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StreamLink® CC 15 – Novel Sequential High-Throughput Clarification and Purification of Monoclonal Antibodies for Cell Line Development

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Abstract

One of the main challenges in cell line development is increasing the speed at which candidate cell lines can be screened. The industry is constantly looking to remove bottlenecks in upstream and downstream processing to increase throughput and bring forward project delivery dates. StreamLink® CC 15 brings an innovative breakthrough to the cell line development (CLD) sample-processing space, drastically improving the speed at which batches of 5 - 15 mL samples can be clarified and/or purified, ready for downstream analysis. Once a process has begun, StreamLink® CC 15 can manage variations in sample characteristics and automatically recover from otherwise problematic errors (e.g., high clarification pressure) without user intervention. The robust nature of StreamLink® CC 15's software architecture assures consistent performance and aims to revolutionize the future of high-throughput processing of CLD samples.

Here, we present findings from a customer development partnership showing the recovery performance of the StreamLink® CC 15.

Introduction

In recent years, multi-parallel, high-throughput bioreactor platforms, such as the Ambr® 15, have accelerated cell line development (CLD) by dramatically increasing the available bioreactor positions and, therefore, capacity for the rapid screening and evaluation of clones. In addition, screening data from Ambr® 15 runs are more representative of production scale cultures compared to shaken cultures, as the system provides conditions and control aligned with manufacturing scale bioreactors.[1] Although multi-parallel bioreactors reduce CLD timelines and save on full-time equivalent (FTE), their full potential is limited by other bottlenecks in the CLD workflow.

The Ambr® 15 has moved this bottleneck from running cell cultures to the end-point sample preparation for downstream analytics. Currently, CLD operators will typically clarify the cultures with centrifugation and syringe or vacuum filtration steps, which are manually intensive, time-consuming, and produce significant differences in sample hold times throughout processing. Purification is then performed with plate-based drip columns or semi-automated purification systems, which are better suited to process development rather than high-throughput sample preparation. Custom workflows using expensive liquid-handling solutions can be set up to perform both clarification and purification. However, these solutions are temperamental, expensive, manually intensive, and require extensive technical training.

The Sartorius StreamLink® CC 15 is a fully automated system for rapid clarification and purification of up to 48 cell culture samples. Walkaway operation with fast and easy setup minimizes FTE requirement to eliminate the CLD workflow bottleneck and further unlock the potential of Ambr® 15. The StreamLink® CC 15 is also applicable to process development (PD) and process characterization (PC) workflows by efficiently preparing mid-point samples from scale-down bioreactors (such as Ambr® 250) for product quality analysis (PQA). The speed and ease of sample preparation can enable more regular PQA and provide better process resolution while shortening PD feedback time, speeding up process adaptation, and reducing the total number of experiments required.

In this application note, we demonstrate that as well as enabling faster processing, the StreamLink® CC 15 supports consistently high recovery of monoclonal antibody products after clarification, purification, and combined processes.

Materials

Hardware

StreamLink® CC 15 is the first fully automated, benchtop, high-throughput system capable of streamlining clarification and/or purification bioprocess steps. The system is suitable for sample preparation of 5-15 mL cell cultures from Ambr® 15 or other suspension cultures and has a maximum possible input volume of 30 mL. It can clarify and purify $24 \times 10^{\pm 5}$ mL culture samples in 2 hours, with a capacity to run 48 samples in 4 hours.

StreamLink® CC 15 is comprised of a robotic liquid handler and two filter stations (FS). The liquid handler (LH) is responsible for transferring samples from the input labware to the filter stations for processing and then from the FS to the output labware once the clarification and/or purification is complete. The filter stations are modular units of hardware that connect to the base of the system. A FS is driven by a peristaltic pump which can pull liquid from one of the eight supply valves or directly from the FS input cup.

Sensing

The liquid flow path has two pressure sensors for monitoring purification device backpressure and clarification differential pressure control. The flow path also contains two liquid sensors to position liquid and ensure that the purification device remains bubble-free. The in-line UV sensor downstream of the purification device enables the estimation of the eluted IgG and collection of concentrated eluates via UV peak cutting. The clarification filter clamp can complete the flow path with or without a Sartoclear® Disc. In addition, there is an optional purification bypass to divert around the Sartobind® Rapid A nano; this allows for separate or combined clarification and purification processes.

Consumables

Clarification is carried out using the Sartoclear® Disc, a single-use multi-layer clarification filter. The Sartoclear® Disc has a surface area of 20 cm² and is made up of four layers of synthetic non-wovens and a final 0.2 µm membrane. This novel combination has been optimized for centrifuge-free clarification of mammalian cell cultures and has been sized to process at least 15 mL of a typical fed-batch mammalian culture. Purification is completed using the new, multi-use Sartobind® Rapid A nano, Protein A membrane adsorber with a membrane volume of 1.2 mL and a binding capacity of <35 mg of IgG. The high flow rate capacity of the device and consumables enable the StreamLink ® CC 15 to complete 24 samples within 2 hours for a standard process.

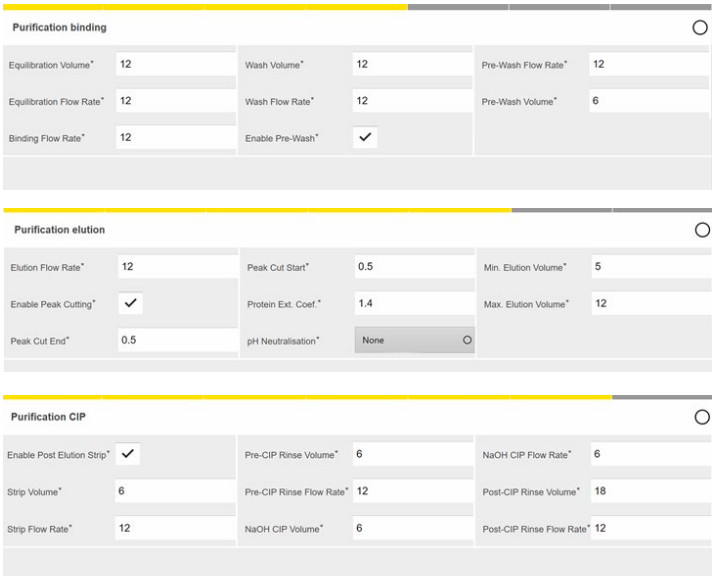
Software

The StreamLink® CC 15's easy-to-use software interface uses wizard-style templates and defined processes to simplify user interaction. The complex process control is fully automated, unlocking the potential for differential pressure control and UV peak cutting for small-volume workflows. In addition to enhanced sensing and process control, the software can automatically recover an experiment in the event of an error.

Methods

Performance data was generated both internally and in collaborative trials with customer development partners.

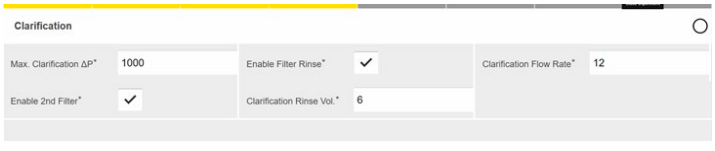
Figure 1: *Screen Capture From the StreamLink® CC 15 Purification Template Creation Wizard.*



For the purification trial detailed in Figure 4, the parameters were defined as displayed in Figure 1. The sample material was taken from a 500 mL shake flask harvest of a CHO cell line (harvested at 1.81×10^6 cells/mL and 66% viability on day 14). Before loading, material was clarified and sterilized with a 15-minute (min) centrifugation step, followed by 0.45 µm and 0. 2 µm vacuum filtration steps, and loaded onto the system in 15 mL falcon tubes. Sample volumes were adjusted to ensure the total product input was below the 35 mg capacity limit of the Sartobind® Rapid A nano filter.

The combined clarification and purification process used the parameters shown in Figure 2 for clarification alongside the purification parameters displayed in Figure 1.

Figure 2: *Clarification Parameters Displayed From the Template Creation Wizard Found Within the Main UI, Guiding the User Through to Beginning a Tailored Process.*



Under these operating conditions, the average time per sample for purification is 12 mins, and 17 mins for a clarification and purification process.

Customer Development Trial

Material was generated from shake flask, Ambr® 15, and 50 L harvests in an on-site trial led by GSK. The samples were pooled and redistributed before each trial to ensure homogeneous input. The input culture had a viable cell density (VCC) of 1.81×10^6 cells/mL, 66% viability, and an average cell diameter of ~14 µm.

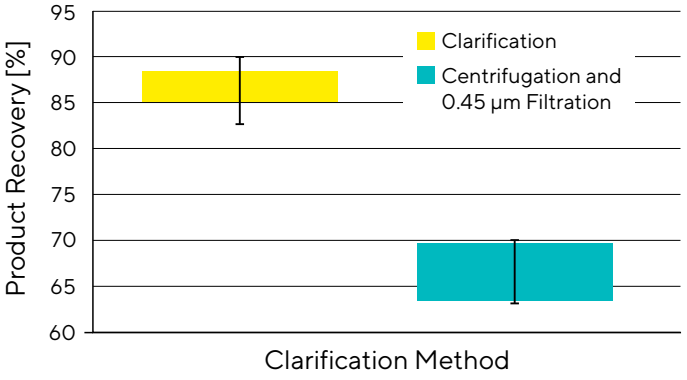
To calculate the IgG recovery, the input and output material were weighed to determine the volume (assuming 1 mg/mL density), and the input material was adjusted for purification and combined processes to compensate for wet cell weight.

Titer was measured using the Cedex BioHT, and product quality data for all samples was generated using HPLC.

Results

The following data demonstrates the StreamLink® CC 15's robust product recovery performance across run types. This data was produced both internally and in collaboration with customer development partners. The aim was to observe the system's performance without prior process optimization, its robustness to variations, and the processing speed.

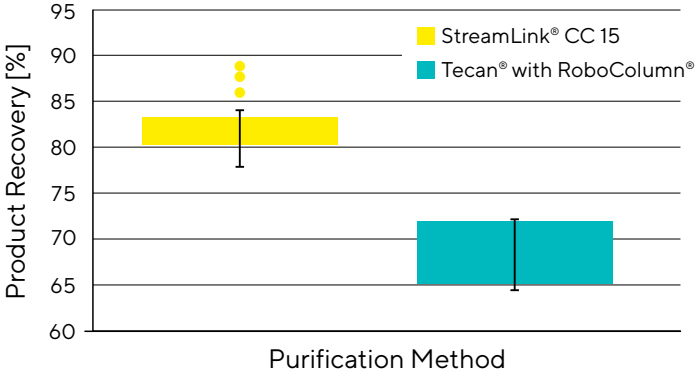
Figure 3: *Clarification Performance – Comparison of Product Recovery Performance Between StreamLink® CC 15 and a Typical Industry Method.*



Note. 5 - 15 mL harvest samples were processed, and the average processing time per sample was ~9 min.

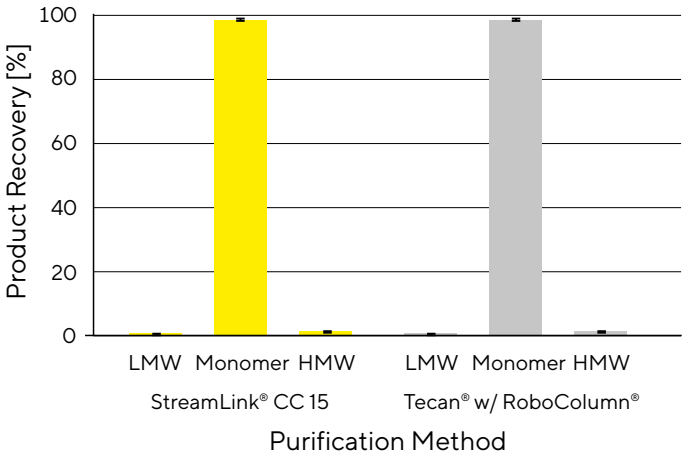
Figure 3 demonstrates excellent clarification performance, indicating minimal product loss during clarification on StreamLink® CC 15. Automated processing offers reduced timelines and the opportunity for multi-stage processing. The equivalent industry-standard method using centrifugation and 0.45 µm syringe filtration yielded a product recovery of 70%, primarily due to volumetric loss instead of concentration.

Figure 4: *Purification Performance – Average % IgG Recovery and Standard Deviation on the Streamlink® CC 15 Alongside a Typical Industry Method.*



Note. n=24 for StreamLink® CC 15 data n=4 for Tecan® with RoboColumn® data. Pooled input material taken from 50 L SS CHO harvest.

Figure 5: *Product Quality Showing Comparable Output Material Between StreamLink® CC 15 and the Tecan® With a RoboColumn®.*

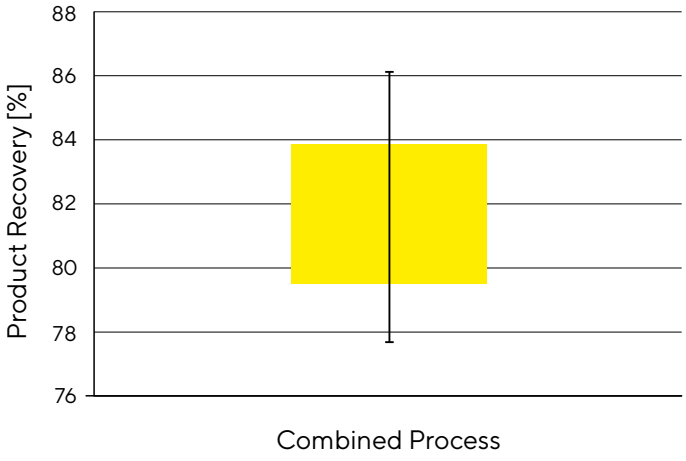


Note. The proportion of low molecular weight (LMW), monomer, and high molecular weight (HMW) products was measured. With StreamLink® CC 15 n = 24, and n = 4 for Tecan® with RoboColumn®. Error bars represent one standard deviation.

Data from purification trials indicate StreamLink® CC 15 achieves consistently high IgG product recovery (~82%). A typical manual, multi-stage purification method (Tecan® with RoboColumn®) was used as a benchmark, with the StreamLink® CC 15 showing a higher total product recovery and an improved sample-to-sample consistency in recovery performance. The drip-wise nature of the RoboColumn® method can induce variability.

Product quality analysis (PQA) (Figure 5) shows statistically comparable post-purification product quality for StreamLink® CC 15 and a typical industry method for all observed species.

Figure 6: *Combined Process (Clarification & Purification) Recovery Performance.*



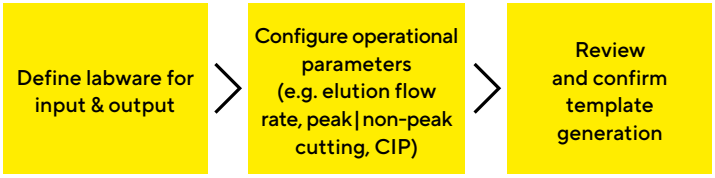
Note. Process from harvested culture broth through to purified, concentrated eluate. Average sample processing time ~17 min per sample.

The combined clarification and purification process begins with culture broth samples through to clarified, purified eluate ready for further analysis such as product quality, total protein, and DNA analysis. StreamLink® CC 15 shows high and reliable recovery in this application, with consistent sample-to-sample performance, highlighting the system's reliability.

Discussion

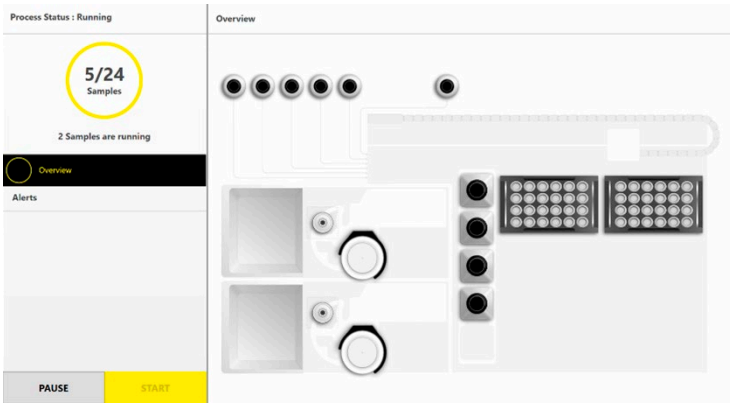
The StreamLink® CC 15 provides a concise user experience with a simple and intuitive interface. Users can tailor platform processes to best suit their operational needs (input/output volumes, flow rates, etc.) within the template wizard.

Figure 7: *The StreamLink® CC 15 Template Wizard.*



StreamLink® CC 15 takes these user-defined parameters and generates consistent sample-to-sample performance with the integrated platform clarification and purification processes. Run progress and alerts throughout the process are clearly displayed to the user on the primary interface (Figure 7). Any actions that may require manual intervention are also displayed here.

Figure 8: *Screen Capture Showing the Primary Interface Visible During Operation.*



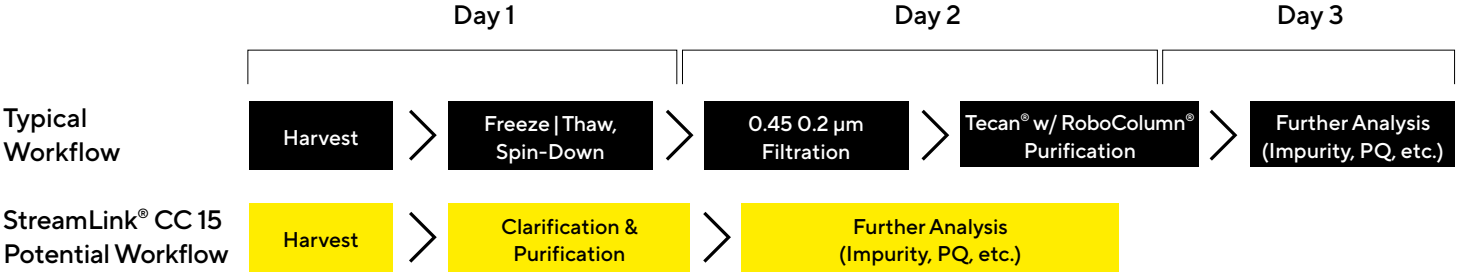
After loading input and output labware on the system deck, users can step away from the system with confidence, knowing StreamLink® CC 15 will deliver output material ready for further analysis. Using a combination of sensing and automation, variations in sample-to-sample characteristics (i.e., viability), which may otherwise cause stoppages because of filter blockages, are managed using integrated error recovery pathways - ensuring efficient, automated processing in a timely manner. The sensing capabilities of StreamLink® CC 15 also safeguard against extreme pressure generation that may otherwise be a concern with automated pumping.

With an increased throughput compared to typical processing methods, StreamLink® CC 15 reduces the time required to generate a batch of clarified, purified samples (e.g., 48 samples from Ambr® 15 harvests) and eases hold time concerns of harvested samples prior to processing. The StreamLink® CC 15 removes the need for intermediate freeze | thaw holding steps.

Using StreamLink® CC 15 to process batches of samples accelerates the CLD workflow, allowing users to analyze samples more quickly while reducing the FTE. Sampling can also be carried out at multiple time points, giving users the opportunity to assess product quality attributes and feed this data into the run to modify conditions in real time. This offers deeper insights into the batch, including how quality attributes change throughout the process.

Given the small volumes being handled upon the harvest of Ambr® 15 cultures, users must often compromise product recovery and concentration of eluate (and vice versa) when using traditional liquid handling methods. StreamLink® CC 15 allows users to remove this trade-off and improve the overall utility of samples prior to downstream analysis.

Figure 9: *Typical Workflow Downstream of Ambr® 15 Harvest With a Streamlined Solution Proposal Using StreamLink® CC 15.*



Conclusion

The StreamLink® CC 15 accelerates and simplifies progress following the harvest of cells from the Ambr® 15 and other small-scale culture formats, taking as little as 12 min for a purification process and 17 minutes for a dual clarification | purification process. The quick and easy-to-use interface paired with automated, consistent performance gives users confidence that samples will be processed uniformly, driving faster downstream analysis with a reduced manual burden. The data shown here demonstrates the significant processing time and consistency improvements offered by the StreamLink® CC 15 compared to typical industry methods.

Technologies such as the in-line UV probe provide additional features such as peak cutting estimation of eluted product, while pressure sensors can gauge the clarification differential pressure and switch out Sartoclear® Disc clarification filters mid-processing to protect against blockages. Overall, the package of features allows users to tailor processes to best suit their needs. The automated error recovery pathways integrated into the software architecture allow the system to rectify issues that may otherwise pause automated downstream systems and ensure the smooth processing of all inputs.

References

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2. Bollmann, F., Riethmüller, D., Johansson, E.S., & Tappe, A. (2019). Optimization of HEK293T suspension cultivation with a DoE-approach in Ambr®15 microbioreactor.

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