IVI Technology Transfer Program:

Cholera, Typhoid vaccines and future Tech Transfers

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International Vaccine Institute

Workshop on technology transfer for local manufacturing capacity of vaccines
30 November – 1 December 2010
Cholera cases are under reported
– WHO estimates only 5 - 10% of cases reported
– Likely to exceed 1 million cases annually
– Estimated 120,000 deaths annually

Cholera Outbreak In Haiti – The World Health Organization has announced today that an unknown type of cholera has killed dozens of people in Haiti in the last few days.

Need for a safe, high quality affordable vaccine
<table>
<thead>
<tr>
<th>Dukoral</th>
<th>ORC-Vax</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crucell / SBL Vaccin AB</strong></td>
<td><strong>VaBiotech</strong></td>
</tr>
<tr>
<td>Inactivated Inaba and Ogawa cells plus recombinant CTB</td>
<td>Inactivated Inaba and Ogawa (O1) plus O139 cells</td>
</tr>
<tr>
<td>Oral delivery</td>
<td>Oral delivery</td>
</tr>
<tr>
<td>3.0 ml liquid per dose</td>
<td>1.5 ml liquid per dose</td>
</tr>
<tr>
<td>Requires buffer made up to 150 ml with water</td>
<td>Buffer not required</td>
</tr>
<tr>
<td>Licensed 1991</td>
<td>First licensed 1997</td>
</tr>
<tr>
<td>WHO prequalified</td>
<td>No WHO prequalified vaccines</td>
</tr>
<tr>
<td>&gt; 40 Euro for 2 doses</td>
<td>Around US $1.00 per dose</td>
</tr>
</tbody>
</table>
Was the vaccine suitable for transfer?

Were there any problems with Safety, Potency?
Reference: Draft WHO guidelines for production and control of inactivated cholera vaccine

Two significant problems identified
1. Antigen quantification method was not accurate.
2. Inconsistent removal of cholera toxin and no assay to detect residual toxin.

Steps taken to improve safety and quality

Reformulation
• Removed toxin hyper-producing strain and replaced with an equivalent serogroup (O1 Inaba).
• Increased the dose of the O1 Ogawa component.

Quality Control
• Introduced an ELISA to quantify O antigen component of LPS.
• Introduced an ELISA to quantify residual cholera toxin.
**Process**

**Oral inactivated Cholera vaccine**

- **Seed bank**
- **Expansion of Cell numbers in Shaker Flask Culture**
- **Seed Fermentation**
- **Production Fermentation**
- **Inactivation**
- **Concentration Diafiltration**
- **Bulk monovalent concentrates**
- **Formulation**
- **Fill and Finish**

**Improvements**

- **Poor passage history**
  - Serial passage at high dilution to ensure absence of BSE
- **Eliminated passage on solid agar**
  - No longer use horse blood
- **Increased washing during diafiltration**
  - Removal of small amounts of toxin
  - Eliminated centrifugation
- **New formulation**
  - More antigen accurately quantified
  - No residual cholera toxin
- **New QC assays**
  - LPS (Antigen) and Toxin
Phase II Studies: Safety and Immunogenicity

- Safety: Vaccine safe
- Immunogenicity: Superior vibriocidal responses compared to existing Vietnamese vaccine and Internationally licensed Swedish vaccine.
- Results similar in non-endemic (SonLa) and endemic (Kolkata) setting.
Does the Vaccine work?
Efficacy trial (Protection from disease)

- **Phase III trial in Kolkata (65,000 persons)**
  Vaccine provided 70% protection (for at least 3 years) and was shown to be safe.
  Protection was conferred even among children aged 1 – 4.9 years of age.

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**THE LANCET**

Efficacy and safety of a modified killed-whole-cell oral cholera vaccine in India: an interim analysis of a cluster-randomised, double-blind, placebo-controlled trial

Technology Transfer to Shantha Biotechnics (India)

Training at IVI (2 weeks duration)
- 2 Production staff
- 1 Quality control
- 1 Project manager

Production Staff
- Production process
- In process control
- Formulation

Quality Control Staff
- Lot release assays
- Formulation
Scale up successful

Testing is compliant with WHO recommendations for Oral Inactivated Cholera Vaccine

Manufacturing method and facility meets WHO standards for cGMP

Government of India
Central Drugs Standard Control Organisation
Directorate General of Health Services
FDA Bhawan, New Delhi – 110 002 [India]

Form-46
(See rules 122-B and 122-D and 122-DA)
Permission/approval for manufacture of new drug formulation
***************
Number of the permission and date of issue: MF-176/09

M/s. Shantha Biotechnics Limited, Survey No. 274, Athveli Village, Medchal Mandal, Ranga Reddy Dist., Andhra Pradesh (address) is hereby granted permission/approval to manufacture the following new drug formulation under rule 122-B/122-D/122-DA of the Drugs and Cosmetics Rules, 1945, namely:

1. Name of the drug: Killed Bivalent (01 & 0139) whole cell Oral Cholera Vaccine.

2. Dosage Form: Liquid vaccine for Oral Administration.

3. Composition: As per Batch.

4. Indication: For active Immunization against Vibrio cholera.

Date: 15 FEB 09

Signature: (Dr. Surinder Singh)
Drugs Controller General (India)
[Name & Designation of Licensing Authority]

Contd.....2
Transfer of quality control assays to VaBiotech

New ELISA based assays
- O antigen quantification
- Cholera Toxin assay
Ivanoff et al. (1994) 17 million cases and 600,000 deaths
Crump et al. (2004) 21.6 million cases and 216,000 deaths
IVI Goal
to make available

• High quality, safe and efficacious typhoid fever vaccine
• Targeting populations most at risk from typhoid infection
• Affordable
• Delivered with other EPI vaccines

High yield, high recovery, cGMP compliant processes for Vi and Vi conjugate vaccine
ANTIGEN PRODUCTION
Upstream process
1. Optimize production of Vi during growth in Bioreactor
Purification
New method developed at IVI

PROCESS DEVELOPMENT

2. Downstream processing (purification of Vi)
Removal of impurities, maximize recovery of Vi.

Seed bank
Local Indian Isolate

Fermentation
Inactivation

Clarification of Vi

Concentration
Diafiltration

Cetavlon precipitation

Wash with 20% ethanol

Dissolve in 60% ethanol

Precipitate with 75% ethanol
Wash with 75% ethanol

Dissolve in water

\((\text{NH}_4)_2\text{SO}_4\)
Precipitate impurities

Concentration / Diafiltration

Sterile filtration

No centrifugation / no phenol extraction
Follow up at Shantha Biotechnics
5 litre and 10 litre fermentation batches

<table>
<thead>
<tr>
<th>Batch number</th>
<th>Protein %</th>
<th>Nucleic acid %</th>
<th>O-acetyl content &gt;mmol/g</th>
<th>Endotoxin EU/µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO Specification</td>
<td>&lt;1</td>
<td>&lt;2</td>
<td>&gt;2.0</td>
<td>25</td>
</tr>
<tr>
<td>Run 1 5 litres</td>
<td>0.2</td>
<td>0.5</td>
<td>4.7</td>
<td>1</td>
</tr>
<tr>
<td>Run 2 10 litres</td>
<td>0</td>
<td>0.5</td>
<td>3.2</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Both batches complied with WHO specifications for Vi polysaccharide vaccine.
**Vi polysaccharide (T-cell independent response)**
- Poor anti-Vi antibody responses
- No response in infants (< 2 years of age)
- No memory response and no boosting
- Generally relatively short lived immunity

**Vi conjugate (T-cell dependent response)**

Recruitment of T helper cells
- Higher antibody responses in all age groups
- Infants less than 2 years now respond to the polysaccharide
- Induction of memory and boosting on revaccination
- Duration of immunity much longer
- Could be delivered with other EPI vaccines
Why DT as the carrier protein?

- Very low cost of manufacture
- High yields and no supply constraints
- Quality control well established and accepted by regulators
- Produced by many developing country manufacturers
- Compatible with pH requirements in the conjugation process
1. Derivatization of Diphtheria Toxoid (DT)

\[
\begin{align*}
\text{DT}^- \text{COOH} & \quad + \quad \text{NH}_2\text{NHCO(CH}_2)_4\text{CONHNH}_2 \\
& \quad \text{EDAC} \quad \text{DT}^- \text{CONHNHCO(CH}_2)_4\text{CONHNH}_2
\end{align*}
\]

2. Conjugation

\[
\begin{align*}
\text{DT}^- \text{CONHNHCO(CH}_2)_4\text{CONHNH}_2 & \quad + \quad \text{HOOC}^- \text{Vi} \\
& \quad \text{EDAC} \quad \text{DT}^- \text{CONHNHCO(CH}_2)_4\text{CONHNHOC}^- \text{Vi}
\end{align*}
\]
Control the number of ADH molecules bound by adjusting EDAC concentration - the more ADH bound the more efficient the binding of DT to Vi.

The quality of the DT also affects the amount of ADH bound - the more cross linking due to formalin the less ADH bound?

After derivatization about 10 ADH spacer molecules bound to the DT.

**PROCESS CONTROL**

ADH:Protein 2.0 to 4.0% (w/w)

7 to 14 spacer molecules per DT.
Conjugation to Vi
Effect of varying DT concentration

Increasing DT concentration in reaction mixture

Size exclusion chromatography of conjugates – Sephacryl S1000
Red line – Abs at 206 - polysaccharide
Blue line – Abs at 280nm - protein
Immunogenicity of conjugates

Anti Vi IgG response post vaccination with three doses of various Vi-DT conjugates

Increasing DT concentration in reaction mixture

Dose 0, 4, 20 weeks

Bleeds 1, 2, 4, 5, 6, 8, 10, 20, 21, and 22 weeks
Technology Transfer
to Shantha Biotechnics (India)

Initial training at IVI
Follow up at Shantha

60,000 dose pilot lot of Vi-DT produced at Shantha

Vi and Vi-DT production at Shantha

Successful adoption of technology at Shantha Biotechnics.
Clinical trials planned for 2011.
Chemistry of OSP

Salmonella paratyphi A polysaccharide component of LPS

Structure of Lipopolysaccharide

Structure of S. paratyphi A O antigen

Toxic lipid A must be removed before conjugation.

Partial O-acetylation

Single repeat unit of para A O antigen

Note the absence of carboxyl groups.
### Development stage

<table>
<thead>
<tr>
<th>Development stage</th>
<th>Progress</th>
</tr>
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<tbody>
<tr>
<td>High yielding fermentation</td>
<td>OD$_{600}$ in the range 18 – 20 consistent with <em>S. typhi</em></td>
</tr>
<tr>
<td>Purified LPS from para A</td>
<td>Purification method: High yield of LPS, Adequate removal of nucleic acid and protein</td>
</tr>
<tr>
<td>Detoxified LPS</td>
<td>Detoxification (acetic acid and heat). Endotoxin levels down to 0.3 E.U./µg OSP</td>
</tr>
<tr>
<td>Conjugated OSP to DT</td>
<td>Produced para A OSP-DT conjugate chemically and physically characterized.</td>
</tr>
<tr>
<td>Immunogenicity</td>
<td>Immunogenicity of conjugate currently being evaluated</td>
</tr>
</tbody>
</table>
Requirements for IVI to perform technology transfer

“To promote the health of people in developing countries by the development, introduction and use of new and improved vaccines”

- From: Constitution of IVI (1996)

- Manufacturer operates in compliance with WHO cGMP standards.
- Manufacturer has the capacity to achieve WHO pre-qualification.
  - NRA in country of manufacturer needs to have met WHO requirements
- Manufacturer should have capacity to produce or acquire bulk components.
- Demonstrated capacity to scale up process from pilot scale and convert into a product.
- Commitment to public health and to supply market demand
Some thoughts on why IVI has been successful in technology transfer

• Thoroughly understand the process and the equipment used in manufacturing.
• High level of process control and lot release assays in place.
• Detailed SOPs for production and quality control.
• Good working relationship between technology provider and commercial partner.
• Commitment to make it happen from both sides.
• Adequate funding for process development and clinical evaluation and transfer.
Vaccines don’t save lives
Vaccination does

Thank you