Production of Avian Influenza H5N1 Virus Using the TideCell Bioreactor System

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A Production Case Study Using MDCK Cell Line
Comparison Study

- Due to significant differences in virus strain and host cell line characteristics, the optimal process for virus production can significantly differ.
- The following slides compare process condition requirements for the production of the Avian Influenza H5N1 virus, based on the comparison of the TideCell & BelloCell and other commonly used systems.
High cell density is required to achieve high titer, and reduce media consumption.

- TideCell: high surface area of system and its carrier matrix increases cell density up to 10 fold and conserves up to 90% of culture media.
- Micro-carrier system: micro-carrier or bead density is limited
- Other packed bed systems: limited scale
(R2) Low cell attachment efficiency due to long term trypsinization

- TideCell: relative static seeding method enables high attachment rate even if the cells have been over-trypsinized.
- Micro-carrier system: agitation environment heightens the difficulties of cell attachment. Cell attachment efficiency becomes lower when the scale is increased.
- Other fixed bed system: agitation environment increases the challenges of cell attachment.
Cells tend to detach after infection due to trypsin addition

- TideCell: extremely low shear stress provides ideal culture environment allowing cells to remain attached after infection
- Other then roller bottles, other systems require agitation which may cause cell detachment after infection.
(R4) Required low DNA and host cell protein impurities during harvest

- **TideCell**: Low shear stress results in less cell disruption and less impurities in the harvest.
- **Micro-carrier system**: Agitated environment causes cell disruption and release of DNA and host cell proteins.
- **Other fixed bed system**: Same as micro-carrier system
(R5) Required refreshing of culture media before infection

- **TideCell**: cells are immobilized in carrier matrix vessel which is separated from mixing vessel containing culture media. Media exchange is direct but gradual and slow to ensure no loss of cells and no temperature shock.

- **Micro-carrier system**: media can only be partially replaced by settling the carriers. Nutrients may be insufficient to support entire post-infection period.

- **Other fixed bed system**: media must be completely replaced thus temperature shock may occur especially in a large scale system.
H5N1 Avian Flu Virus Production in BelloCell Bioreactor

- Cell line: MDCK, ATCC CCL-34
- H5N1 vaccine virus: NIBRG-14 strain
- Culture media: Plus MDCK
- Seed preparation: roller bottle
- Culture system: BelloCell 500-P
- Matrix to media ratio: 1:5
Results of Cell Growth in BelloCell

<table>
<thead>
<tr>
<th>Day</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.15E+08</td>
<td>1.15E+08</td>
<td>1.15E+08</td>
<td>1.15E+08</td>
</tr>
<tr>
<td>2</td>
<td>6.30E+08</td>
<td>1.41E+08</td>
<td>1.73E+08</td>
<td>2.30E+08</td>
</tr>
<tr>
<td>3</td>
<td>7.71E+08</td>
<td>3.18E+08</td>
<td>5.82E+08</td>
<td>7.68E+08</td>
</tr>
<tr>
<td>4</td>
<td>8.29E+08</td>
<td>9.63E+08</td>
<td>8.54E+08</td>
<td>1.19E+09</td>
</tr>
<tr>
<td>5</td>
<td>1.17E+09</td>
<td>1.58E+09</td>
<td>2.09E+09</td>
<td>2.08E+09</td>
</tr>
<tr>
<td>6</td>
<td>2.69E+09</td>
<td>2.05E+09</td>
<td>1.82E+09</td>
<td>2.02E+09</td>
</tr>
</tbody>
</table>
Virus HA Titer in BelloCell

Final virus titer of 2048 can be achieved.
H5N1 Avian Flu Virus Production in TideCell Bioreactor

- Cell line: MDCK, ATCC CCL-34
- H5N1 vaccine virus: NIBRG-14 strain
- Culture media: Plus MDCK
- Seed preparation: BelloCell 500-A
- Culture system: TideCell
- Matrix to media ratio: 1:10
Process Flow Diagram

Culture Medium
  Plus MDCK, filled in 20 L medium bag

System
  TideCell 2

TC Matrix Vessel
  65 g/L BioNOC II

Medium Bag
  20 L LDPE bag

Seed Preparation
  BelloCell-500AP

Batch Run

WCB
  0
  1x T-75

3
  1x T-150

6
  1x RB-850

9
  1x BelloCell-500AP

16
  1x TideCell 2

23
  Virus Infection

27
  Harvest

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Cell Growth Before Infection
Morphology After Infection

1. Cell debris remained mostly in the matrixes with very few virus leftover. Beneficial for downstream purification process
2. DNA content can be reduced.
HA Profile

![Graph showing HA profile over time](image)

- **HA**
- **Post-Infection Time (hr)**
- **Axes:**
  - Y-axis: HA
  - X-axis: Post-Infection Time (hr)

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Samples in lane 2 ~ lane 4 were produced with Cesco Plus MDCK SFM. Samples in lane 5 and lane 6 were produced with DMEM medium containing 10% FBS. Samples in lane 7 ~ lane 9 were egg-based production process prepared in CDC, Taipei. Lane 10 is the commercial seasonal flu vaccine by Kitasato institute.
# Virus Production in Various Media

<table>
<thead>
<tr>
<th></th>
<th>Cesco Plus-MDCK</th>
<th>Gibco OptiPro SFM</th>
<th>Gibco VP SFM</th>
<th>DMEM w/10% FBS</th>
<th>JRH ExCell MDCK SFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confluent in flask</td>
<td>3 days</td>
<td>4 days</td>
<td>4 days</td>
<td>3 days</td>
<td>Cannot grow</td>
</tr>
<tr>
<td>Confluent in roller bottle (850cm²)</td>
<td>4 days</td>
<td>7 days</td>
<td>Cannot grow</td>
<td>5 days</td>
<td>N/A</td>
</tr>
<tr>
<td>Confluent in roller bottle (1700cm²)</td>
<td>6 days</td>
<td>9 days</td>
<td>N/A</td>
<td>7 days</td>
<td>N/A</td>
</tr>
<tr>
<td>HA titer (1/50 µL)</td>
<td>512</td>
<td>128</td>
<td>N/A</td>
<td>128</td>
<td>N/A</td>
</tr>
<tr>
<td>Virus titer (TCID₅₀/mL)</td>
<td>~10⁸</td>
<td>~10⁷</td>
<td>N/A</td>
<td>~10⁷</td>
<td>N/A</td>
</tr>
<tr>
<td>HA yield (µg/mL)</td>
<td>80~110</td>
<td>30</td>
<td>N/A</td>
<td>40</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Comparison Result from NHRI**

- **✓** Whole culture process save 7~8 days compared with Invitrogen OptiPro
- **✓** Immunogenesity is 40% higher than OptiPro, 5% higher than serum containing culture medium
Final virus titer of 9E8 pfu/ml can be achieved.
HA Profiles

![Graph showing HA profiles over time.](image)

- HA profile increases significantly at 24 hours post-infecion, reaching a peak at 96 hours and stabilizing by 120 hours.
# Summary of Results

<table>
<thead>
<tr>
<th>Culture System</th>
<th>Roller bottle</th>
<th>NBS BioRo Bioreactor</th>
<th>BelloCell</th>
<th>TideCell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scale</td>
<td>850 cm²</td>
<td>1 L</td>
<td>100 ml matrix/500 ml medium</td>
<td>5L matrix/50 L medium</td>
</tr>
<tr>
<td>Carrier Weight</td>
<td>5 g</td>
<td>5.5 g</td>
<td>550 g</td>
<td></td>
</tr>
<tr>
<td>Type</td>
<td>Cytodex I</td>
<td>BioNOC II</td>
<td>BioNOC II</td>
<td></td>
</tr>
<tr>
<td>Cell Density (cells/g carrier)</td>
<td>5.54×10⁸</td>
<td>2.36×10⁸</td>
<td>3.08×10⁸</td>
<td></td>
</tr>
<tr>
<td>Cell Density (cells/ml)</td>
<td>5×10⁵</td>
<td>2.77×10⁸</td>
<td>1.3×10⁷</td>
<td>2×10⁷</td>
</tr>
<tr>
<td>Virus titer (HA/50 μl)</td>
<td>256</td>
<td>1024</td>
<td>2048</td>
<td>1024</td>
</tr>
<tr>
<td>Virus titer (pfu/ml)</td>
<td>1×10⁵</td>
<td>1×10⁵</td>
<td>9.2×10⁴</td>
<td>7×10⁵</td>
</tr>
<tr>
<td>Harvest volume (ml)</td>
<td>200</td>
<td>1000</td>
<td>500</td>
<td>50000</td>
</tr>
<tr>
<td>Equivalency</td>
<td>1/1000</td>
<td>1,50</td>
<td>1,50</td>
<td>1</td>
</tr>
</tbody>
</table>

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