

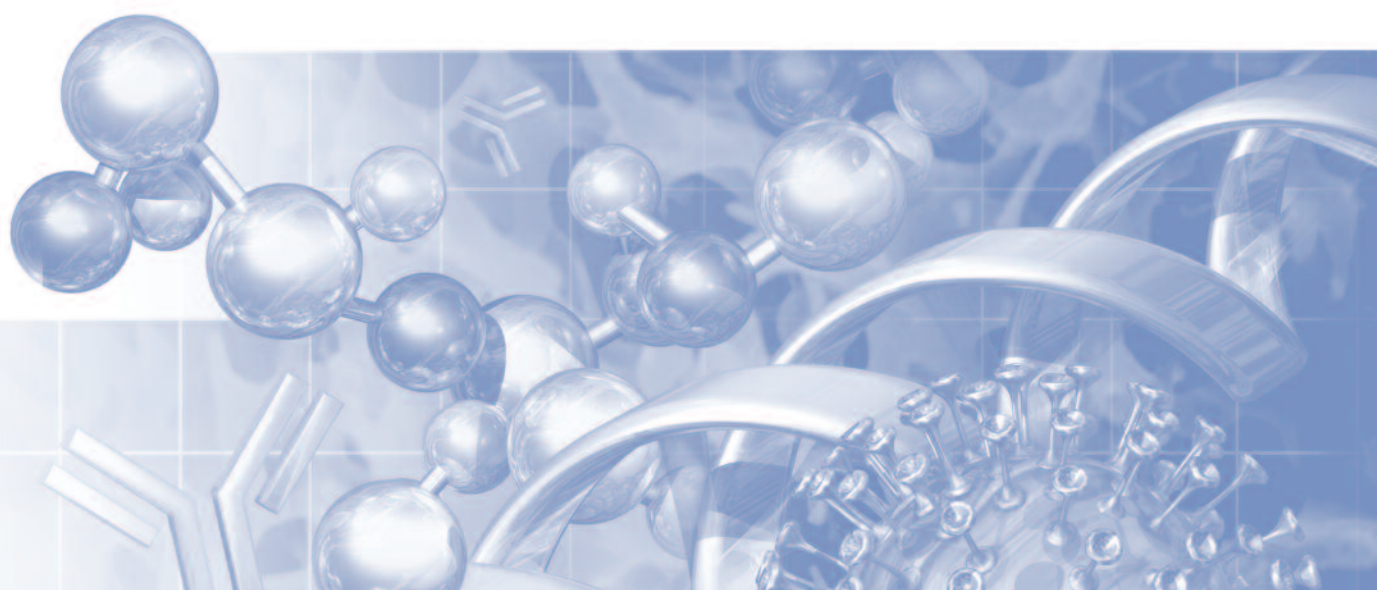


Life Sciences

Validation Guide

USTR 2777a

Pall Allegro Single-Use Mixers



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1. Validation Overview

1.1 Introduction

This guide contains data applicable to the original Allegro™ 200 L single-use mixer and the work conducted on the upgraded 200 L mixer, and new 500 L and 1000 L mixers, including jacketed versions. The purpose of this report is to document testing that has been performed to demonstrate the suitability of Allegro single-use mixers for use in biotechnology and pharmaceutical applications. As the mixer biocontainer has the same manufacturing process and identical materials of construction as the existing range of standard Allegro 3D biocontainers, some of the information related to validation can also be found in the latest revision of the validation guide for Allegro 3D biocontainers and totes (Pall reorder number USTR 2527).

Allegro single-use mixer biocontainers are made of a low density polyethylene (LDPE) fluid contact film that meets the rigorous quality requirements of the biotechnology and pharmaceutical industry. The Allegro single-use mixer biocontainers and the Allegro 2D and 3D biocontainers are made from the same film; therefore the biocontainer film validation is the same for the products. Additional materials included in the single-use mixer product have been tested accordingly and results are detailed in this validation guide. Please refer to the latest revisions of the Pall Allegro 2D biocontainer validation guide (USTR 2475) and the validation guide for Allegro 3D biocontainers and totes (USTR 2527) for further information relevant to the mixer product.

The mixer biocontainer includes some unique design features that significantly improve the ease of use and robustness of the single-use system while enabling efficient mixing of a wide range of solutions and high product recovery. There are three inlet/outlet ports, two for controlling the air flow to and from the mixer biocontainer and one for liquid filling or a combination of liquid filling and recirculation line which can be used to introduce on-line sensors. The outlet/drain port incorporates a flush valve, to ensure that when the outlet port is closed, it is flush with the bottom of the biocontainer. Thus, no solids or concentrated solution can get trapped in the outlet line.

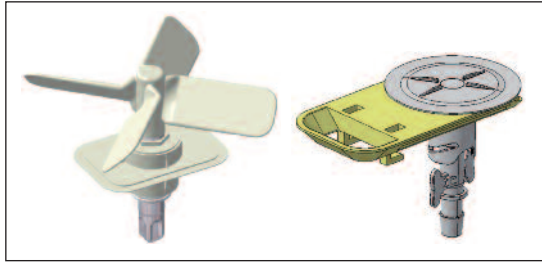
The single-use mixer (Figure 1) comprises a 200 L Allegro 3D single-use biocontainer, a metal tote to support the single-use system and user control interface (UCI) to control the direction and speed (rpm) of impeller rotation as well as control biocontainer inflation, flow rate, and pressure. The biocontainer portion consists of an impeller, an inlet port plate (for gas inlet/outlet and liquid additions), an impeller cover (to protect during transportation) with powder port and cap (to allow powder addition), and a flush drain port/valve. The impeller is over-molded onto a stainless steel shaft, which extends through the seal/bearing housing to the tube support below the tote, which in turn is mechanically coupled to the gear box and motor drive unit. An extension arm is mounted on the tote, with the purpose of securing the fully-inflated biocontainer by its powder port.

These design principles are also consistent with the 500 L and 100 L mixers. In addition, jacketed versions are now also available at all scales.

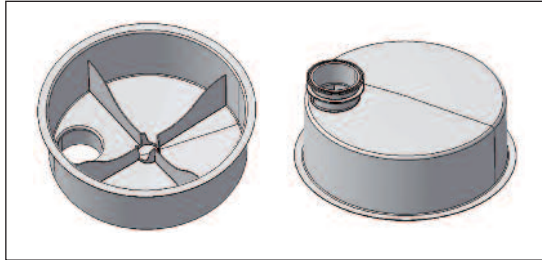
As an option, there are three baffles that can be placed in the assigned slots in the tote to minimize vortex formation and air entrapment during mixing. Also, various powder addition bags are available along with a powder bag support frame. Details on the operation of the Allegro single-use mixer are provided in USD 2743: Instructions For Use.

NOTE: Sections 1 - 9 of this validation guide refer to tests carried out on the original version of the Allegro 200 L mixer. All these tests are deemed relevant to the upgraded version of the 200 L mixer, and the new 500 L and 1000 L mixers (including jacketed versions), due to identical components, manufacturing methods and materials of constructions.

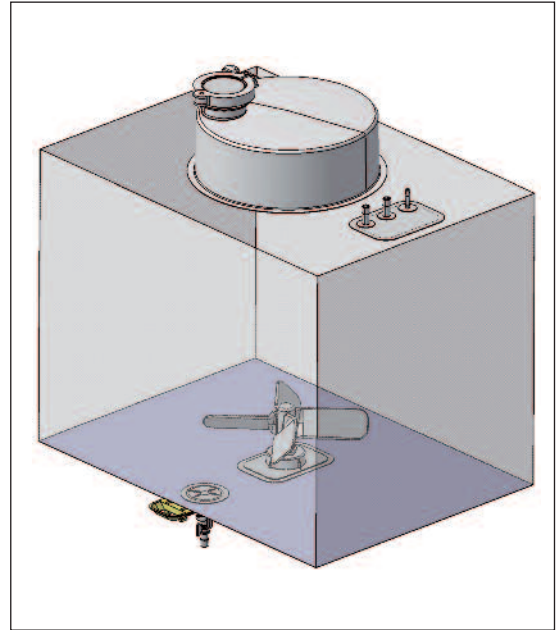
Figure 1
Schematics of the single-use mixer and biocontainer



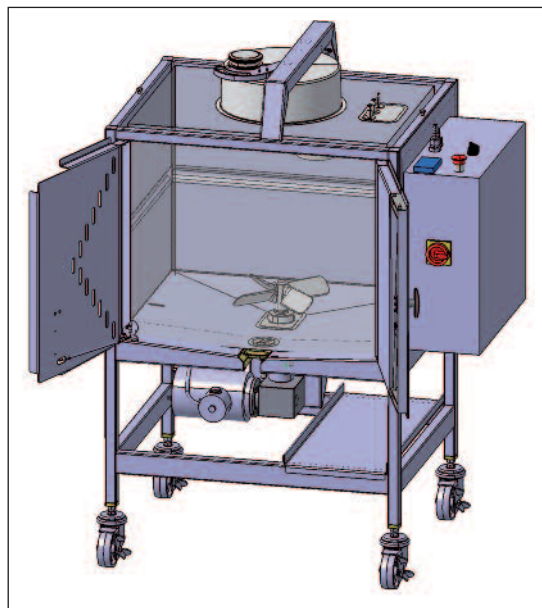
*Impeller and drain port with flush valve**
**New 200 L version and 500/1000 L versions have standard hose barb outlets.*



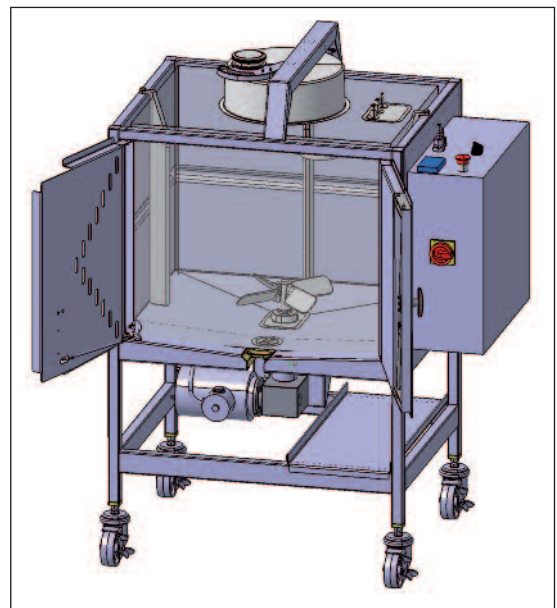
Powder addition port and impeller cover



200 L Single-use mixer biocontainer



200 L Single-use mixer biocontainer and tote without baffles



200 L Single-use mixer biocontainer and tote with baffles

1.2 Summary of Conclusions

1.2.1 Water Leak Test

This test allows us to claim a service lifetime of 3 days. The leak tests confirmed that the manufacturing process produces Allegro 200 L single-use mixer biocontainers that are leak-proof and able to withstand normal use for at least 6 days continuous service, which is twice the claim of 3 days continuous mixing at maximum impeller speed with appropriate mixer totes.

1.2.2 Drainage and Drain Valve Operation

The drain valve operation test showed good drainage and a high level of product recovery (water) from the biocontainer. There are no flow restrictions caused by the drain valve. The liquid volume hold-up during gravity drainage was less than 190 mL without baffles, 260 mL with baffles, and when drained with manual aid it was reduced to 40 mL or less.

1.2.3 Resistance to Gamma Sterilization

The tests conducted for the Allegro 3D biocontainer, which uses the same materials and construction of the film as the mixer biocontainers, confirmed that the welds maintained their tensile strength after being subjected to gamma-irradiation up to 100 kGy.

The tests conducted on the 200 L single-use mixer biocontainers showed that the biocontainers were leak proof after gamma irradiation up to a maximum dose of 55 kGy.

1.2.4 Connections Testing

The tests conducted for the Allegro 3D biocontainer verified that the tubing attached to the molded connection piece of the biocontainers does not pull off when attached using an appropriately tensioned cable tie or BarbLock* tubing retainer. No signs of leakage were detected at the connection when subjected to an applied pressure of 1.2 barg (17.4 psig) for 15 seconds before and after gamma irradiation to a maximum dose of 55 kGy. The connections withstood 5 Kg pull test force for 15 seconds before and after gamma irradiation.

On the Allegro 200 L single-use mixer biocontainers the drain valve flange connection was tested. It worked properly without leaks when tested before and after gamma irradiation up to a maximum dose of 55 kGy. Testing was conducted during mixing mode and static mode.

1.2.5 Extractables Study

The purpose of this study was to quantify and characterize the chemicals that may be extracted out from typical Allegro mixer biocontainers when exposed to different solutions and time periods. The results after 14 days (welded parts) and 91 days (biocontainer film) indicate that the level of extractables for tested contact fluids was extremely low and was close to the detection limit of the analysis techniques. Identified extractables are detailed in Tables 8 and 9.

1.2.6 Biological Safety Tests

The materials used in the Allegro mixer biocontainers meet the requirements of the USP <88> Biological Reactivity Tests *In Vivo* for Class VI Plastics. The materials used also meet the requirements of the USP <87> Biological Reactivity Tests *In Vitro*, Cytotoxicity.

1.2.7 Physico-chemical Tests

The purpose of these tests was to evaluate the physico-chemical suitability of the Allegro mixer biocontainer for USP <661> Containers - Plastics, USP <788> Particulate Matter in Injection, and USP <85> Bacterial Endotoxins. The components of the Allegro mixer biocontainers meet the requirements of all those standards.

1.2.8 Shelf Life Studies

Samples of representative Allegro 200 L single-use mixer biocontainers were subjected to a leak test, tensile strength test of the outer welds, and peel test on welded connections after 6 months of accelerated aging. The tests indicate that the functionality of the Allegro mixer biocontainers remained intact after 6 months of accelerated aging.

A three (3)-year full shelf life study is currently underway. Interim reports for longer time periods will be available upon request as developed.

2. Water Leak Test

2.1 Overview

This testing has been performed as part of the qualification of the Allegro 200 L single-use mixer biocontainers. The aim of the test is to establish that the manufacturing process has produced biocontainers that are leak-free and able to withstand normal use. For this test, five different manufacturing batches were tested.

2.2 Summary of Methods

The Allegro 200 L single-use mixer biocontainers were leak tested with the running impeller. Sixty (60) biocontainers from 5 batches were tested. All biocontainers were gamma irradiated up to the maximum irradiation levels indicated in Table 1. The biocontainers were then filled with water at levels between 50 L (minimum acceptable volume) and 205 L (a safety factor of 5 L was added to the claimed maximum volume of 200 L). The biocontainers were run with and without baffles, to ensure that baffles did not affect the operation of the mixer. The impeller speed was set between 75 and 150 rpm and run for a total of 144 hours, with periodic checks for leaks.

Table 1

Water leak test summary

Batch Number	Biocontainers per Batch	Gamma Irradiation Dosage (kGy)	Baffles Yes/No	Fill Volume (L)	Mixing Speed (rpm)	Testing Time (hours)
1	13	30 - 50	8 Yes; 5 No	2 (50 L); 2 (100 L); 9 (205 L)	4 (75 rpm); 9 (150 rpm)	144
	2	50 ± 5	1 Yes; 1 No	1 (50 L); 1 (205 L)	1 (75 rpm); 1 (150 rpm)	144
2	13	30 - 50	9 Yes; 4 No	1 (50 L); 2 (100 L); 10 (250 L)	3 (75 rpm), 10 (150 rpm)	144
	1	50 ± 5	Yes	205 L	150 rpm	144
3	11	30 - 50	4 Yes; 7 No	2 (100 L); 9 (205 L)	1 (75 rpm); 10 (150 rpm)	144
4	13	30 - 50	6 Yes; 7 No	1 (50 L);	3 (75 rpm);	144
				3 (100 L); 9 (250 L)	10 (150 rpm)	

Table 1 (continued)
Water leak test summary

Batch Number	Biocontainers per Batch	Gamma Irradiation Dosage (kGy)	Baffles Yes/No	Fill Volume (L)	Mixing Speed (rpm)	Testing Time (hours)
5	6	30 - 50	2 Yes; 4 No	All (205 L)	All (150 rpm)	144
	1	50 ± 5	No	205 L	150 rpm	144

2.3 Results

There were no leaks seen at any time point or at any locations on the mixer biocontainer during the 144 hours of testing in the mixing mode, as summarized in Table 2 below.

Table 2
Water leak test results

Batch Number	Biocontainers per Batch	Gamma Irradiation Dosage (kGy)	Water Leak Test – Pass/Fail
1	13	30 - 50	Pass
	2	50 ± 5	Pass
2	13	30 - 50	Pass
	1	50 ± 5	Pass
3	11	30 - 50	Pass
4	13	30 - 50	Pass
5	6	30 - 50	Pass
	1	50 ± 5	Pass

2.4 Conclusions

All 60 biocontainers over 5 manufacturing batches that have been leak tested with water at room temperature in the fluid fill range of 50 to 205 L of water (as detailed in Table 1 above) have passed. Following irradiation up to a maximum of 50 or 55 kGy (as detailed in Table 2 above), no leaks were seen during the entire 6 day mixing period. This allows us to have confidence for the claimed service lifetime of 3 days continuous operation.

3. Drainage and Residual Drainage Testing

3.1 Overview

Testing was performed to observe the drainage of the mixer biocontainers, using water, to confirm that there were no restrictions in the flow through the drain valve, and to measure the remaining fluid volume in the biocontainer after gravity and manually assisted drainage.

3.2 Summary of Methods

Sixty (60) Allegro 200 L single-use mixer biocontainers from 5 batches were filled with water at levels between 50 and 205 L as detailed in Table 1 and were subsequently drained. All biocontainers were gamma irradiated up to a maximum of 55 kGy (as detailed in Table 1). During the drainage the drain valve was monitored for any blockage or flow restrictions. Of these 60 biocontainers, 4 biocontainers from 3 batches were further tested to determine the residual fluid volumes in the biocontainer following gravity drainage and further manually assisted recovery (see Table 3). The 4 biocontainers tested for residual volumes were irradiated to a maximum dose of 50 kGy as shown in Table 3 below. The biocontainers were run with and without baffles (as detailed in Table 3), to determine the difference in final fluid volume within the

biocontainer if using baffles. After the drain valve operation was monitored, the final fluid volume using only gravity drainage was measured. Then the operator aided the drainage process and the final fluid volume after manual assistance was also measured.

3.3 Results

During the drainage process for all 60 Allegro single-use mixer biocontainers from 5 different manufacturing batches, the drain valve was seen to have no restrictions of the flow during the drainage process. Table 3 shows the final fluid volume of gravity drainage and manually assisted drainage of the biocontainers with and without baffles.

Table 3
Residual volume determination

Biocontainer Number	Gamma Irradiation Dosage (kGy)	Baffles – Yes/No	Gravity Drainage Volume (mL)	Manually Assisted Volume (mL)
1	30 - 50	No	120	20
2	30 - 50	Yes	260	40
3	30 - 50	No	120	40
4	30 - 50	No	190	40

3.4 Conclusions

The biocontainers all conform to acceptance criterion of no restrictions in the drain valve during drainage. With gravity drainage (no manual assistance) the approximate hold-up volume was 190 mL with no baffles and 260 mL with baffles. With manual assistance the hold-up volume was reduced to approximately 40 mL or less in all 4 biocontainers tested, with and without baffles.

4. Resistance to Gamma Irradiation

4.1 Overview

Validation for the mixer biocontainer's resistance to gamma irradiation is confirmed based on work conducted previously on the standard Allegro 3D biocontainer products and from additional testing carried out specifically on the 200 L Allegro single-use mixer biocontainers during design verification.

The next paragraph includes summary details of the validation work from the Allegro 3D biocontainer validation guide for the standard 3D biocontainer range of products. For more information, please refer to the latest revision of validation guide for Allegro 3D biocontainers and totes (USTR 2527).

The material of construction and manufacturing process of the biocontainer film and LDPE connection pieces (seal housing, drain valve flange) of the Allegro 200 L single-use mixer biocontainers are the same as for the standard Allegro 3D biocontainers which are used in general purpose fluid management applications. As part of the validation for the 3D biocontainers, tensile strength testing was conducted on the polymer film and the biocontainer welds after gamma irradiation up to 100 kGy. The tensile strength of the welded seal of 10 biocontainers was checked using 25 mm wide sample strip and elongation rate of 200 mm/min. All welds and film exceeded 70 Newtons.

Further resistance to gamma irradiation validation testing was completed on the Allegro 200 L single-use mixer biocontainers to ensure that irradiated biocontainers were free from any leaks, following irradiation and continuous operation for 6 days. Using the same testing as outlined in section 2, sixty (60) Allegro 200 L single-use mixer biocontainers from 5 different manufacturing batches were water leak tested after gamma irradiation. 4 biocontainers were irradiated up to a maximum dose of 55 kGy and 56 biocontainers were irradiated up to 50 kGy, as detailed in Table 1.

4.2 Summary of Methods

Visual observation for any fluid leaks was conducted during the test period as well as periodic pressure decay measurements on the seal housing assembly (with the motor running). 60 biocontainers from 5 batches were tested. All biocontainers were gamma irradiated up to 55 kGy. The biocontainers were then filled with water at levels between 50 L (minimum acceptable volume) and 205 L (a safety factor of 5 L was added to the claimed maximum volume of 200 L). The biocontainers were run with and without baffles, to ensure that baffles did not affect the operation of the mixer. The impeller speed was set between 75 and 150 rpm and run for a total of 144 hours, with periodic checks for leaks. All details of irradiation levels, water fill levels, baffles and impeller speed are detailed in Table 1.

4.3 Results

Resistance to gamma irradiation was confirmed through observation of no leaks during the test period, thus all 60 biocontainers from the 5 different manufacturing batches passed gamma resistance testing.

There were no leaks seen at any time point or any locations on the mixer biocontainer during the 144 hours when the motor/impeller was run, mixing mode as indicated in Table 4 below.

Table 4

Water leak test results

Batch Number	Biocontainers per Batch	Gamma Irradiation Dosage (kGy)	Testing Time (hours)	Gamma Resistance (no leaks) Test – Pass/Fail
1	13	30 - 50	144	Pass
	2	50 ± 5	144	Pass
2	13	30 - 50	144	Pass
	1	50 ± 5	144	Pass
3	11	30 - 50	144	Pass
4	13	30 - 50	144	Pass
5	6	30 - 50	144	Pass
	1	50 ± 5	144	Pass

4.4 Conclusions

The tests confirmed that the Allegro 200 L single-use mixer biocontainers maintain tensile strength having been subjected to gamma irradiation up to 100 kGy and are leak-proof when gamma irradiated up to 55 kGy and operated for up to 6 days. Applying a 100% safety margin on operational life, the maximum continuous operational life for the mixer biocontainers is therefore 3 days.

5. Connections Testing

5.1 Overview

The purpose of this test was to verify that the tubing connections to the molded connection pieces of the Allegro 200 L single-use mixer biocontainers are leak free and strong when using either an appropriately tensioned cable tie or BarbLock tubing retainer.

The material of construction and manufacturing process of the inlet port plate on the Allegro 200 L single-use mixer biocontainers are the same as for the Allegro 3D biocontainers. Thus, tubing attachment to the inlet port plate for the mixer biocontainers is covered in the validation guide for the standard Allegro 3D biocontainer validation guide. Testing performed as part of the validation is summarized as follows: batches of inlet port plates with cable ties and BarbLock tubing retainers were subjected to strength testing and leak testing.

In strength testing, the connection was subjected to a mechanical load of 5 Kg pull for 15 seconds. All connections held securely during this test. The leak test consisted of holding the tubing on the inlet/outlet port under water for 15 seconds with the connection at an internal pressure of 1.2 barg. This test was also repeated on these connections after gamma irradiation up to a maximum dose of 55 kGy. All connections passed with no leakage. For more information on these tests please refer to USTR 2527, validation guide for Allegro 3D biocontainers and totes.

Testing for tubing connections made to the drain valve of the mixer biocontainer using the same procedure as described above is work in progress at the time of writing this validation guide and will be reported in future documentation updates.

In operation, connection validation was confirmed for the outlet drain valve through the extensive operational filling and drainage testing conducted as part of the design verification. This is described below.

5.2 Summary of Methods

Sixty (60) biocontainers were water leak tested, as described in section 2, and then drained, providing 'in operation' testing of the drain valve connection when cable tied with appropriate tension to tubing. During drainage the drain valve/tubing interface was monitored visually to ensure there was no leakage from the drain valve or from the valve/tubing connection. No observed leakage from the interface between the drain valve and the connected tubing confirm a secure connection.

5.3 Results

During leak testing (section 2) and drainage testing (section 3) of the mixer biocontainer all drain valve/tubing connections remained leak proof as detailed in Table 5.

Table 5

Shows whether the drain/tubing connection leaked during drainage.

Batch Number	Biocontainers per Batch	Testing Time (hours)	Drain/Tubing Leak Test – Pass/Fail?
1	13	30 - 50	Pass
	2	50 ± 5	Pass
2	13	30 - 50	Pass
	1	50 ± 5	Pass
3	11	30 - 50	Pass
4	13	30 - 50	Pass
5	6	30 - 50	Pass
	1	50 ± 5	Pass

5.4 Conclusions

When using appropriately sized tubing according to the hose barb sizes on the mixing biocontainer inlets ports and drain valve, the connections are leak free when using cable ties or BarbLock retainers at appropriate tension. All connection ports can withstand normal use for a period of 3 days.

6. Extractables Testing

6.1 Overview

The purpose of the extractables study is to quantify and characterize the volatile, semi-volatile, and non-volatile residues or components that may be extracted out from typical Allegro 200 L single-use mixer biocontainers when exposed to different solutions. Figure 2 shows a schematic of the biocontainer, specifying where specific parts are located. Table 6 explains what each part is made of and the percentage of the total surface area that is in fluid contact.

For information on the biocontainer film (low density polyethylene film) please refer to USTR 2527 (validation guide for Allegro 3D biocontainers and totes). The Allegro single-use mixer biocontainers use the same manufacturing method and materials of construction for the main biocontainer film and LDPE ports as do the Allegro 2D and 3D biocontainers.

Figure 2

Biocontainer schematic

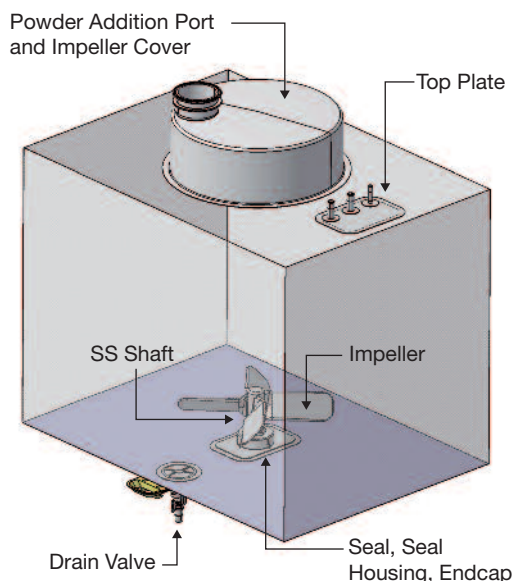


Table 6

Mixer biocontainer materials of construction

Biocontainer Part	Material	% Surface Area in Fluid Contact
Biocontainer Film	Low Density Polyethylene (LDPE)	84.93%
Inlet Port Plate	Low Density Polyethylene (LDPE)	0.80%
Powder Addition Port and Impeller Cover	Low Density Polyethylene (LDPE)	9.59%
Drain Valve Flange	Low Density Polyethylene (LDPE)	0.40%
Seal Housing	Low Density Polyethylene (LDPE)	0.70%
Impeller	Polysulfone	3.05%
Endcap	Polysulfone	0.24%
Drain Valve	Polysulfone	0.12%
Seal	Ultra-high Molecular Weight Polyethylene (UHMW PE)	0.02%
Seal O-Ring	Silicone	0.04%
Drain Valve O-Ring	Silicone	0.02%
Shaft	Stainless Steel 316	0.09%

Note: Percentages shown relate to original 200 L mixer biocontainer. New biocontainer design percentages are shown in Section 10: Appendix A.

6.2 Summary of Methods

Representative parts from the drain valve flange, endcap, seal, and seal o-ring were tested. Each representative part was gamma irradiated to a maximum dose of 55 kGy. The irradiated materials were then submerged into either ethanol or water for injection (WFI). The amount of extraction solvent applied is always equal to 2 cm²/mL (surface area/volume ratio) to allow calculation of extractables from the whole mixer biocontainers. The samples were then stored in a controlled environment of 40 °C for 14 days before testing. The extracted materials were compared with control samples from a glass bottle. The samples were then tested with the analytical tests described in Table 7. Different levels of analytical techniques were applied for each material considering the different percentage of surface area in fluid contact.

Table 7

Analytical methods used for extractables study (these tests are in addition to extractables studies reported in the 3D biocontainer validation guide). Surface area is based on total surface area in fluid contact (as highlighted in Table 6).

	LDPE	Polysulfone		UHMW PE		Silicone	
% of Fluid Contact Surface Area to the Whole Allegro 200 L Single-Use Mixer Biocontainer	11.49%	3.41%		0.02%		0.06%	
Contact Solution	Ethanol, 99%	WFI	Ethanol, 99%	WFI	Ethanol, 99%	WFI	Ethanol, 99%
Contact Time	14 days	14 days	14 days	14 days	14 days	14 days	14 days
pH	–	✗	–	–	–	–	–
Conductivity	–	✗	–	–	–	–	–
TOC	–	✗	–	✗	–	✗	–
Metals (ICP)	✗	✗	✗	–	–	–	–
Volatile Organic Compounds (Headspace GC/MS)	✗	✗	✗	–	–	–	–
Semi-volatile Organic Compounds (GC/MS)	✗	✗	✗	–	–	–	–
Non-volatile Organic Compounds (LC/MS NVR)	✗	✗	✗	✗	✗	✗	✗
Anions (IC)	✗	✗	✗	–	–	–	–

✗: Reflects the scenario for which analysis was performed.

pH Measurement

This test is aimed to detect any substance release from the biocontainer itself that could change acidic/alkaline properties of the aqueous solution.

Conductivity

This test is to detect the presence of ions that could conduct electric current through the fluid, mostly inorganic ions.

Total Organic Carbon (TOC)

This test is to estimate the sum of all the organic components leaching into the contact fluid. This test is aimed at detecting organic molecules.

Metals (ICP)

Metals may come from, for example, the catalysts used for the polymerization processes. They may also come from certain additives used in the polymers. In this test, the presence of metals is analyzed using atomic/optical emission spectroscopy to detect the traces of 23 metals.

Volatile Organic Compounds by Headspace – GC/MS (Gas Chromatography/Mass Spectrometry)

Volatile organic molecules may come from a host of sources, such as monomer and oligomers, residual solvents from various production steps, additives, residues from polymer treatment, and degradation products. The presence of volatile molecules is analyzed by means of headspace gas chromatography coupled with mass spectrograph.

Semi-Volatile Organic Compounds by GC/MS

A large number of potential organic migration products are not volatile enough to be detected in the headspace of a (heated) analytical sample preparation. However, these thermo-stable compounds are still volatile enough to be studied via “standard” GC/MS. These semi-volatile organic compounds may originate from various sources like process lubricants, plasticizers, anti-oxidants, polymer degradation products, and high boiling solvents. The selected analytical method allows identifying target compounds from a combination of its characteristic mass spectrum at its specific retention time.

Solvent Extraction along with LC/MS

If the molecules cannot be properly analyzed in their gaseous state then the compounds are dissolved in a liquid mobile phase: liquid chromatography, coupled with mass spectrograph. Typically, the presence of non-volatile molecules such as BHT and oleamide can be analyzed by this method.

Ion Chromatography

Acetate and formate can be found in small quantities everywhere in plastic products, either coming from raw materials used, or being the smallest degradation particle from organic molecules. The method to analyze their presence is to use their different polarity and thereby their affinity to different polar adsorbents.

6.3 Results

Table 8

Summary of findings from the multi-layer low density polyethylene (LDPE) biocontainer film extractables study from Allegro 3D biocontainers validation guide (USTR 2527).

The samples were extracted in 7 different solvents for 91 days at 40 °C.

Levels	Extractant						
	WFI	PBS-pH 3	PBS-pH 11	3M NaCl	96% Ethanol	1% Tween 80	10% DMSO
2-10 ppm	–	–	–	–	1, 3-di-tret butylbenzene (3.3 ppm)	–	–
1-2 ppm	TOC (1.3 ppm C/L)	TOC (1.1 ppm C/L)	–	–	C ₈ -alkenes (< 2 ppm)	–	–
100 ppb - 1ppm	–	Acetate hexanal	TOC (0.5 ppm C/L)	Fatty acids	Acetate AOx degradation Alkenes (C ₉ +)	1-octene C ₈ -alkenes Methylcyclopentane 1, 3-di-tret butylbenzene	1, 3-di-tret butylbenzene AOx
10-100 ppb	Acetate AOx degradation/ Di-tret butylphenol	AOx degradation/ Di-tret butylphenol	2-methyl-1-propene Di-tret-butylphenol	Di-tret-butylphenol	Alkanes	–	–
5-10 ppb	2-methyl-1-propene 2-octanone	–	Antioxidants	Hexanol	–	–	–
< 5 ppb	Antioxidants	–	–	Antioxidants 2-methyl-1-propene	–	–	–

Table 9a

Summary of findings from polymer material extractables study

	LDPE (Granuflex 4301)		Polysulfone (P1700 NT11 WH6417)			
	T=0	T=3 yrs	T=0	T=3 yrs	T=0	T=3 yrs
Fluid Contact Surface Area (cm ²)	2743		815			
% of Fluid Contact Surface Area to the whole 200 L Disposable Mixer Biocontainer	11.49%		3.39%			
Contact solution	Ethanol, 99%		WFI		Ethanol, 99%	
Time Point	T=0	T=3 yrs	T=0	T=3 yrs	T=0	T=3 yrs
pH	-	-	6.11	5.5	-	-
Conductivity	-	-	<50 µs/cm	<5 µs/cm	-	-
TOC	-	-	0.4 ppm	2.7 ppm	-	-
Metals (ICP)	Al: <0.14 ppm Na: 0.3 ppm	Ba: 0.2 ppm Ca: 1.8 ppm Cr: 0.2 ppm Fe: 0.2 ppm Mg: 0.2 ppm Mn: 0.03 ppm Na: 3 ppm Ni: 0.16 ppm	Ca: 0.94 ppm Mg: 0.04 ppm Na: 3.28 ppm Zn: 0.22 ppm	Ca: 0.18 ppm Na: 0.24 ppm Zn: 0.42 ppm	Al: 0.07 ppm Zn: 0.05 ppm	Cr: 0.05 ppm Fe: 0.03 ppm Mg: 0.01 ppm Na: 0.1 ppm Zn: 0.24 ppm
Volatile Organic Compounds (Headspace GC/MS)	Alkanes: 28.9 ppm	None Detected	None Detected	None Detected	None Detected	None Detected
Semi-volatile Organic Compounds (GC/MS)	4 Hydrocarbons detected with total conc: 38 ppm	13 Hydrocarbons** detected with total conc: 59 ppm	None Detected	None Detected	None Detected	None Detected
Non-volatile Organic Compounds (LC/MS)	None Detected	None Detected	None Detected	Erucamide 0.057 ppm	None Detected	None Detected
Anions (IC)	Acetate: 6 ppm	None Detected	*Formate: 0.1 ppm Phosphate: 0.2 ppm	Acetate: 0.6 ppm Formate: 0.8 ppm	*Acetate: 0.1 ppm	Nitrate: 1 ppm Phosphate: 12 ppm

*Concentration between the detection limit and the quantification limit, therefore only indicative.

**Of the 13 compounds detected 7 compounds were close to the reporting limit of the equipment of 2.4 ppm.

Table 9b

Summary of findings from polymer material extractables study

	UHMW PE				Silicone Seal O-rings			
	WFI		Ethanol, 99%		WFI		Ethanol, 99%	
Fluid Contact Surface Area (cm ²)	4				14			
% of Fluid Contact Surface Area to the whole 200 L Disposable Mixer Biocontainer	0.02%				0.06%			
Time Point	T=0	T=3 yrs	T=0	T=3 yrs	T=0	T=3 yrs	T=0	T=3 yrs
pH	-	-	-	-	-	-	-	-
Conductivity	-	-	-	-	-	-	-	-
TOC	34 ppm	22 ppm	-	-	63 ppm	86 ppm	-	-
Metals (ICP)	-	-	-	-	-	-	-	-
Volatile Organic Compounds (Headspace GC/MS)	-	-	-	-	-	-	-	-
Semi-volatile Organic Compounds (GC/MS)	-	-	-	-	-	-	-	-
Non-volatile Organic Compounds (LC/MS)	None Detected	None Detected	Erucamide: 1.1 ppm *Palmitic acid: 1 ppm	Erucamide: 10 ppm Palmitic acid: 57 ppm Stearic acid: 37 ppm	None Detected	None Detected	Palmitic acid: 12 ppm Stearic acid: 9 ppm	Erucamide: 5.3 ppm Palmitic acid: 8.4 ppm
Anions (IC)	-	-	-	-	-	-	-	-

* Concentration between the detection limit and the quantification limit, therefore only indicative.
 - Not tested due to very small surface area in fluid contact.

6.4 Conclusions

There are 5 different polymer materials, plus stainless steel 316 which come in fluid contact when using the Allegro 200 L single-use mixer. The material with the greatest fluid contact surface area is the biocontainer film and is made from LDPE. This film has been comprehensively tested for extractables. Table 8 presents a summary of the extractables for the film following a 91 day extraction in 7 different solvents at 40 °C. A surface area/volume ratio of 2 cm²/mL of extraction solvent was used in this study.

Summary Tables 9a and 9b show the findings from 2 extractables studies conducted on the other 4 polymeric components used in the construction of the 200 L single-use mixer. The T=0 study was conducted on parts which were irradiated (50 kGy) and sent for analysis. The T=3 yrs study was conducted on parts which were irradiated and subsequently aged, for the equivalent of 3 years, at 40 °C 75% RH prior to analysis. All identified extracted chemical entities are either oligomers from the polymers used or degradation products from antioxidants.

7. Biological Testing

7.1 Overview

The purpose of these tests was to evaluate the biological suitability of the materials of construction of the Allegro 200 L single-use mixer biocontainer. The materials of construction for the mixer biocontainer are detailed in Table 10.

For information on the biocontainer film (low density polyethylene film) please refer to USTR 2527 (validation guide for Allegro 3D biocontainers and totes). The Allegro single-use mixer biocontainers use the same manufacturing method and material of construction for the main biocontainer film as do the Allegro 2D and 3D biocontainers.

Table 10

Materials of construction

Biocontainer Part	Material
Biocontainer Film	Low Density Polyethylene (LDPE)
Inlet Port Plate	Low Density Polyethylene (LDPE)
Powder Addition Port and Impeller Cover	
Drain Valve Flange	
Seal Housing	
Impeller	Polysulfone
Endcap	
Drain Valve	
Seal	Ultra-high Molecular Weight Polyethylene (UHMW PE)
Seal O-Ring	Silicone
Drain Valve O-Ring	
Shaft	Stainless Steel 316

7.2 Summary of Methods

Tests include USP Biological Reactivity Tests, *in vivo*, for Class VI Plastics as described in the current United States Pharmacopeia Chapter <88>, USP Biological Reactivity tests, *in vitro*, as described in the current United States Pharmacopeia Chapter <87>, and tests on Bacterial Endotoxins as described in USP <85>.

7.2.1 USP <88> Biological Reactivity Test, *In Vivo*, for Class VI Plastics

The Biological Reactivity Tests *in vivo* for Class VI-50°C Plastics as described in the United States Pharmacopeia include:

- Injection of extracts of plastic materials
- Implantation of the solid material into animal tissue

The four (4) extracting media listed in the USP simulate parenteral solutions and body fluids. These include:

- 0.9% Sodium Chloride for Injection
- 1 in 20 Solution of Ethanol in Sodium Chloride Injection
- Polyethylene Glycol 400
- Vegetable Oil (sesame or cottonseed oil).

All polymer materials including biocontainer film and molded connection pieces were gamma irradiated (up to 55 kGy) and then extracted with these solutions at 50 +/- 2 °C for 72 +/- 2 hours. The extracts were then used in the following tests to determine the biological effects they have:

- Acute Systemic Injection Tests

An Acute Systemic Injection test was performed to evaluate the potential of a single injection of an extract to produce systemic toxicity. Extracts in Sodium Chloride Injection and 1-in-20 Solution of Ethanol in Sodium Chloride Injection were injected intravenously. Cottonseed oil extract and Polyethylene Glycol 400 extracts were injected intraperitoneally.

- Intracutaneous Tests

An Intracutaneous test was performed to evaluate the potential of a single injection of an extract to produce tissue irritation. All four of the extracts listed above were used for these tests.

- Implantation Tests

Implantation tests were performed, in order to subject the Allegro biocontainer materials of construction to the most stringent conditions included in the USP.

7.2.2 USP <87> Biological Reactivity Test, *In Vitro*, Cytotoxicity

The purpose of this study is to assess cytotoxicity (i.e., the effect of extractables from test material on the test cells) as per USP <87> guidelines. The materials tested under USP <87> are the drain valve flange, endcap, seal, and o-ring. For each material part, an extract of the test article, gamma-irradiated to 50 +/-5 kGy, is prepared using single strength Medium Essential Medium supplemented with 5% serum and 2% antibodies (1X MEM). This test extract was placed onto two separate monolayers of L-929 mouse fibroblast cells propagated in 5% CO₂. Two separate monolayers were prepared for the negative control (high density polyethylene) and the positive control (tin stabilized polyvinylchloride). All monolayers were incubated at 37 °C in the presence of 5% CO₂ for 48 hours and were examined microscopically after 48 hours to determine any change in the cell morphology. After 48 hours, both the negative and positive controls should perform as anticipated. The 1X MEM test extract is analyzed to see if there has been any cell lysis or toxicity.

7.2.3 USP <85> Bacterial Endotoxins Test

This test is to establish that endotoxins level of the disposable mixer in accordance with USP <85> Bacterial Endotoxins Test. The test is to be performed on individual components, which is added to the LAL Endotoxin level from Allegro biocontainers tested to show the result for the 200 L disposable mixer bag. The endotoxin tests were analyzed using chromogenic endpoint techniques. Each component material (low density polyethylene drain valve flange, polysulfone endcap, ultra-high molecular weight polyethylene seal, and silicone o-ring) was extracted with endotoxin free water and the fluid was then tested for endotoxins. As per US Pharmacopeia <85>, the endotoxin level should be < 0.25 EU/mL for each component material. All samples passed the specified tests.

7.3 Results

All materials tested, low density polyethylene drain valve flange, polysulfone endcap, high molecular weight polyethylene seal, and silicone o-ring passed USP <88> Biological Reactivity Test, *In Vivo*, for Class VI Plastics USP <87> Biological Reactivity Test, *In Vitro* and the USP <85> endotoxin test. The film of the Allegro single-use mixer biocontainer has the same manufacturing method and materials of construction as the Allegro 2D and 3D biocontainers, so please refer to USTR 2527 (validation guide for Allegro 3D biocontainers and totes) for biological safety results of the film. Detailed copies of the reports are available upon request.

7.4 Conclusions

All materials used in the Allegro 200 L single-use mixer meet the requirements of the USP Biological Reactivity Tests, *in vivo*, for Class VI Plastics (USP <88>), USP Biological Reactivity Tests, *in vitro*, Cytotoxicity (USP <87>), and the endotoxin determination tests as specified in USP <85>.

8. Physical Testing

8.1 Overview

The purpose of these tests is to evaluate the physico-chemical suitability of Allegro 200 L single-use mixer biocontainers. The purpose of USP <661> and USP <788> are to check that the materials of the Allegro 200 L single-use mixer biocontainers meet their requirements.

For information on the biocontainer film (low density polyethylene film) please refer to USTR 2527 (validation guide for Allegro 3D biocontainers and totes). The Allegro single-use mixer biocontainers use the same manufacturing method and material of construction for the main biocontainer film as do the Allegro 2D and 3D biocontainers.

8.2 Summary of Methods

Test includes USP <661>, Containers - Plastic of the United States Pharmacopeia and USP <788> tests on Particulate Matter in Injections.

8.2.1 USP <661> Containers - Plastic

Plastic containers that are intended for packaging products for parenteral use must meet the requirements of physico-chemical testing - plastics found in the current USP. These tests are designed to measure the properties of impurities extracted from plastics when leached with extraction medium over a specified period and temperature. The value of these tests becomes important to ensure the efficacy of product within the container.

Samples of LDPE drain valve flange, polysulfone endcap, UHMW PE seal, and silicone rubber were irradiated at up to 55 kGy. The irradiated samples were extracted at 70 °C for 24 hours in purified water and isopropyl alcohol. Samples of the liquids were tested under the following conditions under USP <661> guidelines:

- Non-volatile Residue: This test measures organic/inorganic residues soluble in extraction media. A volume of the extract is taken to dryness at 105 °C alongside a blank of the liquid. The individual residue weights are recorded and the difference in weight is calculated. The limit for the test is an NVR of <15 mg.
- Residue on Ignition: This test is only performed when the NVR is greater than 15 mg. The sample and blank residues are heated to 800 °C. The difference in weight of the residues is again calculated. The difference must be less than 5 mg.
- Heavy Metals: This test detects the presence of metals such as lead, tin, and zinc. A volume of the sample extract is brought to a pH of 3.0 – 4.0. The same is done to a dilute lead solution of a known concentration. Both solutions are then treated with hydrogen sulfide TS in separate comparison tubes. Any color produced in the sample tube is compared to the color in the standard tube. The limit is <1 ppm as indicated by the color.
- Buffering Capacity: This test measures the alkalinity or acidity of the extract. A volume of the sample extract is brought to pH of 7.0 using dilute acid or alkali. A blank of purified water is also brought to pH 7.0. The amount of titrant used is recorded for each and compared. There must be no more than 10.0 mL difference in the titrant volume used for each sample.

8.2.2 USP <788> Particulate Matter In Injections

The particle count analysis (or called particulates) based on USP <788> is to determine the particulates level within the Allegro 200 L disposable mixer before, during, and after mixing. The gamma irradiated Allegro 200 L mixer biocontainers were filled with 50 L Water for Injections (WFI) (minimum application volume to represent the worst case scenario) at room temperature. Particulates were measured at T=0, 1 hour, 8 hours (or 2 days) and 3 days mixing, plus negative control samples. Then air was filtered (through a 0.2 µm sterilizing grade Pall Mini Kleenpak™ capsule filter with Emflon® II filter medium, part number KA02V002P2G) and used to inflate and maintain the pressure inside the mixer bag before and during the mixing process. Mixing speed was set at 100 rpm. At each interval, samples of 50 mL taken from the drain valve of the mixer for measurement. The samples were tested to determine if they met with the requirements of USP <788>. The average number of particles of sizes ≥25 µm must be <2 /mL, and for particles ≥10 µm must be <12 /mL.

8.3 Results

All materials of the Allegro 200 L single-use mixer biocontainer tested (LDPE drain valve flange, polysulfone endcap, polyethylene seal, and silicone o-ring) passed all the tests specified under USP <661> physico-chemical Test and USP <788> particulates test.

8.4 Conclusions

The components of the Allegro 200 L single-use mixer biocontainers meet the requirements of the physico-chemical test-plastics USP <661> and USP <788> particulate testing.

9. Shelf Life Studies

9.1 Overview

9.1.1 Early Shelf Life Study

Shelf life studies were set up to establish a six (6) month shelf life for the Allegro 200 L single-use mixer biocontainers subjected to up to 55 kGy gamma irradiation, when stored under controlled accelerated aging conditions at 50 °C (relative humidity not controlled in this test). Tests on the influence of gamma sterilization on the mechanical strength of connectors, seal weld, and cross crease fold were performed. Leak testing was also performed as a further test of the integrity of the seal.

9.1.2 Long Term Shelf Life Study

Full shelf-life studies have been set up to establish a 3-year shelf life for the Allegro 200 L single-use mixer biocontainers subjected to up to 55 kGy gamma irradiation, when stored under controlled real-time (25 °C / 60% RH) and accelerated aging (40 °C / 75% RH) conditions. Interim reports will be available on request as developed.

At each interval during the 3-year shelf life study, tests on the influence of gamma sterilization on the mechanical strength of connectors, seal weld, and cross crease fold are performed. Leak testing is also performed as a further test of the integrity of the seal.

9.2 Summary of Methods

Storage of the real time and accelerated aging biocontainers will be conducted based on ASTM F-1980-07. Devices are subjected to gamma sterilization, and then stored at:

- Early shelf life – 50 °C, no relative humidity control
- Long term shelf life:
 - Real time – 25 °C / 60% RH
 - Accelerated – 40 °C / 75% RH

Following storage (at each shelf life interval) three main tests consisting of mechanical strength, extractables, and mixing/leak tests are conducted.

Table 11
Early shelf-life plan matrix

Sample	Batch Number	Number of Bags and Time at 50 °C (Accelerated Aging)	
		32 Days (Estimated 6 Months)	
Mixer	1	3	
Mixer	2	3	
Mixer	3	3	

Table 12
Long term shelf-life plan matrix - real time

Sample	Batch Number	Number of Bags and Time - Ambient (25 °C / 60% RH)	
		0 Month	36 Months
Mixer	1	1	1
Mixer	1	1	1
Mixer	1	1	1

Table 13
Long term shelf-life plan matrix - accelerated

Sample	Batch Number	Number of Bags and Time - Accelerated (40 °C / 75% RH)	
		0 Month	36 Months
Mixer	1	1	1
Mixer	2	1	1
Mixer	3	1	1

9.2.1 Mechanical Strength Test

This test is conducted for both early and long term shelf life studies.

Both tensile strength and manual peel test are applied to understand the mechanical properties of the mixer biocontainer during 3 years shelf life storage. The following tests are conducted on 1 biocontainer from 3 different manufacturing batches of mixing biocontainers:

- Tensile strength and peel test on connectors (top plate, powder addition port and impeller cover, drain port, and seal housing)
- Tensile strength at seal weld
- Tensile strength at cross crease fold

All the tensile strength tests above are following ASTM D-882 by using Zwick Roell (Z010) Tensile Instrument. The sample strip is 25 mm wide and the tensile force to be applied at a rate of elongation of 1000 mm/min. The force at which the film broke will be recorded. The tensile strength is required to be above 70 N.

9.2.2 Mixing and Leak Test

This test is conducted for both early and long term shelf life studies.

At each interval, mixing and leak test will be performed on the remaining 2 biocontainers from 3 different manufacturing batches. There is no leakage allowed for any mixing biocontainers tested.

9.3 Results

At the time of publication, results for early shelf-life were available. Results for long term testing will be available on request as developed.

9.3.1 Early Shelf Life Studies

All mixer biocontainers were gamma irradiated at 50 ± 5 kGy and then were stored in a 50 °C (relative humidity uncontrolled) oven for 32 days to accelerate to the age of six months.

9.3.1.1 Mechanical Strength Test

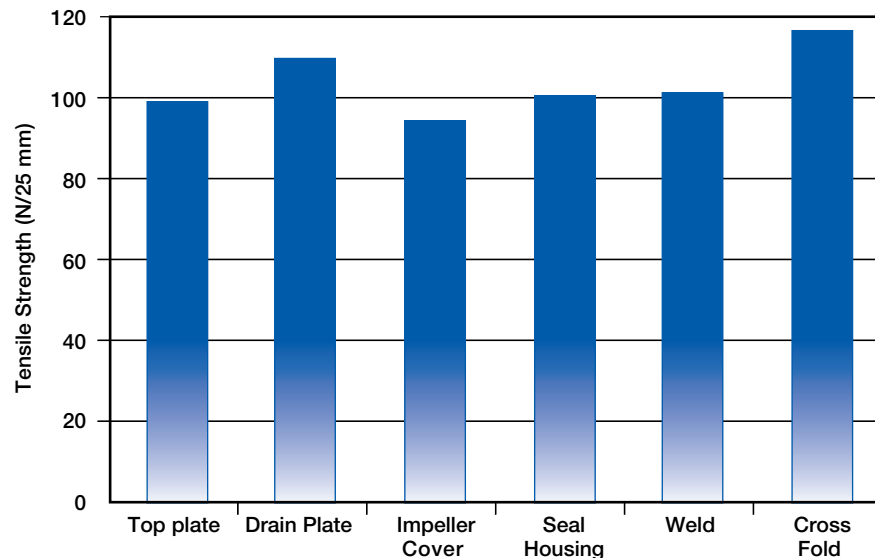
At time T=0 and equivalent to 6 months:

- The tensile strength at the connections was above 70 N / 25 mm. All welds (top plate, drain port, powder port, and seal housing) were not peelable on all biocontainers tested.
- The tensile strength at the seal weld was above 70 N / 25 mm for all biocontainers tested.
- The tensile strength at the cross crease fold was above 70 N / 25 mm for all biocontainers tested.

Figure 3 below shows the average tensile strength comparison at multiple locations on the Allegro 200 L single-use mixer biocontainers after 6 months accelerated shelf-life.

Figure 3

Average tensile strength at 6 months accelerated shelf life.



9.3.1.2 Extractables Testing

Extractables testing was not conducted for early shelf life studies. See Section 6 for extractables data representative of early shelf life.

9.3.1.3 Mixing and Leak Test

At time, T=0 and equivalent to 6 months accelerated time, all 6 (from 3 different manufacturing batches) single-use mixer biocontainers passed the mixing mode leak test as described in Section 2, without any leakage.

9.3.2 Conclusions

No leakage was detected for any of the 6 biocontainers tested at accelerated time of T=0 and 6 months. The average tensile strength for all of the biocontainers at the three different locations up to 6 months of accelerated aging exceeds 70 N. The tests indicated that the functionality of the Allegro 200 L single-use mixer biocontainer remains intact after six (6) months. A three year shelf life study is currently underway. Contact Pall for current information.

10. Appendix A: Additional Test Work for 200 L Upgrade, 500 and 1000 L Allegro Single-Use Mixers

Summary

This appendix has been written to cover extra test work performed during the upgrade of the Allegro 200 L single-use mixer, the addition of the Allegro 500 L and 1000 L single-use mixers to the range and also the addition of Allegro jacketed mixers at 200 L, 500 L and 1000 L sizes.

Introduction

The extra functionality of the mixers and the associated test work performed is summarized in the table below:

Test	Extra Features/Functionality
Water Leak Tests	Extra bag sizes and their operation at temperature
Residual Hold Up Volume Tests	Residual fluid volume following draining of systems
Jacket Performance Tests	Jacketed totes at each size
Connection Tests	Additional connections for acid/base addition, sensor manifold and drain

The surface areas table for extractables has also been updated to reflect the new ratio of surface areas for each material following the addition of new ports and sizes of bag. The material of construction for each of the new ports is identical to the material of construction for the original ports on the Allegro 200 L single-use mixer.

All other test material from the main section of the validation guide is considered to still apply to the new sizes and upgraded mixer products as the materials of construction, component designs, assembly techniques and manufacturing location are all identical to the original Allegro 200 L single-use mixer.

10.1 Water Leak Tests

Overview

This testing has been performed as part of the qualification of the upgraded Allegro single-use mixers to confirm the performance at larger bag sizes and at high and low temperatures are in line with the findings of the original Allegro 200 L single-use mixer qualification.

The aim of these tests, as with the original qualification, was to establish that the manufacturing process had produced biocontainers that are leak-free and able to withstand normal use. For this testing, Allegro single-use mixers of 200 L, 500 L and 1000 L sizes were tested at 2 °C and 40 °C. The different sizes were tested to prove performance of the biocontainer seals at the new larger sizes. The tests were performed at the high and low end of the operating temperature range to prove performance of the bag if held at temperature within an Allegro jacketed tote.

Summary of Methods

The Allegro single-use mixers were leak tested with the impeller running. All Allegro biocontainers were gamma irradiated prior to testing. The biocontainers were then filled with a mixture of water (90%) and propylene glycol (10%) to the maximum operating level for the size of biocontainer under test. This fluid was then recirculated through a heat exchanger and the temperature adjusted to the desired test temperature. The biocontainers were then run for a total of 144 hours with checks being made regularly for leaks. Temperature of the fluid was monitored with a thermocouple to ensure the test temperature was maintained throughout the test. Any leak from the biocontainer was to be considered a failure. One biocontainer was tested at each size and temperature.

Results

The table below summarizes the results of the Water Leak tests for the Allegro single-use mixers:

<u>Single-Use Mixer Biocontainer</u>	<u>Test Temperature 2 °C</u>	<u>Test Temperature 40 °C</u>
200 L	PASS	PASS
500 L	PASS	PASS
1000 L	PASS	PASS

Conclusions

The biocontainers all conform to the acceptance criteria of no leaks from any of the ports, welds or seals.

10.2 Residual Hold Up Volume Tests

Overview

Testing was performed to observe the drainage of the mixer biocontainers, using water, to confirm that there were no restrictions in the flow through the drain valve, and to measure the remaining fluid volume in the biocontainer after gravity and manually assisted drainage.

Summary of Methods

Allegro 500 L and 1000 L single-use mixer biocontainers were filled with test fluids to the design volume and then drained. During the drainage the outlet was monitored for any blockage or flow restrictions. A total of 3 Allegro single-use mixer biocontainers were tested at each size to determine the residual fluid volumes in the biocontainer following gravity drainage.

Results

The table below summarizes the range of the residual hold up volumes for the Allegro single-use mixers and for clarity the 200 L result from the original test work is repeated here. Similar hold-up volumes are seen across the whole range, as indicated:

Single-use Mixer Biocontainer	Residual Hold Up Volume
200 L	50 - 200 mL
500 L	
1000 L	

10.3 Jacketed Performance Tests

Overview

The performance of each size of Allegro jacketed tote was tested to establish heating/cooling rates and differential pressure within the jacket. The aim of the tests was to determine whether the totes met their design criteria and to provide useful data for designing systems using the jacketed mixer totes.

Summary of Test Methods

The tests were performed to the same method on each of the 3 sizes of Allegro jacketed mixer tote:

1. 200 L jacketed tote
2. 500 L jacketed tote
3. 1000 L jacketed tote

For the heating test, hot water was recirculated through the jacket at around 70 °C. For the cooling test, cold water was recirculated at around 7 °C. The heating test measured time taken to raise the fluid inside the biocontainer from 20 °C to 45 °C. The cooling test measured the time taken to lower the temperature inside the biocontainer from 37 °C to 15 °C.

The flow rate of the recirculating fluid was constant for both fluids at 2 m³/hr. Measurements were taken during the test of differential pressure across the thermo channels and also the inlet and outlet temperature of fluid within the channels. The temperature of the fluid within the biocontainer was also measured. In all cases the impeller speed was set to 45 rpm.

Results

The heating and cooling performance of the Allegro jacketed totes is given in the table below:

	Heating Phase Duration (hrs:mins)	Cooling Phase Duration (hrs:mins)
200 L Mixer	00:29	01:04
500 L Mixer	00:48	01:13
1000 L Mixer	01:03	01:35

The differential pressure across the thermo channels was as follows:

	Differential Pressure (Bar)
200 L Mixer	0.66
500 L Mixer	0.54
1000 L Mixer	0.52

Conclusions

The heating and cooling performance of the Allegro jacketed totes meet their specification and are fit for use with biopharmaceutical applications. Detailed heat and cooling curves can be obtained by contacting Pall.

10.4 Connection Testing

Overview

The purpose of this testing has been to verify that the extra ports added to the Allegro single-use mixers are fit for purpose and make strong and leak free connections. The Allegro single-use mixer uses several connections that have already been tested as part of the original release of the 200 L single-use mixer and the Allegro 3D biocontainers. That data is still relevant because no change has been made to those components. The material of construction of the new ports is the same as those already used on the Allegro single-use mixer.

Summary of Methods

The testing has been performed according to a standard test method that applies to all Allegro single-use systems. The test involves making the connection with the preferred tubing size and material and then securing with cable ties as per normal production selection and tensions. The joint is then tested by applying a mechanical pulling load and then performing a water leak test. The ports have been tested with the specific end use in mind – for example the ports intended for use as probe ports have been tested with the components used in the probe assemblies.

Test parameters are summarized in the table below :

	Pull Test	Leak Test
Acid addition port	2 kg / 15 secs	1 bar (15 psi) /30 secs
Base addition port	2 kg / 15 secs	1 bar (15 psi) /30 secs
Sample port	2 kg / 15 secs	1 bar (15 psi) /30 secs
Thermowell	6.5 kg / 15 secs	1 bar (15 psi) /30 secs
Aseptic probe assembly	2 kg / 15 secs	0.5 bar (8 psi) /30 secs
Non-aseptic probe assembly	6.5 kg / 15 secs	1 bar (15 psi) /30 secs

All assembled junctions were irradiated to minimum 50 kGy prior to testing. A junction was considered to have passed the test if the leak test showed the junction to be free of leaks.

Results

The results of the testing are summarized in the table below:

	Pull Test	Leak Test
Acid addition port	PASS	PASS
Base addition port	PASS	PASS
Sample port	PASS	PASS
Thermowell	PASS	PASS
Aseptic probe assembly	PASS	PASS
Aseptic probe assembly	PASS	PASS

Conclusions

The additional ports included on the Allegro single-use mixer are fit for purpose and make strong, leak free joints.

10.5 Extractables Testing

Overview

The upgrade to the Allegro single-use mixers does not use any new materials of construction. However, new ports have been added and these have used the existing port material of construction and so therefore the percentage of this material present in the mixer has changed within the 200 L mixer. The materials of construction were each tested individually during the original release of the Allegro 200 L single-use mixer and therefore the table of results has been updated to reflect the new percentage areas on the 200 L mixer and the new 500 L and 1000 L sizes.

Summary of Method

The method for the extractables test is the same as noted in the original validation guide – no new testing has been performed for these products. The table of results has been updated with new information regarding the surface areas.

Results

The table below shows the new surface areas of the components added to the Allegro single-use mixers and their relation to the whole mixer biocontainer.

	Mixer Size	Mixer Biocontainer Contact Material				Biocontainer Film
		LDPE	Polysulfone	UHMW PE	Silicone Seals	
Fluid Contact Surface Area (cm ²)	200 L (upgrade)	2969	785	2	5	20300
	500 L	2969	785	2	5	38233
	1000 L	2969	785	2	5	62362
% Fluid Contact Surface Area to the whole Mixer Biocontainer	200 L (upgrade)	12.34	3.26	0.0084	0.02	84.37
	500 L	7.07	1.87	0.0048	0.011	91.04
	1000 L	4.49	1.19	0.003	0.007	94.31

Conclusion

The results above should be read in conjunction with the data already published in the extractables testing section of this validation guide. The data for the original Allegro 200 L single-use mixer still applies to the additional products added to the product range. For the upgraded 200 L product and all 500 L and 1000 L products the data should be interpreted with this table above.



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