

## PRODUCTION AND PURIFICATION OF INFLUENZA VIRUS LIKE PARTICLES USING SINGLE-USE TECHNOLOGIES

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There is an increasing trend in the biopharmaceutical market towards the implementation of single-use technologies (SUT) in bioprocesses. These technologies address the needs of bio therapeutic development and manufacturing with appealing advantages over the conventional systems. SUT can be operated at lower costs, eliminating the need for cleaning and regeneration of components, improving process-to-process turnaround, being more flexible enabling modular facilities, and easy to scale up.

This work describes the up and downstream processing of Influenza Virus-like particles (VLPs), produced using the baculovirus expression system with High Five cells. The single-use Mobious®Bioreactor is compared with a glass stirred tank in terms of growth kinetics, cell viability, stability and VLPs production, showing comparable results. The use of this single-use bioreactor was already reported by our group for the successful production of a hepatitis C VLP vaccine candidate.

Moreover, we report on the development of a single-use platform process for purification of Influenza VLPs. We have undertaken an effort to replace chromatographic steps from our platform, with the ultimate goal of an all filtration purification process. The proposed process employs either normal or tangential flow filtration for the clarification stage, followed by a cascade of ultrafiltration steps with different pore sizes and a sterile filtration step to achieve the needed concentration and purity specifications. Efforts to clear nucleic acid without the use of an endonuclease digestion step and the impact on the downstream unitary operations will also be described. By optimizing the filtration mode of operation we were able to achieve product recoveries of 80%. Globally, we have about 1.8 log reduction value (LRV) of DNA and total protein removal and a baculovirus' LRV of 4. Overall, using SUT across all biomanufacturing operations we are able to speed up the process, to improve the scale-up and to reduce costs due to the removal of chromatographic and cleaning and validation steps.