

## FLAVIVIRUS PRODUCTION IN PERFUSION PROCESSES USING THE EB66® CELL LINE

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The outbreak of mosquito-borne yellow fever virus (YFV) in Angola 2016 rapidly spread to urban regions and other countries. Vaccination campaigns were subsequently intensified, but the increased vaccine demand led to depleted stockpiles. Current yellow fever vaccine manufacturing processes rely on embryonated chicken eggs, which are strongly limited with respect to flexible capacity increase in emergencies. The global vaccine demand is estimated by the WHO to 1.38 billion doses needed to eliminate epidemics. Thus, an urgent need for an improved production platform is needed, ideally transferable to new vaccine developments against emerging flaviviruses, such as Zika virus.

Here we present a cell culture-based YFV 17D and Zika virus (ZIKV) production process using the EB66® cell line. The avian EB66® suspension cell line grew fast and stable in chemically defined medium to cell concentrations of  $1.8 \times 10^7$  cells/mL in shake flasks and batch mode. Seed virus was prepared from Vero-derived YFV and ZIKV material over five passages in EB66® cells. Thereby, infectious virus titers successfully increased by one log unit and maximum titers of  $1.4 \times 10^8$  PFU/mL (infectious virions per mL) and  $8.0 \times 10^7$  PFU/mL were obtained two days post infection for YFV and ZIKV, respectively.

The process was intensified using perfusion bioreactors to increase cell concentrations. Therefore, EB66® cells were cultivated in 1 L benchtop bioreactors equipped with an alternating tangential flow filtration (ATF2) perfusion unit. Perfusion rates were adjusted to maintain glutamine concentrations above 1 mM and cells grew up to  $9.5 \times 10^7$  cells/mL. A maximum YFV titer of  $7.3 \times 10^8$  PFU/mL was achieved. The cell-specific virus yield (CSVY) was 8 PFU/cell, similar to shake flask experiments.

For ZIKV production, another approach aimed at the use of on-line capacitance sensors to control cell-specific perfusion rates (CSPRs) based on cell concentrations. This automated system was set to a CSPR of 0.017 and 0.034 nL/cell/day leading to maximum cell concentrations of  $8.9 \times 10^7$  cells/mL and  $1.6 \times 10^8$  cells/mL. ZIKV titers peaked after three to four days post infection with  $2.6 \times 10^9$  PFU/mL and  $1.0 \times 10^{10}$  PFU/mL, respectively. CSVYs increased from 5 PFU/cell (shake flask experiments) to 30 PFU/cell and even above 64 PFU/cell in this set-up. The increased CSPR resulted in an improved volumetric productivity by factor three compared to the lower CSPR.

Further process intensification was achieved by direct cell inoculation to the ZIKV production bioreactor. A 15 mL cryo-bag was thawed with  $8.5 \times 10^8$  cells and cell viabilities of 90% after inoculation quickly increased over the cultivation period.

Taken together, EB66® suspension cells can be grown to concentrations exceeding  $1.5 \times 10^8$  cells/mL in perfusion bioreactors, and cells are highly permissive for YFV and ZIKV. YFV production using perfusion systems generated virus material equivalent to 10 Mio vaccine doses (4.7 log infectious units per dose) in less than two weeks operation time. With the use of on-line sensors to adjust CSPRs meeting cellular nutrient demands, ZIKV titers exceeding  $1.0 \times 10^{10}$  PFU/mL were obtained for the first time. Direct cryo-bag inoculation shortened the seed train phase, and virus production was initiated with full flexibility. This is a powerful demonstration on how next generation flavivirus vaccine production can be realized.