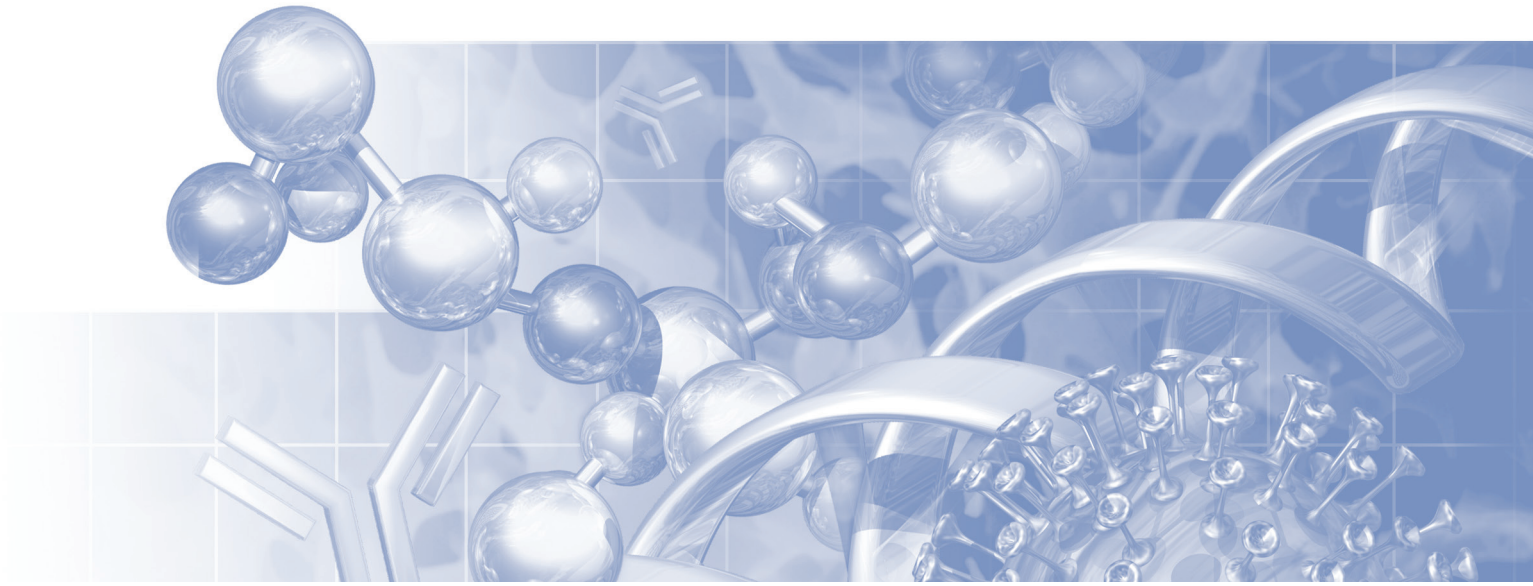
PALL Biotech

Validation Guide

USTR 2900[1]

Allegro™ XRS 25 Biocontainer



*Filtration. Separation. Solution.*SM

Table of Contents

1. Validation Overview

1.1 Introduction

This guide contains data applicable to the Allegro XRS 25 biocontainers designed specifically for use with the Allegro XRS 20 bioreactor, and the Allegro XRS 25 bioreactor platform.

The Allegro XRS 25 bioreactor, and its predecessor the Allegro XRS 20 bioreactor, is a disposable cell culture system that consists of three major components:

1) Rocker platform

2) Disposable biocontainer

3) Control system

This validation guide focuses on the second component: The Allegro XRS 25 biocontainer, which is a flexible, gamma irradiated and single-use biocontainer used to culture up to 25 L of mammalian cells. The rocking motion of the platform induces fluid motion inside the biocontainer which enables cell suspension and efficient mixing of gases and liquids.

Allegro biocontainers are made of a low density polyethylene (LDPE) film and high density polypethylene (HDPE) ports, for which Pall has characterized the extractable's profile as per recommendation of BPOG (www.biophorum.com), to demonstrate the biocontainers comply with the very high standards of quality required for biotechnology and pharmaceutical applications. The Allegro XRS 25 biocontainer includes unique design features that significantly improve the design and robustness of single-use systems while enabling high cell culture performance.

The purpose of this guide is to document extractables/leachables studies, biological safety tests and physico-chemical tests that have been performed to demonstrate the suitability of Allegro XRS 25 biocontainers, for use in cGMP processes for biotechnology and pharmaceutical applications.

IMPORTANT NOTE: As indicated in the text, some data has been used from the original qualification of other Allegro biocontainers (Pall publications Allegro 2D Biocontainers Validation Guide, document reference USTR 2475, and Validation Guide for Allegro 3D Biocontainers and Totes, document reference USTR 2527) because of the similar materials and methods of manufacture.

Please contact Pall for copies of these other validation guides. The confidential reports on extratable profile characterization for the Allegro film and HDPE ports are also available on request.

Cell culture data that proof the suitability of the Allegro XRS 20 bioreactor and the Allegro XRS 25 bioreactor platform for mammalian cell culture from 2 to 25 L can be found in Application Note CHO Cell Cultivation in the Allegro XRS 25 Bioreactor System with Working Volumes from 2 to 25 L, document reference USD 3100.

2. Summary of Conclusions

2.1 Extractables/Leachables Study

The purpose of this study was to quantify and characterize the chemicals that may be extracted/leached out from typical Allegro biocontainers when exposed to different solutions, temperatures and time periods. The results after 30 and 91 days exposure 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).

2.2 Biological Safety Tests

Fully fabricated components used in all Allegro biocontainers (polyethylene connector piece and ethylene-vinyl alcohol copolymer [EvOH] biocontainer) have been tested and met the requirements of the United States Pharmacopeia (USP) Biological Reactivity Tests (*in vivo*) for Class VI-50 °C plastics under USP <88>, which included the Systemic Toxicity Test, the Intracutaneous Test, and the Implantation Test. In addition, the materials used in the biocontainer components 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).

Materials used to fabricate Allegro XRS 25 biocontainer system components which were not covered by the original Allegro biocontainer tests (high density polyethylene [HDPE], polysulfone, silicone elastomers and polycarbonate) have also been tested and met the requirements under USP <88> for Class VI-50 °C plastics and the USP Biological Reactivity Tests (*in vitro*), under USP <87> (*in vitro*) testing was per the Minimum Essential Medium (MEM) Elution Cytotoxicity Test.

2.3 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.

In addition to the above, testing was also performed on materials that are used on the Allegro XRS biocontainer but not covered by the original Allegro biocontainer tests. These include HDPE ports, polysulfone, silicone elastomers and polycarbonate.

3. Extractables/Leachables Study

3.1 Introduction

The purpose of the extractables/leachables study was to quantify and characterize the volatile and non-volatile residues or components that may be extracted/leached out from typical 2D and 3D Allegro biocontainers when exposed to different solutions.

Allegro 2D and 3D biocontainers use the same manufacturing method as well as the same materials of construction. Therefore, the extractables/leachables test results are applicable for both types of biocontainer which includes the Allegro XRS biocontainer. The data presented is taken from the validation of 2D and 3D Allegro biocontainers.

Additional work has been performed on the Allegro XRS 25 biocontainer system which includes components not present in the 2D biocontainer, such as tubing, sterilizing grade filters and disposable sensors.

3.2 Summary of Extractables/Leachables Methods for Allegro 2D and 3D Biocontainers

Gamma irradiated 26.6 – 32.0 kGy samples of Allegro biocontainers and the polymer used in the Allegro XRS molded connection pieces were extracted for 30 and 91 days in conditions and fluids described in Table 1.

Table 1

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 ºC (104 °F) / 75% RH	A	A	A
2	WFI	40 ºC (104 °F) / 75% RH	A	A	A
3	PBS – pH11	40 ºC (104 °F) / 75% RH	A	A	A
4	NaCl 3M	40 ºC (104 °F) / 75% RH	B	B	B
5	Tween 80 – 1% in WFI	40 ºC (104 °F) / 75% RH	B	B	B
6	Ethanol 96%	25 ºC (77 °F)	B	-	B
7	DMSO 10%	-20 ºC (-4 °F)	B	-	B

A and B refer to test packages A and B as detailed in Table 2: Summary of analytical tests performed on Allegro biocontainers.

Five liter Allegro biocontainers were filled with 3.25 L contact fluid via a peristaltic pump to provide an extraction ratio of 2 mL per cm^2 of test material.

The samples extracted/leached from the Allegro biocontainers were compared with control samples from a glass bottle. The samples from the Allegro biocontainers were tested using the analytical tests described in Table 2.

Table 2

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
Derivization GC-MS	X	X

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

- **Allegro biocontainer filling and recording of weight (empty + filled):** The purpose of this test is to detect any loss of the solution during storage.
- **pH measurement:** The purpose of this test to detect any substance release from the bag itself that could change acidic/alkaline properties of the test 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.
- **TOC:** The purpose of this test is to estimate the sum of all the organic components leaching into the contact fluid.
- **Metal (ICP-OES):** Metals may come from the catalysts used for polymerization processes. They may also come from certain additives used in the polymers. The purpose of this test is to determine the presence of metals 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, or from 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 mean of headspace gas chromatography coupled with mass spectrograph.
- **Solvent extraction along with GC/MS:** Many compounds are not sufficiently volatile 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.
- **Derivization 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.

3.3 Summary of Additional Extractable/Leachables Methods for the Allegro XRS 25 Biocontainer System

Typical Allegro XRS biocontainer systems, irradiated between 25 - 40 kGy, were filled with 20 L of ultrapure water and then rocked continuously on an Allegro XRS bioreactor platform at 37 °C for periods of 1, 7, 14 and 28 days.

The following analytical methods were then performed on the extraction fluids collected:

- Non volatile residue (NVR)
- TOC
- Volatile organic compounds by headspace GC/MS
- Fourier Transform Infrared (FTIR)
- Metal (ICP-MS)

3.4 Extractables/Leachables Results for Allegro 2D and 3D Biocontainers

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 exposure to the contact fluids. The analysis after 91 days revealed low concentration of extractables in comparison to the glass bottle used as a control (see detailed report for control results). The summary of these tests results is provided in Table 3.

Table 3

Analytical results of extraction fluids following 91 days exposure to irradiated Allegro biocontainers and connection pieces.

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 (5.3 ppm)		
1-2 Ppm	TOC (1.3 ppm C/L)	TOC (1.1 ppm C/L)			C8-alkenes (< 2 ppm)		
0.1-1 Ppm		Acetate hexanal	TOC (0.5 ppm C/L)	Fatty acids	Acetate, AOx degradation, Alkenes (C9+)	1-octene C8-alkenes methylcyclo pentane 1-3-di-tertbuthy lbenzene	1-3-di-tert-buthylben zene 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			

Virtually all identified extracted chemical entities are explainable as either oligomers and polymer used, or degradation products from the antioxidants used. All chemical entities identified were at concentrations <1 ppm.

3.5 Results for the Additional Extractables/Leachables for Allegro XRS 25 Biocontainer System

- Non Volatile Residue – NVR (Table 4)
- TOC (Table 4)
- Headspace GC/MS (Table 4)
- FTIR (Table 5)
- Inductively Coupled Plasma Mass Spectrometry – ICP/MS (Table 6)

Table 4

Results of NVR, TOC, and Headspace GC/MS testing for Allegro XRS 25 biocontainers following exposure to 20 L of ultra pure water on a rocking Allegro XRS 25 platform at 37 °C.

Analytical Method	1 Days	7 Days	14 Days	28 Days
Non Volatile Residue - NVR	8.4 mg	4.5 mg	5.9 mg	2.6 mg
Total Organic Carbon - TOC	731 ppb	1.66 ppm	1.68 ppm	2 ppm
Headspace GC/MS	4.07 mins	4.36 mins	4.34 mins	4.38 mins

Table 5

Results for Fourier Transform Infrared Test for Allegro XRS 25 biocontainers following exposure to 20 L of ultra pure water on a rocking Allegro XRS 25 platform at 37 °C.

Sample	Likely Source Material	Approximate IR wavenumber (cm-1) associated with species
Allegro XRS 25 biocontainers Day 1, day 7, and day 14	Peaks due to interactions with glassware during storage / analysis	1056 - 1031 (C-O)
	Likely due to the polyethylene bio-container	2924 (C-H)
Allegro XRS 25 biocontainers Day 28	Most likely due to the polyethylene bio-container	2956 - 2922 (C-H), 1359 - 1184 (C-H)
	Glassware interactions	1080 - 1045 (C-O)
Negative control day 28	Poor spectrum – background noise	
Negative controls spectra from day 1, day 7 and day 14 were not possible due to lack of NVR		

Table 6

Results for ICP/MS for Allegro XRS 25 biocontainers following exposure to 20 L of ultra pure water on a rocking Allegro XRS 25 platform at 37 °C.

Element	Mass	Mode	DL	Day 1	Day 7	Day 14	Day 28
Li	7	Cool	0.04	0.13	0.13	0.14	0.13
B	11	Normal	0.08	20.8	17.3	8.09	25.1
Na	23	Cool	0.11	34.5	28.6	23.3	45.4
Mg	24	Cool	0.06	0.42	0.33	2.08	0.25
Al	27	Cool	0.07	0.10	0.69	1.34	2.23
K	39	Cool	0.10	3.33	2.98	2.97	5.35
Ca	40	Cool	0.16	12.8	18.9	24.9	13.8
Cr	52	Cool	0.07	< DL	< DL	< DL	< DL
Ti	47	Normal	0.06	< DL	0.09	0.10	0.11
Mn	55	Cool	0.05	< DL	0.05	0.06	0.06
Fe	56	Cool	0.08	< DL	0.19	0.50	0.62
Co	59	Cool	0.04	< DL	< DL	< DL	< DL
Ni	60	Cool	0.05	< DL	< DL	0.05	< DL
Cu	63	Cool	0.03	0.77	0.47	0.31	0.32
Zn	66	Normal	0.02	< DL	0.57	18.1	0.83
Ag	107	Cool	0.07	< DL	< DL	< DL	< DL
Sn	118	Normal	0.03	0.20	0.21	0.21	0.21
Ba	137	Normal	0.05	0.16	0.20	0.20	0.20
Pb	208	Cool	0.08	< DL	< DL	< DL	< DL

Please note for day 14 the results for Mg, Al, and Zn spiked at high levels that did not fall in line with the other Mg, Al, and Zn results. On investigation this was determined to be most likely caused by sample handling/preparation.

3.6 Extractables/Leachables Testing 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, including Allegro XRS 25 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).

The results after actual service of 28 days of an Allegro XRS 25 biocontainer tested as a whole system were also positive. They also show very low levels of extractables/leachables for the ultra pure water contact fluid. The extractables/leachables found are judged to be acceptable for cell culture applications due to the several successful studies of cell culture performance carried out with the Allegro XRS 25 biocontainer.

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, liquid purity, etc. Evaluation under actual process conditions is therefore also recommended.

4. Biological Safety Tests

4.1 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 as follows:

Inner and outer layers:	Low Density Polyethylene (LDPE)
Oxygen and CO_2 barrier layer:	Ethylene-Vinyl Alcohol Copolymer (EvOH)
Molded connection pieces:	High Density Polyethylene (HDPE)

4.2 Summary of Methods

Tests include USP Biological Reactivity Tests, *(in vivo)* for Class VI Plastics (50 °C (122 °F)) as described in the current United States Pharmacopoeia Chapter <88> 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 (at 50 kGy) biocontainers film and molded connection piece were extracted with these solutions at 50 +/- 2 °C for 72 +/- 2 hours.

The extracts were then used in the following tests to determine the biological effects they have:

Acute Systemic Injection Tests

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

Intracutaneous Tests

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

Implantation Tests

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

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, e.g. 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, e.g. the effect of extractable 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 ± 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.

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

The purpose of this study was to assess cytotoxicity (e.g., 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 2% antibiotics. 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 ºC 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.

4.3 Biological Testing Results

The Allegro biocontainer components passed USP <88> Biological Reactivity Tests (*in vivo*) for Class VI-50 °C Plastics. In addition, the materials used to fabricate these components passed testing described under ISO 10993 Biological Evaluation of Medical Devices as detailed above. The Allegro XRS 25 biocontainer system component materials passed the Elution Test USP <87> Biological Reactivity Test (*in vitro*). Copies of test reports are available upon request.

4.4 Biological Testing Conclusions

Allegro 2D and 3D biocontainers use the same manufacturing method as well as the same materials 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 and of the ISO 10993 Biological Evaluation of Medical Device in section 4, 5, 6, 10 and 11.

5. Physico-Chemical Tests

5.1 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 Section 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.

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, 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 Section 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 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 moulded 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, zinc, etc.

European Pharmacopoeia (3.1.5)

Irradiated samples (at a dose of 26.6 – 32.0 kGy) from the biocontainers film and the moulded 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
- Sulphated ash – Detect the presence of sulphated ash in the extracts

Japanese Pharmacopoeia (Section 61 Part 1)

Irradiated samples (at a dose of 26.6 – 32.0 kGy) from the biocontainers film and the moulded 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 to validate endotoxin determinations in biocontainers of volumes in accordance with the European and US Pharmacopoeia (respectively Section 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 larger size, eight (8) small biocontainers were made in a clean room environment by randomly cutting 100 cm^2 films 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.

5.3 Physico-Chemical Test Results

USP <661>, European, and Japanese Pharmacopoeia

The Allegro biocontainers passed all the tests specified under USP <661>, European Pharmacopoeia (Section 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 physiochemical test results are applicable for both types of biocontainers.

Endotoxin Tests

Table 7

Data on endotoxin concentration measured from 100 L Allegro XRS biocontainers.

Biocontainer Number	Average Endotoxin Concentration (n= 8) of Extraction Volume for 40 mL for 100 cm² 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

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.

5.4 Physico-Chemical Testing Conclusions

The components of the Allegro biocontainers meet the requirements of the Physiochemical 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 tests 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>).

Copies of the test reports are available by contacting Pall Corporation.

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