**Goal: Develop a system that enables 19 or fewer vials to be utilized for protocol development.**

**Experimental Group # 1 Water**

**Full tray in REVO Vs 19 Vials in REVO**

**Theory: A small batch will freeze dry faster than a large batch in a lab freeze dryer.**

***First Experimental Set: A full tray in a lab freeze dryer and then run 19 vials in the same dryer using the same cycle (using CM-Pirani convergence to indicate end of primary drying).***

**Run a REVO with a full tray of vials and time**
Experimental Design
3ml fill in a 10 ml vial
Water
Freezing step ramp 0.5C/min to-40C, hold 60 mins
Primary drying shelf set point +20C
Vacuum set point 100 mT

REVO Full Tray
***636 minutes*** in primary drying

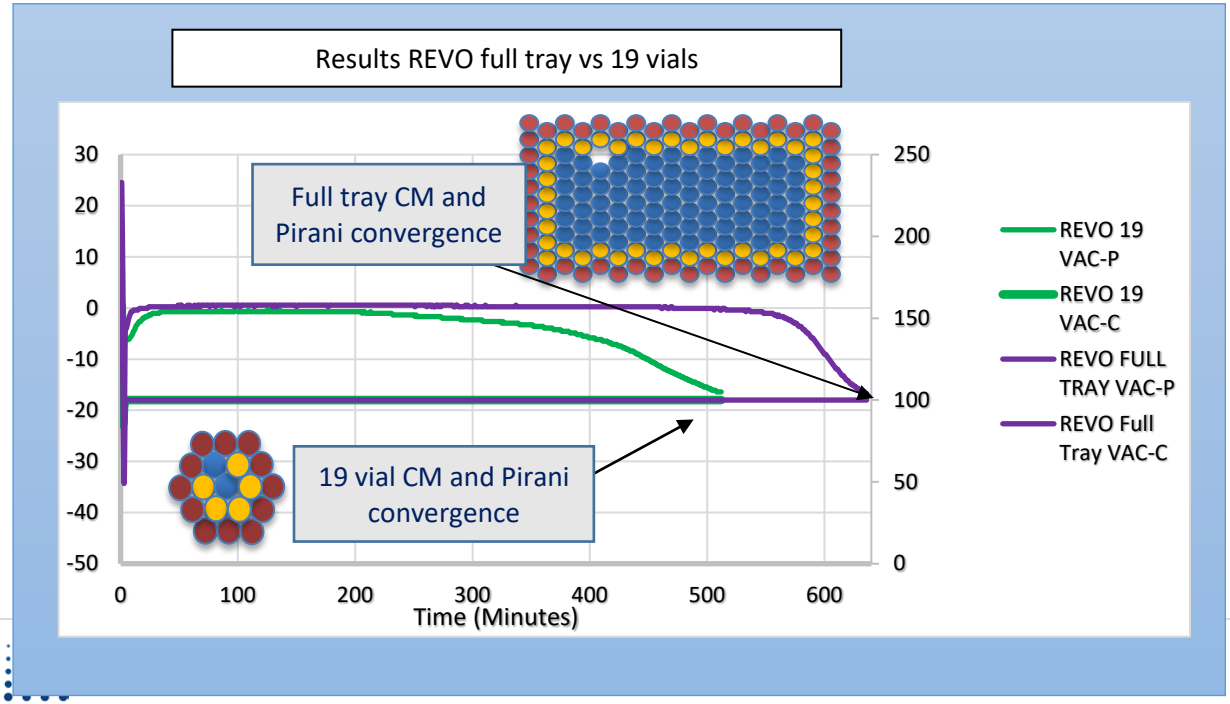
**636 minutes is the target** for all other water runs

**Run a REVO with a 19 vials and time**
Experimental Design
3ml fill in a 10 ml vial
Water
Freezing step ramp 0.5C/min to -40C, hold 60 mins
Primary drying shelf set point +20C
Vacuum set point 100 mT

REVO 19 Vials
***512 minutes*** in primary drying

**Results**

**19 vials dry quicker than a full tray based on end of primary drying being detected by CM vs Pirani convergence**




**Results: 19 Vials Take less time. Why?**
*Assumption: Radiant energy has a significant effect on the freeze drying process via edge vial effect. Smaller batch runs are more susceptible to this effect because a larger percentage of the vials are "edge effect vials".*

MILLROCK TECHNOLOGY

**Experimental Group # 1**

**5% Sucrose**

**Full tray in REVO**

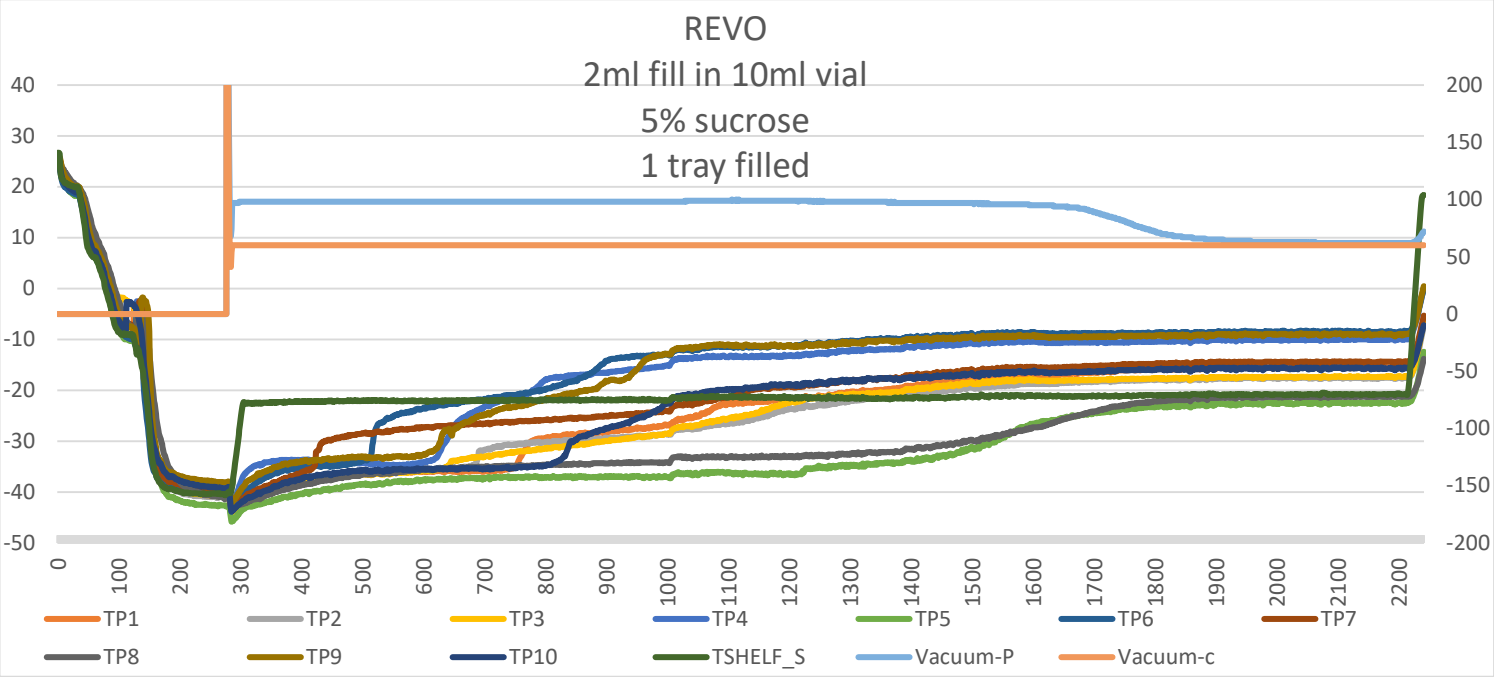
**Run a REVO with a full tray of vials and time**
Experimental Design
2ml fill in a 10 ml vial
5% sucrose by weight
Freezing step -10 hold 30 mins, -40C hold 120 mins
Primary drying shelf set point -20C
Vacuum set point 60 mT

REVO Full Tray
***2026 minutes*** in primary drying

**2026 minutes is the target**
for all other sucrose runs

**Results**

**Full tray took 2026 mins based on end of primary drying being detected by CM vs Pirani convergence**






MILLROCK TECHNOLOGY

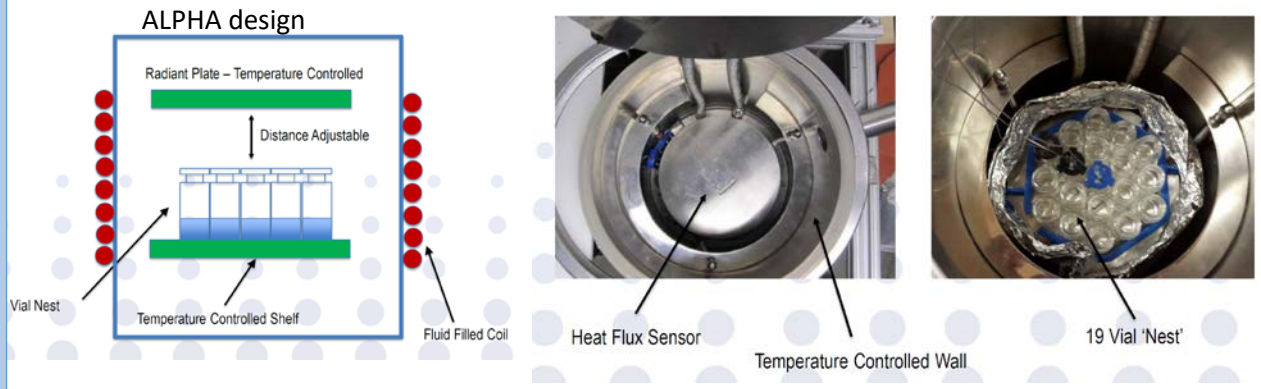
**Experimental Group #2**

**Water**

**Eliminate radiant energy to minimize edge effect vials by cooling and/or shielding the chamber walls of the freeze dryer.**

**Theory: Eliminate radiant energy, and thus edge effect vials, and your small batch runs will be more like the larger batch runs.**

***Second Experimental Set: Eliminate radiant energy from the freeze dryer when using 19 vials. Build a small freeze dryer with the ability to control wall temperature. Note primary drying time.***




Made controlled temperature walls. Also tried adding commercial insulation and aluminum shield to minimize edge effect.

**Run ALPHA with a 19 vials and wall at +20C**
Experimental Design
3ml fill in a 10 ml vial
Water
Freezing step ramp at 0.5C/min to -40C, hold 30 mins
Primary drying shelf set point 20C
Vacuum set point 100 mT

ALPHA with +20C Walls ***532 minutes*** in primary drying. (reminder: target is 636 mins)

**Run ALPHA with a 19 vials and wall at -20C**
Experimental Design
3ml fill in a 10 ml vial
Water
Freezing step ramp at 0.5c/min to -40C, hold 30 mins
Primary drying shelf set point 20C
Vacuum set point 100 mT

ALPHA with -20C Walls ***557 minutes*** in primary drying (reminder: target is 636 mins)

**Results**

**The minimization of radiant energy does not eliminate edge effect.**

**Experimental Group #2**

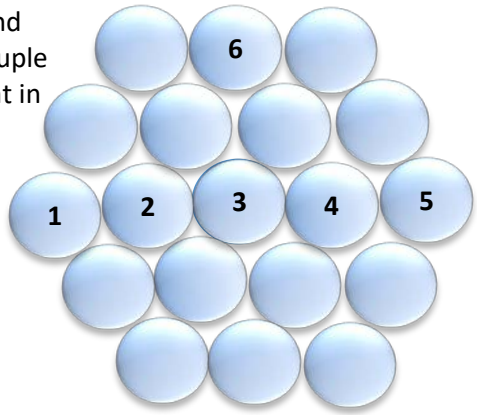
**5% Sucrose**

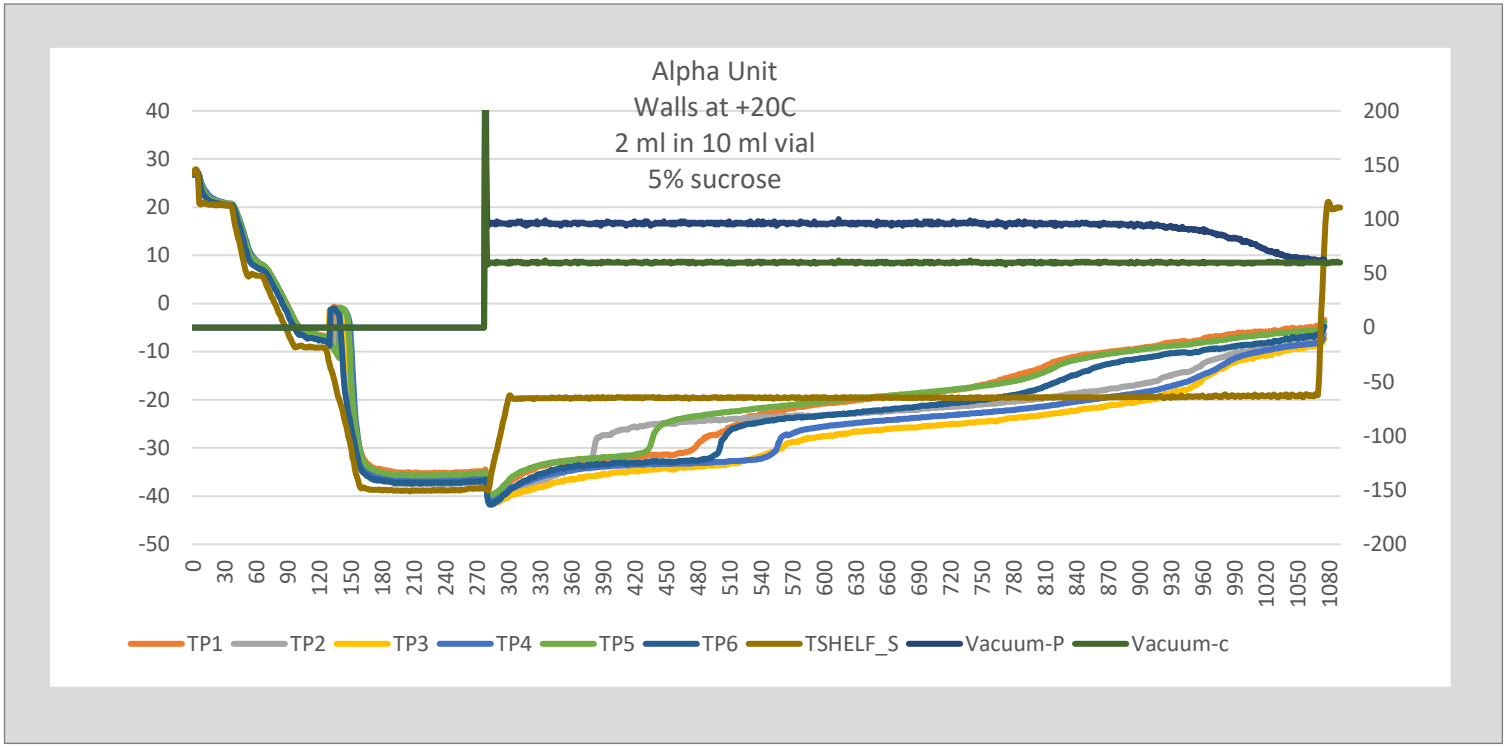
**Eliminate radiant energy to minimize edge effect vials by cooling and/or shielding the chamber walls of the freeze dryer.**

**Run ALPHA with a 19 vials and wall at +20C**
Experimental Design
2ml fill in a 10 ml vial
5% sucrose by weight
Freezing step -10 hold 30 mins, -40C hold 120 mins
Primary drying shelf set point -20C
Vacuum set point 60 mT

ALPHA, wall at +20C
***789 minutes*** in primary drying

Array and thermocouple placement in Alpha




MILLROCK TECHNOLOGY

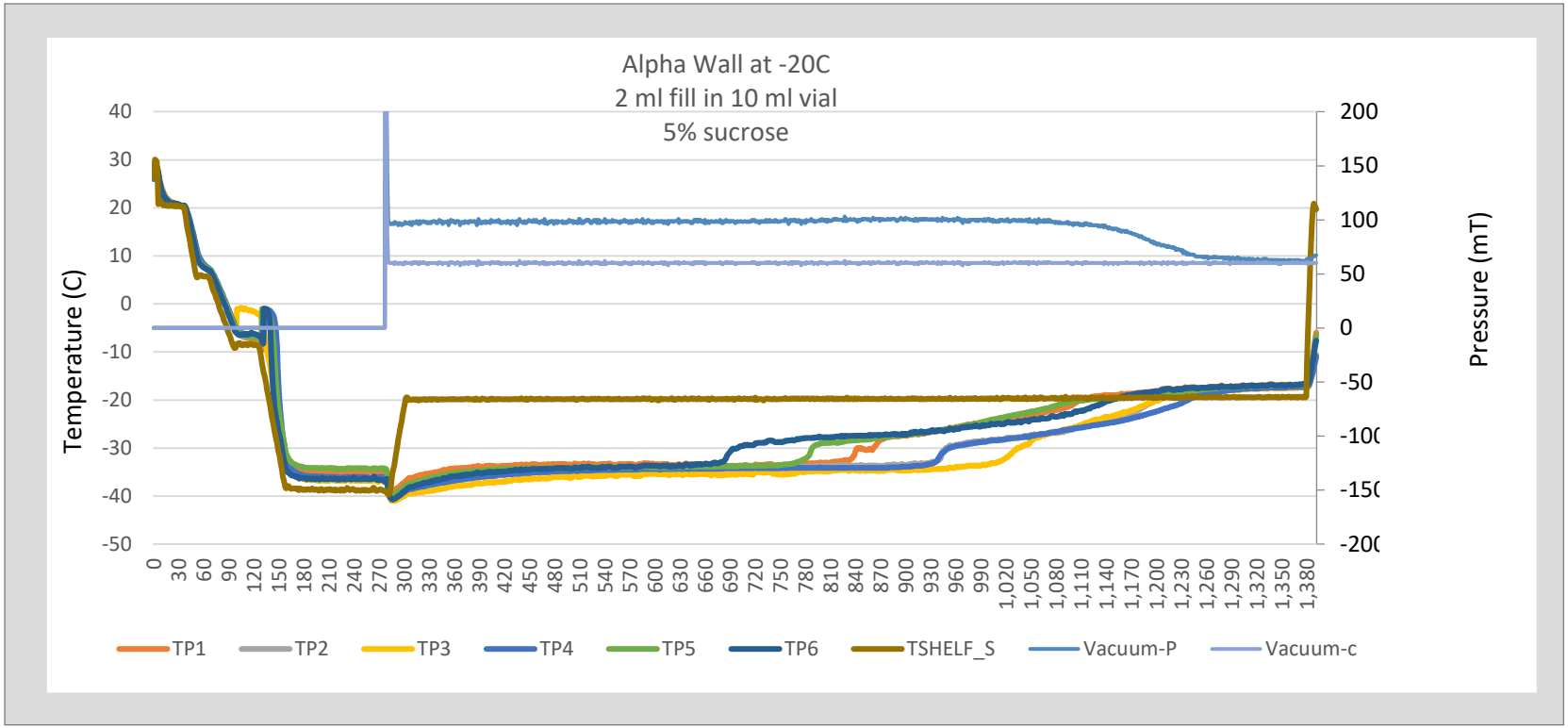
**Experimental Group #2**

**5% Sucrose Continued**

**Run ALPHA with a 19 vials and wall at -20C**
Experimental Design
2ml fill in a 10 ml vial
5% sucrose by weight
Freezing step -10 hold 30 mins, -40C hold 120 mins
Primary drying shelf set point -20C
Vacuum set point 60 mT

ALPHA, wall at -20C
***1096 minutes*** in primary drying

**Results: Cooling the chamber wall to -20C appeared to make some difference but the elimination of radiant energy does not appear to be the answer to simulating a larger freeze dryer. Why?**






MILLROCK TECHNOLOGY

**Experimental Group #3**

**Water**

**Run systems with wall at -40C. Study the rate of sublimation in individual vials in a freeze dryer that has eliminated radiant energy to gain a better perspective on what is happening in the dryer.**

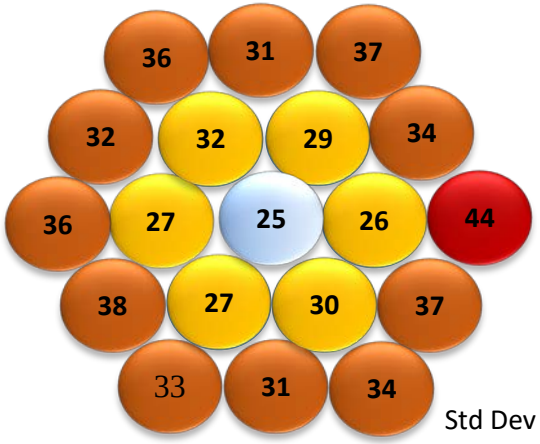
**Results**

**Low temperature chamber walls does not eliminate "edge effect"**

**Theory: Eliminating radiant energy did not appear to eliminate edge effect in the 19 vial array. Taking a closer look at how the individual vials are responding may help us understand this further. The common perception is that when we eliminate radiant energy the edge effect should be eliminated.**

***Third Experimental Set: Utilizing the gravemetric measurement method determine the rate of freeze drying by various vials within the array. Do perimeter vials freeze dry differently than center vials even minimal radiant energy is present?***

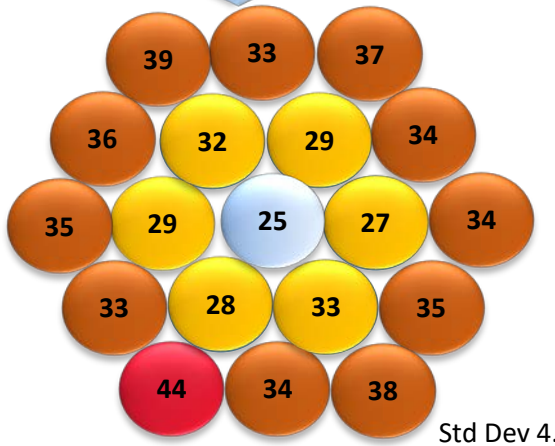
**Run ALPHA with a 19 vials and wall at -40C**
Experimental Design
3ml fill in a 10 ml vial
Water
Freezing step at 0.5 to -40C, hold 30 mins
Primary drying shelf set point +20C
Vacuum set point 100 mT




Std Dev 4.9

% dry by weight 25% of the way into primary drying.

**Run ALPHA with a 19 vials and wall at -40C with aluminum shield and additional insulation. (10+ runs conducted)**
Experimental Design
3ml fill in a 10 ml vial
Water
Freezing step at 0.5 to -40C, hold 30 mins
Primary drying shelf set point +20C
Vacuum set point 100 mT




Std Dev 4.4

**Results: Edge effect NOT eliminated by lowering the temperature of the chamber wall. If the presumption that radiant energy caused edge effect and radiant energy is eliminated, then edge effect should be eliminated. This experiment does not verify this assumption. Gravemetric studies indicate that edge vials are drying faster than the other vials, even when there is minimal radiant energy**

**Experimental Group #3**

**5% Sucrose**

**Run systems with wall at -40C. Study the rate of sublimation in individual vials in a freeze dryer that has eliminated radiant energy to gain a better perspective on what is happening in the dryer.**

**Results**

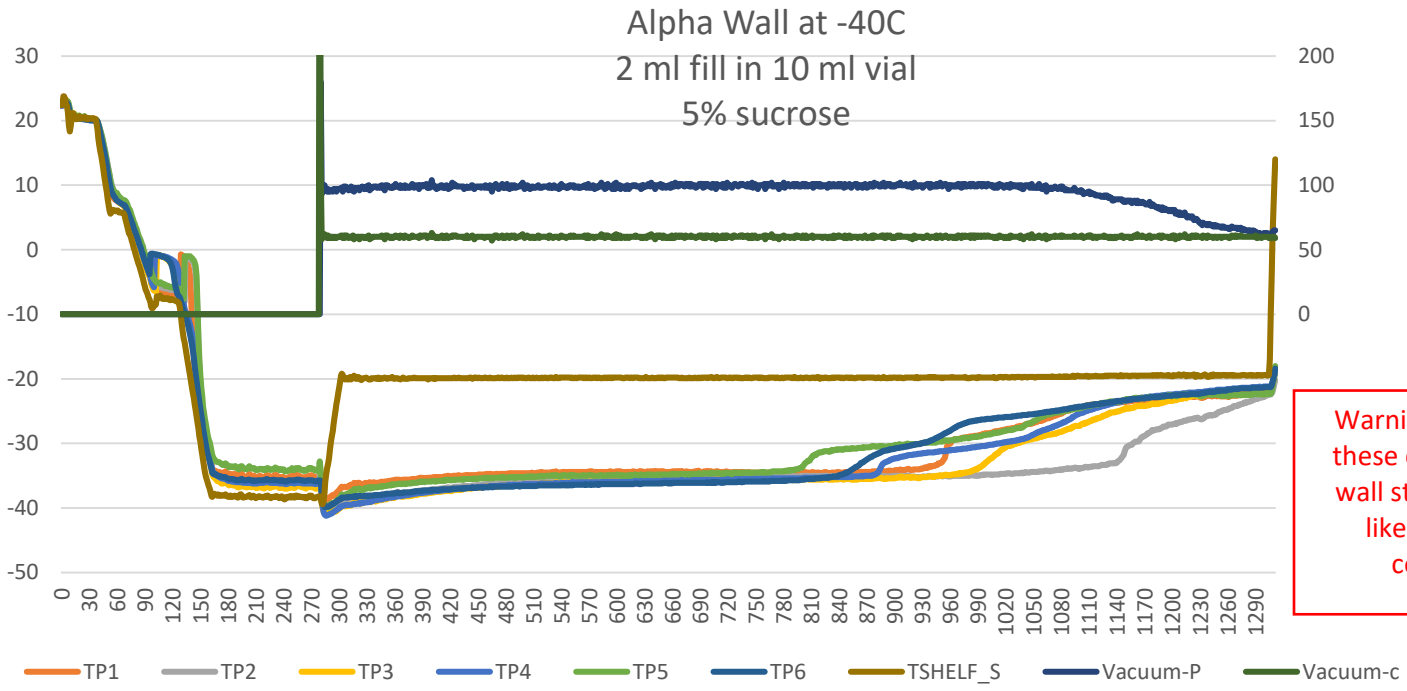
**Low temperature chamber walls does not eliminate "edge effect" as evidence by run time not approaching REVO run time of 2026 mins**

**Run ALPHA with a 19 vials and wall at -40C**
Experimental Design
2ml fill in a 10 ml vial
5% sucrose by weight
Freezing step -10 hold 30 mins, -40C hold 120 mins

Primary drying shelf set point -20C
Vacuum set point 60 mT

Gravemetric tests not yet conducted.

ALPHA, wall at -40C
***1042 minutes*** in primary drying




Warning: At -40C in these conditions the wall starts behaving like an internal condenser



MILLROCK TECHNOLOGY

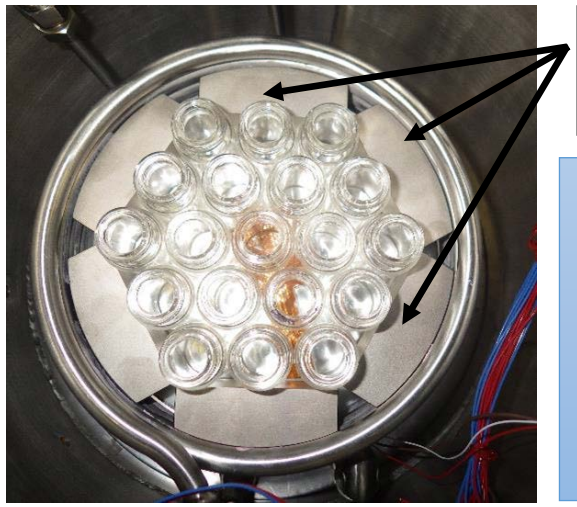
**Experimental Group # 4**

**Water**

**Adjacent vials affect the thermal characteristics of one another. Since edge vials do not have the same number of adjacent vials they sublimate differently. Have the controlled temperature wall come in contact with the vials to eliminate edge effect.**

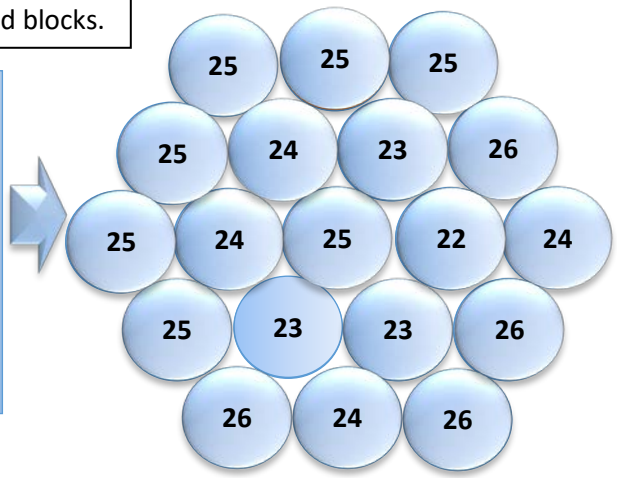
**Theory: The product vials have a thermal effect on one another (essentially acting as cold sinks and hot sinks). Simulate adjacent vials by directly contacting the edge vials and controlling the temperature.**

***Fourth Experimental Set: Find a way to contact the cold temperature walls to the outside edge vials. If "edge effect" is eliminated this indicates that vial to vial contact has a significant effect in "edge effect".***



No longer using the Alpha unit. Designed and build the MicroFD with "thermal simulator" temperature controlled blocks.

**Run MicroFD with a 19 vials and wall at -40C**
Experimental Design
3ml fill in a 10 ml vial
Water
Freezing step ramp 0.5 to -40C, hold 30 mins
Primary drying shelf set point +20C
Vacuum set point 100 mT




Std Dev 4.4

MicroFD with simulator blocks (aka LyoSim™)
***633 minutes*** in primary drying
ONLY 3 MINUTES DIFFERENT THAN THE FULL TRAY RUN DONE IN THE REVO

**EDGE EFFECT ELIMINATED**
**ALL VIALS NOW BEHAVE LIKE CENTER VIALS**
**The use of thermal simulator blocks in the MicroFD created an environment where the sublimation rate was very similar (essentially the same) in every vial. The elimination of edge effect indicates the MicroFD can be utilized for protocol development for larger freeze dryers based on its ability to simulate center vials.**

**Summary**

**Water**

| *Experiment #* | *Product* | *Conditions (see experiments for details)* | *Time* | *Vial variation in sublimation rate* |
|---|---|---|---|---|
| 1 | Water | REVO full tray | 636 mins | Not studied |
| 1 | Water | REVO 19 vials | 512 mins | High disparity, data not reported here |
| 2 | Water | Alpha 19 vials +20C walls | 532 min | High disparity, data not reported here |
| 2 | Water | Alpha 19 vials -20C walls | 557 mins | High disparity, data not reported here |
| 3 | Water | Alpha 19 with -40C wall | Not timed | High disparity, edge effect seen (see pg 3) |
| 3 | Water | Alpha 19 vials with -40C wall and additional radiant energy shielding | Not timed | High disparity, edge effect seen (see pg 3) |
| 4 | Water | MicroFD with LyoSim (thermal sink emulators touching vials) | 633 mins (successfully simulates REVO run with full tray | Variation in vials minimized, edge effect eliminated (see pg 4) |

***(see specific experiments for additional details)***

*Patent # 9121637 Accuflux Heat flux sensor in a freeze dryer*
*LyoSim Patent Pending*

**Summary**

**5% Sucrose**

| *Experiment #* | *Product* | *Conditions (see experiments for details)* | *Time* | *Vial variation in sublimation rate* |
|---|---|---|---|---|
| 1 | 5% Sucrose | REVO full tray | 2026 mins | Not yet studied |
| 2 | 5% Sucrose | Alpha 19 vials +20C walls | 789 mins | Not yet studied |
| 2 | 5% Sucrose | Alpha 19 vials -20C walls | 1096 mins | Not yet studied |
| 3 | 5% Sucrose | Alpha 19 with -40C wall | 1042 mins | Not yet studied |
| 4 | 5% Sucrose | MicroFD with LyoSim (thermal sink emulators touching vials) | Not yet studied | Not yet studied |

Product Experiments with customer

***Additional & Subsequent Studies.***

**Big Pharma Collaboration**: As part of our on-going work we conducted a blind study with a large pharma group. They created a cycle in a SP Scientific LyoStar. They supplied us with vials and product that had a critical temperature of -24C. Within 3 runs we were able to fine tune the Micro FD to simulate their LyoStar freeze dryer run to their satisfaction. They have subsequently purchased a unit for further study.