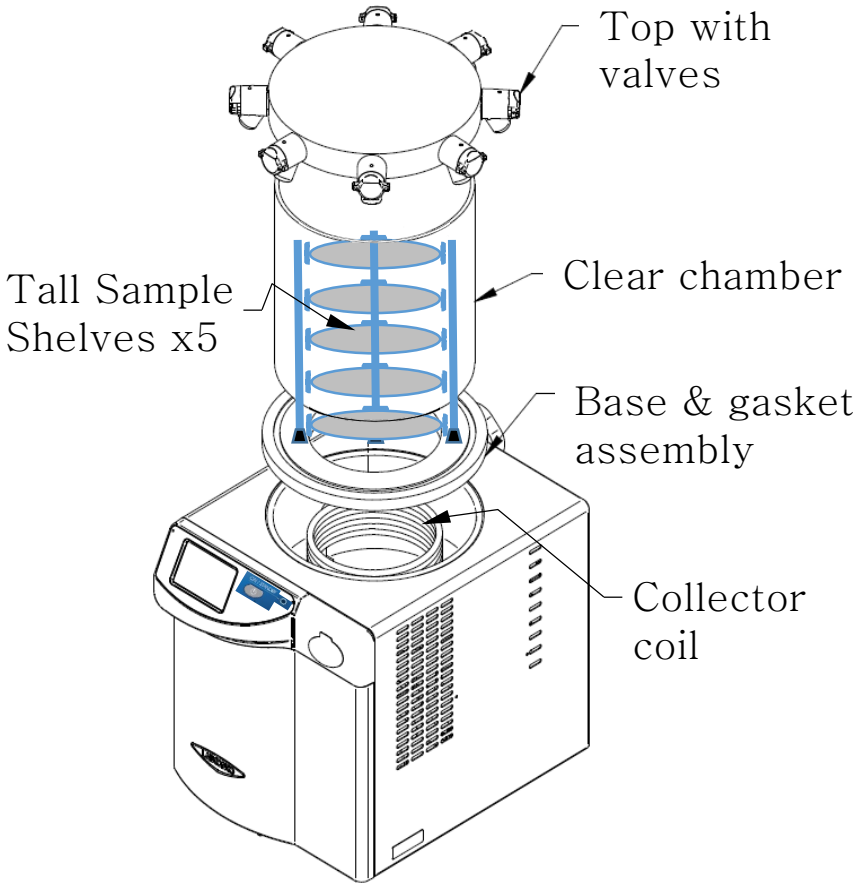
B20 Labconco FreeZone 4.5L Freeze Dryer XL, Training Protocol 2021

User Responsibilities:

- Do not use unless you have been specifically trained by CEST staff.
- For this freeze dryer, any user with samples drying in the **chamber** chooses the timing of system shut down.
- This model is only for "aqueous" samples and will be damaged by exposure to volatile solvents, acids, buffers.
- Users may not add more than 2.25L (2250g) of water to the system or the coil could be damaged.
  - *A 50mL centrifuge tube with 25g of tissue (~80% moisture content) would add 20g of water, so ~113 similar tubes would be max capacity during a single drying event.*
- Users will only utilize the defrost cycle to thaw the coil.

Daily check:

- ❑ Verify the interior of the **collector** is dry & clean; wipe down with paper towels to remove as much moisture from the coil as possible before use.
- ❑ Check that the **base/gasket** is clean and free of dust, debris and grease*.
- ❑ Ensure all sample valves (=ports) on the **top** are closed (notch up for Vent position).
- ❑ After usage and a hot gas defrost cycle, the drain hose must be disconnected *before* next use.
  - *Operation with hose left attached will not achieve consistent vacuum.*



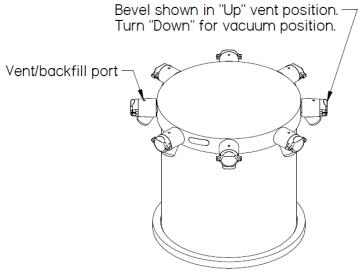
*NO VACUUM GREASE IS NEEDED FOR OPERATION, *please don't make us throw something at you*.

Operation:

- 1st steps:
  1. The **base & gasket** has two different sides, only the side with 2 red rings will catch on the **collector** chamber; mount this side, face-down.
  2. Load pre-frozen samples (ENTIRELY frozen, no liquid moisture) onto **sample shelves**, remove a shelf as needed to accommodate vial/tube height; set frame carefully on clear area of base.
  3. Place **clear chamber** over sample shelves *using caution*; this should settle onto the lone red gasket.
  4. Lift **top** with external valves (HEAVY!) and place onto the clear chamber, this will fit centered into the red gasket track; verify all external valves are closed!
  5. The system will already be in Standby mode, touch the screen or push the ON/STANDBY button.

- Home screen start-up:
  1. Press COLLECTOR>Start to turn on the refrigeration.
     - COLLECTOR will start immediately; temp values will turn **red** ---> **green** when they reach ≤-40° C; this could take ~8min. Proceed when temp values are **ready**.
  2. Press VACUUM>Enter Set Point (**0.133**)>Apply
     - Pressure values will begin at **5.000** mbar and take several minutes to go lower, also with a **red** ---> **green** transition, when **ready**.
  3. As on the older system, we're looking for the VACUUM to reach a "magic number," **0.133 mbar** or lower; given the size of the **chamber** this takes time.
  4. With both the COLLECTOR and VACUUM values in **green**, samples will begin the freeze drying process.
     - *Because you cannot touch an internal sample flask to determine when samples are dried, rely on the change in visual appearance of tubes/vials and your pre-planned duration.*

- Adding Samples to external valves:
  - As on older model, only add pre-frozen samples to a freeze flask (2-3X volume of samples); connect to a closed valve via SS adapter, with paper filter installed.
  - Turn knob slowly (using two hands) until notch points down, to Vacuum position. Wait until system returns to ≤ **0.133mbar** before adding additional flasks.



Shutdown:

1. On the home screen, press VACUUM>Stop to turn off the pump.
2. Slowly vent the **clear chamber** and the **collector**, by opening an unused sample valve on the top (use two hands, SLOWLY!).
   If you vented too fast, you have a mess to clean up, as the **sample shelves** will FLY OFF!
3. Press COLLECTOR>Stop to turn off the refrigeration system.
   - Defrost coil as needed (COLLECTOR>Defrost Options>START).
   - Attach the drain hose at this point, ensure it drains to an open vessel large enough to capture all of the ice melt/sample condensate.
   - The unit will go to STANDBY mode on it's own; no other intervention is needed.
4. Clean up after yourself, rinse any soiled materials, rinse out any freeze flasks or accessories & hang to dry in B19A.
5. Gently place the **clear chamber, sample shelves** and **top** onto a nearby open table.

- Removing Samples advice:
  - External samples should always be removed from the **top** first, after slowly venting the **chamber**.
  - Internal samples should be removed after carefully removing the **top** & **clear chamber**; **sample shelf** users make all operational decisions.