, or similar).
   Note: Please consult safety protocols of individual laboratories.

9. Nitrile or latex disposable gloves

10. 10% bleach solution

11. 70% ethanol

12. Tween® 80

13. Sodium polyphosphate (NaPP), (Thermo Scientific, Item # 390930010)

14. Antifoam Y-30 Emulsion. Prepare your own Y-30 solution by mixing three parts of antifoam A concentrate (Sigma, Item # A6852-100G) in seven parts of propylene glycol (Sigma, Item # 398039).

15. Sterile stir bars, one per sample

16. 500 mL bottle, sterile, one per sample

17. 1 Liter bottle, sterile, one per sample

18. Sterile deionized water

19. 5-10 mL serological pipette

20. Bench scale with 0.1 g sensitivity

21. Peristaltic Pump (Geotech Environmental Equipment; http://www.geotechenv.com/ Geopump™ Peristaltic Pump Series II Package, Geotech Environmental Equipment, Item # 91352123 or equivalent)

22. Construction of Pump Platform: Adjustable height jack, (e.g., Synthware™ Item # J112028; Fisher Scientific, Item # 31-502-290), or a rods and clamps assembly to elevate pump for ease in backflushing filters.
    a. Eisco Aluminum Rod 1/2 in. Diameter x 12 in. Length - Round Bar (0.50 in. x 12 in.); (Fisher Scientific, Item # S24256) (need four per set up)
    b. Troemner™ Stainless Steel Support Rod, Length: 22.76 inches, Diameter: 0.51 inches; (Fisher Scientific, Item # 02-300-253) (need four per set up)
    c. Troemner™ Two-Prong Single Adjust Swivel Clamp; (Fisher Scientific, Item # 02-216-212) (need two per set up)
    d. Troemner™ Talboys™ Labjaws™ Regular Clamp Holder; (Fisher Scientific, Item # 02-300-201) (need eight per set up)
    e. Bel-Art™ SP Scienceware™ Compact Support Stand, 21 x 16cm L x W footprint; (Fisher Scientific, Item # 12-947-976) (need one per set up)

6. FIELD FILTRATION AND SHIPPING PROCEDURES

Please see Fig. 8 in Appendix B to see ultrafilter components and port locations for the various filters.

1. Blood outlet port
2. Dialysate inlet port
3. Dialysate outlet port

4. Blood inlet port

Since these filters need to be primed with solution prior to use, please read through the instructions below before beginning the filtration process and review the figures (11 - 14) in Appendix E and F.

6.1 Put on gloves.

6.2 Place clean bench protector(s) on the area(s) where sampling will be performed.

6.3 Remove ultrafilter from packaging. **Note**: Clearum H22 and Xevonta Hi23 come without dialysates port caps, (Fig. 8, Appendix B). Dialysate storage caps must be attached and secured before starting the procedure (See Fig. 9, Appendix C). Secure the storage dialysate caps with a plastic hose clamp SNP-12 using needle nose pliers.

6.4 Write the date, time of collection, your initials, and sample number and place ultrafilter on the bench protector. **Note**: filters should be primed before the sampling procedure with 1L of sterile water or 1% sodium thiosulfate solution when the water to be collected contains free chlorine or chlorination is suspected. (Mix 10 g sodium thiosulfate and 1L deionized water, shake to dissolve). It is preferred that priming take place immediately before sampling is initiated.

6.5 Disinfect scissors with 70% ethanol and allow to dry for 30 seconds on a paper towel. Then, after locating one end of the tubing inside the bag, cut open the bag and cut approximately 2 ft of tubing for use in priming. Place it on the bench protector. All remaining tubing should stay in packaging to the greatest extent possible.

6.6 Remove the ultrafilter blood outlet port cap, screw in the DIN adapter and connect one end of priming tubing.

6.7 Secure the DIN adapter with the SNP-8 plastic hose clamps or SNP-04 plastic hose clamp if small DIN adapter MPC-855 is used. **Note**: zip ties can be used alternatively for both DIN adapters.

6.8 Place the other tubing end into the bottle of priming liquid and align the center portion of the tubing into the peristaltic water pump head. See Fig. 11, Appendix E. Make sure the pump flow direction “Forward/Reverse” setting is from liquid to ultrafilter, remove the blood inlet port cap and turn on the peristaltic pump at low speed (200-300 rpm) until the liquid emerges from the blood inlet port.

6.9 Secure the blood inlet port with the storage blood port cap, then carefully detach and discard the tubing from the DIN adapter, positioning the filter such that the DIN adapter won’t become contaminated while sitting on the bench paper.

6.10 Using a permanent marker, indicate by writing an arrow on the filter exterior, the direction of water filtration as confirmation. Water should be entering thru the blood outlet port. See Appendix D Fig. 10.

6.11 Attach a new tubing end to a sampling telescoping pole with a zip tie. Leave

around three inches off the telescoping pole and approach your water source leaving the remainder of the tubing in the packaging. Place influent tubing into the body of water and ensure the end of the tubing will stay below the surface of the water and away from plant material or other large debris. Be cautious not to disturb the area (especially any sediment) near the filtration site.

If debris or material that may cause premature filter clogging is present, a sterilized mosquito/insect mesh can be attached to the tubbing end with a rubber band or zip tie to avoid clogging the tubing and ultrafilter with debris.

6.12 Using disinfected scissors, cut the tubing remaining in the packaging to the desired length.

6.13 Insert the tubing into the DIN adapter and secure it with SNP-8 plastic hose clamps or SNP-4 for small DIN adapter (MPC-855).

6.14 Connect the remaining tubing from the original packaging to the dialysate fluid outlet and direct the effluent downstream from sample intake.

If a flow meter will be used to measure the volume of water filtered, cut the effluent tubing in half after it is connected into the dialysate fluid outlet and screw into the 3/4" GHT water flow meter adapter onto each end. (See Fig. 12 Appendix E). Ensure directional flow of the meter is correct. No clamps are needed.

If flow meter is not used, place the tubing into a 20-L cubitainer or other container to measure the total volume that has passed through the filter. Return the measured water downstream far from the influent water source to avoid disturbing the water near intake. Do this 5 times for a 20 L sample. Adjust accordingly for containers of different volumes.

6.15 Verify set up is correct. See Appendix F. Make sure the tubing is passing through the water pump head without pinching the tubing and review the flow direction on the “Forward/Reverse” setting.

6.16 Start the pump by flipping the switch to ON and turning the knob gradually to maximum speed of 600 rpm.

Note: with the Geotech peristaltic pump, the easy-load II pump head can be in one of two positions (Series #1 Drive or #2 Drive) and either will work fine for the purposes of this method. However, the position to the right (Series #2) will result in faster pumping speeds and thus, shorter sampling times. A #1 Phillips head screwdriver is needed to move the easy-load II pump head from one position to another.

6.17 The pump will begin pumping the water through the influent port tubing into the filter and out the side port through the filtrate port tubing. Make sure no ports on

the ultrafilter are leaking water.

6.18 If sampling outdoors and the ultrafilter and pump is in the sun, cover the ultrafilter and pump with a clean bench protector, large umbrella, or other device to shield it from the sun and prevent pump overheating.

6.19 When sampling is completed and the pump is stopped, slowly open the pump head to release any pressure and remove the filtration tubing from the pump head.

6.20 Unplug the pump from the battery. Remove all tubing from the ultrafilter and place end caps and storage caps on the ports, being careful not to allow water in the ultrafilter to escape.

IMPORTANT Be sure endcaps are attached as snug as possible as they tend to leak if not secure. See Fig. 9, Appendix C. Be sure that water does not exit the filter as you are removing the tubing and securing the caps.

Used blood port caps, DIN adapters, and rubber storage caps can be returned to laboratory for cleaning and disinfecting before reuse or dispose of material in appropriate trash or biohazard bag.

6.21 Tubing that came in contact with the water source should be disposed of and not reused to collect another sample.

6.22 Dispose of trash (tubing, gloves, bench protectors, paper towels, etc.) in trash or biohazard bag as per individual laboratory or onsite guidelines.

6.23 Label a sealable 2.5 gallon bag (e.g., Ziploc® bag) with the Sample ID, date, time, and your initials.

6.24 Place the filter inside the bag, seal the bag, and gently place in a chilled cooler (wet ice / freezer packs) or in a refrigerator until shipment or processing.

7. TROUBLESHOOTING

7.1 Pulling air instead of water through tubing.

Symptom: Pump is struggling to pull water from source

Remedy: Stop pump, **slowly** release pressure in tubing by lifting the pump head clamp and resume pumping after checking to be sure intake tubing end is submerged under the water

7.2 Clogging of tubing or filter

Symptom: Debris is observed in tubing near connection with filter inlet or flow of water out of filter visibly decreasing

Remedy: Stop pump, consider if area where debris is can be simply cut out and remainder of tubing reattached; if filter is clogging, it may be a hard stop and end to sampling with current ultrafilter. Note the volume of water filtered on the ultrafilter and on any field datasheets. If debris is noticed before it reaches the filter, the tubing should be disconnected at the DIN adapter and tubing flushed with source water until the debris is removed. Then, reconnect everything and proceed with filtration.

7.3 Split tubing

Symptom: Water is leaking where tubing is threaded thru pump head due to crack in tubing

Remedy: Stop pump, remove tubing from pump head and replace influent tubing piece, clear out any visible sand or debris that might be causing friction in pump head and resume pumping.

8. BACKFLUSH OF ULTRAFILTERS FOR BACTERIAL PATHOGENS PROCEDURE

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.

8.1 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* ***should be discarded after 48 hours****.*

8.1.1 Make 10 mL of a 10% NaPP/1% Antifoam Y-30 stock solution in a 15 mL conical tube. Note that the use of a larger volume tube facilitates the mixing process. It is fine to prepare this stock solution ahead of time and store it for up to one week at 4°C.

8.1.1.1 Add 1g NaPP to 10 mL DI water. Shake vigorously to dissolve the NaPP.

8.1.1.2 Add 100 µL of Antifoam Y-30 to the solution and gently mix by inverting repeatedly.

8.1.2 Add 500 mL of DI water to a 500 ml or 1 L bottle.

8.1.3 Add 2.5 ml of Tween 80 with a 5-10 mL serological pipette.

8.1.4 Stir with a sterile stir bar until the Tween 80 is dissolved. If stir bar is not used, 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.

8.1.5 Add 500 µL of the 10% NaPP/1% Antifoam Y-30 stock solution to the water

8.1.6 Set aside for later use or store at 4°C for no more than 48 hours.

8.2 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.

8.3 Record all pertinent details of the ultrafilter sample on your 1-Liter sample collection bottle.

8.4 Place clean bench protectors on the lab bench where backflushing will be performed.

8.5 Position the peristaltic pump on a level surface or on a lift at least 20” above the bench surface.

8.6 Secure filter vertically using the stand clamps pointing the sample inlet port downwards, and the sampling effluent side port facing thru the water pump as shown in Fig. 16 and 17 in Appendix G. **Note**: confirm the filtration direction by locating an arrow written on the filter surface by the collector. The water flow should have occurred through the blood outlet port. See Appendix B Fig. 8. If filtration happened instead through the blood inlet port, the backflush process should occur with this port facing downwards (i.e., backflush should always occur in the reverse direction from sample filtration).

8.7 Position a 1-L bottle directly under the end port, making sure the ultrafilter doesn’t touch the opening of the bottle.

8.8 Unscrew and remove the cap from the filter end port facing downwards (blood outlet port). Backflush solution will exit this port and into the 1-Liter bottle upon backflushing.

8.9 Disinfect the scissors with 70% ethanol and cut around 3ft section of L/S 36 tubing. Remove the cap from the top side port (dialysate outlet port). Push one end of the L/S 36 tubing fully onto the port as much as possible.

8.10 Feed the tubing through the peristaltic pump head and place the other end into the 500 mL backflush solution.

8.11 Secure the tubing attached on the upper side port (dialysate outlet port) with either a hose clamp SNP-12 or zip-tie. This tubing can slip off the side port when under pressure and must be secured.

8.12 The blood inlet port and dialysate inlet port (top port and bottom side port, respectively) must remain secured. Check to ensure they are finger tightened and ensure that a SNP-12 hose clamp, or a zip tie was applied to the bottom side port (dialysate inlet port) adequately to prevent the rubber cap from leaking or coming off.

8.13 Put on face shield if part of laboratory safety requirements.

8.14 Close the pump head lever.

8.15 Ensure pump flow direction is set from backflush solution to ultrafilter. Pump backflush solution through the ultrafilter at 650 mL/min (or 250-300 rpms), capturing backflush solution into the bottle. If the pump doesn't have a digital display set the dial to a slow pumping rate.

8.16 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 depleted to get as much liquid out of the filter as possible. Do not pump air into the filter for more than approximately 10 seconds.

8.17 Turn off the pump.

8.18 Release the L/S 36 tubing opening the pump head slowly, with one hand on the pump and the other hand releasing the lever gradually. **BE VERY CAREFUL - THE SYSTEM IS UNDER PRESSURE.**

8.19 Replace the lid on the 1-Liter bottle and ensure the bottle is properly labeled. This is your sample. Refrigerate until analysis. Measure and record backflush sample volume, if necessary. Backflush sample volume is typically between 500-700 mL.

8.20 Remove the tubing from the ultrafilter by releasing the SNP-12 hose clamp with a needle nose pliers.

8.21 After backflushing, the ultrafilter may be stored if required by individual laboratory protocols for sample remains. 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.

8.22 Discard bench protector. Disinfect area with 10% bleach followed by 70% ethanol.

8.23 Proceed to pathogen-specific detection method or desired secondary concentration method.

Appendix A

Pictures of Hose Clamp, Blood and Dialysate Port Cap and DIN Adapters

Fig 1. Hose clamp (Cole-Parmer # 6832-04)

Fig 2. DIN adapter (Molded Products #MPC-855)

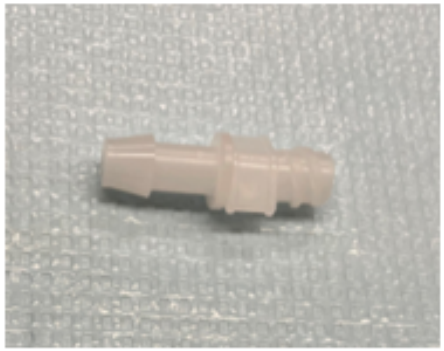


Fig 3. DIN adapter (Molded Products #MPC-855NS.375)

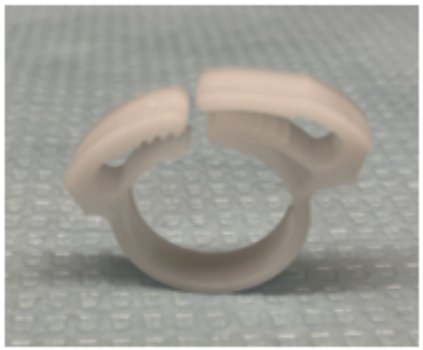


Fig 4. SNP-8 Plastic hose clamp (Cole Parmer EW06832-08 for DIN Adapter 855NS.375)

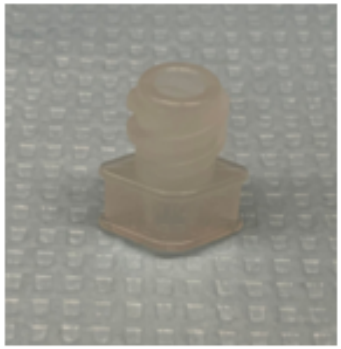


Fig 5. Blood Port cap (Molded Products # MA-40)

Fig 6. Dialysate port storage cap (Molded Product #MPC-60D)

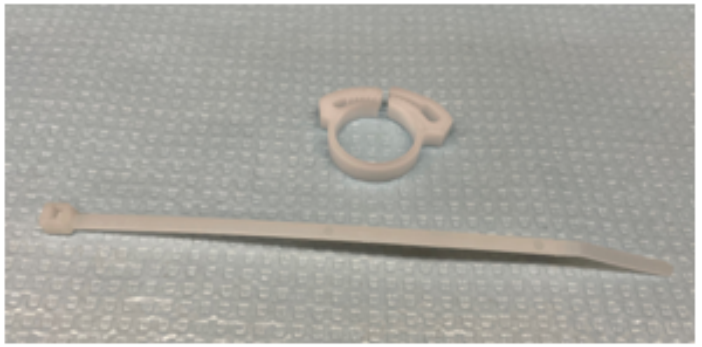
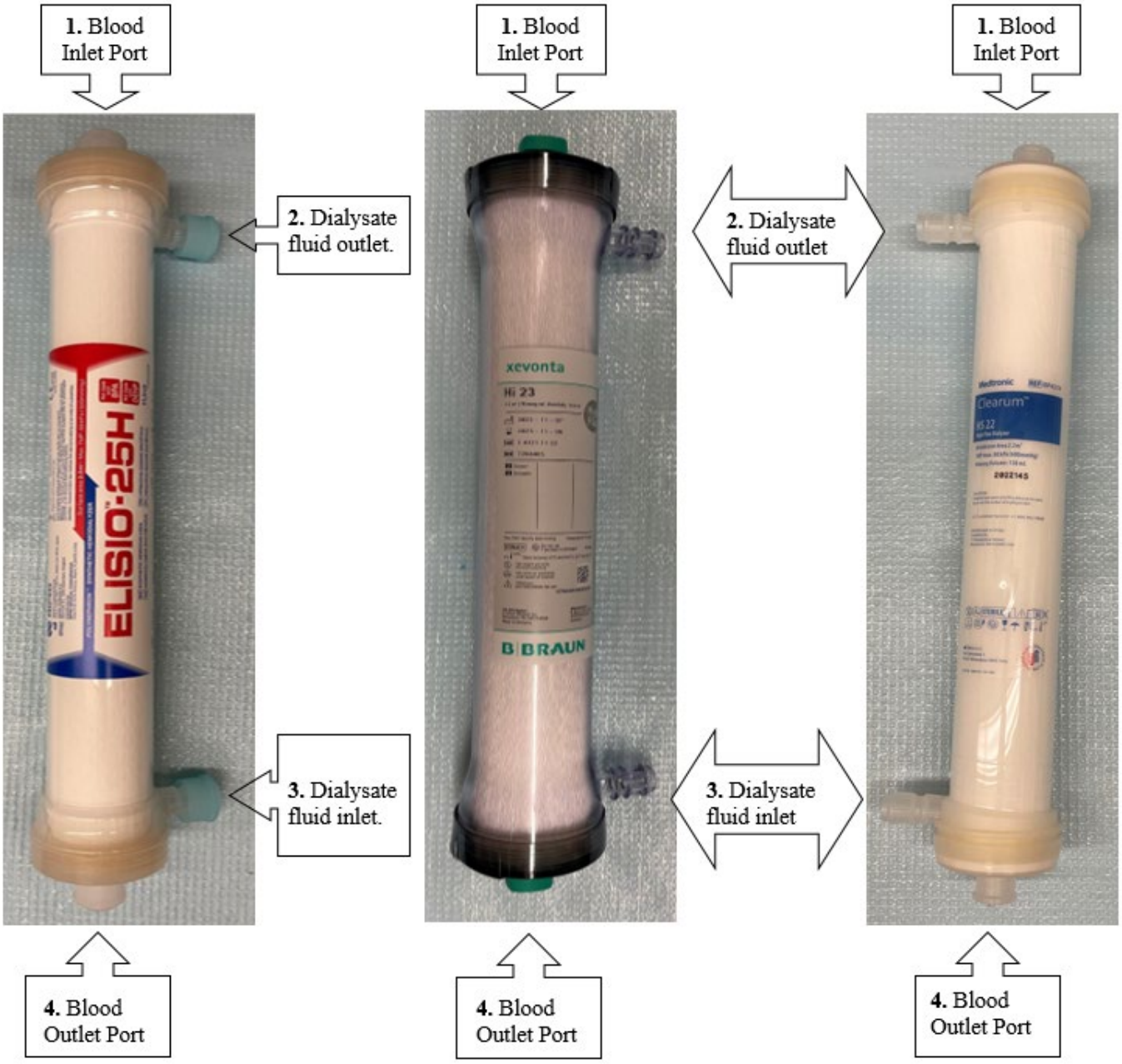


Fig 7. SNP-12 Plastic hose clamp (Cole Parmer EW06832-12) and zip tie (Fisher Scientific Cat. NC9269761) for dialysate side ports.

Appendix B

Fig 8. Ultrafilters with designated ports and outlets.

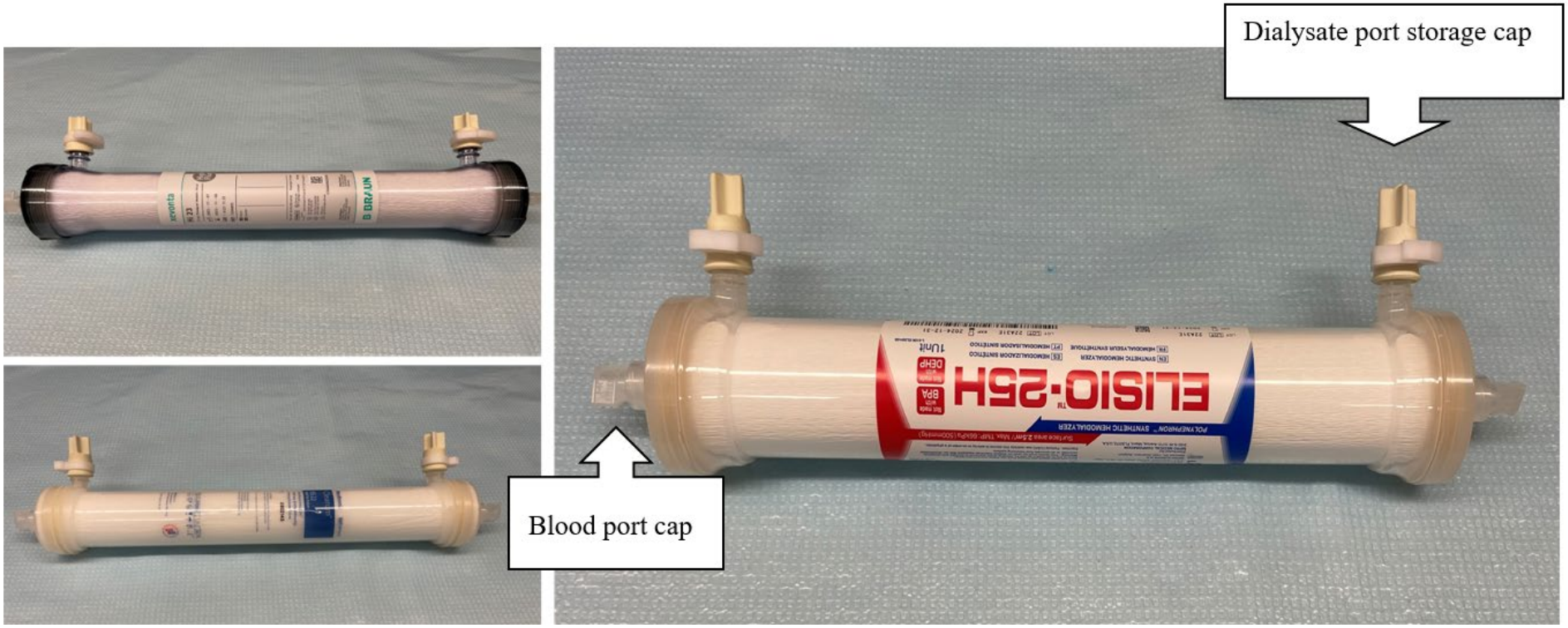


Appendix C

List of supplies in ultrafiltration kit

Each kit contains: 2 bench protectors; one clean/unused 50 mL conical tube with one DIN adapter, 2 rubber storage caps and 2 blood port caps; one SNP-04 hose clamp to secure the MPC-855 DIN adapter or SNP-12 hose clamp/ zip tie for MPC-855NS.375 DIN adapter; 2 SNP-12 Plastic hose clamp or zip tie to secure the two rubber storage caps; 2 zip ties to secure the tubing at the telescoping pole; one roll of tubing; one ultrafilter; and one 2.5-gallon sealable bag (for storage of the ultrafilter after sample collection).

Fig 9. Original caps removed and replaced with storage caps.

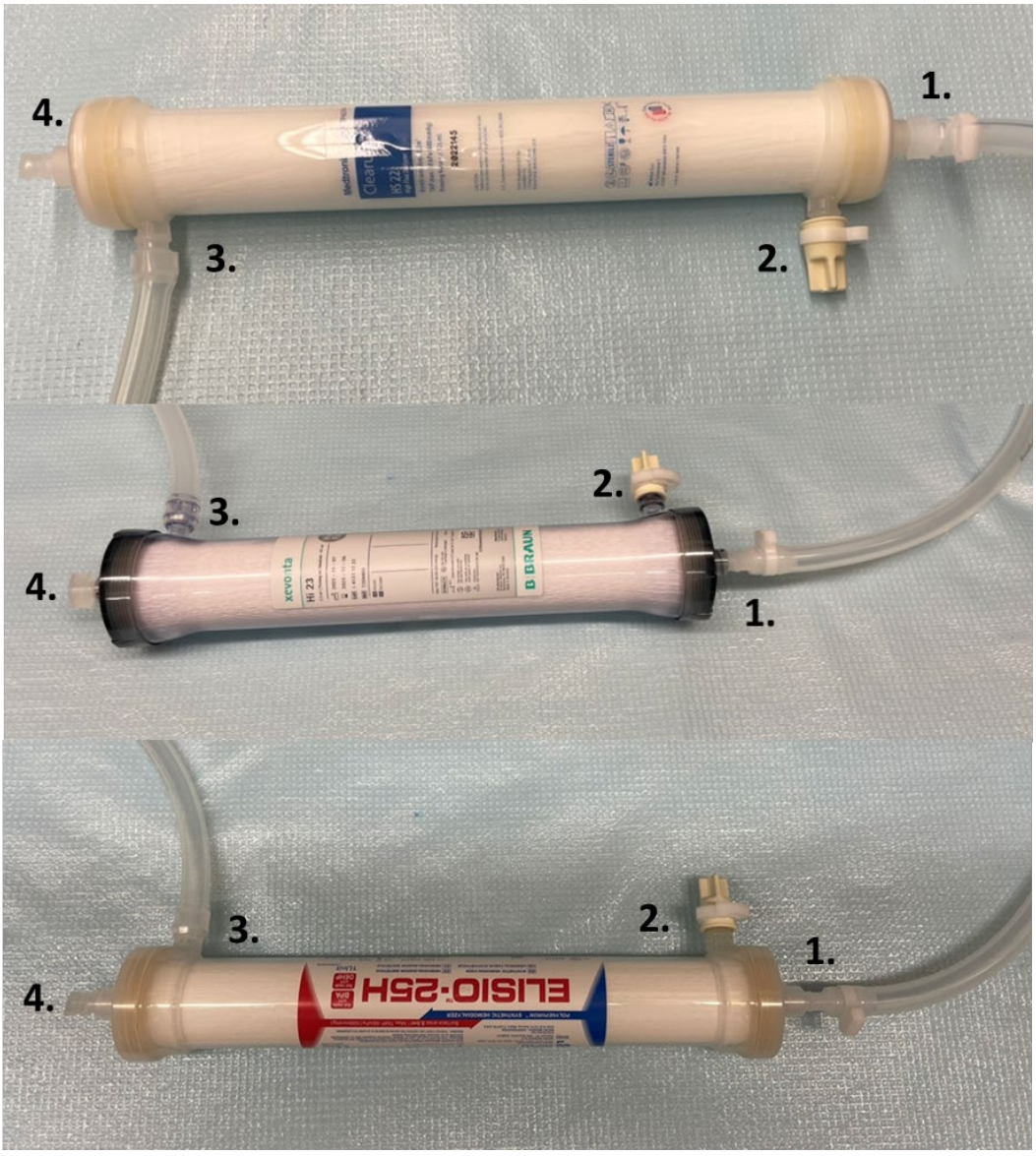


Appendix D

Illustrations of tubing connection to different ultrafilter brands

Fig 10. Ultrafilters tubing connection configuration.

1. Tubing connected onto DIN adapter and secured with plastic hose clamp SNP-8 and needle nose pliers.
2. Dialysate port storage cap secured with a plastic hose clamp SNP-12 and needle nose pliers.
3. Tubing connected to the dialysate fluid outlet and effluent directed away from water sample intake. No clamps are needed.
4. Blood inlet port secured with the storage blood port caps.



Appendix E.

Ultrafilter priming and flow meter configuration

Fig 11. Set up for ultrafilter priming.

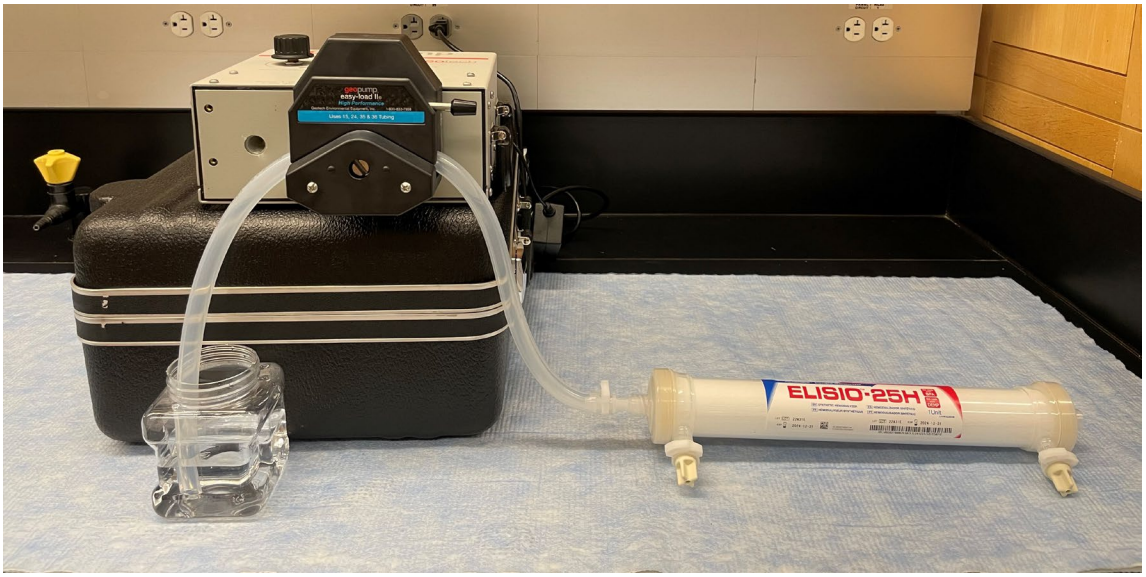
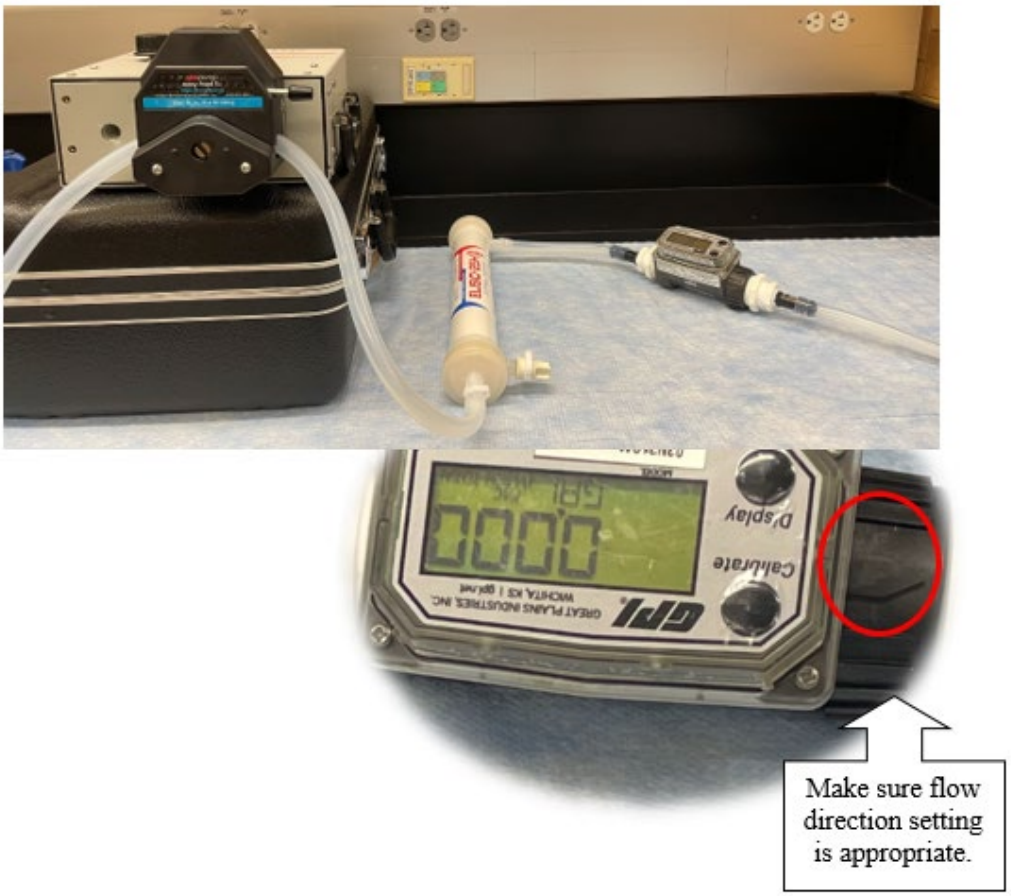


Fig 12. Appropriate configuration of effluent tubing and water flow meter.

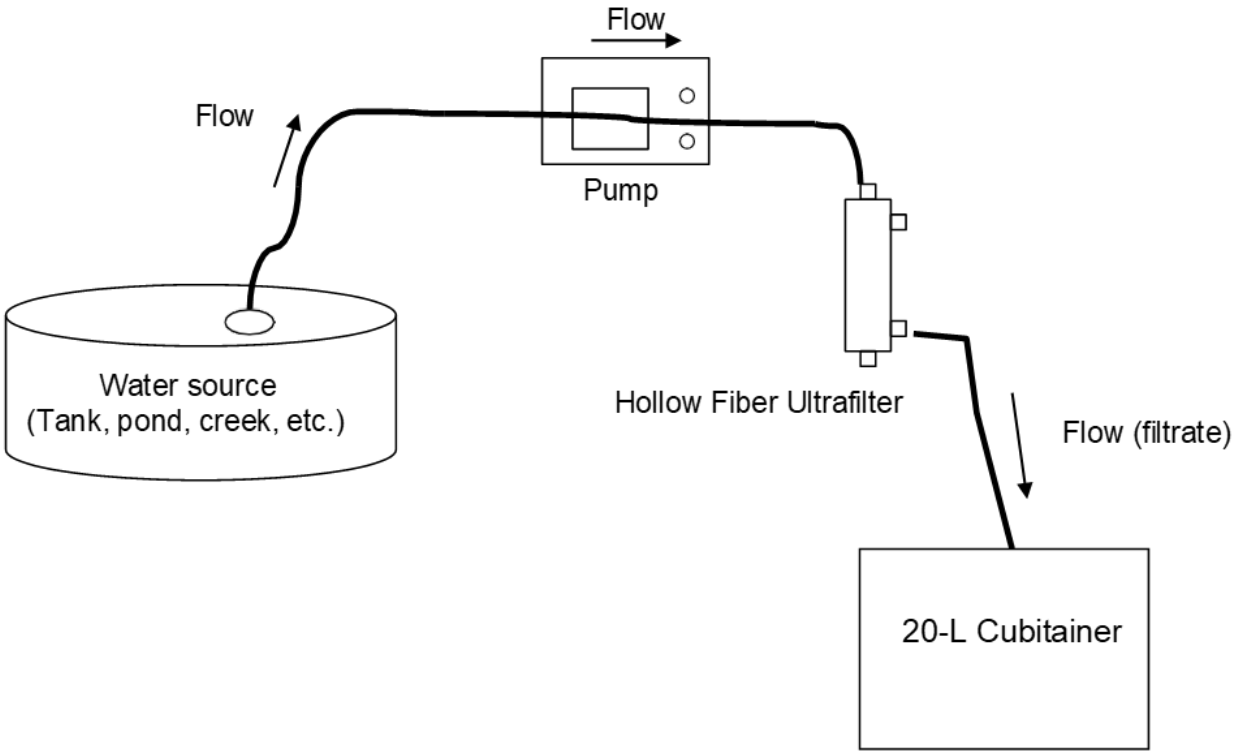


Appendix F

Water sample collection

Fig 13. Example of dead-end ultrafiltration in field.

Fig 14. Dead end ultrafiltration field arrangement diagram.



Appendix G

Backflush procedure.

Fig 15. Ultrafilter upon receipt.

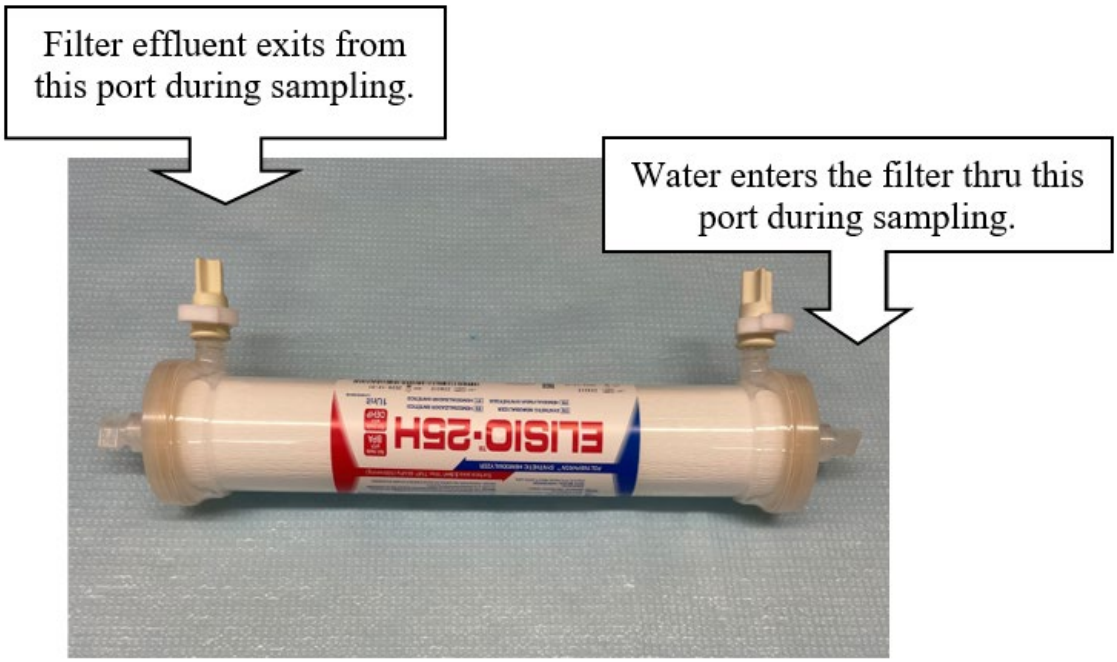


Fig. 16. The filter is secured vertically using the stand clamps with the sample inlet port pointing downwards, and the effluent side port oriented towards the pump.

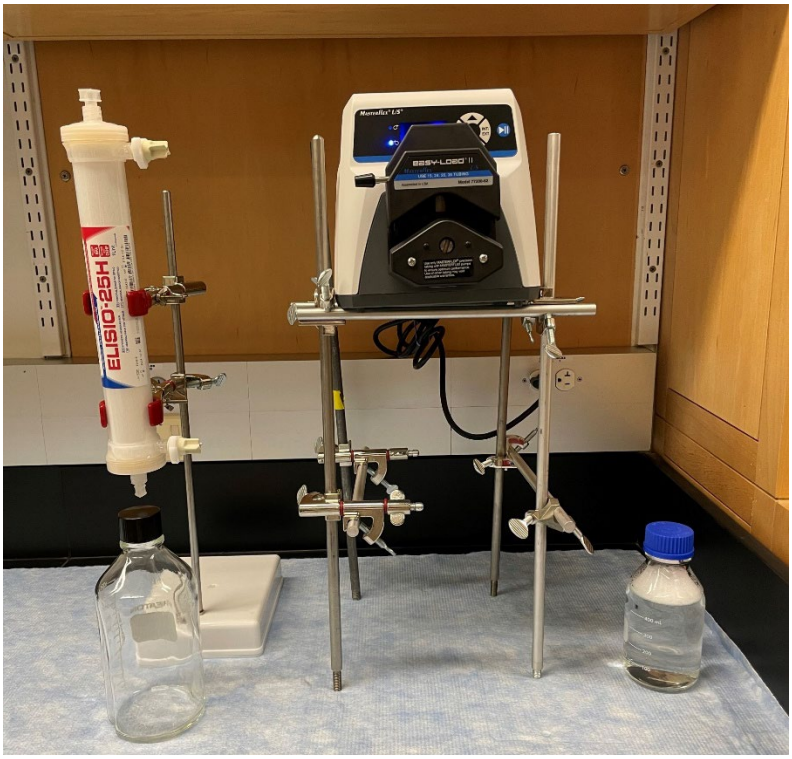


Fig. 17. During the filter set up, the sample inlet port points downwards, and the effluent side port faces the pump. The sample receiving bottle is placed under blood return port without touching the bottle surfaces. The tubing is attached to the dialysate outlet port by pushing onto the port and is secured with a SNP-12 clamp or a zip tie.