ACCELERATED MASS PRODUCTION OF INFLUENZA VIRUS SEED STOCKS IN HEK-293 SUSPENSION CELL CULTURES BY REVERSE GENETICS

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Despite major advances in developing capacities and alternative technologies to egg-based production of influenza vaccines, responsiveness to an influenza pandemic threat is limited by the time it takes to generate a Candidate Viral Vaccine (CVV) as reported by the 2015 WHO Informal Consultation report titled “Influenza Vaccine Response during the Start of a Pandemic”. In previous work, we have shown that HEK-293 cell culture in suspension and serum free medium is an efficient production platform for cell culture manufacturing of influenza candidate vaccines. This report, took advantage of, recombinant DNA technology using Reverse Genetics of influenza A/Puerto Rico/8/34 H1N1 strain, and advances in the large-scale transfection of suspension cultured HEK-293 cells. Transfection in shake flasks was performed using 1ug of total plasmid and $1x10^6$ cells/mL. The supernatant was harvested after 48 hpt and used to infect a new shake flasks at $1x10^6$ cells/mL for virus amplification. 3-L bioreactor was inoculated and transfected at $1x10^6$ cells/mL with 1ug of total plasmid and harvested after 48hpt and the virus generated was amplified in shake flask. Quantification by TCID50, SRID, Dot-blot and TRPS were performed as well as characterization by TEM and HA and NA sequencing. Small-scale transfection in shake flasks generated $1.5x10^5$ IVP/mL after 48 hpt and $1x10^7$ IVP/mL after 96 hpi. For large-scale experiment a 3-L controlled stirred tank bioreactor resulted in supernatant (P0) virus titer of $5x10^4$ IVP/mL and $2.8x10^7$ IVP/mL after only one amplification (P1) in HEK-293 suspension cells. We demonstrate the efficent generation of H1N1 with the PR8 backbone reassortant under controlled bioreactor conditions in two sequential steps (transfection/rescue and infection/production). This approach could deliver a CVV for influenza vaccine manufacturing within two-weeks, starting from HA and NA pandemic sequences. Thus, this innovative approach is better suited to rationally design and mass produce the CVV within timelines dictated by pandemic situations and produce effective responsiveness than previous methodology