Medicines & Healthcare products Regulatory Agency

Development of an immunoassay for potency testing of veterinary tetanus vaccines

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

25 March 2026

Vaccine Potency Testing

- A potency assay is a regulatory requirement for the release of every lot of 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**s – i.e. that new vaccine lots are comparable to those originally used in clinical studies for which efficacy in the target species was demonstrated

Current situation for potency testing of tetanus vaccines

- Potency testing for routine batch release of tetanus vaccines relies on the use of *in vivo* models

- The animal models have significant limitations:
  - Ethical concerns
  - High cost
  - Prolonged testing period
  - High variability / poor discriminative power

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

Coen A.L. Stalpers, Irene A. Retmana, Jeroen L.A. Pennings, Rob J. Vandebriel, Coenraad F.M. Hendriksen, Arnoud M. Akkermans, Marcel H.N. Hoefnagel

Vaccine 39(18), April 2021, 2506-2516

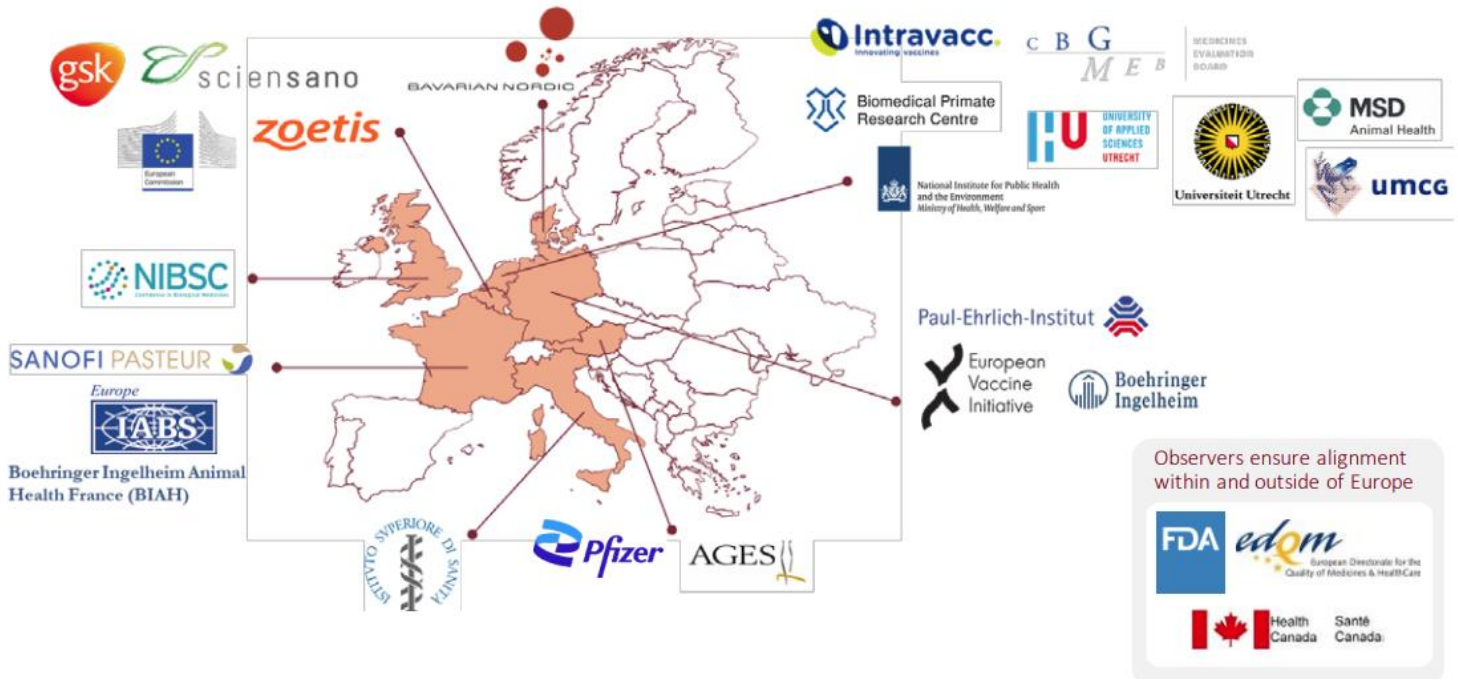
https://doi.org/10.1016/j.vaccine.2021.03.078

Their use in routine quality control testing has become questionable versus more scientifically relevant *in vitro* methods

VAC2VAC

Vaccine batch to Vaccine batch comparison by consistency approach (2016-2022)

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



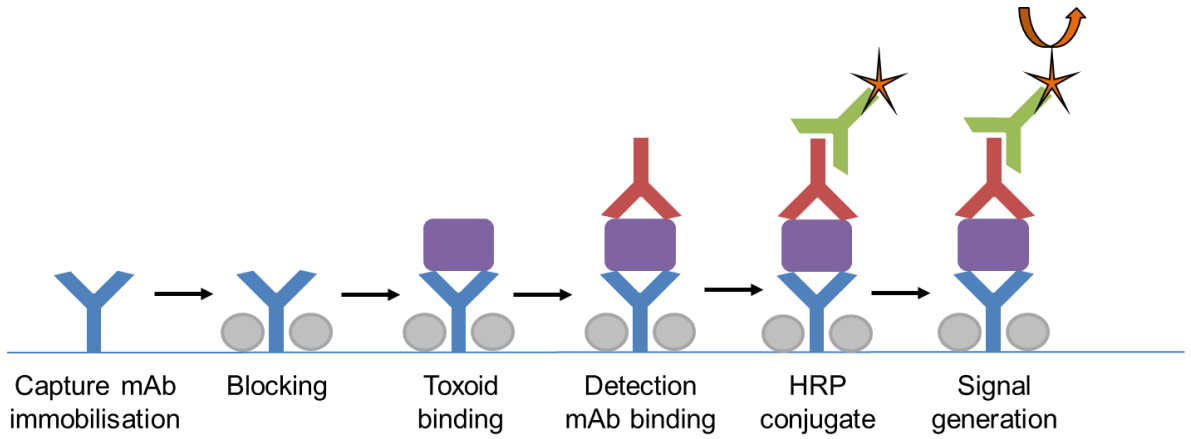
22 partners from veterinary and human vaccine fields

1. Develop and evaluate in vitro analytical methods for the quality control of existing vaccines
2. Define procedural guidance for regulatory approval and routine use

VAC2VAC newsletter Volume V, July 2021 (Newsletters | Vac2Vac)

VAC2VAC outputs – Development of an immunoassay for tetanus antigen

- Demonstrated proof of concept for use of an ELISA **for the quality control of human and animal tetanus vaccines**



Development of a monoclonal antibody sandwich ELISA for the quality control of human and animal tetanus vaccines

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

Altex 41(4), 2024
https://doi.org/10.14573/altex.2401171

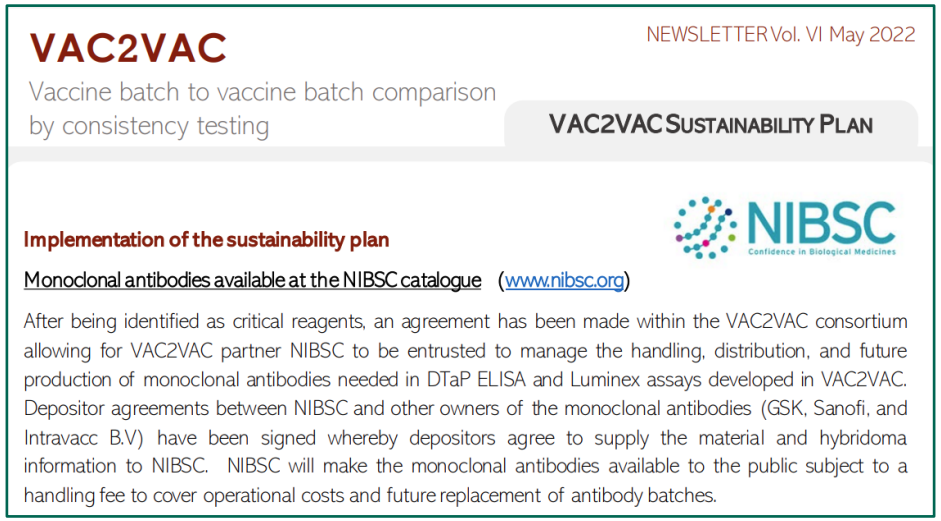
VAC2VAC outputs - Reagents for a tetanus immunoassay

- A pair of **well characterised monoclonal antibodies** was selected for the target antigen
  - Directed against functionally relevant epitopes
  - Bind native, detoxified and adsorbed antigen
  - Sensitive to changes in the quality/integrity of the antigen

- The “VAC2VAC” antibodies are all available from the public catalogue at **https://nibsc.org/products.aspx**
  - Removes one of the most common barriers to development of alternative methods – restrictions on the availability and use of critical reagents

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

R. Riches-Duit et al. Biologicals 71, 2021
https://doi.org/10.1016/j.biologicals.2021.04.002



VAC2VAC

NEWSLETTER Vol. VI May 2022

Vaccine batch to vaccine batch comparison by consistency testing

VAC2VAC SUSTAINABILITY PLAN

NIBSC
Confidence in Biological Medicines

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.

Principle of the *in vitro* approach

- The developed assay is intended to provide a quantitative (in relative terms) estimate of antigen content in the vaccine that reflects both the amount of antigen and its quality / integrity
- Similar approaches have been developed and implemented for other antigens, although never before for a toxoid antigen such as tetanus toxoid
- An *in vitro* approach for the routine monitoring of vaccine potency will have a significant positive impact on animal usage for vaccine QC and, because of superior performance in terms of variability, will provide a higher level of assurance regarding batch-to-batch consistency

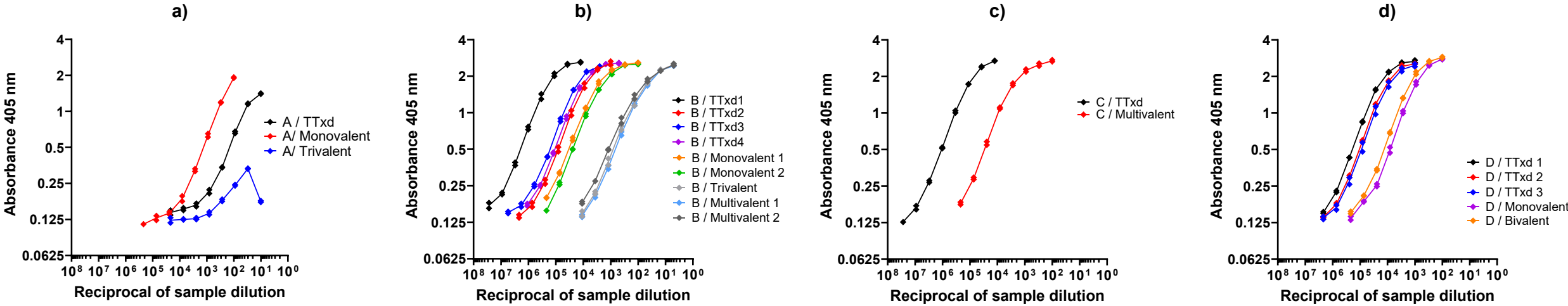
Representative examples from validation and development of the tetanus ELISA

Focus on **veterinary vaccine products**. All studies were done with a wide variety of human and veterinary vaccines.

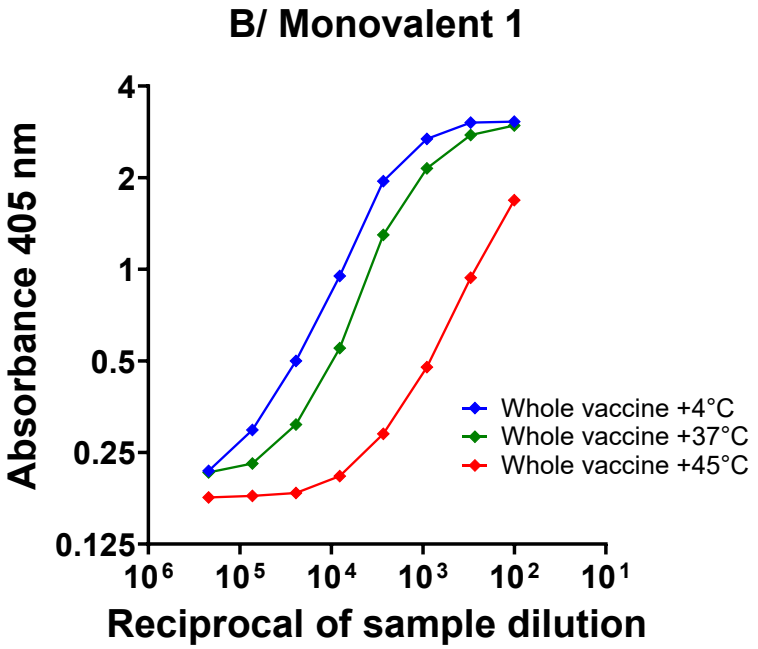
Represent a range of antigen combinations, formulations and adjuvant types – all examples shown here are for testing the “whole vaccine” – i.e. **in the presence of adjuvant**

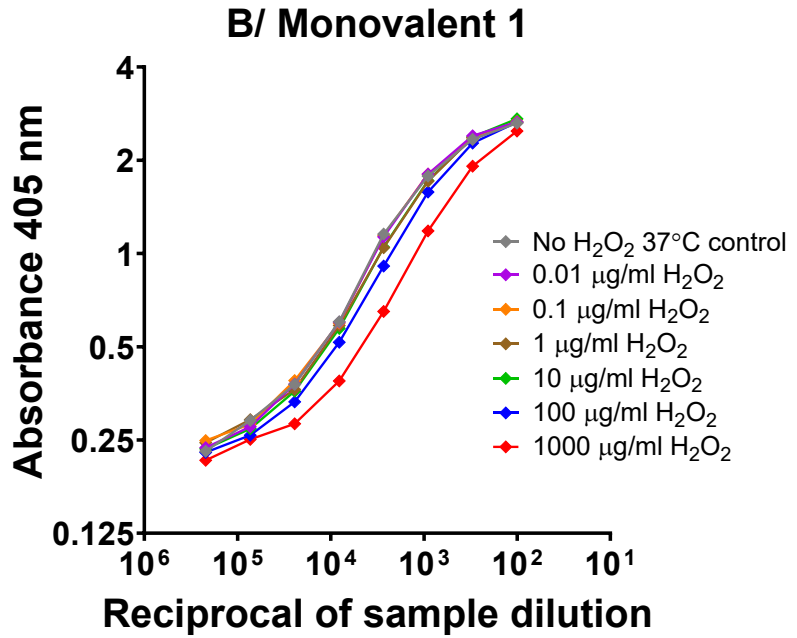
Applicability to different products

- ❑ Results for veterinary vaccine products containing a range of aluminium ($Al(OH)_3$, $KAl(SO_4)_2$, $AlPO_4$) and non-aluminium adjuvants (ISCOMS, Carbomer based, Saponin)
- ✓ Good dose response for **wide range of product types** except for Veterinary company A trivalent product which requires a desorption step to obtain a suitable curve
- ✓ Generally, a clear distinction between high antigen concentration drug substances and vaccine products



Ability to detect changes in antigen **quality**

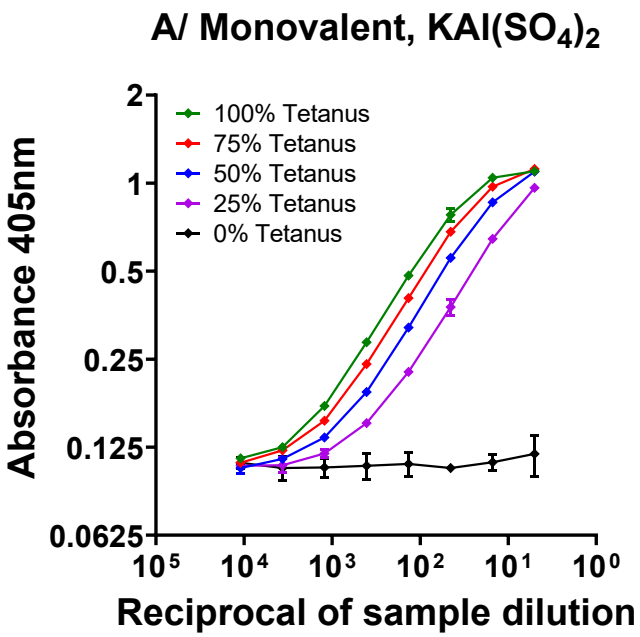


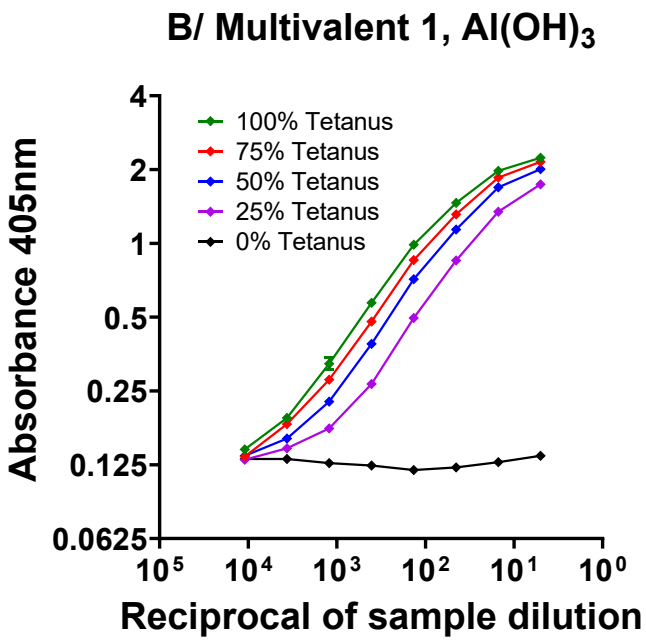


- ❑ Samples **incubated for 8 weeks at elevated temperatures**, with control sample held at the normal storage temperature of +4°C
- ❑ Sample **incubated with increasing concentrations of H_2O_2** for 1 week at 37°C

✓ ELISA is sensitive to antigenic changes caused by temperature and oxidative stress

Ability to detect changes in antigen **content**

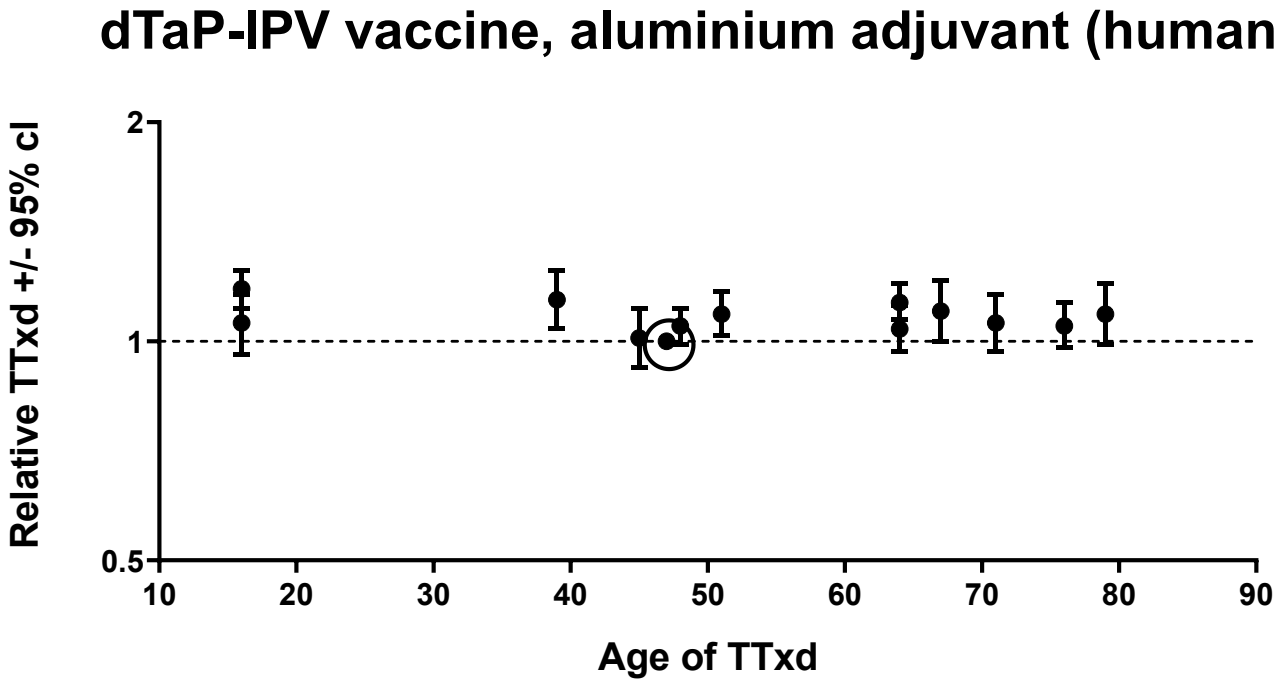




- ❑ Graded dose samples formulated by mixing drop out samples (0% tetanus) with the normal vaccine (100% tetanus) in different ratios
- ✓ ELISA is **sensitive** to relatively small changes (25%) in the amount of antigen present in a combined vaccine
- ✓ The 0% sample contains all other vaccine components, adjuvant and excipients and highlights the **specificity** of the assay

Consistency with real world samples

- Investigating the effect of antigen “age” on measurement of relative antigenicity.



Age refers to the time from the date of manufacture of the final bulk to the date of testing.

- 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
- ✓ No evidence of toxoid-age dependent impact on estimates of relative antigenicity

Transfer to other laboratories

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

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

- ✓ Successful transfer demonstrated to both laboratories (and to other human vaccine manufacturers)
- ✓ Excellent intermediate precision across a range of different product types

Considerations for further validation and implementation

- The published “VAC2VAC” method shows **proof of concept**
- Validation will be done by product manufacturers
  - Work is ongoing in some companies
- Considerations for further validation and implementation include:
  - Choice of reference vaccine / antigen
  - Need (or absence of need) for a desorption step

*

Choice of reference antigen / vaccine

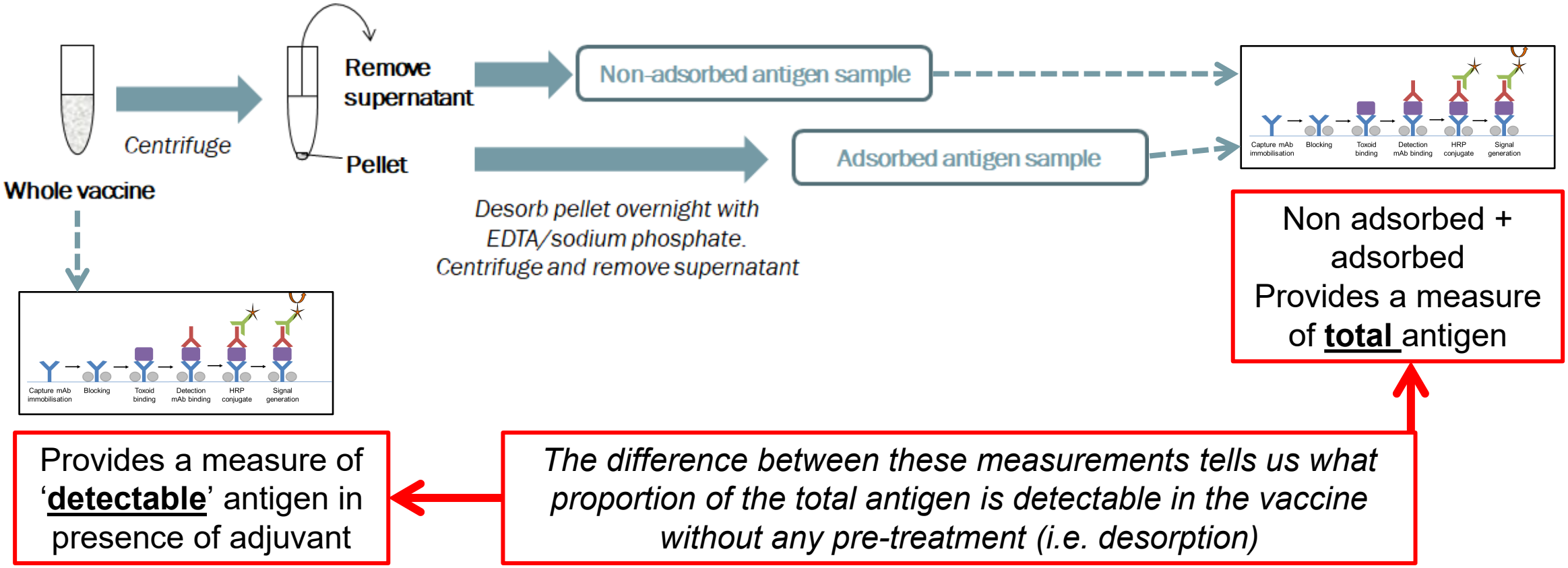
- A stable, qualified reference antigen (or vaccine) will be needed to provide a measurement of relative antigen content (and quality)
- A suitable reference antigen (or vaccine) will fulfil assay validity criteria for linearity and parallelism
- For tetanus, there are several potential options that can be considered for use as a reference antigen (or vaccine) including:
  - WHO standards **16/302** (non-adsorbed tetanus toxoid) and **08/218** (adsorbed tetanus toxoid)
  - Manufacturer matched drug substance
  - Manufacturer matched drug products
- Work performed with a limited range of products suggests that **more than one option will work in most cases**

Need (or absence of need) for a desorption step

- The target antigen in aluminium adjuvanted vaccines will be partially or completely adsorbed to the adjuvant
- Adsorption may affect epitope availability for mAb binding to some extent – and *whether this occurs* and *the extent to which this occurs* will **vary from product to product and will be dependent on the mAbs used in the assay**
  - Investigated the extent to which adsorption impacts detection of tetanus antigen by the VAC2VAC mAbs for a small selection of products
- In terms of monitoring consistency of production, it may not be necessary for 100% of the antigen to be detected

Desorption protocol

How we estimated the **proportion** of total antigen detected in a vaccine sample:



Desorption data

- Desorption increased the amount of tetanus antigen available for antibody detection in the veterinary products containing a $KAl(SO_4)_2$ or $Al(OH)_3$ adjuvant (Approx. 40% of the antigen detected in the whole vaccine sample compared to after a desorption step)
- Most or all the antigen was detected in the whole vaccine samples containing an $AlPO_4$ adjuvant

Sample	Adjuvant	Proportion of antigen detected (%)	Degree of adsorption (%)
A / Monovalent	$KAl(SO_4)_2$	37	74
B / Trivalent	$Al(OH)_3$	35	100
B / Multivalent 1	$Al(OH)_3$	47	90
B / Multivalent 2	$KAl(SO_4)_2$	39	100
D / Monovalent	$AlPO_4$	92	21
D / Bivalent	$AlPO_4$, Quil A ISCOMS	107	14

Desorption step conclusions

- For products where you can demonstrate that all or nearly all of the antigen can be detected in the whole vaccine (i.e. without any desorption step) then it seems clear that no desorption step is needed

- But at what threshold (i.e. at what % of antigen detection) will it be deemed necessary to include a desorption step?
  - Ultimately a question to be answered in validation and in discussion with National Regulatory Authority

- Key considerations during validation if a desorption step is necessary
  - Impact of the desorption process on antigen integrity
  - Impact of the desorption step on overall variability of the assay

Final thoughts

- ❑ Work done in the VAC2VAC project has highlighted the potential of using immunoassays to substitute for current *in vivo* potency tests for tetanus vaccines
- ❑ Significant potential advantages in terms of:
  - ✓ Precision of assays (improved discriminative power for monitoring batch quality)
  - ✓ Significantly reduced time needed for testing each batch
  - ✓ Removing the need for animals for routine potency testing
- ❑ Moving to a new approach creates some challenges in terms of validation and implementation but experience from other similar efforts and the expertise available across different stakeholders means that these challenges can be met

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http://www.imi.europa.eu/ http://www.vac2vac.eu/ www.nibsc.org

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