Ultracentrifugation: Probably the best downstream processing tool for Large Scale Viral Vaccines.

Sandra Meriño
Global Project Director
Alfa Wassermann BV
Zonal Ultracentrifugation

1966

1968

1973

2001

2006

Separation Technologies
KII Continuous Flow Ultracentrifuge

**KII system includes:**
- KII Rotor Tank
- Control Console
- Rotor Lift
- Air / Electric Drive Motor
- Rotor Assembly
- Rotor Cart
Alfa Wassermann Global Group

- AW POLAND Warsaw
- AW HOLLAND Woerden
- AW ROMANIA Bucharest
- AW USA West Caldwell
- BIOSAUDE Lisbon
- BAMA-GEVE Barcelona
- AW TUNISIA Tunis
- AW CHINA Beijing

Separation Technologies
AWST is the creator of the *Continuous Flow Zonal Ultracentrifuge* technology using a vertical rotor

AWST has 45 years’ experience with continuous flow Ultracentrifugation in the vaccine manufacturing industry

AWST offers a remarkably robust Ultracentrifuge design, many units are running >30 years

AWST sold worldwide >300 continuous flow Ultracentrifuges (Jan 11)

AWST Ultracentrifuges are being used in the production of 85% of global flu vaccine doses, every year
<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1969</td>
<td>K Ultracentrifuge commercially produced (by ENI, New Jersey)</td>
</tr>
<tr>
<td>1970</td>
<td>Influenza vaccine marketed, purified using K Ultracentrifuge</td>
</tr>
<tr>
<td>1975</td>
<td>Introduction of the RK and KII Ultracentrifuge, operated by analogue console</td>
</tr>
<tr>
<td>1995</td>
<td>RK and KII made compliant with CE and CSA</td>
</tr>
<tr>
<td>1998</td>
<td>Introduction of Computer Control and GAMP compliant software</td>
</tr>
<tr>
<td>2002</td>
<td>PK and KII Ultracentrifuges enhanced with clean room and BL2+ features for cGMP vaccine manufacturing</td>
</tr>
<tr>
<td>2006</td>
<td>Electric Drive Promatix and eKII.</td>
</tr>
</tbody>
</table>
Continuous Flow Zonal Ultracentrifuge Range

Promatix 1000 R&D
PKII Pilot Scale
KII Manufacturing Scale

Separation Technologies
Why select Ultracentrifugation?
# How Ultracentrifugation Compares

<table>
<thead>
<tr>
<th></th>
<th>Ultracentrifuge</th>
<th>Filtration</th>
<th>Chromatography</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purity</td>
<td></td>
<td>Cost</td>
<td>Purity</td>
</tr>
<tr>
<td>Yield</td>
<td></td>
<td>Scalability</td>
<td>Scalability</td>
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<tr>
<td>Small Particles</td>
<td></td>
<td>Large Particles</td>
<td>Small Particles</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td></td>
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<td>Purity</td>
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<td></td>
<td>Yield</td>
<td>Yield</td>
</tr>
</tbody>
</table>
Bioprocessing – The Ultracentrifuge Fit

1. Cell Harvest
2. Cell Lysis
3. Clarification (post-TFF or centrifuge)
4. Bioburden Reduction
5. Filling
6. Concentration/Dialfiltration
7. Chromatography
8. Sterile Filtration
9. Vent

Separation Technologies
Chromatography compared to Ultracentrifugation – KII Process Efficiency

**Egg Harvest Fluid / Bioreactor Fluid**

- Concentration
- Diafiltration 1
- Column 1
- Diafiltration 2
- Column 2
- Concentration / Buffer Exchange
- Final Formulation

End of day 2

**Final Formulation**

End of day 3

Less Process Steps => Less Product Loss => Higher Yield

A Quicker KII process has a higher production capacity per day
KII Reduced Process time leads to better product quality by reducing Bio-burden build up
The Implication of Multiple Step Processing

<table>
<thead>
<tr>
<th>Step Yield</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>90</td>
<td>90</td>
<td>81</td>
<td>73</td>
<td>66</td>
<td>60</td>
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<tr>
<td>80</td>
<td>80</td>
<td>64</td>
<td>51</td>
<td>41</td>
<td>33</td>
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<tr>
<td>70</td>
<td>70</td>
<td>49</td>
<td>34</td>
<td>24</td>
<td>17</td>
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<tr>
<td>60</td>
<td>60</td>
<td>36</td>
<td>22</td>
<td>13</td>
<td>8</td>
</tr>
</tbody>
</table>

At the best scenario only one step is needed with 100% recovery. With each step and with normal efficiencies a 10% loss will incur as a minimum. It can be seen that a minimum of purification steps is preferable for purification.
Standard Process Steps – Clarification and Concentration

**CLARIFICATION**
- Harvest Volume 400 L
- Low speed centrifuge 4000 rpm
- Followed by Microfiltration
- Final volume 440 L
- Yield 100%
- Time 1-2 hours

**CONCENTRATION**
- Start Volume 440L
- Final Volume 88L
- Concentrate 5 times
- Yield 95%
- Time 3 hours

**KII ULTRACENTRIFUGATION**
- Start volume 88L
- Rotor Volume
- Waste Volume 88L
- Gradient 60% Sucrose
- Flow Rate 15L/h
- Speed: 35 000 rpm
- Time 1 runs of 7h
- Gradient collected:
- Final Volume 600ml per run
- Concentrate 75x
- Yield 80%
How it works...inside the rotor.
K3 Core

For gradient separation of viral particles, viral sub-units, ribosomes and other cellular subunits. Also suitable for Pelleting and clarifying protocols.

K6 Core

K3 performance but with integral pre-clarifier for initial capture of heavy particles such as whole cells and cell debris.

K10 Core

For large volume gradient separation, Pelleting and clarifying.
Scaleable Centrifugation through Rotor Technology

Rotor Assembly
Core – Noryl
Rotor Bowl – Titanium
Rotor End caps - Titanium

KII Rotor Assembly
Promatix Rotor Assembly
R&D Scale Rotor Technology

Promatix 1000 Research Scale Continuous Flow Zonal Ultracentrifuge

Promatix Rotor Assembly
AW KII Separation Technique – Reorienting Gradient

1. Load the Gradient in the stationary rotor.
2. KII accelerates slowly and the gradient becomes vertical.
3. At 35,000 rpm flow allantoic fluid into the rotor to capture the virus in the Density Gradient. All allantoic fluid waste flows out of the rotor.
4. The sample sediments radially into the gradient of increasing density. The particles eventually band in cylindrical zones where the gradient density equals a particle’s buoyant density.
5. Set on the brake and the vertical gradient becomes horizontal again.
6. The layers of virus remain separate in the density gradient.
7. Collect the gradient using a pump from the bottom of the rotor. Select virus fractions using UV monitor.
Particle Separation during Continuous Flow Operation

Waste: Allantoic Fluid without Virus Particles

Influenza Viral particles remain in the stationary gradient phase (purification and concentration)

Ovalbumin waste leaves the rotor in the mobile phase

Product Feed: Clarified Allantoic Fluid
Alfa Wassermann Ultracentrifuges are used globally for manufacture of:

- Influenza vaccine
- Rabies vaccine
- Hepatitis B Vaccine
- Meningitis Vaccine
- Japanese Encephalitis Vaccine
An Example - Influenza Virus Purification
Typical Process Flow for Allantoic Influenza Manufacturing

- **Egg Inoculation**
- **Incubation**
- **Harvesting**
  - Clarification of fluid: Clarified volume 440 Litres
  - Concentration – Cross Flow Filtration: Concentrate (5x) to 88 Litres
  - Virus Continuous Flow (Gradient) Ultracentrifugation
  - Harvest virus fractions of choice
  - Bulk blending and dilution to final product concentration

40,000 / 50,000 eggs, harvest 400 Litres

Isolate purified concentrated virus fractions in 600mL 40,000 / 50,000 eggs, harvest 400 Litres
HA activity and optical density profiles of influenza virus purified by continuous-flow ultracentrifugation. Twenty-six liters of pre-clarified virus fluid were applied to the RK-3 rotor.

PK3 rotor
34,000 rpm
Product 42% sucrose
Virus (fractions 12-17)
Yield 74%
Sedimentation coefficient 722S
Flow rate 6 L/hr
6x10^4 HA units/mg
AW KII and PKII Typical Yield Influenza Vaccine

Source: J. Hinz et al
Extract of Spectra 2000 No. 4
European Symposium of Zonal Centrifugation
Influenza Vaccine Trend in China

- Initial manufacture of vaccines using chromatography provided very low yield.
- Small centrifuges provided a better solution but too many units are required to make a large volume of vaccine.
- KII Ultracentrifuge has revolutionized the China Flu Vaccine Industry:
  - Improved the vaccine yield
  - Reduced equipment cost by using less units with fewer process steps
  - Improved bio-burden by speeding up the process
  - Improved the vaccine quality by improving the purity.
Rabies Purification
Rabies Vaccine

KII Ultracentrifuge KII
RK3 Rotor – 1.6L
Process Speed: 35 000 rpm
Process Centrifugal Force: 90000 xg
Gradient: 0-55% w.w Sucrose
Product flow rate: 7 L/h
Virus fractions: 30-35% sucrose
Yield up to 90% in the gradient
Adenovirus Purification
Adenovirus Purification

**Gradient:** 1:1 (v/v) gradient of 50% (wt/v) Nycodenz: buffer

**Speed:** 40 500 rpm

**Flow rate:** 100 ml/min

**Sample size:** 7 litres of clarified supernatant from Adenovirus production

**Time to band:** 1.0 hrs

ELISA Detection by Hexon Antibody (monoclonal)

4.2x10^{12} CFU obtained from the harvested supernatant of about seven cell factories.

**Result:** Product seen to band at the isodense point in the gradient at 45% Nycodenz (~1.24 g/cm^3). Banding time reduced showing an asymmetrical product peak.

Fraction 9

Fraction 10
SW41 process used cell factory of HEK-293 cells using analytical cesium chloride density gradients. Cells were infected with Ad5-GFP and were harvested after there was approximately 60% CPE observed in the cell monolayer. The cell pellet was lysed by three freeze-thaw cycles and the resultant viral lysate was purified using two sequential cesium chloride density gradients in a SW-41 Beckman rotor.
What Range of Viral Particles can be Purified?
Vaccine Technologies for Virus Like Particles (VLPs)

Over 20 RNA, DNA viruses and bacteria being tested as vectors

- Poxvirus
- Baculovirus
- Canary Pox
- Adenovirus
- AAV
- Lentivirus
Established Protocols for Virus Families

AWST continuous flow ultracentrifuges is used to purify virus particles from all virus families in the diagram for manufacture of viral vaccines.

<table>
<thead>
<tr>
<th>Virus Family</th>
<th>Representative Protocol</th>
<th>Sedimentation range</th>
<th>Flow Rate</th>
<th>Gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoviridae</td>
<td>Adenovirus (AWI data)</td>
<td>650 to 865S</td>
<td>26-35 L/hr</td>
<td>40% Nycodenz</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>Coronavirus (Lisbon 2004)</td>
<td>320-490S</td>
<td>10 – 15 L/hr</td>
<td>0-55% Sucrose</td>
</tr>
<tr>
<td>Hepanaviridae</td>
<td>Hepatitis B</td>
<td>280-310S</td>
<td>8 – 9 L/hr</td>
<td>0-55% Sucrose</td>
</tr>
<tr>
<td>Herpesviridae</td>
<td>HBLV</td>
<td>720-775S</td>
<td>22 – 23 L/hr</td>
<td>0-45% Sucrose</td>
</tr>
<tr>
<td>Orthomyxoviridae</td>
<td>Influenza</td>
<td>700-900S</td>
<td>22 – 30 L/hr</td>
<td>0-45% Sucrose</td>
</tr>
<tr>
<td>coronaviridae</td>
<td>HPV (unpublished data)</td>
<td>255-315S</td>
<td>8 – 9 L/hr</td>
<td>0-45% Sucrose</td>
</tr>
<tr>
<td>Paramyxoviridae</td>
<td>NDV, Mumps</td>
<td>1000-2000S</td>
<td>30 – 60 L/hr</td>
<td>0-60% Sucrose</td>
</tr>
<tr>
<td>Retroviridae</td>
<td>RSV, MuLV, MoMLV, AKRMLV, KiMSV, FeLV, FSV</td>
<td>585-650S</td>
<td>18 – 20 L/hr</td>
<td>0-55% Sucrose</td>
</tr>
<tr>
<td>Flaviviridae</td>
<td>Japanese Encephalitis</td>
<td>180-220S</td>
<td>5 to 7 L/hr</td>
<td>0-55% Sucrose</td>
</tr>
<tr>
<td>Picornaviridae</td>
<td>Polio</td>
<td>160-180S</td>
<td>5 L/hr</td>
<td>0-65% Sucrose</td>
</tr>
<tr>
<td>Poxviridae</td>
<td>Vaccinia</td>
<td>4900-5200S</td>
<td>60 L/hr</td>
<td>0-65% Sucrose</td>
</tr>
<tr>
<td>Togaviridae</td>
<td>Semliki Forest Virus</td>
<td>280-650S</td>
<td>8 – 9 L/hr</td>
<td>0-60% Sucrose</td>
</tr>
<tr>
<td>Rhabdoviridae</td>
<td>Rabies Virus</td>
<td>650-1025S</td>
<td>20 – 31 L/hr</td>
<td>0-55% Sucrose</td>
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Thank You for Attending!

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