BIOPROCESS INTENSIFICATION FOR PRODUCTION OF A PESTE DES PETITES RUMINANTS VIRUS (PPRV) VACCINE

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Peste des Petites Ruminants Virus (PPRV) is a highly contagious disease affecting small ruminants in Africa and Asian countries, with negative/significant economic impact. Aiming to eradicate the disease, targeted by the Food and Agriculture Organization for 2030, a novel and scalable PPRV vaccine production process is clearly needed. Built upon work previously done at iBET, a new production process is herein proposed using Vero cells growing on microcarriers, serum-free medium (SFM) and stirred-tank bioreactors (STB). This includes a new method for cells detachment from microcarriers, and perfusion culture for reducing turnaround time.

The PPRV vaccine production process was developed in the 2L BIOSTAT® DCU-3 and the 20L BIOSTAT® Cplus STB (both from Sartorius) using Nigeria 75/1 strain. Engineering correlations (energy dissipation rate, shear stress and Kolmogorov Eddy size) were used to optimize culture conditions in the 2 L STB and to scale-up the process to the 20 L STB. Vero cells were adapted to grow in ProVero™-1 SFM (Sartorius). A new enzymatic and mechanical method for *in situ* cell detachment from microcarriers was designed. Perfusion was evaluated in the 2 L STB (equipped with internal spin-filter) in order to reduce seed-train preparation time. PPRV were clarified using depth filtration (Sartopure PP3, Sartorius). Process scalability was validated in the 20 L STB.

Vero cells were adapted to ProVero™-1 SFM, reaching growth rates similar to serum-containing cultures (0.03 h^{-1}). The new *in situ* cell detachment method was successfully implemented, with yields above 80%. A two-fold increase in maximum cell concentration was obtained using perfusion when compared to batch culture. Combining perfusion with the new *in situ* cell detachment method enabled the scale-up to 20 L STB directly from a 2 L STB, surpassing the need for a mid-scale platform and thus reducing seed-train preparation time. Infectious PPRV titers increase over culture time in both 2 L and 20 L STBs, reaching maximum values of 4.5-4.9x10^6 $TCID_{50}$/mL at day 4-5 post-infection. The potential of depth filtration for PPRV clarification was confirmed; comparable PPRV recovery yields after clarification (85-90%) were obtained in both STBs.

Overall, the novel and scalable vaccine production process herein proposed has the potential to assist the upcoming PPR Global Eradication Program (PPR GEP), to which iBET already contributes as partner in the PPR Global Research and Experts Network (PPR GREN), and thus support the One Health concept.

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