thermo scientific

Controlled thaw of ultra-cold bulk product to refrigeration temperature in environmental chambers

Introduction

The World Health Organization (WHO) began tracking data on SARS-CoV-2 infections on December 31, 2019, and in a few short years, over millions of deaths globally were directly attributed to the spread of the virus. Remarkably, within the first two years of global spread, numerous SARS-CoV-2 vaccines were developed, tested, and administered to broad populations. Within two years, there were nine SARS-CoV-2 vaccines given Emergency Use Listing (EUL) by the WHO, three of which were also approved by the US-FDA, and over 150 more vaccine candidates undergoing investigational trials.

While the rapid development and swift distribution of coronavirus vaccines proved decisive to the ability of high-income countries to protect their populations against severe SARS-CoV-2 infections, there continues to be a deficiency in vaccine absorption in lower-income countries. With the knowledge that vaccination of all populations is of global and regional importance, global vaccine providers have pivoted to ensure equitable access to SARS-CoV-2 vaccines around the world.

This rapid, globalized response to the spread of SARS-CoV-2 virus produced a range of novel and innovative vaccine formulations. However, vaccines designed with these new technologies, like mRNAs

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encapsulated in lipid nanoparticles, require atypical shipping and storage conditions compared to traditional protein-based or inactivated viral particle vaccines. These vaccines require transport and storage at ultra-cold temperatures, below -70 °C for maximum potency. The time above frozen and refrigeration temperatures also need to be controlled closely to safeguard the vaccine.

Production workflow

The traditional end-to-end operation for the large-scale manufacture of drug product (DP), like those in vaccine development research pipelines, consists of two nodes. The first node, termed the formulation or origination site, is where the bulk DP is produced. After formulation and extensive quality checks the bulk DP is then prepared for global transportation. In the case of temperature-sensitive DPs, this will include transfer to sterile, single use cassettes that can be easily stored and shipped at required temperatures. These filled cassettes can be frozen in traditional ultra-low temperature freezers down to -70 °C, followed by pack out to qualified shipping containers for shipment. The cassettes are shipped frozen in controlled and tracked conditions, via truck and/or air freight to the second node, the fill/finish (F/F) site. At the F/F site, the DP cassette is thawed, and the contents from several cassettes pooled to perform remaining downstream operations, including vial filling, inspection, packaging, QA/QC, and final release of product.

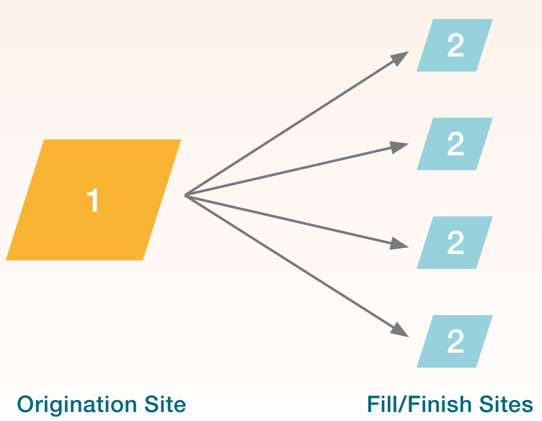
While the sites of formulation are geographically limited, the second node F/F sites are designed to support global operations from distinct localities. This global emphasis lends complexity to the design of standard operating procedures for a downstream process as variances in power supply, water quality supply, and the availability of appropriately graded spaces can vary by locality.

The individual characteristics of a DP also contribute to the complexity of downstream SOPs. Laboratory analysis groups will often perform lab scale thermocycling studies and analytical testing to evaluate the environmental constraints that impact the critical quality (CTQ) characteristics for bulk DP. These CTQ characteristics are tested at various temperatures and times to determine allowable Time in Refrigeration (TIR: 2-8 °C), Time out of Refrigeration (TOR: >8 °C), and frozen (-70 °C) constraints for end-to-end manufacturing to ensure activity is not adversely affected by any conditions during the manufacturing process.

Controlled thaw challenge

For the controlled thaw of ultra-cold DP, undertaken by Thermo Fisher Scientific at the request of a global pharmaceutical customer, it was determined that a thaw time of 15 hours, or less, would provide adequate time for downstream operations while maintaining acceptable TIR/TOR limits. Given these constraints, it was determined a multi-variate-controlled thaw would be preferred to an uncontrolled procedure to help ensure no DP was exposed to conditions that would impact CTQ characteristics.

In response to the challenge of multi-variate-controlled thawing of bulk DP, a Thermo Fisher engineering team proposed and



Manufacture of global temperature-sensitive DP traditionally consists of two nodes.

The First Node, termed the formulation or origination site, is where the bulk DP is produced. After formulation and quality checks, the bulk DP is transferred to sterile, single-use cassettes and frozen to ultra-cold temperatures.

The cassettes are shipped in controlled and tracked conditions to the Second Node, the fill/finish (F/F) site. At the F/F site, the DP cassette is thawed, and the contents from several cassettes pooled to perform remaining downstream operations, including vial filling, inspection, packaging, QA/QC, and final release of product.

validated a fit-for-purpose solution utilizing Thermo Scientific™ Environmental Chambers. These environmental chambers are laboratory standard models, which further fit the directive to be globally available for all second node fill/finish sites that run thawing procedures. To completely fulfill the mandated specifications, the entire contents of 12-liter DP in sealed, sterile cassette must be thawed from ultra-frozen to refrigerated temperature within 15hrs. Additionally, the bulk DP contents cannot exceed 25 °C at any point in the process or spend extended time above refrigeration temperature to preserve product quality.

Thermo Fisher offers a fit-for-purpose solution

Environmental chambers were the right fit for the challenge because they lend the ability to control an environment's temperature and humidity independently, allowing the F/F user to precisely thaw the DP contents in a defined, repeatable manner. To validate the use of the Thermo Scientific Environmental Chamber in the thawing of DP, the requesting company provided Thermo Fisher with empty bulk cassettes and identified distilled water as the appropriate analog to simulate DP. To fully simulate the DP thawing conditions, Thermo Fisher filled the bulk cassettes with 12L of H_2O, and froze the filled cassettes to -70°C. Prior to filling, the bulk cassettes were outfitted with multiple thermocouples to continuously monitor the temperature of the water in the interior middle of the filled cassette, on the top and bottom of the cassette exterior, and the inlet fill tubes.

An important note: the thermocouple in the middle of the bag was placed prior to fill/freeze and was thus susceptible to displacement when frozen. Variability observed in thawing profiles during separate experiments can be attributed to the exact nature of thermocouple placement for each bag and experiment.

As for the thermocouple placed in the product inlet tubing, when the tubes are filled at the site of formulation, residual product hold-up is present from bag filling operation and this DP is susceptible to mix with bag contents during/after thaw. The DP in the fill tube is the most vulnerable to temperature changes and set the standard time DP could remain in process to not exceed the thawing requirement. The group of thermocouples were used to track the thaw rate of the cassettes throughout iterative experiment rounds, with the time-to-thaw being reported as the time until there was no visible ice in the cassette.

Pre-method development for controlled thawing of bulk DP at Thermo Fisher Scientific

To identify the ideal conditions for the controlled thaw of DP in bulk cassettes, Thermo Fisher employed iterative testing with the prepared water cassettes to assist our customer in early method development. While a fast thaw time was the crucial decision factor in method development, it was also key that all bags in the chamber thaw uniformly and in a consistent time. This protects DP from spending variable time at different temperatures, which could impact performance. The iteration of experiments was conducted with input from engineers from both the customer and Thermo Fisher so that the data could inform the next testing steps. In these experiments, water-filled prepared cassettes were frozen to -70 °C for at least 24 hours prior to test start. For testing containing eight (8) cassettes, chambers were loaded with cassette 1 on the bottom shelf and cassette 8 on the top shelf. Melt curves shown in this report were generated from the thermocouples placed at the center of the cassettes before freezing. Most experiments also independently recorded the air temperature of the chamber, to verify testing conditions were maintained throughout the procedure.

The challenge

Thaw 12 L bags filled with DP from ultra-cold -70 °C to refrigeration temperature of 2 - 8 °C within 15 hours. To preserve the CTQ characteristics of the DP, the DP cannot exceed 25 °C, and the dwell time above 10 °C must be minimized.

The answer

Thermo Scientific Environmental Chamber (Cat. No. 3940/3948) with control of temperature and humidity.

Round 1 - Temperature only

The first rounds of testing were designed to utilize the environmental chambers to control temperature only. These tests were meant to determine the impact of air flow on a fully loaded chamber and determine if a closed environment with tight temperature control would be sufficient to speed thawing. Chambers were equilibrated to testing temperature prior to test start. At test time zero loaded with eight (8) frozen cassettes on solid shelves and swiftly closed to maintain air temperature. Figure 1 shows the melt curve for the eight cassettes thawed at 23 °C, while Figure 2 shows the melt curves for the eight cassettes thawed at 40 °C.

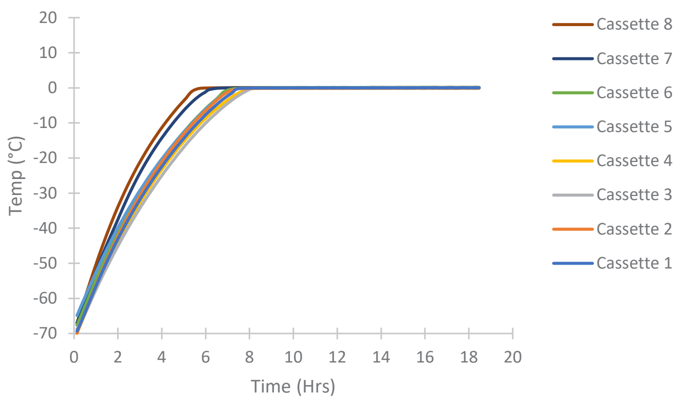


Figure 1. Melt curves of eight (8) frozen bulk cassettes at 23 °C air temperature with no relative humidity. The cassettes were arranged in the environmental chamber so that Cassette 1 was loaded on the bottom shelf and Cassette 8 was loaded on the top shelf.

At the lower end of testing, the temperature only test at 23 °C did not completely thaw any cassette within 18 hours (Figure 1), with interior temperatures on all frozen cassettes remaining at 0 °C, indicating large ice blocks in each cassette.

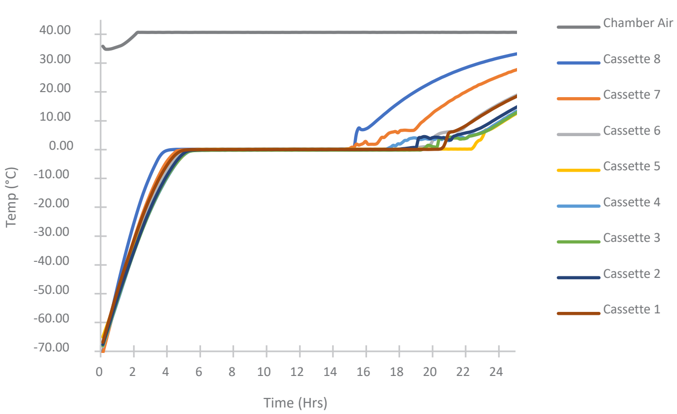


Figure 2. Melt curves of eight (8) frozen bulk cassettes at 40 °C air temperature with no relative humidity. The cassettes were arranged in the environmental chamber so that Cassette 1 was loaded on the bottom shelf and Cassette 8 was loaded on the top shelf.

The temperature only test at 40 °C (Figure 2) was able to thaw some bags within that time, notably those near the top of the cabinet, but the spread for thawing all eight cassettes was around 7 hours and took a total of over 22 hours to complete. This spread of thaw time was determined by the engineering team to be too broad to protect DP activity, and the discussion then turned to how humidity could assist in reducing thaw time and thaw spread.

Round 2 - Temperature and humidity

The next round of testing thus incorporated the independent control of relative humidity (RH) and temperature to begin to determine a programmable set of conditions for use. Figure 3 shows the melt curves of cassettes loaded into 40 °C, 90% RH chamber and held at this warmer temperature for 12 hours, before the temperature was reduced to 23 °C.

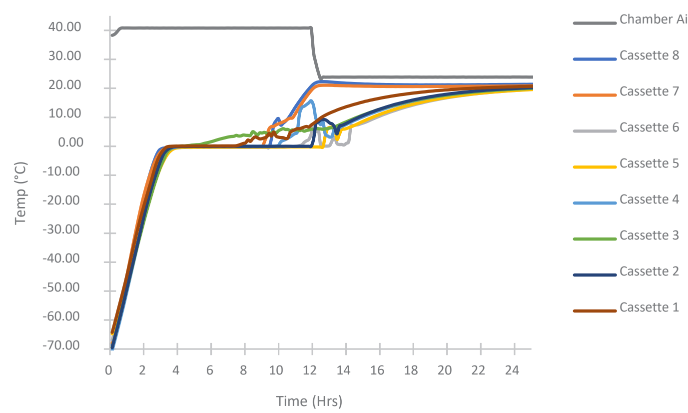


Figure 3. Melt curves of eight (8) frozen bulk cassettes at 40 °C air temperature with 90% RH starting conditions. At 12 hours, conditions were adjusted to 23 °C and 90% RH. The cassettes were arranged in the environmental chamber so that Cassette 1 was loaded on the bottom shelf and Cassette 8 was loaded on the top shelf.

The combination of warm, humid air quickly heated all eight cassettes from -70°C to 0°C, but the time range for a full thaw of all cassettes was still very uneven. While the instability of the melt curves between 0 °C and 10 °C can be attributed to thermocouple placement within the ice block of the frozen cassettes, it still took over 15 hours for the entire contents of all cassettes to thaw, while those cassettes at the top of the chamber thawed to temperatures above refrigeration before 12 hours. The temperature was dropped to 23 °C at 12 hours to protect the contents of thawed bags from reaching undesirable temperatures, but the TOR for several of the cassettes was identified as a concerning outcome of the faster thaw time and warmer temperature.

To determine if dropping the temperature earlier would better protect the product from TOR, the next test utilized the same starting conditions of 40 °C and 90% RH but dropped the chamber temperature to 23 °C after 8 hours. Melt curves shown in Figure 4, with this earlier reduction in chamber air temperature, show an improvement in TOR, but the spread of thaw times was extended.

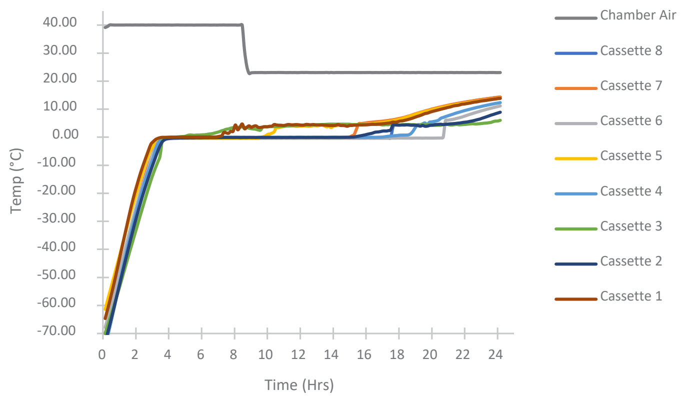


Figure 4. Melt curves of eight (8) frozen bulk cassettes at 40 °C air temperature with 90% RH starting conditions on perforated shelves. At 8 hours, conditions were adjusted to 23 °C and 80% RH. The cassettes were arranged in the environmental chamber so that Cassette 1 was loaded on the bottom shelf and Cassette 8 was loaded on the top shelf.

The experiment detailed in Figure 4 was also the first to incorporate an insulated top shelf to protect the cassettes in the upper portion of the chamber. It was identified in earlier experiments that cassettes 7 and 8 were always among the first cassettes to thaw, so the insulated shelf was intended to protect the upper cassettes from any effect of increased temperature and/or airflow that might be impacting those cassettes at the top of the chamber. The team also decided to switch from solid shelves to perforated shelves to hold the cassettes to promote an increase of airflow beneath each cassette. These decisions were taken together to attempt to reduce the spread of thaw time for all eight cassettes. While the insulated shelf was effective to bring the upper cassettes in line with the group, the wide range of thawing times was not improved.

The initial start temperature of 40 °C was identified by the team as a possible contributor to the thaw time spread, as it could be affecting the rate of thaw for each cassette differently. As a quick test of the effectiveness of elevated temperatures, a test was performed at a steady temperature of 25 °C and 90% RH with the perforated shelving and insulated top shelf included. Figure 5 shows the melt curves of this test.

All cassettes in Figure 5 were thawed within 21 hours, and the TOR for all cassettes was within an acceptable range, but the spread of thawing times was still concerning. The team determined that the cold load in each chamber may be prohibitive to effect controlled thawing and agreed to reduce the load in each chamber from eight (8) frozen cassettes to four (4) frozen cassettes. The engineering teams also decided to study the effects of gentle agitation on the thawing behavior of a cassette in the hope that the agitation would greatly reduce thaw time.

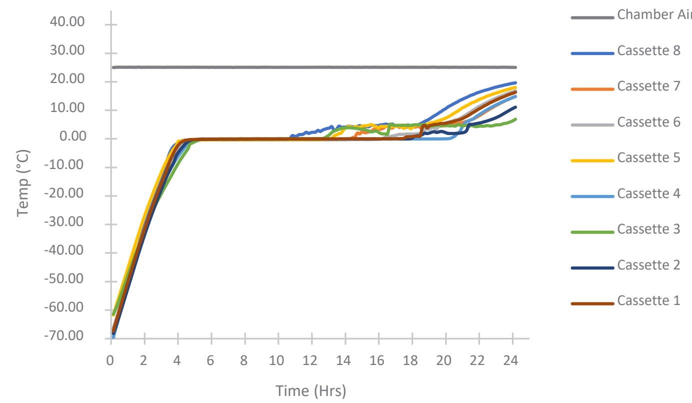


Figure 5. Melt Curve of eight (8) frozen bulk cassettes at 25 °C air temperature with 90% RH on perforated shelves. The cassettes were arranged in the environmental chamber so that Cassette 1 was loaded on the bottom shelf and Cassette 8 was loaded on the top shelf.

Round 3 – Agitation and reduced cold load

In the first of two experiments incorporating gentle agitation into the working protocol, the air temperature of the environmental chamber was set to 25 °C and the humidity to 75% RH. The reduction in humidity was necessary to protect the electronics of the shaker used in the experiments, with the understanding that the final protocol would utilize 90% RH and a shaker designed to handle the condition. To directly compare the result of agitation vs. steady-state thawing, only one cassette per chamber was situated on a shaker. The cassettes were also spaced evenly throughout the chamber, as the reduced load of four cassettes allowed for more room between shelves.

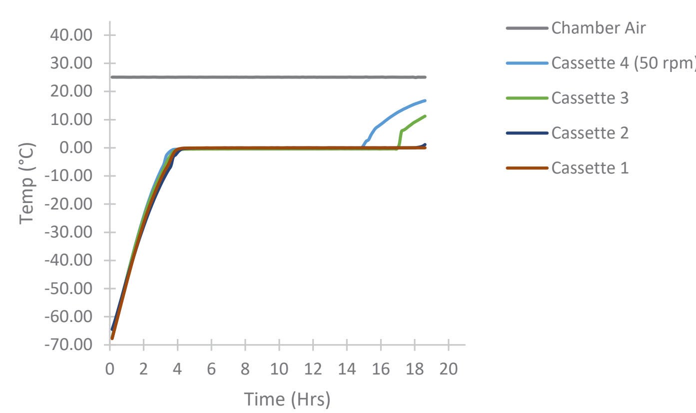


Figure 6. Melt Curve of four (4) frozen bulk cassettes at 25 °C air temperature with 75% RH on perforated shelves. Cassette 4 was arranged on a shaker and gently agitated at 50 rpm for the entirety of the test. The cassettes were arranged in the environmental chamber so that Cassette 1 was loaded on the bottom shelf and Cassette 4 was loaded on the top shelf.

The melt curves in Figure 6 highlight the decreased thaw time needed when gentle agitation at 50 rpm orbital rotation was added to a cassette. The cassette that was thawed with agitation was thawed first and thawed very uniformly. Visual inspection by trained technicians noted key differences in how agitated and steady-state cassettes thawed, with ice in the agitated cassette gently thawing from the outer edges to an inner circle very uniformly. Ice in the steady-state cassettes was more varied, with areas thawing at the top of the cassette first, before moving down towards the bottom.

In the next experiment, the temperature was raised again to 40 °C and the same 75% RH maintained for shaker integrity. The melt curves in Figure 7 again highlight the reduction in thaw time for the cassette under agitation, with thaw being achieved for cassette 4 in 7 hours. While the other three cassettes required around 10 hours to thaw, it was also notable that the spread of thaw time between those three steady-state cassettes was within an hour, validating the choice to switch to four cassettes per chamber to reduce the total cold load.

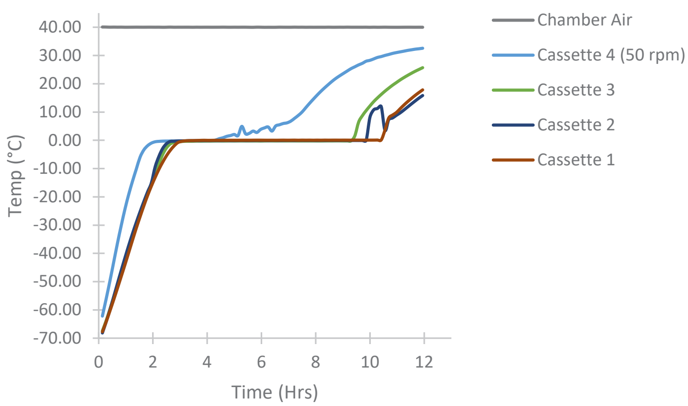


Figure 7. Melt Curve of four (4) frozen bulk cassettes at 40 °C air temperature with 75% RH on perforated shelves. Cassette 4 was arranged on shaker and gently agitated at 50 rpm for the entirety of the test. The cassettes were arranged in the environmental chamber so that Cassette 1 was loaded on the bottom shelf and Cassette 4 was loaded on the top shelf.

While the addition of gentle agitation at 50 rpm on an orbital shaker worked remarkably well to reduce the total thaw time, it was determined that the inclusion of agitation to the final protocol could not be validated quickly enough to meet the time constraints of responding to a global virus crisis. While the data was archived for future use, the team turned to finalizing a pre-method that could be quickly optimized and implemented to fulfil the earlier requirements. This included a total, repeatable thaw time of less than 15 hours and a short TOR for DP in any part of the cassettes.

Round 4 – Final pre-method testing

A final test condition of a pre-warmed 30 °C chamber at 90% RH, with a drop in temperature to 25 °C to protect DP in the tubing of the cassette was tested with a load of 4 cassettes on perforated shelves. To further promote even airflow around the cassettes, and hopefully mimic the thaw pattern of thawing observed with agitation, the cassettes were loaded onto wooden spacers that were mounted on the perforated shelves and held the cassettes about 2 inches off the shelf. The melt curves of a recommended 4 cassette load are presented in Figure 8.

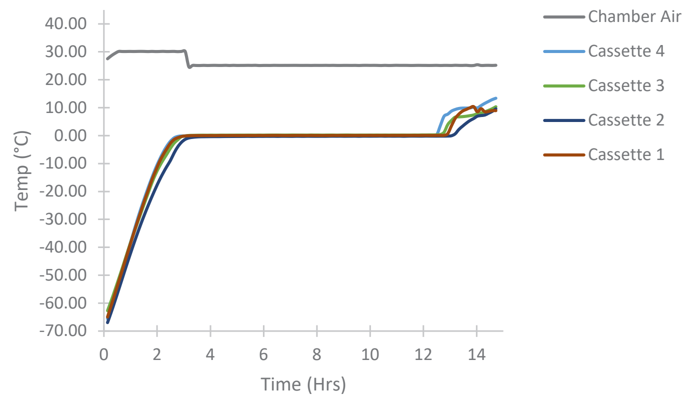


Figure 8. Melt curves of four (4) frozen bulk cassettes at 30 °C air temperature with 90% RH on perforated shelves. The temperature was adjusted to 25 °C when fill-tube temperature reached 24 °C. cassettes were arranged on spacers approximately 2 inches above shelves for maximum air flow. The cassettes were arranged in the environmental chamber so that Cassette 1 was loaded on the bottom shelf and Cassette 4 was loaded on the top shelf.

The final conditions of Thermo Fisher testing resulted in melt curves for the 4 cassettes that were very similar to one another, with a total thawing time of less than 15 hours for full thaw, and a spread of thawing between the cassettes of about 1 hour. In the 15-hour run, the temperatures of the cassettes did not exceed 12 °C, and the temperature in the fill tubing around the cassette did not exceed 25 °C. This test was deemed successful for pre-method transfer to the customer for a 4-cassette method optimization, validation, and implementation.

As a final quick test, Thermo Fisher tested the ability of the chosen final conditions to respond to a minimum DP thawing run, which was defined as a full single cassette. The single cassette was tested at the same starting conditions, but to account for the reduction in cold load, the temperature was dropped to 25 °C after only about 30 minutes during the run, as shown in Figure 9.

The single cassette thawed in about 5 hours, and without the influence of the other ultra-cold cassettes, the thawing was so quick it easily outpaced the chamber with a greater load. It was recommended that a separate minimum run program should be established and validated before use.

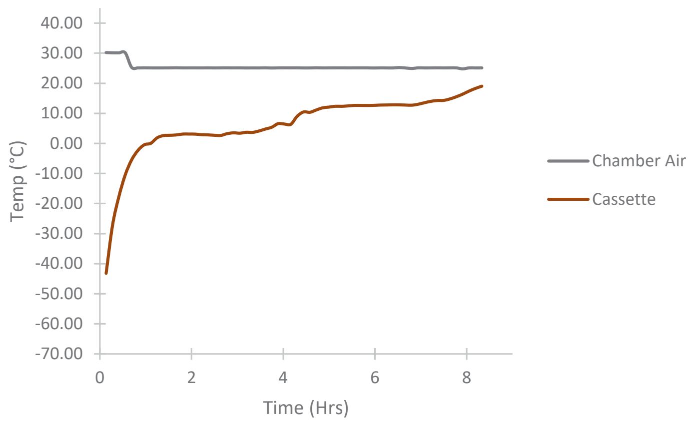


Figure 9. Melt curve of one (1) frozen bulk cassette at 30 °C air temperature with 90% RH on a perforated shelf. The temperature was adjusted to 25 °C when fill-tube temperature reached 24 °C. The cassette was arranged on spacers approximately 2 inches above the shelf in the center of the chamber for maximum air flow.

After the completion of pre-method development, the Thermo Scientific Environmental Chamber was confirmed as a suitable answer to the controlled thawing challenge. The iterative, data-driven decision making utilized by the complementing engineering teams resulted in a designed thaw procedure within identified chamber conditions that met all the pre-defined requirements. The thaw of four ultra-cold DP cassettes from -70 °C to refrigeration temperature was completed within 15 hours, with the four cassettes thawing within an hour of one another. Additionally, the DP was monitored from multiple positions both within and surrounding the cassette with thermocouples, and at no point did any of the DP reach temperatures above 25 °C, ensuring crucial protection of CTQ characteristics. With the pre-method development completed, the final protocol optimization and validation could be completed quickly by the customer at their own origination and fill/finish sites.

Conclusion

As global coronavirus vaccination efforts shift from development research to full-scale manufacturing and deployment, continuous drug product management and repeatable, robust production procedures are key. The Thermo Scientific Environmental Chambers ultimately selected to complete a controlled thawing process in the bulk production of vaccine drug product, provide independent temperature and humidity control in an isolated environment. The international nature of traditional F/F nodes for vaccine rollout adds complexity to the use of standard operating procedures for a downstream process, but the Forma Environmental Chamber is available globally, ensuring that validated protocols can be implemented unchanged by region.

Protecting vaccine viability and immunization potency must be the highest priority, complementary to the WHO directive to help ensure fair and equal access to safe vaccination. While new technology vaccines are safe and effective, meeting the need of global vaccination has introduced new supply chain challenges that require careful thought to ensure the value of vaccination is not compromised by uncontrolled conditions during vital production and deployment steps.

The coordination displayed by the Thermo Fisher team in close collaboration with a critical pharmaceutical customer was crucial in achieving a robust, timely solution to thaw bulk vaccine drug product. The teams met routinely for over three months to select testing options, design experiments, and discuss results. Thermo Fisher was able to provide technical expertise and testing while chambers were being built and shipped worldwide to aid during this unprecedented situation.

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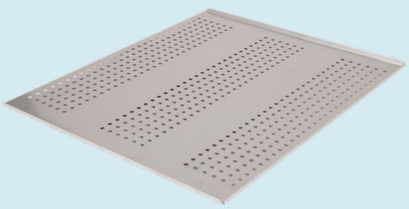
Thermo Scientific Environmental Chambers provide a controlled temperature and relative humidity environment. With a temperature range from 0°C to 60 °C, and a relative humidity range up to 95% RH, these chambers offer the ability to mimic a wide variety of climatic conditions. The Thermo Scientific Directed Airflow System promotes an ideal environment with optimum temperature uniformity and recovery, even when the chamber contains a large product load. Enhanced air flow design includes a positive pressure feed plenum on the right side of the chamber and a negative pressure return plenum on the left to consistently distribute the airflow uniformly throughout the chamber. Even when filled with a large volume of frozen product, each shelf receives a consistent flow of air. Standard 4 to 20 milliamp output connect to most alarm and monitoring systems meet internal and regulatory product documentation.



Ordering information

Product	Description	Cat. No.
Thermo Scientific™ Forma™ Environmental Chamber	821.2 L Stainless steel chamber with infrared CO2 sensor, laminar air flow, humidity control up to 95% RH, and 0 – 60 °C temperature range. Meets IICH, FDA, TAPPI, and ASTM™ standards. Available in the Americas.	16491125
Thermo Scientific™ Environmental Chamber	821.2 L Stainless steel chamber with infrared CO2 sensor, laminar air flow, humidity control up to 95% RH, and 0 – 60 °C temperature range. Meets ICH, FDA, TAPPI, DIN12880 and national testing standards. Available outside of the Americas.	16491125
Stainless steel shelf kit	Solid stainless steel shelf with channels.	15503771
Perforated stainless steel shelf kit	Perforated shelf with channels.	15523771

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