AFSA COLLABORATION

Perspective on the future of wP potency testing

Laura Viviani, SciEthiQ for Humane Society International

January 30th, 2025

Implementation plans for replacement of animal testing for human vaccines

Goal

- Accelerate the implementation and regulatory acceptance of non-animal approaches for human vaccine batch-release testing with a specific focus on developing countries,
- Advance the dialogue toward global regulatory alignment while generating local competencies together with direct experience and confidence in change

Approach

- Creation of stakeholder- and/or country-specific implementation plans, or roadmaps, to include non-animal testing opportunities in the regulations, pharmacopoeias, and in the human vaccine manufacturers' quality-control routine testing

Replacement Implementation Plans
for Human Batch Release Testing

Who is involved

- HSI supports stakeholders from India, Brazil, South Korea, Indonesia to build or review their replacement plans
- International Steering Committee (ISC) with representatives from pharmacopoeias, regulatory agencies, industry associations, international organizations and funding bodies submitting a joint position statement on the transition to innovative non-animal batch release testing

Knowledge Sharing

- 2024 workshops and webinars recordings and presentations available on the Animal Free Safety Assessment Collaboration (Policy and regulations theoretical aspects of the in vivo to in vitro transition; rFC; MAT)
- 2025 workshops and project's final conference

DTaP and DTwP

Globally distributed and biggest animal use

- DTP (acellular and wholecell) combined vaccines are part of immunization programmes worldwide and like few other legacy vaccines relies on animal testing for safety and potency test for the production and release
- Regulatory requirements might slightly vary from TRS to local pharmacopoeias, legislations and regulations but the animal use per batch requires use of mice or guinea-pigs or both up from 2 to 20 animals per safety test (production and/or release) and over 140 for potency tests (challenge) **per batch, if no re-test is needed**
- Hundreds of batches are produced annually
- The time required to performs animal testing requires a minimum of a week time (safety) to over 40 days (potency)

- They are a key example of products that might benefit from a transition to non animal approaches

DTaP and DTwP

Impact of the transition from in vivo to in vitro

- They are a key example of products that might benefit from a transition to non animal approaches. How?

- Reduce reliance on variable animal testing that requires:
  - well trained personnel
  - strong adherence to safety procedures to avoid issues to personnel exposed to toxins and bacteria
- Allow investments on:
  - Methodologies that ensure more specific safety and potency measurements, increasing overall product quality monitoring and the overall product life-cycle
  - Making critical reagents availability, affordability and accessibility
  - Update quality standards and increase personnel's capabilities on innovative and state of the art production and Quality Control for both manufacturers and NCLs
  - Update guidelines, pharmacopoeias and regulations towards a future proof approach allowing innovation to be integrated more easily and benefit vaccines' access

Whocell-Pertussis Potency Testing

Historical vaccine with an historical assay

- For wP vaccines or wP-based combination vaccines (DTwP, DTwP-Hib, DTwP-HB, DTwP-IPV, DTwP-HB-Hib) the Kendrick test is the only internationally agreed official test for batch release
- Kendrick test or mouse protection test (MPT) was developed by Pearl Kendrick in 1947
- The potency is assessed by comparing the dose necessary to protect mice against the effect of a lethal dose of *B. pertussis*, strain B18323, injected i.c., with the quantity of a reference preparation needed to give the same protection
- The vaccine passes the test if the stated potency is not less than 4 IU/ single human dose and the lower confidence limit of the estimated potency is not less than 2 IU/single human dose
- Assay validation is essential and requires good appreciation of assay condition as choice of mice strains, ED50 of immunization dose, and preparation of the challenge dose (LD50)
- It is necessary to evaluate the assay conditions established for mono-components wP vaccines when applying the assay to the evaluation of wP in combined vaccines . Possible interference by the antigen amount, adjuvant, excipient composition between a mono-valent reference preparation and a combined vaccine could potentially affect the potency test.

AFSA COLLABORATION

Why an alternative test to the Kendrick test?

Assay challenges

- Animal welfare: i.c. injection causes pain and distress to mice
- Biohazard: microbiological operations (challenge suspension production and control)
- Technically high demanding which requires highly qualified and trained personnel
- High variability of the test
- Is the strain B. Pertussis 18323 still the right challenge strain, as new clones carrying variants of genes encoding immunogenicity and pathogenicity associated antigens have been evolved in response to vaccination?

Review with extended bibliography in Expert Rev Vaccines, 2014,13:1175-1182

Respiratory challenge test or lung clearance mouse model: mice are infected with an intranasal or aerosol challenge of *B. pertussis*	**Nitric Oxide production by macrophages from mice immunized with wP in response to *in vitro* re-stimulation with bacterial antigens.**	**Pertussis Serological Potency Test in mice**

These methods, even if suitable after appropriate validation, are very difficult to be performed on a QC setting and require special or custom-made equipment

AFSA COLLABORATION

The PSPT Explored

30 years of work

- 1994 described by Van der Ark in Biologicals
- 2000 results of a collaborative study (5 laboratories) published by Van der Ark in Biologicals
- 2 ECVAM studies published in PharmEuropa Bio in 2008
- EDQM BSP104 (closed in 2024, no results published)
- **Developing Countries Vaccine Manufacturers Network PSPT Study (10 laboratories, results available at https://dcvmn.org/pspt-consortium/)**

Conclusion of the DCVMN PSPT assessment:

- ELISA optimization
  - Conjugate dilution (or DOE)
  - Starting dilution of PC
  - Dilution increment
  - Expectations for acceptable performance
  - Use of in-house reference vs International Standard
- *in vivo* optimization
  - Dose-ranging of test vaccine(s)
  - Rules for data screening (no. negatives/missing mice, dose elimination, …)
  - Additional/alternative bases for validity criteria (e.g., USP equivalence approach; vs ANOVA)

Future of the wP Testing

PSPT vs New Method Development?

- Discussions with the stakeholders results in a general interest to continue to explore the PSPT and invest on its product specific optimization but with experts that could guide the laboratories in their investment on the method
  - Short/Medium term strategy (2-5 years)
  - Risk exists to not be successful in further optimizing the method and need to rely on in-house standard and challenge strain
- Invest on a completely new assay, following the work done by the VAC2VAC Consurtium on DTaP
  - Long term strategy (over 5 years)
  - Risk exists to not be able to find the critical quality attributes and a suitable assay
  - Preliminary work has started –> next presentation

- What's your view?


AFSA
COLLABORATION
Thank you!
https://www.afsacollaboration.org/biologicals/