sartorius

# Validation Guide
# MYCAP® CCX Cell Expansion System

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

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# 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 nearly 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 Stedim Biotech'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 cleanroom.

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

## 1.1 Scope Statement

Components and assemblies considered for this Validation Guide are as follows:

**Bottle Closure**

| Manufacturer | Brand | Materials of Construction |
|---|---|---|
| Sartorius Stedim Biotech | 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) |
| Dow Corning® | Pharma-50® | Platinum cured silicone |
| Dow Corning® | Pharma-65® | Platinum cured silicone |

**Connectors & Fittings**

| Manufacturer | Brand\|Description | Materials of Construction |
|---|---|---|
| Sartorius Stedim Biotech | 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-Robers | Robertsite® Luer Activated Access Site | Polycarbonate, platinum cured silicone |

**Venting and Gas Exchange**

| Manufacturer | Brand\|Description | Materials of Construction |
|---|---|---|
| Sartorius Stedim Biotech | MYCAP® CCX Gas Exchange Cartridge | Polycarbonate, polyethersulfone |
| Sartorius Stedim Biotech | 25 mm Minisart® | Polypropylene, 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 upon 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

Sartorius Stedim Biotech's manufacturing resources for MYCAP® CCX bottle closures operate under strictly controlled manufacturing procedures and quality systems.

New Oxford, Pennsylvania -
United States of America
80 Progress Avenue
New Oxford, PA 17350

**Total Facility Size:**
17,000 square feet
ISO 7 Manufacturing & Raw Materials: 3,100 square feet
**Manufacturing Competencies**

- MYCAP® CCX bottle closures
- MYCAP® CCX Gas Exchange Cartridge
- Subcomponent assembly
- Final assembly
- Packaging & Labelling

Stonehouse -
United Kingdom
Sperry Way
Stonehouse Park
Stonehouse GL10 3UT

**Total Facility Size:**
52,800 square feet
ISO 7 Manufacturing & Raw Materials: 2,600 square feet
**Manufacturing Competencies**

- MYCAP® CCX Gas Exchange Cartridge

## 1.4 cGMP Quality Assurance

Our documented quality system is consistent with industry-recognized quality standards including the following:

The FDA current Good Manufacturing Practices (cGMPs)

**Note:**
Sartorius is not a manufacturer of finished pharmaceuticals or finished medical devices, yet we have chosen to align our quality system to the clauses of 21 CFR Parts 210, 211 and 820 that apply to our processes and products.

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

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

## 1.5 Gamma Irradiation

MYCAP® CCX bottle closures are packaged and shipped in cardboard boxes to Steris Isomedix for gamma irradiation.

To assure security of services, three of Steris' sites have been qualified for gamma irradiation services:

- 23 Elizabeth Drive
  Chester, NY 10918
  USA
- 9 Apollo Drive
  Whippany, NJ 07981
  USA
- 435 Whitney Street
  Northborough, MA 01532
  USA

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 & 2-year accelerated aging
- Microbial container closure by aerosol challenge
  Post ≈ 50 kGy irradiation & 2-year accelerated aging
- USP <788>:
  Particulate matter in injections
  Post ≈ 50 kGy irradiation & 2-year accelerated aging
- USP <85>:
  Bacterial Endotoxins Test
  Post ≈ 50 kGy irradiation & 2-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: Cleanrooms and associated controlled environments -- classification of air cleanliness by particle concentration
- Bioburden & Sterility
  - ISO 11137: Sterilization of healthcare products
    Dose Audit: Quarterly
  - ISO 14698: Cleanrooms 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
  - Prior to MYCAP® CCX Closure assembly
  - After MYCAP® CCX assembly
- Pressure decay test of MYCAP® CCX closure and immediate connections*
- Compliance to technical drawing
- Packaging and labeling
- Gamma irradiation

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

# 2. Production and Quality

**2.1 Personnel**

Sartorius Stedim Biotech 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 assure 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 Stedim Biotech 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 & Qualification**

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

- Quality Control System
- Quality Assurance System
- Facility & Cleanroom Controls
- Product & Component Lot Traceability System
- Change Notification Procedures

Suppliers are evaluated and approved according to internal standards.

**2.3.2 Component & 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 are 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 Stedim Biotech 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 upon arrival at Sartorius Stedim Biotech against approved control specifications. Typical testing requirements applied at incoming quality inspection are (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 Stedim Biotech's inventory and quality management system and 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 schedule the calibration plan 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) 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 cleanroom with a schedule of LpH®, Vesphene® and Spor Klenz® occurs per our cleanroom management and cleaning procedures.

Viable organisms are measured quarterly to monitor the effectiveness of the cleanroom 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 cleanroom reduce and control non-viable particles.

Non-viable readings are recorded weekly to ensure 0.5 $\mu/m^3$ and 5.0 $\mu/m^3$ particles are within the ISO Class 7 acceptance criteria, 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 cleanroom per incoming inspection and testing procedures.

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

### 3.4 Traceability & Batch Control

Sartorius Stedim Biotech has a process and maintains an effective traceability system which can be used in the event of product, component or manufacturing issue to alert impacted 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-Stedim. Each component and subassembly has a unique part number. All components and subassemblies are assigned a unique lot number upon 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 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 & 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 assembly, etc.)
- Pressure Decay Test (when applicable)
- Gas Exchange Cartridge Air Flow Rate (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 Stedim Biotech.

#### 3.5.1 Gas Exchange Cartridge Air Flow Rate Test

The gas exchange cartridge undergoes two in-process quality checks. The in-process checks are performed on every gas exchange cartridge before it is installed into the cap closure and on every closure assembly after it has been installed to a flask. The test measures pressure drop across the membrane to confirm membrane integrity and to detect any possible damages or defects.

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 of the bounds of the critical values. Critical values are set to 500 $cm^3$/min – 650 $cm^3$/min.

##### 3.5.1.2 Test Method

Compressed air is regulated and stabilized to 3000 mbar (g)|43.5 psi by a pressure controller. Then the pressure is decreased to 80 mbar (g)|1.2 psi by a second pressure controller (precision pressure controller). The free air flow is restricted to 850 $cm^3$/min. A time period of 5 seconds for stabilization has been proven to be sufficient to detect any defects. The air flow measured on the sample is compared with limit value specified. Passed tests will show a green signal, failed tests will show a red signal.

#### 3.5.2 Pressure Decay Test

MYCAP® CCX bottle closures are leak-tested prior to release. Pressure decay at 2 psi is measured using the TME Worker™, Model W-L-015. Pass|Fail criteria is leak rate less than 0.03 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 0.03 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

MYCAP® CCX bottle closure is a one-piece closure with integral tubing and the 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. 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 & 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 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 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 |
| **Torque specifications** | 6–40 in.-lbs |
| **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 |

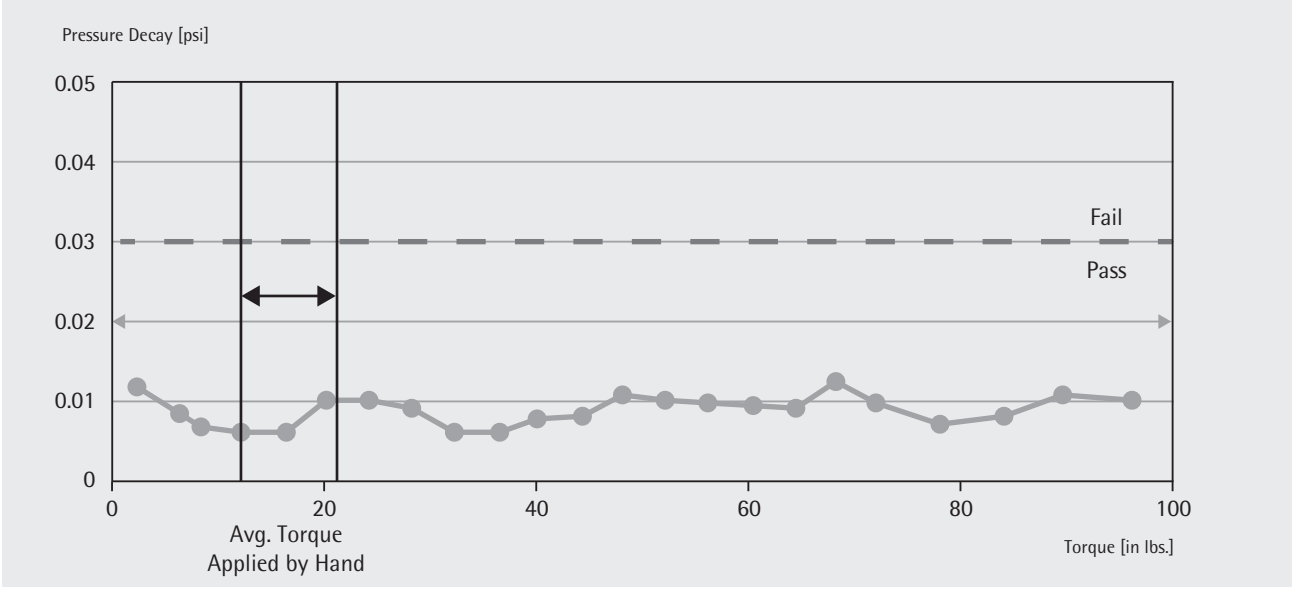
**4.4 Torque Specification**

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 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 0.03 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.

Allowing for a torque factor of safety, Sartorius recommends a minimum|maximum closure torque of 6-40 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 to measure torque applied during installation of MYCAP® bottle closure to containers by Sartorius manufacturing personnel.

The torque applied by a sampling of operators was measured using 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 of 6-40 in.-lbs.

### 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 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 2 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 7 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 MYCAP® CCX indicating that the system may be safely irradiated up to 50 kGy, placed on shelf under ambient conditions for 2 years and the closure maintains 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 on were made after incubation for 72 hours at 37 ± 1 °C with 5 ± 1% $CO_2$.

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

The requirements of 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 7 days, tissue containing the implant was observed for hemorrhage, film, encapsulation, necrosis, discoloration or infections and recorded.

The requirements of 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 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, 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 2 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 effluent collected in a clean container for analysis.

Particulate from the samples were 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' New Oxford facility, including MYCAP® CCX bottle closure to maintain data on particulate manifested on products.

## 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 microorganisms. 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' New Oxford facility, including MYCAP® bottle closure systems to maintain data on endotoxin manifested on products.

The test articles were assembled, 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 2 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 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 the extent of 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 physicochemical 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' CONFIDENCE® Services is 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 byproduct. 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

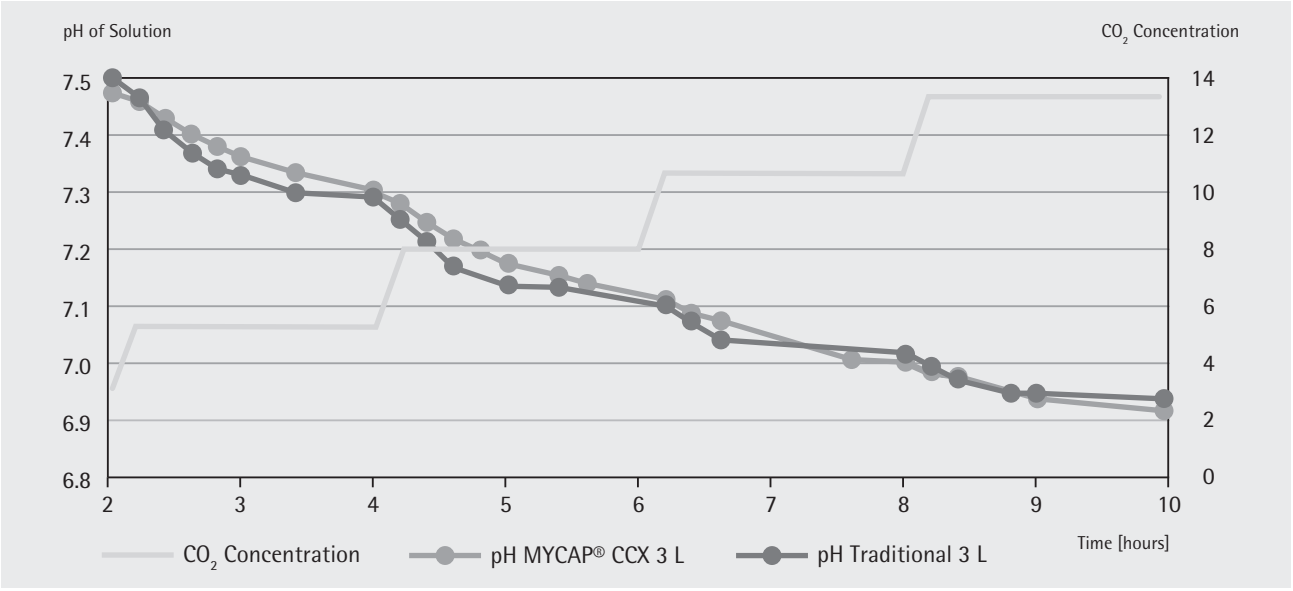
Sartorius performed an evaluation to compare gas exchange across the MYCAP® CCX cap closure to traditional Erlenmeyer flask with vented cap.

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 solution to read pH changes. Flasks were filled with phosphate buffered saline (PBS) solution containing sodium bicarbonate buffer. Test articles were placed in an incubator and $CO_2$ concentrations changed every two hours.

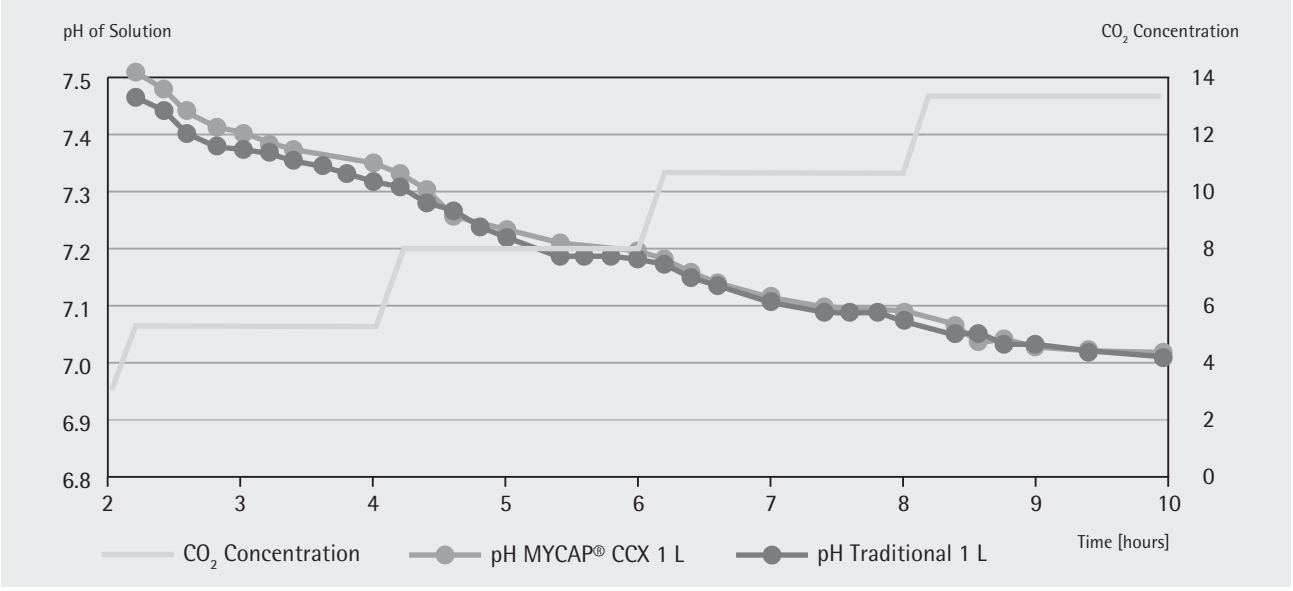
Change in pH of the solution indicates successful gas exchange across the filter membrane.

Comparing of the rates of change of MYCAP® CCX with traditional flasks illustrate 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 MYCAP® CCX cap closure and traditional Erlenmeyer flask with vented cap.

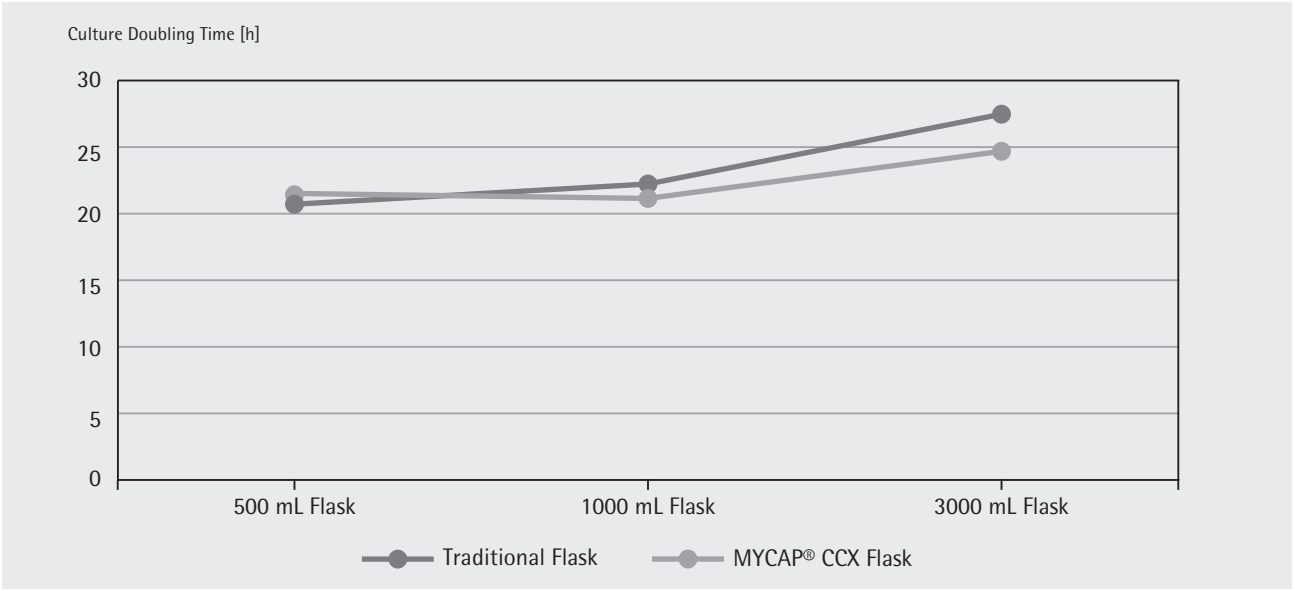
CHO DG44 cells were directly thawed into a traditional flask and then split into two trains. Train 1 utilized MYCAP® CCX flasks; Train 2 utilized traditional flasks. Cells were sub-cultured consecutively for three additional passages in various size flasks up to 3000 mL. Each passage included a 500 mL 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**

Compliance to ISO 11137 allows Sartorius Stedim Biotech to claim (SAL) $10^{-6}$ for all MYCAP® CCX flasks included in the product family.

**7.2.1 Bioburden Evaluation**

As described by the ISO 11137 document, a representative of the product family is defined to carry out the gamma sterilization validation tests.

Product bioburden of the representative assembly is determined. The bioburden evaluation is performed on 10 units selected from 3 different lots.

**7.2.2 Verification Dose Experiments**

Once the bioburden is established (i.e. quantified, identified and assessed for its resistance) the verification dose to be used for the validation is selected. In accordance with the ISO 11137 document, the verification dose is calculated to produce a SAL $10^{-1}$. The verification dose experiment is conducted on 10 units. Sterility test must pass the criteria of ≤ 1 positive.

After a successful verification dose experiment, SAL $10^{-6}$ is verified.

**7.2.3 Maintenance of Sterility**

Environmental monitoring is routinely performed to address potential changes to our manufacturing environment, as described in Section 3.2 Production Environment.

Dosimeters are placed in all sterilization batches to verify that the dose received bags is within specified limits (i.e. greater than 25). Dosimeters are placed at minimum and maximum dose locations based upon the specific loading pattern, including density, of each irradiation batch.

The final critical aspect of maintenance of sterility is the routine characterization of product bioburden. Product bioburden is quantified with dose audits. Dose audits are performed in accordance with ISO 11137. Dose audits verify the initial validation remains applicable and SAL $10^{-6}$ is valid.

**7.3 Product Family Definition**
Product families are defined by the number and types of micro-organisms on the product. The ISO11137 standard offers product related variables to consider when defining a product family.

**7.3.1 Nature and Sources of Raw Materials**
A table of sources of components and raw materials defining the product family is available in section 1.1 Scope Statement. Components or raw materials are polymeric in nature and may be assemblies inclusive of several different materials.

**7.3.2 Components**
A table of sources of components and raw materials defining the product family is available in section 1.1 Scope Statement. Components or raw materials are polymeric in nature and may be assemblies inclusive of several different materials.

**7.3.3 Product Design and Size**
MYCAP® CCX flasks vary in design and size. All systems include at least one MYCAP® CCX bottle closure. MYCAP® bottle closures are unique in the industry in that the general product design is the same regardless of the cap size, tube materials or port configuration. Designs may include several different components; the overall size is restricted by design rules.

**7.3.4 Manufacturing Equipment**
MYCAP® CCX flasks are assemblies of different components or subassemblies. Assembly operations utilize the same equipment (work benches, leak testers, cable tie & oetiker assembly tools, etc.) for all products in the product family. The manufacturing equipment used to produce the MYCAP® CCX flasks are also the same regardless of the final configuration.

**7.3.5 Manufacturing Environment & Location**
All MYCAP® CCX flasks are manufactured and assembled in the same ISO 7 clean room in New Oxford, Pennsylvania. The production environment and controls are described in detail in section 3.2 Production Environment.

**7.4 Results**
The sterility validation for MYCAP® CCX flasks product family has met the requirements set forth in the applicable regulations. SAL $10^{-6}$ is verified.

# 8. Shelf-Life

MYCAP® CCX flasks are validated for a 2 year shelf life post gamma sterilization, using accelerated ageing 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 2 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 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 2 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, per section 4.2 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 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 2 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, 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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