Medicines & Healthcare products Regulatory Agency

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

Laura Hassall, Rebecca Riches-Duit, Daniel Yara,
Paul Stickings

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..

Approach	Animal Assay (**challenge** test)			
Examples	Diphtheria vaccine Tetanus vaccine Pertussis (acellular) vaccine Pertussis (whole cell) vaccine			
Technology				

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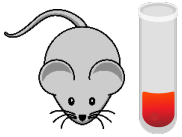
Technology				

Vaccine Potency Testing – Different Approaches are Used

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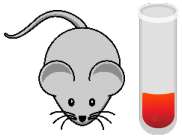
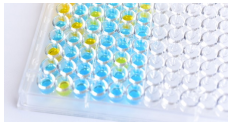
Approach	Animal Assay (**challenge** test)	Animal Assay (**serological** test)	In vitro assay (developed as a **replacement** for an animal assay that was used when the vaccine was first introduced)	
Examples	Diphtheria vaccine Tetanus vaccine Pertussis (acellular) vaccine Pertussis (whole cell) vaccine	Diphtheria vaccine Tetanus vaccine Pertussis (acellular) vaccine	Hepatitis B vaccine Newcastle Disease vaccine	
Technology				

Vaccine Potency Testing – Different Approaches are Used

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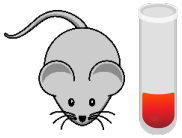
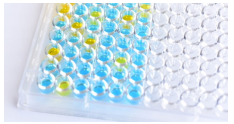
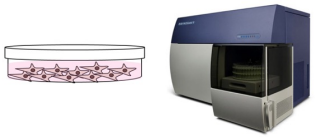
Approach	Animal Assay (**challenge** test)	Animal Assay (**serological** test)	In vitro assay (developed as a **replacement** for an animal assay that was used when the vaccine was first introduced)	In vitro assay (developed and implemented for the **initial release** of the vaccine)
Examples	Diphtheria vaccine Tetanus vaccine Pertussis (acellular) vaccine Pertussis (whole cell) vaccine	Diphtheria vaccine Tetanus vaccine Pertussis (acellular) vaccine	Hepatitis B vaccine Newcastle Disease vaccine	COVID-19 mRNA vaccine
Technology				

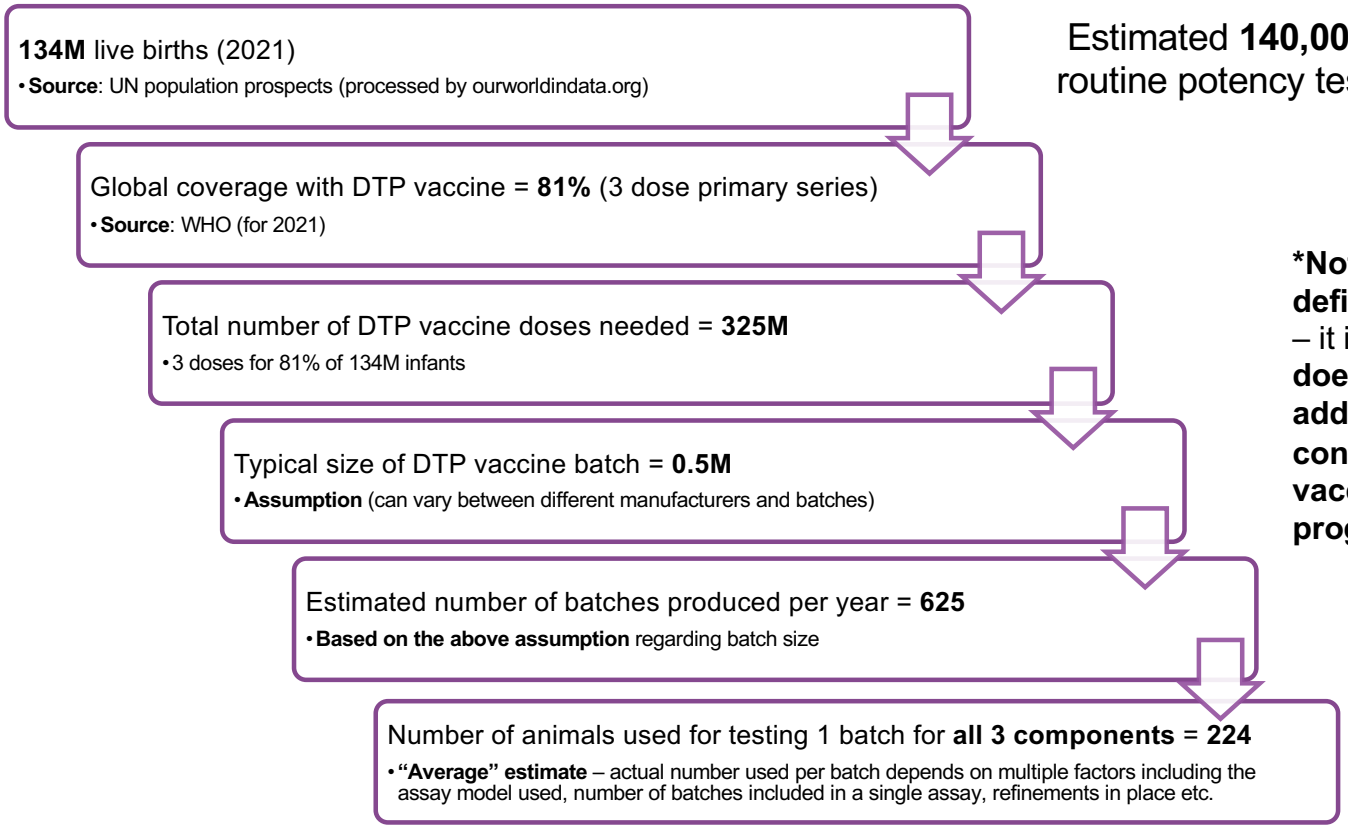
Current regulatory requirements for potency testing of DTP vaccines involves significant use of laboratory animals

- 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

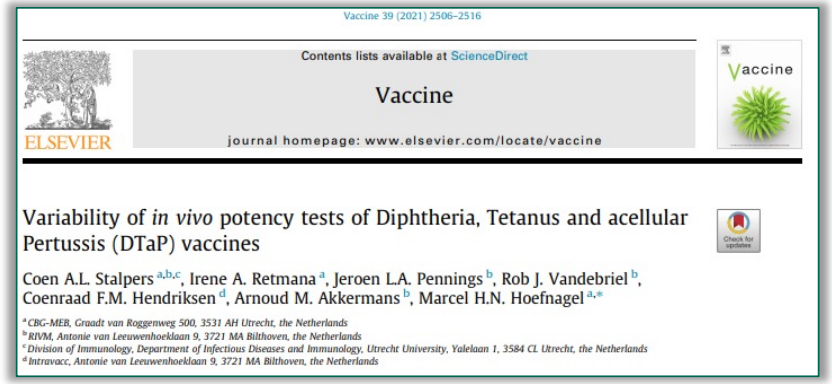


Estimated **140,000 animals** (mice / guinea pigs) needed for routine potency testing of DTP vaccine components **per year**

***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



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,*]

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[d] Intravacc, Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, the Netherlands

AlPO4 adjuvanted combination vaccine

T content Lf/mL	Potency (challenge)
15	Conform
10	Conform
2	Non conform

÷ 7.5

÷ 8

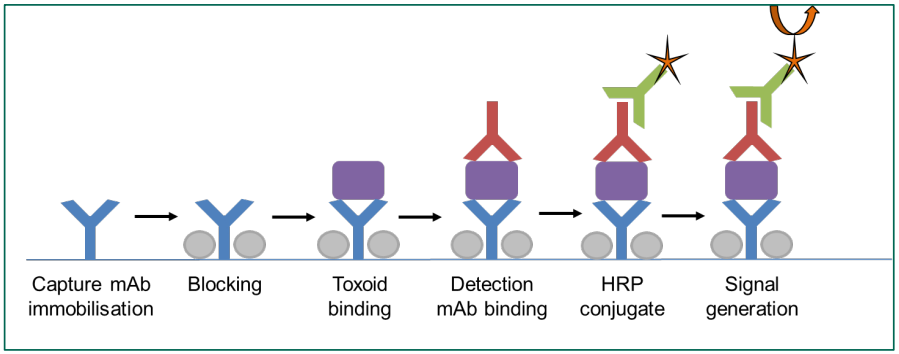
D content Lf/mL	Potency (challenge)
40	Conform
30	Conform
5	Non conform

*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*



	Animal potency test 	VAC2VAC ELISA 
Time required for test	4-6 weeks	2 days
No. animals per assay (for 2 lots)	Assay dependent but can be >200	0
Precision of potency estimate	Assay dependent but typically 70 – 130%	~90 – 110%
Discriminative power	Poor	Good

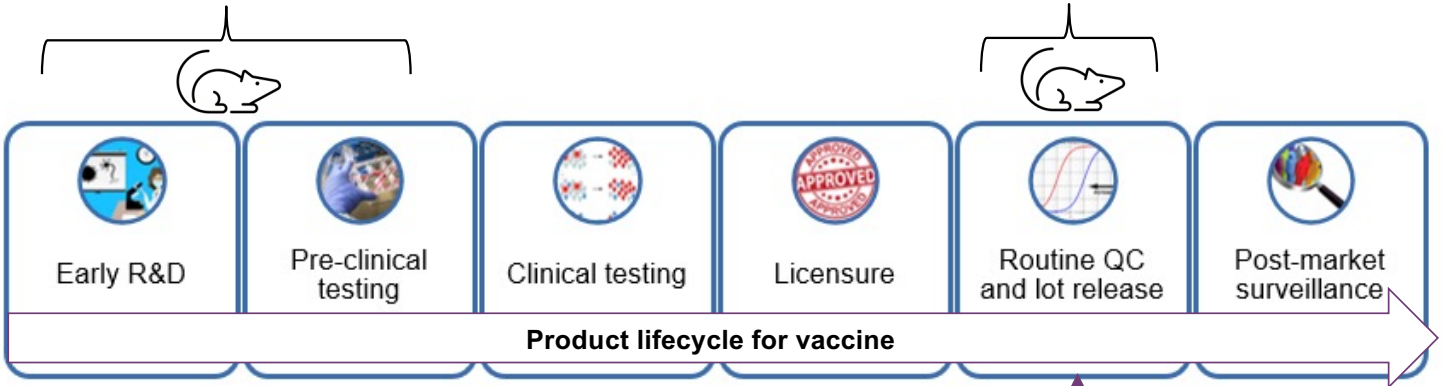
✓ **Save animals**
✓ **Save time**
✓ **Improved ability to identify production/batch issue**

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

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



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

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

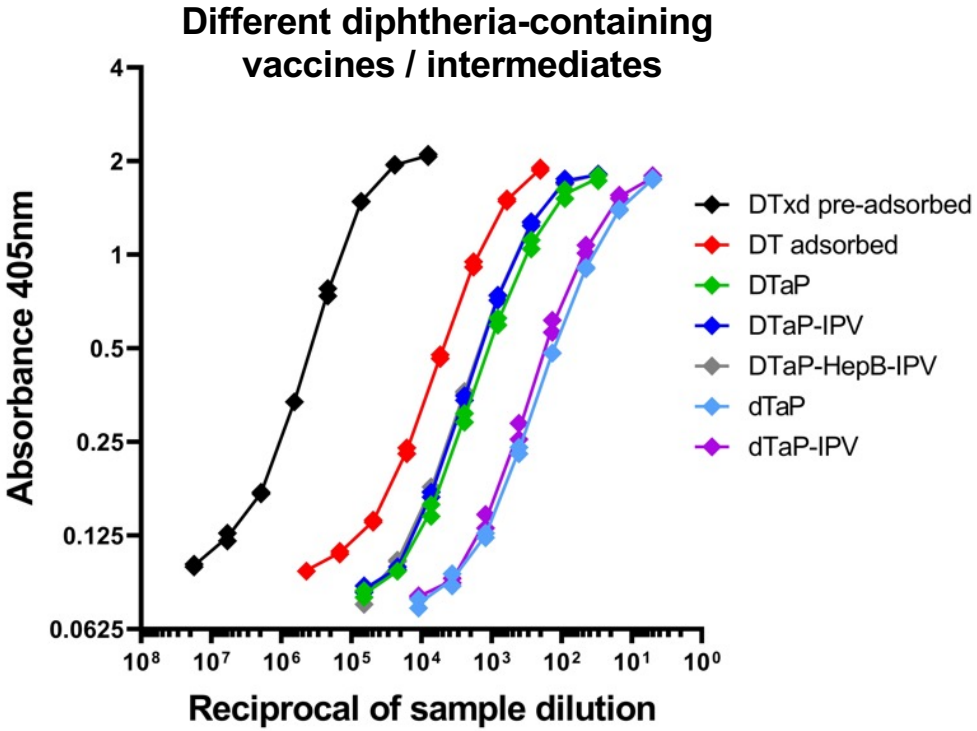
The following slides contain results from the VAC2VAC project **showing how the monoclonal antibody ELISA performs** with **different vaccine products**

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

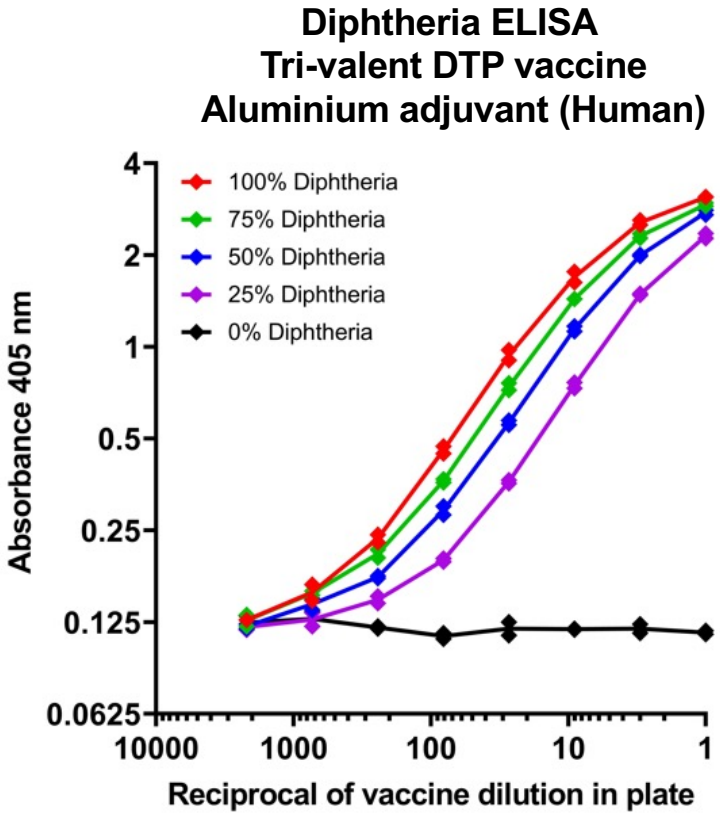


Dose response for **wide range of product types** with clear distinction between:

- 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

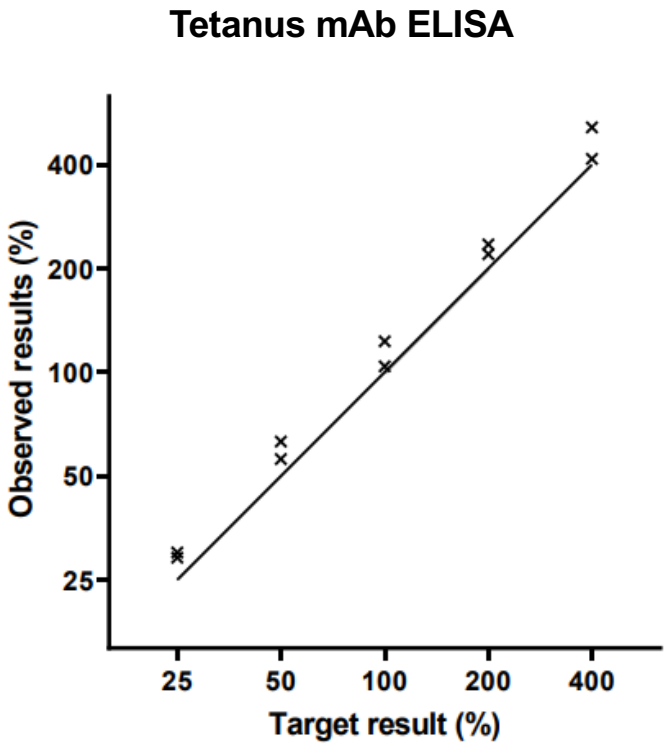
Monoclonal antibody ELISA performance – sensitivity / specificity



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



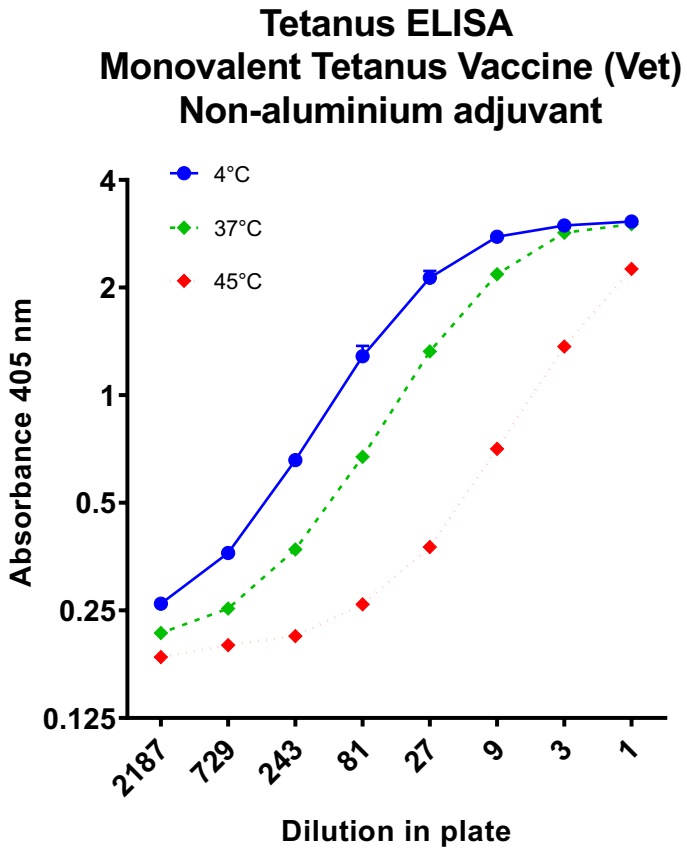
A “drop out” vaccine consisting of **diphtheria** and **acellular pertussis** antigens (plus adjuvant) – **but NO tetanus toxoid (TTxd)**

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

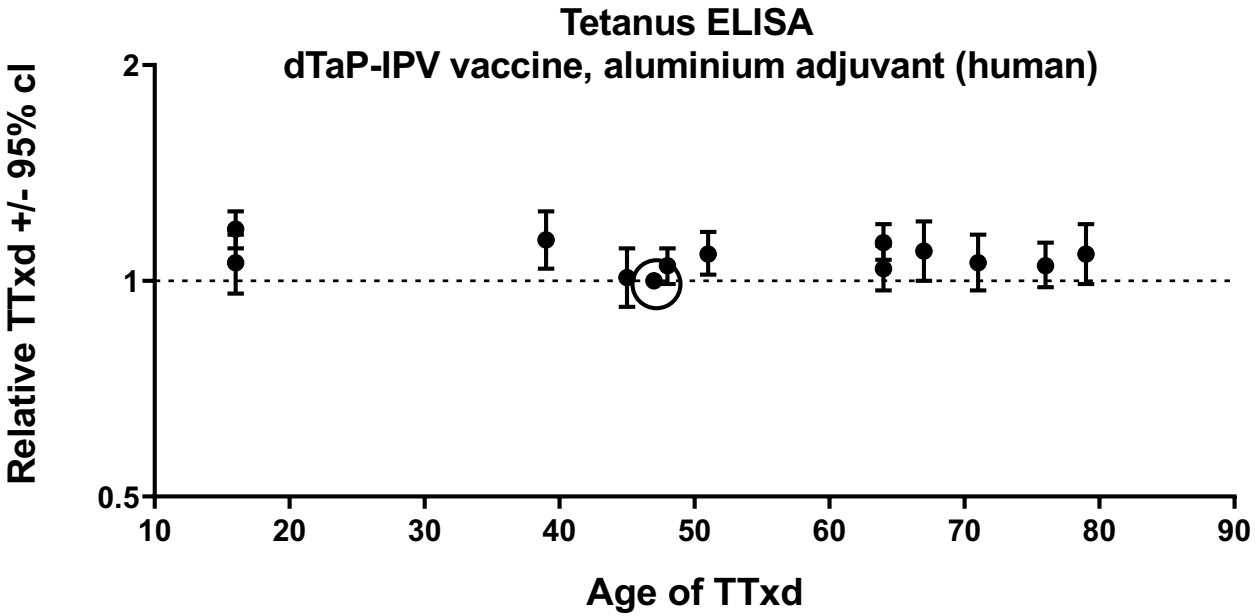


Temperature-dependent antigenic changes detected by mAb ELISA after 8 weeks storage at elevated temperature

+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

Study details	Product + adjuvant type	Intermediate precision GCV%	
		Partner	MHRA
Lab 1 (human)	Tdap + $AlPO_4$	4.5	3.8
Lab 2 (human)	DTaP-IPV-HepB-Hib + $Al(OH)_3$	3.4	4.4
Lab 3 (human)	DTaP + $Al(OH)_3$	2.7	3.9
	dTaP + $Al(OH)_3$	7.2	3.3
Lab 4 (vet)	Ruminant multivalent + Alum	12.3	13.5
	Ruminant multivalent + $Al(OH)_3$	5.3	7.0
Lab 5 (vet)	Ruminant multivalent + Alum	1.8	1.9
	Equine bivalent + $AlPO_4$ / ISCOMS	4.6	6.6

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)

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**

Diphtheria ELISA paper now published – tetanus manuscript submitted

ALTEX, accepted manuscript
published August 7, 2023
doi:10.14573/altex.2305251

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[2], Peter Rigsby[1], Alexandre Dobly[3], Maxime Vermeulen[3], Antoine Francotte[4], Paul Stickings[4]
[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

Multiplex approach using Luminex technology also published

Journal of Immunological Methods 517 (2023) 113483

Contents lists available at ScienceDirect

Journal of Immunological Methods

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

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 [f], Lorenzo Tesolin [a], Wim Van Molle [a], Romain Pizzato [c], Thierry Laurent [d], Charline Hoebreck [e], Paul Stickings [f], Alexandre Dobly [a]

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[c] Sanofi, Analytical Sciences Department, France
[d] GlaxoSmithKline, Belgium
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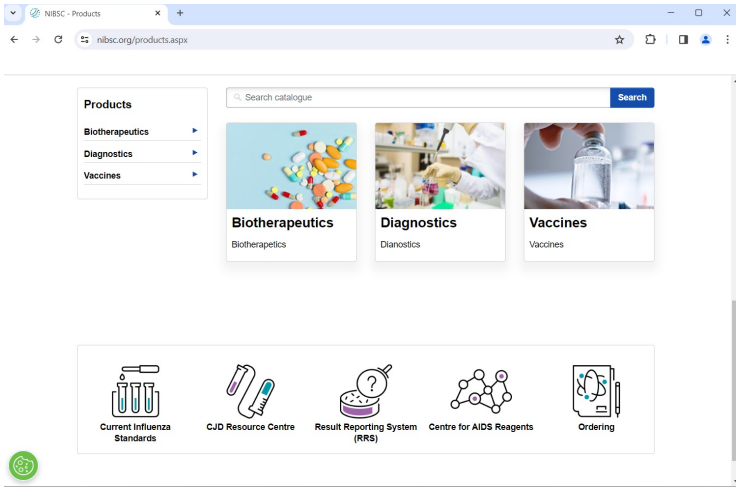
[f] National Institute for Biological Standards and Control, Medicines and Healthcare Products Regulatory Agency, UK

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



Product number: DIM9
Product details
Description: Diphtheria toxoid monoclonal antibody, clone Dim9

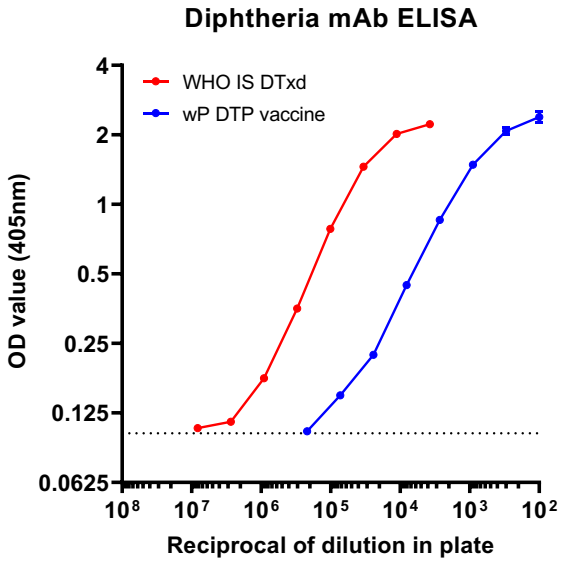
Product number: DT05
Product details
Description: Diphtheria toxoid monoclonal antibody, clone DT05

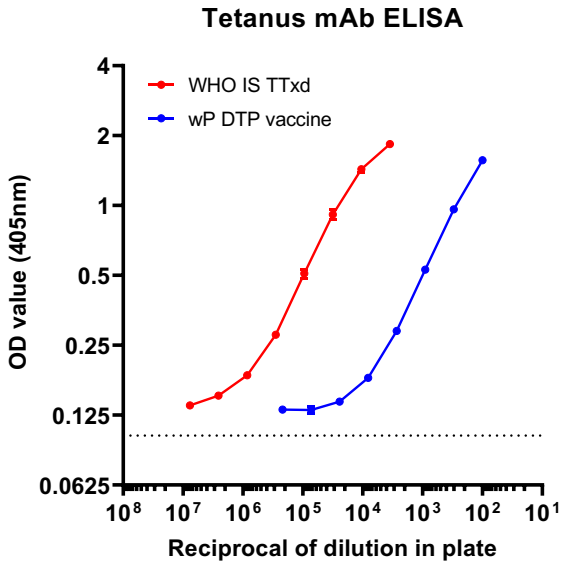
Product number: 8E1-1H1.2.1
Product details
Description: Tetanus toxoid monoclonal antibody, clone 8E1-1H1.2.1

Product number: TT010
Product details
Description: Tetanus toxoid monoclonal antibody, clone TT010

*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 worlds 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!

Acknowledgements

MHRA (UK)

Daniel Yara

Laura Hassall

Peter Rigsby

Rebecca Riches-Duit

Robert Tierney

Shalini Rajagopal

Intravacc (Netherlands)

Amy Kogelman

Bernard Metz

Janny Westdijk

Sciensano (Belgium)

Alexandre Dobly

Antoine Francotte

Maxime Vermeulen

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

VAC2VAC project funded by the EU/EFPIA/Innovative Medicines Initiative 2 Joint Undertaking (Grant No. 115924)

http://www.imi.europa.eu/ http://www.vac2vac.eu/ paul.stickings@mhra.gov.uk www.nibsc.org

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