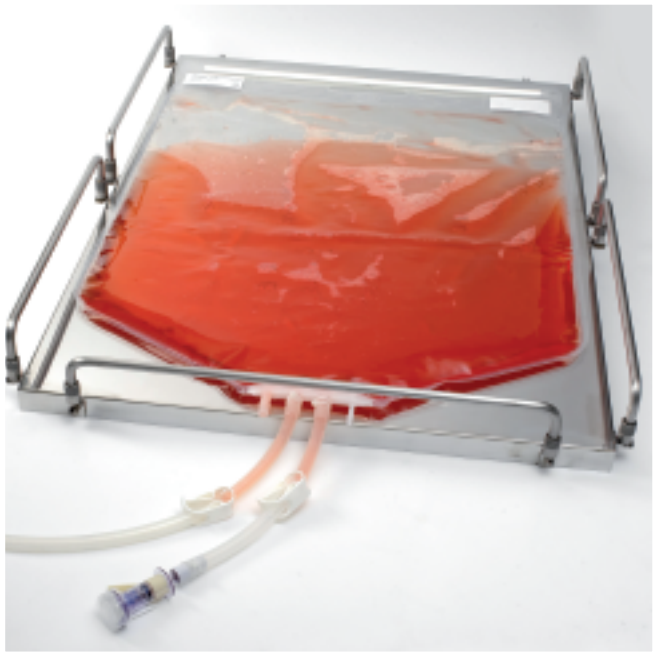
PALL Life Sciences

Validation Guide

USTR 2475a

Allegro™ 2D Biocontainers



*Filtration. Separation. Solution.*SM

Contents

1. Validation Overview

1.1 Introduction

This guide contains data applicable to Allegro™ 2D biocontainers designed specifically for applications where a high quality flexible biocontainer is required. Allegro biocontainers have been developed to replace stainless steel tanks and carboys in numerous applications, thereby reducing the risk of cross-contamination as well as eliminating cleaning and cleaning validation. The purpose of this report is to document testing that has been performed to demonstrate the suitability of Allegro biocontainers for use in biotechnology and pharmaceutical applications.

Allegro biocontainers are made of a low density polyethylene (LDPE) fluid contact and external film with internal ethylene-vinyl alcohol copolymer (EvOH) gas barrier film that comply with the very high standards of quality required for biotechnology and pharmaceutical applications. Allegro biocontainers include unique design features that significantly improve the design and robustness of single-use systems while enabling high product recovery. The polyethylene ports of Allegro biocontainers have been configured so that maximum choice is offered for inlet and outlet connections: there is the choice for tubing of ¼ in., ⅜ in. and ½ in. diameter. A sampling port is available for all options. Pall has designed Allegro biocontainers with a robust, flexible connection capability by utilizing a boat-shaped injection molded multiple port system. The boat connector allows for various size tubing connections but always maintains the same outer size and shape; thus creating consistency and robustness when sealed to the Allegro biocontainer film. Allegro biocontainers of five (5) liters or larger capacity allow the use of a ½ in. port for the inclusion of sensors for process-monitoring.

This validation guide summarizes the tests that were performed to qualify the materials and performance of Allegro biocontainers.

1.2 Summary of Conclusions

1.2.1 Mechanical Tests

Allegro biocontainers were subjected to drop test as per ASTM D4169-05 standards. All of the biocontainers, treated with or without gamma irradiation, passed the test and showed no sign of burst or leaks.

1.2.2 Resistance to Gamma Sterilization/Filling and Transport Test

The polymer film used to manufacture the biocontainers withstood gamma irradiation between 35 – 100 kGy while maintaining tensile strength > 70 Newtons (N). Also, no sign of leaks were visible during either the filling of biocontainers with water or during the transport of these filled biocontainers on a wheeled trolley.

1.2.3 Resistance to Freezing Test

The Allegro biocontainers (1 L, 5 L, and 10 L) both gamma-irradiated and not gamma-irradiated were filled with water and then stored for 7 days at -80 °C. After this period, they were defrosted and checked for the presence of any fluid leaks. The freezing and testing cycles were performed 4 times giving 28 days of freezing in total. After 4 cycles, the Allegro biocontainers showed no signs of leaks after defrosting as tested per drop-testing.

1.2.4 Connections Test

The purpose of these tests is to verify that the tubing attached to the molded connection piece of Allegro biocontainers does not pull off when attached using a cable tie or a BarbLock◆ brand retainer. The tests performed on 50 Allegro biocontainers indicated that the biocontainer, filled with a solution, successfully withstood an applied pressure of 0.5 bar without showing any sign of leakage.

◆ *BarbLock is a trademark of BarbLock Corporation.*

1.2.5 Shelf Life Studies

Samples of Allegro biocontainers with and without exposure to 50 ± 5 kGy gamma irradiation were subjected to a leak test, a tensile strength test on the outer welds, a drop test, and a sterility test on samples as-received and samples after 0, 6, 12, 24, and 36 months equivalent of accelerated aging and 6 months real-time aging. The tests indicate that both functionality and sterility of the biocontainers remained intact after 36 months equivalent accelerated aging, and 6 months real-time aging, respectively. The results for longer periods real-time aging will be shared as they become available over time.

1.2.6 Extractables/Leachables Study

The purpose of this study was to quantify and characterize the components/chemicals that may be extracted/leached out from typical Allegro biocontainers when exposed to different solutions and different storage intervals. The results after 30 and 91 days indicated that the extractables/leachables levels in tested contact fluids were extremely low and were close to the detection limit of the sophisticated analytical techniques applied (with most concentrations in the ppb -1 ppm range).

1.2.7 Biological Safety Tests

The materials used in Allegro biocontainer film (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 that target-monitor 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 (cytotoxicity) and meet the requirements of ISO ISO 10993 Biological Evaluation of a Medical Device (Section 8.2.2: ISO 10993 Biological Evaluation of Medical Devices) in:

- Section 4 (Hemolysis)
- Section 5 (Cytotoxicity)
- Section 6 (Implantation Test)
- Section 10 (Irritation and Sensitization Test)
- Section 11 (Acute Systemic Toxicity)

1.2.8 Physicochemical Tests

The purpose of these tests was to evaluate the physicochemical suitability of Allegro biocontainers per USP <661>, European Pharmacopoeia (Section 3.1.5), and Japanese Pharmacopoeia (Section 61, Part 1) standards, as well as for the presence of endotoxins per USP <85> and European Pharmacopoeia (Section 2.6.14) and particulates per USP <788>. The components of the Allegro biocontainers meet the requirements of all those standards.

2. Mechanical Tests

2.1 Introduction

The following test was carried out on Allegro biocontainers to check their ability to withstand mechanical stress.

2.2 Drop Test

The aim of the drop test is to ensure that the biocontainers do not burst or leak when dropped from a specified height, as per the requirements of the ASTM D4169-05 standard.

2.2.1 Introduction

The purpose of this test was to verify that gamma-irradiated Allegro biocontainers meet the requirements of the ASTM D4169-05. To be accepted, Allegro biocontainers must be dropped from a specified height and must not burst or leak.

2.2.2 Summary of Methods

Four 1-liter and two 10-liter volume Allegro biocontainers were tested for leaks using filtered air at a pressure of 0.5 bar. The biocontainers were supported using a wire mesh during this pressurized test. Then, two 1-liter and one 10-liter biocontainers were gamma-irradiated at 50 kGy ± 5 kGy.

Next, all of these biocontainers were filled with water at a temperature at 15 – 25 °C and sealed off with clamps. The biocontainers were then placed in a freezer set to -40 °C for 7 days. The biocontainers were then taken out and left to defrost back to room temperature. When fully defrosted the bags were examined for any sign of water leaking from the face of the film or the seals of the biocontainer.
After examination the biocontainers were dropped, both horizontally (twice) and vertically, at heights taken from ASTM D4169-05 standards presented in Table 1: ASTM D4169-05 Standards for Drop Test. After the drop test, each biocontainer was inspected for any sign of water leakage.

Table 1

*ASTM D4169-05 Standards for Drop Test**

Biocontainer Volume (Liter)	Height for Drop (mm)
< 9.1	381
9.1 – 18.1	330
18.1 – 27.2	305
27.2 – 36.3	254
36.3 – 45.4	229
45.4 – 90.7	178

** Horizontal and vertical drops must be carried out at the following heights and there must be no damage to the film or the seals that could cause fluid leakage.*

2.2.3 Results

The drop test results for Allegro biocontainers are summarized in Table 2:

Table 2

Drop Test Results for Allegro Biocontainers

Biocontainer Volume	Processing Condition	Drop Test 381 mm Horizontal and Vertical
1 liter	Irradiated 50 kGy ± 5 kGy	No leakage
1 liter	No irradiation	No leakage
Biocontainer Volume	**Processing Condition**	**Drop Test 330 mm Horizontal and Vertical**
10 liter	Irradiated 50 kGy ± 5 kGy	No leakage
10 liter	No irradiation	No leakage

All Allegro biocontainers passed test ASTM D4169-05.

2.2.4 Conclusion

All Allegro biocontainers passed the drop test and showed no sign of burst or leaks when dropped from a specified height, as per requirement of the ASTM D4169-05 standards.

3. Resistance to Gamma Irradiation of the Polymer Film and Welding Technique

3.1 Introduction

The purpose of these tests was to verify that the Allegro biocontainer film will withstand filling with fluid and transport after gamma irradiation. The testing also examines the welding technique used to manufacture the biocontainers.

3.2 Summary of Methods

Ten (10) samples of a 240-liter volume 3-D 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 mixture of hydrogen and nitrogen gases and a hydrogen gas detection device. The biocontainers were then gamma-irradiated between 35 – 50 kGy. Five (5) of the biocontainers were then gamma-irradiated a second time between 35 – 50 kGy (total dose between 70 – 100 kGy).

The biocontainers were placed inside a suitable container and filled with 240 liters of water (temperature between 15 – 25 °C). The aim of this test is to show that the biocontainers can be filled with their working volume of water without any leaks being observed. Each biocontainer was then transported on a wheeled trolley for 5 minutes and then inspected for any leaks. This is to demonstrate the strength of the polymer film and the welded strength of the biocontainer during stress. After this inspection the corner seals of each biocontainer were checked for any leaks with a microscope (magnification 30 times). The seals were inspected for any potential leaks that are not visible to the human eye. The tensile strength of the welded seal of each biocontainer was checked using a tensometer. 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 was recorded. The strength of the weld at break must exceed 70 Newtons (N).

3.3 Results

No leaks were visible from the biocontainers after filling them with water and also during their transportation. The biocontainer seals showed no visible leaks when inspected with a microscope.

3.3.1 Tensile Strength Testing

For each biocontainer used in the test, 14 individual test samples were cut from the biocontainers welded seal and their tensile strength was measured. The minimum, maximum, and average result for each biocontainer is shown in Table 3: Results of Tensile Strength Testing of Biocontainers.

Table 3

*Results of Tensile Strength Testing of Biocontainers**

Biocontainer Test Number	Minimum (N)	Maximum (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

** Force in Newtons to break a sample strip of 25 mm width.*

Table 3 *Continued*

*Results of Tensile Strength Testing of Biocontainers**

Biocontainer Test Number	Minimum (N)	Maximum (N)	Average (N)
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

** Force in Newtons to break a sample strip of 25 mm width.*

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

3.4 Conclusions

The polymer film used to manufacture the biocontainers withstood gamma irradiation between 35 – 100 kGy and had tensile strength > 70 N. There were no visible leakages during either the water filling or after transport of the filled biocontainers.

4. Resistance to Freezing Test

4.1 Introduction

The purpose of this test was to verify that pre and post gamma-irradiated (50 ± 5 kGys) Allegro 2D biocontainers withstand freezing temperature -80 °C whilst filled with their working volume of fluid. This plan of experiments was designed to look at the capacity of the Allegro biocontainers to withstand 4 cycles of 7 days freezing.

4.2 Summary of Methods

The Allegro biocontainers were first tested for leaks using filtered air at a pressure of 0.5 bar. Six biocontainers of each size (1 L, 5 L, and 10 L) were used for this study. For each size, three biocontainers were irradiated at 50.9 kGy, and three were not. Next, they were filled with water at a temperature of 15 – 25 °C and then stored for 7 days at a temperature of -80 °C. After 7 days, they were removed from the freezer and left to defrost at room temperature. When fully defrosted the biocontainers were inspected for leaks. The biocontainers were then placed back into the freezer for another 7 days cycle. The freezing and testing cycle was performed four times giving 28 days of freezing in total. After 4 times freeze and defrost cycles, the defrosted biocontainers were dropped from heights according to ASTM D4169-01. The biocontainers were dropped both horizontally (twice) and vertically (twice) at this height. After the drop the Allegro biocontainers were inspected for any sign of water leakages.

The temperature of the freezer did not exceed -80 °C anytime during the test.

4.3 Results

None of the 1 L, 5 L, and 10 L Allegro biocontainers tested to the above procedure showed any signs of water leaks prior to and after the drop testing at a height of 381 mm or 330 mm according to ASTM D4169-01. Each biocontainer was submitted to 2 horizontal drops plus 2 vertical drops and all of them passed the drop test.

4.4 Conclusions

Allegro 2D biocontainers up to 10 L size can be used for fluid storage at -80 °C.

5. Connection Testing

5.1 Introduction

The purpose of this test was to verify that the tubing attached to molded connection piece of Allegro biocontainers did not pull off when attached using a cable tie or a BarbLock brand molded retainer.

5.2 Summary of Methods

Four (4) configurations of connections exist for Allegro biocontainers:

A: ¼ in., ¼ in., ¼ in., ½ in.

B: ¼ in., ⅜ in., ⅜ in., ½ in.

C: ¼ in., ½ in., ½ in., ½ in.

D: ¼ in., ¼ in., ¼ in.

A, B and C configurations are available for volumes greater than 5 liters.

On the first batch connectors of configuration B (¼ in., ⅜ in., ⅜ in., ½ in.), the following tests were performed on 20-liter volume biocontainers to ensure that the design gave a suitable connection for the application. A total of 50 bags of configuration B were tested.

Out of 50 selected bags, 25 were connected with suitable tubing and BarbLock retainer and the remaining 25 with suitable tubing and a cable tie (width 4.7 mm).

The static tensile strength of all connections was tested with a weight of 5 kg for 15 seconds.

A leak test was performed on Allegro biocontainers and connections at 0.5 bar.

After gamma irradiation of Allegro biocontainers at a dose of 40 – 60 kGy, static tensile strength test was performed on all connections at same conditions as done before gamma irradiation. This test was followed by a dry leak test in the same conditions as performed before gamma irradiation. Gamma-irradiated tested Allegro biocontainers were filled with 24 liters of water at a temperature between 15 to 25 °C and were stored for one week at -20 °C. Connections were checked after defrosting at room temperature for leakage.

5.3 Results

All 50 Allegro biocontainers, showed no sign of leakage at 0.5 bar before and after gamma irradiation and storage at -20 °C for a week. The connections fit well with the C-Flex◆ brand tubing, cable tie (4.7 mm) and BarbLock brand retainers. The connectors also give a good welding with the polymer film.

5.4 Conclusions

The boat connector can be used in combination with the polymer film, C-Flex brand tubing, cable tie and BarbLock brand retainers.

6. Shelf Life Studies

6.1 Introduction

Shelf-life studies were conducted to establish a 3-year shelf life for Allegro biocontainers after gamma irradiation, when stored under ambient controlled conditions.

Shelf life studies are performed as real-time and as accelerated aging tests. There are five objectives for this series of tests:

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

◆ *C-Flex is a trademark of Consolidated Polymer Technologies.*

- To demonstrate that an adequate safety margin is maintained for the tensile test on connections of gamma-irradiated and non gamma-irradiated Allegro biocontainers following storage for up to 3 years.
- To demonstrate that an adequate safety margin is maintained for the tensile strength on outer weld of gamma-irradiated and non gamma-irradiated Allegro biocontainers following storage for up to 3 years.
- To demonstrate that an adequate safety margin is maintained for the drop test of gamma-irradiated and non gamma-irradiated Allegro biocontainers following storage for up to 3 years.
- To demonstrate that Allegro biocontainers are still sterile following storage for up to 3 years after exposure to gamma irradiation.

6.2 Summary of Methods

Three (3) years accelerated aging tests are carried out according to ASTM F-1980-02 on Allegro biocontainers which had been irradiated at a dose of 50 kGy ± 5 kGy. Testing is carried out at time periods that are the equivalent of 0, 6, 12, 24 and 36 months of real-time aging. These time periods are 0, 2.2, 4.3, 8.7 and 13 months of the accelerated aging program respectively. Devices are stored in controlled and monitored cabinets at accelerated aging conditions [40 °C/75% RH].

Three (3) years of real-time aging tests are carried out according to ASTM F-1980-02 on both gamma-irradiated Allegro biocontainers and on non-irradiated Allegro biocontainers. Testing is performed at 0, 6, 12, 24 and 36 months. Devices are stored in controlled and monitored cabinets at real-time conditions (25 °C/65% RH).

On completion of the storage interval, three (3) Allegro biocontainers per time points were subjected to the following tests:

6.2.1 Leak Test

Allegro biocontainers were subjected to the leak test, which is conducted by applying air pressure to a closed Allegro biocontainer followed by measuring any pressure decay with time.

6.2.2 Tensile Test on Connections (min. 50 N, speed 200 mm/min)

Tensile test on connections were determined according to the method described in Section 5.2: Summary of Methods.

6.2.3 Tensile Strength on Outer Weld (strip 25 mm, min. 70 N, speed 200 mm/min)

Tensile strength sample strip has a width of 25 mm. The rate of the testing device is 200 mm/min. The test limit is that the strength of the weld at break must exceed 70 N.

6.2.4 Drop Test

Allegro biocontainers were subjected to the drop test according to the method described previously in Section 2.2: Drop Test.

6.2.5 Sterility

Sterility was tested on three (3) gamma-irradiated samples only per period according to PhEur 2.6.1/ISO11737-2/ASTM F1980-2.

6.3 Results

6.3.1 Thirty six (36) Months Accelerated Aging Test

Leak Test Results

At times, T = 0, 6, 12, 24, and 36 months, no leakage was detected in any of the three Allegro biocontainers selected per batch (total 15).

Tensile strength on outer weld (strip 25 mm, speed 200 mm/min)

Table 4

*Results on Tensile Strength on Outer Weld**

Accelerated-Aging						
	T = 0 month non-irradiated	T = 0 month irradiated	T = 6 month irradiated	T = 12 month irradiated	T = 24 month irradiated	T = 36 month irradiated
Minimum (N)	98.7	95.9	100.3	82.7	89.7	86.4
Maximum (N)	120.4	118.5	111.6	112.1	109.4	111.1
Average (N)	107.7	105.8	106.3	94.7	102.6	103.6

** Force in Newtons to break a sample strip of 25 mm width*

Tensile strength on connections (speed 200 mm/min)

Table 5

*Results on Tensile Strength on Connections**

Accelerated-Aging						
	T = 0 month non-irradiated	T = 0 month irradiated	T = 6 month irradiated	T = 12 month irradiated	T = 24 month irradiated	T = 36 month irradiated
Minimum (N)	81.6	96.1	100.1	106.9	99.8	109.7
Maximum (N)	113.0	114.1	116.5	121.6	116.8	109.9
Average (N)	103.4	106.0	108.9	115.9	107.8	109.8

** Force in Newtons to break a sample strip of 25 mm width*

The average tensile strength for all the Allegro biocontainers welds tested up to 36 months of accelerated aging exceeds 70 N. Tensile strength on connections for all the biocontainers tested up to 36 months of accelerated aging exceeds 70 N.

6.3.2 Six (6) Months Real Time Aging Test

Leak Test Results

At times, T=6 month, no leakage was detected in any of the three Allegro biocontainers selected per batch.

Tensile strength on outer weld (strip 25 mm, speed 200 mm/min)

Table 6

*Results on Tensile Strength on Outer Weld**

Real-Time Aging				
	T = 0 month non irradiated	T = 0 month irradiated	T = 6 month non irradiated	T = 6 month irradiated
Minimum (N)	98.7	95.9	92.8	90.8
Maximum (N)	120.4	118.5	112.4	112.4
Average (N)	107.7	105.8	104.3	102.0

** Force in Newtons to break a sample strip of 25 mm width*

Tensile strength on connections (speed 200 mm/min)

Table 7

*Results on Tensile Strength on Connections**

Real-Time Aging				
	T = 0 month non irradiated	T = 0 month irradiated	T = 6 month non irradiated	T = 6 month irradiated
Minimum (N)	81.6	96.1	99.8	94.4
Maximum (N)	113.0	114.1	116.7	105.3
Average (N)	103.4	106.0	107.1	100.8

** Force in Newtons to break a sample strip of 25 mm width*

The average tensile strength for all the Allegro biocontainers welds tested up to 6 months of real time aging exceeds 70 N. Tensile strength on connections for all the biocontainers tested up to 36 months of accelerated aging exceeds 70 N.

6.3.3 Drop Test

At times, T = 0, 6, 12, 24, and 36 months accelerated aging and 6-month real time aging, all the Allegro biocontainers passed the drop test at both horizontal and vertical drops.

6.3.4. Sterility Test

At times, T = 0, 6, 12, 24, and 36 months accelerated aging and 6-month real time aging, all the Allegro biocontainers passed the sterility tests.

6.4 Conclusion

Samples of Allegro biocontainers were subjected to a leak test, a tensile strength test on the outer welds, a tensile strength test on the connection, a drop test, and a sterility test on as received samples and samples subjected to 36 months of accelerated and 6 month of real time aging. The tests indicate that the functionality of the biocontainers remained intact.
The results of initial testing are presented here and the remaining tests will be conducted in near future.

Interim reports for longer periods will be available as developed.

7. Extractables/Leachables Study

7.1 Introduction

The purpose of the extractables/leachables study was to quantify and characterize the chemicals that may be extracted/ leached out from typical Allegro biocontainers when exposed to different solutions.

7.2 Summary of Methods

Gamma-irradiated (at 26.6 – 32.0 kGy) samples of the Allegro biocontainers and the molding polymer used in the molded connection pieces were extracted for time periods of 30 days and 91 days in the following solutions and under the storage conditions described in Table 9: Summary of the Contact Solutions and Storage Conditions for Extractables/Leachables Study.

Table 8

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

A and B refer to test packages A and B as detailed in Table 9: Summary of Analytical Tests Performed on Allegro Biocontainers.

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

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 9: Summary of Analytical Tests Performed on Allegro Biocontainers.

Table 9

Summary of Analytical Tests Performed on Allegro Biocontainers

Analytical Methods	Package A	Package B
Weight of Allegro Biocontainer (empty and 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
Derivization GC-MS	x	x

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

Weight of Allegro Biocontainer (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 biocontainer itself that could change acidic/ alkaline properties of the aqueous solution.

Conductivity: The purpose of 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 designed to estimate the sum of all the organic components leaching into the contact fluid. This test is aimed at detecting organic carbon present in organic compounds.

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 analysed using atomic/optical emission spectroscopy to detect the traces of 23 metals.

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 compound 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 spectrometer.

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 higher boiling points, lubricants, plasticizers, and 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 compound is dissolved in a liquid mobile phase: Liquid chromatography, coupled with Mass spectrometer. Typically, the presence of non-volatile molecules such as phenolic antioxidants can be analyzed by this method.

Derivitization GC/MS: Some groups of organic compounds, such as 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 a 30-day and 91-day 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 10: Summary of Test Results for Extractables/Leachables.

Table 10

Summary of Test Results for Extractables/Leachables

Levels	WFI Fluid	PBS – pH 3	PBS – pH 11	3M NaCl
2 – 10 ppm				
1 – 2 ppm	TOC (1.3 ppm C/L)	TOC (1.1 ppm C/L)		
0.1 – 1 ppm	Acetate	Acetate Hexanal	TOC (0.5 ppm C/L)	Fatty acids
10 – 100 ppb	Antioxidant degradation/ Di-tert-butylphenol	Antioxidant degradation/ Di-tert-butylphenol	2-methyl-1-propene Di-tert-butylphenol	Di-tert-butylphenol
5 – 10 ppb	2-methyl-1-propene 2-octanone		Antioxidants	Hexanal
< 5 ppb	Antioxidant			Antioxidants 2-methyl-1-propene

Table 10 *Continued*

Summary of Test Results for Extractables/Leachables

Levels	96% Ethanol	1% Tween* 80	10% DMSO
2 – 10 ppm	1-2-di-tert-butyl-benzene (5.3 ppm)		
1 – 2 ppm	C_8-alkenes (< 2 ppm)		
0.1 – 1 ppm	Acetate, Antioxidant degradation Alkanes (C_9+)	1-octene, C8-alkenes Methylcyclopentane 1-3-di-tert-butylbenzene	1-3-di-tert-butylbenzene, Antioxidant
10 – 100 ppb	Alkanes		
5 – 10 ppb			
< 5 ppb			

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 Conclusion

Based on the findings from this study, we can say that the 30 days and the 91 days test results revealed only extractable chemical entities that were expected, with the level of extractables/leachables in tested contact fluids being extremely low (most concentrations in the ppb-1ppm range). All results indicate that extractables/leachables are low and near the detection limit of the analytical techniques.

Actual process conditions may impose different parameters, such as different time, temperature, and process fluid composition. Therefore, evaluation under actual process conditions is also recommended.

8. Biological Safety Tests

8.1 Introduction

The purpose of these tests was to evaluate the Biological suitability of the materials of construction of Allegro biocontainers. The materials of construction of the Allegro biocontainers are as follows:

Table 11

Materials of Construction

Inner and Outer Layers	Polyethylene
Oxygen and CO_2 barrier layer	Ethylene-Vinyl Alcohol Copolymer (EvOH)
Molded connection pieces	Polyethylene

Tests include USP Biological Reactivity Tests, *in vivo,* for Plastics Class VI-50 °C as described in the current United States Pharmacopoeia Chapter <88> and ISO10993 Biological Evaluation of Medical Device.

8.2 Summary of Methods

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

The USP Biological Reactivity Tests, *in vivo,* for Class VI-50 °C Plastics are described in the United States Pharmacopoeia, and include:

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

The four 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 hours ± 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 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 hemolytic 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.

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 hours ± 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 hours ± 2 hours.

8.2.3 USP <87> Biological Reactivity Tests, In Vitro (Cytotoxicity)
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 (elution method). 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 micro-scopically 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.

8.3 Results
The materials used in Allegro biocontainers passed USP <88> Biological Reactivity Tests, *in vivo,* for Class VI-50 °C Plastics, the USP <87> Biological Reactivity Tests, *in vitro,* for Plastics (Cytotoxicity) and the ISO 10993 Biological Evaluation of Medical Devices as described above. The polymer film, connectors, and the connector pieces passed the ISO 10993 standards.

8.4 Conclusion
The materials used in Allegro biocontainers meet the requirements of the USP <88> Biological Reactivity Tests, *in vivo,* for Class VI-50 °C Plastics, the USP <87> Biological Reactivity Tests, *in vitro,* for Plastics and the ISO 10993 Biological Evaluation of Medical Device in Section 4, 5, 6, 10 and 11.

9. Physicochemical Tests

9.1 Introduction
The purpose of these tests was to evaluate the physicochemical suitability of Allegro biocontainers. The purpose of the USP <661> test, of the European Pharmacopoeia Guidelines (Section 3.1.5) test, of the Japanese Pharmacopoeia Guidelines (Section 61 Part 1) test, of USP <85> test, of the European Pharmacopoeia Guidelines (Section 2.6.14) and of USP <788> test was to check that the materials of Allegro biocontainers meet their requirements.

9.2 Summary of Methods
Tests include USP Physicochemical Tests for Plastics, as described in the United States Pharmacopoeia (Chapter <661>), the USP <661>, European Pharmacopoeia (Section 3.1.5), the Japanese Pharmacopoeia (Section 61, Part 1), Endotoxins (USP <85> and European Pharmacopoeia Section 2.6.14) and Particulate (USP <788>).

9.2.1 United States Pharmacopoeia (Chapter <661>)
Plastic containers that are intended for packaging products for parenteral use must meet the requirements of Physicochemical 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 Metal Content — detects the presence of metals such as lead, tin, and zinc.

9.2.2 European Pharmacopoeia (Section 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 the guidelines in the European Pharmacopoeia (Section 3.1.5), "Polyethylene with additives for containers for parenteral and ophthalmic preparations."

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 Endotoxins

Samples of Allegro biocontainers were subjected to validated endotoxin determinations in biocontainers of volumes in accordance with USP <85> and the European Pharmacopoeia (2.6.14, current edition). The endotoxin tests were validated using chromogenic endpoint techniques. 1 L and 20 L biocontainers were tested. The 1 L biocontainers were filled with 100 mL of endotoxin free water and the

fluid tested for endotoxin content. Due to its larger volume, 20 L biocontainer were tested differently; Samples Item Portion (SIP) of 5 cm x 5 cm were cut from the biocontainer, and then extracted with 50 mL of endotoxin free water, the fluid was subsequently tested for endoxin. As per the European and US Pharmacopoeia, the endotoxin level for the filled 1 L bag and 20 L biocontainers should be < 0.25 EU/mL of application volume.

9.2.5 Particulate Test

Samples of Allegro biocontainers were tested for particulate cleanliness. In this test, the biocontainer bags have been spiked with known amounts of defined particle sizes (25 microns), followed by flushing it with water for extraction of the particles. The extract was then filtered over a membrane and the number of particles left on the membrane was counted using a light microscope. The tests were performed on 1 liter and 20 liter biocontainer volumes. In order to meet with the requirements of USP <788> test, average number of particles of sizes ≥ 25 microns must be < 2/mL.

9.3 Results

9.3.1 USP <661>, European Pharmacopoeia 3.1.5 and Japanese Pharmacopoeia

The Allegro biocontainers passed all the tests specified under USP <661>, European Pharmacopoeia (Section 3.1.5) and the Japanese Pharmacopoeia (Section 61, Part 1). The test results for endotoxin determination as per guidelines of USP <85> and European Pharmacopoeia 2.6.14 and USP <788> particulate testing test for particulate sizes 25 µm are presented in Table 12: Data on Endotoxin Concentration Measured from 1 L and 20 L Allegro Biocontainers.

9.3.2 Endotoxin Determination Tests (USP <85> and European Pharmacopoeia 2.6.14)

Table 12

Data on Endotoxin Concentration Measured from 1 L and 20 L Allegro Biocontainers

Bag Number	Biocontainer Volume (1 L)		Biocontainer Volume (20 L)	
	Endotoxin concentration (EU/biocontainer)	Extrapolated endotoxin concentration in 1 L filled biocontainer (EU/mL)	Endotoxin concentration volume of 50 mL for 50 cm^2 SIP from 20 L biocontainer (EU/SIP)	Extrapolated endotoxin concentration in 20 L filled biocontainer (EU/mL)
1	≤ 0.5	≤ 0.0005	≤ 0.34	≤ 0.002
2	≤ 0.5	≤ 0.0005	≤ 0.25	≤ 0.0015
3	≤ 0.5	≤ 0.0005	≤ 0.25	≤ 0.0015
4	≤ 0.5	≤ 0.0005	≤ 0.25	≤ 0.0015
5	≤ 0.5	≤ 0.0005	≤ 0.26	≤ 0.0015
6	≤ 0.5	≤ 0.0005	≤ 0.29	≤ 0.0018
Maximum value reported	**≤ 0.5**	**≤ 0.0005**	**≤ 0.34**	**≤ 0.002**

For 1 L biocontainers, the maximum endotoxin value of the biocontainers tested (a total of 6) was ≤ 0.5 EU/biocontainer, and equivalent to ≤ 0.0005 EU/mL for 1 L application volume being within the preset specification of ≤ 0.25 EU/mL. For 20 L biocontainers, the maximum endotoxin value of the SIP tested (a total of 6) was ≤ 0.34 EU/SIP, and equivalent to ≤ 0.002 EU/mL for 20 L application volume being within the preset specification of ≤ 0.25 EU/mL. So, the endotoxin concentration for 1 L and 20 L biocontainers falls within the specification.

9.3.3 USP<788> Particulate Testing for Particle Sizes 25 µm

Table 13

Data on Particulate Concentration Measured from 1 L and 20 L Allegro Biocontainers

Bag Number	Extrapolated 25 µm Particulate Value for 1 L Biocontainer		Extrapolated 25 µm Particulate Value for 20 L Biocontainer	
	Particulate concentration (count/ biocontainer)	Particulate concentration (count/mL)	Particulate concentration (count/ biocontainer)	Particulate concentration (count/mL)
1	620	0.62	8500	0.43
2	490	0.49	7650	0.38
3	520	0.52	5250	0.26
4	670	0.67	6200	0.31
5	630	0.63	7750	0.39
6	645	0.65	9300	0.47
7	565	0.57	7100	0.36
8	540	0.54	7800	0.39
9	630	0.63	6500	0.46
10	595	0.60	9200	0.47
Mean	**590.5**	**0.59**	**7525**	**0.39**

For the 1 L and 20 L biocontainers, the mean value for all the tested biocontainers are 0.59 and 0.39 particles/mL, falling within the preset value of less than two (2) 25 µm particles/mL.

9.4 Conclusions

The components of the Allegro biocontainers meet the requirements of the Physicochemical 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 1 L and 20 L bags, as per the specifications of USP <788> particulate testing tests for particulate sizes, 25 µm as well as endotoxin determination tests as specified under European and US Pharmacopoeia (respectively 2.6.14 and USP <85> current edition).

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