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Application Note

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Cell Culture Expansion in Fully Closed Erlenmeyer Shake Flasks Outside the Biosafety Cabinet with Mycap® CCX

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Abstract

Expansion of suspension cell culture from cell banks to seed bioreactor is performed through passages of successively larger Erlenmeyer shake flasks. The traditional cap of an Erlenmeyer flask is unscrewed for each fluid transfer. Risk of contamination is mitigated by performing these fluid transfers in a biosafety cabinet (BSC) or laminar flow hood.

Work in a BSC is not preferred because of; high maintenance and operating costs, intensive cleaning and decontamination procedures, and the risk and inconvenience of performing operations in the BSC.

Despite working in a BSC, expansion processes include passages with back-up flasks to be used in the case of contamination. Back-up flasks are a material waste and multiply labor-intensive BSC work.

Sartorius' Mycap® CCX includes integral tubing and a specially-designed gas exchange cartridge. Integral tubing supports good aseptic technique to prevent contamination. All fluid transfers are done outside the BSC. The gas exchange cartridge has a high filter surface area to support passive gas exchange and vibrant cell growth in the incubator.

Find out more: www.sartorius.com/mycap-ccx

Introduction

Bottle closures with integral tubing are widely used in bioprocessing because they reduce or eliminate the risk of contamination from poor aseptic technique.
Good aseptic technique is especially important upstream where preserving axenic, or monoculture conditions is compulsory.

Tubing materials of the cap closure are commonly thermoplastic elastomer (e.g. C-Flex®) which can be aseptically welded to another tube of the same size. Alternatively, aseptic connecting devices (Sartorius Opta®, Colder Aseptiquik®, Pall Kleenpak®, etc.) may be installed at the tube ends. In either case, the bottle can be aseptically connected to receive or dispatch fluids in non-classified spaces without the risk of introducing a contaminant.

Key customers approached Sartorius to improve aseptic technique in cell expansion with the following objectives:
- Eliminate contamination risk
- Enable fluid transfers in non-classified spaces
- Reduce waste from requisite back-up passages
- Achieve comparable culture growth rates & doubling times to incumbent expansion methods

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 exchange of O_2 and CO_2 across the filter membrane is critical to cell growth.

It is customary to attach a disc filter to integrated tubing in a cap for air venting during fluid transfer. Early testing of closures on Erlenmeyer flasks with a 50 mm disc filter showed slowed or fully halted cell growth. Despite the large filter surface area (3 in² | 20 cm²) of the 50 mm disc filter the gas exchange across the membrane was inadequate for cell growth. Air flow through the membrane is restricted at the 8 in. (3.2 mm) orifice of the hose barb on the filter housing. Cell growth resumed once the culture was moved to a flask with the traditional flask cap.

Traditional flasks have a filter membrane embedded in the cap. The arrangement allows for unrestricted air flow across the entire filter surface. However, the filter membrane occupies the entire cap surface leaving no room for integral tubing for aseptic fluid transfers.

Sartorius' Solution
The manufacturing process of the patented Mycap® bottle closure is an enabling technology. Components, usually tube assemblies, are inserted into pre-formed holes. Silicone elastomer is dispensed into the cap to hermetically seal the installed components in place and to create the highly compliant, plasticizer-free bottle closure.

Inserted components are not restricted to tube assemblies. Sartorius developed the Mycap® CCX gas exchange cartridge with the following objectives:
- Provide adequately large filter surface area
- Allow unrestricted air flow across filter membrane
- Reduce the filter footprint allowing space for integral tubing

The Mycap® CCX gas exchange cartridge is a three dimensional, stadium shaped part. Two generous 0.2 µm, hydrophobic filter membranes extend into the neck of the flask. The orientation of the filter membranes protects and places them in position for unrestricted gas exchange between the culture and the incubator environment. The stadium shape conserves space on the cap for integral tubing for media addition, inoculum addition, sampling and transfer.

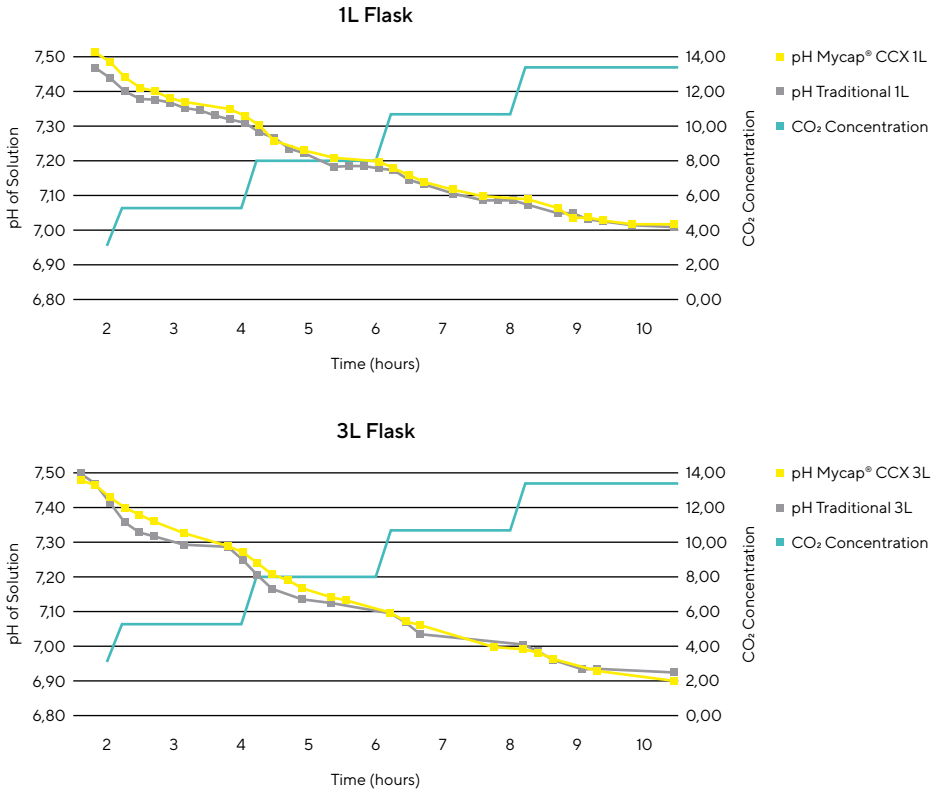
Gas Exchange Study

Sartorius performed an evaluation to compare gas exchange across the Mycap® CCX cap closure and the traditional vented cap closure.

1L and 3L 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 gas exchange across the filter membrane.

The pH change of the solution on flasks with the Mycap® CCX cap and flasks with the traditional vented cap are virtually identical.



Cell Growth Study

Sartorius performed a study comparing cell growth in flasks with the Mycap® CCX cap to flasks with the traditional 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.

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.

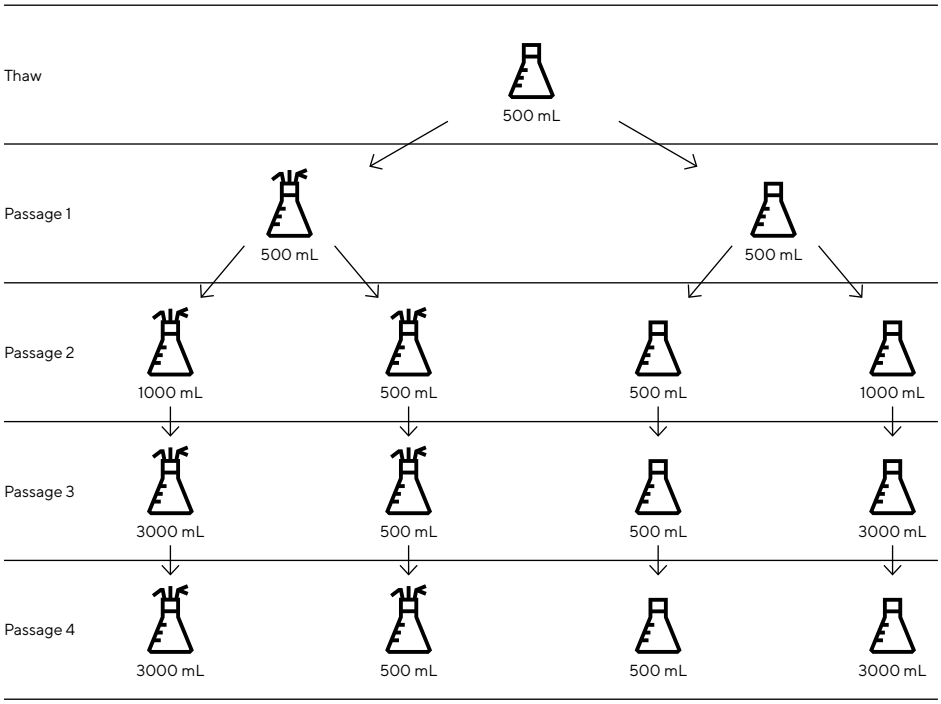
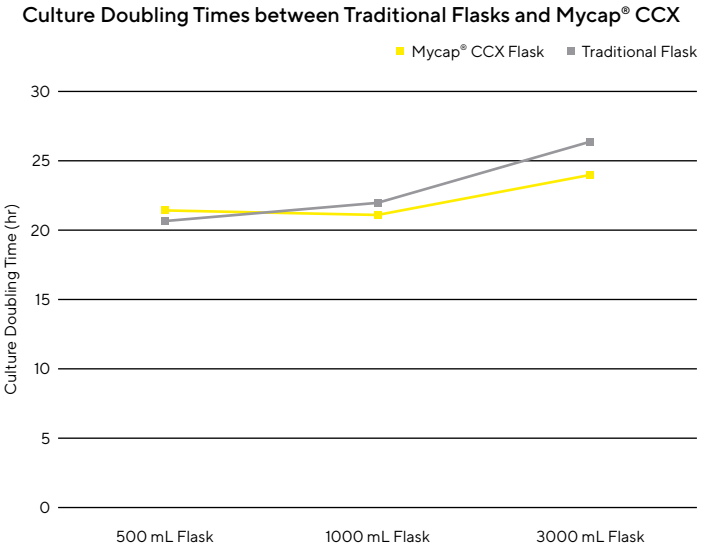


Fig. 1: Cell Growth Study Process Diagram

Incubator Parameter	Description | Set Point	
Temperature	36.8°C	
Carbon Dioxide %	7.5%	
Agitation	500 mL, 1000 mL 3000 mL	120 RPM 80 RPM

Table 1: Process Parameters

Average culture doubling times for each flask size were graphed. The graph illustrates the comparability of doubling times for Mycap® CCX flasks and traditional flasks.



Conclusion

Expansion of suspension cell cultures using Erlenmeyer flasks in a BSC is a labor-intensive process. The flask's cap is removed at each passage and fluid transfers including media addition, inoculation and sampling are done, typically by hand-pipetting. These operations are performed under laminar flow in the BSC to prevent contamination. Yet, contamination risk persists so back-up flasks are maintained for use in the event of a contamination. In a GMP seed expansion process, a typical passage requires three to four operators; the hood technician, hood assistant and data/batch record recorder(s).

Mycap® CCX has integral tubing allowing for aseptic fluid transfers in the open space of a workbench. The number of operators is cut in half, contamination risk is eliminated and wasteful back-up flasks are not necessary.

Carefully controlled conditions for cell growth in a shake flask in an incubator are required. In particular, the unrestricted exchange of CO_2 and O_2 between the cell culture and the incubator environment is critical to achieving targeted cell density and viability.

Gas exchange, as measured by a change in pH of solution in response to a change in CO_2 concentration, between Mycap® CCX and traditional flasks was compared and found to be substantially equivalent.

Successful passages in an expansion process are benchmarked by cell growth rates and cell culture doublings. A comparison of cell culture doublings between Mycap® CCX and traditional flasks across 4 passages were found to be equivalent.

Mycap® CCX should be considered a suitable replacement for traditional Erlenmeyer flasks to reduce waste, eliminate contaminations and streamline cell expansion operations.

Recommendations

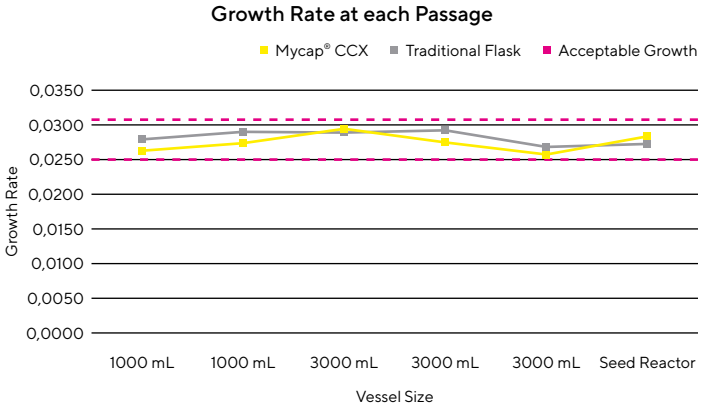
A validation study comparing rates of growth in Mycap® CCX flasks with traditional flasks should performed before implementing. Sartorius offers the Mycap® CCX Validation Template Tool to streamline experimental design, data collection and data analysis.

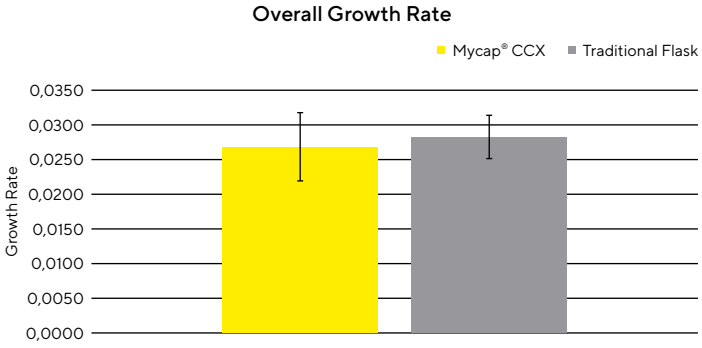
The Tool generates charts to visualize growth rates and performs the Student's T-Test to compare the datasets.

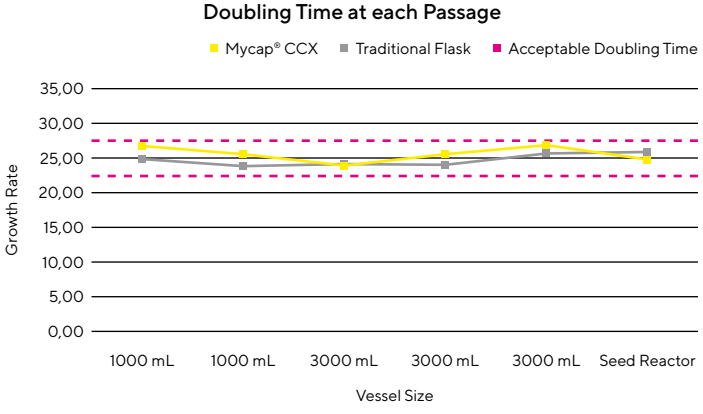
Mycap® CCX Validation Template Tool makes it quick and easy to make a scientifically sound and informed decision if Mycap® CCX is an acceptable replacement of incumbent technology for use in a production process.

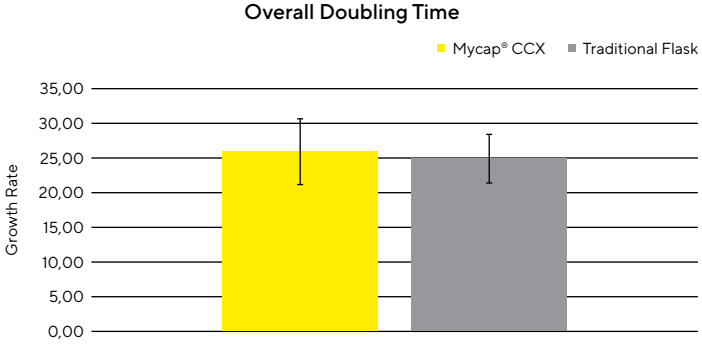
Mycap® CCX Validation Template Tool:

- Supports up to 6 Passages
- Complete Mycap® CCX materials list including 'Where Used Guide'
- Record and maintain experimental conditions; flask size, culture volume, shaker speed, incubator temperature, CO_2 concentration
- Compare against required performance criteria; growth rate, cell count targets, cell viability
- Visual and Statistical Analysis including:
- Doubling Time and Growth Rate Graphs at each Passage
- Overall Doubling Time and Growth Rate Graphs
- Student's T-Test









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