Process development for cell-based influenza virus

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Background

- Influenza virus for vaccines is traditionally produced by infection of fertilized hen eggs.
- This is labor intensive and requires large facilities, which has led to a rapid development of large-scale mammalian cell culture methods for future virus vaccine production processes.
- Downstream processes for cell-based influenza virus require multiple steps to fulfill regulatory requirements.
Background

**Figure 1.** Schematic overview of an influenza virus.
**Background**

**Cell culture**
MDCK cells on Cytodex™ 3 carriers
116444000 ng DNA

**Clarification**
ULTA™ Prime GF 2 μm + ULTA Prime GF 0.6μm
39304000 ng DNA

**Ultrafiltration/Diafiltration**
Cross flow filtration, UFP-500-C
9499000 ng DNA

**Non-binding AEX chromatography**
Capto™ ViralQ
5000 ng DNA

**Ultrafiltration/Diafiltration**
Cross flow filtration, UFP-500-C
4000 ng DNA

**Sterile filtration**
ULTA™ Pure SG 0.2 μm
2000 ng DNA

*Figure 2. Overview of the cell-based Influenza process showing typical reduction of total DNA content (purple) from an 8-L cell culture.*

*Imagination at work*
Background

Aim

• This work describes the development of a process for removal of genomic DNA from live influenza virus cultured in Madin-Darby Canine Kidney (MDCK) cells.

• A main focus was also to use established scalable techniques that can be used in industrial production of live influenza vaccines.
Background
Virus production strategy

- Processes for vaccine production will depend largely on the chosen vaccine strategy.

- Production processes for whole, split, subunit or virus-like particle (VLP) vaccines, with the exception of live attenuated viruses, normally includes a virus inactivation step at some point in the process.
Background
Virus strains

• Four strains were tested with only minor process adjustments, including one attenuated pandemic strain and three seasonal 2007/2008 influenza strains.

<table>
<thead>
<tr>
<th>Influenza A</th>
<th>Influenza B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puerto Rico (PR8)</td>
<td>Malaysia</td>
</tr>
<tr>
<td>Solomon Islands</td>
<td></td>
</tr>
<tr>
<td>Wisconsin</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Strains tested in the process.
Background
Dose and purity considerations

- The route for vaccine administration determines the requirements for residual DNA. The recommendation by WHO for parenteral vaccines is 10 ng DNA/dose. The recommendation for protein is 100 μg/strain (300 μg/dose for a trivalent vaccine).
Results & Considerations

- The reduction of DNA for influenza strain A Solomon Islands over the Capto™ ViralQ step is 3.2 log.
- The remaining DNA from the process is roughly twice the requirement for vaccines (Table 2), while the protein content per dose is below limit.
Results & Considerations

• The total recovery of hemagglutinin (HA) generally ranges from 15% – 60% between runs and strains. Loss of measurable HA during initial micro and ultrafiltration (UF) is the main bottleneck in the process, but no loss of HA in the permeate was detected during UF.

• The retained live virus recovery was determined by TCID<sub>50</sub> to 12%. However, TCID<sub>50</sub> analysis suffers from a very high variability and recoveries can range from below 1% to over 20%. 
Results & Considerations

Figure 3. Removal of DNA from live influenza virus by anion exchange chromatography using Capto™ ViralQ.
# Results & Considerations

<table>
<thead>
<tr>
<th>Strain</th>
<th>HA Yield (Biacore)</th>
<th>DNA/dose(^1) (qPCR)</th>
<th>Protein/Dose(^2) (Bradford)</th>
<th>TCID(_{50})/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Solomon Islands</td>
<td>19%</td>
<td>22.5 ng</td>
<td>52.5 (\mu)g</td>
<td>9.5</td>
</tr>
</tbody>
</table>

\(^1\)10 ng DNA/45 \(\mu\)g HA for parenterally administered vaccines.  
\(^2\)100 \(ug\)/15 \(ug\)
Results & Considerations

**Figure 4.** Example of a SDS-PAGE comparing patterns of PR8 during different steps of the purification procedure compared to virus purified by sucrose gradient centrifugation and identification of HA protein by anti-HA Western Blot. Sample order on the gels: Rainbow™ Marker (Lane 1), Harvest (Lane 2), ULTA™ Prime GF 2 \( \mu \)m + ULTA Prime GF 0.6 \( \mu \)m Filtrate (Lane 3), UF/DF Retentate UF 10x (Lane 4), UF/DF Retentate UF 10x/DF 6x (Lane 5), Capto™ ViralQ flow-through (Lane 6), ULTA Pure SG 0.2 \( \mu \)m filtrate (Lane 7), Sucrose gradient purified influenza virus (Lane 8).
HA analysis

- Influenza vaccine potency is mainly determined by quantitation of HA using the single radial immuno diffusion (SRID) assay. This method, though approved by both FDA and EMEA, is labor intensive and suffers from low precision and sensitivity.

- The Biacore™ biosensor assay is an alternative to SRID for vaccine development and manufacturing.

Figure 5. Biacore T100 processing unit.
HA analysis

- Biacore™ quantitation of influenza HA is performed in an indirect manner as an inhibition assay.

- A comparison of Biacore and SRID is seen in Table 3.

Figure 6. Inhibition assay principle (A, B). HA is first immobilized on a dextran matrix (red-filled circles). Virus is then mixed with a fixed concentration of serum and injected over the surface. Free antibodies (not bound to virus at equilibrium) bind to the surface HA, giving a response. Low concentration of virus in the sample (A) gives high antibody binding, while high virus concentration (B) results in low binding level.
HA analysis

Biacore™ biosensor quantitation of influenza HA shows significantly higher sensitivity and precision as compared to SRID, the method mainly used today. In addition, the analysis time is shorter.

<table>
<thead>
<tr>
<th>Analytical performance</th>
<th>Biacore</th>
<th>SRID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard curve range</td>
<td>0.5-10 μg/ml</td>
<td>5-30 μg/ml</td>
</tr>
<tr>
<td>Sensitivity:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOD&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.3 μg/ml</td>
<td>5 μg/ml</td>
</tr>
<tr>
<td>LOQ&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.8 μg/ml</td>
<td>10 μg/ml</td>
</tr>
<tr>
<td>Precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no. of samples CV &lt; 5%</td>
<td>97%</td>
<td>18%</td>
</tr>
<tr>
<td>Time for 100 samples:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hands-on</td>
<td>1 - 2 h</td>
<td>6 – 8 h</td>
</tr>
<tr>
<td>Total</td>
<td>~15 – 16 h</td>
<td>~20 – 22 h</td>
</tr>
</tbody>
</table>

<sup>1</sup> LOD = limit of detection  
<sup>2</sup> LOQ = limit of quantitation

Table 3. Comparison of analytical performance for Biacore assay and SRID
Conclusion

A process has been developed for the removal of genomic DNA and host cell derived impurities from cell-based influenza.

The process demonstrates:

Efficient removal of genomic DNA

Processing of live influenza virus

Biacore™ biosensor assay as an alternative to SRID
Acknowledgments

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