Regulatory Compliance and Validation Summary for Aramus™ Single-Use 2D Bag Assemblies

High-grade, gamma-stable fluoropolymer film providing higher purity and greater reliability

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1. Introduction

Aramus™ single-use 2D bag assemblies are made of a high-grade, gamma-stable fluoropolymer, providing higher purity, greater compatibility, and increased safety for critical process fluids and final products. With a new single-layer technology that contains no curing agents, antioxidants, plasticizers, or adhesives, the number of potential contaminants is greatly reduced. These assemblies offer a wide operating range and are durable in frozen applications (-196° to 40°C [-321° to 104°F]). This guide pertains only to Aramus gamma-stable, fluoropolymer 2D assemblies.

2. Scope

The scope of this document includes the standard Aramus product assigned to part number format SU-2D-XXXXXX-XXXXX (see part number explanation below). For custom part numbers that use customer specified components following part number formats SU-ADM-XXXXXX and SU-2D-XXXXXX, elements in this validation summary may or may not apply. Contact your Entegris sales representative for regulatory compliance information and qualification data for custom part numbers.

Aramus Assemblies (3-port): part number

SU - 2D - _____ __ __ _____ - __ __

Fasteners
O = Oetiker SS ear clamps
B = BarbLock® clamps
Z = Zip ties*

Sample connectors
1 = Luer female
2 = Luer male

Main connectors
CFM = MPC – *A:* female *B:* male
CFF = MPC – *A:* female *B:* female
CMF = MPC – *A:* male *B:* female
CMM = MPC – *A:* male *B:* male

Note: See drawing below for A and B indicators for part numbers

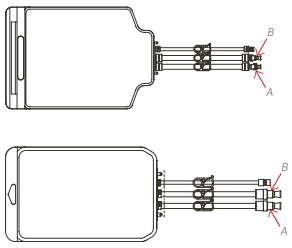
Tube length
0 = 6"
1 = 12"
2 = 18"
3 = 24"

Tube material
F = AdvantaFlex® thermoplastic elastomer
P = AdvantaSil® silicone
S = Tygon® silicone
T = C-Flex® 374 elastomer

Volume
00.5 = 500 mL
0001 = 1 liter
0002 = 2 liter
0005 = 5 L
0010 = 10 L
0020 = 20 L
0050 = 50 L*

Product type
2D = 2D bag

Product family
SU = Single-use



Notes: Assemblies sold sterilized and are individually double bagged.
**Not a standard product, but is available upon request.*

Aramus Assemblies (2-port): part number

SU - 2D - _____ __ __ -

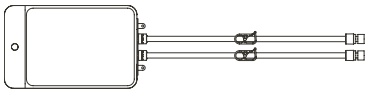
Fasteners
O = Oetiker SS ear clamps
B = BarbLock clamps
Z = Zip ties*

Main connectors
L11 = Luer – 1: female 1: female
L12 = Luer – 1: female 2: male
L22 = Luer – 2: male 2: male

Tube length
0 = 6"
1 = 12"
2 = 18"
3 = 24"

Tube material
F = AdvantaFlex thermoplastic elastomer
P = AdvantaSil silicone
S = Tygon silicone
T = C-Flex 374 elastomer

Volume
0.020 = 20 mL
0.050 = 50 mL
0.100 = 100 mL
0.250 = 250 mL



Product type
2D = 2D bag

Product family
SU = Single-use

Notes: Assemblies sold sterilized and are individually double bagged.
**Not a standard product, but is available upon request.*

3. Regulatory Overview

Entegris has a long history of environmental compliance in countries of operation/distribution. Entegris actively reviews products for compliance and conformance with government and customer requirements affecting raw materials/substances that may be used in manufacturing processes. Entegris relies on information provided by the suppliers as the foundation for the listed regulatory statement, and Entegris makes no representation or warranty about any such testing.

Ultimately, customers must determine that use of this product is safe, lawful, and technically suited for their intended application and purpose, and Entegris assumes no liability for any loss or injury that may result from the use of the information contained in this overview. Table 1 represents compliance claims for fluid path items acquired at the time of publishing this validation summary for components used in the standard Aramus assembly.

Table 1. Fluid path compliance

COMPONENT	LATEX	ADCF	BPA	PHTHALATES	GMO	ALLERGENS
Aramus bag subassembly	Free	Free	Free	Free	Free	Free
C-Flex tubing	Free	Free	Free	Free	Free	Free
Tygon tubing	Free	Free	<DL[1]	Free	Free	Free
MPC connectors	Free	Free	Free	Free	Free	Free
AdvantaFlex tubing	Free	Free	Free	Free	Free	Free
Nordson luers	Free	Free	Free	Free	Free	Free
APST silicon tubing	Free	Free	Free	Free	Free	Free

COMPONENT	REACH	21CFR 177	USP <661>	USP <85>	USP <87>	USP <88>/ CLASS VI	ISO 10993
Aramus bag subassembly	Meets	Pass[2]	Pass	Pass	Pass	Pass	Pass
C-Flex tubing	Meets	Pass	Pass	Pass	Pass	Pass	Pass
Tygon tubing	Meets	Pass	Pass	Pass	Pass	Pass	Pass
MPC connectors	Siloxanes[3] >0.1% by weight	Pass	Pass[4]	Pass	Pass	Pass	Pass
AdvantaFlex tubing	Meets	Pass	Pass	Pass	Pass	Pass	Pass
Nordson luers	Meets	Pass	Pass	Pass	Pass	Pass	Pass
APST silicon tubing	Siloxanes[3] >0.1% by weight	Pass	Pass	Pass	Pass	Pass	Pass

[1]Detection Limit

[2]The resin (film and fitment) used to manufacture subassemblies passes 21CFR177.1550

[3]According to documentation provided by suppliers on file, the silicone elastomers are expected to contain the below-listed siloxanes above the allowable limit of 0.1% w/w: Octamethylcyclotetrasiloxane (D4) (CAS 556-67-2), Decamethylcyclopentasiloxane (D5) (CAS 541-02-6),Dodecamethylcyclohexasiloxane (D6) (CAS 540-97-6)

[4]The resin used to manufacture MPC connectors passes USP<661>

Medical Device Disclaimer

Aramus is not approved/cleared as a medical device by any regulatory authority, therefore cannot be used as a delivery device to inject drug and/or administer therapy directly into a human unless validated for the specific intended use. Entegris can assist in providing all the appropriate documentation as well as support any additional testing, if required, to assist in any required regulatory filings, in conjunction with our customers in the US, Europe, and extended through international CDMO, CRO networks.

4. Certificate of Analysis

Aramus single-use assemblies will be shipped with a Certificate of Analysis and Gamma Irradiation Certificate (if product is irradiated).

5. Manufacturing Environment

Aramus subassemblies are manufactured in an ISO Class 5 cleanroom. Final assembly occurs in a minimum ISO Class 7 cleanroom. Entegris certifies each batch has been manufactured and tested according to approved procedures and specifications under a quality system certified to ISO 9001.

6. Materials of Construction

Aramus bags are made of a single material that is a consistent base polymer. No other materials/substances are added to the subassemblies by Entegris in the manufacturing process.

This base polymer, used in the boat fitments and used to extrude Aramus film, which is provided to Entegris or its supplier(s), is formulated/manufactured to Entegris' specifications.

In the manufacturing of the film no curing agents, antioxidants, plasticizers, adhesives, or colorants of any type are intentionally used by the film extruder. Furthermore, the vendor certifies that they do not expect those above substances would be formed as byproducts or would otherwise be present in the film.

The Aramus film is an 8-mil fluoropolymer film that was selected for use in the biopharmaceutical industry. The material features excellent chemical compatibility, low temperature performance, and gamma radiation stability.

Aramus single-use 2D bags are typically provided to customers after gamma irradiation, therefore it is of paramount importance that the film be compatible with this energy source and not exhibit degradation of physical change. The materials testing summary (Table 2) provides the evidence of the film's gamma stability. Here samples of film and welded film underwent a variety of physical measurements and results have been compared pre- and post-gamma to demonstrate Aramus film's compatibility with the gamma sterilization process.

Table 2 shows that the Aramus film mechanical properties, including welded film, have maintained their values after gamma processing. Gas permeation and optical properties of the bags have also been maintained pre- and post-gamma. The results of permeation testing demonstrate that Aramus bags will maintain their container properties and not expose a contained fluid to the environment post-gamma processing. Optical properties further indicate that the material remains stable and is minimally impacted by gamma irradiation.

Table 2. Materials of construction testing

TEST	SPECIFICATION	PRE-GAMMA[1]	POST-GAMMA
Mechanical testing			
Puncture resistance	ASTM F1306	9.3 lbf	9.6 lbf
Tensile test - MD[2]	ASTM D882	7880 psi	6450 psi
Tensile test - TD	ASTM D882	7940 psi	7440 psi
Elongation - MD	ASTM D882	677%	597%
Elongation - TD	ASTM D882	590%	602%
Seal strength[3]	ASTM F88	32.3 lbf	32.0 lbf
Permeability testing			
CO_2 permeability	ASTM F2476[4]	1082 cc/(m² day)	864 cc/(m² day)
H_2O permeability	ASTM F1249	0.81 g/(m² day)	0.70 g/(m² day)
O_2 permeability	ASTM D3985	234 cc/(m² day)	219 cc/(m² day)
Optical testing			
Haze	ASTM D1003	11.7%	14.2%
Luminous transmittance	ASTM D1003	92.7%	92.7%
Diffuse transmittance	ASTM D1003	10.8%	13.1%

[1]Pre- vs. post-gamma testing was performed on a different set of samples from the main testing and thus the values listed may differ slightly from those presented in the main text where applicable.

[2]MD: Machine Direction, TD: Transverse Direction

[3]Seal strength test samples failed by cracking or tearing and are thus not truly measures of seal strength. This is discussed in the report text.

[4]All conditions of the standard were maintained except the test area was modified: Test area – was Larger than typical, but results presented on per area basis.

Aramus single-use 2D bag assemblies are designed to provide exceptional temperature stability, making them an ideal solution for fill, freeze, thaw, storage, and distribution. Therefore, it is important that the film and welds' mechanical strength be maintained over a wide temperature range. Table 3 provides evidence of the film's temperature stability. The Aramus film maintains its seal and film strength across a wide range of temperatures. It was not possible to determine the glass transition of the Aramus bag due to it maintaining a fully crystalline structure throughout the evaluation.

Table 3. Temperature stability testing

TEST	SPECIFCATION	PRE-GAMMA
Seal strength @ 40°C (104°F)	ASTM F88	26.5 lbf
Seal strength @ -20°C (68°F)	ASTM F88	25.7 lbf
Seal strength @ -85°C (-121°F)	ASTM F88	22.5 lbf
Seal strength @ -196°C (-321°F)	ASTM F88	39.5 lbf
Glass transition	ASTM D3418-12	N/A

7. Country of Origin

The Aramus product was developed and is manufactured in the United States of America.

8. Animal Derivative Content and TSE/BSE Risk

Based on information provided by suppliers, no bovine or animal derived materials are used in the manufacture of the Aramus product fluid path raw materials. The Aramus fluid path materials are not intentionally exposed to animal content during the Entegris manufacturing process. Non-fluid path peripheral items such as clamps and packaging either do not contain added animal derivatives or meet TSE/BSE treatment and manufacturing requirements defined in EMEA/410 rev 3; therefore, these items are not considered at risk for transmitting BSE/TSE as certified by the supplier.

9. U.S. Food and Drug Administration

The material used to produce the Aramus subassembly wetted surface meets FDA 21CFR 177.1550 food contact requirements. Where silicone tubing is used on the Aramus assembled product, our supplier certifies the tubing meets the FDA requirements outlined in the Code of Federal Regulations 21 CFR 177.2600(a) and (b). This conformance includes all ingredients used in the product formulation. These ingredients are compliant to their specific regulations and 21 CFR 177.2600(c). This product can be used for food contact applications with food types I, II, IV B, VI, VII-B, and VIII of Table 1 and under conditions of use C through I-I of Table 2 in 21 CFR 176.170(c).

10. Shelf Life

Aramus assemblies carry a two-year sterility and functional shelf life. Shelf life study design includes real-time and accelerated studies using aging factors and calculations per ASTM F1980. Entegris only certifies product contact surfaces as sterile. Procedures are in place to ensure the appropriate shelf life specification is printed on the packaging. Entegris does not recommend use of Aramus product beyond the product expiration. The expiration date is generated based on the date of manufacture of the assembly and not based on date of irradiation.

The shelf life samples are from the Aramus product family covering various sizes (50 mL to 20 L) of final assemblies and components. The components selected are representative of the family and assume that similar components of the same materials that are packaged, processed, and stored as specified for Aramus products will also meet the stated two-year shelf life. Entegris does not repeat aging studies on purchased components by qualified suppliers. Subcomponents used in the manufacture of Aramus assemblies can be used until their manufacturer's stated date of expiration.

Validated Aramus products that are irradiated carry a sterile shelf life claim. Any Aramus product that is sterile will have "sterile" clearly stated on the packaging and certification. Custom Aramus assemblies with non-validated components that are irradiated do not carry a "sterile" claim but will have gamma dots showing exposure to gamma radiation.

Non-irradiated product expiration dates are a mechanical functional two-year shelf life only with no sterility claims. These part numbers end in -U.

11. Gamma Sterilization

Final routine assemblies were sampled for bioburden prior to sterilization. Total bioburden is determined by summing the aerobic and yeast/mold counts. Testing was performed in accordance with ISO 11737 and ISO 11137-2; and bioburden levels met the VDmax[25] method validation requirements.

A VDmax[25] radiation validation was performed per ANSI/AAMI/ISO 11137-2; Sterilization of Health Care Products – Radiation – Establishing the sterilization dose – Method VDmax, to substantiate Entegris' minimum exposure of 25 kGy provides a Sterility Assurance Level (SAL) of 10^{-6}. Triplicate dose mapping studies were performed using packaged product to validate that the dose of 25 kGy can consistently be achieved. Entegris performs dose auditing in accordance with ANSI/AAMI/ ISO 11137-2. The SIP (sample item portion) used for the dose audits are routinely reviewed and updated with new components to ensure validated sterilization of custom assemblies, and expansion of the Aramus component library.

For sterilized shipments, the certificate of processing is provided with each shipment where the gamma exposure between 25 – 40 kGy is displayed.

12. USP <71> Sterility Testing

During the VDmax[25] radiation validation, bacteriostasis and fungistasis testing were performed prior to sterility testing to ensure the product did not adversely affect the outcome of the sterility test. The product did not exhibit any bacteriostatic or fungistatic activity and units tested post gamma verification dose passed ANSI/AAMI/ISO 11137-2 requirements.

13. USP <87> Biological Reactivity Tests, In Vitro

Cytotoxicity testing assesses the potential of a given material to have a toxic effect on living cells.

Test method: Samples of Aramus subassembly were tested by Toxikon in accordance with USP 40, NF 35, 2017; <87> Biological Reactivity Tests, In Vitro.

The test article sample was extracted in 10% Fetal Bovine Serum for 24 hours at 37 ±1°C (34°F), negative and positive controls were prepared similarly. Samples were placed directly onto mammalian monolayers of L929 mouse for 48 hours. Cultures were monitored for cellular degeneration and malformation and rated on a scale of 0 (no biological reactivity) to 4 (severe biological reactivity).

Results: The test article samples scored a grade 0 for biological reactivity after 48 hours. The negative and positive controls confirmed system suitability. The test samples were deemed to meet USP <87> criteria and not considered cytotoxic.

14. USP <88> Biological Reactivity Tests, In Vivo, Class VI Test

The USP Class VI testing assesses the potential toxicity of extracts from the Aramus subassembly into live animal models. Dosing is performed systemically, intracutaneously, and implanted. Test animals are monitored for irritation or toxicity.

Test method: The post gamma sterilized test article was exposed at a ratio of 120 cm^2 product per 20 mL extract medium that included USP 0.9% sodium chloride for injection, cotton seed oil, 1:20 ethanol in sodium chloride and polyethylene glycol 400 at 50°C ±2° (122°F ±36°) for 72 hours under dynamic conditions. These extracts were injected intracutaneously in rabbits and systemically in mice, and animals were observed for a biological response. Test article implantation into the paravertebral muscles of rabbits was observed for seven days for signs of irritation or infection.

Results: Aramus products meet criteria established per USP guidelines for Class VI Plastics 50°C (122°F).

15. USP <85> Bacterial Endotoxins Test, EP 2.6.14, JP

Endotoxins are lipopolysaccharide complexes found in gram negative bacterial cell walls. They can cause fever in humans.

Test method: The testing was performed by Pace Analytical per the Kinetic Endotoxin Test method. The test article fluid path was flushed with sterile water for injection (WFI) at 37° to 40°C (99° to 104°F). A positive product control was prepared using the test article extract and the endotoxin standard. Sterile WFI and LAL reagent water (endotoxin-free) were used as negative controls. LAL reagent was added to all test and control samples, which were then incubated and read via a plate reader.

Results: Based on the positive control, negative control, and positive product control data; the system was suitable, and the product did not interfere with the test system. The endotoxin level was determined to be below the limit of detection of the standard curve and reported as <0.0050 EU/mL.

16. USP <661.2> Physiochemical Test, Plastic Packaging System for Pharmaceutical Use

Physiochemical testing was performed to assess the safety aspects of a packaging system based on appropriate chemical assessments. The Aramus fluoropolymer does not fall under the scope of specifications for plastic materials of construction listed in USP <661.1>, which includes cyclic olefins, polyethylene, polypropylene, polyethylene terephthalate, polyethylene terephthalate G, and plasticized polyvinyl chloride; therefore, it is considered an "unaddressed material". In addition to physiochemical testing, unaddressed materials should also be tested for extractables and biocompatibility to further satisfy USP <661> requirements; refer to the applicable sections of this validation guide for the appropriate summaries (sections 13, 14, 18, 19, and 20). Alternatively, materials of a packaging system are deemed to be well characterized and appropriate for use if the requirements of USP <661.2> are met.

Test method: The test article was immersed in USP purified water at 70°C (158°F) for 24 hours. The extract was then tested for appearance, absorbance, acidity or alkalinity, and total organic carbon (TOC) per USP <661.2>, (Table 4).

Results: The Aramus products meet the requirements of USP 40-NF 35, 2017, USP <661.2>.

Table 4. Physiochemical test

TEST	CRITERIA	RESULT
Appearance of solution	Clear colorless as purified water	Clear colorless as purified water
Absorbance	Absorbance between 230 nm and 360 nm NMT 0.20 AU	<0.20 AU
Acidity or alkalinity	Solution is clear after the addition of 0.1 mL phenolphthalein	Colorless pH 6.65
	Solution is pink after the addition of 0.4 mL of 0.01 N sodium hydroxide	Pink pH 8.70
	Solution is orange-red or red addition of 0.8 mL of 0.01 N hydrochloric acid and 0.1 mL of methyl red TS	Red pH 3.56
TOC	NMT 8 mg/L	<0.01 mg/L

17. USP <788> Particulate Matter in Injections

USP <788> Particulate Matter in Injections defines testing for injectable solutions. Entegris Aramus subassembly bags and final assemblies do not fall directly under the scope of these tests since they are a container and the industry standards for particulate testing is defined for the actual filled injectable solution. Entegris understands the concern that the container may contribute particles to the customer's filled solution, so testing is executed to provide comparable evaluations as set forth in USP <788>. Entegris routinely performs this sub-visible particulate test on representative subassemblies. These representative samples are tested by introducing DI water into the subassembly and then the fluid is evaluated using USP<788> Method 1, Light Obscuration per the specifications defined in USP<788>.

VISIBLE PARTICULATE MATTER

For visible particle contamination, bags are 100% visually inspected during manufacturing. Bags are visually inspected to internal criteria which includes both loose contamination and embedded particle specifications.

18. ISO 10993-4 Hemolysis: Direct Contact

The Hemolysis test examines the potential for contact of a product sample with blood to cause the rupture of erythrocytes (red blood cells).

Test method: Samples of Aramus subassembly were tested by Toxikon in accordance with ISO 10993-4, 2002, Biological Evaluation of Medical Devices – Part 4: Selection of Tests for Interactions with Blood and ATSM F756.

Phosphate buffered saline was added to the test article at a ratio of 6 cm^2 and then incubated with rabbit blood for three hours, and then incubated at 37°C ±2° (99°F ±36°), in triplicate.

Results: The percent hemolysis resulting from direct contact of the product with rabbit blood was 0.26% above the negative control. Per ISO 10993-4, a test article is considered non-hemolytic if its percent hemolysis is <5.0% above the negative control. The test article was therefore deemed non-hemolytic.

19. ISO 10993-4 Hemolysis: Indirect Contact

The Hemolysis test examines the potential for indirect contact of a product sample with blood to cause the rupture of erythrocytes (red blood cells).

Test method: Samples of Aramus subassembly were tested by Toxikon in accordance with ISO 10993-4, 2002, Biological Evaluation of Medical Devices — Part 4: Selection of Tests for Interactions with Blood and ATSM F756.

The test article was extracted phosphate buffered saline (PBS) for 24 hours ±two hours at 70°C ±2° (158°F ±36°), and then the extract was incubated with rabbit blood for three hours at 37°C ±2° (99°F ±36°), in triplicate.

Results: The percent hemolysis resulting from direct contact of the product with rabbit blood was 0.19% above the negative control. Per ISO 10993-4, a test article is considered non-hemolytic if its percent hemolysis is <5.0% above the negative control. The test article was therefore deemed non-hemolytic.

20. Extractables

An extractables study was performed by an independent laboratory following BPOG recommended protocol. The specific time points evaluated, extraction solvents and conditions, and analysis performed are listed in Tables 5 and 6. Table 5 provides a convenient overview summary of the study results. Please contact your Entegris sales representative to request a copy of the full report as required.

Table 5. Model solvents and time points summary

SOLVENT	VOLUME	TIME INTERVALS AND CONDITIONS
50 EtOH	~ 140 mL[1]	30 minutes at 25°C (77°F) 1 day 50 rpm at 40°C (104°F) 21 days 50 rpm at 40°C (104°F) 70 days 50 rpm at 40°C (104°F)
1% PS-80		
5 M NaCl		
0.5 N NaOH		
0.1 M H_3PO_4		
WFI		

[1]*Reference included in full report*

Table 6. Analytical method and model solvent summary

	HPLC-DAD/MS							
SOLVENT	ESI (±)	APCI (±)	DI-GC/MS	HS-GC/MS	ICP/MS[1]	TOC	PH	NVR
WFI	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50% EtOH	Yes	Yes	Yes	Yes	No	No	No	Yes
1% PS-80	Yes	Yes	Yes	Yes	No	No	No	No
5 M NaCl	Yes	Yes	Yes	Yes	No	Yes	Yes	No
0.5 N NaOH	Yes	Yes	Yes	Yes	No	Yes	Yes	No
0.1 M H_3PO_4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No

[1]*Reference included in full report*

21. Design Validation

Design validation was executed to demonstrate product adherence to functional requirements. All bags tested were exposed to a minimum gamma radiation dose of 42 kGy, as a worst case, prior to testing. A cold chain shipping study is also presented here with Aramus 2D bags that were gamma irradiated above 25 kGy. The design validation included the following tests:

VOLUME CAPACITY TEST

INTRODUCTION

Aramus bags are designated a rated fill volume – the volume to which the bag can be filled and frozen without secondary containment (unconstrained freeze). Aramus bags are tested (filled and frozen) at 10% over their rated fill volume. While not recommended, volume capacity testing provides confidence that slightly over-filled Aramus bags should not result in a bag failure (leak) during an unconstrained freeze application. Custom designs will either call out the rated unconstrained volume or will have a suggested constrained freeze range on the drawing.

TEST METHOD

Tested product is filled to 110% of the rated unconstrained volume with room temperature water. The sample is later drained and assessed for leakage via pressure decay leak testing to verify the stress of over-filling has not compromised the bag.

RESULTS

All Aramus products were able to withstand filling to 110% of the rated volume. No instances of leakage were observed.

Table 7. Volume capacity test

Aramus bag size	Sample size	Volume capacity result
20 mL	30	100% Pass
36 mL	11	100% Pass
50 mL	11	100% Pass
80 mL	5	100% Pass
90 mL	11	100% Pass
100 mL	12	100% Pass
250 mL	12	100% Pass
500 mL	15	100% Pass
1 L	15	100% Pass
2 L	12	100% Pass
5 L	12	100% Pass
10 L	12	100% Pass
20 L	12	100% Pass
50 L	10	100% Pass

HANGING TEST

INTRODUCTION

The Aramus single-use 2D bags up to 20L are designed with a cut-out feature in the header for hanging the bags while full. This test demonstrates that the hanging feature can support the filled bag weight. The 50 L bag is designed to be used in a lay-flat state and does not contain any hanging features and therefore was not included in this testing.

TEST METHOD

Aramus Assemblies filled to 100% of the rated unconstrained volume with room temperature water are hung on a hook for 72 hours. The products are then inspected for film degradation or tearing and assessed for leakage via pressure decay leak testing. Samples were selected to demonstrate compliance in the worst-case configuration. All testing was conducted with at least a confidence/reliability level of 80/85.

RESULTS

No film degradation or tearing was observed and 100% of product tested passed the post-test pressure decay leak test.

Table 8. Hanging test

Aramus Bag Size	Sample Size	Hanging Result
20 mL – 250 mL	11[1]	100% Pass
0.5 L – 2 L	12[2]	100% Pass
5 L – 20 L	12[3]	100% Pass

[1]20 mL to 250 mL Aramus bags have the same header/hole design and therefore testing the 250 mL captures all smaller sizes.

[2]0.5 L, 1 L and 2 L were all tested individually at a sample size of 15 for each size.

[3]5 L to 20 L Aramus bags have the same header/handle design and therefore testing the 20 L captures all smaller sizes.

BURST TEST

INTRODUCTION

The Aramus single-use 2D bags of differing volumes do not burst at the same pressure; this is because the stress of pressure within the container is transferred across the restrained length/width of film, therefore larger bags accumulate more stress and burst at a lower pressure than smaller bags. Most of burst testing is performed as part of process validation. Burst testing is also performed in lower sample sizes during the design validation to assess the effects of gamma irradiation. Burst testing is also performed on a sampling basis in production, providing confidence that bags can remain integral if inadvertently overfilled.

50 L bags are not burst tested due to their size. 50 L bag robustness is assessed via weld tensile strength in production.

TEST METHOD

This process fills the connected bag and ramps pressure until it detects a pressure drop indicating product failure.

RESULTS

The design validation burst test results for all Aramus products are shown in Table 9.

Table 9. Burst test

Aramus bag size	Sample size	Average burst pressure
20 mL	72	35.3 psi
50 mL	96	31.8 psi
100 mL	96	24.9 psi
250 mL	96	22.0 psi
500 mL	90	16.7 psi
1 L	90	12.8 psi
2 L	90	10.2 psi
5 L	135	6.6 psi
10 L	135	5.0 psi
20 L	135	4.1 psi

FREEZE/THAW TEST

INTRODUCTION

The Aramus single-use 2D bags are designed to handle the stress of freeze/thaw cycles. Fluids expand during freezing and can expand around folds and concentrates stress at these points. A freeze/thaw cycle ensures Aramus 2D bags are able to be frozen to -85°C (-121°F).

TEST METHOD

Bags are filled to rated unconstrained volumes and frozen in a conventional freezer to -85°C (-121°F). This temperature is held for 18 hours to ensure that a steady-state temperature is achieved. The products are then removed from the freezer and air thawed until completely liquid, drained, inspected, and subjected to a pressure decay leak test further described in section 22. A passing result indicates that no leaks or other visible signs of bag damage were detected after the freeze/thaw cycles.

RESULTS

All 198 of the Aramus single-use 2D bags that were evaluated passed the criteria indicating the capability of the bags to handle the rigors of the freeze/thaw cycle.

Table 10. Freeze/thaw test

Aramus bag size	Sample size	Freeze/thaw results
20 mL	30	100% Pass
50 mL	24	100% Pass
100 mL	24	100% Pass
250 mL	24	100% Pass
500 mL	15	100% Pass
1 L	15	100% Pass
2 L	24	100% Pass
5 L	6[1]	100% Pass
10 L	6[1]	100% Pass
20 L	30	100% Pass
50 L	12	100% Pass

[1]Due to equipment limitations, sample sizes for 5 L to 20 L Aramus bags freeze/thaw testing were biased to the largest 20 L size due to this representing the worst-case configuration. 5 L and 10 L samples were also tested but at a lower sample size.

FREEZE/THAW TEST – VAPOR (NITROGEN) PHASE

INTRODUCTION

This test subjected water filled bag assemblies to a freeze/thaw cycle to ensure that the process does not impact bag integrity. All bag assemblies were subjected to -85°C passive freeze conditioning prior to being subjected to liquid nitrogen (LN_2) freezing for 24 hours.

TEST METHOD

Bag assemblies were placed into a fluorinated ethylene propylene (FEP) overwrap bag and heat sealed. After FEP bag was heat sealed, the bag assembly was placed into an aluminum cassette. Each FEP bag was enclosed in a cassette and initially subjected to -85°C passive freeze conditioning for a minimum of 2 hours, then transferred into a liquid nitrogen dewar where it was suspended in LN_2 vapor (-150°C) for a minimum of 24 hours.

RESULTS

No damage was visually observed after freeze thaw and the Aramus Assemblies passed pressure decay leak test

Table 11. Freeze/thaw test

Aramus bag size	Sample size	Freeze/thaw results
36 mL	22	100% Pass
50 mL	22	100% Pass
80 mL	22	100% Pass
90 mL	22	100% Pass
250 mL	22	100% Pass
500 mL	22	100% Pass

FREEZE/THAW TEST – LIQUID (NITROGEN) PHASE

INTRODUCTION

This test subjected gamma-irradiated Aramus bag assemblies, overwrapped in heat-sealed EVA bags, to a freeze/thaw cycle to ensure bag integrity when submerged in liquid phase nitrogen. The EVA over-wrap pouches offer the Aramus assemblies an additional layer of protection during freeze/thaw testing when submerged in liquid nitrogen. EVA Overwraps protect the bag and componentry from direct exposure to LN_2 and act as a secondary containment for the Aramus assembly.

TEST METHOD

Bag assembles were placed in an EVA overwrap and heat sealed. The bag assembly was then placed into an aluminum cassette. Each filled cassette was placed in a -85°C passive freezer for a minimum of 2 hours and then submerged in liquid nitrogen for a minimum of 24 hours.

RESULTS

Testing was completed on 29 representative samples using EVA overwraps. Integrity of the Aramus bag assembly was not affected by the liquid nitrogen. No damage or potential failure was visually observed during testing.

LN_2 ASSEMBLY CONFIGURATIONS

The following table is a summary of components included on assemblies that were exposed to one or more freeze/thaw cycles in liquid (-196C) and/or vapor (-192C) nitrogen environments. The Aramus assemblies maintain the ability to pass integrity testing after returning to room temperature.

Table 12.

Component/Material
Aramus subassemblies
Polypropylene connector
Stainless steel and nylon tubing retainer
EVA overwrap
TPE, EVA, PVC tubing
Polycarbonate connector

Note: Not all combinations in the table were individually tested. Contact your Account Manager for any specific information on components.

Entegris does not guarantee any third-party component claims post exposure to LN_2. End-user is responsible for verifying critical material specifications with manufacturer in the event of change notifications. Change notifications provided to Entegris will be handled as per Entegris' quality management system.

LN_2 ASSEMBLY PERFORMANCE

Entegris has performed testing on representative Aramus bag assemblies for use in LN_2 applications. This testing was performed to demonstrate the ability of the Aramus bag subassemblies and assemblies to maintain functionality after repeated exposure to LN_2 freezing, thawing, and handling processes.

The results of this testing demonstrated that repeated LN_2 exposure did not cause a negative impact to product performance and critical to quality attributes. Integrity testing performed before and after testing ensured that no bag assembly failures occurred at any point during bag assembly testing.

With the test results and determinations made in the previous paragraphs, Entegris can offer the below statements regarding the performance of the Aramus bag assemblies in LN_2 applications:

1. Aramus bag assemblies maintained integrity when subjected to multiple iterations of freezing/thawing at LN_2 temperatures (-150°C to -196°C).
2. Aramus bags should not be filled beyond their recommended fill volume for LN_2 applications.

DMSO COMPATIBILITY

Entegris has performed testing to determine chemical compatibility of the Aramus bag subassembly via mechanical testing (burst and tensile strength) after exposure to 100% DMSO at different lengths of time (T=0 (control), 24 hours, 7 days, and 21 days) at room temperature. Aramus™ 50mL bag subassemblies were burst and tensile strength tested before and after exposure to 100% DMSO. All bags were exposed to 42 kGy gamma irradiation prior to beginning the test.

Aramus™ bag integrity was found to not be signifi-cantly affected by exposure of 100% DMSO for up to 21 days. In addition, visual inspection yielded no discoloration after exposure to 100% DMSO after 21 days. The following table is a risk assessment of Aramus subassemblies and components compatible with DMSO. Component level DMSO compatibility is based on public information or vendor documentation

Table 13.

Component/Material	DMSO compatibility
Aramus subassemblies	Extremely low risk
Polypropylene connector	Extremely low risk
Stainless steel and nylon tubing retainer	Non product contact
EVA overwrap	Non product contact
TPE, EVA, PVC tubing	Low risk
Polycarbonate connector	Moderate to high risk

Note: Not all combinations in the table were individually tested. Contact your Account Manager for any specific information on components.

DRAINABILITY TEST

INTRODUCTION

Entegris recognizes that recovery from single-use containers is a critical concern to practical use. To document potential holdup, all Aramus bag sizes were assessed for drainability in both passive and active methods, defined as the ratio of dispensed liquid relative to the original volume. Since Aramus material has an extremely low surface energy compared to other materials, Aramus bags do not have a true "holdup" volume and theoretically 100% recovery is possible given enough time and/or manipulation. Table 15 documents an anticipated residual volume, by bag size, given a reasonable amount of manipulation.

TEST METHOD

Products are filled to their rated volumes and the total weight is recorded. The fluid is dispensed via unassisted gravity drain by opening one tubing line and waiting until liquid is no longer draining. The line is then closed, and the weight of the resultant bag is recorded. Drainability is calculated by comparing the delta in product weight against the rated volume.

RESULTS

The results are reported in terms of drainability % as defined above as well as holdup volume, which is defined as the amount of fluid left in the product after gravity drain. For bags smaller than 500 mL, a manually assisted drain is also performed in which the operator coaxes further dispensed volume by manipulating the film over the boat fitment. Active pumping would likely allow for more recovery out of the bag; therefore, it is anticipated that potential holdup for all subassemblies is <2 mL. Since holdup volume will depend on final assembly, please contact Entegris with questions about theoretical holdup volumes related to specific assemblies.

For 50 L bags, gravity drainability was performed via placing the filled bag on a 6° incline. After gravity drainability was performed, an operator picked up the bag to assist in draining the remaining volume to gain a value for manual drainability.

Table 14. Drainability test

		DRAINABILITY		HOLDUP VOLUME	
Aramus bag size	**Sample size**	**Gravity**	**Manual**	**Gravity**	**Manual**
20 mL	10	87.7%	96.1%	2.4 mL	0.8 mL
50 mL	12	88.1%	98.2%	5.95 mL	0.9 mL
100 mL	12	93.4%	98.8%	6.6 mL	1.2 mL
250 mL	12	87.8%	99.4%	30.5 mL	1.5 mL
500 mL	15	97.5%	—	12.5 mL	—
1 L	15	99.0%	—	10 mL	—
2 L	12	98.9%	—	22 mL	—
5 L	12	99.3%	—	35 mL	—
10 L	12	99.7%	—	30 mL	—
20 L	12	99.9%	—	20 mL	—
50 L	10	99.4%	99.9%	318 mL	24 mL

COLD CHAIN SHIPPING STUDY

INTRODUCTION

The Aramus single-use 2D bags are designed to contain frozen biopharmaceuticals and protect them during storage and transport at -80°C (-112°F). Bags were packed into single-use polyurethane insulated corrugate shippers with dry ice to maintain this low temperature environment during the duration of the shipment. Additional sensors were also used to verify product temperatures.

TEST METHOD

Standard Aramus 2D bag assemblies that were gamma irradiated above 25 kGy were filled and frozen to -85°C (-121°F) with deionized water from 500 mL to 10 L sizes. These were then transported with dry ice in insulated shippers from Minnesota to Massachusetts, one-way truck and one-way air, for a round trip of 2800 miles (4500 km). Additionally, high density polyethylene (HDPE) with stainless steel (SS) freezing shells and aluminum cassettes were used in some configurations to provide additional protection over transporting bags directly inside shippers. Temperature sensors were also used to monitor the shipments. Shippers were inspected upon receipt at both locations. Post shipping, the bags were thawed, drained, and visually inspected for damage. Bags were also integrity tested by pressure decay at a detection sensitivity of 30 µm. The list of materials used is shown in Table 15.

Table 15. Materials used

Part number	Description
SU-FS-0.50-C1	Cassette, 500 mL
SU-FS-0001-01	Freezing shell, 1 L
SU-FS-0005-01	Freezing shell, 5 L
SU-FS-0010-01	Freezing shell, 10 L
EPS126UPS	Small shipper
EPS731UPS	Medium shipper
PHT230	Large shipper
TempTale® dry Ice	Temperature sensor

Images of the materials and configurations that were the subject of this study can be seen in Figure 1. Details of bag fill volumes, shipper size, and dry ice packing quantity are listed in the accompanying table.

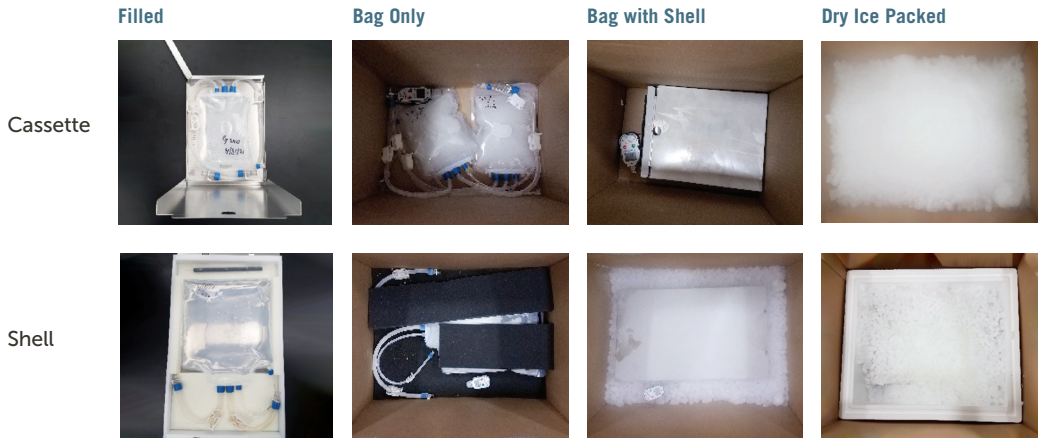


Figure 1. Shipping configurations

Table 16. Shipper configuration summary

Aramus bag size	Quantity	Fill volume	Compatible shipper	Minimum dry ice
500 mL	4	0.42 L	Small	22.7 kg (50 lbs)
1 L	3	0.9 L		
5 L	3	4.2 L	Medium	36.3 kg (80 lbs)
10 L	3	8.1 L	Medium and Larger	36.3 kg (80 lbs) and 52.1 kg (115 lbs)

RESULTS

Temperature

All shipments successfully maintained internal temperatures below -60°C (140°F) for up to one week during the distribution cycle. Most shipment durations were longer and had average temperatures above -70°C (158°F). The fluid volumes per shipper ranged from 820 mL up to 24.3 L. Figure 2 shows the shipper internal temperature throughout the distribution cycle from packing out to unpacking on receipt and is summarized in Table 17.

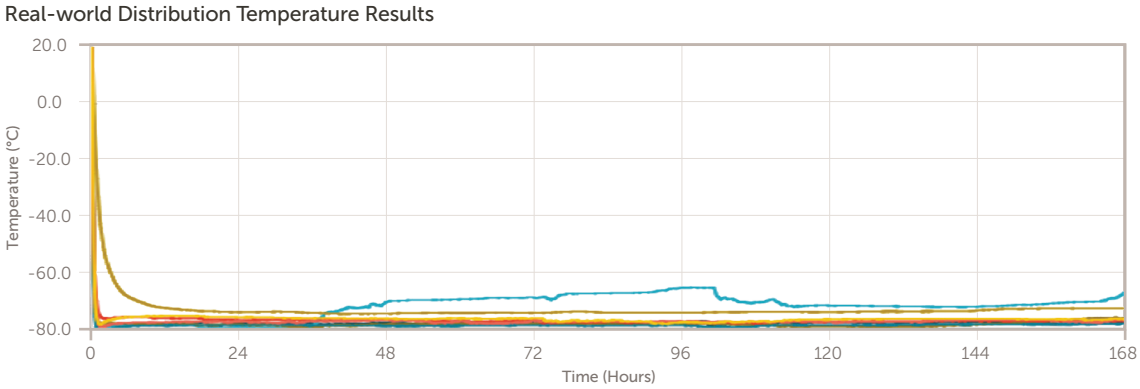


Figure 2. Shipper temperature monitoring

Contact your Entegris sales representative if you have questions regarding the temperature results from this study.

Table 17. Summary of shipping contents and temperatures

Shipper contents	Quantity	Duration	Average temperature	Fluid volume
500 mL bag	2	11 days	-78.2°C (-108.8°F)	0.82 L
500 mL bags and cassettes	2	11 days	-77.5°C (-107.5°F)	0.82 L
1 L bag, 1 L bag and shell	1, 2	8 days	-70.9°C (-95.6°F)	2.7 L
5 L bag	1	11 days	-73.4°C (-100.2°F)	4.2 L
5 L bag and shell	1	11 days	-77.7°C (-107.9°F)	4.2 L
5 L bag and shell + CCU	1	7 days	-77.7°C (-107.9°F)	4.2 L
10 L bag and shell	3	11 days	-72.9°C (-99.2°F)	24.3 L

Bag integrity

The bags encased in shells or cassettes had no visual defects or damage on the assemblies and passed pressure decay integrity testing post thaw and drain. The shipments with bags packed directly into the shippers were also successful. Results are shown in Table 18.

Table 18. Summary of pressure decay test

	BAG ONLY		BAG AND SHELL/CASSETTE		TOTAL
Aramus bag size	Quantity	Visual plus integrity test results	Quantity	Visual plus integrity test results	Quantity
500 mL	2	Pass	2	Pass	4
1 L	1	Pass	2	Pass	3
5 L	1	Pass	2	Pass	3
10 L	N/A	N/A	3	Pass	3

CONCLUSION

The results of this study demonstrate that the Aramus 2D bag design successfully meets the rigorous challenges of shipping bulk frozen fluids in a variety of ship system formats. Additionally protecting Aramus bags during frozen distribution is recommended using a cassette or shell.

22. Leak Testing

INTRODUCTION

The leak test performed on the Aramus bags aligns with ASTM F 2095-01: "Standard Test Method for Pressure Leak Decay Test for Nonporous Flexible Packages with and without Restraining Plates." System validation testing was conducted on samples that span the range of product the test equipment encounters during production. For leak testing, the validation sample group included products with a range of bag sizes. However, creating product with a targeted leak size is very difficult to manufacture, therefore, fixed orifices of known sizes (50 µm and 30 µm) were introduced into the leak detection process to simulate nonconforming samples. The 50 µm and 30 µm defect sizes were determined as the targeted sensitivity for this testing based on review of approach in the single-use market for pressure decay testing method. In practice, and based on units manufactured and customer complaint data, this selected test sensitivity has demonstrated an excellent quality control to ensure that non-integral bags are not released as product.

TEST METHOD

Each bag assembly was introduced into the leak system 24 times and resulting evaluation was recorded. To determine if the bag had a leak, the tester calculated the decay rate or the rate of decreasing pressure. The system measures a decay rate and determines if the bag fails (had a decay rate higher than tolerable threshold indicates leak) or passes (had a decay rate below a threshold). To test the full range of product evaluated during operations, leak-free Aramus bag samples were tested either with or without the orifice inline. Aramus bags can be safely retested at least 24 times before the weld may be affected. Each bag sample was tested without an orifice four times to demonstrate that it does not leak, tested eight times with the use of each orifice size, and then tested again four times without an orifice to verify that the leak condition of the bag itself had not been altered. This final test demonstrates that the only leak exhibited is due to the use of an orifice.

The conforming sample group consisted of eight evaluations of conforming bags without an orifice for each bag assembly used. This was conducted by sampling the conforming bags both before and after testing with the orifices. The conforming and non-conforming sample groups were tested in the same manner.

RESULTS

Table 19. Leak test summary

Criteria	Result pass/fail
All conforming samples without an orifice must have a leak rate <0.01 psi	Pass
All 30 µm and 50 µm orifice sample results must have a leak rate >0.01 psi.	Pass
All 30 µm orifice sample results must be in a definitive grouping between the conforming samples and the 50 µm orifice samples.	Pass

All acceptance criteria were met. The Aramus assembly leak test is considered validated.

23. Process Validation

Manufacturing equipment was validated as a system to provide evidence that the process, as defined using equipment specified, can consistently make product that meets final specifications.

Test method: Process parameters where variation may impact product quality were identified and challenged. Ranges in process parameters were challenged in the operational qualification (OQ), product was run using the high and low ends of the accepted process ranges of welding equipment. Non-variable parameters were set at nominal.

Leak, burst, and visual testing was conducted on units manufactured from the high/low settings to verify products are integral and meet acceptance criteria.

Following the OQ, PQ (process qualification) testing was executed. Three runs of the subassemblies for each of the various bag sizes were run using normal production settings and procedures. PQ tests included visual, dimensional, leak, burst, particulate (LPC), endotoxin, and bioburden, (Table 20).

Table 20. Operational and process qualification testing

Evaluation	Acceptance criteria	OQ results	PQ results
Leak test	≤0.01 psi	>1000 samples tested of various sizes and passed	>1500 samples tested of various sizes and passed
Burst test	≥12 psi (50 mL to 250 mL) ≥4.5 psi (0.5 L to 5 L) ≥3.5 psi (10 L) ≥3 psi (20 L)	>900 samples tested of various sizes and passed	>1000 samples tested of various sizes and passed
Visual inspection	No loose contamination, surface defects, or weld defects; embedded particles must be <3 and smaller than 0.5 mm	>1000 samples tested of various sizes and passed	>1500 samples tested of various sizes and passed
Dimensional inspection	Per approved drawing	22 samples tested of various sizes and passed	>500 samples tested of various sizes and passed
Subvisible particulate	Meets USP <788>	Evaluated in PQ	36 samples tested of various sizes and passed
Endotoxin	<0.125 EU/mL	Evaluated in PQ	Representative samples were tested per USP <85> and EP 2.6.14 and met the Entegris acceptance criteria
Bioburden	ISO 11137-2 bioburden levels must meet the VDmax[25] method validation requirements	N/A	Representative samples were tested in accordance with ISO 11737 and ISO 11137-2; bioburden levels met the VDmax[25] method validation requirements

Results: OQ and PQ testing confirmed Entegris' manufacturing process can consistently and reliably manufacture Aramus bags that meet product specifications.

24. Packaging – Shipping Validation

Product packaging was tested per the ISTA 2A, Testing for Packaged Products, standard. ISTA 2A is designed to provide a standard set of tests that can be used to verify the performance of individual packaged products weighing 68 kg (150 lbs) or less in relation to the risks involved during the distribution cycle. ISTA 2 Series tests include at least one element of a 3 Series type test in addition to basic elements of a 1 Series type test. Entegris chose the ISTA 2A Series because the packaging format most closely aligns with how Aramus assemblies are packaged and shipped, and is representative of the majority of stresses that Aramus packaging would experience. In addition, Entegris has an established history of using the ISTA 2A Series test standard for shipping and packaging validation.

Prior to testing, all assemblies were exposed to gamma irradiation at the required dosage window of 25 kGy to 40 kGy. The packaged product was exposed to the conditions shown in Table 21.

Table 21. Packaged product testing

Test	Conditions
Climatic conditioning	23°C ±2.0° (73°F ±36°), 50% ±5.0%, 72 hours
Compression	182 kg (401.8 lbs)
Vibration	60 mins, 1 – 200 hertz
Shock and drop	10 drops at differing orientations
Vibration	60 mins, 1 – 200 hertz

Test method: Following exposure, assemblies were tested for visual and functional criteria. All acceptance criteria were met.

Results: Testing demonstrated that the final packaging protects the product during shipping.

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