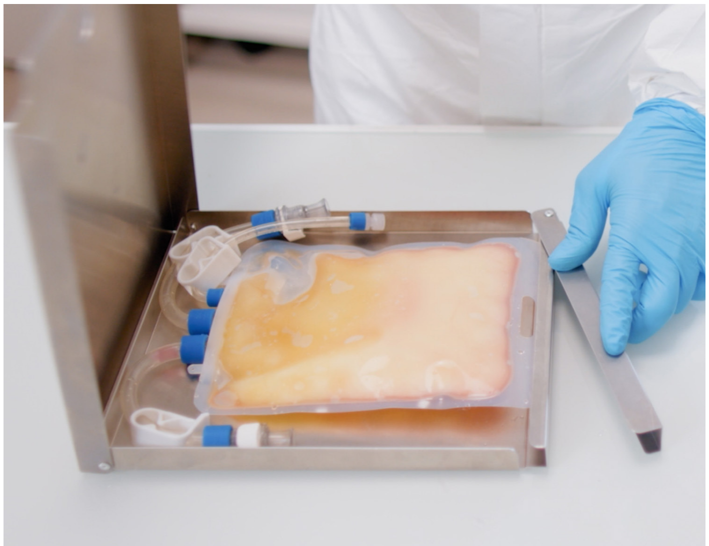
Aramus™ Single-Use Bag Assemblies

Cryogenic temperature compatibility in vapor phase liquid nitrogen



OBJECTIVE

Determine the cryogenic temperature compatibility of the Aramus™ bag assembly material through freezing and drop testing after 24-hour minimum immersion in the liquid nitrogen (LN_2) vapor phase.

SUMMARY

Aramus 50 mL, 250 mL, and 500 mL bags were tested for frozen compatibility at LN_2 temperatures (-196°C [-321°F]) by multiple freeze/thaw cycles and a single frozen drop test. In addition, the freeze rates and temperature profiles were monitored. All bags were exposed to above 25 kGy gamma irradiation prior to testing.

The results demonstrate Aramus bags:

- Achieving near -1°C/min (-1.8°F/min) optimal cooling rates while in cassettes
- Enduring 3x freeze/thaw cycles at -196°C (-321°F)
- Resisting frozen expansion at 110% of their recommended fill volume
- Surviving -196°C (-321°F) frozen drop tests from 16" (40 cm)

This study expands upon Aramus bag use in cell and gene therapy (CGT) applications and complements the study on Aramus single-use bags chemical compatibility with 100% dimethyl sulfoxide (DMSO), a common cryoprotectant.[1]

MATERIALS AND EQUIPMENT

12 Aramus bag assemblies of each of the following sizes: 50 mL, 250 mL, and 500 mL, with Oetiker retainers and EVA tubing were gamma irradiated above 25 kGy. Each bag was filled with DI water and tested with its corresponding aluminum cassette. See appendix for details.

Table 1. Equipment used

Instrument	Calibrated date	Calibration due
-85°C (-121°F) freezer	03/12/2020	03/12/2021
Liquid nitrogen dewar	N/A	N/A
T-type thermocouples	03/12/2020	03/12/2021
Pressure decay integrity test	01/27/2020	01/27/2021
Meter stick	N/A	N/A

RESULTS

Cooling Rates

One bag of each size was selected, and a surface thermocouple was placed on the middle of each. The bags were filled to their recommended volumes, placed within aluminum cassettes, and horizontally stored into a -85°C (-121°F) lab freezer with the thermocouple oriented on the bottom face of the bag, the last point to freeze in this case.

Table 2. Aramus bag assembly cooling rates

Bag size	Fill volume	Surface area: volume ratio (Sa:V)	Cooling rate 20° to -40°C (68° to -40°F)
50 mL	50 mL	3.20 cm²/mL	-1.46°C/min (-2.63°F/min)
250 mL	200 mL	1.91 cm²/mL	-1.25°C/min (-2.25°F/min)
500 mL	350 mL	1.44 cm²/mL	-0.99°C/min (-1.78°F/min)

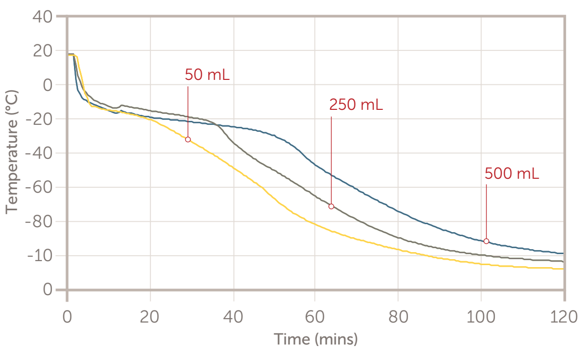


Figure 1. Time vs. temperature profile for freezing Aramus bag assembly in a cassette.

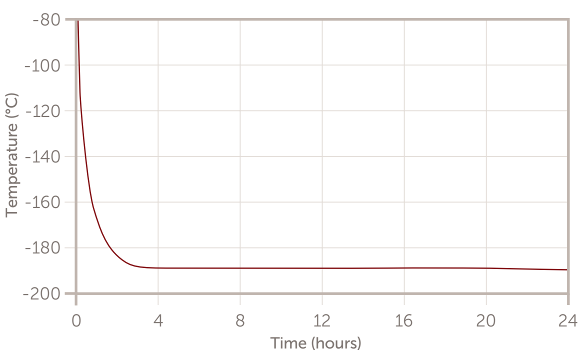


Figure 2. Time vs. temperature profile for 50 mL bag in LN_2 dewar.

All bags had cooling rates close to the desired -1°C/min (-1.8°F/min), a rate known to be optimal for cell survival, Table 2. The overall time vs. temperature profile can be seen in Figure 1. In addition, we see the trend that the higher fluid surface area to volume (SA:V) ratio leads to a faster cooling rate. Higher SA:V means a lower fill volume so this can be used to optimize cooling rates and ensure consistent scalability from 50 mL to 500 mL for specific applications. Note, though a standard lab freezer was used, cooling rates can vary by size of freezer, number of samples, and storage location within freezer.

For reference, the time vs. temperature profile was monitored for the 50 mL bag when stored in the LN_2 dewar after freezing in a -85°C freezer, Figure 2.

3x Freeze/Thaw Cycle

12 Aramus bags for each bag size were filled to its tested fill volume, which was at least 110% of its recommended maximum fill as a worst-case scenario. The bags were then frozen in aluminum cassettes at -85°C (-121°F) and then stored inside a liquid nitrogen dewar at -196°C (-321°F) for >24 hours. Afterwards the bags were thawed (ambient 20°C (68°F) with circulating air) and inspected. This was repeated three times then the bags were drained, and integrity tested by pressure decay method with a 30 µm leak detection limit.

All bags passed the three freeze/thaw cycles with no visual damages or defects and they passed the integrity test at the end of all three cycles.

Table 3. Results of 3x freeze/thaw cycles at -196°C (-321°F)

Bag size	Recommended fill	Tested fill	Visual inspection	Integrity test
50 mL	50 mL	55 mL		Pass
250 mL	200 mL	225 mL		Pass
500 mL	350 mL	400 mL		Pass

Robustness Tests

The 10-12 bags used for the 3x freeze/thaw cycles were reused for a worst-case drop test. The bags were filled to their recommended fill volumes, frozen in cassettes first at -85°C (-121°F) for at least four hours, then at -196°C (-321°F) for >24 hours. Upon removal, the bags were immediately subjected to a 16” (40 cm) drop test onto the floor. This drop height was determined to simulate accidentally dropping a cassette from chest height onto a bench. This was measured at about 14-16” (36-40 cm) and the higher end of the measurement was taken for the test, Figure 3. Afterwards, the tests were repeated with bags from the 3x freeze/thaw study but at a drop height of 20” (50 cm) as a more severe proof point. All bags were inspected post drop, post thaw, and integrity tested.

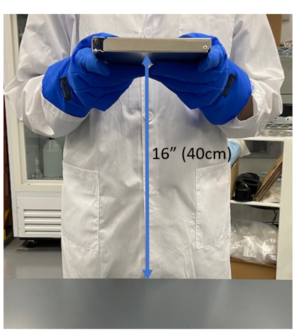


Figure 3. Drop height reference.

Table 4. Frozen drop test results

Bag size	16" (40 cm): % pass	20" (50 cm): % pass
50 mL	6/6 (100%)	3/5 (60%)*
250 mL	5/5 (100%)	5/5 (100%)
500 mL	7/7 (100%)	3/5 (60%)

**One failure due to improper clamp positioning*

All bags dropped at 16” (40 cm) had no visual damage to the bag or cassette and passed integrity testing. As a result, these bags were subject to a cumulative of 4x Freeze/thaw cycles (3x at >110% fill) and a 16” (40 cm) drop test and still maintained their integrity. At the 20” drop height, some failures were observed for the 50 mL and 500 mL sizes but not for the 250 mL bags. The failures were clearly visible post drop in the form of film breaks near the bag welds. One 50 mL bag failure was from improper positioning of the tubing clamp on top of the bag seam, which on impact created a crack in the film.

APPENDIX

Bag size	Bag part number	Cassette part number
50 mL	SU-2D-0.050	SU-FS-0.05-C2
250 mL	SU-2D-0.250	SU-FS-0.25-C2
500 mL	SU-2D-00.5	SU-FS-0.50-C2

REFERENCES

[1] *Aramus Single-use Bags Compatibility with Dimethyl Sulfoxide (DMSO) Technical Note.* (2019, December 16). Retrieved from https://www.entegris.com/content/dam/product-assets/aramus2dsingleuseassembly/technote-aramus-single-use-bags-10849.pdf

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