PALL Life Sciences

Validation Guide

USTR 2924

Allegro™ STR 200 Biocontainer

*Filtration. Separation. Solution.*SM

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1. Introduction

This guide contains data applicable to the Allegro STR 200 single-use biocontainer designed for use with the Allegro STR 200 system. This stirred tank reactor is a single-use, cell culture system that consists of three major components: 1) an Allegro STR 200 Tote, 2) a single-use Allegro STR 200 Biocontainer and 3) an Allegro STR 200 Controller. This validation guide focuses on the second component, the biocontainer, which is a flexible, gamma irradiated plastic bioreactor used to culture up to 200 L of mammalian cells. The biocontainer is equipped with a pitched blade impeller driven by a motor at the base of the tote. The rotation of the impeller induces fluid motion inside the biocontainer and facilitates cell suspension, efficient gas exchange, and liquid mixing. The purpose of this guide is to document the validation testing that has been performed to demonstrate the suitability of the Allegro STR 200 biocontainer to culture cells for use in the biotechnology industry.

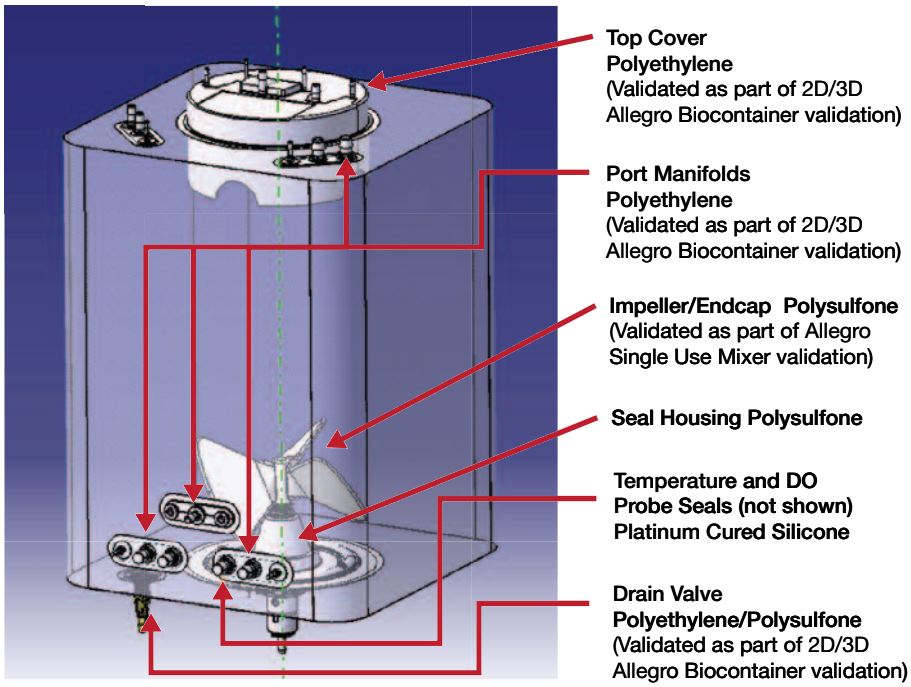
The Allegro STR 200 biocontainers are made of a low density polyethylene (LDPE) film which complies with the very high standards of quality required for biotechnology and pharmaceutical applications. The component parts of the Allegro STR 200 biocontainer are shown in Figure 1 and Table 1. These are also made from very high quality materials and tested as per the guidelines for biotechnology and pharmaceutical applications.

This validation guide summarizes the tests performed to qualify the Allegro STR 200 biocontainers for use in cell culture applications.

IMPORTANT NOTE: As indicated in the text, some of this validation data was generated during the original qualification of other Allegro biocontainers (Pall publications USTR 2475 Allegro 2D Biocontainers Validation Guide, USTR 2527 Validation Guide for Allegro 3D Biocontainers and Totes, USTR 2900 Allegro XRS 20 Biocontainer Validation Guide and USTR 2777 Pall Allegro Single-Use Mixers) as the materials and methods of construction are the same. To have copies of these validation guides please contact Pall at bioreactors@pall.com.

Figure 1

Allegro STR 200 Biocontainer Schematic



2. Summary of Conclusions

Extractables/Leachables Study

The purpose of this study was to characterize the chemicals that may be extracted/leached from typical Allegro biocontainers when exposed to different solutions and at specified temperatures for 30 and 90 days. All other Allegro STR 200 biocontainer parts in direct contact with cell culture fluid were tested separately (see Table 1). The results indicate low extractables/leachables levels when tested under specified conditions.

Biological Safety Tests

The materials used in all Allegro biocontainers [polyethylene and ethylene-vinyl alcohol copolymer (EvOH)] meet the requirements of the USP Biological Reactivity Tests (*in vivo*) for Class VI - 50 °C plastics. Specifically these tests assess the effect of biocontainer extracts for systemic toxicity, tissue irritation, and biocompatibility upon implantation. The materials used in the biocontainers meet the requirements for ISO 10993 Biological Evaluation of Medical Device in section 4 (Hemolysis), 5 (Cytotoxicity), 6 (Implantation test), 10 (Irritation and sensitization test) and 11 (Acute systemic toxicity).

Additional parts unique to the biocontainer used in Allegro STR 200 bioreactor, which were not covered by the original Allegro biocontainer tests, namely the impeller (polysulfone), sparger, (polyethylene), seal housing (polysulfone), and temperature and DO probe seals (platinum cured silicone elastomers) all meet the requirements of USP<88> Biological Reactivity Tests (*in vivo*) for Class VI-121 °C Plastics Testing and USP <87> Biological Reactivity Tests (*in vitro*).

Physico-chemical Tests

The purpose of these tests was to evaluate the physico-chemical suitability of the Allegro biocontainers for USP <661> Containers - Plastics, European Pharmacopoeia (sec. 3.1.5), and Japanese Pharmacopoeia (Sec. 61 Part 1) standards, USP<788> Particulate Matter in Injection and USP<85> Bacterial Endotoxins. The components of the Allegro 3D biocontainers meet the requirements of all these standards.

In addition to the above, testing was also performed on materials used for construction of the Allegro STR 200 Biocontainer, but not included in the original Allegro biocontainer tests. The results show that the impeller (polysulfone), sparger (polyethylene), seal housing (polysulfone), and temperature and DO probe seals (platinum cured silicone elastomers) all meet the requirements of USP <661> Containers – Plastics, USP<788> Particulate Matter in Injection and USP<85> Bacterial Endotoxins.

Shelf Life Tests

Nine fully assembled Allegro STR 200 biocontainers were subjected to shelf life testing immediately after gamma irradiation and again after accelerated aging equivalent to 6 months of real time aging. Stirring and water leak tests, peel tests on connectors, and tensile strength tests on the outer welds, top plate, bottom drain port, and cross crease fold, were performed. The results demonstrate that functionality of the biocontainers remained intact after gamma irradiation and 6 months of accelerated aging. A three year shelf life study is currently underway. Interim reports for longer time periods will be available upon request (bioreactors@pall.com) as data become available.

Table 1
Results Summary for all Allegro STR 200 Parts Tested

Fluid Contact Part*	Material	% of Total Biocontainer Surface Area	E/L tests	USP <85>	USP <88>	USP <87>	USP <661>	USP <788>
Biocontainer Film	Polyethylene	80.5	Y	Y	Y (Class VI-50 °C)	Y	Y	Y
Molded Connection Pieces (Drain Valve Flange/ Port Manifolds /Topcover)	Polyethylene	11.75	Y	Y	Y (Class VI-50 °C)	Y	Y	Y
Endcap/Impeller/ Drain Valve Spigot	Polysulfone	4.90	Y	Y	Y (Class VI-50 °C)	Y	Y	Y
Sparger Rings	Polyethylene	1.70	Y	Y	Y (Class VI-121 °C)	Y	Y	Y
Seal Housing	Polysulfone	0.90	Y	Y	Y (Class VI-121 °C)	Y	Y	Y
Seal around Temperature/ DO Probe	Platinum Cured Silicone Rubber	0.05	Y	Y	Y (Class VI-121 °C)	Y	Y	Y

** group of parts to which the material test applies*
E/L = extractables/leachables
Y = completed tests that meet indicated standards

3. Extractables/Leachables Study

3.1 Introduction

The purpose of the extractables/leachables study was to characterize and semi-quantify the volatile and non-volatile residues or components that may be extracted/leached from typical Allegro biocontainers, which includes the Allegro STR 200 biocontainer, when exposed to different solutions.

All Allegro 2D and 3D biocontainers use the same manufacturing method and materials of construction for the film and molded connection pieces. Therefore, the extractables/leachables test results are applicable for all types of Allegro biocontainers, including the Allegro STR 200 biocontainer. The data presented is taken from the validation of 2D and 3D Allegro biocontainer.

The additional fluid contact parts unique to the construction of the Allegro STR 200 biocontainer were also tested as part of this validation. A schematic of the biocontainer, showing the additional components and their location within the container, can be seen in Figure 1. These components are listed described in Table 1, along with information about the type of material, the percentage of total fluid contact surface area comprised by the specific part(s), and the test results.

3.2 Summary of Extractables/Leachables Methods for the Allegro Biocontainer Film and Polyethylene Material used for Molded Connection Pieces.

The Allegro biocontainer with molded connection pieces were gamma irradiated at a dose ≥26 kGy. Subsequently, samples of the biocontainers and additional components were extracted for 30 and 90 days as described in Table 2.

Table 2

Contact Fluids and Tests Applied to Allegro Biocontainers

	Contact Fluid						
Analytical Test Methods	**PBS–pH3**	**WR**	**PBS–pH11**	**NaCl 3M**	**Tween 80–1% in WR**	**Ethonal 96%**	**DMSO 10%**
Allegro biocontainer filling and recording of weight (empty + filled)	ABC	ABC	ABC	ABC	ABC	BC	BC
pH Measurement	ABC	ABC	ABC	ABC	ABC	BC	BC
Conductivity	ABC	ABC	ABC				
Total Organic Carbon (TOC)	ABC	ABC	ABC				
Metals (ICP-OES)	ABC	ABC	ABC	ABC	ABC	BC	BC
Headspace GC-MS	ABC	ABC	ABC	ABC	ABC	BC	BC
Solvent Extraction + GC-MS	ABC	ABC	ABC	ABC	ABC	BC	BC
Solvent Extraction + LC-MS	ABC	ABC	ABC	ABC	ABC	BC	BC
Ion Chromatography	ABC	ABC	ABC	ABC	ABC	BC	BC
Derivization GC-MS	ABC	ABC	ABC	ABC	ABC	BC	BC
	40 ºC 75% RH	40 ºC 75% RH	40 ºC 75% RH	40 ºC 75% RH	40 ºC 75% RH	25 ºC	25 ºC
	Storage Conditions						

Biocontainer – 30 Day Incubation = A, Biocontainer – 90 Day Incubation = B, Control – Gas Bottle = C

PBS is phosphate buffered saline, WFI is water for injection, DMSO is dimethyl sulfoxide, and RH is relative humidity, ICP is inductively coupled plasma, GC is gas chromatography, LC is liquid chromatography, MS is mass spectrometry.

Seven test bags were constructed from the Allegro biocontainer film and the polyethylene material used for Allegro molded connection pieces. These bags, with an internal surface area of 1625 cm^2 and a maximum volume of 5 liters, were each filled with 3.25 L of contact fluid (table 2) via a peristaltic pump to provide an extraction ratio of 2 mL per cm^2 of test film material. Once filled, they were stored under conditions specified in Table 2 for 30 or 90 days.

The extractable/leachable test fluids were analyzed as described in Table 2 and data from the bags made using the Allegro biocontainer film were compared with control samples from a glass bottle. The glass bottle control samples were stored at the same conditions as the test samples.

3.3 Summary of Extractable/Leachables Methods for Additional Materials used in the Allegro STR 200 Biocontainer

Extractables/leachables tests for Allegro STR 200 biocontainer components (sparger, impeller*, seal housing and silicone probe seals) were conducted by submerging each material part in contact fluid, either ethanol or water for injection (WFI), at 40 °C for 30 days. Reference extractions were set up in parallel, using glass bottles. Test materials and glass bottles were irradiated at 50 +/-5 kGy prior to the test. The same surface area/volume ratio of 2 cm^2/mL of extraction solvent for each material was applied. The samples were then subjected to the analytical tests described in Table 3.

**Impeller study done as part of Allegro single-use mixer validation*

Table 3

Experimental Design for Extractables Testing on Additional Allegro STR 200 Biocontainer Components

Fluid Contact Part	Endcap/Impeller		Sparger Ring		Seal Housing		Seal around Temperature/DO Probe	
Extraction Solution	WFI	Ethanol 96%	WFI	Ethanol 96%	WFI	Ethanol 96%	WFI	Ethanol 96%
pH Measurement	✔		✔		✔		✔	
Conductivity	✔		✔		✔		✔	
Total Organic Carbon (TOC)	✔		✔		✔		✔	
Metals (ICP)	✔	✔	✔	✔	✔	✔	✔	✔
Headspace GC/MS	✔	✔	✔	✔	✔	✔	✔	✔
GC/MS	✔	✔	✔	✔	✔	✔	✔	✔
LC/MS	✔	✔	✔	✔	✔	✔	✔	✔
Residue on Evaporation (NVR)	✔	✔	✔	✔	✔	✔	✔	✔
Anions (IC)	✔	✔	✔	✔	✔	✔	✔	✔

3.4 Extractables/Leachables Testing Results

Extractables/Leachables Results for Allegro Biocontainers

The bags made using the Allegro biocontainer film, together with polyethylene material used for the molded connection pieces (after gamma irradiation at ≥26 kGy), were filled with 7 different contact fluids. No significant loss in weight (evaporation), change in pH or change in conductivity was observed after 30 days and 90 days exposure to the contact fluids. The fluid analysis after 90 days of contact revealed low concentrations of extractables in comparison to the glass bottle used as a control. Virtually all identified extracted/leached chemical entities attributed to the biocontainer (Table 4) are explainable as either oligomers of polymer used or degradation products from the antioxidants used.

Table 4

Analytical Summary of Extractables/Leachables Analysis for Allegro Biocontainers, Comprised of Allegro Film and Molded Connection Pieces

Contact Fluid / Extractant							
Level	**WFI**	**PBS-pH3**	**PBS-pH 11**	**3M NaCl**	**96% Ethanol**	**1% Tween 80**	**10% DMSO**
2-10 ppm					1-3-di-tert-buthylbenzene		
1-2 ppm	TOC	TOC			C8-alkenes		
0.1-1 ppm		Acetate Hexanal	TOC	Fatty acids	Acetate, AOx degradation, Alkenes (C9+)	1-octene C8-alkenes Methylcyclo pentane 1-3-di-tert-buthy lbenzene	1-3-di-tert-buthyl benzene AOx
10-100 ppb	Acetate AOx degradation/ Di-tert-butylphenol	AOx degradation/ Di-tert-butylphenol	2-methyl-1-propene Di-tert-butylphenol	Di-tert-butylphenol	Alkanes		
5-10 ppb	2-methyl-1-propene 2-octanone		Antioxidants	Hexanal			
<5 ppb	Antioxidant			Antioxidants 2-methyl-1-propene			

ppm is parts per million, ppb is parts per billion

Extractables//Leachables Results for the Additional Materials Used in the Allegro STR 200 Biocontainer.

Contact fluids did not show a significant change in pH or conductivity after 30 days of contact. Additionally, virtually all identified extracted/leached chemical entities are explainable as oligomers of polymer used, polymer additives, or degradation products from the antioxidants used (Table 5).

Table 5

Summary of Extractables/Leachables Data for Additional Parts Used for the Allegro STR 200 Biocontainers

	Contact Fluid / Extractant	
Level	**WFI**	**Ethanol**
>100 ppm		Alkanes
10 ppm – 100 ppm	TOC	1,3-Di-tert-butylbenzene fatty acids
5 ppm – 10 ppm		2,4-Di-tert-butylphenol, Ethyl hexadecanoate AntiOx degradation products
1 ppm – 10 ppm	Chloride, Nitrate, Acetate, Formate	Acetate, Formate, TOC Chlorobenzene
0.1 ppm – 1 ppm	AntiOx degradation products Ca, Na	2-methyl-1-propene (1), Hydrocarbons Ca, Al, Fe, K, Na, Zn
1 ppb – 100 ppb	2-methyl-1-propene (1), AntiOx degradation Products, 2-methyl-1-propanol, 2,2-Dimethylpropanoic acid (1), Octanone Amide (2) Polyethoxylated Compound (2) Fatty acids Al, Cu, Fe, K, Mg, Zn	Cu, Mg Isomer of Chloromethylphenol (1) Amide (2)

(1) Most probable compound
(2) Tentatively Identified

Extractables/Leachables Testing Conclusions

The results from the Allegro biocontainer testing after 30 and 90 days indicate that the level of extractables/leachables for tested contact fluids was extremely low (Tables 4 and 5), especially for the WFI and PBS contact fluids, which most closely match actual application conditions.

Additional extractables/leachables test results from parts used in the construction of the Allegro STR 200 biocontainers also show low levels of extractables. Virtually all identified extracted chemical entities are explainable as oligomers from the polymers, polymer additives, or degradation products from the antioxidants.

As expected, the ethanol extraction showed higher concentrations of the detected compounds for most parts tested. The levels seen in the water extract were much lower, which suggests that the compounds have limited solubility in water. The components tested account for a very small proportion of the total surface area of the biocontainer and therefore the concentration of the detected compounds should be viewed in that context. For example, TOC detected for the sparger component was 10 ppm in extraction volume. The area of the sparger ring as a percentage of the whole system is 1.64% and therefore the sparger would contribute only extremely low TOC to the whole biocontainer.

More detailed information on the results of extractables/leachables study is available upon request (at bioreactors@pall.com).

4.0 Biological Safety Tests

4.1 Introduction

The purpose of these tests was to evaluate the biological suitability of the materials of construction of Allegro STR 200 biocontainers. Allegro 2D and 3D biocontainers use the same manufacturing method and same material of construction for the film, drain valve, port manifolds and top cover. Additionally, the endcap and impeller from the Allegro mixer are made of the same material and use the same method of construction. Therefore, the biological safety test results for these products are applicable for all Allegro biocontainers. Table 6 lists the biological safety tests performed on these parts as well as on newly validated parts Allegro STR 200 biocontainer components.

Table 6

List of Tests Applied for Each Allegro STR 200 Biocontainer Fluid Contact Part

Fluid Contact Part	Test Type	Test Results From
Biocontainer Film	USP 88 Class VI-50 °C / USP87 & ISO10993	2D/3D Allegro biocontainer validation
Molded Connection Pieces (Drain Valve Flange/Port Manifolds / Topcover)	USP 88 Class VI-50 °C / USP87 & ISO10993	2D/3D Allegro biocontainer validation
Endcap/Impeller	USP 88 Class VI-50 °C / USP87	Allegro single-use mixer validation
Sparger Ring	USP 88 Class VI-121 °C & USP87	Allegro STR 200 biocontainer validation
Seal Housing	USP 88 Class VI-121 °C & USP87	Allegro STR 200 biocontainer validation
Seal around Temperature/DO Probe	USP 88 Class VI-121 °C & USP87	Allegro STR 200 biocontainer validation

4.2 Summary of Biological Safety Test Methods

Tests include USP Biological Reactivity Tests, *in vivo* for Class VI Plastics (50 °C / 121 °C) as described in the current United States Pharmacopoeia Chapter <88>, USP Biological Reactivity Tests, *in vitro* as described in the current United States Pharmacopoeia Chapter <87>, and ISO10993 Biological Evaluation of Medical Device.

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 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 (50 kGy) biocontainer film, impeller material and molded connection piece were extracted with these solutions at 50 +/- 2 °C for 72 +/- 2 hours.

The additional materials used in the Allegro STR 200 biocontainer, namely the sparger ring, seal housing material, and silicone probe seals, were gamma irradiated (50 kGy) and extracted with these solutions at 121 °C +/- 2 °C for 60 mins.

The extracts were then used in the following tests to assess biological effects:

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.

USP <87> Biological Reactivity Tests After 50 kGy Gamma Irradiation

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, gamma-irradiated to 50 kGy, was prepared using single strength medium essential medium (1X MEM) supplemented with 5% serum and antibiotics. This test extract was placed onto two separate monolayers of L-929 mouse fibroblast cells. 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 ºC in the presence of 5% CO_2 for 48 hours then examined microscopically to assess changes in cell morphology. The negative and positive controls performed as anticipated. Extracts from test materials showed no evidence of a cell lysis or toxicity effect on the L-929 cells, thus meeting the requirement of the USP <87> standards.

ISO 10993 Biological Evaluation of Medical Devices

Gamma irradiated (≥26 kGy) samples of the film and of the molded connection pieces were tested for the following sections of ISO 10993:

- ISO 10993-4 Hemolysis

This study assesses 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 extractable from test material on the test cells, by adding the extracts to a cell culture. 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 these 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 irritation effects upon intracutaneous injection in animals. The test materials were extracted with sodium chloride for injection and cottonseed oil at 70 ± 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 ± 2 °C for 24 ± 2 hours.

4.3 Biological Testing Results

A summary of the Biological Safety Testing is shown in Table 7. All materials used in the construction of the Allegro STR 200 biocontainer met the test requirements as defined by the indicated protocols.

Table 7

Summary of the Biological Safety Test Results for the Allegro STR 200 Biocontainer

Fluid Contact Part	Test Type	Test Results
Biocontainer Film	USP 88 Class VI-50 °C / USP87 and ISO10993	Met all requirements
Molded Connection Pieces (Drain Valve/Port Manifolds / Topcover)	USP 88 Class VI-50 °C / USP87 and ISO10993	Met all requirements
Endcap/Impeller	USP 88 Class VI-50 °C and USP87	Met all requirements
Sparger Rings	USP 88 Class VI-121 °C and USP87	Met all requirements
Seal Housing	USP 88 Class VI-121 °C and USP87	Met all requirements
Seal around Temperature/DO Probe	USP 88 Class VI-121 °C and USP87	Met all requirements

4.4 Biological Safety Test Conclusions

All materials used in the Allegro STR 200 biocontainer meet the requirements of the USP Biological Reactivity Tests, *in vivo*, for Class VI Plastics (USP <88>) and USP Biological Reactivity Tests, *in vitro*, Cytotoxicity (USP <87>).

5.0 Physico-chemical Tests

5.1 Introduction

The intent of these tests was to evaluate the physico-chemical properties 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 specific physico-chemical test requirements.

Table 8 lists physico-chemical tests used for each of the components in the Allegro STR 200 biocontainer.

Table 8

Physico-chemical Tests Performed for the Allegro STR 200 Biocontainer Components

Fluid Contact Part	Test Type	Dataset
Biocontainer Film	USP<661> EP3.1.5 JP(Sec61Pt1) USP<788> EP2.6.14 USP<85>	Validated as part of 2D/3D Allegro biocontainer validation
Drain Valve Flange/Port Manifolds/Topcover	USP<661> EP3.1.5 JP(Sec61Pt1) USP<788> EP2.6.14 USP<85>	Validated as part of 2D/3D Allegro biocontainer validation
Endcap/Impeller	USP<661> USP<788> USP<85>	Validated as part of Allegro single-use mixer validation
Sparger Ring	USP<661> USP<788> USP<85>	Tested as part of Allegro STR 200 biocontainer validation
Seal Housing	USP<661> USP<788> USP<85>	Tested as part of Allegro STR 200 biocontainer validation
Seal around Temperature Probe	USP<661> USP<788> USP<85>	Tested as part of Allegro STR 200 biocontainer validation

5.2 Summary of Physico-chemical Test 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, and Japanese Pharmacopoeia guidelines section 61 Part 1. Tests on particulates 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.

USP <661>

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 in contact with extraction medium over a specified period and specific temperature.

Irradiated samples (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 these contact liquids are then analyzed following the USP <661> guidelines.

In addition, irradiated samples (50 kGy) of the other Allegro STR 200 biocontainer parts, specifically the sparger, probe seal, and seal housing, were extracted at 70 °C for 24 hours in purified water and isopropyl alcohol. Samples of these contact liquids were then tested following USP <661> guidelines listed below.

- Non Volatile Residue (NVR) - 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 Content- detects the presence of metals such as lead, tin, zinc, etc.

European Pharmacopoeia (3.1.5)
Irradiated samples (> 26 kGy) of film used to construct Allegro biocontainers and material used for the molded connection pieces were extracted under European Pharmacopoeia guidelines section 3.1.5 Polyethylene with additives for containers for parenteral and ophthalmic preparation. The following tests were performed:

- Appearance - extract should be clear and colourless
- Acidity and alkalinity – pH measurement, 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
- Sulphated ash - detect the presence of sulphated ash in the extracts

Japanese Pharmacopoeia (Section 61 Part 1)
Irradiated samples (> 26 kGy) of film used to construct Allegro biocontainers and material used for 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.

Endotoxins
Samples of representative Allegro biocontainers were tested for endotoxin at 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 samples of 100 L representative biocontainers were used. Due to their larger size, eight small bags were made in a clean room environment by randomly cutting 100 cm^2 films from each of the eight 100 L 3D biocontainers. These small biocontainers were extracted with endotoxin free water and the contact 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.

Additionally, irradiated samples (50 kGy) of the sparger, silicone probe seal, seal housing material, impeller material and port manifold were tested for endotoxin in accordance with the US Pharmacopoeia USP <85>, current edition.

- Three samples of the sparger ring were placed in a depyrogenated foil tray with 200 mL of LAL reagent water and incubated at 37 °C +/- 2 °C for a minimum of 15 minutes.
- Three samples of the other components (silicone probe seal, polysulfone seal housing material, impeller material and LDPE port manifold) were placed in a depyrogenated beaker with 2000 mL of LAL reagent water and incubated at 37 °C +/- 2 °C for a minimum of 15 minutes.
- After incubation, 20 mL of LAL reagent water from the foil tray (sparger ring extract) and 20 mL from the beaker (extraction of remaining components) were combined in a depyrogenated container.
- The combined test fluid pool was analysed, in triplicate, for endotoxin as per USP<85>.

USP <788> Particulate Matter in Injections

Testing was performed on three fully assembled STR 200 biocontainers containing 60 L of ultra pure water, mixed at the maximum impeller speed of 150 RPM. The three biocontainers were run for 30 days. Samples of water were removed aseptically at 0, 15, and 30 days. The samples were then analyzed to determine the sub-visible particle count as per USP<788> using the light obscuration particle count test.

5.3 Physico-Chemical Test Results

A summary of the Physico-chemical test results from the Allegro STR 200 biocontainer parts and assembled biocontainer (Table 9), indicating that all requirements were met.

Table 9

Physico-Chemical Test Results Summary for the Validation of the Allegro STR 200 Biocontainer

Fluid Contact Part	Test Type	Test Results
Biocontainer Film	USP<661> EP3.1.5 JP(Sec61Pt1) USP<788> EP2.6.14 USP<85>	Met all requirements
Drain Valve Flange/Port Manifolds/Topcover	USP<661> EP3.1.5 JP(Sec61Pt1) USP<788> EP2.6.14 USP<85>	Met all requirements
Endcap/Impeller	USP<661> USP<788> USP<85>	Met all requirements
Sparger Ring	USP<661> USP<85>	Met all requirements
Seal Housing	USP<661> USP<85>	Met all requirements
Seal around Temperature Probe	USP<661> USP<85>	Met all requirements
Fully Assembled Allegro STR 200 Biocontainers	USP<788>	Met all requirements

USP<661>, European and Japanese Pharmocopoeia

The Allegro biocontainers passed all the tests (Table 9) specified under USP <661>, European Pharmacopoeia (3.1.5), and Japanese Pharmacopoeia (61 part 1). Additionally, the sparger, impeller seal housing and silicone seal components of the Allegro STR 200 L biocontainers met the requirements of the Physiochemical Test-Plastics USP <661>.

Endotoxin Tests

Results from the endotoxin tests are shown in Tables 10 and 11, below. In all cases endotoxin levels were below the limit of quantitation for these assays, and thus within an acceptable range for single-use biocontainers. Copies of the detailed test reports are available from Pall (bioreactors@pall.com).

Table 10

Endotoxin Concentration Data 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)
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

Table 11

Endotoxin Results: Two Separate Endotoxin Tests with Specific Components used in the Allegro STR 200 Biocontainers

Sample	Dilution	Lot	Results EU/mL	EU/device
Kit Components	Neat	Batch One	< 0.01	< 20
(18 Pooled)	1:2	Batch One	< 0.02	< 20
	1:4	Batch One	< 0.04	< 20
	1:8	Batch One	< 0.08	< 20
	Neat	Batch Two	< 0.01	< 20
	Neat	Batch Three	< 0.01	< 20

USP <788> Particulate Matter in Injections

Table 12 shows the particulate data from replicate samples taken from three Allegro 200 STR biocontainers. The results show particulate counts of less than 25 particles per mL at ≥10 µm and less than 3 particles per mL at ≥25 µm. Thus, Allegro STR 200 biocontainers met the requirements of USP <788> Particulate Matter in Injections. All samples at each time point passed the requirements of USP <788>.

Table 12

Particulate Test Results from Allegro STR 200 Biocontainer

			Counts per mL			
STR Biocontainer	Time Point (Days)	Sample	≥ 10 µm Blank	Biocontainer	≥ 25 µm Blank	Biocontainer
1	0	1	3	10	0	0
		2		2		0
2	0	1	4	2	0	0
		2		3		0
3	0	1	8	1	0	0
		2		1		0
1	15	1	1	3	0	0
		2		12		1
2	15	1		6		0
		2		8		1
3	15	1		12		1
		2		14		1
1	30	1	1	4	0	0
		2		5		0
2	30	1		3		0
		2		6		0
3	30	1		3		0
		2		5		0

Physico-Chemical Testing Conclusions

The components of the Allegro biocontainers met the requirements of the Physiochemical Test-Plastics USP <661>, the European Pharmacopoeia Guidelines (section 3.1.5) and the Japanese Pharmacopoeia guidelines (section 61 part 1), as well as endotoxin determination tests as specified under European Pharmacopoeia (PhEur. 2.6.14) and US Pharmacopoeia (USP <85>).

Components used specifically in the construction of Allegro STR 200 biocontainers, namely sparger, impeller seal housing and silicone seal also met the requirements of the Physiochemical Test-Plastics USP <661>.

The Allegro STR 200 biocontainers met the requirements of particulate testing, performed on fully assembled and irradiated biocontainers, as per the specifications of USP <788> particulate testing for particulate sizes ≥ 10 µm and ≥ 25 µm.

In addition, irradiated samples of the sparger, probe seal, seal housing and port manifold (from 3 batches) met the requirements of US Pharmacopoeia USP <85>.

Copies of the test reports are available by contacting Pall (bioreactors@pall.com).

6.0 Shelf Life Studies

6.1 Introduction

Full shelf-life studies have been set up to establish a 3-year shelf life for the Allegro STR 200 biocontainer. Shelf life testing is performed on biocontainers after gamma irradiation and storage under controlled real-time and accelerated aging conditions. At the time of publication of this guide only six month accelerated shelf life results were available. Reports for longer time periods will be available on request (bioreactors@pall.com).

There are three objectives for this series of tests:

- To demonstrate that an adequate safety margin is maintained for the water leak test of gamma-irradiated Allegro STR 200 biocontainer, 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 STR 200 biocontainer, 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 STR 200 biocontainer, following storage for up to 3 years.

6.2 Summary of Methods

Full shelf-life study using real time and accelerated aging has been carried out on three different lots of the Allegro STR 200 biocontainer, based on ASTM F-1980-07 and internal procedures. Biocontainers were subjected to gamma sterilization then stored in a warehouse for three years at real time (ambient) and accelerated aging conditions. Table 13 shows a detailed matrix plan for the full shelf life study.

Table 13

Description of Shelf Life Tests Performed

	6 Month Equivalent*	3 Year Equivalent*	3 Year Real Time
Sample Number	9 pieces, 3 ea from 3 lots	3 pieces, 1 ea from 3 lots	3 pieces, 1 ea from 3 lots
Gamma Dose	50 kGy	30-50 kGy	30-50 kGy
Storage Temperature	50 °C	40 °C	Ambient
Storage Humidity	Ambient	75% relative humidity	Ambient

** accelerated aging*

At each time point indicated above, tests of the mechanical strength on connectors, seal weld, and cross crease fold were performed. Leak testing was also performed as a further test of the integrity of the seal. These tests are described below:

6.2.1 Leak Test

The leak test was carried out by filling the nine biocontainers supported in the metal tote with 200 L of water. The impeller was set to run at 150 RPM, all gas inlet ports were set to flow at 20 SLM (standard liters/min) with total gas flow of 60 SLM, the water temperature was set to 40 °C. The biocontainer was visually inspected for any signs of leakage during filling and for 30 days of running at maximum gas flow, impeller speed and water temperature.

6.2.2 Tensile Strength Test and Peel Test

Tensile strength and peel testing was conducted on biocontainers at each shelf life interval. The strength of the film to film seal welds, cross crease fold, film only and peel strength of the film to molded connector welds was checked using a Tensile Test Instrument.

The test required three samples from ten locations on each of three biocontainers tested.

Each test strip was 25 mm wide and taken using a sample cutter from the following locations:

Film only – (Tensile Test),

Film on film weld – (Peel Test – Top, side, bottom and cross crease welds),

Top manifold to film weld – (Peel Test),

Top cover to film weld – (Peel Test),

Sensor manifold to film weld – (Peel Test),

Base plate to film weld – (Peel Test),

Drain port to film weld – (Peel Test)

The tensile force was applied at a rate of elongation of 200 mm/min. To pass this test, the force at which the film breaks must exceed 70 N (newtons) and there should be no peeling away of the film from the molded connector parts.

Figure 2
Connector Peel Test method

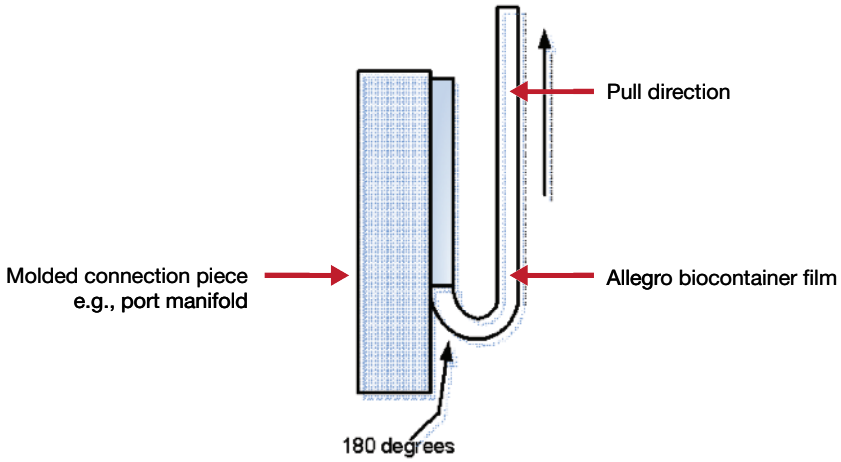


Figure 3
Film On Film Weld Area Peel Test method

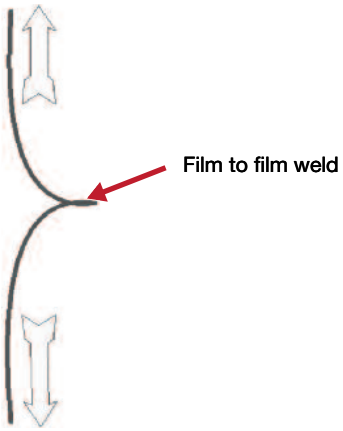


Figure 4
Film Only Tensile Test method

6.3 Shelf Life Testing Results

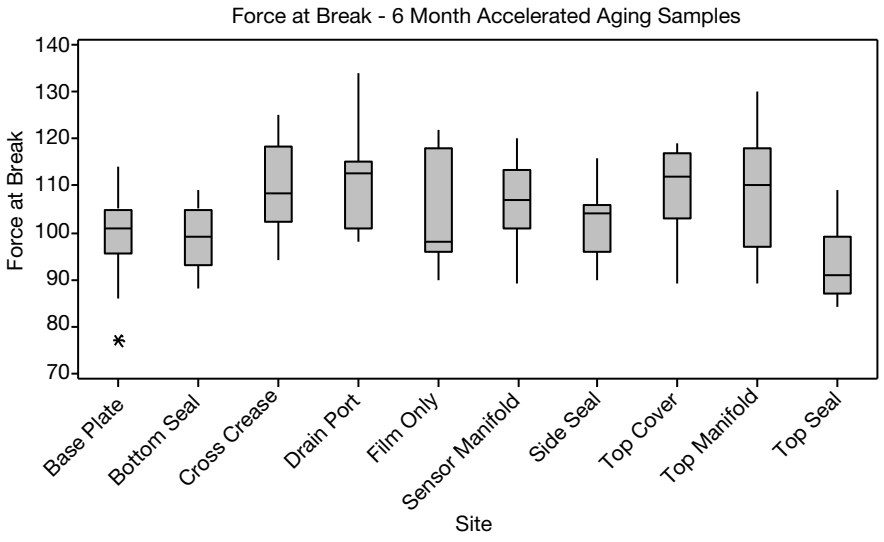
Leak Test Results

All biocontainers, immediately after irradiation and after accelerated aging equivalent to six months, passed the leak test as described in Section 6.2.1. None of them showed any sign of leakage during the 30 day test period.

Tensile Strength and Peel Test Results

Figure 5 shows the average tensile strength comparison at 10 locations, after accelerated aging equivalent to 6 months real time aging. All test samples required more than 70 N of force to break the film, thus passing the minimum requirement for this test. There was no peel away from any of the manifolds or the film to film welds.

Figure 5
Results of Tensile Strength Testing after 6 Month Equivalent Accelerated Aging



Base Plate N=13, Bottom Seal N=15, Cross Crease N=10, Drain Port N=12, Film Only N=15, Sensor Manifold N=30 , Side Seal N=15, Top Cover N=15, Top Manifold N=15, Top Seal N=15

6.4 Conclusions

At the time of publication, shelf life testing from accelerated aging equivalent to 6 months was available. Data at this time point showed that the Allegro STR 200 biocontainer passed all tests. No leakage was detected for any of the biocontainers tested. The average tensile strength for all the biocontainers tested at all 9 locations, plus film only, exceeded 70 N. The results indicate that the functionality and integrity of the Allegro STR 200 biocontainer remained intact after 50 kGy irradiation and 6 months accelerated aging.

A three year real-time shelf life study is currently underway. Interim reports will be available upon request at bioreactors@pall.com as data becomes available.

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