2018/05/18 Experiment 235.1 – RACE Human Sepsis Serum Ultrafiltration after MeOH Precipitation

Supplies:

- D_2O (Deuterium Oxide)
- Micropipetter and repeatting pipetters
- 2.0-mL red-top cryovials (labeled)
- Ultrapure (Millipore) water
- Pall Nanosep 3K Omega Centrifugal Devices (Filters) (Fisher 0D003C34)
- Cryotubes (Fisher 02-912-729 or similar)

Procedure:

1. Turn on the centrifuge and set the temperature to 4 oC. Have the lid closed so that the temperature can go down.
   Note: when prepping filters, it is fine to start the centrifuge before it reaches temperature, but when actually filtering samples, the centrifuge should be at 4C
2. With a repeating pipette, pipet 500uL of ultrapure (Milli-Q) water onto each filter.
3. Close the cap of the filter. Place the filters in the centrifuge so that each filter faces across from another filter. This is to balance the centrifuge.
   a. Ex. If there are 12 filters, place 6 filters on one side and the other 6 on the positions across from them.
   b. Close the lid of the centrifuge. Run the centrifuge at 14.000g for 4 mins at 4oC.
4. Discard filtrate, which contains glycerine/glycerol trapped on the filters.
   Wash 4 more times with ultrapure water (total of 5 water rinses).
   After the fifth water rinse, discard all filtrate and remove excess water from the top and bottom of the filter.
5. Next, there will be 2 washes with D2O. After each rinse remove filtrate and discard.
   The filters can be stored the 4 oC until use, but for no longer than 1 month. **Make sure there is D_2O on top of filters before storage so they do not dry out during storage.**
   If you are using filters the same day they are prepped, rinse once more with D_2O before use.
6. Before using stored filters rinse once more with D_2O before use. **Remove D_2O from the top of the filter before use!**
7. Add resuspended samples to the filter with a micropipetter.
   a. Add no more than 500uL of the resuspension to the filter.
   b. **Record this volume**. This is the pre-filter volume.
8. Spin each sample at 14,000xg at 4oC for 20 minutes.
9. After the spin, remove the filtrate from the bottom of each filter and transfer each sample to a clean, labeled cryovial (red top for serum).
10. To the top of each filter add 50uL of D_2O.
    a. There is a vortex with a big blue pad with holes to fit the filters in. **Be very careful using this vortex unless caps are tightly sealed!** If you're working with a partner, it's fine to just give samples a quick pop on a regular vortex.

b. Turn the vortex off, place the filters in the blue pad, then turn the vortex to "Auto" so that it turns on only when you press on it.

Sample ID	Pre-filter vol (µL)	Post filter vol (µL)	D_2O added (µL)	Internal Std added (µL)	Total final vol (µL)
R.001					
R.002					
R.003					
R.004					
R.005					
R.006					
R.007					
R.008					
R.009					
R.010					
R.011					
R.012					
R.013					
R.014					
R.015					
R.016					
R.017					
R.018					
R.019					
R.020					
R.021					
R.022					
R.023					
R.024					

c. Place your hand over the filters so that your hand generally keeps the filters in the blue pad.
d. Vortex for about 10 seconds (be sure to hold caps shut during vortexing).

11. Take the filters out of the vortex and centrifuge again at 14,000xg at 4oC for 25 minutes.
12. Discard filters and transfer the filtrates to the corresponding labeled cryovials.
13. Measure and **record the total filtrate volume**. This is the post-filter volume.
14. Add D_2O to samples so that the final volume is 500 uL.
15. Do not add D2O to samples that are already over the set final volume.
16. To this, add 50uL of **formate internal standard.**
17. **Check the concentration of Ca Formate used in each experiment.**

Actual concentration of formate used: