General notes

➢ The goal of the tank rotation and autoclaving regime is to improve sorter sterility and to prevent biofilm growth at the bottom of the tank. Please do not refill the tank under non-sterile conditions like previously done.

➢ Workflow:

     a. Use tank until software asks for a tank refill. Do NOT refill tank at the instrument as last user of the day.

     b. The user receiving the software prompt to refill sheath fluid disconnects the empty tank, prepares the autoclaved backup tank with sterile PBS and connects it to the sorter.

     c. The old empty tank is emptied completely and autoclaved after removing the sheath level sensor by the same user.

➢ A tank can be exchanged while running the sorter. Simply pause your sort and sample acquisition, switch off the stream, disconnect the empty tank (air & sheath lines + sheath sensor), connect a new tank and switch the stream back on.

Important note: If the stream stabilizes at very similar values for drop one as before, the drop delay calibration is still valid after switching to a new tank. Otherwise rerun drop delay.

I. How to fill and assemble a sterile tank

- An empty autoclaved tank is stored inside the biosafety hood opposite the Aria (check that tape indicates autoclaving). No tank? → Check autoclave in PATHG43

- Remove tank from hood to insert the sheath level sensor, this is best done at the sink.

- Find screw-wrench (size 16) and Teflon tape (on shelf above sink)

- Remove sheath level sensor from 70% EtOH measuring cylinder and wrap screw thread of sensor with ca 2-3 layers of Teflon tape.

- To ensure that the sensor is clean inside (hollow rod), spray 70% EtOH into the inverted sensor until it flows out of the vent holes of the sensor.

- Turn sensor back upright and allow EtOH to flow out. Wipe outside of sensor with EtOH tissue.

- Remove autoclave tape from sensor hole in the tank and quickly insert sensor into tank (mind sterile conditions when inserting the sensor) and gently tighten it with the screw-wrench on the **lowest nut** of the sensor to secure it.
  Don't overtighten! Sensor only has to be secured and sealed for pressure build up in tank.

- Return tank to hood. Turn on hood and open and angle tank lid far enough to insert tubing of PBS bag (spray tubing with 70% EtOH before inserting) and fill tank with sterile PBS inside the hood.
  Note: Rest PBS bag on a chair set to the highest possible position to make refilling easier.
  An empty tank will take a complete 10L PBS bag.

- Close tank lid under sterile conditions.

- Remove tank from hood and connect sheath level sensor plug (arrow mark on plug facing up), sheath line and air pressure line to the tank at the sorter. Check for leaks on lid and sheath level sensor after pressurising the tank.

II. How to autoclave an empty tank

- ➢ Disconnect sheath line, air pressure line and sheath level sensor cable from the tank
- ➢ Depressurise tank!
- ➢ Unscrew sheath level sensor via the bottom nut using the size 16mm screw-wrench (located above the sink).
- ➢ Store sheath level sensor in cylinder with 70% EtOH
- ➢ Open tank lid and discard remaining PBS.
  Wipe bottom of tank with tissue paper and EtOH 70%.
- ➢ The autoclave is in room PATH G43, left corner next to the biosafety cabinet.
  Please note: PATHG43 and the autoclave belong to the Manz haematology lab. Please treat all equipment with the utmost care and respect that you are a guest in this room. In case of any questions or issues regarding the autoclave please contact facility staff or Syndi Uhlig (PATH G37) from the Manz lab directly.
- ➢ Remove all wire baskets from the autoclave:
- ➢ Check that the water level in the autoclave is just below the bottom plate and refill with DI water if necessary.
- ➢ Place tank on the tip boxes used as spacers at the bottom of the autoclave.
- ➢ Close lid of autoclave and slide door lock to closed position.

- ➢ Switch on auotclave.
- ➢ Press "Mode" to select the "Solid" option.
- ➢ Press "Start"