Title: Operation of the Thermo/Forma Liquid Nitrogen Storage Systems

SOP Number: 12207

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Approval/Date:

Approval/Date:

Table of Contents

1.0 Purpose

This SOP outlines the operation and use of the Thermo Forma CryoPlus Liquid Nitrogen Storage Systems.

2.0 Scope

This procedure applies to all Biopharmaceutical Development Program (BDP) Manufacturing and Materials Management and Inventory Control (MMIC) personnel, as well as Offsite facility personnel, using the CryoPlus storage systems.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract

3.0 Authority and Responsibility

3.1 The Director, Technical Operations, Biopharmaceutical Development Program (BDP) has the authority to define this procedure.

3.2 BDP personnel are responsible for training on this procedure and documenting this training to Biopharmaceutical Quality Assurance (BQA).

3.3 BDP personnel are responsible for the performance of this procedure.

3.4 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this procedure.

3.5 The operation instructions in this SOP will start with the freezer connected to a liquid nitrogen supply per the manufacturer's instructions, turned on, and filled with liquid nitrogen for vapor phase use.

3.6 BDP personnel are responsible for setting up maintenance schedules and overseeing repairs to the equipment with FME or an outside contractor.

4.0 Warnings

4.1 The extremely low temperature can freeze human flesh very rapidly. When spilled on a surface, the liquid tends to cover it completely, cooling a large area. The gas resulting from the liquid is also extremely cold. Delicate tissue, such as that of the eyes, can be damaged by an exposure to the cold gas which would be too brief to affect the skin of the hands or face.

NOTE: Never allow any unprotected part of your body to touch objects cooled by liquid nitrogen.

4.2 Wear protective clothing.

4.2.1 Protect your eyes with a face shield or safety goggles (safety glasses without side shields do not give adequate protection).

4.2.2 Always wear gloves when handling anything that is, or may have been, in immediate contact with liquid nitrogen. Insulated gloves are recommended, but heavy leather gloves may also be used. The gloves should fit loosely, so that they can be thrown off quickly if liquid should splash into them.

NOTE: Do not cover or plug the entrance opening of any liquid nitrogen refrigerator or dewar. Do not use any stopper or other device that would interfere with venting of gas. These cryogenic liquid containers are generally designed to operate with little or no internal pressure. Inadequate venting can result in excessive gas pressure which could damage or burst the container. Use only the loose-fitting neck tube core supplied or one of the approved accessories for closing the neck tube. Check the tank vent periodically to be sure that venting is not restricted by accumulated ice or frost (see **Attachment 4**).

4.3 Nitrogen gas can cause suffocation without warning. It is colorless, odorless, and tasteless, and will displace normal air. Be extremely cautious to avoid asphyxiation, and be sure appropriate ventilation and Oxygen detection equipment is in place and functioning before using this unit.

NOTE: The cloudy vapor that appears when liquid nitrogen is exposed to the air is condensed moisture, not the gas itself. The issuing gas is invisible.

5.0 Monitoring

5.1 The bar graph on the front of the unit displays the chamber liquid level and low and high level setpoints (see **Attachment 1**). The left side of the graph is the high scale, for use with liquid phase operation, and ranges from 1-25 inches in 1 inch increments. The right side of the graph is the low scale, for use with vapor phase operation, and ranges from 2-7 inches in ¼ inch increments. To toggle between scales, press the scale select button. Unless a change is specified by the supervisor, the unit will be run in vapor phase, with the corresponding scale selected.

5.2 The LED colours on the bar graph represent the following (see Attachment 2):

Colour	State	Significance
Orange	Steady	Remaining space above high level setpoint
Orange	Flashing	High and Low level (stop and start fill) setpoints
Green	Steady	Actual liquid Nitrogen level
Red	Steady	Amount of space above or below setpoint from actual level
Red	Flashing	Liquid level is above or below level setpoints. Possible Alarm
Red	Single Flashing	Liquid level is at the high level setpoint after a fill. Not an Alarm.

5.3 When the key switch on the control panel (see **Attachment 3**) is in the Lock position, the display will show the chamber temperature directly under the unit's lid.

6.0 Programming

NOTE: The Key Switch must be in the Programming Access position to program or to access the Touch Pad functions. If no entry is made within 4 minutes, the Controller will return to the lock position even if the key is in the Access Position. On the Bar Graph, the high and low level setpoints must be at least 3" apart for the high scale and 1.25" apart on the low scale.

6.1 High Level (Stop Filling) Setpoint

6.1.1 Verify the correct scale is selected on the bar graph

6.1.2 Turn the key switch to the Programming Access position, press the High-Level button, and then press and hold the up or down arrow to raise or lower the flashing orange LED to the desired setpoint. Set the level at **5 inches** unless validation data suggests use of an alternate value to achieve peak performance.

6.1.3 Press Enter to store the new setting, and then turn the key switch to the Lock position.

6.2 Low Level (Start filling) Setpoint

6.2.1 Verify the correct scale is selected on the bar graph

6.2.2 Turn the key switch to the Programming Access position, press the High-Level button, and then press and hold the up or down arrow to raise or

lower the flashing orange LED to the desired setpoint. Set the level at **3.5 inches** unless validation data suggests use of an alternate value to achieve peak performance.

6.2.3 Press Enter to store the new setting, and then turn the key switch to the Lock position.

6.3 High Temperature Alarm Setpoint

6.3.1 Turn the key switch to the Programming Access position. The display will indicate the current High Temperature Alarm setpoint.

6.3.2 Press the High Temp button. Raise or lower the setpoint with the up or down arrow, then press Enter and turn the key switch to the Lock Position.

6.4 Setting the Microprocessor's Internal Clock

6.4.1 The "real time clock" internal clock enables alarms, program changes, and current system status to be printed relative to the actual time and date of occurrence. This information is made available through the RS-232 data port.

6.4.2 To set the clock, start with the unit turned off.

6.4.3 Turn the key switch to Full Access. Press the High Level button on the key pad while turning on the power switch located on the back of the unit.

6.4.4 Starting from the bottom of the bar graph, the first LED on the bar graph lights and the numeric display on the control panel shows the current time hundredths of seconds. Press the Enter key to lock in the value and advance to the next setting. The chart below shows the settings in their sequence.

LED Lights *	Clock Setting
1	Hundredths of seconds
2	Seconds
3	Minutes
4	Hours (24 hour clock)
5	Day of the week
6	Day of the month
7	Month
8	Year

*LED on the Bar Graph, starting at the bottom of the graph and counting upward

7.0 Use

7.1 Complete segregation of Quarantine and Released materials in separate units is preferred. If this is not possible segregation will be handled by racks such that a stack/rack is dedicated to one or the other. All Quarantine racks should be denoted with a temperature compatible tag affixed on the handle.

7.2 Verify the liquid level is within parameters.

7.2.1 Unlock the tank and open the lid.

7.2.2 Pull out the desired stack and place or remove desired vials/boxes in/from the stack.

7.2.3 Return the stack to the tank, then close and lock the lid.

7.2.4 The manual fill button may be used to replenish liquid nitrogen lost as part of extended opening or addition of new material.

7.3 Potentially applicable only to units outside the [redacted]. The unit is equipped with an automatic switching mechanism in order to avoid an interruption in LN supply. Monitor the LN cylinders on a routine basis (with the frequency determined by the level of usage) and replace any empty tanks as necessary.

7.4 Freezers residing within offsite facilities will be maintained by the staff at those facilities per the governing contract.

8.0 Troubleshooting and Cleaning

NOTE: Internal adjustments and maintenance must be performed by qualified service personnel and documented in the equipment logbook.

8.1 Defrosting

8.1.1 If the freezer needs to be defrosted, quickly move the contents to a back-up liquid nitrogen freezer.

8.1.2 Turn the power off.

8.1.3 Remove the liquid nitrogen supply by allowing it to evaporate if the freezer is in a large room, or dipping it out into another freezer.

8.1.4 Allow the freezer to warm to room temperature.

8.2 General Cleaning: The outside cabinet can be washed with a mild detergent, while the interior can be cleaned after defrosting with a mild soap solution or 70% Isopropyl or ethyl alcohol. Do not use a cleanser with chlorine.

8.3 To troubleshoot when the freezer is in an alarm condition (See **Attachment 5**), reference the unit's Operator's Manual.

9.0 Documentation

Document the use of the Liquid Nitrogen Freezer in the logbook, being sure to include the following: Date, Time, Temperature, Samples added/removed, and Initials.

10.0 References and Related Documents

10.1 Model 7400 Series, CRYO Plus 1, 2, 3, and 4, Liquid Nitrogen Storage System Operating and Maintenance Manual 7007400 Rev. 9

10.2 Forma Scientific, Inc., Liquid Nitrogen Storage System, Manual No. 7007400 Revision 4. 3/8/00, For Models 7400, 7402, 7404, 7406, and 7407.

11.0 Attachments

11.1 Attachment 1, Level Indicators and Setpoints

11.2 Attachment 2, Bar Graph Colours

11.3 Attachment 3, Control Panel Elements

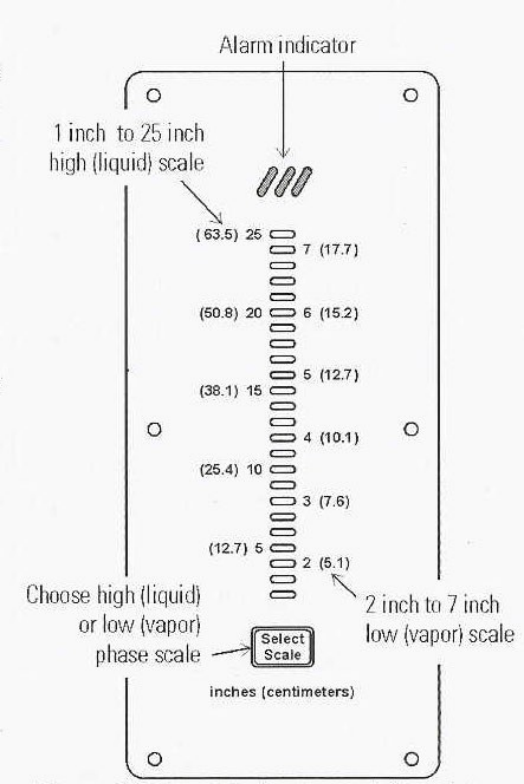
11.4 Attachment 4, The Vapor and Liquid Phase Storage

11.5 Attachment 5, Troubleshooting Alarms

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

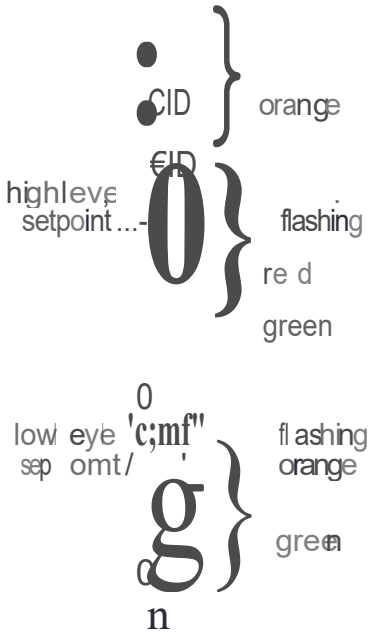
ATTACHMENT 1

Level Indicators and Setpoints



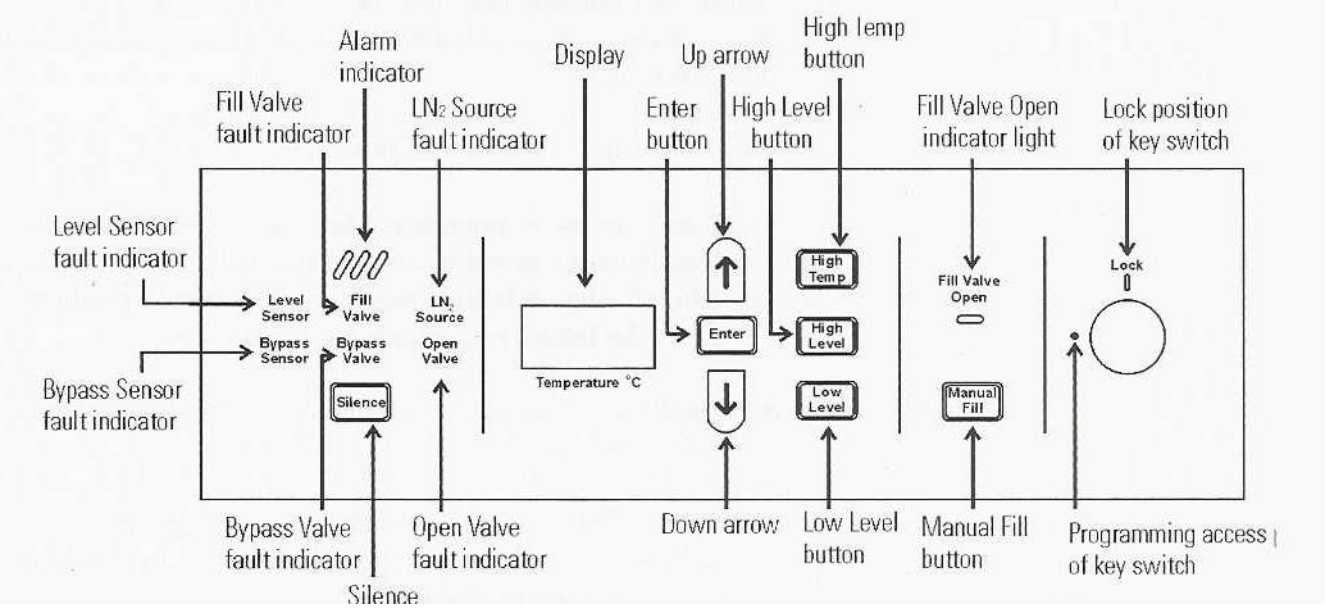
ATTACHMENT 2

Bar Graph Colours



ATTACHMENT 3

Control Panel Elements



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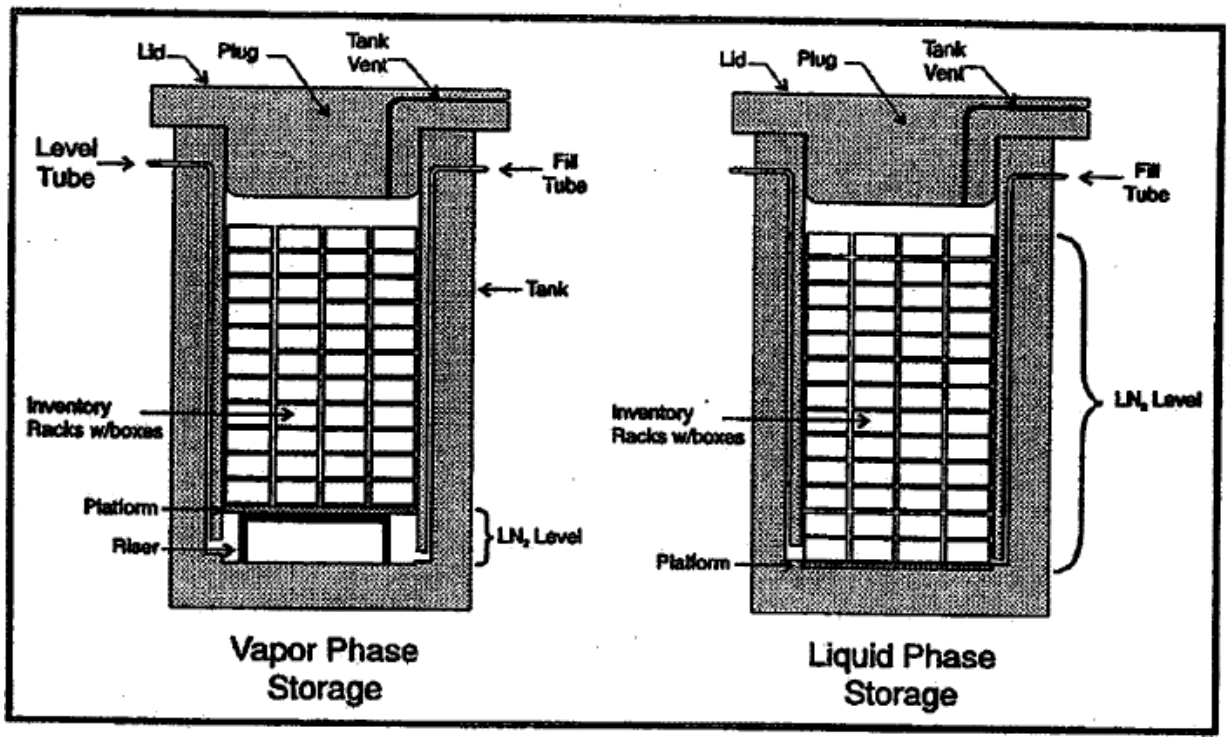
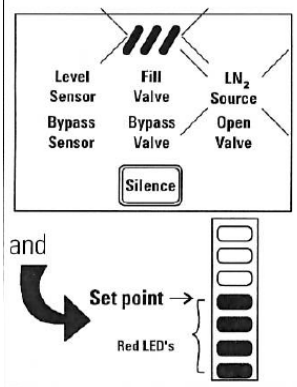
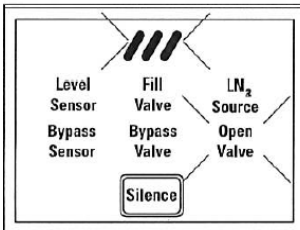
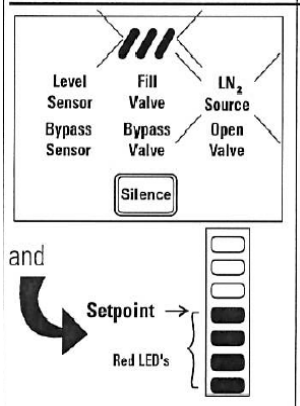
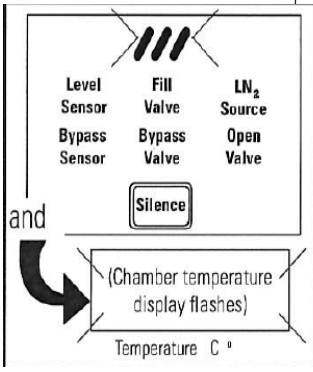
ATTACHMENT 4

The Vapor and Liquid Phase Storage



ATTACHMENT 5
Troubleshooting Alarms

Front Panel Alarm	Alarm Description / System Response	Probable Cause	Corrective Action
	LN2 Source warning lamp and the alarm bars light, indicating that no liquid nitrogen is flowing to the unit. Fill and Bypass valves close. The audible alarm sounds. The remote alarm contacts set to activate in 30 minutes. This alarm will ring back in 30 minutes if the alarm condition is not corrected.	Liquid Nitrogen tank is empty. Bypass valve is open but the system does not see a ¼-inch rise of LN2 in 20 minutes	Listen for flow of gas or liquid. Check the supply tank for LN2. Review any changes or conditions which may have impact on the system, such as: Longer hoses or pipes installed. Flow obstructing or restricting fittings installed. Heat emitting source moved to proximity of system. Note: An LN2 Source Alarm is normally a LN2 supply problem and not a fault of the Cryo Plus Storage System. Refer to Section 1.2 of this manual and make sure that the unit is receiving *liquid* nitrogen and not just nitrogen *gas*.
	Open Valve warning lamp and the alarm bars light. The audible alarm sounds. Fill and Bypass valves attempt to close. The Remote alarm contacts activate immediately Alarm system resets if condition corrects.	The command to close has been given to the fill valve but the level of LN_2 in the unit continues to rise. The system will go into alarm if the liquid nitrogen rises more than 1/4-inch above the high limit setpoint within 3-10 minutes after the fill valve closes.	See if LN2 or gas is still being injected into the tank. If so, turn off the tank. Disassemble and check the fill valve for ice, dirt, or other contaminates. (Refer to Section 4) If LN2 is NOT being injected into the tank and the fill valve appears to be closed, verify that the LN2 supply tank pressure is 22 psi or less. A high pressure LN2 source may cause the unit to overfill past the high level set point. Note: This condition can result when several storage racks are placed into the container, raising the level of the liquid nitrogen above the High Level set point, within 10 minutes of a fill.
	LN_2 Source warning lamp and the alarm bars light, indicating that no liquid nitrogen is flowing to the unit. Fill and Bypass valves close. The audible alarm sounds. The remote alarm contacts set to activate in 30 minutes. This alarm will ring back in 30 minutes if the alarm condition is not corrected.	LN_2 level does not reach the setpoint in 60 minutes. The Bypass Sensor does not see the supply line temp drop below -130°C in 60 minutes.	Listen for flow of gas or liquid. Check the supply tank for LN_2. Review any changes or conditions which may have impact on the system, such as: Longer hoses or pipes installed. Flow obstructing or restricting fittings installed. Heat emitting source moved to proximity of system. Note: An LN_2 Source Alarm is normally a LN_2 supply problem and not a fault of the Cryo Plus Storage System. Refer to Section 1.2 of this manual and make sure that the unit is receiving *liquid* nitrogen and not just nitrogen *gas*.
	High temperature alarm: The alarm bars light and the temperature display flashes the thermocouple temperature. The audible alarm sounds. Remote alarm contacts set to activate in 30 minutes. Alarm system resets if condition corrects.	The thermocouple temperature is warmer than the high temperature setpoint. The lid has been open too long. An excessive heat load (warm product) has been placed into the chamber.	Make sure the temperature sleeve is properly installed to lower the chamber temperature. Raise the high temperature setpoint. The temperature is measured using a T-type thermocouple with an accuracy of ±3C for 196C. The temperature gradient within the tank is dependent upon changes to the LN2 which affects it's stabilization (fill, evaporation, introduction of warmer product, etc.). The higher the level of LN2, the colder the unit will be. Make sure the high temperature alarm set point is not lower than the height of the LN2 is capable of maintaining.