VAC_08 - Downstream establishment of the chimeric live-attenuated Zika virus vaccine

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Introduction: Zika virus (ZIKV) outbreak in Brazil, in 2016, was deemed a global public health emergency by the World Health Organization (WHO) due to its association with fetus's neurological abnormalities in infected pregnant women. Since then, the development of Zika vaccines has become crucial to prevent ZIKV infection and consequently maternal-fetal transmission. Downstream corresponds to an essential step of vaccine development, whose main objective is to reduce impurities to a safe and acceptable level by regulatory agencies. Purification techniques can encompass filtration, centrifugation, and chromatography, which must be standardized according to the antigen's physicochemical properties.

Objectives: The purpose of this work is to establish the downstream steps for a chimeric live-attenuated vaccine candidate against ZIKV and assess the efficiency of this process from purifying viral batches obtained with different human recombinant albumin (rHSA) concentrations.

Methodology: To optimize the reduction of host cell DNA, we tested different concentrations of endonuclease at different times. Purification involved tangential flow filtration (TFF), with assessments of flow rate (ml/min), the number of membrane depolarizations and the ideal antigen concentration. Process efficacy was evaluated by titration to assess viral stability throughout the process; quantification of host cell protein (HCP) using commercial ELISA kits and RT-qPCR to quantify residual DNA content after TFF.

Results: The results indicate approximately 90% reduction in residual DNA and 80% reduction in HCP levels, with a reduction up to 0.5log in viral mass, acceptable for titration assays, in both batches produced with different concentrations of rHSA. Based on the data of the ELISA (HCP) and RT-PCR (DNA), the effectiveness of the process was confirmed and meet the regulatory requirements for impurities, specifically a maximum of 10 ng/dose of DNA and 1.5 µg/dose of HCP.

Conclusion: In conclusion, it can be considered that the purification process of the chimeric antigen has been successfully established in bench-scale, allowing the project to advance to non-clinical and formulation studies.

Keywords: Vaccine; Zika; Downstream