# Adaptation of an Adherent hck (MDCK) cell line to a Serum-Free Suspension RCB for MCB Production

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## 1. Abstract

An engineered Madin-Darby canine kidney (MDCK) cell line that is more suitable for human influenza virus isolation and propagation was developed by Takada et al. By altering the expression levels of α-2, 6-sialoglycans and α-2, 3-sialoglycans of the host cell. However, the adherent nature of this humanized MDCK (hCK) cell line limits the scalability of ist use in large-scale production of influenza vaccines. Over the span of 10 months, we adapted this adherent serum dependent hCK cell line to a serum free suspension cell line without altering ist engineered characteristic. The adaptation study was performed in three phases: 1) adaptation from growth in serum–containing media to serum-free media in flatware, 2) adaptation from adherent serum free to suspension serum free in shake flasks, and 3) the generation of a stable (progenitor) cell bank with optimal growth. After testing multiple serum free media and methods of motion (for developing growth in suspension), we successfully generated a Research Cell Bank with ≥ 80% viability, ≤20% aggregation, and ≤ 30 hour doubling time and were able to successfully tech transfer the process to Manufacturing for production of a GMP Master Cell bank. This modified serum free suspension MDCK cell line provides an ideal platform for isolation of human influenza viruses from clinical specimens, and production of influenza vaccines at an industrial scale.

## 2. Materials and Methods

**Adaptation of adherent hCK from growth in serum containing medium to serum free medium (hCK-SF) in T flasks (5 passages):**
Parental hCK was propagated in MEM (Gibco) containing 5% FBS and 2 mMGlutamine and gradually adapted to grow in OptiPro (Gibco) Serum free medium in Tissue Culture Flask 225 cm². For each passage, the trypsinized cells were centrifuged at 300 x g for 10 mins and re-suspended in the media containing the conditioned medium and OptiPro SFM at a 1:3 ratio. As a result, FBS concentration was sequential decreased from 5%, 1.667%, 0.556%, 0.185%, 0.062%, 0.021% to 0% at the end of adaptation.

A total of 5 passages were performed to fully adapt the cells in OptiProSFM (0% FBS) with seeding densities between 1.0-3.0E+04 vc/cm2 for 48-72 hrs. An additional 3 passages in SFM occurred before the preparation of the hCK-SF Research Cell bank (RCB). The critical parameters to proceed per passage was ≥80% confluency (48-72 hrs) and ≥ 80% viability following the change of medium composition.

**Adaptation adherent hCK-SF cells to suspension hCK-Sfsus cells (66 passages):**
hCK-SF cells grown in OptiPro SFM + 2mM Glutamine were gradually adapted to grow in suspension in SFM4BHK SFM (Cytiva) + 2 mMGlutamine in vented non-baffled Erlenmeyer shake flask.

The parental hCK-SF cells are grown in T-flasks, trypsinized, centrifuged at 300 x g 10 min and re-suspend in OptiPROand SFM4BHK21 at a ratio of 75:25. Subsequent adaptation were performed in the media ratio of 75:25, 50:50, 25:75, 10:90, and 1:99 for a total of 16 passages. An additional 50 passages were performed in 100% BHK4SFM to fully adapt for growing in suspension. For each passage, cells were seeded at 5.00E+05vc/mL in a total working volume of 30 mL and incubated at 37°C and rotating speed of 140±5 rpm for 72-96 hrs.

**Propagation of hCK-Sfsus for pre-MCB preparation (3 passages):**
Stable Propagation of the serum free suspension cell line began with fully adapted hCK-SF cells in 99% SFM4BHK21, 2mM Glutamine in 125mL vented non-baffled Erlenmeyer flask.

Our target to generate a RCB to Pre-MCB was ≤30hrs of doubling time, ≥70% viability and ≤20% aggregation. Passages were seeded between 4.0-6.0E+05 vc/mL .Each passage was centrifuged at 300gX 5-10mins, re-suspended in fresh media and counted prior to seeding. A lag phase of no growth occurred;cells were then centrifuged and re suspended in fresh media for an additional 72-96 hrs. Once a stablegrowth was observed (consistent doubling time) cells were expanded in 500mL to 1000mL vented non-baffled Erlenmeyer flasks to generate the RCB used to create the pre-MCB.

Figure 2: Cell harvest density and aggregation percentage during adaption in shake flasks

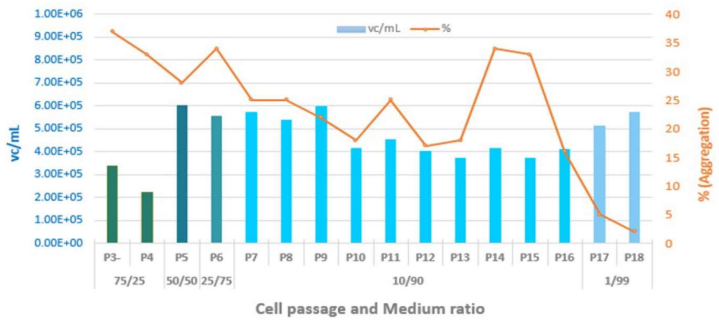


**Fig.2:** Graph showing passages Opti/SFMBHK21 ratios with harvest densities and % aggregation

Figure 3: Cell Density and cell doubling time of culture grown in 100% SFMBHK21

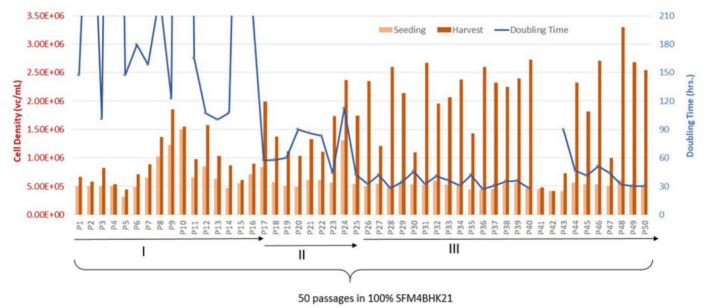


**Fig.3:** Graph showing passages in 100% SFMBHK21 in suspension with seeding, harvest densities and doubling time per passage.

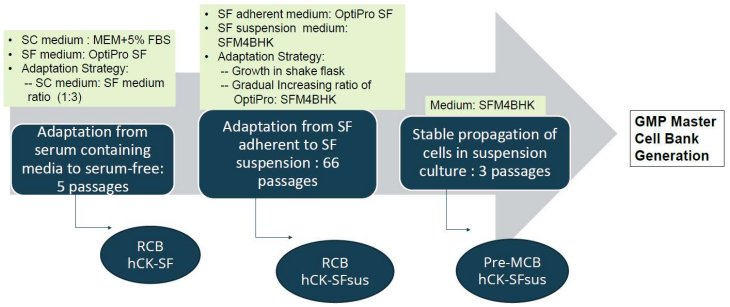


**Fig.1:** Process Diagram of the three phases on the adaptation of serum containing adherent cell line to serum free suspension MCB.

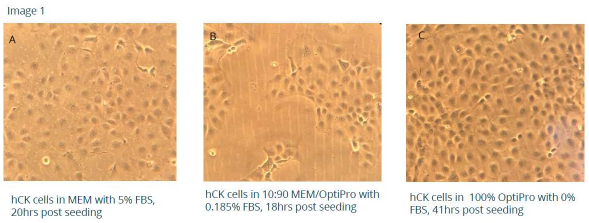


**Image.1:** *hCK cell line in various FBS concentrations using MEM/OptiPro. All images were taken in a Tissue Culture Flask 225cm² at 20x magnification.*

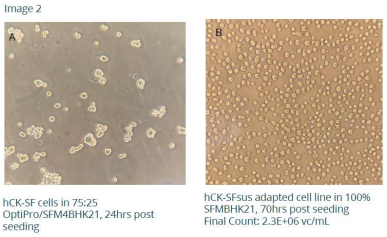


**Image.2:** *hCK cell line grown in shake flasks at 20x magnification*

## 3. Observations and Results

Serum free adaptation began with hCK cells in OptiPro, 5% FBS seeded between 1.0-1.5E+04 vc/cm2. The hck propagated similar to parental cell line in FBS concentrations 0.062%-5.00% with comparable doubling time of (21-25 hrs). When the FBS % dropped below 0.021% the hCk doubling time increased to 32-37hrs, however the morphology of the cells were very similar to the parental thaw passage.

Adapting the serum free hCK line proved to be a longer process than adapting to serum free conditions. SFM4BHK21 was chosen due to rounded single cells observed from the first phase that supported suspension growth and high viability.

- First two passages in 75%/25% of OptiPro/SFM4BHK21 growth medium, >30% of cell aggregation was detected and cell density decreased to (2-4)E+05 vc/mL.
- Next two passages in 50%/50% and 25%/75% of OptiPro/SFM4BHK21 medium, the viable cell count increased to ~5E+05vc/mL, despite the high percentage of cell aggregation
- 10 passages in 10%/90% Opti/SFM4BHK21 medium, cell harvest density fluctuated between (3-5)E+05vc/mL and aggregation rate ranged from16-33%.
- Two passages in 1%/99% OptiPro/SFM4BHK21 medium, cell aggregation decreased drastically to <10%. Despite little cell growth and high cell aggregation rate, the cell viability stayed above 90% during the growth in the various ratio of mixed medium.

Once the cells were transferred to 100% SFM4BHK21 medium, the passage number was restarted as passage 1. A total of 50 serial passages were performed in 100% SFM4BHK21 growth medium and can be divided to 3 phases based on the growth characteristic.

- Phase I (passage 1-16): After cell propagation in shake flasks for 3-4 days, cell aggregation percentage in this phase ranged between 8-30% (The cell growth remained minimal, i.e., there was little increase of cell density from seeding to harvest.
- Phase II (passage 17-24): Cell growth consistently reached >1.0E+06vc/mL at the end of each passage, and less cell aggregation was observed. However, cell doubling time remained high (~57-120 hours).
- Phase III (after passage 25): Cell doubling time decreased to < 50 hours for most of the passages. Once the doubling time of two consecutive passages reached ~30 hours (passage 48, and 49), cells were expanded for banking.

To prep for the mini bank Passage 50 was expanded in a total working volume of 200mL with a seeding density of 5.00E+05 vc/mL and reached a cell density of 2.54E+06 vc/mL, viability of >99% after 71hrs (~30hr doubling time). The adapted mini bank was sent out for testing to ensure expression of the linked sialoglycans and replication of human influenza virus. A panel of seasonal influenza virus were selected and shown to replicate in hCK-Sfsus and parental hCK cells with equivalent titer. (NIAD). The mini bank was frozen down in vials at 1.00E+07 vc/mL and used for the production of the Pre-MCB. The Pre-MCB was generated after 3 passages using same parameters and was used in the production of the Master Cell bank.

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## Contact

**www.idt-biologika.com**

## References

Takada et al. 2019. A humanized MDCK cell line for the efficient isolation and propagation of human influenza viruses. Nature Microbiology 4: 1268-1273.