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Resolute® BioSC Pilot: Designed for Effective And Consistent Chemical And Microbiological Cleaning

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Abstract

One of the most frequent issues raised in the Warning Letters issued by the FDA concerns cleaning or cleaning validation. Besides ensuring compliance with current good manufacturing practice (cGMP) regulations, cleaning operations allow manufacturers to produce multiple products, improve product qualityconsistency, and prevent cross-contamination and adulteration of drug products. "Cleaning and cleaning validation considerations for continuous manufacturing equipment and systems are primarily the same as those for non-continuous manufacturing equipment and systems."[1]

This application note demonstrates how Resolute® BioSC Pilot can help drug manufacturers ensure effective and consistent chemical and microbiological cleaning and pass their cleaning validation tests.

For further information, visit
www.sartorius.com/multi-step-chromatography

Introduction

Resolute® BioSC Pilot Facilitates Cleanability

Establishing effective procedures is not enough to perform efficient and safe cleaning. Selecting a cleaning-friendly equipment is also essential.

Making cleaning-friendly equipment starts right at the component selection stage. As a multicolumn system, valves are key components of Resolute® BioSC Pilot design. Diaphragm valves are widely used and recognized in biopharmaceutical process equipment due to the absence of dead spaces and the easy flushing. All Resolute® BioSC Pilot valves were specifically designed in blocks to minimize dead volume and allow efficient column inter-connections.

Pumps are another key component. Resolute® BioSC Pilot uses best-in-class QuattroFlow® diaphragm pumps of PSG Germany GmbH to facilitate cleaning.

Components such as pressure, flowmeter, conductivity, pH and UV sensors have been selected and fit onto dedicated flow cells, in accordance with good sanitary practices.

Resolute® BioSC Pilot is designed to perform the entire downstream process on a single machine, and can operate simultaneously and continuously 3 chromatography separations, including viral inactivation and buffer preparation. The system is thus smaller than traditional batch units, with a reduced surface area to volume ratio, making the cleaning process easier. It can also operate in a closed system to reduce the risk of external contamination.

The cleaning method is automated by the Resolute® BioSC Pilot control software and follows a series of steps defined in the recipe editor. Sartorius collaborated with a big pharma company to establish standard methods and recommendations to configure and set recipes with a view to facilitate and secure cleaning operations.

QuattroFlow® is a registered trademark of PSG Germany GmbH

Methods

Manufacturers are required to document their cleaning processes for all kinds of equipment parts. In order to evaluate the cleaning process, they must first define acceptance criteria and use samples | testing programs to check these have been met. Ideally, a single cleaning process is applied to each equipment, although this can vary depending on the product.

Cleaning operations must be performed regularly and are highly dependent on production strategies.

For example, a cleaning process may be required between:
- bioprocessing steps of the same product
- production batches of the same product
- production batches of different products
- production campaigns

The method and acceptance criteria are also dependent on the production strategy. FDA does not establish acceptance specifications due to the high diversity of equipment and products.

Acceptance Criteria and Analysis

As mentioned above, the FDA does not set acceptance criteria and analytical methods. It is up to each manufacturer to define their own criteria, as they have an in-depth knowledge of their particular production environment (machines and materials involved) and are therefore best placed and able to define limits that are appropriate, feasible and verifiable. Nonetheless, certain reference points can be found in the literature.

Table 1: *General Examples of Acceptance Criteria*

Contaminant	Analysis Method	Acceptance Criteria
Cleaning agent (e.g. 0.5 M Sodium hydroxide NaOH)	Conductivity	<4.3 µS/cm
Bacterial contamination	Bioburden	≤1 CFU/mL
Endotoxin level	LAL	<0.5 EU/mL
Organic residues	TOC	<0.50 ppm

For analysis, samples can be taken from the last rinse water and | or by swabbing the internal surface of the equipment when possible (e.g. tank surfaces). Samples analysis will demonstrate the level of cleanliness by reaching the defined acceptance criteria.

- Visual inspection is an active observation of the accessible product contact surfaces of the equipment. It will determine whether the equipment is free from any visible residues.
- Conductivity is used to evaluate the effectiveness of rinsing procedures by detecting trace quantities of cleaning chemicals during water rinse.
- The removal of residues of organic origin can be determined by total organic carbon analysis (TOC analysis) and | or by SDS-PAGE Silver stain (applicable for pre-clinical activities). These tests are performed on surface swabs amples and | or on the last rinse water and | or on the elution buffer of the blank run (if applicable).
- The absence of bacterial contamination is demonstrated by a bacterial load count (bioburden) and measuring the endotoxin level (LAL) in the last rinse solution (if applicable).

The procedure described below was designed and executed by the customer on the Resolute® BioSC Pilot system. This method is just one example in order to demonstrate the effectiveness of the cleaning.

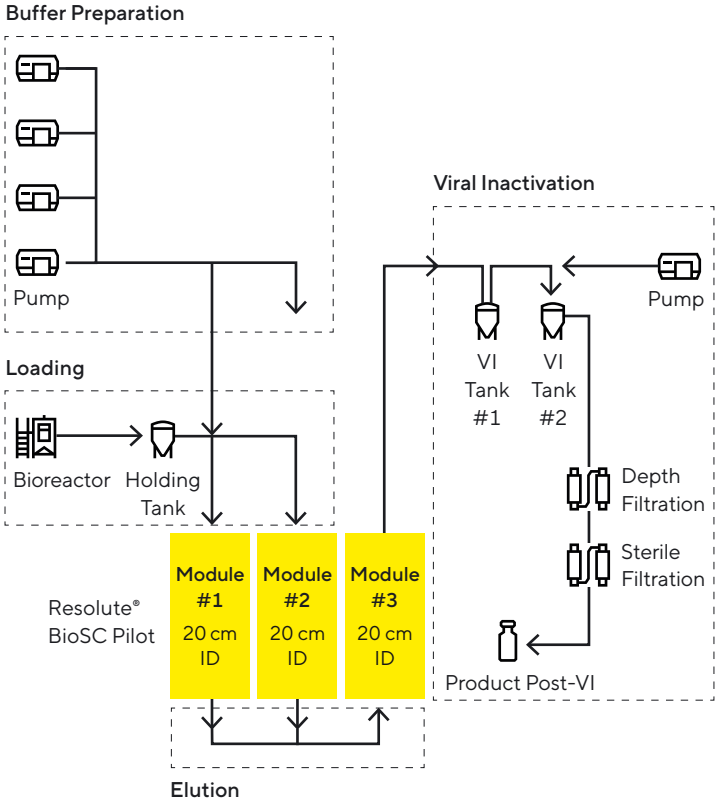


Figure 1: *Capture System*

Process Overview

- Parallel Batch Capture cycles on 20 cm diameter columns on modules 1 and 2 (including in-line dilution of concentrated buffers).
- Accumulation of protein A elution surge tank.
- After 2 capture elutions: Automated transfer of the protein A elutions in VI tank and launch of Viral Inactivation step followed by Depth Filtration step and Sterile Filtration step on Module 3.

The customer designed a 12-hour process to purify a 2,000 L bioreactor, from capture to post-VI. Cleaning was evaluated during and after the run. All buffers were first filtrated and processed into single-use bags. Chromatography columns were sanitized and stored as prepacked columns. Gamma-irradiated filters were installed before columns and after depth filtration.

After the production run described above, Resolute® BioSC Pilot was completely sanitized with 0.5 M NaOH (Sodium hydroxide) for 30 minutes at a high flowrate (80 L/h) to create a turbulent regime. It was then rinsed with purified water until conductivity fell below 1 mS/cm.

Sterility Monitoring

Two types of samples were taken at different stages to monitor sterility.

- Equilibration fractions after each capture cycle. For each capture cycle, the last mL of equilibration was pooled in a single bag placed on capture system Outlet 2 (Capture step system). This pooled fraction is called "EQ_FC_Capture".
- Samples from the post-VI: pool of two cycles of viral inactivation, depth filtration and 0.2 µm filtration. This pooled fraction is called "VI_FI_Final".

Acceptance Criteria

Specifications for this experiment on a process intermediate were as follows:

- Bioburden quantification. In CFU/mL.
  Specs: ≤ 1 CFU/mL (or ≤ 10 CFU/10mL)
- Endotoxin quantification (LAL). In Endotoxin Unit/mL.
  Specs: ≤ 0.5 EU/mL (for information the LAL sensitivity for a process intermediate is 0.5 EU/mL)

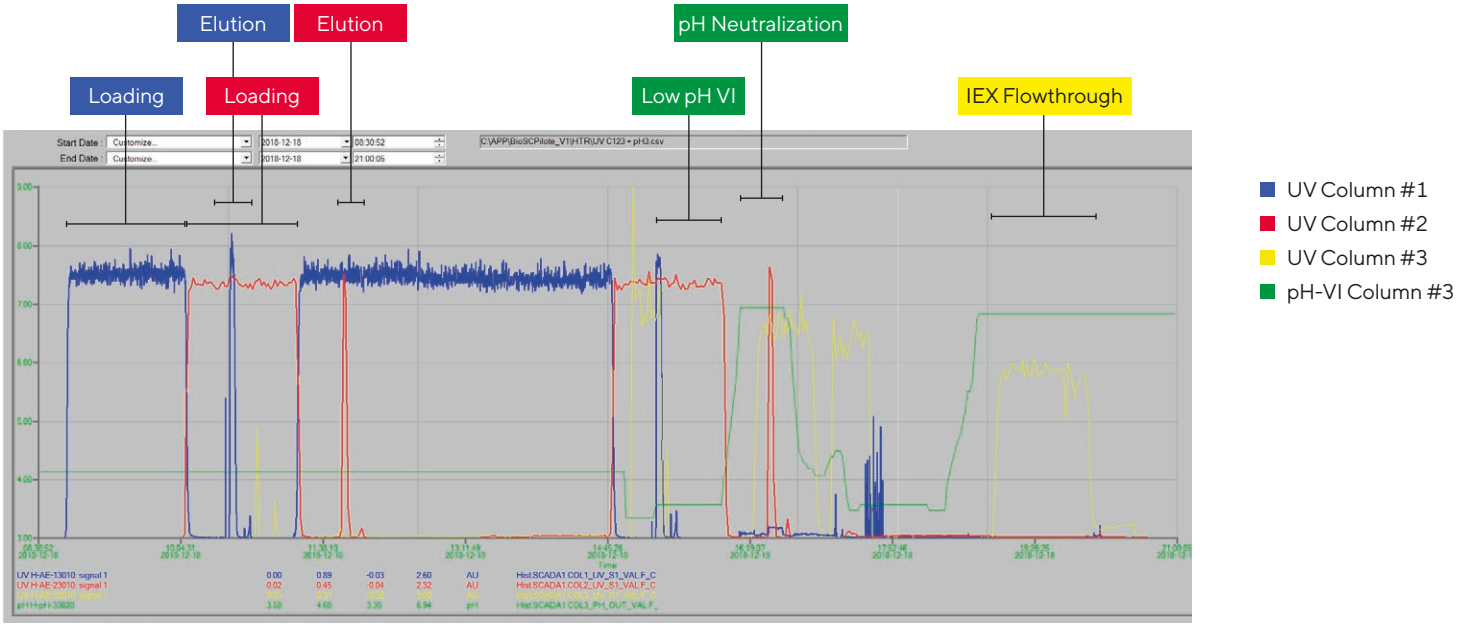


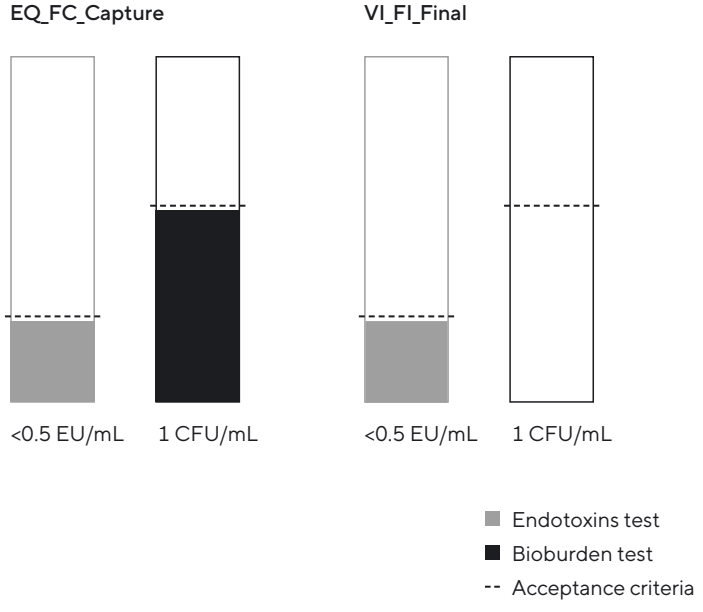
Figure 2: *Production Run Chromatogram*

Results

The results obtained for both the pool of equilibration fractions (EQ_FC_Capture) and the final product fraction (VI_FI_Final) are within specifications for bioburden and endotoxin contents for such process intermediate.

Table 2: *Results Obtained for the Pool of Equilibrations and the Final Product Fractions*

Sample name	Tests	Results
EQ_FC_Capture	Endotoxins	<0.5 EU/mL
VI_FI_Final	Endotoxins	<0.5 EU/mL
EQ_FC_Capture	Bioburden	1 CFU/mL
VI_FI_Final	Bioburden	0 CFU/mL



Conclusion

The cleaning of equipment in the pharmaceutical industry is a major issue.

This application note shows that Resolute® BioSC Pilot was successfully cleaned after a 12 h-production run, designed to purify a 2,000 L bioreactor, thus demonstrating the ability capability of the system to comply with biopharma standards in terms of residual bioburden and endotoxin levels.

Recommendations

We recommend cleaning the Resolute® BioSC Pilot system using purified water first, followed by 0.5 M NaOH sanitizing solution. The entire flow path, from inlet to outlet, is flushed at a high flow rate using both solutions to create turbulence and remove residues. Inlet and outlet manifolds are supplied with the equipment to connect all inlets and outlets. The cleaning recipe is designed to cover all piping, valves and instrumentation components. The flow is then put on hold for one hour to increase contact time and effectiveness of the sanitizing solution. After this static cleaning, the system is flushed with purified water at the maximum flow rate until at least 3 times the hold-up volume is achieved or until conductivity falls below a given threshold. Collection of the flushed water at predefined points of the flow path is performed to analyze and validate the cleaning procedure.

References

[1] Khinast, J., Rantanen, J. Continuous Manufacturing of Pharmaceuticals. John Wiley & Sons Ltd 121, (2017)

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