Medicines & Healthcare products Regulatory Agency

Antigen ELISA for D and T vaccine potency – key features and considerations for validation and implementation

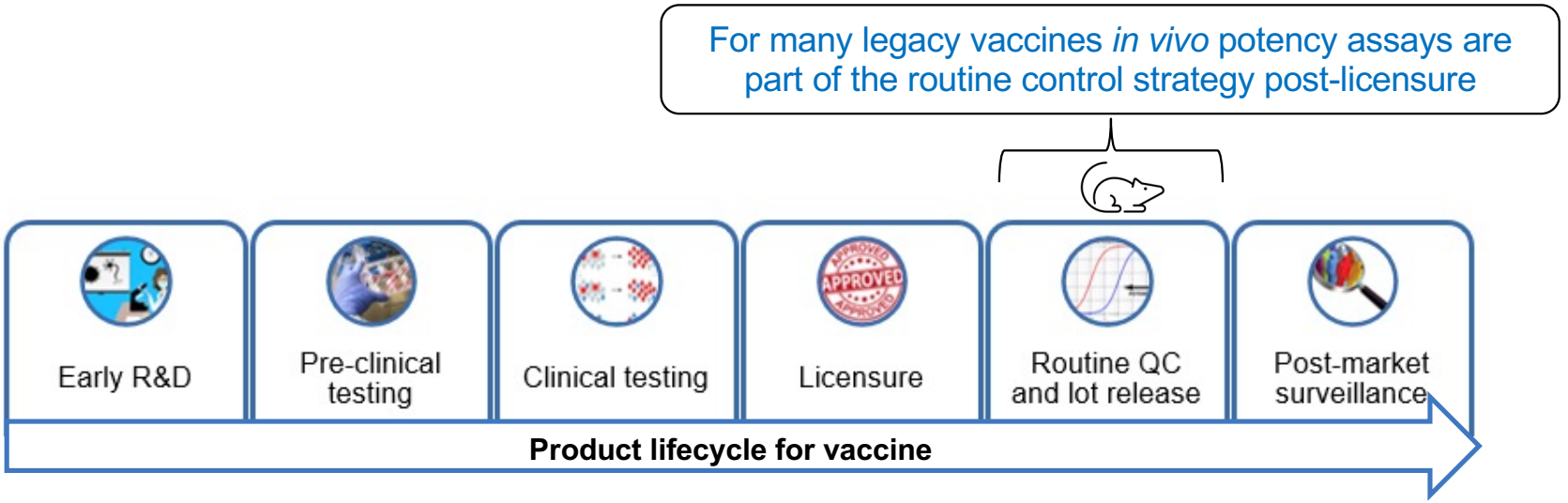
Paul Stickings and Laura Hassall

MHRA

AFSA-HSI Webinar

Virtual meeting Jan-2025

Potency assays used for routine vaccine QC



However, we should also acknowledge that, in many cases, the ***in vivo* assays** currently used in routine control strategies **may not be the best tool** for the purpose of providing assurance that new batches are consistent with those shown to be safe and effective in clinical studies (or in clinical use)

High variability of current *in vivo* potency assays limits their utility in a routine control strategy

AIPO4 adjuvanted combination vaccine

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

÷ 7.5

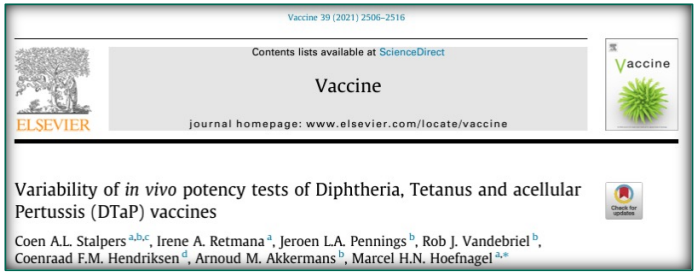
D content Lf/mL	Potency (challenge)
40	Conform
30	Conform
5	Non conform

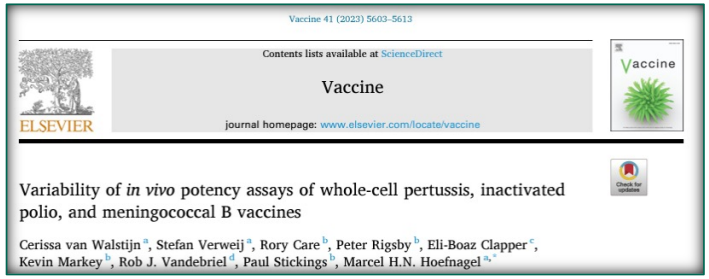
÷ 8

*Data from **Emmanuelle Coppens (Sanofi),** presented at an IABS 3Rs & consistency approach in vaccine lot release testing conference 2015*

Data presented at a previous meeting that provides an indication of the poor discriminative power of *in vivo* potency assays for D and T

Recent publications that quantify the high variability of current *in vivo* potency assays for DT and other vaccines



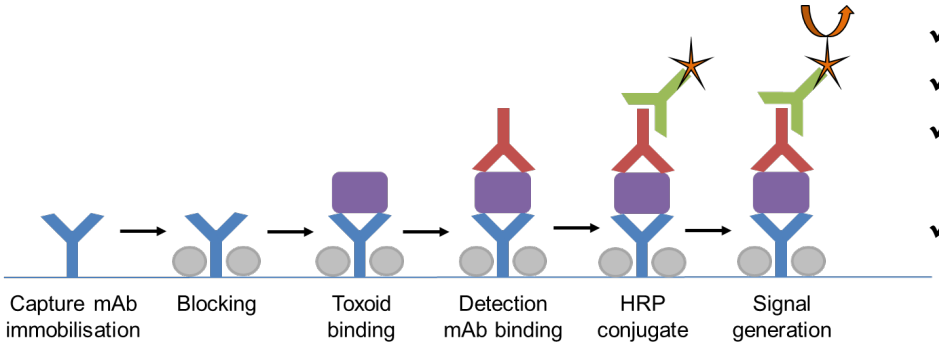


Development of a monoclonal antibody sandwich ELISA for the determination of antigen content and quality in diphtheria vaccines

Laura Hassall et. al. (2024) *Altex – Alternatives to Animal Experimentation*, 41(1), pp. 57-68
https://doi.org/10.14573/altex.2305251

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

Laura Hassall et. al. (2024) *Altex – Alternatives to Animal Experimentation*, 41(4), pp. 588-604
https://doi.org/10.14573/altex.2401171



One of the main objectives for the **VAC2VAC** project was to develop **immunoassays** that could potentially **substitute** for current *in vivo* potency tests for the **routine QC** of DTaP vaccines

MHRA developed ELISAs for **Diphtheria** and **Tetanus**, both of which are published and demonstrate **proof of concept** for the approach:

- ✓ **Wide applicability** to different products (inc. vet tetanus)
- ✓ **Specific**, **sensitive** and **precise**
- ✓ Good evidence that the assays are likely to be **stability indicating**
- ✓ **Successful transfer** to other laboratories

Considerations for further validation and implementation..

- The published “VAC2VAC” methods show **proof of concept**
  - it was not within the scope of the VAC2VAC project to validate these assays
- Validation will be done by product manufacturers
  - work is ongoing in some companies
- Considerations for further validation and implementation include:
  - Availability of critical reagents (antibodies)
  - Choice of reference vaccine / antigen
  - Need (or absence of need) for a desorption step

- ❑ **Availability of critical reagents (antibodies)**
- ❑ **Choice of reference vaccine / antigen**
- ❑ **Need (or absence of need) for a desorption step**

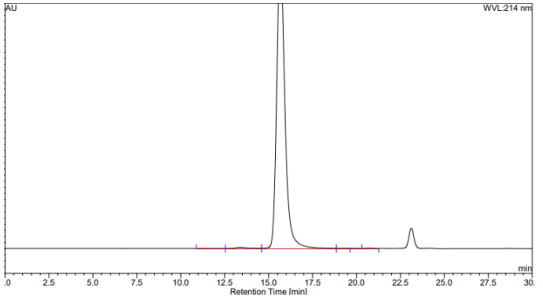
Availability of critical reagents (antibodies)

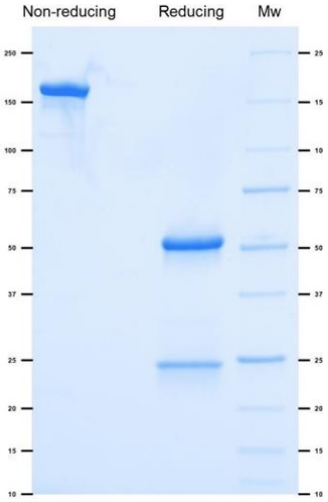
- The “VAC2VAC” methods use well characterised **monoclonal antibodies*** for antigen capture and detection
  - In some cases, manufacturers may decide to characterise and use different monoclonal antibodies for some antigens (as well as other potential manufacturer specific adaptations to the published methods)

- The “VAC2VAC” antibodies are all available from the public catalogue at **https://nibsc.org/products.aspx**

Clone ID	Antigen
DT05	Diphtheria toxoid
Dim9	
8E1-1H1.2.1	Tetanus toxoid
TT010	
629E1	Pertussis toxoid
PS21C2.2.1	
3-5	Pertactin (69K)
69K/16	
FHADETOX/6	FHA
32-1	
G10F8C3	Fimbrae
1-7	

Purity data for purified mAb (example)





* https://doi.org/10.1016/j.biologicals.2021.04.002 (tet mAbs) https://doi.org/10.1016/j.biologicals.2020.12.002 (dip mAbs)

- Availability of critical reagents (antibodies)
- **Choice of reference vaccine / antigen**
- Need (or absence of need) for a desorption step

Choice of reference antigen / vaccine

- The VAC2VAC immunoassays provide a measure of ***relative*** antigen content (and quality) and a **stable**, **qualified reference antigen** (or vaccine) will be needed
  - A suitable reference antigen (or vaccine) will fulfil assay validity criteria for **linearity** and **parallelism**

- For D and T, there are a number of potential options that can be considered for use as a reference antigen (or vaccine) including:
  - *WHO Standard (adjuvanted or non-adjuvanted)*
  - *Manufacturer drug substance (adjuvanted or non-adjuvanted)*
  - *Manufacturer drug product (adjuvanted – same as product being tested)*
  - *Manufacturer drug product (adjuvanted – "similar" to product being tested)*

- For D and T ELISAs we investigated this for a selection of different human vaccines..

Diphtheria mAb ELISA – evaluating reference antigen options

Reference antigen / vaccine	**Product (test sample) – whole vaccine**						
	1	2	3	4	5	6	7
WHO IS Toxoid (non-adjuvanted)	×	×	✓	×	×	×	×
Manufacturer DS (non-adjuvanted)	×	× / ✓	✓	× / ✓	✓	×	×

Non-adjuvanted

Reference antigen / vaccine	**Product (test sample) – whole vaccine**						
	1	2	3	4	5	6	7
WHO IS Toxoid (adjuvanted)	✓	✓	✓	✓	✓	✓	✓
Manufacturer DS (adjuvanted)	✓	✓	✓	✓	✓	✓	✓
Manufacturer DP (specific)	✓	✓	✓	✓	✓	✓	✓
Manufacturer DP (“similar”)	✓	✓	✓	✓	✓	✓	✓

Adjuvanted

× Indicates substantially different slope and/or asymptote

✓ Indicates comparable slope and/or asymptote

Tetanus mAb ELISA – evaluating reference antigen options

Reference antigen / vaccine	**Product (test sample) – whole vaccine**						
	1	2	3	4	5	6	7
WHO IS Toxoid (non-adjuvanted)	✓	✓	✓	×	×	× / ✓	✓
Manufacturer DS (non-adjuvanted)	✓	✓	✓	✓	✓	× / ✓	✓

Non-adjuvanted

Reference antigen / vaccine	**Product (test sample) – whole vaccine**						
	1	2	3	4	5	6	7
WHO IS Toxoid (adjuvanted)	✓	✓	✓	×	×	× / ✓	✓
Manufacturer DS (adjuvanted)	✓	✓	✓	✓	✓	× / ✓	✓
Manufacturer DP (specific)	✓	✓	✓	✓	✓	✓	✓
Manufacturer DP (“similar”)	✓	✓	✓	✓	✓	✓	✓

Adjuvanted

× Indicates substantially different slope and/or asymptote

✓ Indicates comparable slope and/or asymptote

Choice of reference antigen / vaccine – conclusions

- Work performed in VAC2VAC project (albeit with a limited range of products) suggests that **more than one option will work in most cases**
  - Number of factors to consider for the choice of reference including availability, stability, qualification and maintenance over time
  - Need to be mindful of potential for “drift” when a reference has relatively short shelf life and requires relatively frequent replacement

- Availability of critical reagents (antibodies)
- Choice of reference vaccine / antigen
- **Need (or absence of need) for a desorption step**

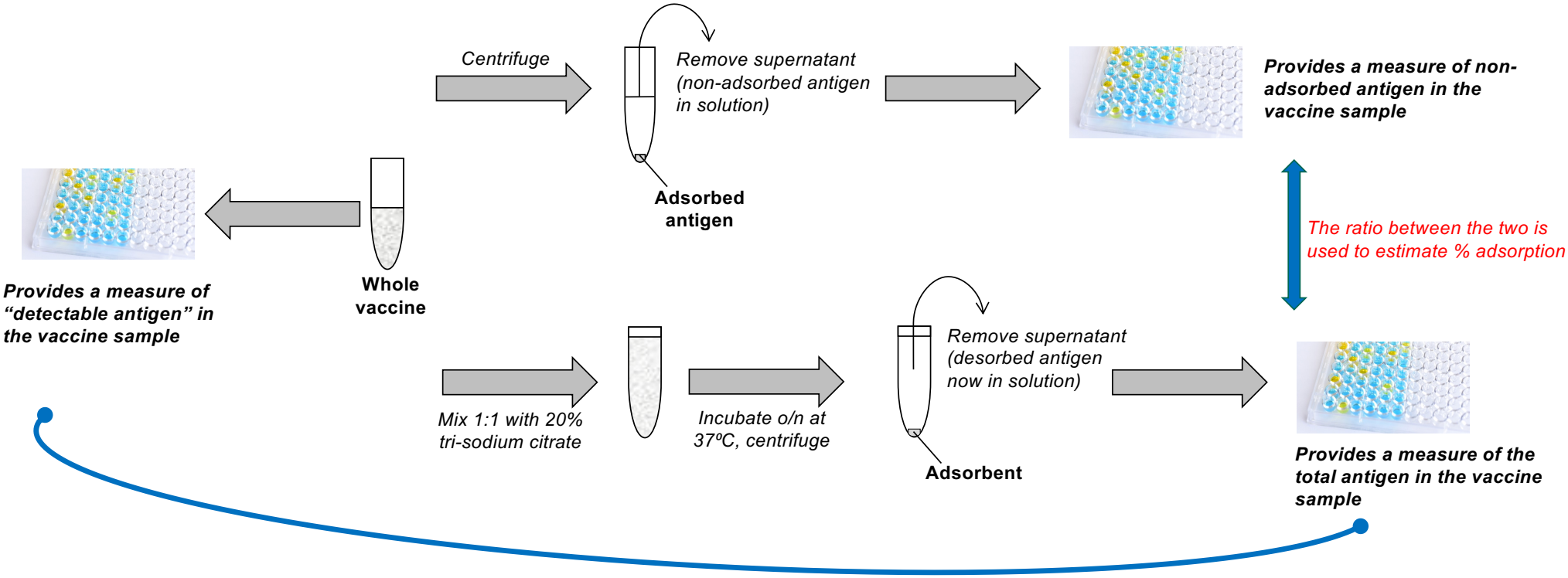
Need (or absence of need) for a desorption step

- 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 antigen to antigen) and will be dependent on the mAbs used in the assay**

- We investigated this for the D and T ELISAs for a small selection of products during the VAC2VAC project (data is published)

How we estimate the amount of adsorbed and non-adsorbed antigen in a vaccine sample using the D or T ELISA



The difference between these two measurements tells us what proportion of the total antigen is detectable in the vaccine – i.e. without any pre-treatment (i.e. desorption) of the vaccine sample

Desorption step – data from VAC2VAC project

Manufacturer	Product	Adjuvant	Proportion of antigen detected (%)[a]	Degree of adsorption (%)[b]
HuA	dTaP	$AlPO_4$	98	84
	DTaP-IPV-HepB-Hib	$Al(OH)_3$	88	12
HuB	dTaP	$Al(OH)_3 + AlPO_4$	103	100
	DTaP	$Al(OH)_3$	59	100

TETANUS

Manufacturer	Product	Adjuvant	Proportion of antigen detected (%)[a]	Degree of adsorption (%)[b]
HuA	dTaP	$AlPO_4$	67	89
	DTaP-IPV-HepB-Hib	$Al(OH)_3$	56	58
HuB	dTaP	$Al(OH)_3 + AlPO_4$	63	100
	DTaP	$Al(OH)_3$	43	100

DIPHTHERIA

EXAMPLE: the highlighted product is a dTaP vaccine with hydroxide and phosphate aluminium adjuvant
D and T toxoids are completely adsorbed
If we run the whole vaccine in the tetanus ELISA we detect all of the antigen that is in the vaccine
If we run the whole vaccine in the diphtheria ELISA, we detect ~63% of the antigen that is in the vaccine

Desorption step – to desorb or not desorb?

- 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 fairly 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 NRA

- 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

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  - Ultima…
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MHRA performed limited studies during VAC2VAC which suggest that, while adding a desorption step increases the total assay time (by ~24h), it does not significantly affect antigen integrity and does not significantly increase variability of the assay..

Implications of a switch from *in vivo* to *in vitro* potency

- Compared to current (WHO/EP) potency tests for D and T, *in vitro* assays will not be calibrated and traceable to a common higher order standard
  - As discussed earlier, **current WHO standards may be suitable for use in these assays**, **but will be used differently** to how they are currently used for *in vivo* potency assays
  - Comparing potency of different products (through laboratory testing) will not be possible following a switch to *in vitro* immunoassays
  - Specifications are likely to be based on consistency and therefore product specific

- Where the "VAC2VAC methods" are taken up, manufacturers may make modifications prior to validation – even using different antibodies in some cases
  - As discussed earlier, some products may require a desorption step, others may not

- NCLs may therefore prefer to adopt and validate a "universal" protocol that can be applied to multiple products (provided it is shown to work for those products)

Final thoughts

- Work done in the VAC2VAC project has highlighted the potential of using immunoassays to substitute for current *in vivo* potency tests for DTaP 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!

Acknowledgements

MHRA (UK)

Daniel Yara

Laura Hassall

Paul Stickings

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

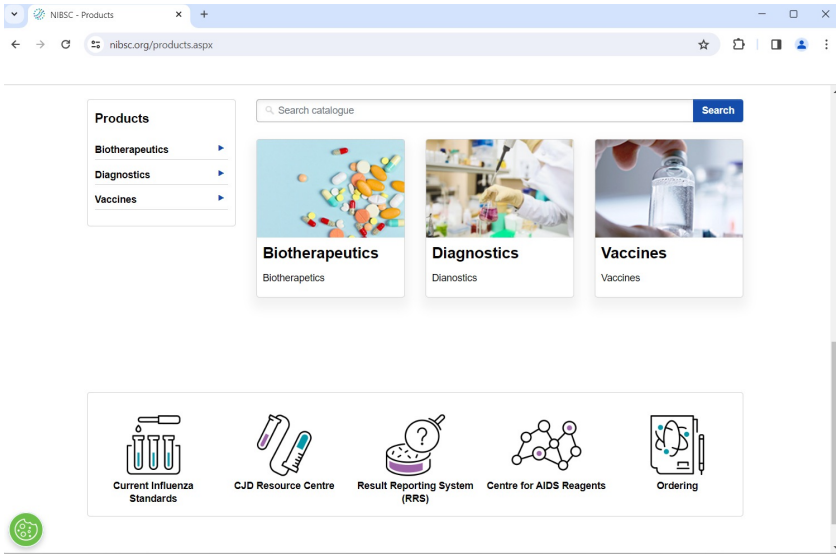
VAC2VAC project funded by the EU/EFPIA/Innovative Medicines Initiative 2 Joint Undertaking (Grant No. 115924)

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

Further information

- Queries related to assay, critical reagents and standards:
  bacvac@mhra.gov.uk

- VAC2VAC monoclonal antibodies and WHO reference materials from https://nibsc.org/products.aspx



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