scale-X™ fixed-bed bioreactor for vaccine manufacturing

Process transfer of four viral vaccine candidates using WI-38, Vero and CEF substrates.

Application note

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Introduction

Cell based vaccines manufacturing traditionally rely on adherent cell lines for producing target antigens. Conventional technologies such as eggs or roller bottles that scale-out rather than scale-up are still widely used but require vast amounts of cleanroom space and numerous manual operations. The use of such technologies results in both high capital and operating expenses ultimately impacting the vaccine affordability and hence its availability. In contrast with static technologies stirred-tank bioreactor technologies with microcarriers offer the advantage of a scalable and automated manufacturing. Nevertheless, such processes suffer from development and operational complexity such as bead-to-bead cells transfers, management of shear forces, large operating volumes and burdensome seed trains. Fixed-bed bioreactors have revolutionized bioprocessing by offering the same level of automation as stirred tank bioreactors with a significantly reduced footprint, a gentle growth environment suitable to sustained productivity and the opportunity to reduce the seed train.

The scale-X™ bioreactor range from Univercells Technologies embodies a novel generation of structured fixed-bed bioreactors that offer scalability from the laboratory (scale-X hydro, 2.4 m² cell growth surface, used in this study) through to clinical and commercial production (scale-X carbo, with 10 & 30 m² cell growth surface) and the NevoLine Upstream platform featuring a scale-X nitro (200 & 600 m²). The manufacturing systems developed by Univercells Technologies offer the possibility of process chaining by integrating automated in-line TFF in the same unit operation as the bioreactor, thereby producing a highly concentrated harvest that reduces both the footprint and time required to process the material. This study evaluates the suitability of the scale-X hydro technology for producing four viral vaccine antigens for pediatric and adult use using three different cell lines (primary chicken embryonic fibroblasts (CEF), human diploid fibroblasts (WI-38) and Vero cells). The culture conditions were directly reproduced from pre-established processes developed in a multi-layer bioreactor and roller bottles. We have established the viral antigen productivity as well as cell growth performance.

Material and Methods

Materials

Cell culture

The cell culture is performed within the scale-X™ hydro bioreactor system (developed by Univercells Technologies). The system is composed of a control system and an autoclavable single-use bioreactor.

The latter features a spiral wound structured fixed bed (proprietary rolled & hydrophilized PET non-woven fibers with polypropylene spacer layers) with a surface area for cell anchorage of 2.4 m².

scale-X™ [hydro]

Figure 1: scale-X™ hydro bioreactor system (2.4 m² of cell growth surface)

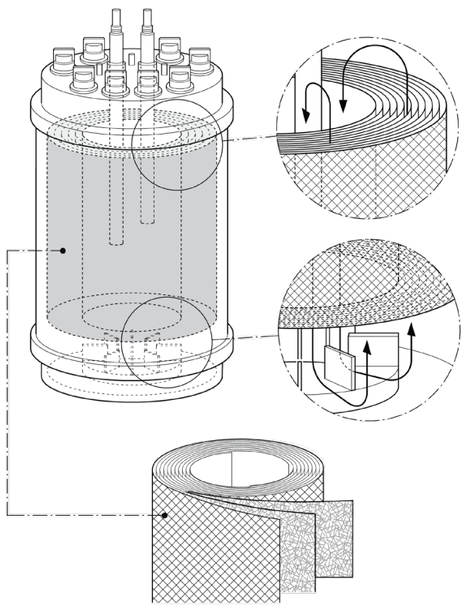

Figure 2 : Proprietary fixed-bed structure and media flow in the scale-X bioreactor series

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Culture media circulation through the fixed bed is enabled by a magnetic impeller which pushes liquid upwards through the bed at a set linear velocity. Once the liquid emerges at the top of the bed, it overflows into the outer perimeter of the vessel. Operating the bioreactor at a working volume which creates a height difference between the top of the fixed-bed and the main liquid level in the vessel creates a larger surface area for gas exchanges.

Pre-cut fixed bed strips (n = 12) can be extracted for off-line cell analysis. pH and DO measurement are achieved using re-usable probes (respectively EasyFerm Plus HB Arc and VisiFerm Arc DO autoclavable probes, Hamilton Bonaduz AG).

Liquid flow into and out of the bioreactor is achieved via 4 peristaltic pumps (Watson Marlow 114, Watson Marlow Fluid Technology Group).

Where non-primary cells were used, inoculum was prepared in a series of multi-layer cell culture dishes (2- and 10- layer CellSTACK® (CS), Corning) from frozen stock to reach the targeted cell quantity required to seed the bioreactor in the following fashion: vial thaw > 1 × CS2> 1 × CS10 > scale-X hydro. The reference processes against which scale-X results were compared are a 850 cm2 roller bottles (for antigens C and D) and a multi-layer disk bioreactor system (undisclosed, for antigens A and B).

Cell measurement

Biomass measurement was achieved via two methods.

- Method 1: The cell count was measured by extracting pre-cut strips of the non-woven fixed bed fabric from the bioreactor for off-line analysis. Where fixed-bed strips were used, cells were enzymatically detached, the nuclei stained using crystal violet and counted using a hemocytometer.
- Method 2: A 150 cm² control T-flask (Corning, Edison (NJ), USA) seeded at the same time as the bioreactor as a representative measurement of the cells inside the bioreactor was used. Where a control T-flask was used, the cell density was measured both visually (confluence) as well as via nuclei staining following enzymatic detachment (TrypLETM select, ThermoFisher Scientific). The harvested cell suspension was counted with an automated cell analyser (ViCell XR Cell Viability Analyzer, Beckman Coulter).

Metabolite measurement

At fixed time points, liquid samples were manually extracted from the bioreactor for off-line analysis. Selected metabolites (calcium, iron, glucose, glutamine, glutamate, lactate, and ammonia) were measured using an automated bioprocess analyzer (Cedex Bio Analyzer, Roche CustomBiotech)

Infectious titer measurement

- All but one antigen studied here (A, B and C) were measured using a TCID50 assay from a liquid sample taken at a time of interest in the culture harvest.
- Antigen D was measured using a plaque assay from a harvest sample.

Method

This proof-of-concept study evaluated four nondisclosed vaccine antigens (referred to here below as A, B, C and D) for viral production in the scale-X hydro bioreactor. Antigens A, B and C are targeted for pediatric conditions while antigen D is targeted for emergent adult use. Pre-set seeding densities, media usage (mL/cm2) and process timing (for infection) were transferred directly from a reference process to the fixed bed bioreactor without optimization.

Antigens A & B (CEF)

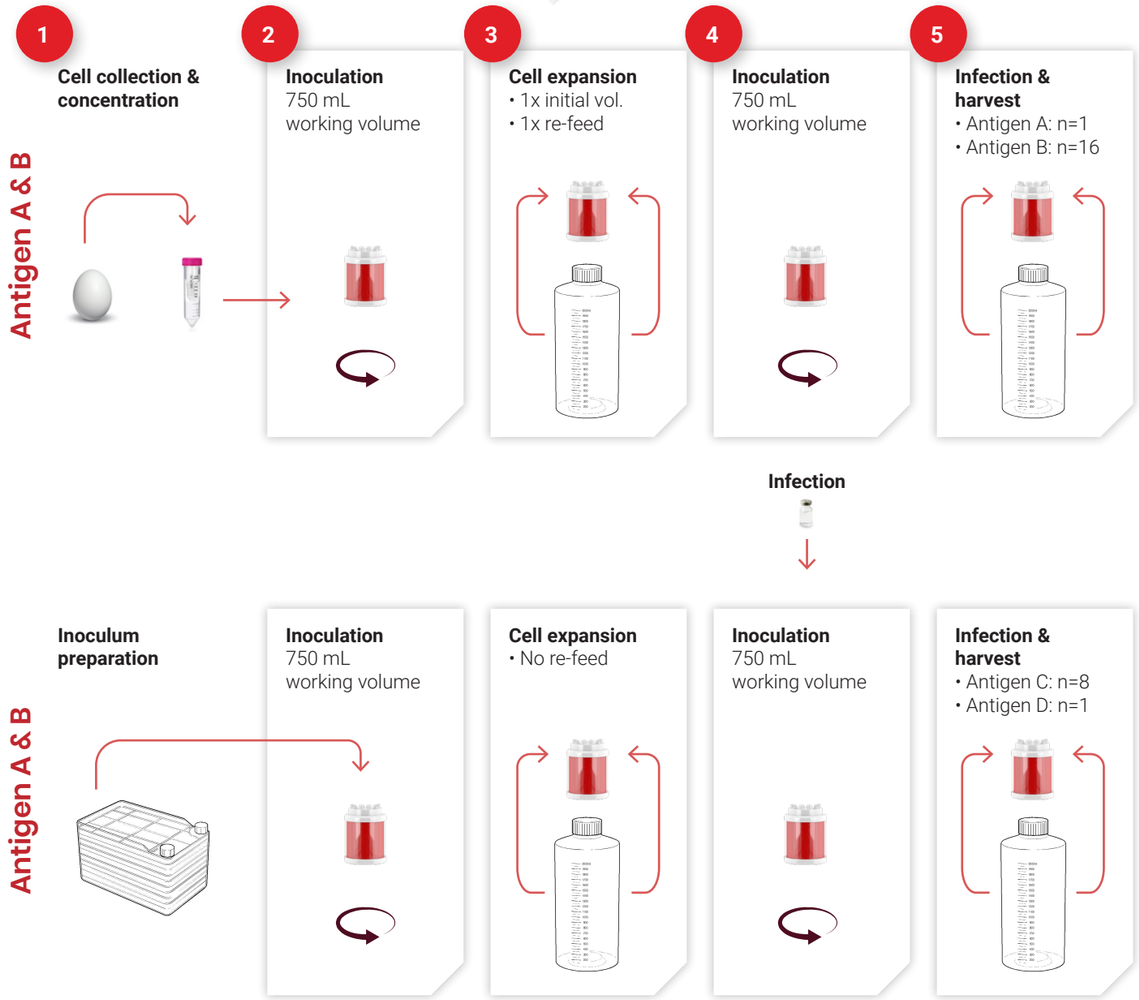
Antigens A and B were produced in primary chicken embryo fibroblast (CEF) cells. The cells were concentrated via bucket centrifugation and then seeded at an inoculation density of 7.4 × 105 and 3.2 × 105 cells/cm2, respectively. These were left to circulate through the fixed bed in batch mode to ensure maximum cell attachment (as estimated by measuring remaining suspended cells in liquid sample extracted from the vessel). Once this was achieved, serum containing media was added to reach a final working concentration of 0.5 mL/cm2. Post inoculation, during cell expansion and for infection the media working concentration was reduced to 0.35 mL/cm2 except for one of the antigens where the media volume target was further reduced during the harvest stages to 0.08 mL/cm2 per harvest. Media samples were collected from each of the aforementioned process steps for metabolite (glucose and lactate) analysis. In all instances, media samples were also collected at the harvest steps to measure infectious titer.

Antigens C (WI-38)

One of the antigens studied (antigen C) was produced using human diploid fibroblast (WI-38) as the cell substrate. WI-38 cells were expanded in static cell culture stacks to obtain the sufficient cellular material to seed the hydro fixed bed reactor at 2.4 × 104 cells/cm2 initially at a reduced working volume and then increased a target media volume of 0.44 mL/cm2 and maintained as such during cell expansion. At re-feed and during the multiple harvest operations this was reduced to 0.15 mL/cm2. Media samples were retained for metabolite analysis (glucose and lactate) from each process step: (inoculation, re-feed, and harvests) and infectivity measurement was carried out on the harvest material.

Antigens D (Vero)

The fourth antigen studied (antigen D) was produced using in Vero cells. Inoculum was expanded in static cell culture stacks to a final seed concentration of 4.0 × 104 cells/cm2. As for other experiments inoculation was carried out at a reduced working volume (750 mL) and then increased to a target media volume of 0.24 mL/cm2 for cell expansion and during infection. Samples were collected at the harvest step only for infectivity measurement.



Antigen type	Cells substrate	Seeding density (cells/cm²)	Growth media (mL/cm²)	Infection media (mL/cm²)	Number of harvests	pH setpoint	DO setpoint	Linear velocity
A	CEF	7.4×10^5	0.50 (initial) > 0.35 (re-feed)	0.35	1	7.2	50%	2 cm/s (inoculation & infection) > 1 cm/s (expansion, harvests)
B	CEF	3.2×10^5	0.50 (initial) > 0.35 (re-feed)	0.08	16			
C	WI-38	2.4×10^4	0.44	0.15	8			
D	Vero	4.0×10^4	0.24	0.24	1			

Figure 3: process flow diagrams for antigens A & B (using chicken embryonic fibroblasts) as well as C (using WI-38 cells) & D (using Vero cells). A rinse step prior to wash out the serum-containing growth media in the production of antigens A, B and C scenarios is not shown.

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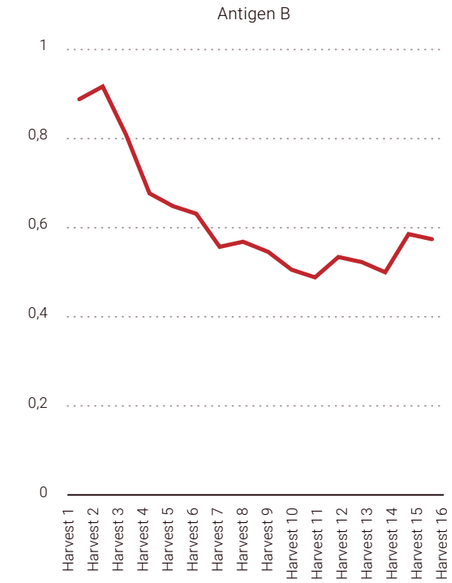
In all cell cultures, the pH was maintained at a fixed set point of 7.2 using carbon dioxide and sodium hydroxide additions as acidic and basic controls respectively. The dissolved oxygen set point as maintained at 50%. During the cell attachment and infection phases the agitation was set to meet a linear velocity of 2 cm/s, while agitation to meet a linear velocity of 1 cm/s was used during all other processing steps.

Results and Discussion

Through this proof-of-concept study the main target was to establish the feasibility of producing antigens A, B, C and D in the scale-X hydro system and this was measured first by the infectious titers. For all experiments described in this paper, the process conditions used in the scale-X hydro was a direct copy-paste of those used in a reference process, with no optimization. For antigens A and D the harvest was carried out as a single step while for antigen B and C the harvest was carried out as multiple steps. Each infectious titer (Figure 1) was normalized against averaged control values from an existing production-scale manufacturing process (undisclosed).

Antigens A and D feature single harvest steps, where the bulk liquid is collected at the end of the infection process. In both cases, the infectious titer was comparable to the reference process. Antigens B and C feature multiple harvest steps, respectively 16 and 8. In this case, at each harvest step, the bulk liquid is collected as a batch and a new culture media container is connected to the bioreactor to continue with the harvest. This is repeated until the final number of desired harvest steps is reached. While the initial harvest steps for antigen B did match the reference process (normalized infectious titer >0.85), the observed infectious titer dropped thereafter, which indicates that further process optimization would be required to ensure titers are maintained. In the production of antigen C, the target infectivity (>0.85) was reached up until the 4th harvest. It is very common for process optimization to be required when changing from one production platform to another and the results observed here are considered a success in this regard (it is not uncommon to yield non-measurable infectious titers in proof-of-concept studies in such technology transfers).

In addition to the active monitoring of pH, dissolved oxygen, and temperature (data not shown), media samples were taken at different processing steps to establish metabolites profiles: glucose and lactate are chosen as representative of cell growth for antigens A, B, and C (data for antigen D not available). For each antigen studied where results are available, the amount of glucose and lactate in the spent media indicate cell growth prior to infection, and post inoculation showed decreased consumption of glucose and decreased production of lactate as expected with viral propagation. This trend confirms the experimental measure observed in the reference process.



	Antigen A	Antigen B
Normalized infectivity (Single harvest)	0.899	1.041

Figure 4: Normalized Infectivity from proof-of-concept case study for Antigens A, B, C, D. Experimental Infectivity divided by representative manufacturing infectivity. The reference process for antigens A and B is an un-disclosed multilayer disk bioreactor system while antigens C and D are compared against a 850 cm² roller bottle process.

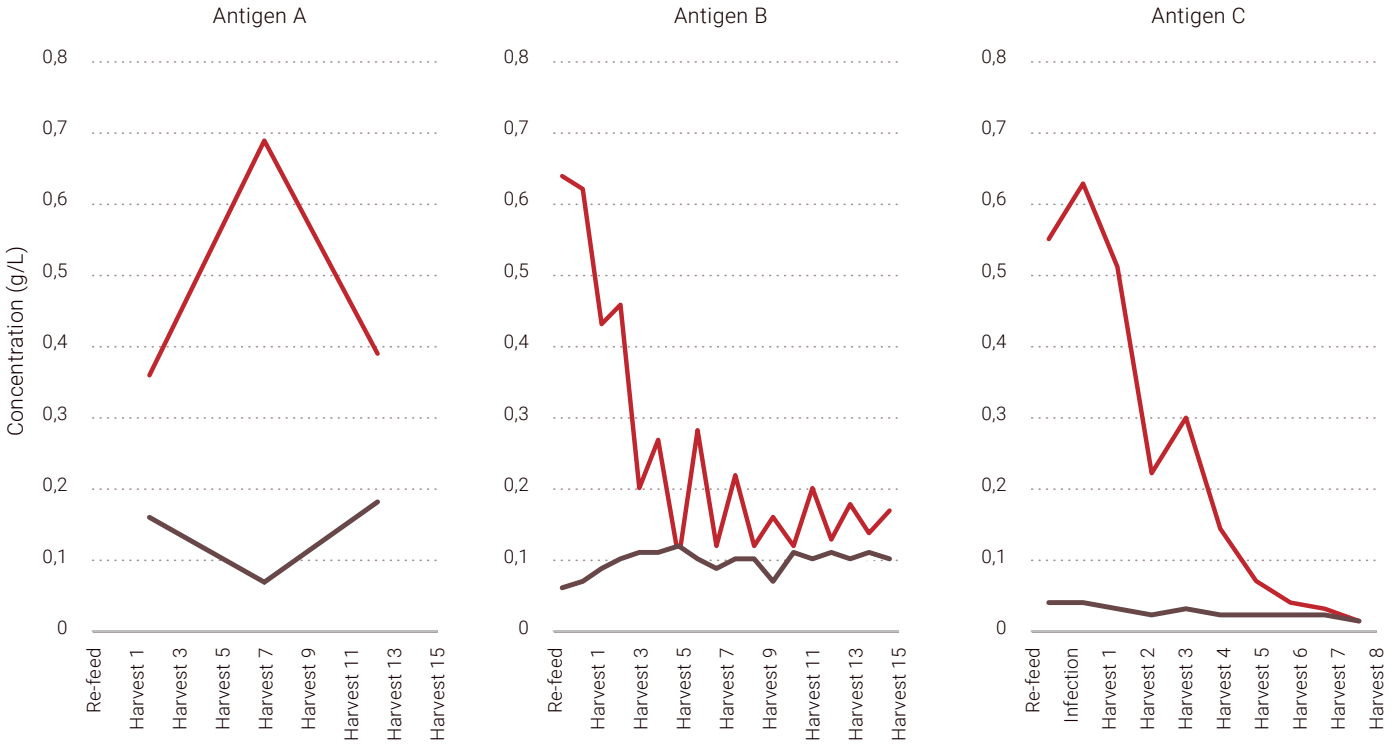


Figure 5: media values for glucose (•) and lactate (•) for Antigen A, B and from scale-X hydro fixed bed reactor experiments. Data not available for experiment with antigen D.

Conclusion and perspectives

This study demonstrates how a process using adherent cell lines for vaccine production can be transferred to the innovative structured scale-X fixed-bed bioreactor system. As this was a proof-of-concept study no process optimization was applied for direct comparison to the reference processes roller bottle for antigens C and D and an undisclosed multilayer disk-bioreactor system for antigens A and B. Antigens A and D yielded infectious titers within specification of the reference process (>0.85) while results obtained for antigens B and C show promise in early harvest stages and offer scope for optimization in the later ones.

The study is considered a success as it provides the foundation to a scalable platform from which commercial quantities of antigens could be produced when translated to the larger versions of the scale-X range (the scale-X carbo system and the NevoLine Upstream platform). The next steps in process development will focus on optimizing the culture conditions to further improve titers and could integrate novel pH, DO and temperature set-point strategies and optimized media supply. A scale-up from the scale-X hydro system to the scale-X carbo system could also be of interest to evaluate the use of in-line TFF concentration for an improved harvest schedule.

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