Vaccine production: improved supply in the region through collaborations

by Dr. Nora Dellepiane

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Taipei, Taiwan 6 to 10 March 2017
Vaccine types

√ Bacterial vaccines:
  • Killed (chemical and/or heat), e.g. whole cell pertussis
  • Toxoids, e.g. tetanus and diphtheria
  • Atenuated: live modified micro-organisms in which the virulent properties have been modified. They are able to replicate and infect cells in the organism but they do not cause the disease, e.g. BCG
  • Subunits: polysaccharide vaccines (meningococcal, pneumococcal), acellular pertussis vaccines

√ Viral vaccines:
  • Killed/ inactivated, e.g. Rabies, polio (Salk)
  • Atenuated e.g. YF, JE, measles, rubella, mumps and polio (Sabin)
  • Subunits: purified protection conferring antigens, e.g. influenza vaccine
Recombinant DNA

- Identification of genes
- Transfer from one organism to another
- Expression vector for protein synthesis

Using genetic engineering techniques, a gene codifying for the relevant antigen is isolated and introduced into another organism or cell that will express the protein, which following the required purification steps will constitute the vaccine. Usually such technique will render virus like particles, e.g. hepatitis B and HPV.
Recombinant HPV L1 VLP Vaccine

Courtesy of Dr. Umesh Shaligram, SIIPL
Live attenuated recombinant vaccine (dengue vaccine)

- The active substances contained in the CYD-TDV dengue vaccine are 4 live attenuated recombinant viruses representing serotypes 1, 2, 3, and 4.

- Each monovalent CYD recombinant is obtained separately by replacing the genes encoding the prM and E proteins of the attenuated yellow fever (YF) 17D virus genome with the corresponding genes of the 4 wild-type dengue viruses.

- The final formulation contains 4.5–6.0 log10 median cell-culture infectious doses (CCID50) of each of the live attenuated dengue serotype 1, 2, 3 and 4 vaccine viruses.
Conjugate vaccines

Polysaccharide vaccines are not immunogenic in young infants, usually under the age of two. The method of conjugation has overcome this difficulty.

Immune response is improved by chemically linking the polysaccharide to a protein ‘carrier’. The carrier is often either highly purified tetanus toxoid, or diphtheria toxoid (CRM)

Examples of conjugate vaccines are *haemophilus type b* vaccine, meningococcal A,C, W,Y and also pneumococcal vaccines (PCV 10 and 13)
Vaccine combinations

Individual antigens can be combined in order to provide protection against several diseases, thus minimizing the number of injections and interventions. Examples of combos are:

- DTP-Hepatitis B
- DTP-Hib
- DTP-Hepatitis B – Hib
- DTP-Hepatitis B-Hib-IPV

Measles, mumps and rubella
Summary type of vaccines

- Bacterial vaccines: Killed, attenuated and subunits
- Toxoids: D and T
- Viral vaccines: Killed, attenuated and subunits
- Recombinant vaccines: Hepatitis B vaccine, HPV
- Live attenuated recombinant virus vaccine, dengue
- Conjugated vaccines: Hib, pneumococcal, meningococcal
- Combined vaccines
The vaccine development cycle

**Average development time for a vaccine:** 12 years

**Overall cost to develop a vaccine investment:** More than half a billion US dollars

**70%** of a vaccine’s production time dedicated to quality control.

**Source:** SANOFI Pasteur website

**RESEARCH & DEVELOPMENT**
- **Exploratory stage:** 2 to 4 years
  - Identifying antigens to prevent or treat a disease. Selected candidate vaccines will continue the process.
- **Pre-clinical stage:** 1 to 2 years
  - Assessing antigens’ safety in animals and selecting the best candidate vaccine to continue the process.
- **Clinical development:** 6 to 8 years
  - Evaluating the immune response in 100 to 3,000 subjects
  - Testing the candidate vaccine in humans
  - Phase I: test of safety on 10 to 100 subjects
  - Phase II: evaluation of the immune response in 100 to 3,000 subjects
  - Phase III: large-scale tests of the vaccine’s efficacy and tolerance on 3,000 to 40,000 subjects

**INDUSTRIAL OPERATIONS**
- Germ culture
- Harvesting
- Purification
- Inactivation
- Valence assembly
- Formulation
- Filling
- Freeze-drying
- Packaging
- Batch release
- Transport

**COMMERCIAL OPERATIONS**
- Market research
- Assessment of the market needs
- Target profile of the product:
  - Target populations
  - Candidate vaccine features

**Pharmacovigilance**
- Quality: quality control, quality assurance

**Sales:**
- Private Market
- International Market
- Health Organizations (UNICEF, WHO...)

**Continent:**
- **Exploratory stage:** 2 to 4 years
- **Pre-clinical stage:** 1 to 2 years
- **Clinical development:** 6 to 8 years
- **Phase II:** Evaluation of the immune response in 100 to 3,000 subjects
- **Phase III:** Large-scale tests of the vaccine’s efficacy and tolerance on 3,000 to 40,000 subjects
- **Registration:** Synthesis stage from 12 to 18 months
  - All the data that have been collected during the preceding stages are gathered in a file and submitted to the health authorities in order to obtain a marketing authorization.
- **The first batches are clinical batches and industrial batches of compliance.**
- **The vaccines are filled,**
  - Primarily in vials and syringes and then packed.
  - When the manufacturing process is complete, the cold chain must be constantly maintained during all stages, from distribution to vaccine administration to patients.
Steps involved in vaccine production

**Steps:**
- Inactivation
- Valence
- Assembly
- Formulation
- Filling
- Germ culture
- Harvest
- Purification
- Inactivation
- Valence Assembly
- Formulation
- Filling
- Freeze drying

**Processes:**
- Bioreactors
- Centrifugation
- Precipitation
- Chromatography
- Tangential filtration
- Heat, Inactivating agents (formaldehyde, B-propiolactone, etc)
- Mixing serotypes
- Mixing of Active ingredients with excipients including adjuvants, stabilizers, etc

**Quality Controls:**
- IPC
- Control of intermediates

**GMP compliance:**
- Process validation for each step
- Cleaning validation
- Preventive maintenance
- Environmental monitoring
- Data trending and analysis
- Media fills
- Line clearance

**Steps:**
- (1) Bulk production
- (2) Final bulk
- (3) Final product
Steps involved in vaccine production

- Germ culture
- Bacteria, Strain certification for bacteria and viruses (historical records, origin Seed lot system)
- Viruses
- Fermentation
- Cell culture
- Controlled culture media, GPP, sterility, etc
- Cell bank system
  - Fully characterised cells, controlled culture media
  - Controlled culture conditions
  - Controlled virus multiplication conditions
Viruses cannot grow on their own, they require a host cell for multiplication.

Courtesy: GTN Lot Release Course CDL India
Cell Bank System

Original source of cells → testing → Master cell bank

Working cell bank → testing → Cells for production

Courtesy: GTN Lot Release Course CDL India
Virus Seed Lot System

Production cells

Infected cells

Virus strain

Testing

Master virus seed lot

Testing

Working virus seed lot

All lots start with this virus

Courtesy: GTN Lot Release Course CDL India
WHO references

✓ TRS No 978, Annex 3: 2013. Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. Replacement of annex 1 of WHO TRS 878


✓ Vaccine specific requirements.
Example of Hib polysaccharide conjugated vaccine

WHO Reference

© World Health Organization

Annex 1

Recommendations for the production and control of Haemophilus influenzae type b conjugate vaccines
Example of a polysaccharide conjugate and non-conjugate vaccine

- Polysaccharide coat only
  - Polysaccharide vaccine
    - e.g. Meningococcal A,C,W,Y vaccine
- Conjugated polysaccharide vaccine
  - e.g. Hib vaccine, conjugated pneumococcal or conjugated meningococcal vaccine

Purification
Purification and conjugation

Bacterial growth

Courtesy: GTN Lot Release Course CDL India
**Haemophilus influenzae** type b capsular polysaccharide (PRP)

Formulation of different Hib vaccines

Table A1
Formulation of some currently available *H. influenzae* type b conjugate vaccines\(^{a,b}\)

<table>
<thead>
<tr>
<th>H. influenzae polysaccharide material</th>
<th>Polysaccharide per single human dose (µg)</th>
<th>Nature of carrier protein</th>
<th>Protein per single human dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polysaccharide (size-reduced)</td>
<td>25</td>
<td>Diphtheria toxoid</td>
<td>18</td>
</tr>
<tr>
<td>Polysaccharide (low relative molecular mass)</td>
<td>10</td>
<td>Diphtheria CRM 197 protein</td>
<td>25</td>
</tr>
<tr>
<td>Polysaccharide (size-reduced)</td>
<td>7.5</td>
<td>Outer membrane protein complex of <em>Neisseria meningitidis</em> group B</td>
<td>125</td>
</tr>
<tr>
<td>Polysaccharide</td>
<td>10</td>
<td>Tetanus toxoid</td>
<td>20</td>
</tr>
</tbody>
</table>

\(^{a}\) For guidance only.

\(^{b}\) *H. influenzae* type b conjugate vaccine is a preparation of capsular polysaccharide from *H. influenzae* type b covalently linked to carrier protein.
Bulk conjugate

Final bulk

Final Product

fermentation

Hib-working seedlot

Crude polysaccharide

Purified polysaccharide

Purification

Consistency: Growth, pH and polysaccharide yield
Bacterial purity
Identity
Molecular size distribution
Moisture content
Polysaccharide composition: ribose and phosphorous content
Protein
Nucleic acid
Endotoxin

Modification

(number of functional groups per repeat unit of polysaccharide
Molecular size distribution)

(modified) Polysaccharide

Protein Carrier

Consistency: Growth rate, pH and protein yield
Purity and concentration

Conjugation

Residual reagents
Conjugation markers
Residual reactive functional groups
PRP content
Conjugated and unbound PRP
Protein content
PRP-to-protein ratio
Molecular size distribution
Sterility
Specific toxicity of the carrier protein

Identity
Sterility
PRP content
Residual moisture
Pyrogen content
Adjuvant content
Preservative content
General safety test
pH

Sterility

Formulation

Filling
## Control of the Polysaccharide Specifications Summary

<table>
<thead>
<tr>
<th>Process step</th>
<th>“Component”</th>
<th>Assay</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hib fermentation</strong></td>
<td>Strain</td>
<td>NMR</td>
<td>Type b</td>
</tr>
<tr>
<td></td>
<td>Seedlot system</td>
<td>x</td>
<td>Consistency</td>
</tr>
<tr>
<td></td>
<td>Culture media</td>
<td>x</td>
<td>No human blood-group antigen-like material and no high-molecular-weight polysaccharide</td>
</tr>
<tr>
<td></td>
<td>Harvest</td>
<td>pH, OD, polysaccharide</td>
<td>Consistency</td>
</tr>
<tr>
<td></td>
<td>Contamination</td>
<td>Gram-smear</td>
<td>Pure</td>
</tr>
<tr>
<td><strong>Polysaccharide purification</strong></td>
<td>Identity test</td>
<td>NMR</td>
<td>PRP</td>
</tr>
<tr>
<td></td>
<td>Molecular size distribution</td>
<td>HP-GPC</td>
<td>Consistency</td>
</tr>
<tr>
<td></td>
<td>Moisture content</td>
<td>Karl Fisher</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Ribose</td>
<td>Orcinol</td>
<td>&gt;32% dry weight</td>
</tr>
<tr>
<td></td>
<td>Phosphorus</td>
<td>Ames</td>
<td>6.8%-9% dry weight</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>Lowry</td>
<td>&lt;1% dry weight</td>
</tr>
<tr>
<td></td>
<td>Nucleic acid</td>
<td>UV260</td>
<td>&lt;1% dry weight</td>
</tr>
<tr>
<td></td>
<td>Endotoxin</td>
<td>LAL Rabbit test</td>
<td>&lt;10 IU/ µg PRP 1 µg PRP / Kg</td>
</tr>
<tr>
<td><strong>Polysaccharide modification</strong></td>
<td>Degree of activation</td>
<td>TNBS</td>
<td>Consistency</td>
</tr>
<tr>
<td></td>
<td>Molecular size distribution</td>
<td>HP-GPC</td>
<td>Consistency</td>
</tr>
</tbody>
</table>
## Control of the carrier protein
### Specifications Summary

<table>
<thead>
<tr>
<th>Process step</th>
<th>“Component”</th>
<th>Assay</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fermentation</strong></td>
<td>Seedlot system</td>
<td>x</td>
<td>Consistency</td>
</tr>
<tr>
<td></td>
<td>Culture media</td>
<td>x</td>
<td>Free from substances likely to cause toxic or allergic reactions in humans</td>
</tr>
<tr>
<td></td>
<td>Harvest</td>
<td>pH, OD, Protein</td>
<td>Consistency</td>
</tr>
<tr>
<td></td>
<td>Contamination</td>
<td>Gram-smear</td>
<td>Pure</td>
</tr>
<tr>
<td><strong>Protein purification</strong></td>
<td>Purity</td>
<td>LF test, HPLC or SDS-PAGE</td>
<td>D&amp;T-toxoid: &gt;1500 LF/mg protein N&lt;br&gt;CRM197: &gt;90%&lt;br&gt;Outer-membrane complex of MengB: &lt;8% lipopolysaccharide/weight + rabbit test</td>
</tr>
<tr>
<td><strong>Protein modification</strong></td>
<td>Extent of derivatization</td>
<td>x</td>
<td>Consistency</td>
</tr>
</tbody>
</table>
Table A2
Methods currently used for conjugation of *Haemophilus influenzae* type b polysaccharide and control of conjugates

<table>
<thead>
<tr>
<th>Method</th>
<th>Procedure</th>
<th>Assay for conjugation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reductive amination</td>
<td>Combine carrier protein and aldehyde form of polysaccharide in presence of reducing agent</td>
<td>Formation of unique amino acid and gel filtration</td>
</tr>
<tr>
<td>Reductive amination and attachment of spacer linked to active ester</td>
<td>Selective reducing end group activation and coupling to carrier protein through spacer</td>
<td>Gel filtration or SDS-PAGE</td>
</tr>
<tr>
<td>Carbodiimide-mediated coupling</td>
<td>Combine reactants in presence of carbodiimide</td>
<td>Gel filtration</td>
</tr>
<tr>
<td>Cyanogen-bromide activation of polysaccharide</td>
<td>Addition of carrier protein to cyanogen-bromide-activated polysaccharide</td>
<td>Gel filtration and assay for bound polysaccharide</td>
</tr>
<tr>
<td>Thioether bonding</td>
<td>Combine haloacetyl polysaccharide with protein thiol</td>
<td>Formation of unique amino acid and assay for bound polysaccharide</td>
</tr>
</tbody>
</table>

* For guidance only.
## Control of bulk conjugate Specifications Summary

<table>
<thead>
<tr>
<th>Process step</th>
<th>“Component”</th>
<th>Assay</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polysaccharide-protein conjugation</td>
<td>Residual reagents</td>
<td>x</td>
<td>Removal to be confirmed</td>
</tr>
<tr>
<td></td>
<td>Conjugation markers</td>
<td>PRP:Protein</td>
<td>consistency</td>
</tr>
<tr>
<td></td>
<td>Residual reactive functional groups</td>
<td>x</td>
<td>No residual reactive group</td>
</tr>
<tr>
<td></td>
<td>PRP content</td>
<td>Orcinol</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Conjugated and unbound PRP</td>
<td>Orcinol, sample pretreatment</td>
<td>&lt;40% free PRP</td>
</tr>
<tr>
<td></td>
<td>Protein content</td>
<td>BCA</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Polysaccharide-protein ratio</td>
<td>To be calculated</td>
<td>Diphtheria &amp; tetanus toxoid: 0.3-0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CRM197: 0.3-0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OMC: 0.05-0.1</td>
</tr>
<tr>
<td></td>
<td>Molecular size distribution</td>
<td>HP-GPC</td>
<td>Consistency</td>
</tr>
<tr>
<td></td>
<td>Sterility</td>
<td>Bacterial &amp; mycotic</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Specific toxicity</td>
<td>guinea-pig test</td>
<td>Absence of specific toxicity</td>
</tr>
</tbody>
</table>
### Control of final product Specifications Summary

<table>
<thead>
<tr>
<th>Process step</th>
<th>“Component”</th>
<th>Assay</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polysaccharide-protein conjugation</td>
<td>Identity</td>
<td>Immunological test</td>
<td>PRP</td>
</tr>
<tr>
<td></td>
<td>Sterility</td>
<td>Bacterial &amp; mycotic</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>PRP content</td>
<td>Orcinol and/or chromatographic</td>
<td>±20% of stated PRP content</td>
</tr>
<tr>
<td></td>
<td>Residual moisture</td>
<td>Karl Fisher</td>
<td>&lt;2.5%</td>
</tr>
<tr>
<td></td>
<td>Pyrogen content</td>
<td>LAL or rabbit test</td>
<td>Acceptable</td>
</tr>
<tr>
<td></td>
<td>Adjuvant content</td>
<td>Spectroscopy</td>
<td>&lt;1.25 mg aluminium or 1.3 mg calcium per s.h.d.</td>
</tr>
<tr>
<td></td>
<td>Preservative content</td>
<td>UV</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>General safety</td>
<td>General safety test</td>
<td>Animals should survive for at least 7 days</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>pH test</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Inspection</td>
<td>visual</td>
<td>No clumping, lack of integrity and/ or particles</td>
</tr>
</tbody>
</table>
The vaccine development cycle

**Average development time for a vaccine:** 12 years

**Overall cost to develop a vaccine:** More than half a billion US dollars

**70%** of a vaccine’s production time dedicated to quality control.

**Source:** SANOFI Pasteur website
Key factors to consider before launching vaccine production

- Cost of development
- Time for development
- Cost and difficulties of technological know how, commercial scale, consistency of production, GMP compliance
- Cost and difficulties for testing
- Technical difficulties to get appropriately characterized production strains
- IP related matters
- Cost and timeframe for non-clinical and clinical development
- Registration related issues and timelines
- Size of market for cost recovery and further profit
Fostering collaborations between DCVMN members

- Information exchange
- Support to acquire specific technologies (freeze drying, cell culture, other)
- Support to acquire testing methodologies
- Sources of strains for vaccine production
- Sources of formulated bulk ready for filling, labelling and packaging
- Sources of concentrated bulk material for formulation, filling, labelling and packaging
- Full transfer of technology from seed

USE THE NETWORK FOR MUTUAL BENEFITS
THANK YOU