

THE EB66[®] CELL LINE FOR YELLOW FEVER VACCINE PRODUCTION AT HIGH CELL CONCENTRATIONS

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The global threat of the emerging yellow fever disease can be effectively countered by vaccination. First vaccines against yellow fever have been developed in embryonated chicken eggs in the 1930s and this production platform remained almost unchanged until today. However, recent outbreaks revealed vaccine supply shortages due to limiting options to ramp up production.

Here, we present a cell culture-based process using EB66[®] cells for production of a live, attenuated yellow fever vaccine (YFV; WHO 17D-213/77 strain). The duck embryo-derived EB66[®] suspension cell line showed good growth performance in batch mode achieving up to 1.8×10^7 cells/mL and doubling times of less than 17 h in shake flasks in the chemically-defined CDM4Avian medium at 37°C. The seed virus material was adapted by five serial passages to the cell substrate, which resulted in an 8-fold increase in virus titer to 1.3×10^8 PFU/mL (infectious virions per mL). Changes in process temperature and cell disruption to facilitate virus release did not improve final virus titers. In a next step, the process was transferred into benchtop bioreactors equipped with an alternating tangential flow filtration unit (ATF2) operating at a working volume of 700 mL. An on-line conductivity probe was implemented, which enabled cell growth monitoring in real-time. This setup allowed to achieve high cell densities of up to 9.5×10^7 cells/mL resulting in a further increase of YFV titers up to 7.3×10^8 PFU/mL. Based on an input of 4.7 log infectious units per dose, raw virus material equivalent to 10 Mio vaccine doses was produced in less than two weeks operation time.

Taken together, EB66[®] suspension cells can grow to very high cell densities in perfusion systems. Present process intensification clearly demonstrated the potential to produce millions of YFV vaccine doses from small scale cultures in a controllable and scalable manner.