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Operation of the Millrock Quanta Series Freeze Dryer PC/PLC: Production of Freeze Dried Standard Reference and Control Materials (SRMs/CMs) at the NIST Reference Material Production Facility

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Production of Freeze Dried Standard Reference and Control Materials (SRMs/CMs) at the NIST Reference Material Production Facility

The National Institute of Standards and Technology (NIST) maintains a Reference Material Production (RMP) Facility at the Hollings Marine Laboratory (HML) in Charleston, South Carolina. The RMP Facility is co-housed with NIST's Marine Environmental Specimen Bank (Marine ESB), a cryogenic facility that stores biological and environmental specimens collected as part of monitoring and research programs supported by several government agencies, such as the National Oceanic and Atmospheric Administration (NOAA), US Geological Survey (USGS), and US Fish and Wildlife Service (USFWS), as well as non-profit and academic organizations.

Co-locating the RMP Facility and Marine ESB within one complex allows for efficient use of the cryogen delivery system and ISO certified clean air room conditions required by both facilities.

The RMP Facility - Marine ESB complex consists of: the 73.2 m² (788 sq. ft.) ISO Class 5 Clean Room, the 79.4 m² (855 sq. ft.) ISO Class 7 RMP Facility, the 146.3 m² (1,575 sq. ft.) ISO Class 7 cryogenic specimen storage room, two ISO Class 6 ante rooms for entry into the complex, as well as an associated 46.8 m² (504 sq. ft.) office space. Specimen banking and clean room protocols for the Marine ESB are described in the NIST Internal Report, **NISTIR 7389** (Pugh et al., 2007)¹.

Reference material production activities conducted at the facility involves the development, certification and dissemination of certified reference materials for various analytical analyses. The RMP Facility has been equipped to produce large quantities of frozen and freeze dried biological and environmental materials. Fresh frozen SRM/CMs are produced using a Palla VM-KT Vibrating Cryomill while lyophilized (freeze-dried) materials are produced using a Millrock Quanta Series Freeze Dryer PC/PLC. The protocol for the production of lyophilized material is described in detail herein.

Equipment

A Millrock Quanta Series Freeze Dryer PC/PLC (Figures 1 and 2) is maintained in the NIST RMP Facility for preparing freeze-dried materials. The freeze dryer temperatures and pressures are controlled using the Opti-Dry software program (Figure 3).



Figure 1. Millrock Quanta Series Freeze Dryer Product Chamber



Figure 2. Vacuum Pumps and Condenser

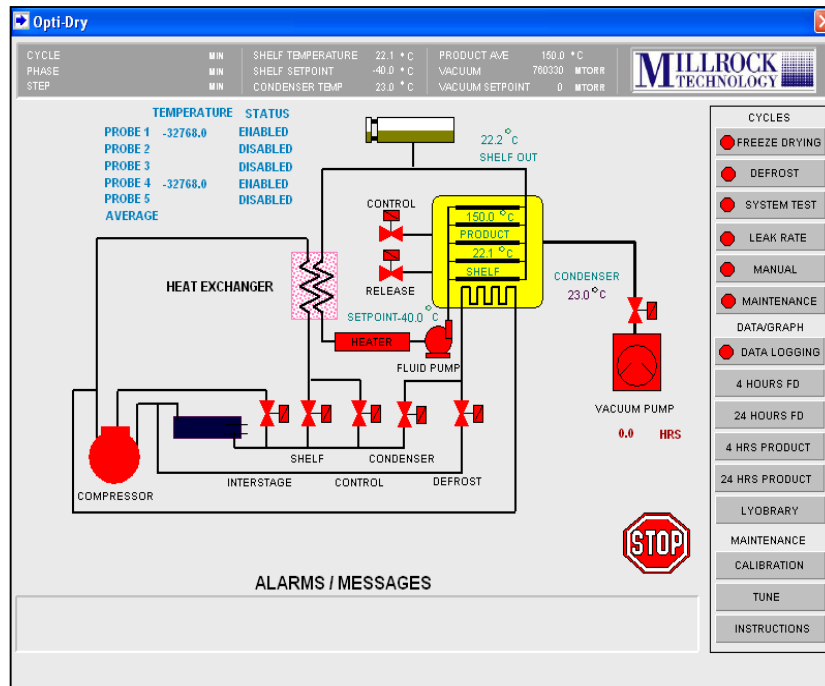


Figure 3. Opti Dry Main Screen

Lyophilization using Opti-Dry Software

The purpose of lyophilization is to freeze moisture in a material then slowly warm the material under a controlled vacuum. The moisture content within the sample is removed through sublimation. Sublimation is a process where the frozen moisture in the product vaporizes without passing through the liquid phase. This allows the chemical integrity of the sample to be maintained during the moisture removal process. Lyophilization using the Millrock Quanta Series Freeze Dryer Opti-Dry software program has three cycles; a Freeze Cycle, a Primary Drying Cycle, and a Secondary Drying Cycle. The Freeze Cycle is used to freeze the product at a controlled rate under a vacuum set by the operator. The Primary Drying Cycle operates as the drying step within a controlled vacuum and temperature set-points, and is where sublimation occurs. In the Primary Cycle, the pressure is decreased and the temperature is slowly raised. The Secondary Drying Cycle is the final step used to complete the freeze drying process at a controlled temperature and vacuum. The Secondary Cycle allows the ionically-bound water molecules to be removed from the material. The temperature in the Secondary Cycle is raised higher than in the Primary Cycle and the bonds are broken between the water molecules and the material.³ These three steps, the Freeze Cycle, the Primary Cycle, and the Secondary Cycle, combine to complete a “recipe.” Each material is freeze dried according to specific parameters in the recipe created by the operator. As shown in the recipe in Figure 4, the temperature and pressure are manipulated and controlled to achieve a freeze dried product. A recipe is created based on the material weight, moisture content, and biological composition. Additionally, alarm conditions are also set to prevent overheating and loss of product.

Opti-Dry

CYCLE PHASE STEP MIN MIN MIN SHELF TEMPERATURE 22.1 °C PRODUCT AVE 150.0 °C
SHELF SETPOINT -40.0 °C VACUUM 760330 mTorr
CONDENSER TEMP 23.0 °C VACUUM SETPOINT 0 mTorr

PRODUCT NAME Enter Product Name
PRODUCT # Enter Product Number
OPERATOR Enter Operator Name

FREEZE DRYING

RECIP Water-Nov 2011.rcp

FREEZE

	1	2	3	4	5	6	7	8	9	10
SHELF SETPT °C	-10	-15	-20	-25	-30	-35	-40	-45	0	0
TIME MIN	10	20	30	40	50	60	70	80	0	0
FINAL FREEZE °C	-50									
EXTRA FREEZE MIN	60									
PRI VAC STARTMT	100									

NEW RECIPE
EXISTING
DELETE
SAVE

PRIMARY

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
SHELF SETPT °C	-40	-35	-30	-25	-20	-15	-10	-5	0	5	10	15	20	25	30	35
TIME MIN	0	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
VACUUM SETPT MT	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260

SECONDARY

SHELF SETPT °C	20
TIME MIN	30
VACUUM SETPT MT	100
FINAL SETPT °C	25

ALARMS

COND OVERLOAD °C	-40
VACUUM OVERLOAD MT	500
POWER OUTAGE MIN	10

START

CYCLES

- ☒ FREEZE DRYING
- ☐ DEFROST
- ☐ SYSTEM TEST
- ☐ LEAK RATE
- ☐ MANUAL
- ☐ MAINTENANCE
- DATA/GRAPH**
 - ☒ DATA LOGGING
 - 4 HOURS FD
 - 24 HOURS FD
 - 4 HRS PRODUCT
 - 24 HRS PRODUCT
- LYOBINARY
- WINDOW
- PRINT
- INSTRUCTIONS
- MAIN

Figure 4. Opti Dry Example Recipe

Freeze Cycle

The Freeze Cycle is a 10 step process used to freeze the material in the product chamber under controlled conditions set by the operator in the recipe. During the Freeze Cycle, the product chamber is maintained under a constant vacuum generated manually after placing the freeze dryer in the “operating state”, as described in further detail within the Operation Guide below. In the representative recipe shown in Figure 4, the product has an initial shelf set-point of -10 °C, which is established by the operator within the Freeze section of Opti-Dry program. The condenser will cool the shelf to -10 °C and hold it at this temperature for 10 minutes. After satisfying each set point in Step 1, the freeze dryer will move to Step 2. The condenser will cool the shelves until all of the shelf set-points in steps 1 through 10 of the Freeze Cycle have been satisfied. The freeze dryer then moves to Final Freeze step. In the Final Freeze step in Figure 4, the shelves will hold a temperature of -50 °C for 60 minutes under a 13.33 Pa (100 mTorr) vacuum. After satisfying this step, the vacuum will begin to achieve the Primary Vacuum Start set-point. In example recipe, Figure 4, the Primary Vacuum Start is 13.33 Pa (100 mTorr). The Freeze Cycle can be an optional step if the product has previously been frozen and is placed into the freeze dryer in a frozen state. If a freeze step is not required, “0” is entered in all of the TIME fields. Once all set-points of the Freeze Cycle have been satisfied, the freeze dryer begins the Primary Cycle.

Primary Cycle

The Primary Cycle is a multi-step shelf temperature and vacuum controlled program. In the Primary Cycle, the product must not be raised higher than its eutectic (melting) point. Each step of the Primary Cycle will be satisfied before moving ahead to the next step. For example, in Step

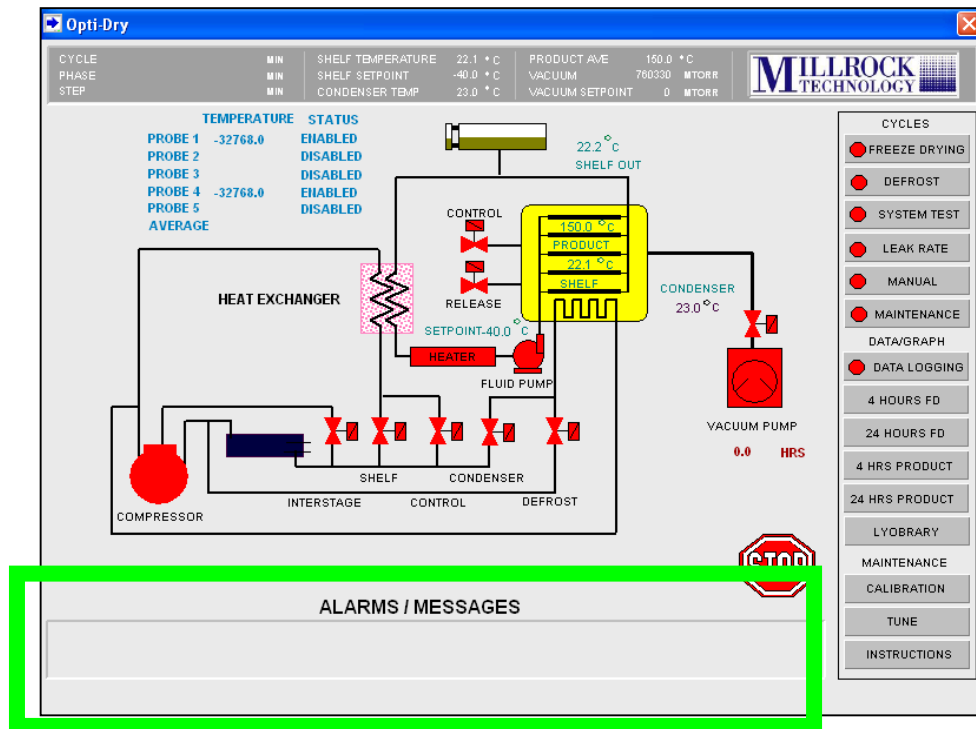
2 of the Primary Cycle, the material was warmed to -35 °C and held at this temperature for 10 minutes under a vacuum of 120 mTorr. Once all set-points in Step 2 are satisfied, the freeze dryer moves to Step 3. If all 16 steps of the Primary cycle are not needed or necessary, “0” is entered into the fields that are unnecessary. After completing all of the steps in the Primary Cycle, the Secondary Cycle begins.

Secondary Cycle

The Secondary Cycle is the final step in the freeze drying process that provides a controlled shelf temperature and vacuum. It is the final step used to eliminate any moisture that remains in the material. In the Secondary Cycle (Figure 4) the product will hold at 20 °C for 30 minutes under a vacuum of 100 mTorr. After the set-points have been satisfied, the shelves will move as fast as possible to the final shelf set-point. In this example the final shelf point is 25 °C. It is recommended that the final set-point of the Secondary cycle be warmer than the initial Secondary shelf set point. An increased final shelf set point ensures that any water molecules that remain in the material will be eliminated. This step may also be called the desorption step.

Alarm Conditions

Alarm conditions are defined in the user’s recipe prior to beginning a freeze dry cycle. There are three alarms that are user defined: the condenser alarm, vacuum overload, and power outage. If an alarm is triggered during the freeze drying process, details about the alarm are displayed on the Main Opti Dry screen in the “Alarms/Messages” box (Figure 5). If the condenser warms above the set temperature, the system will go into alarm and all components will shut down until the condenser has recovered. If the freeze dryer is in the Primary Cycle and the condenser fails to recover after 30 minutes, the system will return to the final freeze step of the Freeze Cycle. The vacuum overload alarm indicates when the vacuum within the system has risen above the defined vacuum limit set by the user. If the vacuum in the chamber starts to lose suction, the vacuum system will try to recover and restore the vacuum. If after a vacuum alarm occurs in the Primary Cycle and the vacuum fails to recover after 30 minutes, the system will return to the final freeze step of the Freeze Cycle. The power outage alarm notifies the user when the allotted time for which the system may be without power is exceeded, at which time the system will return to the final freeze step. Power outage problems at our RMP Facility have been minimized by connecting the Millrock Freeze Dryer to the HML building back-up generator and the Millrock operations computer to an Uninterruptible Power System (UPS), see details below.



Uninterruptible Power System

The Millrock operations computer is connected to an Uninterruptible Power System (UPS) to insure that the computer remains in the “on” position in the event of a power outage (Figure 6). The UPS provides an approximate power time of 47 minutes to the computer in the event of a power outage.



Figure 6. Uninterruptible Power System

Water Temperature

The temperature of the water circulating in the Millrock Freeze Dryer is critical to the operation of the equipment. The normal temperature of the HML source water is $23.3\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$, however during the summer months the water temperature may be higher. Such an increase may cause the freeze dryer to be inoperable as it will not cool down during the freeze cycle. To verify the temperature of the water the HML maintenance staff should be contacted. It is recommended that freeze drying be conducted during the cooler months. In the future, if the workload for the freeze dryer increases, additional water cooling equipment may have to be purchased.

Completion

Once the Secondary Cycle steps have been satisfied, a FINISHED message will flash on the MAIN screen. The freeze dryer will wait for the operator to complete the lyophilization cycle and manually release the vacuum.

The final freeze dried product should be removed immediately, weighed, and stored in an air tight container or desiccator. Upon completion of a freeze drying process, the product will have retained its original form, physical, chemical, and biological properties.

Located below the product chamber is the condensation drain valve that allows the moisture from the temperature coils to drain (Figure 7). This valve must be opened by turning the blue lever perpendicular to the floor, to the ON position.



Figure 7. MillRock Drain Valve

Conversion Factor and Percent Moisture Loss

After freeze drying, a conversion factor and the percent moisture loss is calculated to determine if the material has freeze dried completely. To calculate the conversion factor, the final sample mass is divided by the starting sample mass. The resulting number rounded to the fourth decimal place is the conversion factor. This conversion factor is used to convert wet weight data to dry weight. To calculate the percentage moisture loss, the conversion factor is multiplied by 100 and the resulting number subtracted from 100. This is the percent moisture removed from the sample. The percent moisture loss is used to determine the amount of moisture that was drawn out from a sample. A thoroughly freeze dried sample will have an average of 85 % less moisture than the starting sample, however this can vary depending on sample type.

Operation of the Freeze Dryer located at the RMP

The steps below are a guide to using the MillRock Quanta Series Freeze Dryer located at the RMP Facility:

1. Determine the mass of the material to be freeze dried. This will be necessary to determine the moisture content drawn out from the material. Record the mass of the empty containers, ensuring that the appropriate containers (e.g. stainless steel trays, Teflon jars, etc.) are used and are compatible with the overall goals of the project. Place the material to be freeze dried into the pre-weighed containers and record the mass of the containers with the material to be freeze dried. The mass of the material can be accurately calculated by subtracting the mass of the empty container from the mass of the container plus the material. Figure 8 is an example of a candidate SRM ready to be freeze dried.



Figure 8. SRM Candidate ready for freeze drying

Ensure that water is running from the HML main water supply. Water is circulated to the freeze dryer through the copper pipe attached at the ceiling. If the water is not circulating through the freeze dryer, turn the red valve at the top of the copper pipe to the “ON” position, Figure 9

2. Figure 9. The water circulates to cool the machine during a freeze dry cycle then flows from a waste pipe into the drain in the floor.



Figure 9. HML Main Water Supply ON Position

3. The drain valve underneath the vacuum chamber should be in the “OFF” position during the freeze dry cycle. The handle should be parallel to the floor (Figure 7).
4. Create a recipe using the Opti Dry software (Figure 4). It is recommended to save a hard copy of your recipe before entering the information into the Opti Dry program. In addition, save your recipe in the Opti Dry program before moving on. For steps that are unnecessary in the Freeze, Primary, or Secondary Cycles, enter zero in the appropriate locations. Alarm conditions must also be entered before moving on to the next step.
5. Place the material in the product chamber and plug the temperature monitoring probes into the probe monitoring ports inside the top of the product chamber, Figure 10. Place the temperature monitoring probe(s) into the material or onto the shelf. Depending on the operator’s probe placement, the material or shelf temperature will be displayed on the Opti Dry “MAIN” screen (Figure 11).

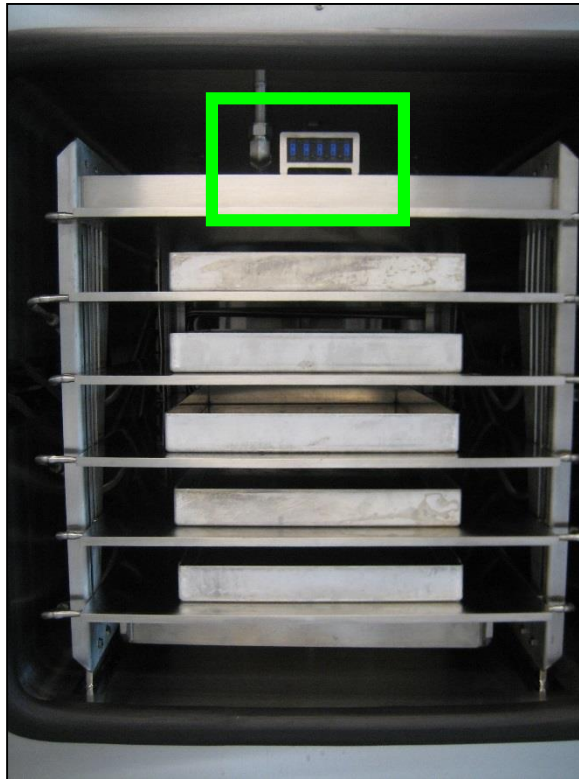


Figure 10. Probe Monitoring Port

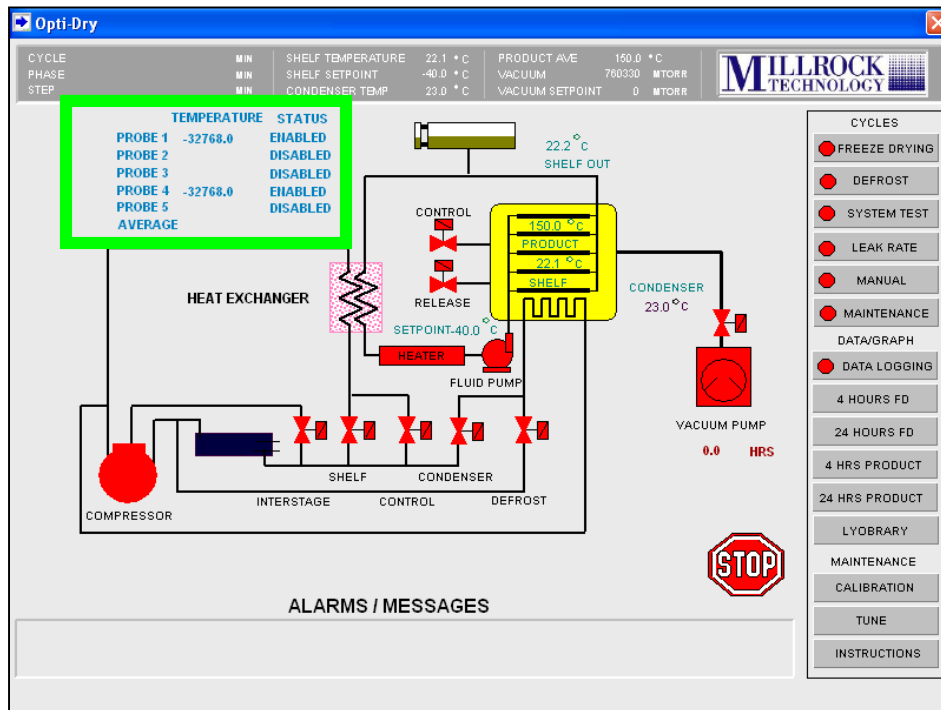


Figure 11. Probe Monitoring Port Temperature Display

6. Close the freeze dryer chamber door and tighten the large wing nuts (Figure 12). Be careful not to tighten the wing nuts *after* a vacuum has been created. This will result in the inability to reopen the chamber door once the freeze dry cycle is complete and the pressure has been released.

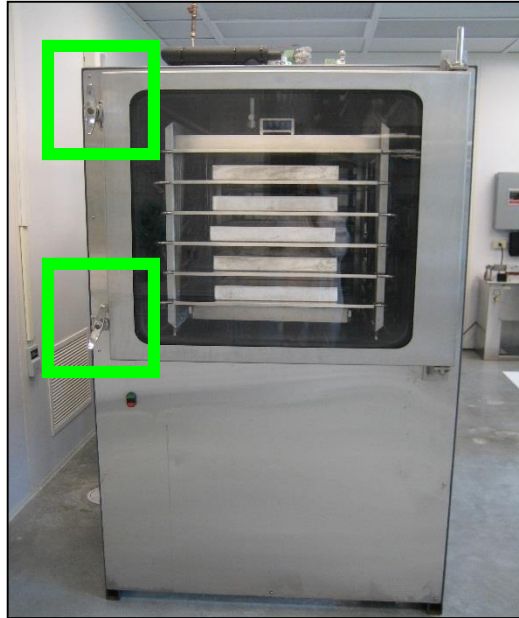


Figure 12. Wing Nuts on Chamber Door

7. Initially, the freeze dryer must be manually placed in an “operating state” by generating a low vacuum inside the product chamber prior to beginning the freeze drying process. To do this, select the “MANUAL” tab in the Opti-Dry program’s main menu, Figure 13. Turn the power ON, followed by turning ON the vacuum, Figure 14. The two large vacuum pumps will turn on to create a vacuum. (Note: the vacuum pumps are initially very loud and become quiet as the vacuum increases.) The atmospheric pressure inside of the drying chamber is indicated in the Opti Dry program in the top portion of the display screen. If a vacuum does not begin to initiate, press the product chamber door until it seals and a vacuum is initiated. Continue to monitor the vacuum and only initialize a vacuum seal of $\sim 53,300$ Pa (400,000 mTorr). Atmospheric vacuum pressure is 101,325 Pa (760,000 mTorr). After a sufficient vacuum has been created, turn the vacuum OFF by clicking the vacuum “OFF” button on the MANUAL screen. This does not release the vacuum, but only turns the vacuum pumps off. Next, turn the manual power OFF. This does not shut down power to the freeze dryer; it only turns the manual power off placing the freeze dryer in the “operating state” and allows the operator to begin the recipe.

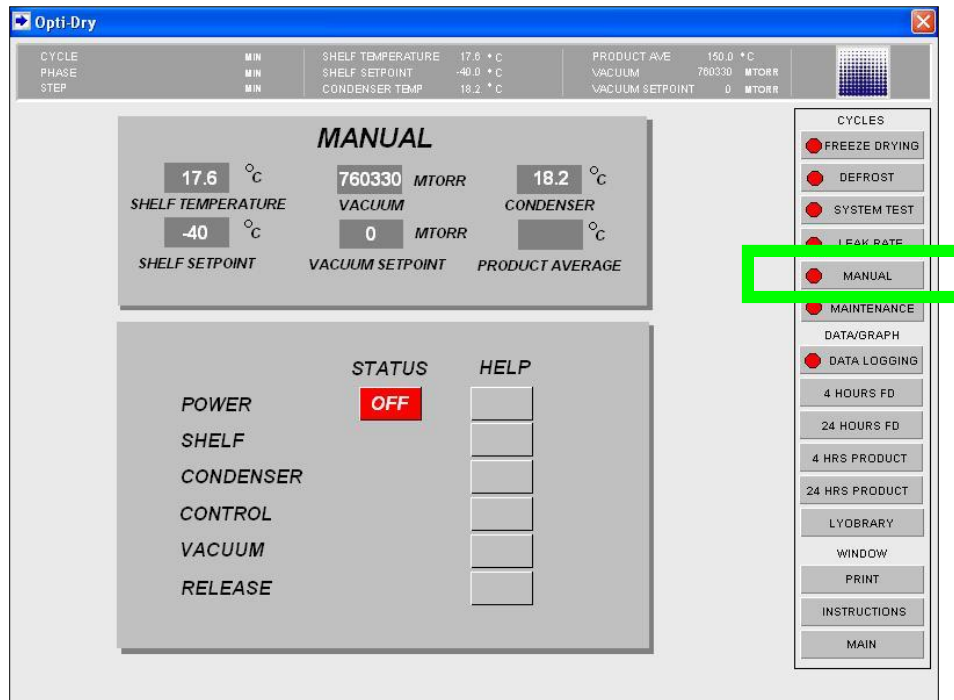


Figure 13. Opti Dry Manual Operation Screen

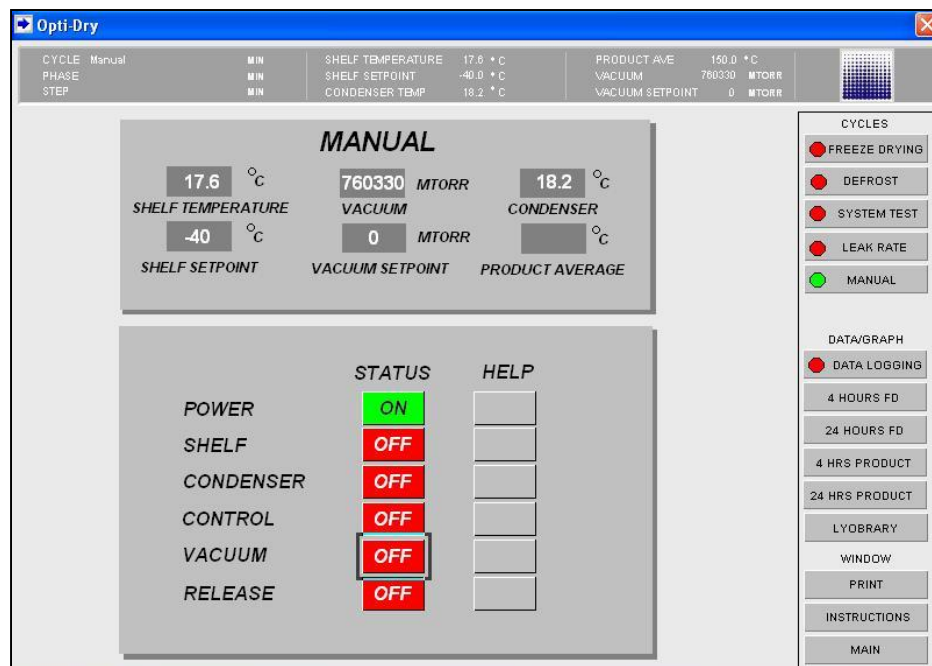


Figure 14. Opti Dry Manual Vacuum Operation

8. Open the “Freeze Dry” tab to begin the saved freeze dry recipe, Figure 15. Make sure the recipe is visible on the screen. If it is not visible, click “SAVED RECIPES” for the saved recipes in the Opti Dry program. To start freeze drying, click “START”. A prompt screen will open before the machine starts; click YES, to start the machine.

Opti-Dry

CYCLE	MIN	SHELF TEMPERATURE	22.1 °C	PRODUCT AVE	150.0 °C
PHASE	MIN	SHELF SETPOINT	-40.0 °C	VACUUM	760330 MTORR
STEP	MIN	CONDENSER TEMP	23.0 °C	VACUUM SETPOINT	0 MTORR

PRODUCT NAME: Enter Product Name
 PRODUCT #: Enter Product Number
 OPERATOR: Enter Operator Name

FREEZE DRYING
 RECIP: Water-Nov 2011.rcp

FREEZE

	1	2	3	4	5	6	7	8	9	10
SHELF SETPT °C	-10	-15	-20	-25	-30	-35	-40	-45	0	0
TIME MIN	10	20	30	40	50	60	70	80	0	0
FINAL FREEZE °C	-50									
EXTRA FREEZE MIN	60									
PRI VAC STARTMT	100									

NEW RECIPE
 EXISTING
 DELETE
 SAVE

PRIMARY

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
SHELF SETPT °C	-40	-35	-30	-25	-20	-15	-10	-5	0	5	10	15	20	25	30	35
TIME MIN	0	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
VACUUM SETPT MT	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260

SECONDARY

SHELF SETPT °C	20
TIME MIN	30
VACUUM SETPT MT	100
FINAL SETPT °C	25

ALARMS

COND OVERLOAD °C	-40
VACUUM OVERLOAD MT	500
POWER OUTAGE MIN	10

START

CYCLES

- ☒ FREEZE DRYING
- ☐ DEFROST
- ☐ SYSTEM TEST
- ☐ LEAK RATE
- ☐ MANUAL
- ☐ MAINTENANCE

DATA/GRAPH

- ☒ DATA LOGGING
- 4 HOURS FD
- 24 HOURS FD
- 4 HRS PRODUCT
- 24 HRS PRODUCT
- LYOBRARY

WINDOW

- PRINT
- INSTRUCTIONS
- MAIN

Figure 15. Freeze Drying Recipe Operation

9. The freeze drying recipe will begin and meet all of the set-points indicated in the Freeze Cycle, the Primary Cycle, and the Secondary Cycle.
10. Upon satisfying all steps of all cycles in the recipe, the freeze dryer MAIN screen will flash a FINISHED message. The freeze dryer will not shut down upon completion. The vacuum will remain and the final temperature will remain constant until the user completes the recipe by going through a manual shut down.
11. Click the “STOP” button on the FREEZE DRY cycle to stop the recipe.
12. Next, click on the “MANUAL” cycle button, Figure 13. Turn the power ON, and then turn the RELEASE ON, Figure 16. This will release the vacuum from the drying chamber. Once the vacuum has been completely released, approx. 101300 Pa (760,000 mTorr), the wing nuts can be released and the chamber door opened.

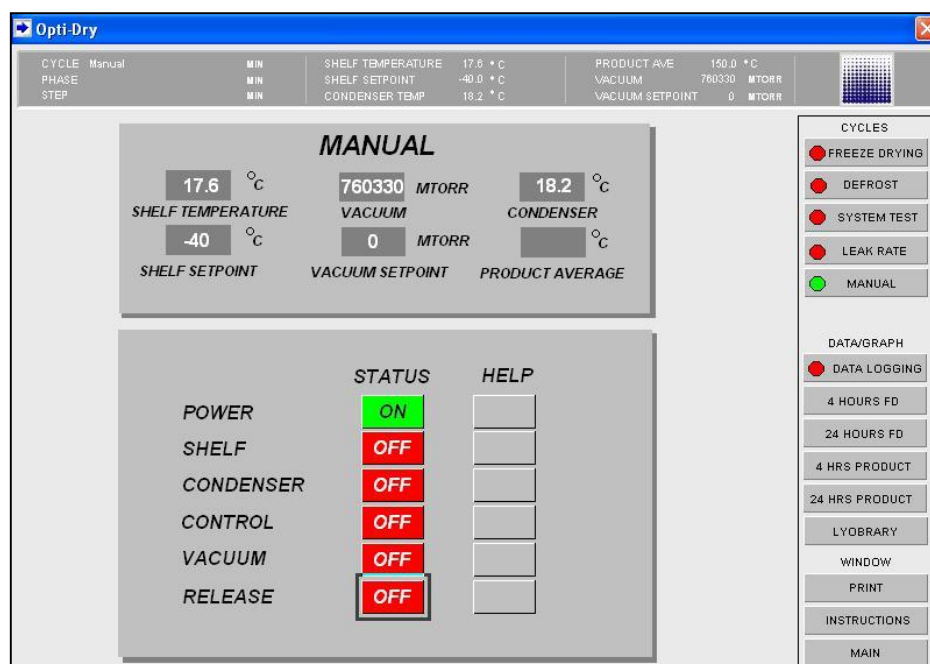


Figure 16. Vacuum Release Manual Operation

13. Remove and weigh the final freeze dried material. Immediately cap or place the sample in an air tight container or desiccator for storage. Using the final mass of the freeze dried material the moisture values can be calculated as described within the Conversion Factor and Percent Moisture Loss section.

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