**Supplemental Document**

# Emergency Production and Collection of Dialysate for CVVHD during the COVID-19 pandemic

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## S1. Methods

### S1.1 Producing the Dialysate (with Pictures)

The following method was used to produce Dialysate using a Fresenius 2008T machine to bag dialysate in a Baxter Exactamix TPN bag through a Fresenius Optiflux high-flux dialyzer.

1. Power the machine ON

2. Hook up acid connectors and the bicarbonate solution.

3. Put the machine on dialysis mode (choose the concentrate being used). You do NOT need to hook up any bloodlines.

4. Set your bicarbonate level

5. Run 800 mL/min flow and set preferred temperature of 36C.

6. Wait for machine to reach appropriate stable conductivity (around 13.7 – 13.9)

7. Once machine has stable conductivity:

7.1. Take out blue Hansen (shunt interlock), sterilize it with chlorhexidine swabs, and connect to the dialyzer inflow port. Remove the cap from the effluent port.

7.2. Avoid moving the dialyzer or the Hansen hose (shunt interlock) to prevent the machine from activating the Transmembrane Pressure (TMP) alarm.

7.1.2. If a TMP error comes on, just clear it, and the machine should continue making dialysate.

7.2. The button needs to be continuously pressed inside the slot where the head of the blue Hansen usually hits so the machine doesn't go on bypass mode. We used a piece tubing that is cut to the right fit.


7.3. Close the shunt interlock cover. The machine should make dialysate nonstop but will stop from time to time to cycle.

8. Once the dialysis machine begins to output dialysate open the shunt interlock door to stop the flow.

9. Unscrew the adapter adapter and scrub it with a chlorhexidine swab

10. Aseptically attach the larger opening of the adapter to the effluent port of the dialyzer by screwing it on.

    10.1. Avoid directly touching the effluent port and the inside of the adapter in order to maintain sterility.

    10.2. Avoid moving the dialyzer to prevent a TMP error.

11. Aseptically attach the collection bag to the smaller end of the adapter by screwing it on. We use the ExactaMix 5000 mL TPN bag. If you intend to use a different bag, it may not be compatible with the adapter. For help solving this problem, please email us with the bag you would like to use and we will help you modify the adapter.

11.1. Avoid touching the threads on the bag's fill port and the inside of the adapter.

11.2. Make sure that the connection between the TPN bag screw and the adapter is secure, the flange of the TPN bag screw should sit flush with the adapter's flat surface with no room for further travel.

11.3. Avoid moving the dialyzer to prevent a TMP error.

12. Close the shunt interlock door to resume flowing dialysate, thus filling the bag. It is advised to place the bag on a table or chair to support the bag's weight as it fills.

13. Confirm that the bag is filling and monitor for leaks around the adapter.

14. Once the bag is full, stop the dialysate flow by opening the shunt interlock door, then clamp the bag's tubing. This clamp will help maintain the sterility of the dialysate as it is being removed from the adapter.

15. Unscrew the full dialysate bag from the adapter to remove it and then put the original cap back on the dialysate bag's screw.

16. While the adapter is still attached to the dialyzer, use a chlorhexidine swab to scrub both the inside and outside of the adapter.

17. To fill more bags, repeat steps 11-15 with a new bag. Repeated use of the same adapter may result in a loss of sterility. Remember to scrub the adapter with chlorhexidine prior to connecting a new bag.

**S1.2 Preparing the Connector**

The connector is cylindrical with female threads on both ends, with one end that is compatible with the effluent port of the Dialyzer and another that is compatible with the Inlet port of the collection bag. The connectors are designed such that they can be easily printed on desktop 3D

printers with materials that can sustain multiple cycles of common sterilization methods like EtO sterilization (please refer Supplementary Section S1.1). The most up-to-date CAD drawings, STL files and printing instructions for the connector can be obtained at the link and description shared in the supplementary document (Section S1.1).

**S1.3 Laboratory Assessments**

At the beginning of our experiment, we used the above procedure to collect two full bags of dialysate in the ExactaMix TPN bags. One was stored in a 4°C refrigerator and the other was stored at room temperature. The following is the testing procedure used.

1. Two samples were received (room temperature and 4°C) at two-hour intervals over 12 hours.

2. Total of six room temperature and six 4°C samples were received.

3. All fluids were centrifuged for 5 minutes at 3,000 RPM before plating.

4. The supernatant was removed leaving approximately 0.25 mL to re-suspend the sediment/pellet prior to inoculation.

5. Using a sterile, individually wrapped pipette, one drop of the fluid was plated to blood agar and chocolate agar plates for the aerobic culture and one drop to a brucella blood agar plate for the anaerobic culture. Then, plates were streaked for isolation.

6. Aerobic plates were incubated in a CO2 incubator at 37°C ± 2°C and anaerobic plates were incubated in anaerobic condition at 37°C ± 2°C.

7. Aerobic plates were checked at 24 and 48 hrs.

8. Anaerobic plates were checked at 48 and 96hrs.

The results of the culture tests showed that there was no growth on any of the plates throughout the experiment, suggesting that the bagged dialysate fluid was safe for use. Produced dialysate was stored at room temperature or refrigerated over approximately 24 hours. Laboratory measurements of calcium, chloride, bicarbonate, glucose, potassium, lactate, magnesium, and sodium were performed on a Roche cobas® 8000 modular instrument within the Core Laboratories of the Johns Hopkins Hospital.

**S2. 3D-printing**

The files for the connector can be downloaded at the following link:

http://bit.ly/3pKlT78

Please note that this is not an FDA approved medical device and the files should not be used for any clinical tests or use without the appropriate permissions.

**S2.1. Print Settings**

The test pieces were printed using an Ultimaker 3 Extended printer with Ultimaker branded Transparent Polycarbonate (PC) filament using Cura Version 4.5.0 software. Following key settings were used to print the adapters:

1. Layer height: 0.15mm

2. Infill density and pattern: 50% (Cubic)

3. Printing Temperature: 280 C

4. Build Plate Temperature: 107 C

5. Generate support: Unchecked

6. Enable prime blob: Unchecked

A clean buildplate was used with no additional material to support adhesion, the adapter was printed with the, smaller opening (for the TPN bag) as the bottom face. Default settings were used for all other parameters.

**S3. Performance under Sterilization and Leak Test**

The custom 3D printed adapter, printed on a commercial Ultimaker 3D printer using off-the-shelf Ultimaker branded Polycarbonate (PC) Filament, maintained dimensional stability for at least seven consecutive EtO Sterilizaiton cycles. The dimensions of both ends of the adapter before and after sterilization can be seen in Table S1.

**Table S1. Dimensions of the connector before and after sterilization for the Polycarbonate (PC) connector**

| PC Sample # | Type | Number of Cycles | Before Sterilization | | | | After Sterilization | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Inner Diameter (mm) [bag end] | Inner Diameter (mm) [dialyzer end] | Height (mm) | Fit | Inner Diameter (mm) [bag end] | Inner Diameter (mm) [dialyzer end] | Height (mm) | Fit |
| 1 | EtO | 1 | 10.57 | 15.01 | 31.54 | Yes | 10.56 | 15.03 | 31.53 | Yes |
| 2 | EtO | 1 | 10.58 | 15 | 31.53 | Yes | 10.55 | 15.01 | 31.55 | Yes |
| 3 | EtO | 3 | 10.59 | 15.03 | 31.55 | Yes | 10.56 | 15.04 | 31.53 | Yes |
| 4 | EtO | 3 | 10.6 | 14.91 | 31.52 | Yes | 10.6 | 14.94 | 31.55 | Yes |
| 5 | EtO | 7 | 10.61 | 14.97 | 31.53 | Yes | 10.54 | 15.01 | 31.52 | Yes |
| 6 | EtO | 7 | 10.59 | 14.94 | 31.51 | Yes | 10.59 | 15.02 | 31.53 | Yes |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 7 | Steam | 1 | 10.59 | 14.99 | 31.56 | Yes | NA | NA | 32.31 | No |
| 8 | Steam | 1 | 10.62 | 14.93 | 31.54 | Yes | NA | NA | 32.33 | No |

A special test version of the connector was printed with slots to check for any fluid leakage. Microscopic observation of the Dialyzer-TPN bag interface inside the adapter revealed no leakage or fluid contact with the adapter walls (see Fig.S1). No change was found on chromatography strips placed underneath the interface, indicating that the fluid passes directly from the dialyzer into the TPN bag without making contact with the adapter.

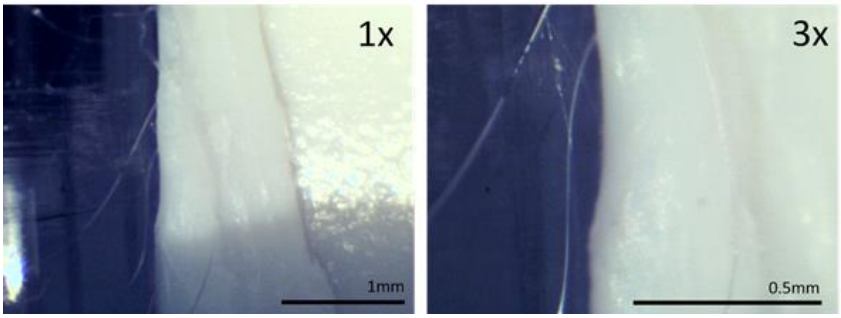


Fig.S1. Dialyzer connection (translucent, right)-TPN bag's screw (white, left). The connector is not pictured, the image is taken through the special slots of the test connector.