Moving towards consistency approach for Diphtheria Tetanus Pertussis potency assays

IABS Conference
3Rs and consistency testing in Vaccine Lot release testing
Egmond aan Zee, September 16-18, 2015

Sylvie Uhlrich
Presentation outline

- Regulatory context and 3Rs status for DTP potency assays
- Diphtheria Tetanus Pertussis potency assays: roadblocks for 3Rs implementation
- Consistency approach (GMTs) for current DTP *in vivo* assays
- Perspectives for the future
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Regulatory context for DTP potency assays

Several methods are required / recommended depending on the destination

**D & T Potency**
- **Challenge tests**
  - Guinea pigs for D, mice and guinea pigs for T
    - **Multi-dilution assay (ED50)**
      - Ph. Eur.; WHO; JP; Chinese Ph.
    - **One-dilution assay (limit test)**
      - Ph. Eur.; WHO
    - **In vivo toxin neutralisation test**
      - US NIH
  - **Immunogenicity tests**
    - ELISA or Vero cell assay (for D) or ToBI (for T)
      - **Guinea Pig model**
        - multi- or one-dilution assay
          - Ph. Eur.
      - **Mouse model**
        - Chinese Ph.

**Pertussis Potency**
- **Intracerebral Challenge tests**
  - **Mouse model**
    - JP; Chinese Ph.
  - **Immunogenicity tests (mouse or Guinea Pig)**
    - **Relative potency**
      - Ph. Eur.
    - **GMTs/GMUs**
      - WHO, US

Several methods are required / recommended depending on the destination.
Diphtheria Tetanus Pertussis potency assays: current 3Rs advances

- **REFINEMENT**
  - Challenge tests ➔ Immunogenicity assays

- **REDUCTION**
  - Multidose assays ➔ Unidose assays

- **REFINEMENT & REDUCTION**

<table>
<thead>
<tr>
<th>Current tests</th>
<th>Single test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria Challenge test</td>
<td>Single immunogenicity assay (multiplexing)</td>
</tr>
<tr>
<td>Tetanus Challenge test</td>
<td></td>
</tr>
<tr>
<td>Pertussis Immunogenicity assay</td>
<td></td>
</tr>
</tbody>
</table>
Presentation outline

- Regulatory context and 3Rs status for DTP potency assays

- Diphtheria Tetanus Pertussis potency assays: roadblocks for 3Rs implementation
  - Capability to discriminate subpotent batches
  - Assay variability
  - Difficulties related to the use of reference standards

- Consistency approach (GMTs) for current DTP in vivo assays

- Perspectives for the future
Roadblocks for 3Rs implementation
Limited Discriminative power of challenge tests to detect subpotent lots

- **AIPO4 adjuvanted combination vaccine**

<table>
<thead>
<tr>
<th>T content Lf/mL</th>
<th>Potency (challenge)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Conform</td>
</tr>
<tr>
<td>10</td>
<td>Conform</td>
</tr>
<tr>
<td>2</td>
<td>Non conform</td>
</tr>
</tbody>
</table>

\[ \div 7.5 \]

<table>
<thead>
<tr>
<th>D content Lf/mL</th>
<th>Potency (challenge)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>Conform</td>
</tr>
<tr>
<td>30</td>
<td>Conform</td>
</tr>
<tr>
<td>5</td>
<td>Non conform</td>
</tr>
</tbody>
</table>

\[ \div 8 \]

Need for more than 5 fold less antigen to detect subpotent lots
Roadblocks for 3Rs implementation
Limited Discriminative power of challenge tests to detect subpotent lots

- **AIOOH adjuvanted combination vaccine**
  - Experimental formulations PRP-T depleted
  - Matrix effect for some tests (e.g. Tetanus)

<table>
<thead>
<tr>
<th>tests</th>
<th>lots</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Challenge T</td>
<td>Lot 100%</td>
<td>Invalid (lin.)</td>
</tr>
<tr>
<td></td>
<td>Lot 60%</td>
<td>Invalid (lin.)</td>
</tr>
<tr>
<td>Challenge D</td>
<td>Lot 100%</td>
<td>Conform</td>
</tr>
<tr>
<td></td>
<td>Lot 60%</td>
<td>Conform</td>
</tr>
<tr>
<td>Immunogenicity mice FHA</td>
<td>Lot 100%</td>
<td>Conform</td>
</tr>
<tr>
<td></td>
<td>Lot 30%</td>
<td>Invalid (NR)</td>
</tr>
<tr>
<td>Immunogenicity mice PT</td>
<td>Lot 100%</td>
<td>Conform</td>
</tr>
<tr>
<td></td>
<td>Lot 30%</td>
<td>Conform</td>
</tr>
</tbody>
</table>

2-3 fold less antigen not detected, neither in current tests nor in single immunogenicity assay in Guinea pig
Assay variability

- **Lot to lot Vaccine titer (log)**
  ![Graph showing lot to lot variability](chart1.png)

- **Reference Std titer (log)**
  ![Graph showing reference std variability](chart2.png)

<table>
<thead>
<tr>
<th>Dilution 1/5</th>
<th>GMTs Ref Std %RSD</th>
<th>GMTs Batches %RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>36%</td>
<td>42%</td>
</tr>
<tr>
<td>D</td>
<td>34%</td>
<td>37%</td>
</tr>
<tr>
<td>FHA</td>
<td>36%</td>
<td>35%</td>
</tr>
<tr>
<td>PT</td>
<td>42%</td>
<td>41%</td>
</tr>
</tbody>
</table>

As much Lot to Lot variability as day to day variability of Ref Std
Difficulties with the use of reference standards

Correlation between Vaccine and Reference Standard GMTs

- Diphtheria
  \[ R^2 = 0.25 \]

- FHA
  \[ R^2 = 0.43 \]

- Tetanus
  \[ R^2 = 0.25 \]

- PT
  \[ R^2 = 0.53 \]

No or poor correlation between Vaccine and Ref Std GMTs
Relevance of relative potency assay using an homologous Ref Std?
Difficulties with the use of reference standards 2/2

- Replacement of homologous reference standards
  
  - Workload / Number of animals for the qualification of new homologous reference standards
  
  - For Relative potency assays, difficult to have similar response to previous reference standard for all antigens
    - Need for implementation of correction factors
  
  - For D&T, calibration of homologous Reference Standard using challenge tests
    - Calibration using immunogenicity assay in the future?
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Consistency approach: immunogenicity assay (GMTs) for Pertussis

Relative potency assay (Ph. Eur.)
- Cumulative variability of both vaccine & Ref Std
- Difficulties with replacement of Ref standards

Immunogenicity assay GMTs (WHO TRS 979)
Monitoring of GMTs of positive control

Response

Dose

ED50 product
ED50 reference

Vaccine not less than Reference standard

Acceptance criteria on GMTs of test Vaccine
Consistency approach: immunogenicity assay (GMTs) for D&T

**D & T immunogenicity assays**

- Titer calculation is based on relative activity
  
  \[ \text{Product Titer (IU/mL)} = \text{R.A.} \times \text{Ref Std Titer (IU/mL)} \]

- Workload related to replacement of in-house Reference standards
- Independent behavior of test vaccine and reference vaccine
- Cumulative variability of both vaccine and Reference standard

- Interest for consistency approach (GMTs)?

**Relative activity (R.A.)** = \( \frac{\text{ED}_{50 \text{ product}}}{\text{ED}_{50 \text{ reference}}} \)
### Pros and Cons of Consistency approach (immunogenicity GMTs) for DTP

<table>
<thead>
<tr>
<th>PROS</th>
<th>CONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Described in WHO TRS 979 anx 4 for Pertussis and already implemented at SP for Pertussis immunogenicity assay in US and Canada</td>
<td>Not described for Diphtheria and Tetanus</td>
</tr>
<tr>
<td>Implies changing the specifications</td>
<td>Implies changing the specifications</td>
</tr>
<tr>
<td>- Small change for Pertussis</td>
<td>- Major change for Diphtheria &amp; Tetanus</td>
</tr>
<tr>
<td>Assay variability = variability of test batch response only (instead of cumulative variability of test batch and Ref standard responses)</td>
<td></td>
</tr>
<tr>
<td>No need for a reference vaccine; only a positive control is monitored that ensures validity and consistency of the assay</td>
<td>No direct link with clinical batches</td>
</tr>
<tr>
<td>No more risk of non relevant OOS result due to Reference standard variability and independent behavior of test vaccine and reference standard</td>
<td>However clinical batches to be used to establish relevant Product specific acceptance criteria on GMTs</td>
</tr>
</tbody>
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Perspectives for the future

Current

- In vivo Challenge
  - Multidose assay

Future

- In vivo Immunogenicity
  - Serology by ELISA, TobI, or Vero cells...

In vitro Assays specific for each antigen

DTP Potency assays

- In vivo challenge
  - Single dose assay

Vaccine manufacturing process

- Emphasis on
  - Final Product testing

- More emphasis on in process control of antigen production & formulation

Emphasis on Final Product testing

More emphasis on in process control of antigen production & formulation

In vitro Assays specific for each antigen

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Perspectives for the future *in vitro* analytical tools

**Standardized Processes**

- **Purified antigens**
  - Antigenicity
  - Structure
  - Residual toxicity
  - Aggregation
  - Purity

- **GMP**

- **Product Consistency**

- **Enabling technologies**
  - Better knowledge & monitoring

**Seeds**
- Sequencing
- PFGE
- Toxinotyping

**Final formulated products**
- Antigenicity / content
- Interac° with Adjuvant
- Residual toxicity
- MoA

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SANOFI PASTEUR
Future *in vitro* approaches

- *In vitro* tests is the future of quality control of vaccines in the frame of consistency approach
  - No animals
  - Less variability
  - Better control of product consistency

- Difficulties
  - Not direct surrogate markers of potency
  - Need for information on antigen itself and on its environment
  - Interpretation of many and more specific information
  - Product specific criteria to be defined
  - Worldwide Acceptability
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Thank you