Insulin increases influenza virus yield in HEK293 cells for vaccine production

OBJECTIVES

1. Evaluate the effect of insulin on HEK293SF-3F6 cell growth in two types of media.
2. Boost influenza production with addition of different concentrations of insulin.
3. Quantify influenza using a robust method.

1) EFFECT OF INSULIN ON CELL GROWTH
- HEK293SF-3F6 is a suspension-adapted cell line (a GMP cell bank is available).
- HEK293SF-3F6 cells have successfully been used for the production of influenza virus, virus-like particles, lentiviral vectors, adenovirus and adeno-associated virus.
- HEK293SF-3F6 can be cultured in an in-house media (IHM-03) and in commercially-available serum-free media (Media 1) but weak growth profiles are obtained in chemically-defined media.

1.1) Growth limitations observed in a chemically-defined media (Media 1) were alleviated by insulin:

![Figure 1: By adding 10-20mg/L insulin every 72hrs, the maximal viable cell density of HEK293SF-3F6 cells was increased by 4-fold in Media 1.](image)

1.2) Growth kinetics were improved by insulin in an In-House Media (IHM-03):

![Figure 2: By adding 10-20mg/L insulin every 72hrs, the maximal viable cell density of HEK293SF-3F6 cells was reached 3 days sooner in IHM-03, a media designed specifically for HEK293SF-3F6 cells.](image)

2) EFFECT OF INSULIN ON INFLUENZA PRODUCTION IN HEK293SF-3F6 CELLS
- Cell-culture based vaccines are a valuable alternative to egg-produced vaccines: the equivalent of 1500 influenza vaccine doses can be produced in a 1L bioreactor within 48 hours with HEK293 cells.

2.1) Insulin is a strong activator of the PI3K/Akt pathway, which plays a key role in influenza production:

![Figure 3: A) Overview of signaling pathways modulated by influenza infection: The replication of influenza virus hijacks the cellular machinery and involves multiple signaling pathways including Akt. B) Akt is activated by viral NS-1.](image)

2.2) Insulin increases the yield of influenza virus in a 24-well microbioreactor:

![Figure 4: Addition of 25-100 mg/L insulin at time of infection increases the yield of influenza H1N1 A/Puerto Rico 8/34 by almost 2-fold in Media 1 (A), without affecting the total cell count at harvest (B). The virus was added at an MOI of 0.01 at 35°C in the presence of 1ug/ml tropsin-TPCK. HA concentration was measured by dot-blot using pan-HA antibodies developed in-house (See panel 3).](image)

- Increase in total HA was not due to increased cell density
- Similar results were obtained with H3N2 A/Avian/2/88 treated with 5 and 25 mg/L insulin at time of infection

2.3) Akt activity is increased by influenza infection and insulin addition in HEK293SF-3F6 cells:

![Figure 5: HEK293 cells were infected in a shake flask. The control was treated with 1 µg/ml tropsin-TPCK (without virus). Phospho-Akt was measured by flow cytometry after infection and representative results are shown (A-B) and summarized (C). The addition of insulin further activates Akt (D). Results were obtained by dividing the fluorescence signal obtained for the infected sample by the untreated control (m2).](image)

3) INFLUENZA CAN BE QUANTIFIED WITH PAN-HA ANTIBODIES IN A DOT-BLOT ASSAY
- Quantification of influenza is an enduring challenge:
  - Measures of infectivity are highly variable
  - Physical methods to count viral particles can be confounded by the presence of non-viral particles
  - Antibody-dependent methods to quantify surface proteins such as hemagglutinin (HA) rely on strain-specific antibodies

- A pan-HA antibody cocktail was generated using a highly conserved peptide sequence found within the HA molecule (the fusion peptide):