Benchmarking the scale-X™ Bioreactor System for Production of Influenza Virus

Lenny Godinho[1], Heather Branscome[1], Zachary Cuba[1], Firo Boskani[1], TD Khang[1], Yasmeen Zoubi[1], Nikhita Puthuveetil[1], David Yarmosh[1], Heather Couch[1]

[1]American Type Culture Collection, Manassas, Virginia, USA

INTRODUCTION

Influenza virus remains a persistent global health threat, driving the need for continuous surveillance, diagnostics, and vaccine development. This demand necessitates production of high-quality virus stocks for research and manufacturing. Mammalian cell culture, particularly using Madin-Darby Canine Kidney (MDCK) cells, is a well-established platform for influenza virus production due to its scalability, regulatory acceptance, and adaptability to various strains. To address the growing need for scalable and efficient virus production, ATCC has recently evaluated a novel fixed-bed bioreactor system, the scale-X™ hydro. This platform was assessed for its ability to support large-scale production of seasonally relevant influenza strains, including B/Michigan/01/2021 (B/Victoria lineage) and A/District of Colombia/27/2023 (H3N2 subtype). Using real-time process monitoring, we tracked key parameters such as dissolved oxygen (DO), pH, and temperature to monitor cell health and virus growth. Compared to traditional stationary flask cultures, the scale-X™ demonstrated improved MDCK growth kinetics and enhanced viral yield and infectivity, as measured by hemagglutination (HA) and Tissue Culture Infectious Dose at 50% ($TCID_{50}$) assays. Virus produced in the bioreactor also had higher titer relative to its original source material as measured by $TCID_{50}$. Importantly, next-generation sequencing (NGS) analysis of eight key influenza genes revealed no significant increase in single nucleotide polymorphism (SNP) frequency in bioreactor-derived virus compared to the original source material. These findings support the use of the scale-X™ hydro as a scalable, high-yield platform for influenza virus production, with the potential to strengthen pandemic response capabilities and ensure a reliable supply of high-quality virus stocks.

METHODS

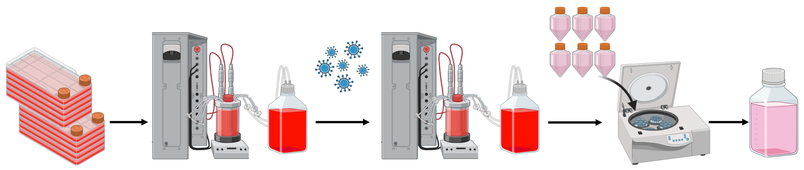

Figure 1. Large scale influenza production. MDCK cells are scaled-up and seeded in the bioreactor. After approximately 24 hours, the cells are infected with influenza virus and then incubated for an additional 48 to 72 hours. The viral material (~5 L) is harvested from the bioreactor, pooled, and clarified by centrifugation. The resulting supernatant is aliquoted and frozen prior to quality control testing.

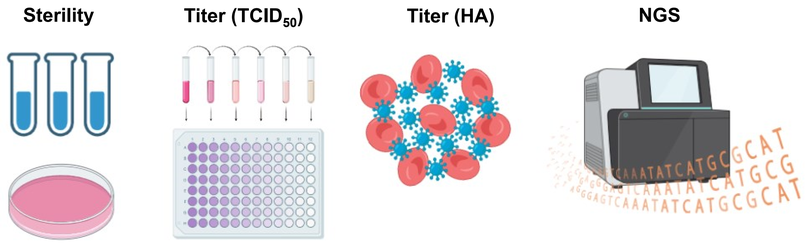

Figure 2. Post-freeze quality control testing. Following harvest and preservation of influenza virus, multiple quality control assays are performed to assess product integrity and suitability for downstream assays. Sterility testing ensures the absence of microbial contamination, $TCID_{50}$ quantifies infectious virus titer using an ELISA-based readout, hemagglutination (HA) assay measures the concentration of viral particles based on their ability to agglutinate red blood cells (RBCs), and next-generation sequencing (NGS) is used to confirm viral genome identity and to detect potential mutations.

RESULTS

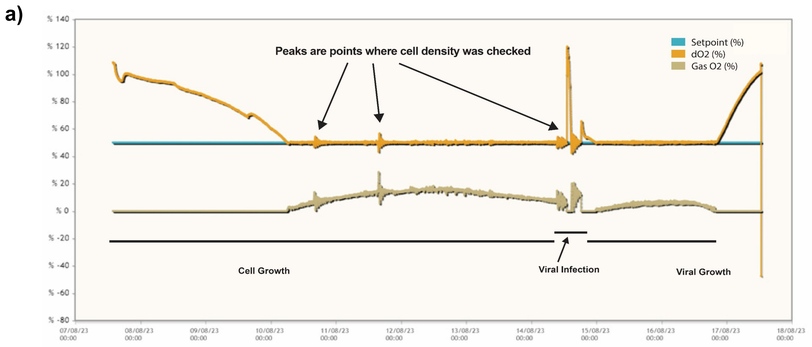

Figure 3. Virus growth. a) Dissolved oxygen (DO) levels were continuously monitored by the scale-X™ system to assess metabolic activity and oxygen demand during cell expansion and viral infection. The bioreactor was programmed to maintain a DO set point of 50% and the corresponding traces reflect dynamic changes in oxygen consumption throughout the production run. Other parameters that were monitored but not shown include temperature and pH. **b)** T-flasks were seeded with MDCK cells and infected in parallel to the bioreactor to serve as a control. Representative photos show **b)** confluent cells prior to infection and **c)** cells 72 hours post-infection exhibiting signs of cytopathic effect (CPE).

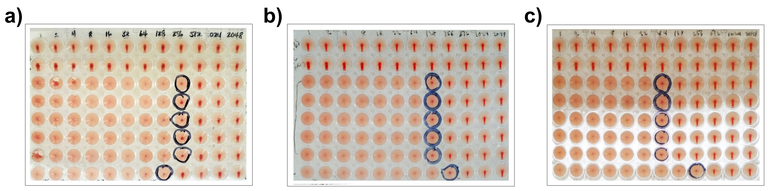

Figure 4. Hemagglutination assay. The HA assay was performed by serially diluting virus-containing samples in a 96-well V bottom plate, followed by addition of RBCs. Hemagglutination was assessed visually after incubation and the highest dilution showing agglutination was recorded as the HA titer. Each plate had two negative controls (rows A-B), five diluted test samples (rows C-G), and one positive control (row H). Representative images from Influenza B testing show HA assay results for **a)** original source material, **b)** virus produced in the bioreactor, and **c)** virus produced from stationary/T-flasks. Data for both influenza A and B viruses are summarized in **Table 1** below.

Table 1. HA assay results for Influenza A and B viruses grown in bioreactor.

Virus	Condition	HA Titer
Influenza B	Source material	256
Influenza B	Bioreactor	128
Influenza B	T-flask	128
Influenza A	Source material	128
Influenza A	Bioreactor	128

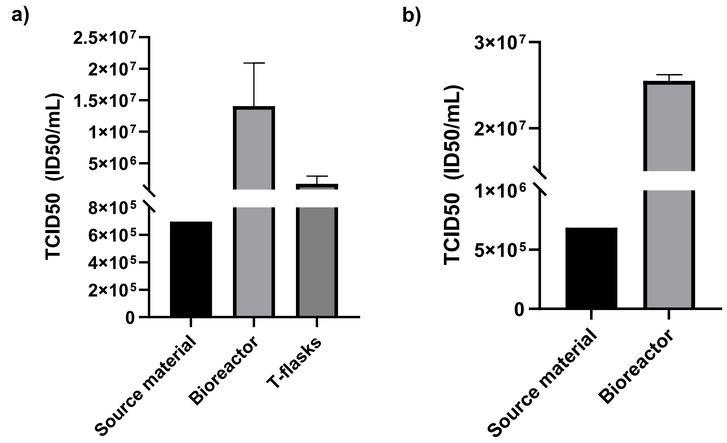

Figure 5. $TCID_{50}$ Assay. a) Influenza B virus harvested from the bioreactor was compared to the original source material as well as the same virus harvested from a proportional volume grown in T-flasks. **b)** Influenza A virus harvested from the bioreactor was compared to the original source material. Samples were serially diluted and used to infect MDCK cells in a 96-well format. After an 18-hour incubation, viral antigen was detected using an ELISA-based assay and $TCID_{50}$ was calculated based on the dilution at which 50% of wells showed positive signal. The bioreactor and T-flask assays were run in duplicate. Data represents the mean with standard deviation error bars.

Table 2. NGS results and variant analysis.

Virus	Condition	Identification	Variant Analysis
Influenza B Virus, B/Michigan/01/2021 (BV)	Source material	Identity confirmed	6 variants, all SNPs <20%
Influenza B Virus, B/Michigan/01/2021 (BV)	Bioreactor	Identity confirmed	4 variants, all SNPs <20%
Influenza B Virus, B/Michigan/01/2021 (BV)	T-flask	Identity confirmed	1 variant, SNPs <20%
Influenza A Virus, A/District of Colombia/27/2023 (H3N2)	Source material	Identity confirmed	2 variants, all SNPs <20%
Influenza A Virus, A/District of Colombia/27/2023 (H3N2)	Bioreactor	Identity confirmed	1 variant, SNPs <20%

CONCLUSIONS

ATCC continues to advance scalable, high-quality solutions to meet the growing demands of influenza virus production. In this study, the scale-X™ hydro fixed-bed bioreactor demonstrated strong performance for scalable production of Influenza B/Michigan/01/2021 (B/Victoria lineage) and Influenza A/District of Colombia/27/2023 (H3N2 subtype) using MDCK cells. The system supported increased viral titers and maintained the genetic integrity of the virus, as confirmed by HA, $TCID_{50}$, and NGS analyses. These results highlight the scale-X™ hydro as a promising platform for efficient, large-scale virus production. By actively evaluating innovative technologies like this, ATCC strengthens its role as a trusted partner in supporting seasonal vaccine programs, pandemic preparedness, and global influenza research.

Acknowledgements This work was funded by the ATCC Internal Research and Development (IRAD) Program. We thank Rebecca Bradford and all members of the IRAD committee. We also thank Univercells Technologies for providing support for the scale-X™ bioreactor.

AFS 10801 University Boulevard, Manassas, VA 20110-2209 **Phone:** 800.359.7370 **Email:** atccfederalsolutions@atcc.org **Web:** www.atcc.org