From *in vivo* to *in vitro* for DTaP (Diphtheria, Tetanus, acellular Pertussis) potency testing

Emmanuelle Coppens, Global Analytical Expert, Global Analytical Sciences, Sanofi, Marcy l'Etoile

December 03, 2025

AGENDA

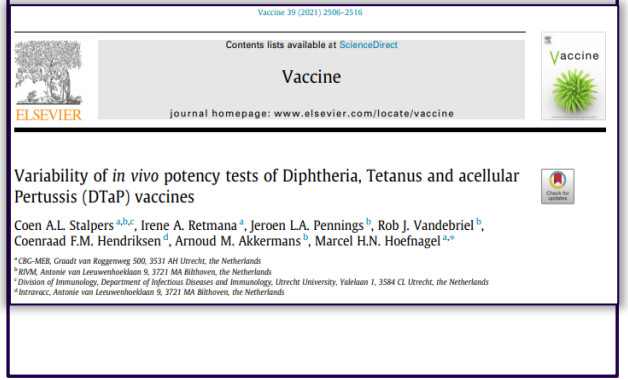
1 Current *in vivo P*otency Testing

2 VAC2VAC Collaboration

3 Sanofi Antigenicity Assays

Current *in vivo* potency testing for DTaP vaccines : Are they still scientifically appropriate?

High variability of in vivo potency testing of DTaP vaccines



Vaccine 39 (2021) 2506–2516

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Variability of *in vivo* potency tests of Diphtheria, Tetanus and acellular Pertussis (DTaP) vaccines

Coen A.L. Stalpers [a,b,c], Irene A. Retmana [a], Jeroen L.A. Pennings [b], Rob J. Vandebriel [b], Coenraad F.M. Hendriksen [d], Arnoud M. Akkermans [b], Marcel H.N. Hoefnagel [a,e]

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- These ***in vivo*** assays (on guinea pigs or mice) :
  - are **labor intensive**, **costly, lengthy**
  - remain **an ethical concern**
  - have **high inherent variability**
  - show **poor discriminative power**
  - show high **invalidity rate**
  - can lead to **false out of specification** results

Their use in routine batch release testing is now questionable versus more scientifically relevant *in vitro* methods

From *in vivo* to *in vitro* for DTaP potency testing (only remaining *in vivo* potency test)

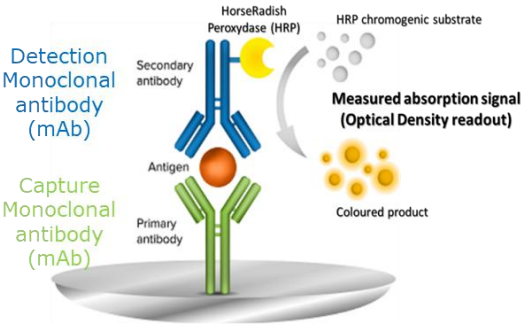
CURRENT

- different antigen and product -specific tests

	US	EU/ International		
D	G.Pig serum neutralization (USPHS)	G.Pig Serological	G.Pig intradermal challenge	G. Pig Single Immunogenicity Assay
T	G.Pig serum neutralization (USPHS)	G.Pig Serological	Mouse paralytic challenge	
aP	Mouse Serological			

FUTURE

Sandwich ELISAs for all antigens and all markets



Moving to *in vitro* is the opportunity to move away from market specific testing and complex testing profiles

D: Diphtheria antigen
T: Tetanus antigen
aP: pertussis antigens

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VAC2VAC Collaboration

IMI Vac2Vac

Vac2Vac stands for Vaccine batch to Vaccine batch comparison by consistency testing*

Industry, Academia & Regulators working together to substitute animal assays for established vaccines

http://www.vac2vac.eu/

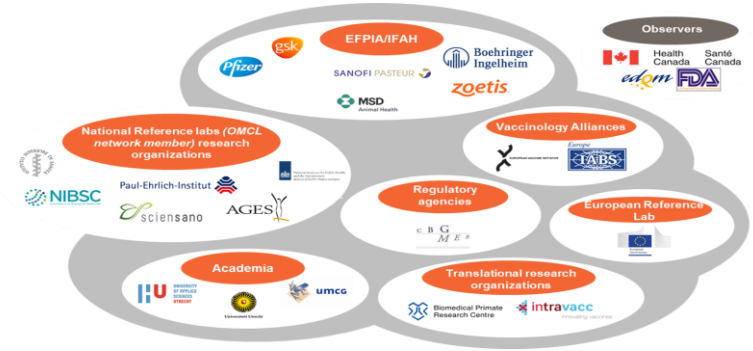
23 European Partners

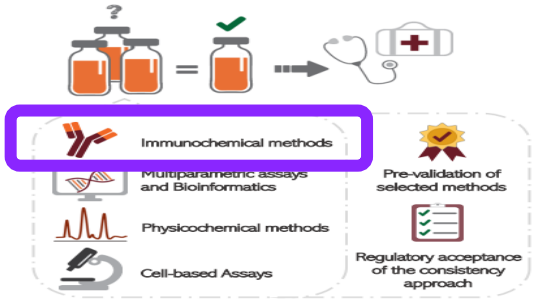
5 years project (Mar 2016 – Feb 2022)

16 M€ total budget

Products: **7 Vaccine Franchise**
5 veterinary + 2 human + 1 adjuvant

33 tasks organised in **4 technical work packages** to replace animal assays in Quality Control





Objective

- **Demonstrate proof of concept of novel physicochemical, immunochemical or biological *in vitro* tests for vaccines**
- **Facilitate regulatory acceptance**

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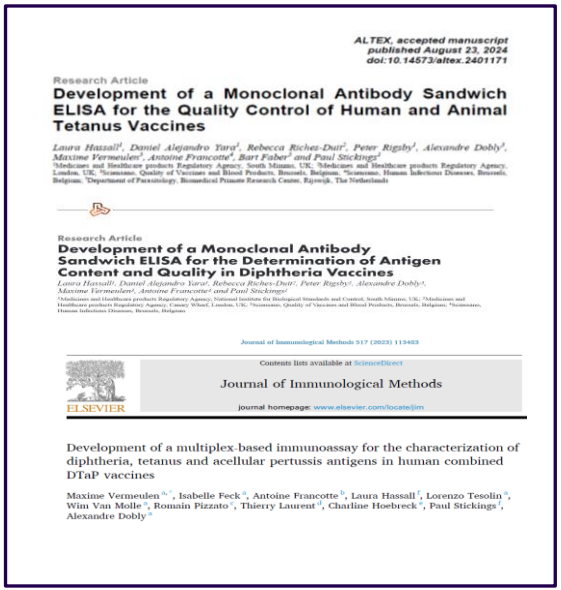
VAC2VAC Outputs : Immunoassays for DTaP vaccines & available reagents

Immunoassays



ALTEX, accepted manuscript published August 23, 2024 doi:10.14573/altex.2401171

Research Article

Development of a Monoclonal Antibody Sandwich ELISA for the Quality Control of Human and Animal Tetanus Vaccines

Laura Hassall[1], Daniel Alejandro Yara[1], Rebecca Riches-Duit[1], Peter Rigsby[1], Alexandre Dobly[1], Maxime Vermeulen[2], Antoine Francotte[2], Bart Faber[2] and Paul Stickings[1]

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Research Article

Development of a Monoclonal Antibody Sandwich ELISA for the Determination of Antigen Content and Quality in Diphtheria Vaccines

Laura Hassall[1], Daniel Alejandro Yara[1], Rebecca Riches-Duit[1], Peter Rigsby[1], Alexandre Dobly[1], Maxime Vermeulen[2], Antoine Francotte[2] and Paul Stickings[1]

[1]Medicines and Healthcare products Regulatory Agency, National Institute for Biological Standards and Control, South Mimms, UK; [2]Medicines and Healthcare products Regulatory Agency, Canary Wharf, London, UK; [3]Sciensano, Quality of Vaccines and Blood Products, Brussels, Belgium; [4]Sciensano, Human Infectious Diseases, Brussels, Belgium

Journal of Immunological Methods 517 (2023) 113403

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

ELSEVIER

Development of a multiplex-based immunoassay for the characterization of diphtheria, tetanus and acellular pertussis antigens in human combined DTaP vaccines

Maxime Vermeulen [a,*], Isabelle Feck [a], Antoine Francotte [b], Laura Hassall [a], Lorenzo Tesolin [a], Wim Van Molle [a], Romain Pizzato [a], Thierry Laurent [a], Charline Hoebreck [a], Paul Stickings [a], Alexandre Dobly [a]

Characterization of mAbs

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

ELSEVIER

Research paper

Characterisation of diphtheria monoclonal antibodies as a first step towards the development of an *in vitro* vaccine potency immunoassay

Rebecca Riches-Duit [a,1], Laura Hassall [a,1], Amy Kogelman [b], Janny Westdijk [b], Alexandre Dobly [c], Antoine Francotte [c], Paul Stickings [a,*]

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

ELSEVIER

Research paper

Characterisation of tetanus monoclonal antibodies as a first step towards the development of an *in vitro* vaccine potency immunoassay

Rebecca Riches-Duit [a,1], Laura Hassall [a,1], Amy Kogelman [b], Janny Westdijk [b], Shalini Rajagopal [a], Bazbek Davletov [c], Ciara Doran [a], Alexandre Dobly [d], Antoine Francotte [d], Paul Stickings [a,*]

Sustainability plan

VAC2VAC

NEWSLETTER Vol. VI May 2022

Vaccine batch to vaccine batch comparison by consistency testing

VAC2VAC SUSTAINABILITY PLAN

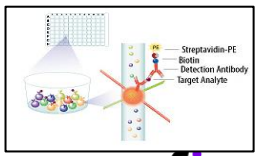
NIBSC

Implementation of the sustainability plan

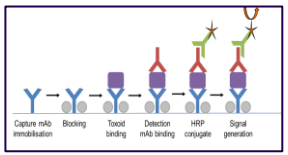
Monoclonal antibodies available at the NIBSC catalogue (www.nibsc.org)

After being identified as critical reagents, an agreement has been made within the VAC2VAC consortium allowing for VAC2VAC partner NIBSC to be entrusted to manage the handling, distribution, and future production of monoclonal antibodies needed in DTaP ELISA and Luminex assays developed in VAC2VAC. Depositor agreements between NIBSC and other owners of the monoclonal antibodies (GSK, Sanofi, and Intravacc B.V) have been signed whereby depositors agree to supply the material and hybridoma information to NIBSC. NIBSC will make the monoclonal antibodies available to the public subject to a handling fee to cover operational costs and future replacement of antibody batches.

MULTIPLEX



ELISA



- **Proof of concept for DTaP immunoassays**
- **A pair of monoclonal antibodies (mAbs) was selected for each antigen**
- **A model was created for sustainable supply of these critical reagents through MHRA (Medicines and Health care products Regulatory Agency)**

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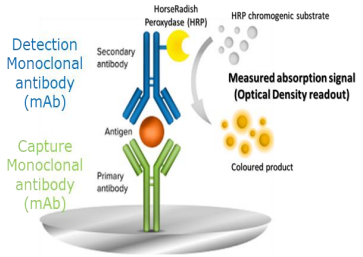
Remaining activities for manufacturers



- Further **development** and **optimization** of assays to **specific products,** including the potential selection of alternative mAbs as analytical tool, that allow for **optimal assay performance.**

- Demonstration of **suitability** further to V2V deliverables, including:
  - mAbs characterization
  - Studies demonstrating assays' capacity to :
    - detect changes in antigen quantity and quality
    - serve as stability indicators (ability to detect product degradation)
  - Comparison to *in vivo* assays.

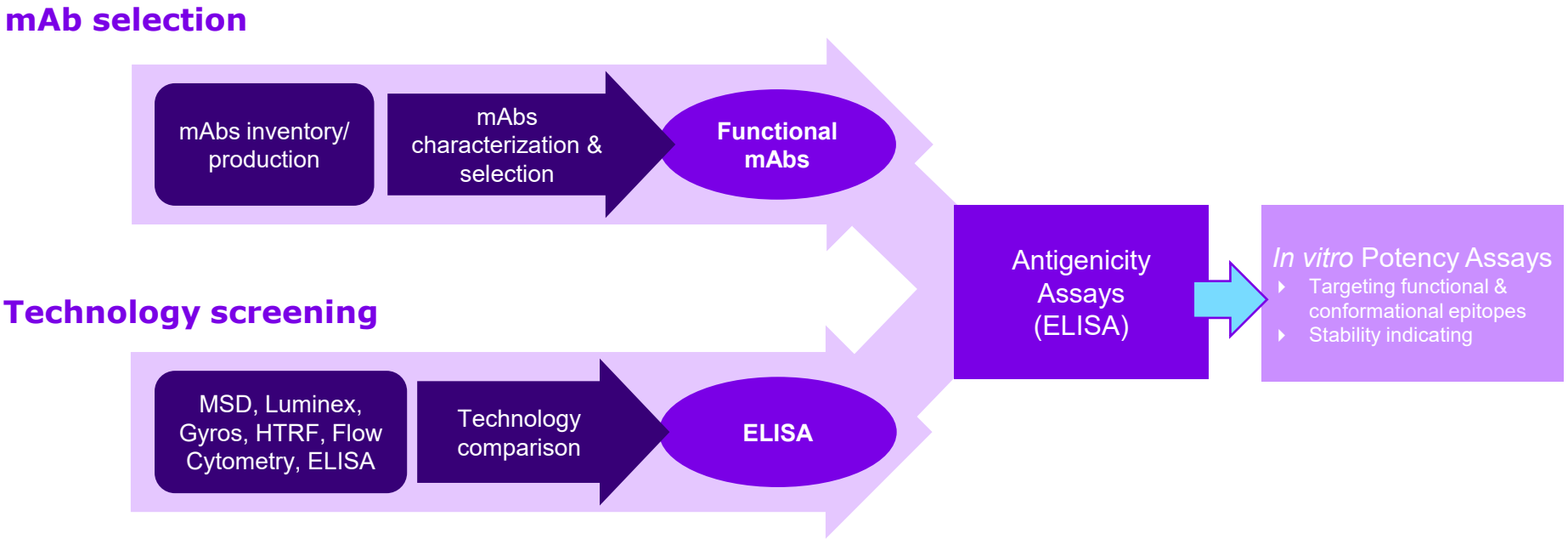
- Full **method validation.**

- Setting **product acceptance criteria**

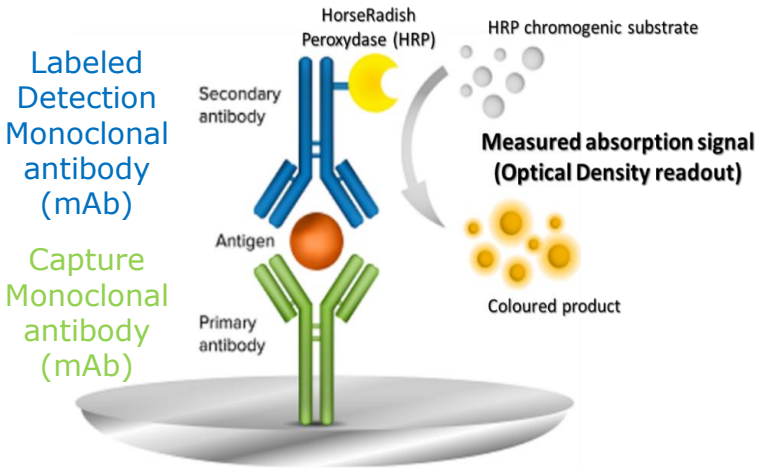
- **Publication** of additional data (e.g.: pertussis mAbs characterisation and assay development).

Sanofi Vaccines Antigenicity Assays

Overall strategy for the development of in vitro assays to substitute the in vivo potency assays

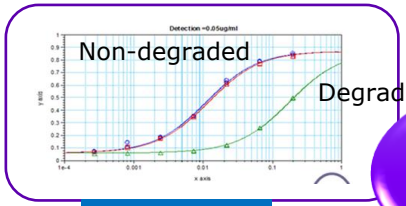


Antigenicity ELISA assays_One Assay principle for all DTaP combinations



ELISAs ID CARD	
ELISA format	Sandwich ELISA (96well plate)
Readout	Colorimetric (HRP)
In-house Reference standard (RefStd)	Non-homologous (non adsorbed DS)
Unitage	Arbitrary Antigenicity Units/mL (AU/mL)
Curve-fitting model	4PL
Parallelism stat model	Equivalence
Acquisition and analytical Software	SoftmaxPro

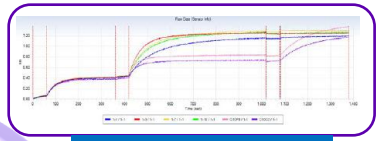
Suitability demonstration : Characterization of mAbs & Stability indication studies



ELISA

Stability Indication

Specificity and Affinity



BLI, SPR: Epitope binning

mAbs

Function

TOXINS

PT DT TT

e.g. NEUTRALIZATION
--IN VITRO
- IN VIVO

ADHESINS

PRN FIM FHA

e.g. AGGLUTINATION

Epitope mapping

Conformational epitope

HDX-MS Epitope mapping

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Method Validation as per ICHQ2 guideline

Test Method Validation Parameter	Acceptable limit
Accuracy	The average recovery of antigenicity for each theoretical expected concentration level of mock samples should be between 80-120%
Precision **(Intermediate Precision and Repeatability)**	The %CV for repeatability and intermediate precision must be less than or equal to 15% (%CV≤15%) for each of mock sample
Specificity	Mock sample which lacks antigen of interest must not generate a valid result
Range	The reportable range of the assay will be determined with acceptable accuracy, precision for each impacted product.

Inspired from B. Ljutic, WCBP 2025
Pertactin antigen ELISA for Quadracel

ICHQ2(R2) :
Acceptance criteria on linearity no more required
Acceptance criteria applicable to confidence intervals for accuracy and precision : ongoing implementation

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Guidelines for the transition from in vivo to in vitro potency

Chapter 5.2.14 : Substitution of in vivo method(s) by in vitro method(s) for the quality control of vaccines - General Considerations

- Tests methods used for **QC** are intended to monitor **production consistency**
  → the inherent variability of ***in vivo* assays** can make them **less suitable** than appropriately designed *in vitro* assays for that purpose

- ***In vitro* bioassays** can mimic **specific elements** of complex *in vivo* responses :
  - The **quality attribute** of the product will likely be **assessed differently**
  - with generally **lower variability** and **higher sensitivity**

  → a typical one-to-one assay comparison may not be appropriate for reasons unrelated to the suitability the *in vitro* method(s) used

- Assays must be :
  - **fit for purpose** (including stability indicating capacity)
  - **properly validated-**Not necessarily validated through collaborative multicentric studies and widely applicable to a range of products

Guidelines on the replacement or removal of animal tests for the quality control of biological products

World Health Organization

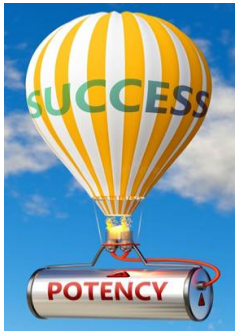
Adopted on the recommendation of the Eighty-first meeting of the World Health Organization Expert Committee on Biological Standardization, 13–16 October 2025

8. Potency testing

Despite the challenges, different approaches can be used to assess the suitability of in vitro potency tests. The goal is to have sufficient data demonstrating that the in vitro assay(s) are capable of measuring CQAs that have been scientifically justified to be relevant both for efficacy and for ensuring that the product quality profile stays within the desired range. Any changes in quality should be assessed in comparison to the expectations defined for the same CQAs of lots shown to be safe and efficacious during clinical studies or routine use.

When replacing an existing in vivo assay for a legacy product, a statistically meaningful number of commercial lots tested with the in vivo assay should be evaluated using the new in vitro test to establish a baseline for specification setting. If available, borderline pass/fail lots, artificially altered lots or specifically manufactured lots are particularly useful in identifying relevant specification limits. For new products, in vitro tests for CQAs should be integrated as

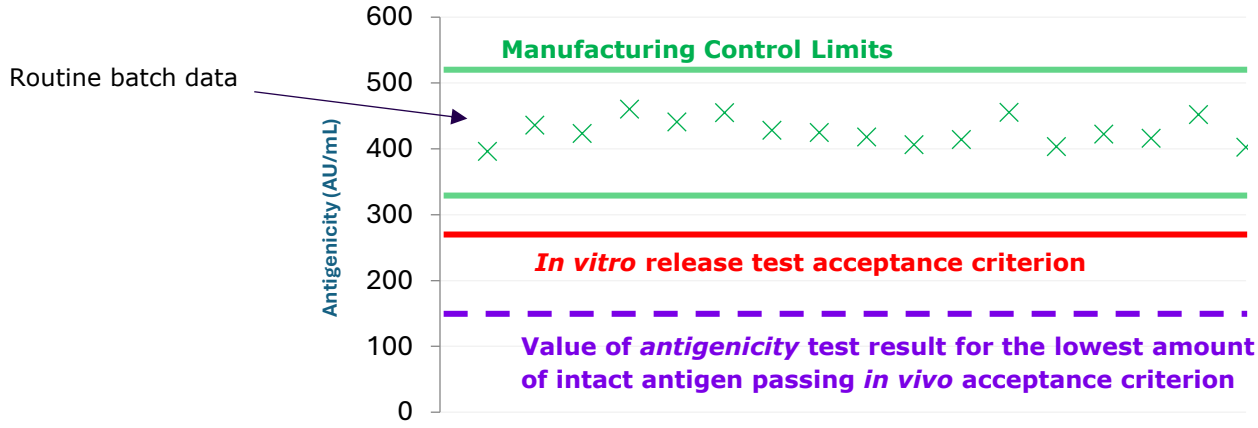
Bridging with current *in vivo* testing and acceptance criteria setting



- Manufacturer continues to supply batches that meet the clinically relevant specifications
- OOT lots are investigated according to GMP system
- Manufacturer has flexibility for Life Cycle Management

OOT: Out of trend = out of control limits

- Comparison of antigenicity assay with current in vivo to demonstrate the alternative method is **at least as discriminative** (use of **purposely manufactured samples** with different concentrations of intact antigen)
- This comparison **could be used** to establish **acceptance criteria**
- In all cases manufacturing consistency is monitored through **product control chart & trend analysis**



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Current status, challenges and next steps

Current status	• **Pre-submission consultations and submissions engaged in 2020** • **Approval granted by FDA & EMA & BRDD (Health Canada), MHRA for some antigens and products** • **Antigenicity assay for PRN (Pertactin) antigen implemented in routine QC testing for some products**
Challenges	• ***In vitro* alternative not yet described in Pharmacopoeias** • **Setting clinically relevant acceptance criteria that are not too stringent versus current *in vivo* assays**
Next steps	• Continue **development & validation for all Sanofi products and antigens** • Continue **scientific consultations & regulatory submissions** • Continue to **contribute to revisions of compendia**

Antigenicity ELISA assays_Publications and Communications

vaccines

MDPI

Article

Development of an In Vitro Test Method to Replace an Animal-Based Potency Test for Pertactin Antigen in Multivalent Vaccines

Jason Szeto [1,*], Aruun Beharry [2,†], Tricia Chen [1], Eric Zholumbetov [3], Emilie Daigneault [4], Marin Ming [1], Iain Lounsbury [1,3], Nelson Eng [1], Nemika Thangavadivel [1,5], Robbie Jin [1,6], Aurélie Denis-Jacquot [1,§], Bahram Behnam Azad [4,‖], Meili Li [4], Diana Keizner [4], Marcus Liu [4], Sophia S. F. Lee [7], Kai He [7] and Beata Gajewska [8]

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‖ Current address: QC Product Testing and Lab Systems, Resilience, 2585 Meadowpine Blvd, Mississauga, ON L5N 8H9, Canada.

check for updates

Citation: Szeto, J.; Beharry, A.; Chen, T.; Zholumbetov, E.; Daigneault, E.; Ming, M.; Lounsbury, I.; Eng, N.; Thangavadivel, N.; Jin, R.; et al. Development of an In Vitro Test Method to Replace an Animal-Based Potency Test for Pertactin Antigen in Multivalent Vaccines. *Vaccines* **2023**, *11*, 275. https://doi.org/10.3390/vaccines11020275

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Published: 27 January 2023

Abstract: There is increasing interest to replace animal-based potency assays used routinely to test vaccines, since they are highly variable, are costly, and present ethical concerns. The development of relevant in vitro assays is part of the solution. Using pertactin (PRN) antigen as an example in DTaP-IPV (diphtheria, tetanus, acellular pertussis, and inactivated poliovirus) vaccines, a PRN antigenicity ELISA was developed using two monoclonal antibodies with a high affinity to unique PRN epitopes, relevance to human immune responses, and evidence of functionality. The ELISA measured consistent PRN antigenicity between the vaccine lots and was validated to demonstrate its accuracy, precision, linearity, and specificity. Notably, the PRN antigenicity ELISA was more sensitive than the mouse-based potency test and could more effectively differentiate between degraded and intact vaccine lots compared to the in vivo test. From these studies, the PRN antigenicity ELISA is proposed as an in vitro replacement for the in vivo potency test for PRN in DTaP-IPV-based formulations. Important considerations in this study included comprehensive antibody characterization, testing of multiple vaccine lots, method validation, and comparison to animal-based potency. Together, these factors form part of an overall strategy that ensures reliable and relevant in vitro assays are developed to replace animal tests.

Keywords: antigenicity; pertactin; ELISA; replacing animal testing; in vitro testing; 3Rs; vaccines

1. Introduction

As part of routine potency testing for vaccine product release, manufacturers continue to rely on animal-based test methods, particularly for well-established vaccines that have

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Emmanuelle Coppens is a Sanofi employee and may hold shares and/or stock options in the company.

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