PALL Life Sciences

Validation Guide

USTR 2527b

Allegro™ 3D Biocontainers and Totes

*Filtration. Separation. Solution.*SM

Contents

1. Validation Overview

1.1 Introduction

This guide contains data applicable to Allegro™ 3D biocontainers and totes. These high quality flexible biocontainers are designed to be able to replace stainless steel tanks and carboys in process applications, to reduce the risk of cross contamination and eliminate cleaning and cleaning validation. The purpose of this report is to document testing that has been performed to demonstrate the suitability of Allegro 3D biocontainers fitted in an Allegro tote for use in biotechnology and pharmaceutical applications.

The Allegro biocontianer film is made of a low density polyethylene (LDPE) film that meets the rigorous quality requirements of the biotechnology and pharmaceutical industry. The Allegro biocontainer includes unique design features that significantly improve the ease of use and robustness of single-use systems while enabling high product recovery. The ports of Allegro 3D biocontainers have been configured so that maximum choice is offered for inlet and outlet connections. There are two inlet ports: one out of the two inlet ports can be used either for the introduction of sensor or as an additional filling line. Both the inlet ports and the outlet ports can be connected to tubing of ⅜ in., ½ in., ¾ in. and 1 in. nominal inner diameter. A ¼ in. sampling port is available for all options which could be kept open or closed depending on the specific application.

This validation guide summarizes the tests that were performed to qualify the performance of Allegro 3D biocontainers in the appropriate Allegro tote for various applications.

1.2 Summary of Conclusions

1.2.1 Filling, Drainage, Transport, and Stacking Tests

Filling Test

In the filling test, the Allegro 3D biocontainer was securely placed in an appropriate size Allegro tote and was filled to its capacity with water. During the filling process, biocontainer expansions and any potential signs of deflections to the tote surface or the biocontainer film were observed. The biocontainers were filled up to the desired fill volume and observed deflections were as expected.

Drainage Test

In the drainage test, the liquid was allowed to drain via action of gravity and the minimum hold-up volume within the Allegro biocontainer was measured. During drainage, the biocontainer collapsed evenly. The minimum hold-up volume after drainage was approximately 100 mL for the 100, 200 and 500 L biocontainers. The hold-up volume after drainage for the 1000 and 1500 L Allegro biocontainers was < 4 L.

Transport Test

In the transport test, a filled Allegro 3D biocontainer placed in appropriate size Allegro tote was safely transported using an Allegro trolley for the 100, 200 and 500 L biocontainers and casters for the 1000 and 1500 L attached to the metal tote over a distance of 100 meters.

Stacking Test

In the stacking test, the maximum stack height of three (3) 100/200 L Allegro totes were safely stacked on top of each other and remained stable. In addition, the maximum stack height of two (2) 500, 1000 and 1500 L Allegro totes were stacked on top of each other and also remained stable during filling and emptying of the appropriate Allegro 3D biocontainer.

1.2.2 Water Leak Test

The tests confirmed that the manufacturing process produces Allegro 3D biocontainers that are leak-proof and able to withstand normal use with appropriate totes.

1.2.3 Resistance to Gamma Sterilization

The tests confirmed that the Allegro 3D biocontainer welds maintained their tensile strength after being subjected to gamma-irradiation between 35 and 100 kiloGray (kGy).

1.2.4 Connections Testing

The tests verified that the tubing attached to the molded connection piece of Allegro biocontainers does not pull off when attached using 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 bar (17.4 psi) for 15 seconds before and after gamma irradiation. The connections withstood 5 Kg for 15 seconds before and after gamma irradiation.

1.2.5 Shelf Life Studies

Samples of the gamma irradiated Allegro 100 L biocontainers were subjected to a water leak test, peel test on connectors, and tensile strength test on the outer welds, top plate, bottom drain port, and cross crease fold on as received samples as well as on samples after 36 months of accelerated aging. The tests indicate that the functionality of the Allegro 3D biocontainers remained intact after 36 months of accelerated aging. A three year real-time shelf life study is currently underway. Interim reports for longer time periods will be available upon request as developed.

1.2.6 Extractables Study

The purpose of this study was to quantify and characterize the chemicals that may be extracted from typical Allegro biocontainers when exposed to different solutions and different time periods. The results after 30 and 91 days indicate that the level of extractables/leachables for tested contact fluids was extremely low and was close to the detection limit of the analysis techniques (with most concentrations less than 1 ppm, i.e. ppb range).

1.2.7 Biological Safety Tests

The materials used in Allegro biocontainers [polyethylene and ethylene-vinyl alcohol copolymer (EvOH)] meet the requirements of the USP <88> Biological Reactivity Tests *(in vivo)* for Class VI-50 °C plastics which target monitoring the effect of the biocontainer's extracts for their systemic toxicity, tissue irritation, and biocompatibility for implantation. The materials used in the Allegro biocontainers also meet the requirements of the USP <87> Biological Reactivity Tests *(in vitro)* for plastics (cytoxicity by MEM elution). The materials used in the biocontainers were also tested for ISO 10993 Biological Evaluation of Medical Devices (Section 7.2.3: ISO 10993 Biological Evaluation of Medical Devices):

- ISO 10993-4 Hemolysis
- ISO 10993-5 Cytotoxicity
- ISO 10993-6 Implantation Test
- ISO 10993-10 Irritation and Sensitization Test, and
- ISO 10993-11 Acute Systemic Toxicity

1.2.8 Physico-chemical Tests

The purpose of these tests was to evaluate the physico-chemical suitability of Allegro biocontainers for USP <661>, European Pharmacopoeia (Section 3.1.5), and Japanese Pharmacopoeia (Section 61 Part 1) standards as well as for presence of endotoxins and particulates. The components of the Allegro 3D biocontainers meet the requirements of all those standards.

2. Filling, Drainage, Transport, and Stacking Tests

2.1 Introduction

Testing was performed to observe the filling and drainage of the Allegro 3D biocontainers with water and to confirm that the 3D biocontainer in an Allegro tote could be transported over a distance without damage and are stable when stacked on top of each other.

2.2 Summary of Methods

2.2.1 Filling Test

For 100, 200, 500, 1000 and 1500 L Allegro 3D biocontainers, the biocontainers were fitted into the appropriate size Allegro tote using a standard adapter located at the bottom of the tote. The selected port (top or bottom) of the biocontainer was then attached to the water hose. The biocontainer was filled up to the pre-selected biocontainer fill volume. During the filling process, the biocontainer expansion was observed for any signs of deflection to either side of the tote surface. Before and after loading with water as well as post discharge any signs of damage to the biocontainer were observed. The acceptance criteria was that the biocontainers should fill up to the desired fluid volume and the deflections to the side walls of the tote were as expected.

2.2.2 Drainage Test

For each of the 100, 200, 500, 1000 and 1500 L Allegro biocontainer sizes, the biocontainer placed in the Allegro tote was filled with water to its capacity. Next, the drain port, located at the bottom of the tote, was opened to drain liquid by action of gravity. During the discharge process, the collapsing of the biocontainer was observed. After complete drainage the final hold-up fluid volume, with and without additional assistance, was recorded. The acceptance criteria were that the biocontainer collapse evenly and the liquid drain continuously.

2.2.3 Transport Test

One each of 200, 500, 1000 and 1500 L Allegro biocontainers were used. The empty biocontainers was fitted into an appropriate sized Allegro tote which was then fitted onto the appropriate Allegro trolley/casters. The biocontainer was filled with water to its capacity and then transported over a distance of 100 meters. During the transport, stability of the tote along with any relative displacement of the biocontainer was recorded. The acceptance criteria were that the biocontainer remain stable during transport over an even floor and the movement of the biocontainer before and after the travel remain less than 30 mm.

2.2.4 Stacking Test

The purpose of this test was to ensure that the 100, 200, 500, 1000 and 1500 L Allegro 3D biocontainer placed in the appropriate Allegro tote could be stacked safely and remain stable. The tests were conducted by taking three (3) water-filled 100/200 L Allegro biocontainers in the appropriate Allegro totes, and stacking them on top of each other using a mechanical fork lift truck. The test was repeated using two (2) water-filled 500 L, two (2) water filled 1000 L and two (2) water-filled 1500 L

Allegro biocontainers in the appropriate Allegro totes. Additional similar tests were conducted without filling the Allegro biocontainers with water. The acceptance criteria was that the stacks remain stable when pushed by hand.

2.3 Results

2.3.1 Filling Test

The Allegro biocontainers were filled up to the desired fluid volume and an adequate safety margin for the deflection to the side walls of the Allegro tote was achieved.

2.3.2 Drainage Test

The Allegro biocontainer collapse did not compromise drainage. In the 100, 200 and 500 L Allegro biocontainer volumes, the final hold-up volume within the bag without assistance was approximately 100 mL, and with assistance was brought down to approximately 10 mL. In the 1000 and 1500 L Allegro biocontainers, the final hold-up volume in the bag without assistance was < 4 L, and with assistance was brought down to < 0.5 L. The biocontainers conform to the acceptance criteria.

2.3.3 Transport Test

The 200, 500, 1000 and 1500 L Allegro biocontainers remained stable during their transport within their appropriate totes and showed no sign of biocontainer movement or damage.

2.3.4 Stacking Test

Both filled and empty 100/200, 500, 1000 and 1500 L Allegro biocontainers in the appropriate Allegro totes remained stable after stacking, filling and emptying.

2.4 Conclusions

All the tests conform to the acceptance criteria. The minimum hold-up volume for the 1000 and 1500 L Allegro biocontainers with and without assistance was < 0.5 L and < 4 L respectively. The minimum hold-up volume for the 100, 200 and 500 L Allegro biocontainers with and without assistance was approximately 10 mL and 100 mL respectively. Transport tests confirmed that a loaded 100, 500, 1000 and 1500 L Allegro 3D biocontainer in the appropriate Allegro tote could be transported on its Allegro trolley or casters over a distance of 100 meters without any damage to either the biocontainer or the tote. Stacking tests confirm that 100/200, 500, 1000 and 1500 L totes could be stacked and remained stable during filling and emptying of installed Allegro 3D biocontainers.

3. Water Leak Testing

3.1 Introduction

This testing has been performed as part of the qualification of the Pall Allegro 3D biocontainers. The same test has been performed on 100, 200, 500, 1000 and 1500 liter 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 with the tote. For each size of Allegro biocontainer, three batches of 20 biocontainers were tested in totes specifically designed for use with that size biocontainer. It is intended that customers use these Pall Allegro totes with the Pall Allegro biocontainers.

3.2 Summary of Methods

60 Pall Allegro 3D biocontainers of each size were tested from 3 batches with 20 per batch. All biocontainers were filled with water at room temperature to the design volume plus 10% in the corresponding sized totes, and checked for leaks. For 100, 200 and 500 L biocontainers ½ in. connections were used, whilst for the 1000 L and 1500 L biocontainers 1 in. connections were used to allow a higher flow rate when filling the biocontainer.

3.3 Results

As shown in Table 1, all 300 biocontainers passed the leakage test.

Table 1
Water Leak Testing Results

Size (L)	Quantity and Batch Number	Result
100	20 pcs, batch no FM15817	Pass
100	20 pcs, batch no FM15818	Pass
100	20 pcs, batch no FM15819	Pass
200	20 pcs, batch no FM15820	Pass
200	20 pcs, batch no FM15821	Pass
200	20 pcs, batch no FM15822	Pass
500	20 pcs, batch no FM15823	Pass
500	20 pcs, batch no FM15824	Pass
500	20 pcs, batch no FM15825	Pass
1000	20 pcs, batch no FM16048	Pass
1000	22 pcs, batch no FM16049	Pass
1000	18 pcs, batch no FM16050	Pass
1500	20 pcs, batch no FM16052	Pass
1500	20 pcs, batch no FM16053	Pass
1500	20 pcs, batch no FM16875	Pass

3.4 Conclusion

For each size of Pall Allegro biocontainers a total of 60 biocontainers over 3 manufacturing batches have been leak tested with water at room temperature to the design volume +10% and all have passed.

4. Resistance to Gamma Irradiation of the Polymer Film

4.1 Introduction

The purpose of the test was to verify that the Allegro biocontainer welds maintained their tensile strength after being subjected to gamma-irradiation between 35 and 100 kGy.

4.2 Summary of Methods

Ten (10) samples of a 240 L volume 3D biocontainer were manufactured for these tests using the polymer film and manufacturing methods used for the manufacture of Allegro biocontainers.

Prior to the gamma irradiation, all of the biocontainers were tested for leaks using a forming gas. The biocontainers were then gamma irradiated between 35 and 50 kGy. Five (5) of the biocontainers were then gamma irradiated for a second time between 35 and 50 kGy (total dose between 70 – 100 kGy).

The tensile strength of the welded seal of each biocontainer was checked using testing device X107. The sample strip was 25 mm wide and the tensile force was applied at an elongation rate of 200 mm/min. During this test the force at which the film broke must exceed 70 Newtons (N).

4.3 Results

For each biocontainer used in the test, 14 individual test samples were cut from the biocontainer's welded seal and their tensile strength was measured. The minimum, maximum, and average result for each biocontainer is shown in Table 2.

Table 2
Results of Tensile Strength Testing of Biocontainer Seals

Biocontainer Test Number	Min (N)	Max (N)	Average (N)
Irradiated 35 – 50 kGy			
1	90.0	129.3	114.7
2	90.6	121.4	111.7
3	102.1	118.4	111.4
4	86.0	124.3	104.5
5	94.3	116.3	106.5
Irradiated 70 – 100 kGy			
1	93.7	103.5	97.7
2	97.3	122.4	109.5
3	86.2	103.9	97.3
4	87.7	106.0	97.8
5	87.7	104.3	96.1

Among all of the samples that were tested, the polymer film material broke before any breakage of the welded seal. All the values found during tensile testing were > 70 N.

4.4 Conclusions

The tests confirmed that the Allegro 3D biocontainer welds maintained their tensile strength after being subjected to gamma-irradiation between 35 – 100 kGy.

5. Connection Testing

5.1 Introduction

The purpose of this test was to verify that the tubing connections to the molded connection pieces of the Allegro 3D biocontainers are leak free and strong when using either an appropriately tensioned cable tie or Barblock tubing retainer.

5.2 Summary of Methods

The 100, 200, 500, 1000 and 1500 L Allegro biocontainers have three (3) ports on the top plate, ¼ in. port for sampling purpose and additional two same size ports available in four sizes, ½ in., ⅜ in., ¾ in. and 1 in.. Selected batches of each port size for top plate and bottom port were subjected to the following functional tests with cable ties and Barblock retainers for silicone tubing and C Flex® thermoplasic elastomer tubing.

5.2.1 Strength Test

For each combination of port, tubing and connector type a total of 5 connections were prepared and submitted for test. It should be noted that Barblock retainers were not available for the connections of ¾ in. size and above. All connections were subjected to a mechanical load of 5 kg for 15 seconds.

5.2.2 Leak Test

All of the connections that had been subjected to the mechanical load are then subjected to various leak tests including being held under water for 15 seconds with an internal pressure of 1.2 bar. This test is then repeated on these connections after they have been gamma irradiated to a dose of 50 kGy ± 5 kGy.

5.3 Results

Table 3
Connections tested prior to Gamma Irradiation

	C - Flex Thermoplastic Elastomer Tubing		Silicone Rubber Tubing	
Component	Cable Tie	Barblock Retainer	Cable Tie	Barblock Retainer
Top plate ¼ in.	passed	passed	passed	passed
Top plate ½ in.	passed	passed	passed	passed
Top plate ⅜ in.	passed	passed	passed	passed
Top plate ¾ in.	passed	n/a *	passed	n/a
Top plate 1 in.	passed	n/a	passed	n/a
Bottom port ½ in.	passed	passed	passed	passed
Bottom port ⅜ in.	passed	passed	passed	passed
Bottom port ¾ in.	passed	n/a	passed	n/a
Bottom port 1 in.	passed	n/a	passed	n/a

** n/a indicates that Barblock connectors at this size were not available at the time of testing.*

Table 4
Connections tested post Gamma Irradiation

	C - Flex Thermoplastic Elastomer Tubing		Silicone Rubber Tubing	
Component	Cable Tie	Barblock Retainer	Cable Tie	Barblock Retainer
Top plate ¼ in.	passed	passed	passed	passed
Top plate ½ in.	passed	passed	passed	passed
Top plate ⅜ in.	passed	passed	passed	passed
Top plate ¾ in.	passed	n/a*	passed	n/a
Top plate 1 in.	passed	n/a	passed	n/a
Bottom port ½ in.	passed	passed	passed	passed
Bottom port ⅜ in.	passed	passed	passed	passed
Bottom port ¾ in.	passed	n/a	passed	n/a
Bottom port 1 in.	passed	n/a	passed	n/a

** n/a indicates that Barblock retainers at this size were not available at the time of testing.*

5.4 Conclusion

The top plates of sizes ¼ in., ½ in., ⅜ in., ¾ in. and 1 in. and the bottom port of sizes ½ in., ⅜ in., ¾ in. and 1 in. have given connections in both silicone and C-Flex thermoplastic elastomer tubing that are secure and leak free when using either cable ties or Barblock retainers.

6. Shelf Life Studies

6.1 Introduction

Full shelf-life studies have been set up to establish a 3-year shelf life for Allegro 3D biocontainers after 50 ± 5 kGy gamma irradiation, when stored under controlled real-time and accelerated aging conditions. At the time of publication of this report, 12 and 36 month accelerated shelf life results were available. Interim reports for longer time periods will be available on request as developed.

Shelf life studies will be performed as real-time and as accelerated aging tests.
There are three objectives for these series of tests:

- To demonstrate that an adequate safety margin is maintained for the water leak test of gamma-irradiated Allegro biocontainers following storage for up to 3 years.

- To demonstrate that an adequate safety margin is maintained for the tensile test on connectors, outer welds, and cross crease fold of gamma-irradiated Allegro biocontainers following storage for up to 3 years.
- To demonstrate that an adequate safety margin is maintained for the peel test on the top plate and bottom drain port of gamma-irradiated Allegro biocontainers following storage for up to 3 years.

6.2 Summary of Methods

Full shelf-life study by real time and accelerated aging has been carried out on the final design of Allegro 3D 100 L biocontainers from 3 different batches based on ASTM F-1980-07 and R&D BPH 104. Devices are subjected to gamma sterilization (50 ± 5 kGy), and then stored in controlled and monitored cabinets at real time conditions (25 °C/60% RH) and accelerated conditions (40 °C/75% RH). Table 5 shows a detailed matrix plan for the full shelf life study.

Table 5
Full Shelf Life Plan Matrix

		Number of Bags and Time @ 25 °C, 60% RH	
Bag Size	Batch	0 Month	36 Months
100 L	FP16169	2	2
100 L	FP16170	2	2
100 L	FP16171	2	2

Accelerated Aging

		Number of Bags and Time @ 40 °C, 75% RH	
Bag Size	Batch	4.3 Months (equivalent to 12 Months)	13 Months (equivalent to 36 Months)
100 L	FP16169	2	2
100 L	FP16170	2	2
100 L	FP16171	2	2

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

In the shelf life study, on completion of the storage interval, Allegro biocontainers will be subjected to the following tests:

6.2.1 Leak Test

At each interval, 3 biocontainers from 3 different batches are selected for water leak test. The leak test was carried out by filling the 100 L biocontainer in the designed metal tote with 110 L of water (10% more than the design volume) in an ambient environment. The biocontainer was visually inspected for any signs of leakage during filling and for 30 minutes after filling.

6.2.2 Tensile Strength Test

Mechanical testing was conducted on the other 3 biocontainers left at each interval. The strength of the seal weld, connectors, and cross crease fold of each biocontainer was checked by using Tensile Instrument and following ASTM D-882. The sample strips were 25 mm wide and taken from three samples for each location of the biocontainer. The tensile force was applied at a rate of elongation of 200 mm/min. The force at which the film broke must exceed 70 N.

6.2.3 Peel Test

For the same three biocontainers used for the tensile strength test, a manual peel test was also applied to check the welding strength between the film and top plate or bottom drain port. Both top plate and bottom drain port must not be peelable.

6.3 Results

At the time of publication, results for 36 months accelerated shelf-life biocontainers were available. Results for longer time periods will be available on request.

6.3.1 Twelve (12) and 36 Month Accelerated Shelf-life Tests

Leak Test Results

At time, T = 0 and equivalent to 12 and 36 months accelerated time, all 9 (from 3 different batches) 100 L 3D biocontainers passed the leak test as described in Section 3 without any leakage.

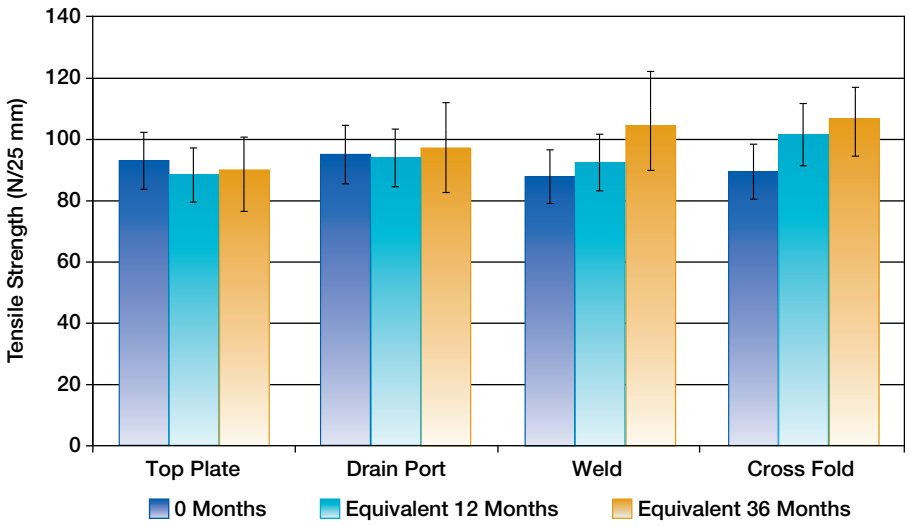
Tensile Strength and Peel Test Results (strip 25 mm, speed 200 mm/min)

At time, T = 0 and equivalent to 12 and 36 months, all the yield tensile strength at top plate area, drain port area, weld, and cross fold crease area was above the low specification limit of 70 N/ 25 cm, and was not peelable at either the top plate or the bottom drain port area for all the 3D biocontainers.

Figure 1 shows the average tensile strength comparison at four locations for Allegro 3D biocontainers after 0 months and accelerated 12 and 36 months shelf life. For the average tensile strength at top plate, drain port, weld, and cross fold crease area, there was no difference between 0 and accelerated 12 and 36 months shelf life time. The average tensile strength for all the biocontainers welds tested up to 12 and 36 months of accelerated aging exceeded 70 N. Both top plate and bottom drain port were not peelable from the biocontainers.

Figure 1

Average Tensile Strength Comparison after 0 and Equivalent to 12 Months and 36 Months.



6.4 Conclusions

No leakage was detected for any of the 9 biocontainers tested at accelerated time of T = 0, 12 and 36 months. The average tensile strength for all the biocontainers at four different locations up to 36 months of accelerated aging exceeds 70 N. The tests indicated that the functionality of the Allegro 3D biocontainer remains intact after 36 months.

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

7. Extractables Study

7.1 Introduction

The purpose of extractables study was to quantify and characterize the volatile, semi-volatile, and non-volatile residues or compounds that may be extracted/leached out from typical Allegro biocontainers when exposed to different solutions. Allegro 2D and 3D biocontainers use the same manufacturing method as well as same material of construction. Therefore, the extractables/leachables test results are applicable for both types of biocontainers.

7.2 Summary of Methods

Samples of the Allegro biocontainers and the molding polymer used in the molded connection pieces were gamma-irradiated at 26.6-32 kGy and then extracted after time periods of 30 days and 91 days in the following solutions and under the storage conditions described in Table 6.

Table 6

Summary of the Contact Solutions and Storage Conditions for Extractables/Leachables Study

	Contact Fluid	Storage Conditions	Glass Bottle	Biocontainer 30 Days	Biocontainer 91 Days
1	PBS – pH3	40 °C/75% RH	A	A	A
2	WFI	40 °C/75% RH	A	A	A
3	PBS – pH11	40 °C/75% RH	A	A	A
4	NaCl 3M	40 °C/75% RH	B	B	B
5	Tween◆ 80 – 1% in WFI	40 °C/75% RH	B	B	B
6	Ethanol 96%	25 °C	B	–	B
7	DMSO 10%	-20 °C	B	–	B

Five (5) liter Allegro biocontainers were filled with 3.25 L contact fluid via a peristaltic pump to reach extraction ratio of 2 mL per cm^2 solution.

The materials extracted/leached from the Allegro biocontainers were compared with control samples from a glass bottle. The samples from the Allegro biocontainers were tested with the analytical tests described in Table 7.

Table 7

Summary of Analytical Tests Performed on Allegro Biocontainers

Analytical Actions	Package A	Package B
Allegro biocontainer filling and recording of weight (empty + filled)	X	X
pH Measurement	X	X
Conductivity	X	
Total Organic Carbon (TOC)	X	
Metals (ICP-OES)	X	X
Headspace GC-MS	X	X
Solvent Extraction + GC-MS	X	X
Solvent Extraction + LC-MS	X	X
Ion Chromatography	X	X
Derivatization GC-MS	X	X

The rationale for using these tests was to look for the following:

Allegro Biocontainer Filling and Recording of Weight (Empty and Filled)
This test is targeted to detect any loss of the solution during its storage.

pH Measurement
This test is aimed to detect any substance release from the bag 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 sum of all the organic components leaching into the contact fluid. This test is aimed at detecting organic molecules.

Metal (ICP-OES)
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 that include Zr, Va, Ti, Si, Hf.

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.

Volatile Organic Compounds by Headspace-GC/MS
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.

Solvent Extraction along with GC/MS
Many compounds are not volatile enough to be analyzed by Headspace GC/MS but are still volatile enough to be analyzed by “standard” GC/MS. These compounds may comprise solvents with high boiling points, lubricants, plasticizers, antioxidants such as octanone and butylphenol.

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.

Derivatization GC/MS
Some group of organic compounds, e.g. organic acids need to be treated for generating sufficient signal to the GC/MS assay. Derivatization comprises treatment with BF3 and butanol and is specifically used to detect the presence of organic acids such as stearic acid, myristic acid, and palmitic acid.

7.3 Results

The polymer film and the molded connection pieces of Allegro biocontainers (after gamma irradiation at 26.6 – 32.0 kGy) were filled with 7 different contact fluids. No significant loss in weight, change in pH and conductivity was observed after 30 days and 91 days time period. The analysis after 91 days revealed low concentration of extractables in comparison to the glass bottle (used as a control). The summary of these tests results is provided in Table 8.

Table 8

Summary of Different Test Results

Extractant						
Level	2 – 10 ppm	1 – 2 ppm	0.1 – 1 ppm	10 – 100 ppb	5 – 10 ppb	< 5 ppb
WFI		TOC (1.3 ppm C/L)	Acetate	Antioxidant (AOx) degradation/ Di-tert-butylphenol	2-methyl-1-propene 2-octanone	Antioxidant
PBS-pH 3		TOC (1.1 ppm C/L)	Acetate Hexanal	AOx degradation/ Di-tert-butylphenol		
PBS-pH 11			TOC (0.5 ppm C/L)	2-methyl-1-propene Di-tert-butylphenol	Antioxidants	
3M NaCl			Fatty Acids	Di-tert-butylphenol	Hexanal	Antioxidants 2-methyl-1 propene
96% Ethanol	1-2-di-tert butylbenzene 5.3 ppm)	C8-alkenes (< 2 ppm)	Acetate, AOx degradation Alkanes (C9+)	Alkanes		
1% Tween 80			1-octene C8-alkenes Methylcyclo pentane 1-3-di-tert-butylbenzene			
10% DMSO			1-3-di-tert-butylbenzene AOx			

Most findings are in the concentration range up to 1 ppm. Virtually all identified extracted chemical entities are explainable as either oligomers and polymer used, or degradation products from the antioxidants used.

7.4 Conclusions

Allegro 2D and 3D biocontainers use the same manufacturing method as well as same material of construction. Therefore, the extractables/leachables test results are applicable for both types of biocontainers.

The results after 30 and 91 days indicate that the level of extractables/leachables for tested contact fluids was extremely low and was close to the detection limit of the analysis techniques (with most concentrations in the ppb-1 ppm range). More detailed information on the results of extractables/leachables study is available upon request.

Actual service life may impose different conditions, such as different exposure times, temperature, and liquid purity. Evaluation under actual process conditions is therefore also recommended.

8. Biological Tests

8.1 Introduction

The purpose of these tests was to evaluate the biological suitability of the materials of construction of Allegro biocontainers. Allegro 2D and 3D biocontainers use the same manufacturing method as well as same material of construction. Therefore, the biological safety test results are applicable for both types of biocontainers. The materials of construction for the biocontainer are detailed in Table 9.

Table 9
Materials of Construction

Inner and outer layers	Polyethylene
Oxygen and CO_2 molded barrier layer	Ethylene-vinyl alcohol copolymer (EvOH)
Molded connection pieces	Polyethylene

8.2 Summary of Methods

Tests include USP Biological Reactivity Tests, ***in vivo***, for Class VI Plastics (50 °C) as described in the current United States Pharmacopoeia Chapter <88>, USP Biological Reactivity tests *in vitro* for Plastics as described in the current United States Pharmacopoeia chapter <87>, and ISO 10993 Biological Evaluation of Medical Device.

8.2.1 USP <88> Biological Reactivity Test *In Vivo* for Class VI-50 °C Plastics

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

Samples of gamma irradiated (at 50 kGy) biocontainers film and molded connection piece were extracted with these solutions at 50 °C ± 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 material of construction to the most stringent conditions included in the USP.

8.2.2 USP <87> Biological Reactivity Tests, *In Vitro*

The purpose of this study was to assess cytotoxicity (i.e., the effect of extractable from test material on the test cells) as per USP <87> guidelines. An extract of the test article, Allegro film gamma-irradiated to 50 kGy, was 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% CO_2. 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% CO_2 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 performed as anticipated, whereas the 1X MEM test extract showed no evidence of causing cell lysis or toxicity and thus met with the requirement of the USP <87> standards.

8.2.3 ISO 10993 Biological Evaluation of Medical Devices

Gamma irradiated (at 26.6 – 32.0 kGy) samples of the film and of the molded connection pieces were tested for the following sections of ISO 10993:

ISO 10993-4 Hemolysis
The purpose of this study was to assess the haemolytic activity; i.e., the effect of test material on the cellular components of the blood, by placing the test material in direct contact with the human blood.

ISO 10993-5 Cytotoxicity
The purpose of this study was to assess cytotoxicity; i.e., the effect of extractables from test material on the test cells, by adding the extracts to a cell culture media on test cells. The samples were tested using a direct contact method. A negative result indicates that a material is free of harmful extractables or has an insufficient quantity of them to cause acute effects under exaggerated conditions with isolated cells. Tests were also performed after gamma-irradiating the sample of polymer film to 50 kGy.

ISO 10993-6 Implantation Test
The purpose of this study was to test and evaluate the test material for the potential to induce local toxic effects after implantation in the muscle tissue of animals during 2 weeks.

ISO 10993-10 Irritation and Sensitization Test
The purpose of this study was to test extracts from test materials for their potential irritation effects as a result of an intracutaneous injection in animals. The test materials were extracted with sodium chloride for injection and cottonseed oil at 70 °C ± 2 °C for 24 ± 2 hours.

ISO 10993-11 Acute Systemic Toxicity
The purpose of this study was to test the extracts from test materials for their potential toxic effects as a result of a single-dose systemic injection in animals. The test materials were extracted with sodium chloride for injection and cottonseed oil at 70 °C ± 2 °C for 24 ± 2 hours.

8.2.4 USP <85> Bacterial Endotoxins (European Pharmacopoeia 2.6.14)

Samples of representative Allegro biocontainers were tested to validate endotoxin determinations in biocontainers of volumes in accordance with the European and US Pharmacopoeia (respectively 2.6.14 and USP <85>, current edition). The endotoxin tests were validated using chromogenic endpoint techniques. The representative biocontainers used the same polymer film, manufacturing process, and tools as the final product. Eight (8) samples of 100 L representative biocontainers were used. Due to their large size, eight (8) small bags were made in a clean room environment by randomly cutting 100 cm^2 pieces of film from the eight (8) of the 100 L 3D biocontainers. These small biocontainers were extracted with endotoxins free water and the fluid was tested for endotoxins. As per the European and US Pharmacopoeia, the endotoxin level for the filled 100 L biocontainers should be < 0.25 EU/mL.

8.3 Endotoxins Determination Tests

Table 9

Data on Endotoxin Concentration Measured from 100 L Allegro Biocontainers

Bag Number	Average endotoxin concentration (n = 8) of extraction volume for 40 mL for 100 cm^2 area (EU/mL)	Extrapolated endotoxin concentration in 100 L filled biocontainer (EU/mL) (Surface area = 14868 cm^2)
1	< 0.005	< 0.0003
2	< 0.005	< 0.0003
3	< 0.005	< 0.0003
4	< 0.005	< 0.0003
5	< 0.005	< 0.0003
6	< 0.005	< 0.0003
7	< 0.005	< 0.0003
8	< 0.005	< 0.0003
Maximum value reported	**< 0.005**	**< 0.0003**

For the 100 L biocontainers the maximum value reported for endotoxin concentration of the biocontainers tested (n = 8) was < 0.0003 EU/mL being within the preset specifications of < 0.25 EU/mL.

8.4 Results

The materials used in Allegro biocontainers passed USP <88> Biological Reactivity Test *in vivo* for Class VI-50 °C Plastics, USP <87> Biological Reactivity tests *in vitro* for Plastics, and ISO 10993 Biological Evaluation of Medical Devices as described above. The polymer film, connectors, and the connector pieces passed the ISO 10993 standards. Detailed copies of the reports are available upon request.

8.5 Conclusions

Allegro 2D and 3D biocontainers use the same manufacturing method as well as same material of construction. Therefore, the biological safety test results are applicable for both types of biocontainers.

The materials used in Allegro biocontainers meet the requirements of the USP Biological Reactivity Tests *(in vivo)* for Class VI-50 °C plastics, the USP Biolological Reactivity Tests *in vitro* for Plastics, and the ISO 10993 Biological Evaluation of Medical Devices (Sections 4, 5, 6, 10 and 11).

9. Physical/Physico-chemical Tests

9.1 Introduction

The purpose of these tests was to evaluate the physico-chemical suitability of Allegro biocontainers. The purpose of USP <661> test, European Pharmacopoeia guidelines, Section 3.1.5 test, Japanese Pharmacopoeia guidelines, Section 61 Part 1, USP<788> and the European and US Pharmacopoeia (respectively 2.6.14 and USP <85>, current edition) standards was to check that the materials of Allegro biocontainers meet their requirements. Allegro 2D and 3D biocontainers use the same manufacturing method as well as same material of construction. Therefore, the physico-chemical test results are applicable for both types of biocontainers.

9.2 Summary of Methods

Tests include USP Physico-chemical Tests for Plastics, as described in Chapter <661> of the United States Pharmacopoeia, European Pharmacopoeia guidelines, Section 3.1.5, Japanese Pharmacopoeia guidelines Section 61 Part 1. Tests on particulate were performed as described in USP <788> and the tests on endotoxin were performed as described in European and US Pharmacopoeia (respectively 2.6.14 and USP <85>, current edition) standards.

9.2.1 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 insure the efficacy of product within the container.

Irradiated samples (at a dose of 50 kGy) from the Allegro biocontainers and the molded connection pieces were extracted at 70 °C for 24 hours in purified water and isopropyl alcohol. Samples of the liquids are then tested for the following under USP <661> guidelines:

- Non Volatile Residue (NVR) — measures organic/inorganic residues soluble in extraction media
- Residue On Ignition — performed when the NVR is greater than 15 milligrams.
- Buffering Capacity — measures the alkalinity or acidity of the extract.
- Heavy Metals Contents — detects the presence of metals such as lead, tin, and zinc.

9.2.2 European Pharmacopoeia (3.1.5)

Irradiated samples (at a dose of 26.6 – 32.0 kGy) from the biocontainers film and the molded connection pieces were extracted under European Pharmacopoeia guidelines, Section 3.1.5, Polyethylene with additives for containers for parenteral and ophthalmic preparation.

- Appearance — extract should be clear and colorless.
- Acidity and alkalinity — measures the alkalinity or acidity of the extract.
- Absorbance — measures absorbance of the extract.
- Reducing substances — measures reducing substances of the extract.
- Soluble substances in hexane — measures soluble substances of the extract.
- Extractable aluminium, chromium, titanium, vanadium, zinc, zirconium — detects their presence in the extract.
- Extractable heavy metals — detects the presence of heavy metals.
- Sulfated ash — detect the presence of sulfated ash in the extracts.

9.2.3 Japanese Pharmacopoeia (Section 61 Part 1)

Irradiated samples (at a dose of 26.6 – 32.0 kGy) from the biocontainers film and the molded connection pieces were tested under Japanese Pharmacopoeia guidelines that relate to plastic containers made from polyethylene that are used for aqueous injections.

- Cytotoxicity — measures the effect of extracts on cell culture growth.
- Extractable cadmium, lead, tin — detects their presence in the extract.
- Heavy Metals – detects the presence of heavy metals.
- Residue on ignition — measures the weight of the residue upon ignition.
- Residue on evaporation — measures the residue weight after evaporation of water.
- pH shift — measures the extent of alkalinity or acidity of the extract.
- Reducing substance — measures reducing substances of the extract.
- UV absorbance — measures UV absorbance of the extract.

9.2.4 USP<788> Particulates in Injectables, Microscopic Particle Count Test

Rinse volume samples from representative Allegro biocontainers were tested in accordance with the US Pharmacopoeia (USP <788>) to characterize biocontainer particulate cleanliness. In this test, the biocontainers were first filled with a known amount of particle-free water, left at the room temperature for 15 – 20 minutes, following which water was removed and tested for particulate level.
Eight (8) samples of 100 L representative biocontainers were used. 100 cm^2 surface area small bags were made by randomly selecting from the master 100 L biocontainers. Care was taken to make sure that no foreign particles were added (particle free cabinets were used). Due to the stickiness of the film there is no chance for foreign particles entering the tested surface area. These small biocontainers were subjected to test for particulate cleanliness. In order to meet with the requirements of USP <788> test, average number of particles of sizes ≥ 25 µm in the drained fluid must be < 2/mL, and for particles ≥ 10 µm must be < 12/mL.

9.3 Results

9.3.1 USP <661>, European, and Japanese Pharmacopoeia

The Allegro biocontainers passed all the tests specified under USP <661>, European Pharmacopoeia (3.1.5), Japanese Pharmacopoeia (61 part 1).
Both Allegro 2D and 3D biocontainers use the same manufacturing method as well as same material of construction. Therefore, the physico-chemical test results are applicable for both types of biocontainers.

9.3.2 Particles Testing

Table 11
Data on Particulate Concentration Measured from 100 L Allegro Biocontainers

	Results per 40 mL/100 cm^2		Extrapolated value for 100 L biocontainer (Surface area = 14868 cm^2)		Extrapolated particulate count/mL in 100 L biocontainer (Surface area = 14868 cm^2)	
Bag Number	Particles ≥ 10 µm	Particles ≥ 25 µm*	Particles ≥ 10 µm*	Particles ≥ 25 µm*	Particles ≥ 10 µm*	Particles ≥ 25 µm*
1	1069	< 3	158939	<446	< 2	< 0.004
2	1253	8	186296	1189	< 2	0.012
3	1331	< 3	197893	<446	< 2	<0.004
4	2189	32	325461	4758	< 4	0.048
5	1576	8	234320	1189	< 3	0.012

Table 11 *continued*

Data on Particulate Concentration Measured from 100 L Allegro Biocontainers

	Results per 40 mL/100 cm²		Extrapolated value for 100 L biocontainer (Surface area = 14868 cm²)		Extrapolated particulate count/mL in 100 L biocontainer (Surface area = 14868 cm²)	
Bag Number	Particles ≥ 10 µm	Particles ≥ 25 µm*	Particles ≥ 10 µm*	Particles ≥ 25 µm*	Particles ≥ 10 µm*	Particles ≥ 25 µm*
6	1571	3	233576	446	< 3	0.004
7	1485	24	220790	3568	< 3	0.036
8	1917	5	285020	743	< 3	0.007
Mean	**1549**	**≤ 11**	**230287**	**≤ 1598**	**< 4**	**≤ 0.016**

** Cumulative values.*

For the 100 L Allegro biocontainer, the mean value for particulate count/mL complies with USP <788> with being ≤ 4 particles/mL for ≥ 10 µm particles and ≤ 0.016 for particle sizes ≥ 25 µm, respectively. These values fall within the preset values of 12 and 2 particles/mL for particulate size ≥ 10 µm and ≥ 25 µm, respectively.

9.4 Conclusions

The components of the Allegro biocontainers meet the requirements of the physico-chemical test-plastics USP <661>. The components of the Allegro biocontainers meet the requirements of the European Pharmacopoeia Guidelines (Section 3.1.5) and of the Japanese Pharmacopoeia guidelines (Section 61 part 1).

The Allegro biocontainers meet the requirements of particulate testing, performed on 100 L biocontainers, as per the specifications of USP <788> particulate testing for particulate sizes ≥ 10 µm and ≥ 25 µm as well as endotoxin determination tests as specified under European Pharmacopoeia (PhEur. 2.6.14) and US Pharmacopoeia (USP<85>).

10. Operation and Storage

Allegro 3D biocontainers up to 500 L in size can be stored and used at temperatures between 4 °C and 60 °C. The 1000 L and 1500 L Allegro biocontainers can be stored and used at temperatures between 4 and 40 °C. For more information on test methods please contact Pall.

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