Influenza Vaccines from Mammalian Cell Culture and Synthetic Seed Viruses

Philip R. Dormitzer, MD, PhD
Global Head of Virology and Head of Research, US
Novartis Vaccines and Diagnostics

New Cells, New Vaccines VII; Wilmington, DE; March, 2013
Novartis V&D is a leader in influenza vaccines

- Egg-based products
- Enhanced products
- Cell culture products
- Pandemic products
The 1960’s – the decade of flu vaccine innovation

Will we enter another period of effective innovation?

- 1930’s and early 1940’s – Flu virus can be grown in eggs
- 1964 - Splitting
- 1965 – Cold adapted live attenuated vaccines
- 1967 – Purification of flu viruses by continuous flow ultracentrifugation
- 1969 – Generation of high growth strains by reassortment
- 1977 – Quantification of HA by SRID
- 1997 – MF59-adjuvanted flu vaccines
- 2007 – Optaflu

Neither Optaflu nor any MF59-adjuvanted vaccine is licensed in the US
Production of Licensed US Influenza Vaccines: by Growing Viral Isolates in Embryonated Chicken Eggs

WHO, World Health Organization.

Egg-based Influenza Vaccine Issues

- Egg Production Requires 900,000,000 Fertilized Eggs Annually
- Egg Production Lead Time ≥9 Months
- Not All Strains Grow Well on Eggs (2003-2004 season A/Fujian/411/02 (H3N2) virus strains did not replicate well in eggs¹)
- Potentially Vulnerable to Avian Flu Zoonotics e.g. Mexico 2012 H₇N₃
- Significant Waste Disposal Requirement
- All Production Strains Must Undergo Egg Adaptation
- Egg Process Largely Unchanged Since 1960s
- No “Plan B”

Highly Pathogenic Avian Influenza in Mexico (H7N3)

- “A significant threat to poultry production not to be underestimated”
- 4.9 million birds slaughtered (out of a total of 9.3 million) in the quarantine area – August
- 22 million birds slaughtered by September
  - Losses > $ 50MM USD
- Sporadic bird-human transmission causing conjunctivitis

FAO. 2012. Highly Pathogenic Avian Influenza in Mexico (H7N3) - A significant threat to poultry production not to be underestimated. EMPRES WATCH, Vol. 26-August 2012. Rome
FLUCELVAX (Influenza Virus Vaccine): the First Cell Culture–Based Influenza Vaccine in the United States

- Approved by the US-FDA on November 20, 2012
- Currently produced in Marburg, Germany
- Expected to be manufactured in the US in 2014-2015 in the Holly Springs Novartis manufacturing facility in North Carolina


US-FDA, United States-Food and Drug Administration.
Cell culture-derived influenza vaccines: advantages and challenges

Current flu cell culture advantages

- Supply of cells more reliable and flexible than supply of eggs
- Can produce during a pandemic before biosafety level dropped – while egg production idle
- Eliminates bioburden during manufacture from chickens
- Adds to thimerosal-free vaccine supply
- Efficacious and well tolerated – OPTAFLU® clinical data

Flu cell culture advantages with all cell process

- Suitable for people with egg allergy
- Every vaccine strain a better match to circulating virus if no egg-adaptation mutations
  - May lead to superior efficacy, and broader coverage
- In some years, a dramatically better match due to higher isolation rates
  - When a good match can’t be found from eggs but can in cells
- Challenge:
  - How to demonstrate clinical superiority?
Why does egg isolation lead to mutation?

*Avian and human viruses use different glycans as cell receptors*

► **Avian viruses** preferentially bind SA$\alpha$-2,3Gal

► **Human viruses** preferentially bind SA$\alpha$-2,6Gal

► **Human viruses** must mutate the receptor binding region of HA to enter egg cells

Influenza virus binds to cells via hemagglutinin

![Diagram showing the interaction between influenza virus and host cell](diagram.png)

**Legend:**
- Neuraminidase (NA)
- Hemagglutinin (HA)
- Sialic acid
- Galactose
- Protein
- Glycan chain of host cell
- Membrane of host cell

*Ito et al. 1997*
Primary isolation of H3N2 viral strains from clinical specimens in NVD MDCK cells or eggs, by WHO CC

**Graph:**

- **Y-axis:** Percentage

**Legend:**
- CDC MDCK
- CDC Eggs
- Melbourne MDCK
- Melbourne Eggs

**Details:**

- 2008: CDC MDCK, Melbourne MDCK, Melbourne Eggs
- 2009: CDC MDCK, CDC Eggs, Melbourne MDCK, Melbourne Eggs
- 2010: CDC MDCK, CDC Eggs, Melbourne MDCK, Melbourne Eggs
- 2011: CDC MDCK, Melbourne MDCK, Melbourne Eggs

**Note:**

Dormitzer | Mammalian Cells and Synthetic Viruses | New Cells, New Vaccines | Wilmington, DE | March 18, 2013
### TABLE 2. Number and percentage receiving 2012–13 seasonal trivalent influenza vaccine among 2,697 outpatients with acute respiratory illness and cough, by influenza test result status, age group, and vaccine effectiveness* against all influenza A and B and against virus types A (H3N2) and B — U.S. Influenza Vaccine Effectiveness Network,† United States, December 3, 2012–January 19, 2013

<table>
<thead>
<tr>
<th>Influenza type/Age group</th>
<th>No. vaccinated/ Total (%</th>
<th>No. vaccinated/ Total (%)</th>
<th>Unadjusted (%)</th>
<th>Adjusted (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Influenza A and B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>367/1,115 (33)</td>
<td>793/1,582 (50)</td>
<td>(51) (43–58)</td>
<td>(56) (47–63)</td>
</tr>
<tr>
<td>Age group (yrs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 mos–17</td>
<td>118/463 (26)</td>
<td>275/565 (49)</td>
<td>(64) (53–72)</td>
<td>(64) (51–73)</td>
</tr>
<tr>
<td>18–49</td>
<td>100/353 (28)</td>
<td>256/604 (42)</td>
<td>(46) (29–60)</td>
<td>(52) (38–79)</td>
</tr>
<tr>
<td>50–64</td>
<td>63/174 (36)</td>
<td>143/248 (58)</td>
<td>(58) (38–72)</td>
<td>(63) (43–76)</td>
</tr>
<tr>
<td>≥65</td>
<td>86/125 (69)</td>
<td>119/165 (72)</td>
<td>(15) (–42 to 49)</td>
<td>(27) (–31 to 59)</td>
</tr>
<tr>
<td><strong>Influenza A (H3N2) only</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>211/544 (39)</td>
<td>793/1,582 (50)</td>
<td>(37) (23–48)</td>
<td>(47) (35–58)</td>
</tr>
<tr>
<td>Age group (yrs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 mos–17</td>
<td>52/179 (29)</td>
<td>275/565 (49)</td>
<td>(57) (38–70)</td>
<td>(58) (38–71)</td>
</tr>
<tr>
<td>18–49</td>
<td>53/183 (29)</td>
<td>256/604 (42)</td>
<td>(45) (21–61)</td>
<td>(46) (20–63)</td>
</tr>
<tr>
<td>50–64</td>
<td>41/96 (43)</td>
<td>143/248 (58)</td>
<td>(45) (12–66)</td>
<td>(50) (15–71)</td>
</tr>
<tr>
<td>≥65</td>
<td>65/86 (76)</td>
<td>119/165 (72)</td>
<td>(–20) (–118 to 34)</td>
<td>(9) (–84 to 55)</td>
</tr>
<tr>
<td><strong>Influenza B only</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>90/364 (25)</td>
<td>793/1,582 (47)</td>
<td>(67) (58–77)</td>
<td>(67) (51–78)</td>
</tr>
<tr>
<td>Age group (yrs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 mos–17</td>
<td>59/230 (26)</td>
<td>275/565 (49)</td>
<td>(64) (49–74)</td>
<td>(64) (46–75)</td>
</tr>
<tr>
<td>18–49</td>
<td>17/79 (22)</td>
<td>256/604 (42)</td>
<td>(63) (35–79)</td>
<td>(68) (40–83)</td>
</tr>
<tr>
<td>50–64</td>
<td>8/40 (20)</td>
<td>143/248 (58)</td>
<td>(82) (59–92)</td>
<td>(75) (39–90)</td>
</tr>
<tr>
<td>≥65</td>
<td>6/15 (40)</td>
<td>119/165 (72)</td>
<td>(74) (24–91)</td>
<td>(67) (–10 to 90)</td>
</tr>
</tbody>
</table>
# HEMAGGLUTINATION INHIBITION REACTIONS OF INFLUENZA H3 VIRUSES

(GUINEA PIG RED BLOOD CELLS) (01/24/13)

## REFERENCE FERRET ANTISERA

<table>
<thead>
<tr>
<th>REFERENCE ANTIGENS</th>
<th>EGG 3C</th>
<th>MDCK 3C</th>
<th>EGG 3C</th>
<th>MDCK 3C</th>
<th>HA GROUP</th>
<th>SEQ CHANGES</th>
<th>DATE</th>
<th>COLL.</th>
<th>PASS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/VICTORIA/361/2011</td>
<td>1280</td>
<td>320</td>
<td>320</td>
<td>320</td>
<td>3C</td>
<td>H156Q, G186V, S219Y¹</td>
<td>10/24/11</td>
<td>E3/E3</td>
<td></td>
</tr>
<tr>
<td>A/VICTORIA/361/2011</td>
<td>40</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>3C</td>
<td></td>
<td>10/24/11</td>
<td>C2/C3</td>
<td></td>
</tr>
<tr>
<td>A/Texas/50/2012</td>
<td>640</td>
<td>1280</td>
<td>640</td>
<td>640</td>
<td>3C</td>
<td>T128N, G186V, S198P, S219F²</td>
<td>04/15/12</td>
<td>E3</td>
<td></td>
</tr>
<tr>
<td>A/Texas/50/2012</td>
<td>40</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>3C</td>
<td>T128N, S198P²</td>
<td>04/15/12</td>
<td>M1/C2</td>
<td></td>
</tr>
<tr>
<td>A/Texas/50/2012 X-223</td>
<td>640</td>
<td>640</td>
<td>320</td>
<td>320</td>
<td>3C</td>
<td>I226N³</td>
<td>REASS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/Texas/50/2012 X-223A</td>
<td>640</td>
<td>640</td>
<td>320</td>
<td>320</td>
<td>3C</td>
<td>I226N³</td>
<td>REASS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## TEST ANTIGENS

<table>
<thead>
<tr>
<th>TEST ANTIGENS</th>
<th>EGG 3C</th>
<th>MDCK 3C</th>
<th>EGG 3C</th>
<th>MDCK 3C</th>
<th>HA GROUP</th>
<th>SEQ CHANGES</th>
<th>DATE</th>
<th>COLL.</th>
<th>PASS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Arkansas/05/2012</td>
<td>80</td>
<td>640</td>
<td>320</td>
<td>320</td>
<td>3A</td>
<td></td>
<td>12/19/12</td>
<td>C2</td>
<td></td>
</tr>
<tr>
<td>A/Virginia/03/2013</td>
<td>80</td>
<td>320</td>
<td>320</td>
<td>320</td>
<td>3C</td>
<td>T128A, R142G, N145S²</td>
<td>01/04/13</td>
<td>C2</td>
<td></td>
</tr>
<tr>
<td>A/New Hampshire/21/2012</td>
<td>40</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>3C</td>
<td>T128A, R142G, N145S²</td>
<td>12/14/12</td>
<td>R1/C1</td>
<td></td>
</tr>
<tr>
<td>A/South Carolina/16/2012</td>
<td>80</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>3C</td>
<td>T128A, R142G, N145S²</td>
<td>11/07/12</td>
<td>M1/C2</td>
<td></td>
</tr>
<tr>
<td>A/Kansas/16/2012</td>
<td>20</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>3C</td>
<td>T128A, R142G, N145S²</td>
<td>12/12/12</td>
<td>C1</td>
<td></td>
</tr>
<tr>
<td>A/Massachusetts/15/2012</td>
<td>40</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>3C</td>
<td>T128A, R142G, N145S²</td>
<td>12/18/12</td>
<td>C1</td>
<td></td>
</tr>
<tr>
<td>A/Cote d'Ivoire/1331/2012</td>
<td>80</td>
<td>160</td>
<td>160</td>
<td>320</td>
<td>3C</td>
<td>N145S³</td>
<td>11/07/12</td>
<td>X/C1</td>
<td></td>
</tr>
<tr>
<td>A/Sapporo/125/2012</td>
<td>40</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>3C</td>
<td>N145S³</td>
<td>11/01/12</td>
<td>C1/C1</td>
<td></td>
</tr>
<tr>
<td>A/Hubei-Hongshan/1368/2012</td>
<td>80</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>3C</td>
<td>N145S³</td>
<td>09/26/12</td>
<td>C4/C2</td>
<td></td>
</tr>
<tr>
<td>A/Laos/717/2012</td>
<td>80</td>
<td>320</td>
<td>160</td>
<td>320</td>
<td></td>
<td></td>
<td>09/17/12</td>
<td>C2/C1</td>
<td></td>
</tr>
<tr>
<td>A/Laos/727/2012</td>
<td>80</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td></td>
<td></td>
<td>09/17/12</td>
<td>C1/C1</td>
<td></td>
</tr>
</tbody>
</table>

2. Substitution in the HAs of the clade 3C viruses compared to cell-propagated A/Victoria/361/2011.
3. Substitution in the HAs of the A/Texas/50/2012 reassortants compared to egg-propagated A/Texas/50/2012.
4. All test viruses were cell-propagated.

Slide from “Information Regarding Seasonal Influenza Viruses,” Presentation of Nancy J. Cox, Ph.D., CDC, to VRBPAC, Feb. 27, 2013
Vaccine only available in substantial quantities after the 2nd pandemic wave peak

"World now at the start of 2009 influenza pandemic"
- WHO Director-General Dr Margaret Chan

Close to 40% of cases occurred in a time when no meaningful vaccine quantities were available

Source: sources include: [http://www.cdc.gov/influenza/pandemic_2009.html](http://www.cdc.gov/influenza/pandemic_2009.html) and [http://www.cdc.gov/flu/weekly/index.htm](http://www.cdc.gov/flu/weekly/index.htm) As of Jan 16, 2010, the CDC estimated that about 77 million people are infected with 2009 H1N1. Weekly data on influenza positive tests reported to CDC by U.S. WHO/NREVSS collaborating laboratories applied to CDC estimate to arrive at the weekly estimate for number of cases in the US.
Early events in the NV&D response to H1N1v
Generated virus in 17 days, made GMP 3 wks later, never used

<table>
<thead>
<tr>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
</tr>
</thead>
<tbody>
<tr>
<td>k 17</td>
<td>Wk 18</td>
<td>Wk 23</td>
<td>Wk 27</td>
</tr>
<tr>
<td>Wk 19</td>
<td>Wk 20</td>
<td>Wk 24</td>
<td>Wk 28</td>
</tr>
<tr>
<td>Wk 21</td>
<td>Wk 21</td>
<td>Wk 25</td>
<td>Wk 29</td>
</tr>
<tr>
<td>Wk 22</td>
<td>Wk 26</td>
<td>Wk 26</td>
<td>Wk 30</td>
</tr>
</tbody>
</table>

- **US epidemic announced**
  - HA, NA gene synthesis
  - HA, NA cloning and subcloning into rescue vectors
  - Rescue of RG seed accomplished under GMP on MDCKs
  - Manufacture with reassortant seed
  - Clinical trial start

- **Viral RNA from CDC arrives**
  - A/Cal HA and NA rescued on a PR8 (standard vaccine) backbone by reverse genetics
  - Calibrated SRID reagents expected

- **Virus from CDC arrives**
  - NV&D and Univ. Marburg culture of wild type virus on MDCKs, followed by transfer to TD
  - Yield and purity too low. Switch to reassortant seed.

- **Reassortant seed from WHO CC arrives in Marburg**
  - Manufacture with wild type seed
  - Manufacture with reassortant seed
Eggs: labor intensive, open process, one dose per egg
Cell lines: semi-automated, closed process, scalable

Production in cell lines
- Scalable, flexible, high-volume output
- Bioreactors replace millions of eggs
- No antibiotics, no preservatives
- Closed process; BSL3 containment
  - Lower bioburden
  - Quicker startup for pandemic vaccine
- If we change to cell derived vaccine strains
  - Potential for shorter lead time – vaccine virus selection later and hence closer to viral circulation
  - Potential in some years for dramatically better strain match
With SGVI and JCVI, established a process for rapid generation of synthetic influenza viruses

- Synthetic biology improves the **speed** of vaccine seed generation
- Enzymatic error correction improves the **accuracy** of seed generation
- Optimized backbones produce superior virus and HA **yield**
- Process **robustness** shown by making >25 synthetic flu A and B virus strains

---

**Currently done by JCVI/SGVI**

- **Step 1**: Identify relevant new HA and NA sequences
- **Step 2**: Design oligos
- **Step 3**: Chemically synthesize oligos
- **Step 4**: Assemble oligos into flu HA and NA gene segments enzymatically
- **Step 5**: Rescue influenza viruses using synthetic HA and NA gene segments and backbone plasmids in vaccine-approved MDCK cells. **(5d)**

**Currently done by NV&D**

- **Step 6**: Amplify recovered viruses in MDCK cells **(3-7d)**
- **Step 7a**: Analyze viruses rapidly and select best for seeds **(3d)**
BARDA’s milestone 1 test for speed was surpassed

Confirmed virus rescue 4 days, 4 hours after starting from sequence

- HA and NA coding sequences of a low-path H7N9 virus from US poultry sent by BARDA
- NCRs selected from the database
- Overlapping oligos were designed and ordered, genes assembled, virus rescued
- Can accelerate further by consolidating functions in one location

<table>
<thead>
<tr>
<th>Oligos ordered</th>
<th>0h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genes received at NVD; virus rescue started 6pm (34h)</td>
<td></td>
</tr>
<tr>
<td>Virus rescue confirmed 12pm (100h)</td>
<td></td>
</tr>
<tr>
<td>Started growth curve 6pm (178h)</td>
<td></td>
</tr>
<tr>
<td>H7N9 sequences confirmed 12pm (244h)</td>
<td></td>
</tr>
</tbody>
</table>

8am

- D1
- D2
- D3
- D4
- D5
- D6
- D7
- D8
- D9
- D10
- D11
- D12

- Oligos received at JCVI 6pm (10h)
- Synthetic genes sent to NVD 6am (22h)
- Virus rescued 6pm (82h)
- P1 started 6pm (106h)
- P2 started 6pm (154h)
- P2 virus sequenced 6pm (202h)
- Analysis of virus yield 12pm (268h)

- HA and NA segments synthesized (22h)
- Virus rescued (82h)
- Confirmed virus rescues (100h)
- Confirmed HA & NA sequences (244h)
Real world example of generating pre-pandemic strains  
Synthetic viruses yield ~2X HA of conventional HGR strains in MDCKs & eggs

- **Early Sept,** MMWR reported swine-origin H3N2v symptomatic infections of humans
- **Shortly after,** A/Indiana/8/2011 sequences found on GISAID after posting by CDC
- **09/20/11** - JCVI delivered synthesized HA, NA, and M genes to NV&D
- **09/23/11** - NVD rescued research-grade viruses

\[
\begin{align*}
&\text{HA yield of sucrose-purified} \\
&\quad \text{CELL- DERIVED virus by HPLC} \\
\end{align*}
\]

\[
\begin{align*}
\text{HA yield (µg/ml)} \\
\text{#21} & \quad 32.3 \\
\text{PR8x} & \quad 38.3 \\
\text{X203 HGR} & \quad 12.9 \\
\text{X213 HGR} & \quad 14 \\
\end{align*}
\]

- **RG backbone with**  
  HA/NA from A/Indiana/8/11

\[
\begin{align*}
&\text{HA yield of sucrose-purified} \\
&\quad \text{EGG- DERIVED virus by HPLC} \\
\end{align*}
\]

\[
\begin{align*}
\text{HA (µg/ml)} \\
\text{PR8x} & \quad 30 \\
\text{X213 HGR} & \quad 18.4 \\
\end{align*}
\]

- **Synthetic virus was passaged 2x in eggs to increase yield**

- **Representative of two equivalent data sets**
Summary of accomplishments to date

- Research process to generate synthetic seeds established
  - Synthetic viruses are **quickly** generated from sequence data – as fast as 4 days and 4 hours
  - Synthetic viruses are **accurately** generated – 100% of viruses generated have expected sequence
  - Synthetic viruses are **reliably** generated – 29/30 strains successfully generated (H1N1, H3N2, B Yamagata, B Victoria, H5N1, H7N9, H3N2v)
  - Synthetic viruses created with new backbones produce **more HA** in MDCK cells and eggs - typically 1.25-15x the yield of conventional seeds

- Improved computational methods to select the strains to synthesize developed

- Process has moved from research to development
The emerging system

More rapid and reliable protection of the public from flu

How the system will work

- Continuous computational monitoring of sequences on the web combined with human intelligence identifies strains of interest as soon as they are posted
- Selected strains synthesized on high-growth backbones within days of sequence posting
- Rapid testing for growth, identity, antigenicity, HA production
- Based on triaging protocol, manufacturing optimization for new strains started at risk
- By the time a seasonal strain has been selected or a pandemic threat has been declared, the manufacturer already has a high growth vaccine seed that produces more HA than any conventional seed, and vaccine production can begin (if not already underway)

System advantages

- Primary synthesis by the manufacturer from posted sequences with no need for reassortment allows faster pandemic responses and earlier seasonal production
- Greater selection of seeds allows better strain match and more reliable production
- The “electronic filter” decreases adventitious agent risk
- Higher yields with new backbones allow more vaccine to reach the population sooner
A Caution: Don’t Put All Your...