UPSTREAM AND DOWNSTREAM PROCESS DEVELOPMENT OF A VERO CELL-BASED YELLOW FEVER VACCINE

Leda R. Castilho, Cell Culture Engineering Lab, COPPE, Federal University of Rio de Janeiro
leda@peq.coppe.ufrj.br
Tania Pato Cunha, Biomanguinhos/FIOCRUZ
Diogo A. Mattos, Biomanguinhos/FIOCRUZ
Marta Cristina O. Souza, Biomanguinhos/FIOCRUZ
Marlon V. Silva, Biomanguinhos/FIOCRUZ
Elena C. C. S. Campos, Biomanguinhos/FIOCRUZ
Luciane P. Gaspar, Biomanguinhos/FIOCRUZ
Marcos S. Freire, Biomanguinhos/FIOCRUZ

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Yellow fever (YF) is a lethal viral disease that is endemic in some tropical regions of South America, Central America and Africa. An attenuated vaccine produced in embryonated eggs is available since the 1930's and is known to be highly effective and safe. However, after large vaccination campaigns in the 2000's, reports of rare, but serious adverse events have stimulated Biomanguinhos/FIOCRUZ, who produces the current attenuated 17DD vaccine, to develop a new, inactivated vaccine.

Over the last years, through a partnership of the Federal University of Rio de Janeiro and Biomanguinhos/FIOCRUZ, both upstream and downstream processes were developed. The upstream process was established based on Vero cell cultivation on microcarriers in serum-free medium, using stirred-tank bioreactors. The first studies were carried out in spinner flasks to select the microcarrier type and the serum-free medium. Also, statistical DOE tools were used to study the infection step, varying the moiety of infection and the time of infection. This process was then scale-up to stirred-tank bioreactors and further optimized regarding microcarrier concentration, stepwise medium addition, dissolved oxygen level/sparging intensity, impeller configuration and time of harvest. The final upstream process that was established results in virus titers of 10^8 pfu/mL within a time frame 144h post inoculation of the cells in the bioreactor.

The downstream process was designed priorizing chromatographic techniques, aiming at achieving high purity levels and extensive removal of process-related critical contaminants, such as DNA and host-cell proteins (HCP), as preconized by the regulatory authorities. For the capture step, both cation- and anion-exchange chromatographies were evaluated. A Q membrane adsorber process was selected and the best operational conditions in terms of pH, temperature, buffers and washing strategies were determined. For the second purification step, three techniques were evaluated: multimodal chromatography, ultrafiltration/diafiltration, and hydrophobic interaction chromatography using a HIC membrane adsorber. The multimodal resin showed the best results, and operational conditions of this step were further optimized. The final 2-step yellow-fever virus purification process resulted in an overall yield of 52% and residual HCP of 350 ppm (0.05%). Residual DNA was 1.2 ng per dose, considering the dose established based on animal studies, and is in agreement with the limit recommended by the World Health Organization (<10 ng/dose). Electrophoretic analysis (SDS-PAGE) of the purified samples showed a band corresponding to 96% of identified proteins with molecular mass of 56 kDa, which is the expected mass for the virus envelope protein (E). Anti-E Western blot (WB) showed a single band, confirming the identity of the samples. No band was revealed in the anti-HCP blot, confirming the low HCP levels quantified.

The developed process allows the production of a new, high-purity yellow-fever vaccine through a scalable technology, which is better suited than egg-based technology to meet emergency demands in case of epidemics and is useful in the current scenario of increasing worldwide demand YF vaccine.