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Application Note

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Accelerating Purification Process Characterization and Optimization for Genetically-Modified Influenza Using the Virus Counter® Plus Platform

Michael Lehrer[1], Yann Rault[2], Ysaline Roland[2], Nathalie Leizorovici[3], Fabian Fillmer[4], Jayson Duval[3], Nemanja Bankovic[4], Frederic Thiebaut[3]
[1] Sartorius Stedim North America Inc., 6542 Fig St, Arvada, Colorado 80004, North America
[2] Naobios, 6 Rue Alain Bombard, 44800 Saint-Herblain, France
[3] Sartorius Stedim France FMT S.A.S., ZI Les Paluds, Avenue de Jouques - CS 71058, 13781 Aubagne Cedex, France
[4] Sartorius Stedim Biotech GmbH, August Spindler Straße 11, 37079 Göttingen, Germany

Contact: nemanja.bankovic@sartorius.com

Abstract

Traditional virus quantification methods, such as qPCR and ELISA, often fail to correlate with the number of intact, functional virus particles and can take weeks to yield results. The Virus Counter® Plus platform is designed for the rapid and direct quantification of viruses, addressing the need for quick and reliable analytics in viral production processes.

In this application note, we demonstrate how the Virus Counter® Plus platform was used to accelerate the characterization and optimization of genetically modified influenza A purification processes at Naobios. We compared the performance of two ion-exchange chromatography columns, Sartobind® Q and CIMmultus® SO3, in purifying influenza A virus. The Virus Counter® Plus provided rapid and precise titer measurements. Results were obtained within 24 hours, highlighting the platform's ability to deliver fast and actionable insights, enhancing the efficiency and effectiveness of process development.

Introduction

The Virus Counter® Plus platform is purpose-built for direct, rapid quantification of viruses. The platform addresses the current lack of analytics that deliver rapid and reliable insights for effective biomanufacturing. It can accelerate process development by delivering analytics quickly and robustly, enabling confident decision-making and optimization of viral production processes.

Current virus quantification methods often measure virus components to derive a titer measurement and can be a significant bottleneck to process optimization. Quantitative PCR (qPCR) enumerates viral genome copy numbers to measure titer, and ELISA methods quantify viral envelope proteins to estimate virion concentration. However, these approaches do not correlate with the number of intact, functional virus particles in a sample. Transducing (functional) titer units (TU) can be measured by infectivity assays, but these are dependent on cell culture processes, which can require multiple weeks to yield results.

The Virus Counter® Plus platform consists of hardware, reagents, and software that deliver a user-friendly experience to acquire titer data more quickly and with less hands-on time than other quantification methods. The Virotag® INVA reagent contains fluorescently labeled monoclonal antibodies specific to H1N1 and H3N2 influenza A strains. The Virus Counter® Plus fluidics directs virus particles in suspension through an optical detection system and quantifies stained virus particles with high precision. The time-to-result ranges from two hours to overnight, depending on the number of samples analyzed. The Virus Counter® Plus software reports the concentration of viral particles in each well (“Result”) as viral particles per mL (vp/mL) and the corresponding titer as the concentration multiplied by the dilution factor of the sample.

Here, we demonstrate that the Virus Counter® Plus accelerated the characterization and optimization of genetically-modified influenza A purification processes at Naobios.

Materials and Methods

Virus production at Naobios begins with clarification and endonuclease treatment of the harvested bulk. The clarified bulk is filtered, purified by ion-exchange (IEX) chromatography, and subjected to a second filtration to obtain the final product (Figure 1).

Clarified, endonuclease-treated, and filtered influenza A bulk material was purified using two different Sartorius ion-exchange (IEX) columns using the Cytiva ÄKTA pure™ chromatography system.

- Sartobind® Q Nano 3 mL (P/N 96IEXQ42EUC11)
- CIMmultus® SO3 1 mL (P/N 311.6157-2)

The same amount of virus was loaded on both devices. However, Sartobind® Q required a threefold higher loading volume due to its larger device volume. Virus elution was performed by applying a NaCl concentration gradient, and samples of the eluate were collected in fractions. Samples from each fraction were stained with Virotag® INVA reagent and analyzed with the Virus Counter® Plus (Figure 2). Samples were prepared by diluting the virus sample into sample dilution buffer (SDB AB) and subsequently adding Virotag® INVA reagent to the tube at a 40:1 ratio, mixing thoroughly. Blanks were prepared by adding SDB AB to a clean polypropylene tube and subsequently adding Virotag® INVA Reagent to the tube at a 40:1 buffer-to-stain ratio, mixing thoroughly by pipette. Blanks and triplicate samples were loaded at 250 µL into the 96-well plate, sealed, protected from light, and incubated at room temperature for at least 30 minutes before being placed inside the chilled autosampler chamber. The plate was then read with the Virus Counter® Plus instrument using the Virotag® INVA stain setting with the chiller turned on.

Figure 2: Sample preparation and instrument operation workflow for the Virus Counter® Plus

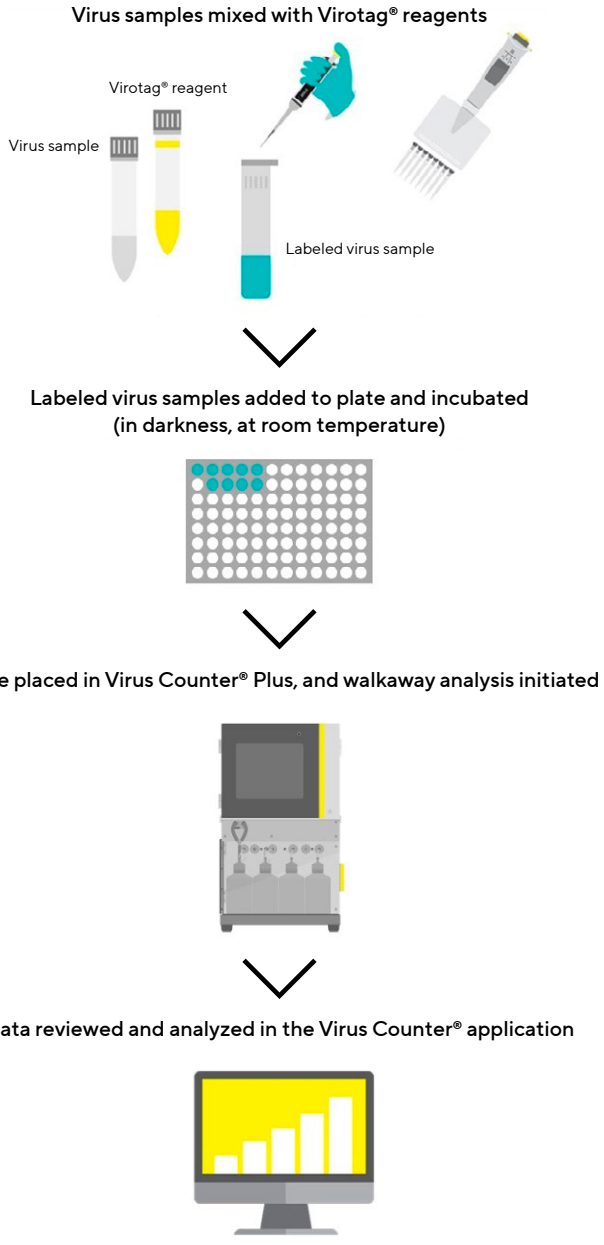
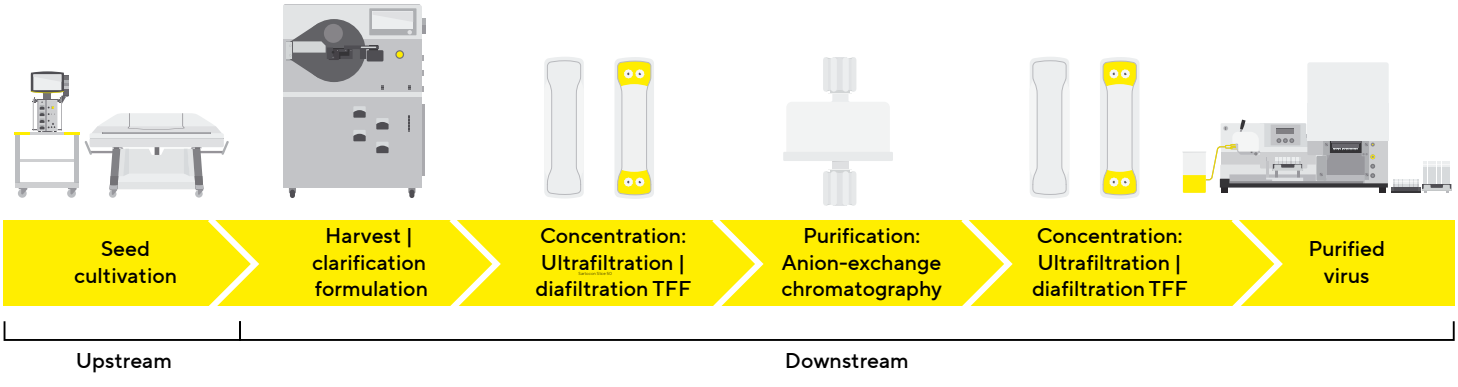


Figure 1: Naobios influenza A manufacturing process



Results and Discussion

The Virus Counter® Plus analysis of filtered influenza A bulk material chromatography fractions was achieved in less than 24 hours from sample preparation to the completion of instrument operations and reporting of the results by the software. The instrument analysis results revealed the differences in influenza A virus titer across the elution fractions.

The viral content in fractions 3 and 4 obtained from the Sartobind® Q was below the quantification limit of the Virus Counter® Plus, and sample titer rose in fraction 5, with the subsequent fractions containing approximately $3-5\times10^8$ vp/mL (Table 1). Variability in the triplicate wells loaded with each diluted fraction was below 5%, indicating excellent instrument and assay performance (Table 1).

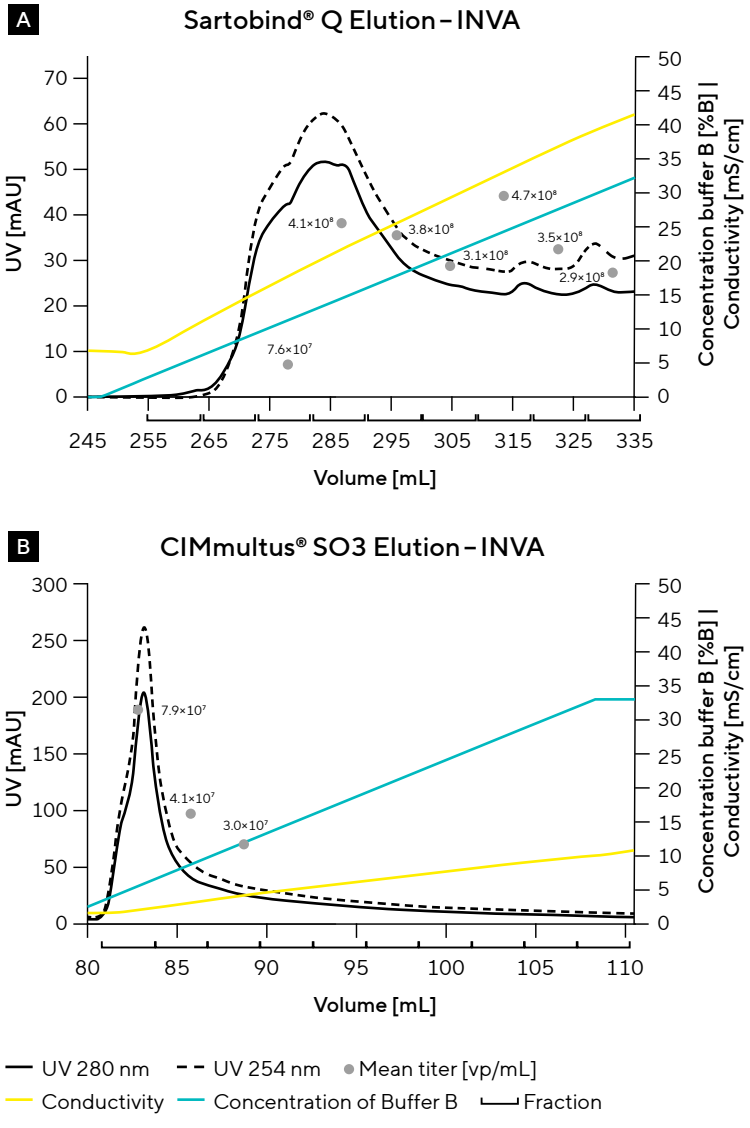
The Virus Counter® Plus results correlated with the 280 nm and 254 nm absorbance (Figure 3), except for fractions 6–8 in the case of the Sartobind® Q. Fractions 6–8 showed the highest protein content, but titer was nearly identical to fractions 9–12, suggesting the presence of damaged virus particles or matrix protein in fractions 6–8. Further characterization of these fractions may be warranted. Virus Counter® Plus results from the fractions collected from the CIMmultus® SO3 column indicate the column-buffer combination requires further optimization, as the obtained elution titer results are lower than those obtained with Sartobind® Q. However, a correlation between the Influenza A virus titer and the UV absorbance profiles is clearly visible.

Table 1: Sartobind® Q fraction titer measured by the Virus Counter® Plus

Fraction	Mean titer [vp/mL]	% Coefficient of variation
3	< IQL	NA
4	< IQL	NA
5	7.6×10^7	4.8
6	4.1×10^8	0.5
7	3.8×10^8	1.0
8	3.1×10^8	1.8
9	4.7×10^8	1.2
10	3.5×10^8	3.8
11	2.9×10^8	2.1

Note. Fractions 3 and 4 results were below the Virus Counter® Plus quantification limit (IQL) and are not reported.

Figure 3: Absorbance, conductivity, and elution Buffer B composition are shown as a function of elution volume in the influenza A purification and have been overlaid by the virus titer measured by the Virus Counter® Plus. **(A)** Purification with Sartobind® Q, **(B)** purification with CIMmultus® SO3



Note. Elution buffer gradient properties are indicated by the conductivity and concentration curves.

The Virus Counter® Plus software reports the concentration of viral particles in each well ("Result") as viral particles per mL (vp/mL) and the corresponding titer as the concentration multiplied by the dilution factor. All fraction samples were diluted 1 : 50 in sample dilution buffer (SDB AB). Titers from fractions with results below the instrument quantification limit are not shown.

Conclusion

The Virus Counter® Plus proved to be a valuable analytical tool for directly comparing two different purification strategies for influenza A. The system also provided detailed insights into the distribution of virus particles across the elution profile. This information could not have been obtained from UV elution profiles alone, as UV signals do not allow differentiation between virus particles and other co-eluting components. By quantifying virus concentrations in individual fractions, the Virus Counter® Plus made it possible to precisely identify the fractions containing the highest virus load. Using the Virus Counter® Plus, reliable conclusions on virus content were obtained within 24 hours at both the individual fraction level and across different purification approaches. This rapid turnaround significantly accelerates data-driven decision-making during process development, in contrast to the standard functional $TCID_{50}$ assay currently performed at Naobios, which requires two weeks to obtain results.

Overall, the Virus Counter® Plus is well suited for process development applications, as it provides fast and direct insights into critical process parameters and product quality attributes. The ability to quickly evaluate purification performance supports informed and efficient process development decisions.

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For more information, visit
sartorius.com

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