Low Risk Frozen Distribution of Single-Use Bags for Bioprocessing

Entegris

INTRODUCTION

As biopharmaceutical therapy development and manufacture proliferates on scale-up in biosimilars and scale out in emerging cell and gene therapies, the successful distribution between process sites becomes vital for the supply chain. These steps are often decentralized for added manufacturing flexibility since final fill and finish processes are not bound by time-dependent active pharmaceutical ingredient (API) product factors and can therefore be utilized at a higher rate than API production processes. As the global clinical pipeline expands with more complex and temperature-sensitive products, the distribution of high value API's between development sites, clinical sites, and to drug manufacturers for further processing will become a more relied upon paradigm. This is the case in the ongoing race to develop treatments for COVID-19, which requires a temperature-controlled distribution network that can quickly scale-up to meet huge demands. Cold chain dependent pharmaceuticals are projected to grow twice as fast as the industry overall with the 2022 expectation that 30 of the 50 top global biopharma products will require cold chain handling.[1] As cold chain grows, effectively managing and mitigating its associated risks will be critical.

Cold chain consists of the freezing of heat liable APIs to below subzero temperatures for storage and transport followed by thawing for their subsequent manufacturing and production. The current implementation of single-use bag technology is reducing operating costs and mitigating the contamination risk of traditional stainless steel freezing tanks and bottles through batch flexibility, lower storage density, and controlled freezing all in a sterile closed system. However, under frozen temperature these single-use components are brittle and prone to failure that can lead to product loss and contamination, especially during transportation. In addition, transport can be a nebulous step where it is the only part of the production process that is outside of the manufacturer's process control. Even within the cold chain end-to-end process (Figure 1) the frozen distribution of product is where significant failure can occur from various unknown mechanical and thermal shocks experienced along its route. Mechanical damage typically results in 3 – 5% product loss from bag breakage in frozen bulk drug cold chain handling while most pharma end users see temperature excursions in their shipments. 43% of those end users see excursions that exceed four degrees, which is enough to harm their product.[2] To further quantify this impact, the industry sees a $35 billion loss annually from failures in temperature-controlled logistics.[2] It is vital then to have a single-use product packaging system that functions under these conditions but often, these systems are not appropriately qualified to avoid failure or maintain temperature because their challenging distribution environments are not fully understood. Fortunately, the risks of single-use bags in frozen shipping can be mitigated by following the approach of:

- employing sensors for monitoring critical shipment parameters
- utilizing more suitable low-temperature materials and packaging
- qualifying and evaluating through simulated standards and testing in real-world transit lanes

Implementing these guidelines increases visibility in your distribution lane, incorporates robust materials, and qualifies them against relevant metrics to ensure consistent product quality in cold chain.

This paper will discuss the results of applying this approach to a frozen shipping study of single-use fluoropolymer bags.

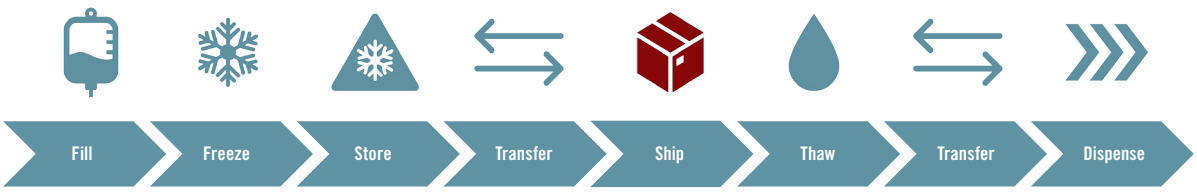

Figure 1. Cold chain end-to-end process workflow

RISK MITIGATION OVERVIEW

The three-step risk mitigation approach can be further defined. The first step is to understand the distribution environment and the specific hazards that a shipment will be experiencing. This is based on the geographical locations of processing sites, transport vehicles, handling points between vehicles, climates, and times of year, etc. Failure to understand these factors poses the worst-case risk of product loss but at minimum, batch-to-batch inconsistency. Temperature, shock/vibration, and location are the most critical parameters to track and using shipment monitors and indicators provide data that brings more control to the frozen distribution step.

The second step is to select the primary packaging for the biological products, the single-use bag assemblies, based on their ability to protect, store, and identify the sensitive product. The bag assembly is the first line of defense so beyond the standard requirements of sterility, low extractables and leachable profile, and chemical inertness, low-temperature functionality and robustness must also be required. This extends to the secondary packaging as well which is typically a shell or holder the bag is encased in to facilitate interfacility handling and shipping. The secondary container is recommended for frozen distribution.

The third step is qualifying the single-use packaging system with test methods that reflect the transit lane the product is expected to be transported through. Regulatory bodies do not provide specific guidance on how to qualify shipments but require the end user to provide evidence with high assurance that the process will maintain product quality. This is typically done through the ASTM D4169 or ISTA 3 series lab simulated test standards and in some cases, monitored real-world shipping tests between the actual processing sites. Both types of tests have limitations and do require input from the distribution environment to select the specific tests that are most relevant to the shipment. With high-value frozen product in a high-risk process step, the combination of both is recommended.

Frozen Distribution

Cold chain distribution occurs at chilled temperatures of 2° to 8°C (36° to 46°F) in some cases and down to cryogenic or below -150°C (-238°F) in others. Most frozen biopharmaceutical storage and transport occurs at approximately -80°C (-112°F), which is where protein-based drugs such as monoclonal antibodies and vaccines are below their glass transition temperature, the point where amorphous motion stops to ensure long-term preservation. Because of their low cost, single-use insulated corrugate shippers are typically used to maintain this low-temperature environment along with phase change materials like ice packs, gels, and dry ice. Dry ice sublimates at -78.5°C (-109°F), which then becomes the perfect shipping medium to hold this temperature range over short periods. It is important to note that from a regulatory perspective, dry ice is considered hazardous material and to safely transport, will require proper documentation and labels, in addition to those for the biological material.

Test Methods and Materials

The principles of the low risk frozen distribution approach were applied to a study of single-use bags. Aramus® 2D single-use bags,[3] from 500 mL to 10 L sizes, were used for their superior low-temperature durability.[4] Additionally, high density polyethylene (HDPE) with stainless steel (SS) freezing shells[5] and aluminum cassettes[6] were used in some configurations as the secondary container to provide additional protection, (Figure 2). Each bag size was tested at least once in a bag only and a bag encased in a shell/cassette configuration for comparison. Gamma sterilized bags were filled, frozen, and tested inside of dry ice-filled insulated shippers by means of a real, temperature-monitored, shipment between two locations as well as a lab simulated ASTM D4169 test. Two sets of bags were used for this evaluation; one for the real distribution and the other for ASTM lab simulation. The test plan overview is shown in Figure 3 and the list of materials used is in Table 1.

Figure 2. Aramus 2D bag assembly, freezing shell, and cassette

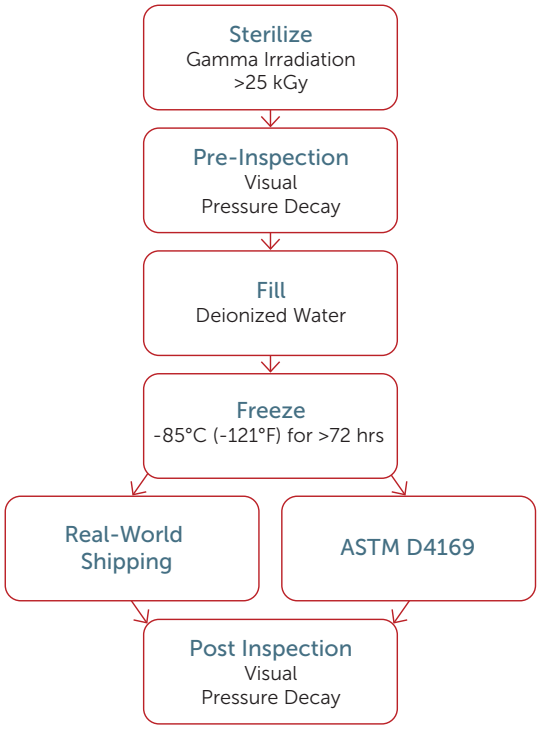

Figure 3. Test plan overview

Material	Brand	Part number
Aramus 500 mL	Entegris	SU-2D-00.5
Aramus 1 L	Entegris	SU-2D-0001
Aramus 5 L	Entegris	SU-2D-0005
Aramus 10 L	Entegris	SU-2D-0010
Cassette, 500 mL	Entegris	SU-FS-0.50-C1
Freezing shell, 1 L	Entegris	SU-FS-0001-01
Freezing shell 5 L	Entegris	SU-FS-0005-01
Freezing shell, 10 L	Entegris	SU-FS-0010-01
Small shipper	ThermoSafe	EPS126UPS
Medium shipper	ThermoSafe	EPS731UPS
Large shipper	Intelsius	PHT230
Temperature sensor	Sensitech	TempTale® dry ice

Table 1. Material list

Test Method Specifics

- All bags were gamma irradiated above 25 kGy and visually inspected pre use. They were also integrity tested using a pressure decay test that has the capability to detect minimum defect size of 30 µm.
- Bags were filled with deionized water and frozen in a standard laboratory freezer at a setpoint of -85°C (-121°F) for a minimum of 72 hours.
  - For shell/cassette configurations, empty bags were placed within the secondary container then filled and frozen *in situ*.
  - Bags were packed by being placed at the bottom of their respective shippers and filled with dry ice until the shipper was full.
  - For bag only configurations above 500 mL, a 1" thick gray urethane packaging foam was placed under and on top of the bags prior to filling box with dry ice.
  - No additional foam was used for shell/cassette configurations.

Real-World Shipping

- Each shipper underwent this procedure for the real-world shipping test:
  - A temperature sensor was placed inside to monitor the shipment
  - Shipped round trip from Bloomington, Minnesota to Billerica, Massachusetts
    - 2800 miles (4500 km), one-way via truck and one-way via air (Figure 4)

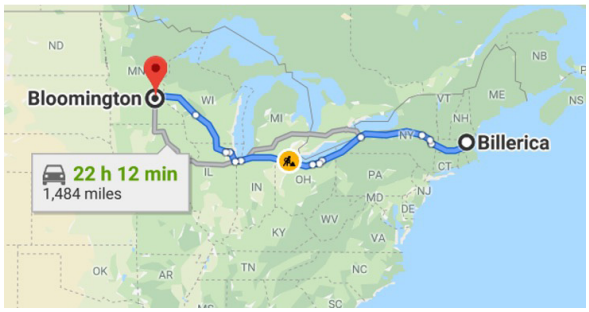

Figure 4. Real-world shipping transit route

ASTM D4169 Assurance Level 1

- Each shipper underwent this procedure for the simulated shipping test:
  - One 18" horizontal drop
  - Two 16" rotational drops on two edges
  - 60 minutes of truck vibration
  - 120 minutes of air vibration
  - Two 16" rotational drops on two edges (remaining two not tested)
  - One 18" horizontal drop
- Shippers were inspected for damage and then unpacked with the bags removed.
  - The temperature sensors were removed from the shipping test packaging
- Bags were set on a bench to thaw at ambient temperatures.
- Bags were drained and visually inspected for damage. Pressure decay integrity testing was repeated to confirm the presence of any leaks or damages.

Table 2 summarizes the test setup with bag sizes, fill volumes, secondary containers, and shippers used. Note that bag sizes that are compatible with a specific shipper can also fit into larger shipper sizes. Also, very large dry ice quantities were used to keep the bags cold for as long as possible. Figure 5 showcases the packing configurations used for the bag inside the insulated shipper.

Bag size	Fill volume	Container	Compatible shipper	Minimum dry ice
500 mL	0.42 L	Cassette	Small	50 lbs (22.7 kg)
1 L	0.9 L	Shell	Small	50 lbs (22.7 kg)
5 L	4.2 L	Shell	Medium	80 lbs (36.3 kg)
10 L	8.1 L	Shell	Medium and large	80 lbs (36.3 kg) and 115 lbs (52.1 kg)

Table 2. Test setup summary

Figure 5. Packing configurations

RESULTS

Real-World Shipping

A total of eight shipments were tested to provide data for shipping between 500 mL and 10 L frozen bags. The total fluid volume per shipper ranged from 820 mL to 24.3 L. Every shipment successfully maintained internal temperatures below -65°C (-85°F) for up to one week during the distribution cycle. Most shipment durations were longer and had average temperatures above -70°C (-94°F). The graph below illustrates the shipper internal temperature throughout the distribution cycle from packing out to unpacking on receipt and can be summarized in the accompanying table.

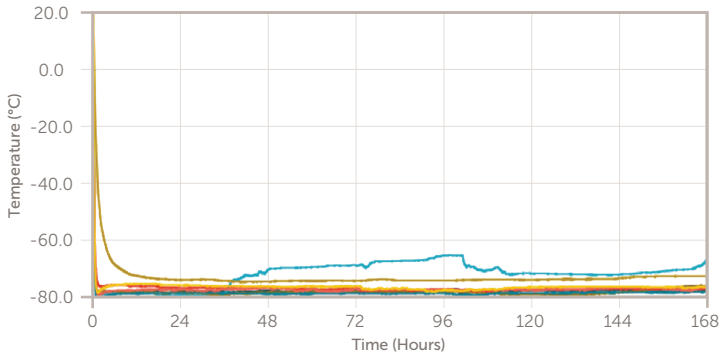

Figure 6. Temperature-time shipment profiles

Shipper	Content(s)	Duration	Average temperature	Fluid volume
Small	500 mL bag	11 days	-78.2°C (-108.8°F)	0.82 L
Small	500 mL bag in cassette	11 days	-77.5°C (-107.5°F)	0.82 L
Large	1L bag and 2× 1L bags in shells	8 days	-70.9°C (-95.6°F)	2.7 L
Medium	5L bag	11 days	-73.4°C (-100.1°F)	4.2 L
Medium	5L bag in shell	11 days	-77.7°C (-107.9°F)	4.2 L
Large	5L bag in shell	7 days	-77.7°C (-107.9°F)	4.2 L
Medium	10L bag	8 days	-77.6°C (-107.7°F)	8.1 L
Large	3× 10L bags in shells	11 days	-72.9°C (-99.22°F)	24.3 L

Table 3. Real-world shipping temperature summary

These observed temperatures are colder for longer than the shipper manufacturer's rating due to over-packing dry ice, however, this creates the most extreme thermal conditions for determining true single-use component compatibility in frozen transport. In addition, pallet damage was noted on several occasions during testing, highlighting the variability of shipping conditions. Severe damage was seen on two occasions to both the pallet and the shipper requiring a new pallet on return in one case, Figure 7.

Figure 7. Damages to pallet on return receipt

Under these conditions the Aramus bag assemblies that shipped in cassettes and shells did not have any observable defects or failures but there was a failure for the 10 L bag-only shipment. This had an approximately 3.3 mm tear on the inner perimeter weld on the upper side of the bag as seen more clearly using red penetration dye, Figure 8. There was also no damage or leaks in the tubing, fittings, or connections used in any of the bag assemblies and apart from this 10 L bag, they all passed pressure leak testing. The most likely cause of the bag failure is severe handling during transport. Figure 7 shows the medium shipper that contained the 10 L bag in the two right images. Upon receipt, it was also observed that the bag and temperature sensors had shifted inside the shipper.

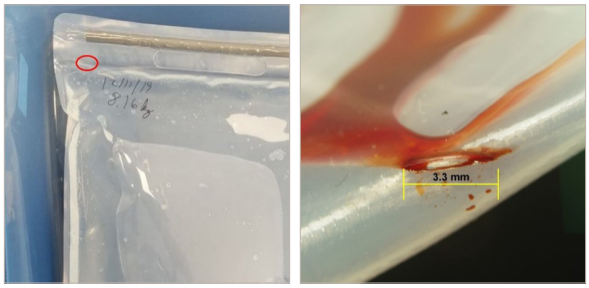

Figure 8. Failure location in 10 L bag

ASTM D4169

The medium and large shipper were used to hold the bag-only and bag in shell/cassette samples respectively. The 10 L bag-only size was not tested due to unavailability at the time of this study. Figure 9 shows images of the drop test and vibration equipment used on the samples inside the medium shipper.

Figure 9. ASTM D4169 AL1 drop and vibration tests

After all samples were tested, the 5 L bag-only configuration failed with a significant film break on one corner of the inner weld, Figure 10. Apart from damage to this 5 L bag, no other damage or leaks in the tubing, fittings, or connections used in the other bag assemblies were observed and these bags passed pressure leak testing.

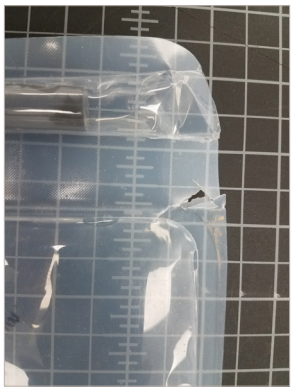

Figure 10. Failure location in 5 L bag

The overall bag integrity results of this study, real-world and simulated ASTM shipping, can be seen in Table 4. All bags encased in a secondary container passed both types of distribution testing. The larger the bag size, the more mechanical and thermal constraints on the container which points to the need for secondary shipping protection at higher volumes.

	REAL-WORLD SHIPPING		MODIFIED ASTM D4169	
Bag sizes	**Bag only**	**Bag in shell/cassette**	**Bag only**	**Bag in shell/cassette**
500 mL	Pass	Pass	Pass	Pass
1 L	Pass	Pass	Pass	Pass
5 L	Pass	Pass	Fail	Pass
10 L	Fail	Pass	N/A	Pass

Table 4. Distribution study results summary

This is reflected in the bag-only test failures with the 5 L bag during the ASTM test, but not during the real-world shipping, which also supports this test being more stringent, especially at assurance level 1. At the same time, the 10 L bag failure during real-world shipping suggests actual transit can run into issues of mishandling that are not represented by simulated standards. Regarding temperature, shipment monitoring confirmed successful temperature maintenance during transport.

Discussion

Drugs that require cold storage and transport must be treated with additional care regarding the materials and methods used to do so. A highly regulated industry such as bioprocessing cannot have gaps in controlling the transportation process. Closing the gaps starts by asking questions like,

- "What are my product's sensitivities (temperature, UV light, pH, etc.)?"
- "What does my distribution lane look like (climate, vehicles, distance, time, etc.)?"
- "Which single-use materials are compatible with my product AND robust at low-temperatures?"
- "What are my filling, freezing, thawing, and dispensing process requirements?"
- "How do I continue to monitor product quality once a shipment leaves a processing site?"

These inquiries help identify a single-use cold chain system that is compatible with a manufacturer's product and processes and reduces the associated risks of frozen distribution. The specific risks include environmental hazards in transit, brittle single-use components, and an improperly qualified product-packaging system. The mitigation strategy presented reduces these risks by recommending parameter monitored shipments, robust, low-temperature materials, and a comprehensive qualification based on input from the shipment's monitored data.

The single-use Aramus bag assembly distribution study connects to this three-part approach:

Study	Distribution Environment	Product Fragility	Relevant Test Methods
Input	Used temperature monitoring	Used robust, low-temperature bags, shells, and cassettes	Conducted real-world shipping and ASTM D4169
Output	Successfully maintained temperature through transit	Shells and cassettes ensured that all bags passed integrity	ASTM more stringent while real world has other variables unseen in lab simulations

Figure 11. Low risk frozen distribution summary

The testing of both real and standardized frozen transport highlighted the gaps of each and the need for using both to comprehensively qualifying a product-packaging system in a distribution lane. The selection of robust, low-temperature bags, shells, and cassettes ensured that the first lines of defense for product integrity were upheld as much as possible. Finally, the use of temperature monitoring confirmed the product would stay at its optimal state to maintain product quality.

This study highlights the steps that can be taken to reduce risk in the single-use bag frozen distribution process and provide reproducibility independent of what may happen in your transportation lane. In addition, it provides guidance on the use of Aramus 2D single-use bag assemblies for cold-chain transport.

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