Developing non-animal methods for potency testing of diphtheria and tetanus vaccines – a VAC2VAC case study

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Transition to non-animal based vaccine batch release testing
Webinar 27th March 2024
Vaccine Potency Testing

- A potency assay is regulatory requirement for the release of every lot of a vaccine

- Potency is a critical quality attribute (typically measured using a single method) and is a test of the functional integrity of the antigen

- The potency measurement provides assurance that the vaccine lot will elicit the desired immunological response in the target species, and is an important indicator of stability

- The potency measurement also provides important evidence and assurance for consistency of the manufacturing process – i.e. that new vaccine lots are comparable to those originally used in clinical studies for which efficacy in the target species was demonstrated
Vaccine Potency Testing – Different Approaches are Used

Different approaches are used for routine quality control potency testing for different vaccine types.

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<td><img src="image1.png" alt="Mouse" /></td>
<td><img src="image2.png" alt="Mouse with blood" /></td>
<td><img src="image3.png" alt="In vitro assay" /></td>
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Tetanus vaccine  
Pertussis (acellular) vaccine  
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Newcastle Disease vaccine | COVID-19 mRNA vaccine |
| Technology | ![Mouse](image1.png)  
![Vial](image2.png) | ![Mouse](image1.png)  
![Test Tube](image3.png) | ![Test Tube](image4.png)  
![Plate](image5.png) | ![Test Tube](image6.png)  
![Petri Dish](image7.png) |
Current regulatory requirements for potency testing of DTP vaccines involves significant use of laboratory animals

- Potency testing is **routine** – **every lot** of vaccine must undergo (and pass!) a potency test before release

- D and T are most widely used in a combination vaccine that also contains Pertussis (P) and other antigens

- As is currently the case for D and T, the potency test for the P component also requires animals
Current regulatory requirements for potency testing of DTP vaccines involves significant use of laboratory animals

134M live births (2021)  
*Source: UN population prospects (processed by ourworldindata.org)*

Global coverage with DTP vaccine = 81% (3 dose primary series)  
*Source: WHO (for 2021)*

Total number of DTP vaccine doses needed = 325M  
• 3 doses for 81% of 134M infants

Typical size of DTP vaccine batch = 0.5M  
*Assumption (can vary between different manufacturers and batches)*

Estimated number of batches produced per year = 625  
*Based on the above assumption regarding batch size*

Number of animals used for testing 1 batch for all 3 components = 224  
*“Average” estimate – actual number used per batch depends on multiple factors including the assay model used, number of batches included in a single assay, refinements in place etc.*

Estimated 140,000 animals (mice / guinea pigs) needed for routine potency testing of DTP vaccine components per year

*Note: figure quoted here is illustrative not definitive and based on a number of assumptions – it is likely to be a conservative estimate since it does not account for re-testing invalid assays; additional testing performed by some national control authorities; potency testing of tetanus vaccine for veterinary use; stability testing programmes etc….*
Current situation for potency testing D and T Vaccines

- Although refined animal models are available, and animal models with a reduction in the total animal number needed can be implemented, these animal models have significant limitations:
  - Ethical concerns
  - High cost
  - Prolonged testing period
  - High variability / poor discriminative power

Data from Emmanuelle Coppens, Sanofi Pasteur, previously presented at an IABS 3Rs and consistency testing in vaccine lot release testing conference 2015
A new approach for testing legacy vaccines

- In the VAC2VAC project, we have developed an ELISA that is intended to provide a quantitative (in relative terms) estimate of antigen content.

- The immuno-detection of the antigen uses well characterised monoclonal antibodies (mAbs), directed against relevant epitopes on the target antigen, that are sensitive to changes in the quality/integrity of the antigen.

<table>
<thead>
<tr>
<th>Animal potency test</th>
<th>VAC2VAC ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time required for test</td>
<td>4-6 weeks</td>
</tr>
<tr>
<td>No. animals per assay (for 2 lots)</td>
<td>Assay dependent but can be &gt;200</td>
</tr>
<tr>
<td>Precision of potency estimate</td>
<td>Assay dependent but typically 70 – 130%</td>
</tr>
<tr>
<td>Discriminative power</td>
<td>Poor</td>
</tr>
</tbody>
</table>

- Save animals
- Save time
- Improved ability to identify production/batch issue

*Riches-Duit and Hassall et. al. Biologicals 2021*
VAC2VAC Immunoassay for DT-Vaccines – position in a product lifecycle

1. *In vivo* assays have an important role in early R&D and non-clinical development.

2. For new vaccines, *in vitro* assays can be developed alongside *in vivo* assays used in early R&D and non-clinical development such that they can be taken forward for use in the routine control strategy post-licensure.

3. For legacy vaccines, such as DTP, the *in vivo* potency assays remain part of the routine control strategy post-licensure.

4. The assays developed in the VAC2VAC project were developed with the aim of substituting existing animal potency tests used as part of the routine control strategy for legacy vaccines – either alone, or in combination with other *in vitro* assays as part of a consistency approach that provides the necessary assurance regarding vaccine safety quality and efficacy.
The following slides contain results from the VAC2VAC project showing how the monoclonal antibody ELISA performs with different vaccine products.

All studies were done with both the diphtheria and tetanus ELISA assay and with a wide variety of vaccine products (including veterinary vaccine products for the tetanus assay).

These products represent a range of antigen combinations, formulations and adjuvant types – all examples shown here are for testing the “whole vaccine” – i.e. in the presence of adjuvant.

In the interests of time, representative examples are shown here.
Monoclonal antibody ELISA performance – product dose response curves

Dose response for wide range of product types with clear distinction between:
- high antigen concentration drug substance (black)
- medium antigen concentration drug substance (red)
- vaccines for primary immunisation (green, blue, grey)
- vaccines for booster immunisation (purple, light blue)

All samples shown here (with exception of the pre-adsorbed DTxd) contain an aluminium adjuvant.
Monoclonal antibody ELISA performance – sensitivity / specificity

Diphtheria ELISA
Tri-valent DTP vaccine
Aluminium adjuvant (Human)

mAb ELISA is **sensitive** to relatively small changes (25%) in the amount of antigen present in a combined vaccine.

Note: the 0% sample contains all other vaccine components, adjuvant and excipients but NO diphtheria antigen and highlights the **specificity** of the assay.
Monoclonal antibody ELISA performance – Dilutional Linearity

A “drop out” vaccine consisting of diphtheria and acellular pertussis antigens (plus adjuvant) – but NO tetanus toxoid (TTxd)

Vaccine was then spiked with increasing amounts of TTxd

Estimates for the spiked samples were calculated relative to the normal drug product vaccine sample

Fitted slope not significantly different to 1.0, and intercept not significantly different to 0
Monoclonal antibody ELISA performance – detection of heat-altered samples

Temperature-dependent antigenic changes detected by mAb ELISA after 8 weeks storage at elevated temperature

+4°C (blue line) is the normal storage temperature for the vaccine

Good example of how the mAb ELISA is able to identify changes in antigen quality induced by temperature stress
Monoclonal antibody ELISA performance – consistency with real world samples

Investigating the effect of antigen “age” on measurement of relative antigenicity in the mAb ELISA

Samples tested were drug product lots (dTaP-IPV) made from different batches of bulk purified tetanus toxoid spanning a wide range of “ages”

Estimates are relative to the batch circled and there is no evidence of toxoid-age dependent impact on estimates of relative antigenicity
Monoclonal antibody ELISA performance – transfer to other laboratories

Transfer study protocol was conducted for both the D and T ELISA (data shown here is for the T ELISA)

Each lab performed 3 assays, 2 plates per run (total of 6 plates per lab)

<table>
<thead>
<tr>
<th>Study details</th>
<th>Product + adjuvant type</th>
<th>Intermediate precision GCV%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Partner</td>
</tr>
<tr>
<td>Lab 1 (human)</td>
<td>Tdap + AlPO₄</td>
<td>4.5</td>
</tr>
<tr>
<td>Lab 2 (human)</td>
<td>DTaP-IPV-HepB-Hib + Al(OH)₃</td>
<td>3.4</td>
</tr>
<tr>
<td>Lab 3 (human)</td>
<td>DTaP + Al(OH)₃</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>dTaP + Al(OH)₃</td>
<td>7.2</td>
</tr>
<tr>
<td>Lab 4 (vet)</td>
<td>Ruminant multivalent + Alum</td>
<td>12.3</td>
</tr>
<tr>
<td></td>
<td>Ruminant multivalent + Al(OH)₃</td>
<td>5.3</td>
</tr>
<tr>
<td>Lab 5 (vet)</td>
<td>Ruminant multivalent + Alum</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Equine bivalent + AlPO₄ / ISCOMS</td>
<td>4.6</td>
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Successful transfer of the D and T ELISA has been demonstrated to multiple laboratories

Excellent intermediate precision across a range of different product types
Conclusions

- Proof of concept has been demonstrated for the D and T ELISA, including evidence that the assay may be stability indicating.

- Tet mAb ELISA shown to be suitable for a wide range of human & veterinary tetanus vaccines.

- Assays are robust and successful transfer to other laboratories has been achieved.

- Vaccine manufacturers from the VAC2VAC consortium are further exploring the utility of a mAb immunoassay approach as a potential substitute for current in vivo potency assays.
Availability of purified mAbs

- The mAb pairs used in the D and T ELISAs shown in this presentation are available from www.nibsc.org for laboratories who want to establish and validate these methods.

- As are the mAb pairs for acellular pertussis antigens used in the multiplex assay developed by Sciensano*

- Removes one of the most common barriers to development of alternative methods – restrictions on the availability and use of critical reagents.

*See Vermeulen et. al. JIM, 2023 for details of the acellular pertussis mAbs used in the multiplex assay.
Example of dose response curve in the diphtheria and tetanus mAb ELISA for a whole cell pertussis DTP combination vaccine

The VAC2VAC project was a European initiative that focused on vaccines used in Europe (which are predominantly acellular pertussis DTaP combination vaccines)

As a result, we did not evaluate performance of the mAb ELISA with whole cell pertussis DTP combination vaccines – although these are the vaccines received by most of the world's infants

Data here is therefore very preliminary but suggests that a wP component does not negatively impact the dose response curve for D or T

More work needed for wP vaccines!
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  Maxime Vermeulen

+all industry partners in the VAC2VAC consortium; project coordinator and other consortium members

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