Mycap® CCX Cell Expansion System

Validation Guide

SARTORIUS

Disclaimer
The results shown in this Validation Guide are indicative of, but do not constitute, product specifications.

Table of Contents

1. Introduction 4
   - 1.1 Scope Statement 5
   - 1.2 Security of Supply 6
   - 1.3 Manufacturing Resources 6
   - 1.4 Quality Management System 6
   - 1.5 Gamma Irradiation 7
   - 1.6 Validation Test Summary 8

2. Production and Quality 9
   - 2.1 Personnel 9
   - 2.2 Facilities 9
   - 2.3 Supply Chain 9
   - 2.3.1 Supplier Evaluation and Qualification 9
   - 2.3.2 Component and Raw Material Qualification 9
   - 2.3.3 Incoming Quality Controls 9

3. Production 10
   - 3.1 Equipment Qualification 10
   - 3.2 Production Environment 10
   - 3.2.1 Viable Organism Control and Monitoring 10
   - 3.2.2 Non-viable Control and Monitoring 10
   - 3.3 Material Receipt 10
   - 3.4 Traceability and Batch Control 11
   - 3.5 In-Process and Product Release Controls 11
   - 3.5.1 Gas Exchange Cartridge Air Flow Rate Test 11
   - 3.5.2 Pressure Decay Test 12

4. Mycap® CCX Bottle Closure Properties 13
   - 4.1 Mycap® CCX Structure 13
   - 4.2 Cap and Closure Sizes 13
   - 4.3 Properties 13
   - 4.4 Torque Specification 14
   - 4.5 Container Closure by Aerosol Challenge 15
   - 4.5.1 Controls 15
   - 4.5.2 Results 15
   - 4.6 Biocompatibility 16
   - 4.6.1 USP <87> 16
   - 4.6.2 USP <88> 16
   - 4.7 Particulates 17
   - 4.7.1 USP <788> 17
   - 4.8 Endotoxin 17
   - 4.8.1 USP <85> 17

5. Leachables and Extractables 18
   - 5.1 Overview 18
   - 5.2 USP <381> 19
   - 5.3 21CFR177.2600 19
   - 5.4 Further Leachable and Extractable Studies 19

6. Cell Expansion Performance Evaluation 20
   - 6.1 Background 20
   - 6.2 Gas Exchange Study 20
   - 6.3 Cell Growth Study 22

7. Gamma Sterilization Validation 23
   - 7.1 Purpose 23
   - 7.2 Method 23
   - 7.2.1 Bioburden Evaluation 23
   - 7.2.2 Verification Dose Experiments 23
   - 7.2.3 Sterility Testing 23
   - 7.2.4 Conclusion 23
   - 7.2.5 Maintenance of Sterility 23

8. Shelf-Life 24
   - 8.1 Verification of Critical Performance Properties 24
   - 8.1.1 Container Closure 24
   - 8.1.2 Leak Rate and Gas Exchange Cartridge Air Flow Rate 24

1. Introduction

Expansion of suspension cell cultures from working or master cell banks to seed bioreactor is commonly performed through passages of successively larger Erlenmeyer shake flasks.

Cellular respiration and growth consumes O_2 and produces CO_2 as a byproduct. Cell cultures starved of O_2 will not propagate. Cultures with an overabundance of CO_2 become acidic and impair cell viability. Thus, the exchange and control of O_2 and CO_2 between the flask contents and the incubator environment is critical for successful cell growth.

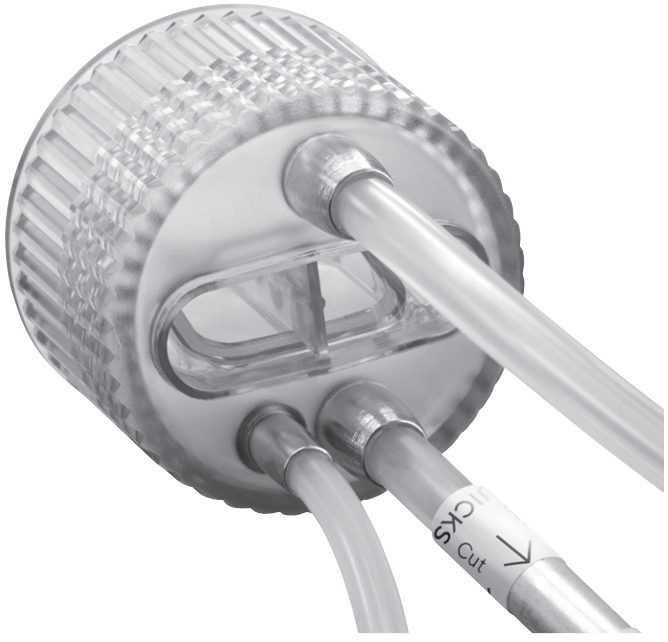
The cap for a traditional Erlenmeyer flask cap is almost entirely occupied by a microporous membrane, leaving no room for integral tubing. Thus, a flask's cap must be unscrewed and the flask's contents exposed to the environment for each fluid transfer including: media addition, inoculation and sampling. Risk of contamination is mitigated by performing these fluid transfers in a biosafety cabinet (BSC) or laminar flow hood. Despite these preventative measures, it is customary to design expansion processes with back-up flasks to be used in the case of contamination. Back-up flasks are a material waste and cause labor-intensive BSC work.

Sartorius's Mycap® CCX is a one-piece closure system with integral tubing and a specially designed gas exchange cartridge. The gas exchange cartridge supports necessary passive gas exchange in an incubator because of its high filter surface area with unrestricted gas flow. The cartridge has a small footprint, leaving room in the cap for integral tubing to enable safe, aseptic, fluid transfer outside a BSC.

Mycap® CCX aims to reduce waste, eliminate contamination risk and streamline operations by avoiding unnecessary work in a BSC.

Mycap® CCX systems are qualified, manufactured and released under a Quality Control system which is compliant to the key principles of cGMP. Assembly is done in an ISO Class 7 Certified clean-room.

This Validation Guide describes qualification of materials, performance specifications, manufacturing conditions and quality control systems of the Mycap® CCX Cell Culture Expansion System.



1.1 Scope Statement

Unless stated otherwise, tests and reports provided in this guide are applicable for Mycap® CCX products constructed from materials and components listed on the table below:

Bottle Closure		
Manufacturer	**Brand**	**Materials of Construction**
Sartorius	Mycap® Bottle Closures	Platinum-cured silicone

Containers		
Manufacturer | Brand	**Sizes**	**Materials of Construction**
Corning Erlenmeyer	125 mL, 250 mL, 500 mL, 1000 mL, 2000 mL, 3000 mL, 5000 mL	Polycarbonate

Tubing		
Manufacturer	**Brand**	**Materials of Construction**
Saint-Gobain Performance Plastics	C-Flex®	Thermoplastic elastomer (TPE)
Dupont®	Liveo® Pharma50	Platinum-cured silicone
Dupont®	Liveo® Pharma50	Platinum-cured silicone
Sartorius	Tuflux® TPE	Thermoplastic elastomer (TPE)

Connectors and Fittings		
Manufacturer	**Brand | Description**	**Materials of Construction**
Sartorius	Anti-Suction Dip Tube Tip	Polypropylene
Nordson Medical	Tube to Tube Fittings	Polypropylene
Colder Products	AseptiQuik® S Aseptic Connecting Device	Polycarbonate, platinum-cured silicone, polyethylene
Halkey-Roberts	Robertsite® Luer Activated Access Site	Polycarbonate, platinum-cured silicone

Venting and Gas Exchange		
Manufacturer	**Brand | Description**	**Materials of Construction**
Sartorius	Mycap® CCX Gas Exchange Cartridge	Polycarbonate, polyethersulfone
Sartorius	25 mm Minisart®	Polycarbonate, polyethersulfone

Unless otherwise stated, tests described in this Validation Guide were performed on the Mycap® CCX bottle closure and may not include all or any of the components, assemblies or accessories described above. Wherever possible, Sartorius refers to our supplier's product validation documentation. Supplier documentation is available on request or by contacting the supplier directly.

1.2 Security of Supply

Assurance and security of supply are significant market requirements for Mycap® CCX bottle closures. The robustness of our supply chain relies on effective supplier management, multiple manufacturing sites with consistent industrial and quality processes, process automation, application of lean manufacturing practices, expertise for designing fluid management systems, close collaborative relationships with customers, and senior management's strong commitment to continuous and dynamic improvement.

1.3 Manufacturing Resources

Multiple manufacturing sites and safety stock all along the supply chain steps provides a robust business continuity plan. The details for manufacturing sites are mentioned in 'Supply Chain Specification' document; it is available upon request.

1.4 Quality Management System

Sartorius' quality system is compliant to ISO 9001 standard for all manufacturing sites. Mycap® CCX is qualified according to the current applicable safety and performance industry standards, as described in this guide.

Sartorius ISO certificates are available on the Sartorius website at: www.sartorius.com/en/legal-documents/quality-management

These quality system processes direct and inform our entire quality system and all the procedures, work instructions, forms, etc. contained therein:

- Management Responsibility and Review
- Document Control
- Records Control and Retention
- Corrective and Preventive Action
- Internal Auditing
- Personnel Training and Competency
- Customer Notification and Recall

1.5 Gamma Irradiation

Mycap® CCX bottle closures are packaged and shipped in cardboard boxes to the sterilization center for gamma irradiation. The sterilizers are qualified according to Sartorius' internal procedures.

Mycap® CCX bottle closures are irradiated at a minimum dose of 25 kGy. The efficiency of the minimum dose of 25 kGy has been validated according to the ISO 11137 standards in order to obtain Sterility Assurance Level (SAL) 10^{-6}.

The certificate of release issued with each lot of products indicates the gamma irradiation run identification number. Each shipment includes a certificate of processing which reports the irradiation dose and lists the lot number(s) of the Sartorius product(s) included in that irradiation run. The two documents may be cross-referenced.

1.6 Validation Test Summary

Qualification Tests

- Biocompatibility testing
  - USP <87>:
    Biological reactivity tests, in vitro
  - USP <88>:
    Biological reactivity tests, in vivo

- Gas Exchange Study

- Cell Growth Study

- Gas Exchange Cartridge Flow Rate Test
  Post ≈ 50 kGy irradiation and two-year accelerated aging

- Microbial container closure by aerosol challenge
  Post ≈ 50 kGy irradiation and two-year accelerated aging

- USP <788>:
  Particulate matter in injections
  Post ≈ 50 kGy irradiation and two-year accelerated aging

- USP <85>:
  Bacterial Endotoxins Test
  Post ≈ 50 kGy irradiation and two-year accelerated aging

- USP <381>:
  Physico-chemical
  (Mycap® CCX Closure)
  Post ≈ 50 kGy irradiation

- 21CFR177.2600:
  Rubber articles intended for repeated use
  (Mycap® CCX Closure)
  Post ≈ 50 kGy irradiation

Monitoring Tests

- Particulate Control
  - USP <788>:
    Particulate matter in injections
  - ISO 14644-1:
    Clean-rooms and associated controlled environments — classification of air cleanliness by particle concentration

- Bioburden and Sterility
  - ISO 11137:
    Sterilization of healthcare products
    Dose qudit: quarterly
  - ISO 14698:
    Clean-rooms and associated controlled environments – biocontamination control

- Endotoxins
  - USP <85>:
    Bacterial Endotoxins Test

Lot Release Tests

- 100 % Visual inspection
  - Visible particulate
  - Component defects

- 100 % Gas Exchange Cartridge Flow Rate Test
  - Before Mycap® CCX closure assembly

- Pressure Decay Test of Mycap® CCX closure and immediate connections*

- Compliance to technical drawing

- Visual inspection of packaging and labeling

- Verification of Gamma Irradiation

* Pressure decay testing is performed only when the system includes a flask.

2. Production and Quality

2.1 Personnel

Sartorius recognizes that human resources and personnel competency are of utmost importance, and have therefore established a comprehensive human resources management program. Stringent selection, motivation, initial and continuous training and qualification of personnel at all levels of the company ensure that every employee is at his or her best at all times for each step of the manufacturing and control processes. Comprehensive training records are kept for all employees.

2.2 Facilities

The buildings, equipment and work environment at Sartorius have been designed to maximize employee comfort and safety while complying with the key principles of cGMP for the manufacture of Mycap® CCX bottle closures destined to the pharmaceutical industry. All infrastructure (equipment, utilities, etc.) that has an impact on the product quality is inventoried and undergoes an appropriate qualification, calibration and maintenance.

2.3 Supply Chain

2.3.1 Supplier Evaluation and Qualification

Suppliers are carefully selected according to internal standards and applicable regulations. Typical requirements for suppliers include the following (not exhaustive list):

- Quality Control System
- Quality Assurance System
- Facility and Clean-room Controls
- Product and Component Lot Traceability System
- Change Notification Procedures

Suppliers are evaluated and approved according to internal standards.

2.3.2 Component and Raw Material Qualification

Each raw material and | or component is qualified. This qualification includes a list of required statements from the supplier that is dependent on the final use of the component and | or raw material. Typical requirements for components that are in contact with the product flow include the following (not exhaustive list):

- USP Class VI and | or ISO 10993 conformity
- TSE | BSE Compliance statement
- EP conformity (if applicable)
- Change notification statement
- REACH Compliance
- Bisphenol A free

Beyond these requirements, Sartorius may perform qualification of the proposed component and | or raw material internally.

For raw materials, the internal qualification will include physical performance of the component made with this raw material.

For components, the qualification will be centered on the testing of the assembly of the new component with other components that will be attached.

2.3.3 Incoming Quality Controls

All raw materials, components and sub-contracted products are inspected on arrival at Sartorius against approved control specifications.
Typical testing requirements applied at incoming quality inspection include (not exhaustive):

- Supplier documentation controls (Certificates)
- Packaging identification and integrity
- Visual inspection
- Dimensional check

Only approved materials will be allowed to be used in production of Mycap® CCX bottle closures. Approved materials are recorded in Sartorius's inventory and quality management system, labeled with the lot number and designated internal part number, and released for use.

3. Production

3.1 Equipment Qualification

All equipment used in production goes through qualification that includes installation qualification, operational qualification and performance qualification. This qualification effort is carried out by a multidisciplinary team and follows the rules described in the corresponding procedure in our Quality System.

Equipment undergoes its applicable calibration plan as described in our Quality System.

3.2 Production Environment

The New Oxford and Stonehouse facilities house engineering, product development, warehousing and manufacturing space. Product assembly occurs in an ISO 7 (Class 100,000 clean-room) as per ISO 14644-1 and in accordance with the key principles of cGMPs.

Contact us for further details or precise questions about our quality and operating systems or to schedule an on-site audit.

3.2.1 Viable Organism Control and Monitoring

In addition to line clearance and weekly cleaning of equipment and work surfaces, monthly cleaning of the clean-room with a schedule of LpH®, Vesphene® and Spor Klenz® occurs as per our clean-room management and cleaning procedures.

Viable organisms are measured quarterly to monitor the effectiveness of the clean-room management and cleaning procedures and to be compliant to EU GMPs and ISO 14698. As of the drafting of this document, viable monitoring is up to date:

- Air viables < 100 CFU
- Surface viables < 25 CFU
- Wall viables < 5 CFU

3.2.2 Non-viable Control and Monitoring

Line clearance, weekly cleaning of equipment and work surfaces, and monthly cleaning of the clean-room reduce and control non-viable particles.

Non-viable readings are recorded weekly to ensure 0.5 μ/m^3 and 5.0 μ/m^3 particles are within the ISO Class 7 acceptance criteria, as per ISO 14644-1. As of the drafting of this document, non-viable monitoring is up to date.

3.3 Material Receipt

Components received at our facilities arrive in two forms: double-bagged and clean or bulk-packed and cleaned. Double-bagged and clean materials (tubing, for example) are received into our Class 7 clean-room as per incoming inspection and testing procedures.

Bulk-packed items are cleaned and transferred into the clean-room as per incoming inspection and testing procedures.

3.4 Traceability and Batch Control

Sartorius has a process and maintains an effective traceability system which can be used in the event of product, component or manufacturing issues to alert affected customers.

Generally, all finished assemblies are composed of components and subassemblies. Subassemblies are built from components or subassemblies. Components are parts that are purchased or manufactured by Sartorius. Each component and subassembly has a unique part number. All components and subassemblies are assigned a unique lot number on receipt or manufacture | assembly. The lot number is recorded in batch records and maintained in our traceability system.

Batch records provide the operators all the necessary instructions, and component and subassembly list to execute the designated procedure. Operators fill in batch records including recording the lot number of components and subassemblies. This data is also entered into the traceability system.

The traceability system and batch record system links all manufacturing steps, components and subassemblies to the final assembly, allowing for complete backward and forward traceability of every assembled product.

3.5 In-Process and Product Release Controls

Quality controls are performed at various stages during the manufacturing process. Some of these controls are listed below. Other specific controls, dependent on the specific application of the products, may be performed but are not listed.

- Product conformity against technical drawing
- Visual inspection (particles or contamination, correctness of assembly, etc.)
- Pressure Decay Test (when applicable)
- Gas Exchange Cartridge Air Flow Rate Test (when applicable)
- Product packaging controls
- Product labeling controls

After production, every batch of finished products is released by Quality Assurance before it can be shipped. The release will be documented in the batch record and in the traceability system.

The system for product release is constructed in such a way that only batches that have been released by quality can have the corresponding shipping and billing documents.

A Certificate of Release is issued for each batch of finished product that is shipped from Sartorius.

3.5.1 Gas Exchange Cartridge Air Flow Rate Test

The gas exchange cartridge undergoes an in-process flow rate test and mulitple visual inspections. The in-process checks are performed on every gas exchange cartridge before it is installed into the cap closure. The test measures the flow rate of air across the membrane at a fixed pressure to confirm the integrity of the cartridge and detect possible damage or defect.

Only devices that pass the test are cleared for continued production and quality release.

3.5.1.1 Selection of Air Flow Rate

Critical values were determined during qualification of the test equipment. The critical values were established by measuring air flow rate with different membrane defects. Defects are identified when the test measures air flows that are outside the bounds of the critical values.

Gas Exchange Cartridge	Minimum Air Flow	Maximum Air Flow
Small (125mL, 250mL Flask)	500 cm^3/min	900 cm^3/min
Large (500mL, 1000mL, 200mL, 3000mL Flask)	300 cm^3/min	550 cm^3/min

3.5.1.1 Test Method

A fitting of the test equipment is connected to the opening of the gas exchange cartridge. Dry and filtered compressed air is introduced through the fitting and into the gas exchange cartridge for 3 seconds. The flow of compressed air is regulated by the test equipment to maintain 45mbar| 0.65psi (±1.5%) pressure on the gas exchange cartridge. The test equipment measures the volume of air passing through the gas exchange cartridge during the 3 second pressure hold and is converted to flow rate (cm^3/min). The tested flow rate is compared to the specified limit values. Devices outside the specified limit values are rejected.

3.5.2 Pressure Decay Test

Mycap® CCX bottle closures are leak-tested before release. Pressure decay at 2 psi is measured using the TME Worker™, Model W-L-015. Pass | Fail criteria is a leak rate of less than 0.02 psi. Only devices that pass the leak test are cleared for shipment.

3.5.2.1 Selection of Leak Rate

Deliberate defects were made on devices. Leak rates detected with TME Worker™, Model W-L-015 at 2 psi pressure on defective devices were noted and compared with leak rates of devices not made deliberately defective. The threshold of 0.02 psi decay was set.

Related validation testing, including bioburden testing and performance in the field, supports that 0.03 psi decay is a suitable threshold for device integrity.

4. Mycap® CCX Bottle Closure Properties

4.1 Mycap® CCX Structure

The Mycap® CCX bottle closure is a one-piece closure with integral tubing and a specialized gas exchange cartridge. Tubing and the gas exchange cartridge are inserted into preformed holes. Platinum-addition liquid silicone is dispensed into the cap, bonding to and encasing the inserted components. The assembly is heat-cured to form the Mycap® CCX bottle closure. The Mycap® CCX is typically installed onto Erlenmeyer flasks.

Only the dispensed liquid silicone, components inserted into the cap, components attached to the tubing or flask should be considered a fluid-contact surface of the Mycap® CCX system.

4.2 Cap and Closure Sizes

Mycap® CCX is available on Erlenmeyer flasks. Cap and closure sizes available are listed below (not exhaustive list):

- 33 mm
- 38 mm
- 43 mm
- 48 mm
- 70 mm
- 100 mm

4.3 Properties

The following table describes general properties of the Mycap® CCX bottle closure only, and does not consider properties of tubing, fittings, container or other components that may be included in the fluid management system with the Mycap® CCX bottle closure.

Cap material (non-fluid contact)	Polycarbonate
Seal material	Platinum-cured silicone
Appearance	Translucent
Maximum use temperature [°C]	121
Minimum use temperature [°C]	0
Brittleness temperature (of cap material) [°C]	-135
Heat deflection temperature (of cap material) [°C]	138
TSE | BSE	Animal derivative component free
Container closure by aerosol	Pass
USP <87>	Pass
USP <88>	Pass
USP <788>	Pass (< 25 particles/mL larger than 10 µm; < 3 particles/mL larger than 25 µm)
USP <85>	Pass (< 0.125 EU/mL)
USP <381>	Pass
21CFR2600.177	Pass

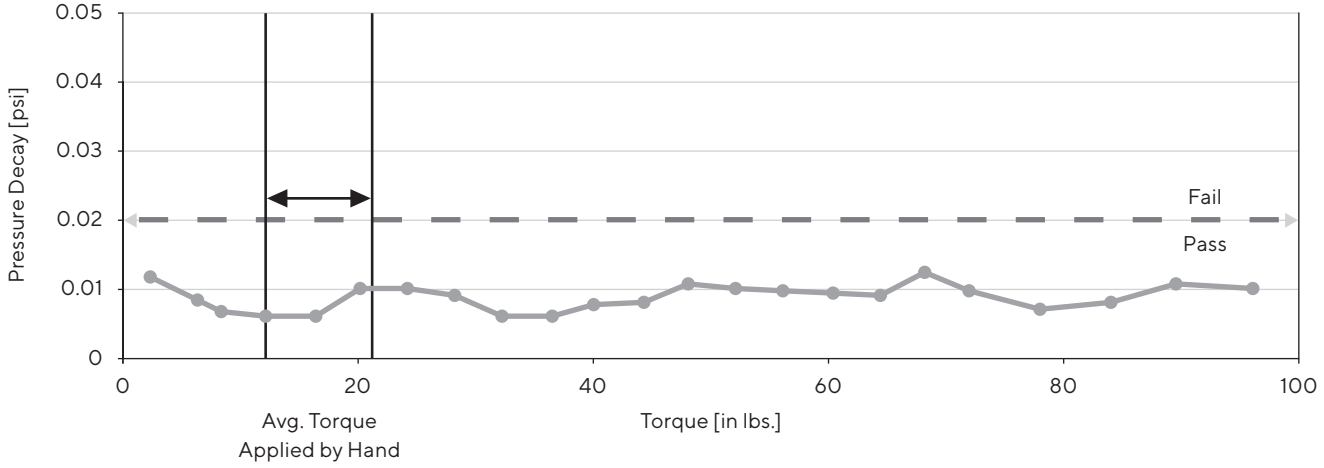
4.4 Torque Specification

Sartorius considers torque specification provided by a container manufacturer important but not applicable. The dimensions and materials of the Sartorius Mycap® CCX cap may be different from the cap supplied by the container manufacturer, and the Mycap® CCX bottle closure includes the robust platinum-cured silicone seal.

A study was executed to affirm Mycap® bottle closures are easily and appropriately installed.

A torque wrench was used to install Mycap® bottle closures at precise torque applications. Once a closure was torqued to a known value, the vessel was leak tested using the TME Worker™, Model W-L-015. Passing criteria is less than 0.02 psi, in accordance with the Mycap® bottle closure leak test. Torque values and corresponding pressure decay results are shown below:

Average Leak Rate vs Torque



An acceptable leak rate was observed with minimal torque applied, 2 in.-lbs. until material failure at 64 and 100 in-lbs.

Torque is not measured during Mycap® CCX system assembly. Tools are not used in manufacturing to install Mycap® CCX bottle closure to the flask. Instead, Sartorius relies on passing leak test results, as described in section 3.5.2 **"Pressure Decay Test"** to confirm correct assembly and installation.

A second study was performed by Sartorius manufacturing personnel to measure torque applied during installation of Mycap® bottle closures to containers.

The torque applied by a sampling of operators was measured using a torque wrench. The data table is shown on the preceding graph.

Torque values confirm operators are able to consistently apply closures within the recommended range that yields a leak-free closure.

4.5 Container Closure by Aerosol Challenge

Bacterial aerosolization tests were conducted on the Mycap® CCX system.

The test articles were assembled and gamma irradiated to a minimum of 50 kGy. The test articles were exposed at 55 ± 4 °C and an ambient temperature of 25 °C for 91 days, which simulated a time period of approximately two years on the shelf. The test articles were aged under 50 ± 20% relative humidity (RH).

Test articles, pre-filled with soybean casein digest broth (SCDB) media were placed in a 1 m^3 glass aerosol exposure chamber. A 60-minute bacterial challenge (Bacillus atrophaeus) followed a 30-minute sterile water preconditioning cycle. Test articles were decontaminated and incubated for a minimum of seven days at 30–35 °C. The media in test articles were inspected for growth of the challenge organism.

4.5.1 Controls

Criteria	Acceptance	Value
Aerosol Challenge Fallout	>100 CFU/cm^2	≈ 6.3 + 10^2 CFU/cm^2
Mean Particle Size	≤ 4.5 µm	2.5 µm
Positive Controls	Growth of organism	Growth observed
Growth Promotion	Growth of organism	Growth observed

4.5.2 Results

Test articles showed no growth in the media, indicating that the Mycap® CCX system can be safely irradiated up to 50 kGy, placed on a shelf under ambient conditions for two years, with the closure maintaining a microbial barrier.

4.6 Biocompatibility

4.6.1 USP <87>

The purpose of this test is to determine if any chemicals that leach or may be extracted from the Mycap® CCX bottle closure are cytotoxic. The study is conducted in accordance with United States Pharmacopoeia (USP) Section 87.

A 5.9 gram sample of article was extracted in 29.5 mL of 1x minimum essential media (MEM) with 5 % bovine serum for 24–25 hours at 37 ± 1 °C, with agitation.

Multiple well cell culture plates were seeded with L-929 mouse cells and incubated until 80 % confluent. Extract solution was added to the wells. Observations for reactivity were made after incubation for 72 hours at 37 ± 1 °C with 5 ± 1 % CO_2.

The requirements of the USP Cytotoxicity Test have been met.

4.6.2 USP <88>

Intracutaneous Reactivity
The purpose of this test is to determine if any chemicals that leach or may be extracted from the Mycap® CCX bottle closure cause local irritation in the dermal tissue of rabbits. The study is conducted in accordance with United States Pharmacopoeia (USP) Section 88.

A 4 gram test article was placed into 20 mL of extraction solution. Extraction of test articles was performed for 72 ± 2 hours at 50 ± 2° C. Extract solutions are: normal saline, cottonseed oil, 5 % ethanol in saline, polyethylene glycol.

Observations of reactivity in the rabbits were made at 24, 48 and 72 hours after intracutaneous injection of test extracts.

The requirements of the USP Intracutaneous Reactivity Test have been met.

Acute Systemic Injection Test
The purpose of this test is to screen extracts from Mycap® CCX bottle closure for potential toxic effects. The study is conducted in accordance with United States Pharmacopoeia (USP) Section 88.

A 4 gram test article was placed into 20 mL of extraction solution. Extraction of test articles was performed for 72 ± 2 hours at 50 ± 2 °C. Extract solutions are: normal saline, cottonseed oil, 5 % ethanol in saline, polyethylene glycol.

Observations of biological reaction in rabbits were made at 0, 24, 48 and 72 hours after intravenous and intraperitoneal administration of test extracts.

The requirements of the USP Acute Systemic Injection Test have been met.

Intramuscular Implant Test
The purpose of this test is to study local effects of Mycap® CCX bottle closure when in direct contact with living skeletal muscle tissue of rabbits. The study is conducted in accordance with United States Pharmacopoeia (USP) Section 88.

Test articles were cut into 3 mm × 10 mm pieces. Test articles were surgically implanted into the paravertebral. After seven days, tissue containing the implant was observed for hemorrhage, film, encapsulation, necrosis, discoloration or infections and recorded.

The requirements of the USP Intramuscular Implant Test have been met.

4.7 Particulates

4.7.1 USP <788>

The purpose of this test is to detect and quantify particulate matter in the Mycap® CCX bottle closure. Particulate matter is defined as extraneous, mobile, undissolved substances, other than gas bubbles, unintentionally present in the device.

The USP <788> test is a destructive test and is done as part of product validation. The study is conducted in accordance with United States Pharmacopoeia (USP) Section 788.

The test articles were assembled and gamma irradiated to minimum of 50 kGy. The test articles were exposed at 55 ± 4 °C and an ambient temperature of 25 °C for 91 days, which simulated a time period of approximately two years on the shelf. The test articles were aged under 50 ± 20 % relative humidity (RH).

The fluid pathway, including the flask of the test article, is flushed and filled with 100 mL of low particulate water. The system was inverted 20 times to mix the solution, and the effluent collected in a clean container for analysis.

Particulate from the samples was measured and enumerated using the HIAC Royco Liquid Particle Counting System. The values obtained were averaged.

Acceptance criteria is ≤ 25 particles per mL larger than 10 µm and ≤ 3 particles per mL larger than 25 µm.

The requirement for USP <788> has been met.

Particulate testing is done routinely on products manufactured at Sartorius's New Oxford facility.

4.8 Endotoxin

4.8.1 USP <85>

The purpose of this test is to detect and quantify bacterial endotoxins in Mycap® CCX bottle closure systems. The Limulus Amebocyte Lysate (LAL) test is an in-vitro, destructive test and is done as part of product validation. The study is conducted in accordance with United States Pharmacopoeia (USP) Chapter <85> and ANSI | AAMI ST72.

Endotoxins are lipopolysaccharides from the cell wall of micro-organisms. In some cases, endotoxins from gram-negative bacteria may be pyrogenic (fever inducing). Clean-room management procedures described in the New Oxford site quality system include strategies to reduce, control and monitor viable organisms.

LAL testing is done routinely on products manufactured at Sartorius's New Oxford facility, including Mycap® CCX bottle closure systems, to maintain data on endotoxin manifested on products.

The test articles were assembled, gamma irradiated to a minimum of 50 kGy. The test articles were exposed at 55 ± 4 °C and an ambient temperature of 25 °C for 91 days, which simulated a time period of approximately two years on the shelf. The test articles were aged under 50 ± 20% relative humidity (RH).

The fluid pathway of the test article is flushed with LAL reagent water heated to 37 ± 1 °C. Fluid was kept in contact with the fluid pathway for > 1 hour at 18–25 °C. The extract solution was then analyzed for endotoxin units (EU).

Detected endotoxin was below detection limits of 0.0050 EU/mL. Sartorius's acceptance criteria is less than 0.125 EU/mL. The requirement for USP <85> has been met.

5. Leachables and Extractables

5.1 Overview

Extractables are compounds that have the potential to leach from the materials of the fluid handling system into the solution. The conditions and solvents used in a study of extractables are more extreme than normal process conditions. Aside from the intrinsic properties of the solvent, exposure time and temperature are manipulated in order to extract the most compounds.

Leachables are the compounds that will actually leach from the materials of the fluid handling system into the process fluid. It is important to understand leachables' effect on the safety, identity, strength, purity or quality of the drug product. Sartorius is not able to provide applicable leachable studies, because the conditions and solutions of our customers' processes are unknown.

A risk assessment is advised to determine whether leachable and extractable studies are required. Considerations should include the production stage, exposure time and temperature, exposure surface area, and the process fluid pH and polarity.

Testing for low-risk profiles may be adequately met by USP <87> and USP <88>. These studies to not identify or quantify compounds leaching from materials. Instead, these studies measure biologic and cytotoxic effects of leachables from the materials under the defined extraction parameters. Per guidelines, extractions are performed under the following conditions:

Extract Solvent	Extraction Time [h]	Extraction Temperature [°C]
Normal Saline	72	50
Cottonseed Oil	72	50
5 % Ethanol in Saline	72	50
Polyethylene Glycol	72	50
1x minimum essential media (MEM) with 5 % bovine serum	24	37

Extracts for all fluid-contact materials of Mycap® CCX bottle closures are found to have no cytotoxic or adverse biological effect.

5.2 USP <381>

Elastomeric closures for containers are made of materials obtained by vulcanization (cross-linking), polymerization, polyaddition, or polycondensation of macromolecular organic substances (elastomers).

USP <381> measures physico-chemical characteristics of extractions from the Mycap® CCX elastomeric closure. Test articles were gamma irradiated to 50 kGy and extracted in purified using an autoclave at 121 °C. The extractions were tested and measured against limits.

Test Description	Result
Acidity or Alkalinity	Pass
Absorbance	Pass
Reducing Substances	Pass
Heavy Metals	Pass

5.3 21CFR177.2600

21CFR177.2600 sets limits for extractables from rubber articles including platinum-cured silicone. Test articles were gamma irradiated to 50 kGy and extracted in distilled water.

The rubber articles of Mycap® CCX bottle closures meet the standards of 21CFR177.2600.

5.4 Further Leachable and Extractable Studies

Further leachables and extractables data may be necessary for components with high-risk profiles. Confidential information about additional leachable and extractable studies may be available from our component manufacturers.

Sartorius's Confidence® services are available to perform customized and confidential extractable and leachable studies on polymer-based process components.

6. Cell Expansion Performance Evaluation

6.1 Background

Cellular respiration consumes O_2 and produces CO_2 as a by-product. Cell cultures starved of O_2 will not propagate. Cultures with an overabundance of CO_2 become acidic and impair cell viability. The transfer of gases across the filter membrane of a flask is passive. There must be unrestricted flow of air across the entire surface area of the membrane to support respiration requirements of most cell lines.

Mycap® CCX gas exchange cartridge design has a large microporous membrane with unrestricted air flow and has a small footprint, so integral tubes can be included for aseptic fluid transfer.

6.2 Gas Exchange Study

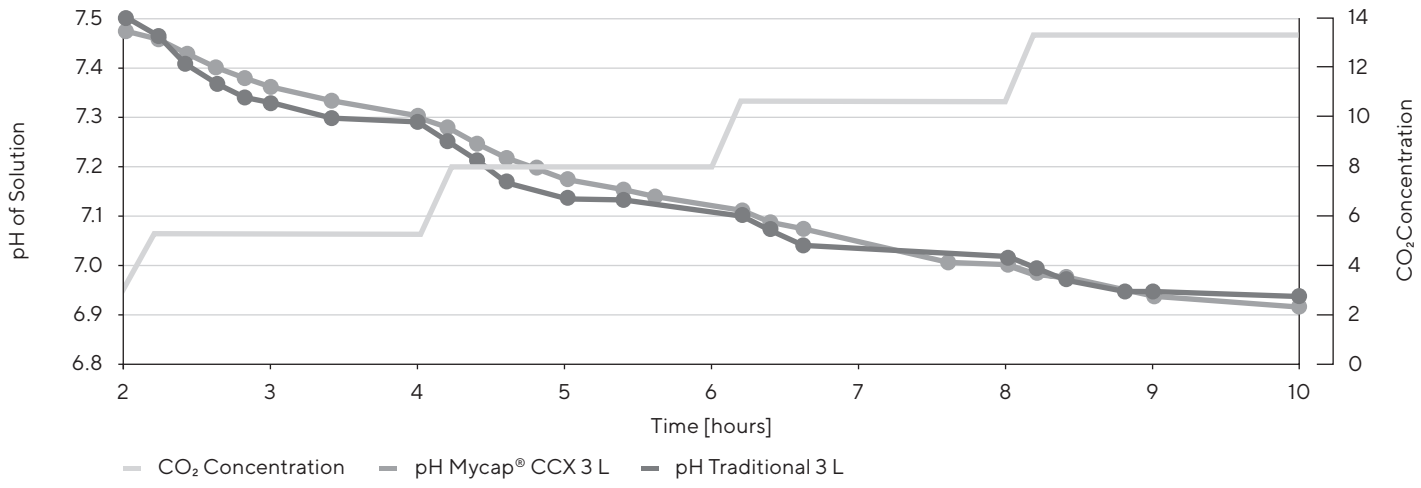
Sartorius performed an evaluation to compare gas exchange across the Mycap® CCX cap closure with a traditional Erlenmeyer flask with vented cap.

1 L and 3 L flasks were modified to accept a pH probe in the side wall so that the probe would be in direct contact with the solution to read pH changes. Flasks were filled with phosphate-buffered saline (PBS) solution containing a sodium bicarbonate buffer. Test articles were placed in an incubator and CO_2 concentrations changed every two hours.

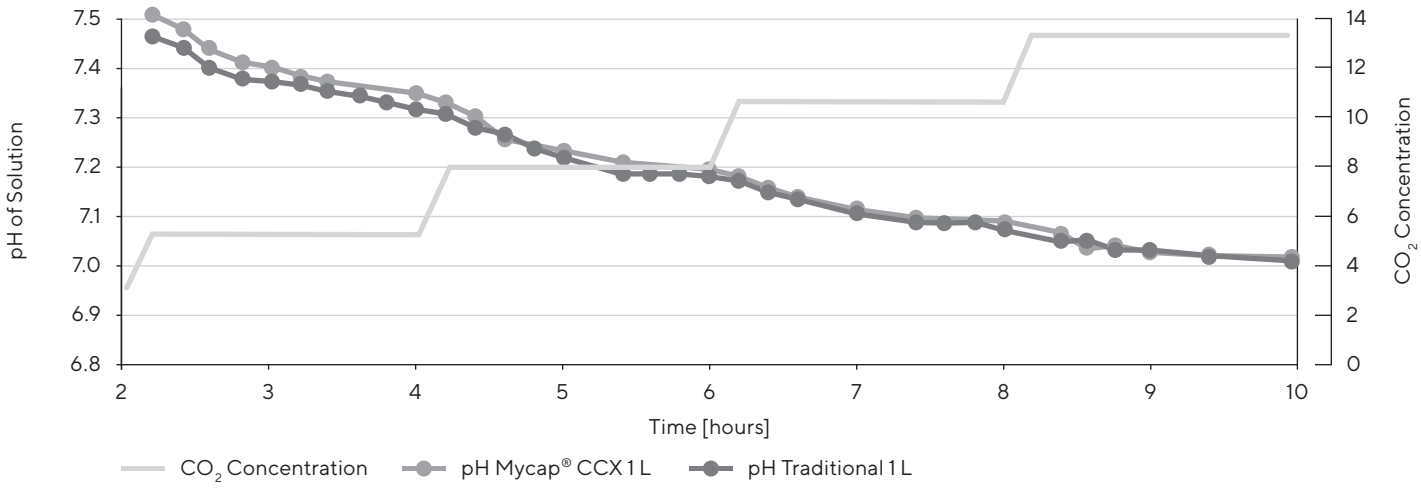
Change in pH of the solution indicates successful gas exchange across the filter membrane.

Comparing the rates of change of Mycap® CCX with traditional flasks illustrates that the rate of exchange is substantially equivalent.

3 L Flask



1 L Flask



6.3 Cell Growth Study

Sartorius performed an evaluation to compare cell growth between a Mycap® CCX cap closure and a traditional Erlenmeyer flask with vented cap.

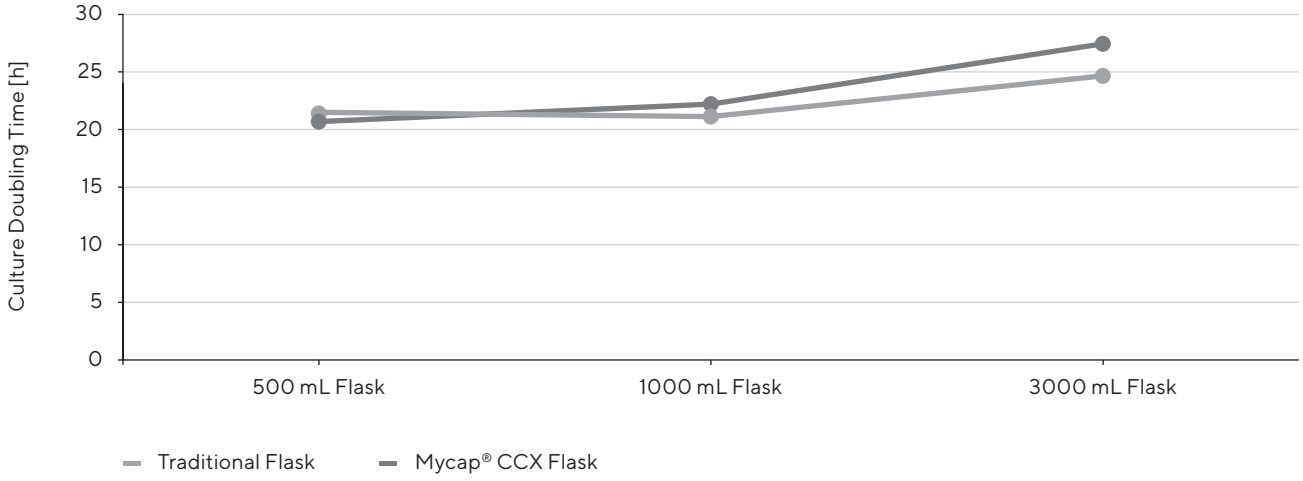
CHO DG44 cells were directly thawed into a traditional flask and then split into two trains. Train 1 uses Mycap® CCX flasks; train two uses traditional flasks. Cells were sub-cultured consecutively for three additional passages in various size flasks up to 3000 mL. Each passage included a 500 mL flask for data generation.

Two-tailed T-tests were performed comparing the doubling times between Mycap® CCX and traditional flasks of the same size. There was no statistically significant difference in growth rates between the two systems, with a 95 % confidence level.

	Traditional Flask	Mycap® CCX
Mean Growth Rate	20.73225	21.5925
Variance	4.630726917	1.253225
Hypothesized Difference of Growth Rates	0	
P (T < = t) two-tail	0.509843261	

The difference between the growth rates are not statistically significant.

Culture Doubling Times between Traditional Flask and Mycap® CCX in Cell Expansion Process



7. Gamma Sterilization Validation

7.1 Purpose

A sterilization validation study has been performed to validate sterility assurance level (SAL) 10^{-6} for the fluid pathway of Mycap® CCX flasks after gamma irradiation to 25 kGy. The method follows the current ISO 11137 guideline.

7.2 Method

VDmax25 for multiple production batches procedure described by the ISO 11137 has been selected for this study. The method for 25 kGy as routine minimum dose is applicable to product having an average bioburden less or equal to 1,000 CFU.

7.2.1 Bioburden Evaluation

Nature of raw material, type of components, product design and size, manufacturing process, manufacturing equipment and manufacturing environment have been considered to define the representative product.

The bioburden study is performed on 3 × 10 units of representative products. Samples have been manufactured from 3 batches and packaged under normal production conditions. The bioburden is evaluated according to ISO 11737-1 requirements.

7.2.2 Verification Dose Experiments

The verification dose is determined to produce a SAL 10-1 according to the rules defined in the ISO 11137 for method VDmax25. It is characteristic of both the bioburden level and the associated maximal resistance. Ten units of representative products have been manufactured and packaged under normal production conditions. They are then irradiated at the selected dose experiment ± 10 % according to the ISO 11137-2 recommendations.

7.2.3 Sterility Testing

Individual sterility testing has been performed on these ten irradiated samples. Sterility test meets the criteria: not more than one positive.

7.2.4 Conclusion

The verification dose experiment is accepted as no growth has been observed on the 10 units tested. Therefore, 25 kGy is safely substantiated as the minimum sterilization dose for Mycap® CCX to obtain SAL of 10^{-6}.

7.2.5 Maintenance of Sterility

Dose audits verifications are performed quarterly on representative products in order to confirm the validity of the 25 kGy minimum dose according to the ISO 11137-2 requirements.

Conclusion
The validation of the sterilization process conducted on Mycap® CCX has met the standard ISO 11137 requirements. Sterility of Mycap® CCX is validated with a SAL of 10-6. An irradiation certificate, in addition to the certificate of release, is provided with each released batch.

8. Shelf-Life

Mycap® CCX flasks are validated for a two-year shelf life post gamma sterilization, using accelerated aging conditions. If a new component with a shorter shelf life is used in a Mycap® CCX system, the whole system will receive the shortest shelf life. Design rules control Mycap® CCX designs.

The critical performance properties and bioburden of the Mycap® CCX has been assessed and compared with original properties after a two-year storage in accelerated conditions.

8.1 Verification of Critical Performance Properties

8.1.1 Container Closure

The test articles were assembled and gamma irradiated to a minimum of 50 kGy. The test articles were exposed at 55 ± 4 °C and an ambient temperature of 25 °C for 91 days, which simulated a time period of approximately two years on the shelf. The test articles were aged under 50 ± 20% relative humidity (RH).

At the conclusion of the aging, the container closure study by aerosol was conducted, as per section 4.5 **"Container Closure by Aerosol Challenge"**.

The samples passed the container closure test affirming closure integrity is maintained after aging.

8.1.2 Leak Rate and Gas Exchange Cartridge Air Flow Rate

The test articles were assembled and gamma irradiated to a minimum of 50 kGy. The test articles were exposed at 55 ± 4 °C and an ambient temperature of 25 °C for 91 days, which simulated a time period of approximately two years on the shelf. The test articles were aged under 50 ± 20 % relative humidity (RH).

The samples passed the critical in-process and final article release criteria, as per 3.5.1 **"Gas Exchange Cartridge Air Flow Rate Test"** and 3.5.2 **"Pressure Decay Test"**, thus affirming the closure is leak free after aging.

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