VAC2VAC

Vaccine batch to vaccine batch comparison by consistency testing

IABS Webinar in collaboration with HSI

Global availability of critical reagents for biologicals testing

Case Study: DTaP monoclonal antibodies

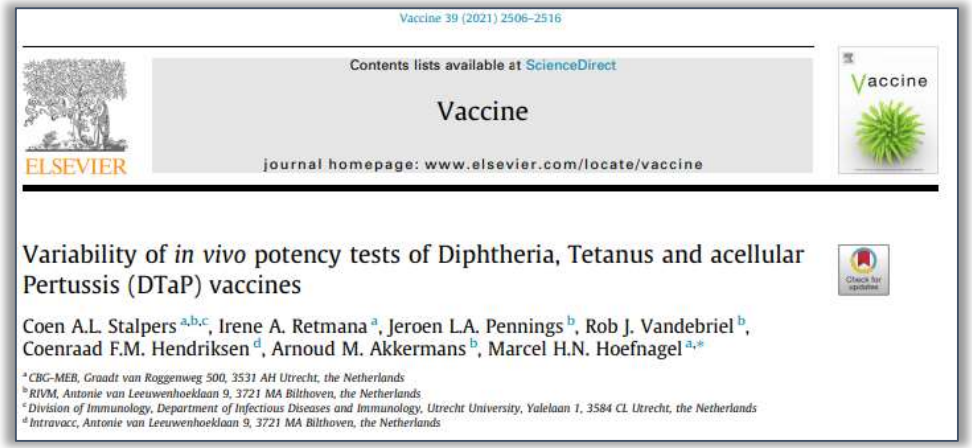
The VAC2VAC project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement N-115924. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

vac2vac

Potency testing of DTaP vaccine

- Potency testing of diphtheria (D), tetanus (T) and acellular pertussis (aP) vaccine components is currently based on ***in vivo*** **assay models** (guinea pigs or mice)

- Although refined models for these animal assays are available, their use in routine batch release testing is questionable because of high inherent variability and poor discriminative power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Vaccine

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

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

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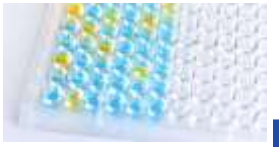
A new approach for testing legacy vaccines

- One output from the VAC2VAC project included the development of immunoassays for DTaP vaccine components

- These immunoassays have the potential to substitute for current animal potency tests in a routine control strategy, potentially improving the ability to detect a batch issue in addition to the ethical advantages and overall efficiency

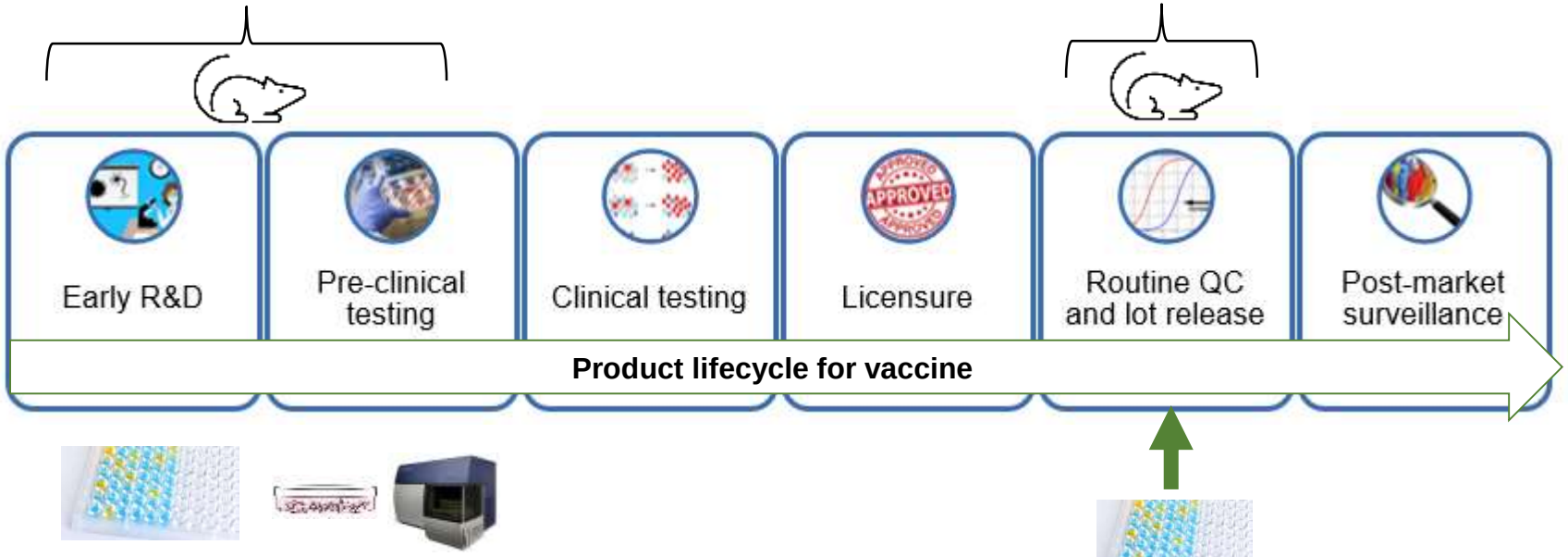
	Animal potency test 	**V2V Immunoassay** 
Time required for test	4-6 weeks	2 days
No. animals per assay (for 2 lots)	Assay dependent but can be >200	0
Precision of potency estimate	Assay dependent but typically 70 – 130%	~90 – 110%
Discriminative power	Poor	Good

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

VAC2VAC Immunoassay for DTaP 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 DTaP, the *in vivo* potency assays **remain part of the routine control strategy post-licensure**



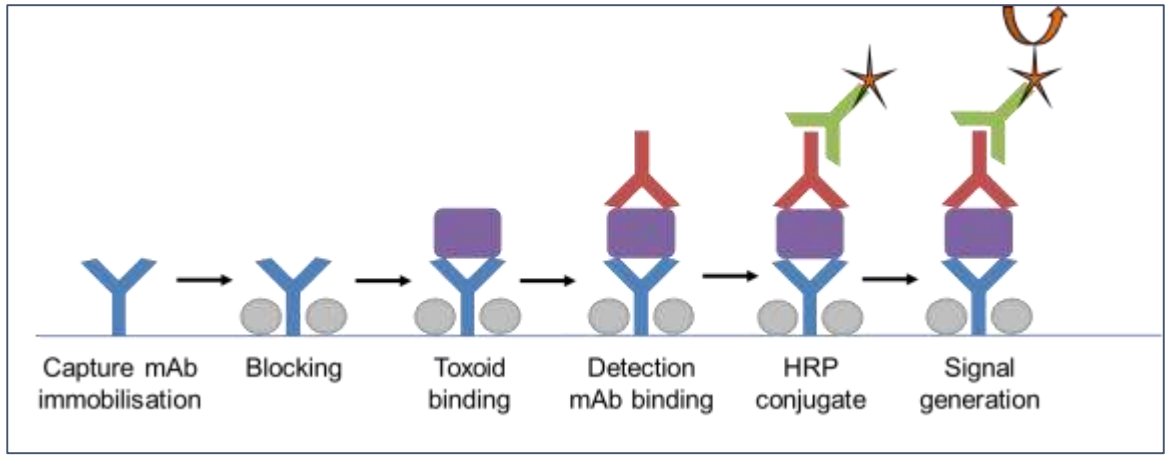
2. For new vaccines, *in vitro* assays can be developed alongside *in vivo* assays used in early R&D and non-clinical development such that they can be taken forward for use in the routine control strategy post-licensure

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**

Immunoassays for DTaP vaccine QC

- Two different immunoassay formats were developed in the VAC2VAC project:

ELISA for D and T (single plex assay)



ALTEX, accepted manuscript
published August 7, 2023
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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[2], Peter Rigsby[1], Alexandre Dobly[3], Maxime Vermeulen[3], Antoine Francotte[4], Paul Stickings[1]

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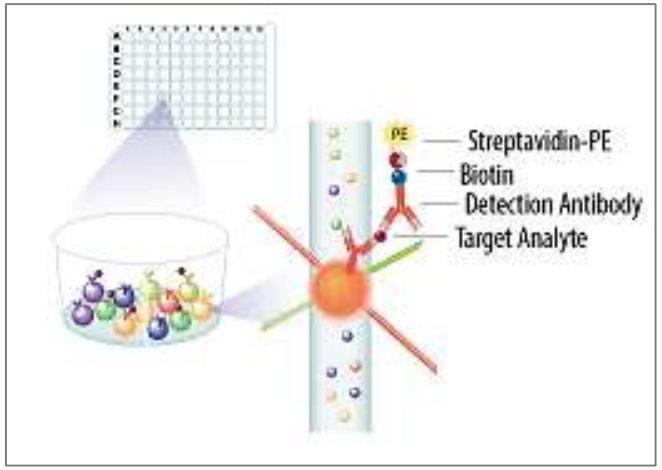
Luminex for DTaP (multiplex assay)



Journal of Immunological Methods

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

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

Immunoassays for DTaP vaccine QC

- The work done in the VAC2VAC project demonstrated proof of concept for the immunoassays for DTaP and showed:

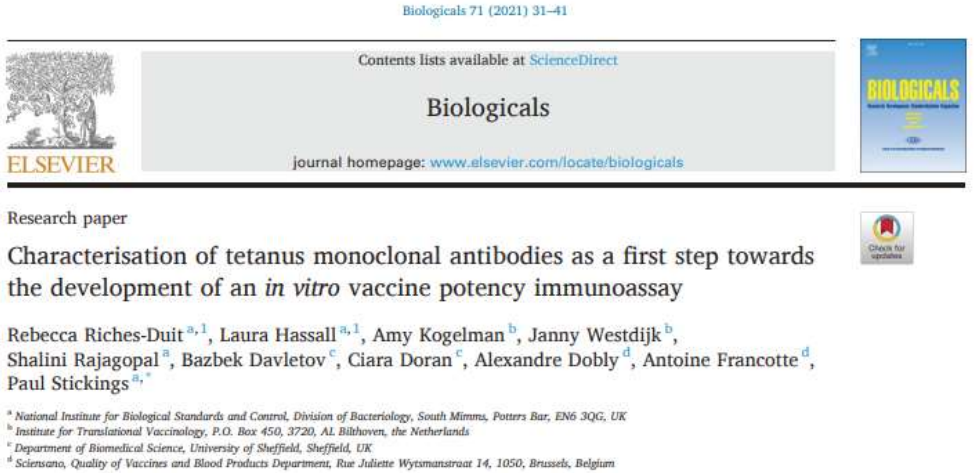
❑ **Wide applicability to different vaccine products (including tetanus vaccines for veterinary use)**
❑ **Excellent precision – including with real world samples**
❑ **Ability to detect relatively small changes in antigen content and quality**
❑ **Successful transfer to other laboratories**

Hassall et. al. ALTEX, 2023 (for diphtheria ELISA) – manuscript for tetanus ELISA currently undergoing peer review
Vermeulen et. al. Journal of Immunological Methods (for DTaP Luminex)

The need for reagents to support introduction of alternative methods

- The DTaP immunoassays developed in VAC2VAC are based on the use of well characterised monoclonal antibodies (mAb) directed against relevant epitopes on the target antigen

- Antibodies were selected based on their ability to bind **native** and **detoxified** antigen and ability to recognise **heat-altered** antigen



Biologicals 71 (2021) 31–41

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Biologicals

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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 [c], Alexandre Dobly [d], Antoine Francotte [d], Paul Stickings [a,*]

[a] National Institute for Biological Standards and Control, Division of Bacteriology, South Mimms, Potters Bar, EN6 3QG, UK
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Research paper

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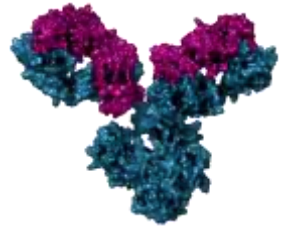
[a] National Institute for Biological Standards and Control, Division of Bacteriology, South Mimms, Potters Bar, EN6 3QG, UK
[b] Institute for Translational Vaccinology, P.O. Box 450, 3720, AL, Bilthoven, the Netherlands
[c] Sciensano, Quality of Vaccines and Blood Products, Rue Juliette Wytsmanstraat 14, 1050, Brussels, Belgium

VAC2VAC mAbs

- A pair of mAb were selected for each antigen in DTaP vaccines

Vaccine component	mAb pair selected for immunoassay
Diphtheria	DIM9 DT05
Tetanus	TT010 8E1-1H1.2.1
Acellular pertussis – pertussis toxoid	629E1 PS21C2.2.1
Acellular pertussis – filamentous haemagglutinin	FHADETOX/6 32-1
Acellular pertussis – pertactin	69K/16 3-5
Acellular pertussis – fimbrae	1-7 G10F8C3



These mAbs are **critical reagents** for the VAC2VAC immunoassays

Needed for capture and detection of the target antigen

VAC2VAC mAbs

- The selected mAbs are owned by 4 different entities

- The close collaboration within the VAC2VAC project and **shared vision** for future implementation of non-animal methods meant that all 4 organisations were able to **agree a model for sustainable supply of these critical reagents** to users who want to further develop, optimise and validate the immunoassays developed within the VAC2VAC project

- **Removes a potential barrier to wider implementation of alternative, non-animal methods**

VAC2VAC mAbs

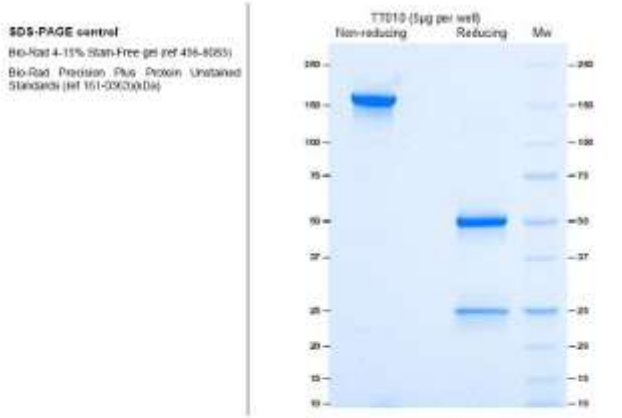
- MHRA is the custodian of the purified mAbs and manages the production, storage and distribution (has the appropriate facilities and expertise as a WHO Collaborating Centre and International Laboratory for Biological Standards)

- The mAbs are accessible through **nibsc.org**
  - https://www.nibsc.org/products.aspx

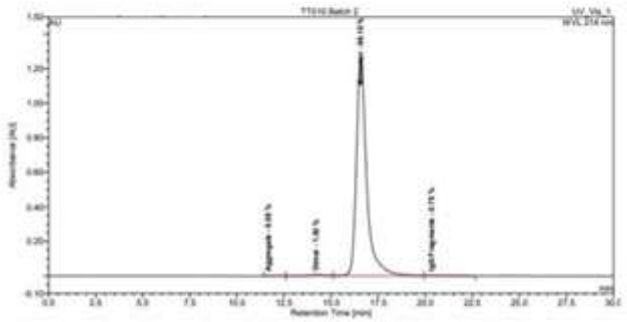
- Small handling fee is charged which covers
  - The production and purification of replacement batches of purified mAb
  - Post-production quality control tests
  - Filling into containers, labelling and storage and curation of the stock

- Customers are required to sign a **Material Transfer Agreement** prior to shipment of the product

SDS-PAGE profile for TT010



SE-HPLC chromatogram for TT010



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Assay development was done at:

MHRA, UK (D and T ELISA) – Daniel Yara, Laura Hassall, Paul Stickings, Peter Rigsby, Rebecca Riches-Duit, Robert Tierney, Shalini Rajagopal

Sciensano, Belgium (DTaP Luminex) – Alexandre Dobly, Antoine Francotte, Maxime Vermeulen

With valuable support for mAb characterisation from:

Intravacc, Netherlands – Amy Kogelman, Bernard Metz, Janny Westdijk

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

And most importantly the 4 organisations who own the selected mAbs for their cooperation ensuring global supply of these critical reagents

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