BIOTECH

Validation Guide

USTR 3473

iCELLis® 500+ Generation R Bioreactors

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1 Introduction

1.1 iCELLis 500+ Generation R Bioreactor

This validation guide contains data applicable to iCELLis 500+ Generation R (GenR) bioreactors. An iCELLis 500+ GenR bioreactor is an automated single-use (SU) bioreactor that provides excellent cell growth conditions for adherent cells. Central to the iCELLis 500+ GenR bioreactor technology is the use of a compact fixed bed, filled with folded macrocarriers.

Evenly distributed media circulation is achieved by a built-in magnetic drive impeller, ensuring low shear stress and high cell viability. The cell culture medium flows through the fixed bed from the bottom to the top. At the top, the medium falls as a thin film down the outer wall where it takes up O_2 to maintain high a Volumetric Oxygen Transfer Coefficient (k_La) in the bioreactor. This unique waterfall oxygenation, combined with a gentle agitation and biomass immobilization, enables the compact iCELLis 500+ GenR bioreactor to achieve and maintain high cell densities, equaling the productivity of much larger stirred tank units.

The iCELLis 500+ bioreactor system consists of two major components:

- A control system
- A single-use (SU) bioreactor

This guide focuses on the second element, which is a gamma-irradiated plastic bioreactor that provides cells with a growth area up to 500 m². This document describes testing demonstrating the suitability of iCELLis 500+ GenR bioreactors to culture cells in the biotechnology industry.

The single-use iCELLis 500+ GenR bioreactor is composed of the following elements (Figure 1):

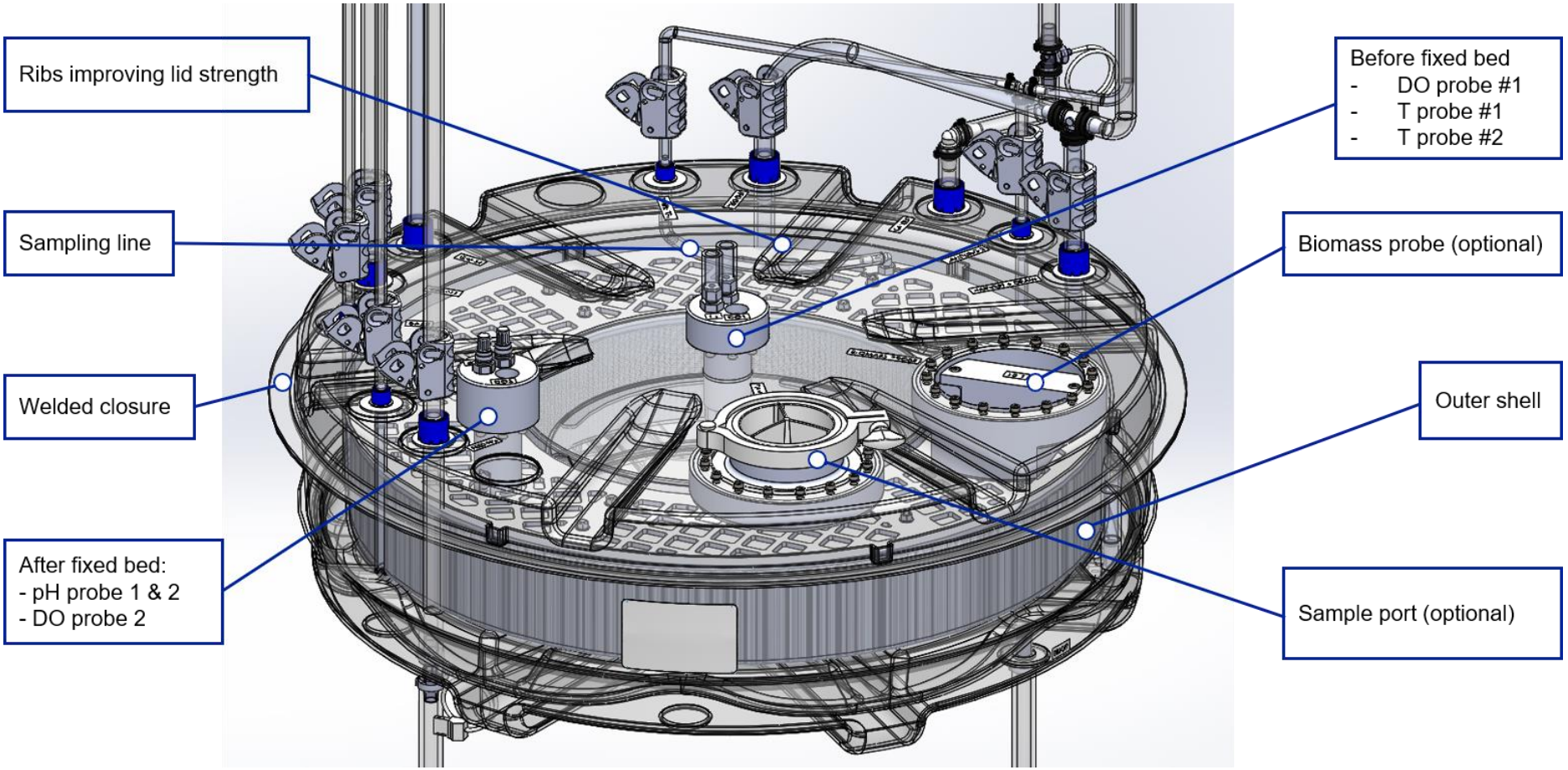
- 2 probe supports for temperature (T), dissolved oxygen (DO) and pH sensors
- Lid containing 8 connections:
    - 5 inlets: gas inlet, 2 feed inlets, inoculum inlet, base inlet
    - 3 outlets: gas outlet, feed outlet, harvest/drain outlet
- A sampling line (allows to sample before and after fixed bed)
- A fixed bed support integrating a pump housing, filled with macrocarriers and a top grid to retain the macrocarriers
- An integrated magnetic-driven impeller and a pump housing ring
- An outer shell including a double jacket at the bottom for temperature regulation
- A biomass probe (optional)
- A sampling port (optional)

This validation guide provides specific instructions that should be followed while using the iCELLis 500+ GenR single-use bioreactors with Hamilton♦ probes with the iCELLis 500+ control system.

In this document, “iCELLis 500+ GenR bioreactor” refers to iCELLis 500+ R Generation single-use bioreactors. Please refer to customer notification CN-RPII-BXGRJ9.

Figure 1

The single-use iCELLis 500+ GenR bioreactor



The bioreactor has two main product contact materials:

- Polyethylene terephthalate (PET) used for the macrocarriers (98% of the total product contact surface area for the 500 m^2 version; 85% for the 66 m^2 version).
- Polyethylene terephthalate glycol (PETG) used for the main housing components (1% of the total product contact surface for the 500 m^2 version; 6% for the 66 m^2 version).

The same manufacturing methodology and same component materials are used to produce different types of iCELLis 500+ GenR bioreactors (66, 100, 133, 200, 333 or 500 m² carriers, with or without a biomass probe). Therefore, the validation results mentioned in this guide are applicable for all container models.

1.2 History

To meet customer requests and to improve the performance of the bioreactor, changes were made to the existing Gen Q disposable bioreactor and a new R Generation of iCELLis 500+ single-use bioreactors was launched.

The main changes include:

- New rotary die cutting machine: the macrocarriers are made using the same raw materials, but on a new machine using a new technique which improves both output and quality.
- New cleanroom and optimized workstations: a new ISO class VI at rest cleanroom was built to house both the new rotary die cutter and the complete/improved workstation.
- New packaging: the wooden crate is replaced with a corrugated cardboard box combined with custom foam parts for improved protection and ease of unpacking at the customer site.
- Lid welding machine: a new machine is operational which welds the vessel to the lid, making the previous closure system (jawbones) obsolete.
- New sample port (optional): to grant the customer access to the bioreactor a non-aseptic sampling port is provided.
- New biomass design: to allow for lid welding the biomass was redesigned slightly.
- New lid design: a new, stronger lid design is implemented to minimize lid deformation.

Note for customers upgrading from all previous generations (pre-Q Generation) to GenR: Q Generation of iCELLis 500+ single-use bioreactors were launched and included the main following changes:

- Complete transition from Polestar to Hamilton pH and DO sensors.
- Addition of a sampling line
- Change to injection molded fitments
- Change of the macrocarrier shape (fold)

2 Material Compliance

2.1 BSE/TSE

All product contact materials of the iCELLis 500+ GenR bioreactor components are certified ADCF (Animal Derived Component Free) or comply with the requirements of EMA 410/01 rev 3- July 2011.

2.2 Biological Safety Tests

The purpose of these tests is to evaluate the biological suitability of the construction materials of iCELLis 500+ GenR bioreactors. All polymer-based product contact materials of the iCELLis 500+ GenR bioreactor are certified USP <88> Class VI. The main product contact materials of the bioreactor (PET and PETG) are also certified USP <87> and USP <661>.

3 Extractables

Extractables are chemical entities that migrate from process equipment under appropriate exaggerated conditions (e.g. solvent, temperature) that exceed worst case process conditions. They represent chemicals that may (or may not) be found in actual process fluid, final formulations, and/or finished dosage.

Polymers, used in the production of pharmaceutical containers and medical devices, cannot be considered as pure compounds. Medical grade polymers should be a blend of base polymers with a broad range of chemicals that may be added for several reasons.

Polymer additives are added to improve the processability of the polymer to enhance its end-use performance in various ways. Moreover, residues of unexpected and undesirable compounds may also be present and can affect the biocompatibility and the toxicity of the materials. Residues can originate from unreacted monomers, solvents used in the process, polymerization catalysts, surfactants, or polymer degradation products.

Both the pharmaceutical and medical device industries require to assess the toxicological risk associated with the use of the product. The Food & Drug Association (FDA) as well as the European Medicines Agency (EMEA) suggest in these cases to perform an extraction study on pharmaceutical containers or medical devices. These studies are performed to determine which chemical species may migrate out of the polymer material and at what concentration level. These data will allow evaluation of the potential toxicological risk cause by the extracted substances.

3.1 Selection of Test Materials

When defining the extractables program for the iCELLis 500+ GenR bioreactor, a materials risk evaluation of the product contact materials was initiated. From a risk evaluation point of view, the most important materials are those with the largest contact surface, largest contact time, and those which might represent a safety risk. This approach reduces the list of materials to be tested to the following 2 materials:

- Polyethylene terephthalate glycol (PETG) - main contact surface in the bioreactor (sum of vessel, pump ring, pump housing basket support, and fixed bed plates and lid).
- Polyethylene terephthalate (PET) - main contact surface in the bioreactor.

For the other product contact materials, the rationale for not testing their extractables is due to their small contact surface and/or short contact time.

3.2 Pre-Treatment Prior to Extraction

Gamma irradiation can potentially increase the amount of extractables, so the test articles should be exposed to this sterilization treatment at the maximum dosage allowed prior to extraction. For this reason, test articles were gamma irradiated at 50 kGy prior to extraction.

3.3 Extraction Conditions

Extractions should be performed at exaggerated conditions, considered to be harsher than normal process conditions. To prevent over-prediction of extractable compounds however, conditions should not be overly extreme.

Table 1

Extraction conditions

Solvent	Temperature and Time
10% ethanol in water*	8-hour, reflux

** This solvent was chosen because the iCELLis 500+ GenR bioreactor is used in applications involving aqueous solutions. Using stronger solvents will generate a far too exaggerated condition.*

3.4 Analysis of the Extract

To identify the extract, the following analytical methods were applied:

3.4.1 Headspace Gas Chromatography/Mass Spectrometry (HS-GC/MS) to Determine Volatile Organic Components (VOC)

Volatile organic molecules which migrate into the contact solution during a prolonged contact step between the test material and the extraction solution, may come from various sources, for example:

- Monomer residues
- Solvent residues from various production or polymer treatment steps (e.g. washing)
- Smaller polymer breakdown products

The selected technique for this analytical method – Purge and Trap coupled to a Gas Chromatography (GC) with Mass Spectrometry (MS) as a detection technique – enables the identification of the target analytes based on both the retention time of the analytes in the chromatogram and the mass spectrum of the eluting compound at this specific retention time. The concentration of detected volatile compounds should be estimated by a semi-quantitative internal calibration method. For this purpose, the relative analytical response of a reported compound was compared to the response of an appropriate internal standard (toluene-d8), added to the samples at a fixed concentration.

3.4.2 Gas Chromatography/Mass Spectrometry (GC/MS) to Determine Semi-Volatile Organic Components (SVOC)

A lot of potential organic migration products are not volatile enough to be detected via Purge and Trap GC/MS. These thermostable compounds can be studied via GC/MS, even though under different experimental and instrument condition than used with Purge and Trap GC/MS. These products are called the semi volatile compounds and may come from various sources, for example:

- Process lubricants
- Plasticizers
- Antioxidants
- Polymer degradation products
- Solvents with a higher boiling point

Dichloromethane (DCM) extraction followed by GC with MS can identify the target analytes based on both the retention time of the analytes in the chromatogram and the mass spectrum of the eluting compound at this specific retention time. The concentration of detected semi-volatile compounds should be estimated by a semi-quantitative internal calibration method. The relative analytical response of a reported compound was compared to the response of an appropriate internal standard (2-Fluorobiphenyl), added to the samples at fixed concentration.

Firstly, the water samples are extracted with an organic solvent with a low boiling point. In general, the solubility of organic compounds is much larger in organic solvents than in water. During the extraction, most organic compounds are concentrated in the organic phase.

3.4.3 Liquid Chromatography/Mass Spectrometry (LC/MS) to Determine Non-Volatile Organic Components (NVOC)

For migration compounds that are non-volatile or non-thermostable (like antioxidants, fillers, plasticizers, polymerization or hydrogenation catalysts, anti-slip agents, and other polymer additives), Liquid Chromatography (LC) is better suited as an analytical tool compared to GC. Mass spectral detection was used because it offers numerous advantages over the traditional Liquid Chromatography/Ultraviolet Visible Spectroscopy (LC/UV-VIS) technique, such as additional molecular and structural information of the compounds, a higher sensitivity of the instrument, and a better identification of the target compounds. The analytes present in the extract are identified from their retention time and the corresponding mass spectrum in a semi-quantitative manner. The concentration of detected non-volatile compounds could be estimated by a semi-quantitative internal calibration method.

The relative analytical response of a reported compound was compared to the response of an appropriate internal standard (Tinuvin♦ 327). A compound specific Relative Response Factor (RRF) is available for several target compounds. This RRF is used for the more accurate quantification of identified target compounds. For other compounds the RRF is assumed to be 1.

Firstly, the water samples are extracted with an organic solvent with a low boiling point. In general, the solubility of organic compounds is much larger in organic solvents than in water. During the extraction, most organic compounds are concentrated in the organic phase.

3.5 Results

The migration behavior was verified and compared with the blank solution, stored in a glass bottle. In this way, it becomes possible to discriminate migration and material degradation compounds from compounds originating from the matrix/solution degradation under test conditions. Tables 2 and 3 show the extractables results for PETG and PET correspondingly.

Table 2

Extractables results for polyethylene terephthalate glycol (PETG), minimum 50 kGy gamma irradiated*

Analysis	Compound	Concentration (µg/g)
Volatile organic compounds (neat). Reporting limit: 0.50 µg/g.	No compounds detected above the reporting limit and different from the blank	N/A
Semi-volatile organic compounds. Reporting limit: 0.050 µg/g.	Unknown	0.11
	Ester of terephthalic acid	0.067
	Bis (2-hydroxyethyl) – terephthalate	0.057
	PET dimer compound	0.38
	PET dimer related compound	0.39
Non-volatile organic compounds. Reporting limit: 0.050 µg/g. Positive ionization mode.	Probably PET related	0.27
	Unknown	0.20
	PET dimer	0.59
	PET dimer	0.75
	Probably PET related	0.053
	Probably PET related	0.081
	PET dimer	0.089
	PET trimer	0.63
	PET related compound	0.055
	PET linear vinyl (CDHM) dimer of PET cyclic (CDHM glycol diglycol) dimer	0.24
	PET (CDHM) cyclic trimer	0.052
	PET cyclic trimer	0.82
	PET linear bisglycol dimer	0.81
	PET linear (monoglycol) dimer methyl ester	0.096
	Unknown	0.12
	Unknown	0.09
	PET dimer	0.12
	PET trimer	2.2
	PET linear (CDHM monoglycol) dimer	0.41
	Unknown	0.055
	Unknown	0.11
	PET trimer	0.15
	PET related compound	0.27
	PET related compound	0.056
	PET related compound	0.062
	PET (CDHM) cyclic trimer	0.39
Non-volatile organic compounds. Reporting limit: 0.050 µg/g. Positive ionization mode.	Unknown	0.052
	PET cyclic trimer	1.5

** Surface to volume ratio: 1 g/10 mL. Results are reported in µg/g units, meaning the amount of extractable compound (µg) per amount of extracted polymer material (g).*

Table 3

Extractables results for polyethylene terephthalate (PET), non-woven, minimum 50 kGy gamma irradiated*

Analysis	Compound	Concentration (µg/g)
Volatile organic compounds (neat). Reporting limit: 0.50 µg/g.	Acetaldehyde	0.93
	Methyl formate	2.3
	Formic acid	1.0
	Acetic acid	2.2
Semi-volatile organic compounds. Reporting limit: 0.10 µg/g.	Diethyl terephthalate	0.23
	Ester of terephthalic acid	0.36
	Ester of terephthalic acid	0.52
	Bis(2-hydroxyethyl)-terephthalate	1.1
	PET dimer	3.4
	PET dimer	5.6
	Cyclic PBT dimer	0.75
	Probably PET related	0.31
	Unknown	0.13
	PET dimer	0.65
	PET dimer	1.2
	PET dimer	7.4
	Probably PET related	0.86
	PET dimer	0.96
	Probably PET related	0.66
	PET dimer	0.82
	PET trimer	0.20
	PET dimer	0.10
	PET trimer	0.11
Non-volatile organic compounds. Reporting limit: 0.10 µg/g. Positive ionization mode.	PET cyclic trimer	4.5
	PET cyclic tetramer	0.27
	PET cyclic pentamer	0.26
	2-hydroxyethyl terephthalate	0.13
	Impurity related to PBT	0.45
	PET dimer	2.6
	PET dimer	0.19
	PET dimer	21
	Unknown	1.4
	PET dimer	0.90
	Probably PET related	0.29
	Unknown	0.92
	Unknown	0.79
	PET trimer	1.2
Non-volatile organic compounds. Reporting limit: 0.10 µg/g. Negative ionization mode.	PBT dimer	0.16
	PET cyclic trimer	6.9

** Surface to volume ration: 1 g/mL. Results are reported in µg/g units, meaning the amount of extractable compound (µg) per amount of extracted polymer material (g).*

4 Cleanliness

To meet the cleanliness specifications for the operational iCELLis 500+ GenR bioreactor, described in US and European Pharmacopoeia, Pall uses internal specifications that must be met by all components of the bioreactor.

To improve cleanliness, the iCELLis 500+ GenR bioreactor is assembled in a qualified ISO class 6 at rest cleanroom.

4.1 Particles

4.1.1 Empty Generation R Bioreactor

A volume (65 L) of test liquid (Isopropanol 20%, Sodium chloride 0.2%) was transferred into an iCELLis 500+ bioreactor using a particle-free 0.1 µm rated filter. After filling the bioreactor, the test liquid was agitated at 400 revolutions per minutes (rpm) for 60 minutes to ensure the liquid was exposed to all surfaces inside the bioreactor.

Particulates were analyzed after samples (5 L) were filtrated through a membrane with maximum 1 µm pore size (as per USP <788>, method 2). The membrane was inspected using a light microscope at 100 x magnification and an external illuminator. The particles were sized and enumerated.

4.1.2 Macrocarriers

Three aliquots of 1 g were analyzed. Each aliquot was placed in 100 mL of particle free water. After 5 minutes of stirring, 10 mL of this solution was diluted to 100 mL. 10 mL of this dilution was filtered and particles were counted in accordance with Ph.Eur 2.9.19, method 2.

Table 4

Particle concentration in filled bioreactor* (particles/mL)

	Particle Size	
	≥ 10 µm	**≥ 25 µm**
Average of 3 samples macrocarriers	488	179
One empty iCELLis 500+ GenR bioreactors	1	1
Sum	489	180

** Considering 3600 g macrocarriers in a volume of 65 L in the iCELLis bioreactor model with 500 m². These results were generated for information purposes. During production no cleanliness data on product level is measured as a release criterium.*

4.2 Endotoxins

4.2.1 Empty Generation R Bioreactor

A volume (20 L) of deionized (DI) water was transferred into an iCELLis 500+ bioreactor using a 0.2 µm rated positively charged inlet filter (KA3NFZP*G), using a maximum flux of 0.5 (mL/min)/cm² for endotoxin removal (for KA3NFZP*G maximum 1.15 L/min).

After filling the bioreactor, an additional 1 L volume of the DI water was filled through the inlet filter into a 1 L Allegro™ biocontainer to use as the negative control.

Care was taken to ensure the liquid was exposed to all surfaces inside the bioreactor.

After agitation was complete, 1 L of the test liquid was transferred into a sterile 1 L Allegro biocontainer. From this volume, 3 x 40 mL test samples were collected for endotoxin analysis.

Additionally, from the 1 L Allegro biocontainer containing the negative control, 2 x 40 mL negative control samples were taken for endotoxin analysis.

4.2.2 Macrocarriers

Three aliquots of 1 g were analyzed. Each aliquot was tested in accordance with USP <85>, kinetic colorimetric method.

Table 5

Endotoxin concentration in filled bioreactor* (EU/mL)

	EU/mL
Average of 3 samples macrocarriers	<0.003
One empty iCELLis 500+ GenR bioreactors	<0.005
Sum	< 0.008

** Considering 3600 g macrocarriers in a volume of 65 L in the iCELLis bioreactor model with 500 m². These results were generated for information purposes. During production no cleanliness data on product level is measured as a release criterium.*

5 Sterility

The iCELLis 500+ GenR bioreactor is not shipped with a sterility claim, but each bioreactor is shipped with an irradiation certificate for minimum 30 – maximum 50 kGy.

5.1 Bioburden Prior to Gamma Irradiation

The bioburden load prior to gamma irradiation of one iCELLis 500+ GenR bioreactor and of the macrocarriers was analyzed in accordance with ISO11737-1.

Table 6

Bioburden content in bioreactor*

	Bioburden Content in Bioreactor* - Colony-Forming Unit (CFU)
Macrocarriers	< 288
One empty iCELLis 500+ GenR bioreactors	56
Sum	< 344

** Considering 3600 g macrocarriers in a volume of 65 L in the iCELLis 500+ GenR bioreactor model with 500 m²*

These results were generated for information purposes. During production no bioburden data on product level is measured as a release criterium.

5.2 Dose Mapping

All iCELLis 500+ GenR bioreactors are shipped with an irradiation certificate of a minimum 30 kGy – maximum 50 kGy. According to ISO11737-2, 30 kGy is the dose needed to reach a sterility assurance level (SAL) of 10^{-6} for items with an average bioburden load < 23000 CFU.

6 Shelf Life

An 18-month shelf life is ensured for gamma-irradiated iCELLis 500+ GenR bioreactors based on aging studies:

- A real time aging study of the plasma treatment confirms hydrophilicity of the macrocarriers.
- A real time aging study of DO and pH sensors, performed by the supplier, which results in a shelf life of 18 months after gamma irradiation. A real time ageing study performed by Pall Life Sciences Hoegaarden confirms this statement.
- An accelerated aging study of iCELLis 500+ GenR bioreactor is performed in accordance with ASTM F1980-16 and demonstrates conformity with the specifications after 18 months for the following parameters:
    - Bioreactor integrity
    - Fitment integrity
    - Sampling line integrity

- Sensor functionality and integrity
- Pump house basket support (PHBS) connection quality
- Fixed bed compaction consistency
- Packaging integrity

7 Transportation Study – Packaging

The bioreactor packaging is tested according to ISTA 3E and confirmed to be compliant. These tests include shock, compression, and vibration tests.

The bioreactor was tested after exposure to the ISTA 3E test and demonstrated conformity with the product specification for the following parameters:

- Bioreactor integrity
- Fitment integrity
- Sampling line integrity
- Sensor functionality and integrity
- PHBS connection quality
- Fixed bed compaction consistency

8 Sensor Accuracy

In case of a one-point calibration in the range from 6.5 to 8.5, an accuracy of ± 0.1 is guaranteed for the pH sensor after gamma irradiation and with product calibration.

For the DO sensor, an accuracy of ± 3.5% between 15% and 75% and of ± 7% between 75% and 150% after one-point calibration is provided after gamma irradiation and with product calibration.

9 Fixed Bed Compaction

Pall provides all bioreactors with a uniform carrier distribution within the fixed bed for optimal cell growth and cell distribution.

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