Development of a cost effective and scalable purification process for Vi polysaccharide.

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Moral obligation

Every child should have the opportunity to receive high quality, safe and efficacious vaccines to protect them from life threatening infectious diseases.
Funding for vaccination is limited so in order to get the broadest coverage in resource poor countries, vaccines must be affordable.
How does VPD address affordability?

**Process development:**
High yielding antigen production
High recovery purification process using cost effective technology
Scalable process compatible with cGMP production

**Technology Transfer:**
High standards for quality, strict adherence to cGMP
Manufacturer(s) committed to low cost production and low profit margins

—Developing Country Vaccine Manufacturers

Manufacturer with capacity to WHO prequalify
Development of a Typhoid Conjugate Vaccine at IVI

Production and purification of Vi Polysaccharide
Vi polysaccharide

O-acetyl groups

N-acetyl-α-D-galactosaminuronic acid
2,000 to 10,000 repeating units
3 factors are important in Vi production

- High density bacterial culture
  - Fed batch culture increased OD₆₀₀ four fold
- Optimal environment for expressing genes coding for Vi
  - High osmolality inhibited Vi gene expression
- Optimal chemical environment for biosynthesis and polymerization of the Vi
  - High concentrations of glucose and high pH inhibited Vi biosynthesis
Purification optimization

Downstream processing (purification of Vi)

Removal of impurities
Maximize recovery of Vi polysaccharide

Seed bank
Local Indian Isolate

Fermentation
Inactivation

Clarification of Vi using TFF

Concentration
Diafiltration

Cetavlon precipitation

Dissolve in 60% ethanol

Precipitate and wash with 75% ethanol

Dissolve in water

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

Concentration / Diafiltration

Sterile filtration
### Consistency lots of Vi

<table>
<thead>
<tr>
<th>Batch number</th>
<th>Protein %</th>
<th>Nucleic acid %</th>
<th>O-acetyl content &gt;mmol/g</th>
<th>Vi ELISA mg/ml</th>
<th>Endotoxin EU/µg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WHO Specification</strong></td>
<td>&lt;1</td>
<td>&lt;2</td>
<td>2.0</td>
<td>NA</td>
<td>25</td>
</tr>
<tr>
<td>Consistency Run 1</td>
<td>0.4</td>
<td>0.3</td>
<td>2.3</td>
<td>2.6</td>
<td>2</td>
</tr>
<tr>
<td>Consistency Run 2</td>
<td>0</td>
<td>0.29</td>
<td>2.1</td>
<td>2.4</td>
<td>3</td>
</tr>
<tr>
<td>Consistency Run 3</td>
<td>0</td>
<td>0.68</td>
<td>2.6</td>
<td>1.9</td>
<td>4</td>
</tr>
</tbody>
</table>

WHO Size specifications: At least 50% of the Vi shall elute before a $K_D$ of 0.25 (Sepharose CL4B)

All three batches comply with WHO specifications
Purification (magnitude of challenge)

In 100 litre fermentation broth

End of fermentation (upstream)

Nucleic acid: 240 g
Protein: 880 g
Endotoxin: 100 g LPS
Vi polysaccharide: 70 g

End of purification (downstream)

Nucleic acid: 0.2 g
Protein: undetectable
Endotoxin: 0.008 g LPS (<100 E.U./dose)
Vi polysaccharide: 30 g (1 million doses)
Motivation for alternative downstream process development

- Improve Vi recovery
- Removal of detergent and ethanol based precipitation steps
- Minimizing number of purification steps
- Reducing production timeline and cost
- Ease of scalability
Alternative Purification optimization

- Seed bank
  - Local Indian Isolate
- Fermentation
  - Inactivation
- Clarification of Vi using TFF
- Concentration
  - Diafiltration
- Cetavlon precipitation
- Dissolve in 60% ethanol
- Precipitate and wash with 75% ethanol
- Dissolve in water
- (NH₄)₂SO₄
  - Precipitate impurities
- Concentration / Diafiltration
- Sterile filtration

Investigate optimizing this step

Investigate replacing this step with chromatography and NFF
Clarification

- Could not achieve clarification using depth filtration with a series of membranes at equivalent bacterial load
- Continued using 0.45µm TFF filtration
Comparison of different 88 cm² pellicon® 3 UFDF Cassettes

- UFDF studies with 100kD Ultracel reduced 96% of the protein and 98% of Nucleic acid contamination from the pool of 0.45μm clarified pooled samples.
- 100kD ultracel UFDF conditions are optimized with 2stage DF with 1M NaCl and Citrate phosphate buffer 6DV’s each
- The Vi recoveries are close to 100%
Ammonium sulphate precipitation and concentration and diafiltration at 100kD

With 10% Ammonium sulphate there is an additional 2% reduction in protein impurities and additional 0.5% reduction in Nucleic acid impurities

Per Vi recovery at 100kD Ultracel without ammonium sulphate precipitation has only 0.01-0.02% higher impurities
Activated carbon pod or 0.45µm Durapore can be used for filtration pre loading in Chromatography column.
Isoelectric point of Vi polysaccharide

- Vi polysaccharide (N-acetyl-a-D-galactosaminouronic acid)
- 2,000 to 10,000 repeating units
- The theoretical pI of the Vi polysaccharide was determined to be around 4-4.2
AEX is evaluated with Weak AE (DEAE) and Strong AE resin (TMAE).
The binding capacities of Vi is higher in Citrate phosphate buffer in comparison to MES and PBS buffer under similar pH conditions. Most optimal pH conditions are pH 6.2.
Elution of Vi from DEAE column

- Vi elution is in a range above 0.25M salt concentration up to 0.55M Salt
- Binding of Vi in Citrate phosphate buffer per ml of DEAE resin is 5.6mg
- Vi yield in elution pool is 78%
Different Hydrophobic, charged, and uncharged filtration membranes were tested for the removal of endotoxin.

Activated carbon pod is able to remove 70-75% of the remaining endotoxin in the pool at 4M salt concentration but at the same time it is also binding ≈80% of Vi polysaccharide.
### WHO specification for Vi polysaccharide

<table>
<thead>
<tr>
<th>Process type</th>
<th>Protein %</th>
<th>Nucleic acid %</th>
<th>O-acetyl content &gt;mmole/g</th>
<th>Endotoxin EU/µg</th>
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<td>&lt;1</td>
<td>&lt;2</td>
<td>2.0</td>
<td>25</td>
</tr>
<tr>
<td>Vi purification (IVI)</td>
<td>0.4</td>
<td>0.3</td>
<td>2.3</td>
<td>2</td>
</tr>
<tr>
<td>DEAE 0.55 pool</td>
<td>15.7</td>
<td>2.3</td>
<td>2</td>
<td>8535.9</td>
</tr>
</tbody>
</table>

- % recovery of Vi has improved with the new process from 40-50% to 77.5%.
- Alternate process still have high protein and endotoxin impurities.
• New Vi purification process is developed without use of any detergents, ethanol precipitation and enzymatic hydrolysis.

• The process is close to the criteria where regulatory authorities foresee the future of Polysaccharide vaccine purification.

• Further work needs to be done to reduce the impurity levels within the WHO specifications for Vi polysaccharide.

• % recovery of Vi polysaccharide is 30-40% higher with new process.

• Future work will focus on additional reduction of impurities specifically endotoxin.
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Thank you

Vaccines don’t save lives, vaccination does