Medicines & Healthcare products Regulatory Agency

Development of an immunoassay for potency testing of tetanus vaccines

A development from the VAC2VAC consortium

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IABS Webinar October 2022

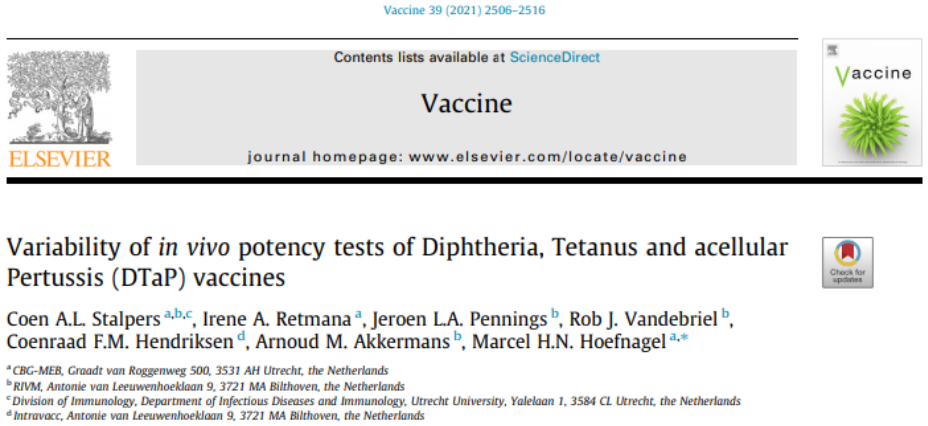
3Rsimplementation in veterinary vaccine batch-release testing: Current state-of-the-art and future opportunities

Problem Statement

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

Although refined models are available, and reduction schemes can be implemented, the 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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AlPO4 adjuvanted combination vaccine

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

÷ 7.5

÷ 8

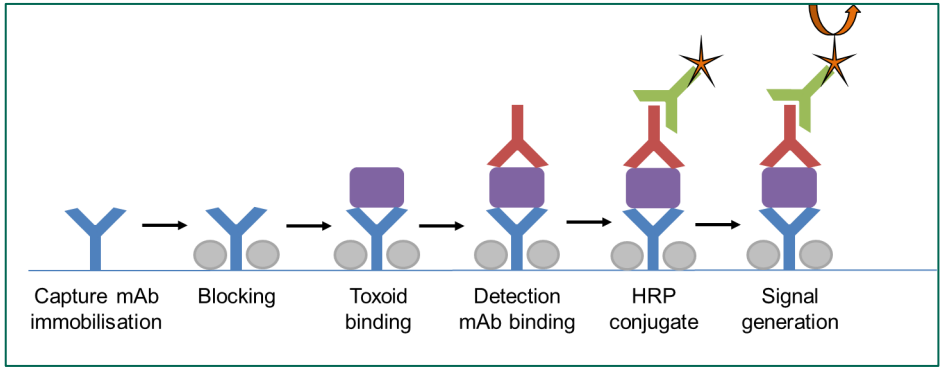
D content Lf/mL	Potency (challenge)
40	Conform
30	Conform
5	Non conform

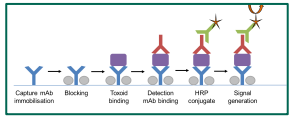
Data from Emmanuelle Coppens, Sanofi Pasteur, previously presented at IABS 3Rs and consistency testing in vaccine lot release testing conference 2015

A new approach to testing legacy vaccines

In the VAC2VAC project, we have developed an ELISA that is intended to provide a quantitative (in relative terms) estimate of tetanus toxoid antigen content that reflects both the amount and quality of the antigen..

..through use of well characterised monoclonal antibodies (mAbs), directed against relevant epitopes on the target antigen, that are sensitive to changes in the amount/quality/integrity of the antigen

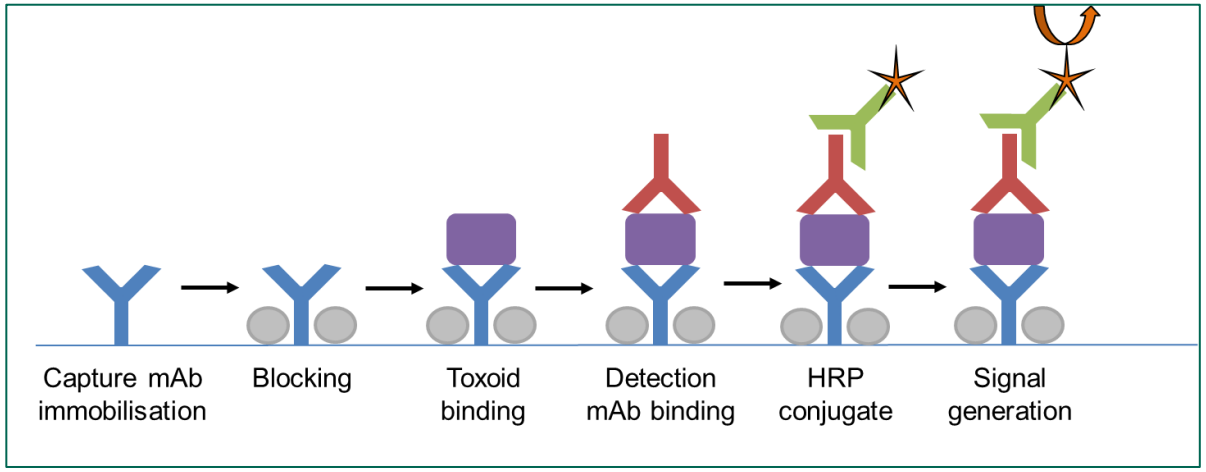


	Animal potency test 	**VAC2VAC Tetanus ELISA** 
Time required for test	>4 weeks	2-3 days
Number of animals per assay (to test 2 batches)	Assay dependent but typically >200	0
Precision of potency estimate	Assay dependent but typically 70 – 130%	~90 – 110%
Variability of assay	~16 – 36%[#]	~10%
Discriminative power	Poor	Good

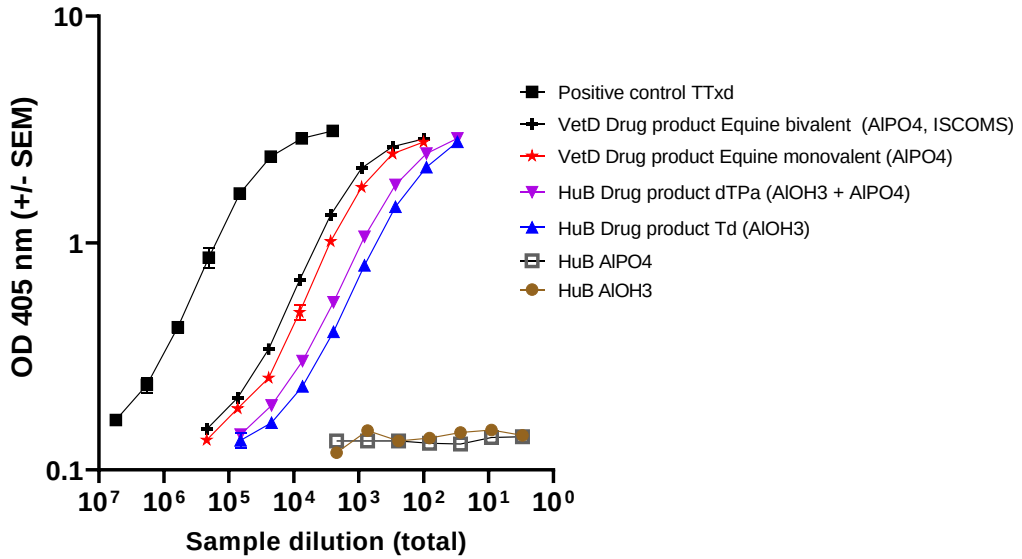
✓*Save animals*
✓ *Save time*
✓ *Improved ability to identify production/batch issue*

[#]*tetanus potency assays evaluated by Stalpers et. al. (2021)*

Monoclonal antibody ELISA – applicability



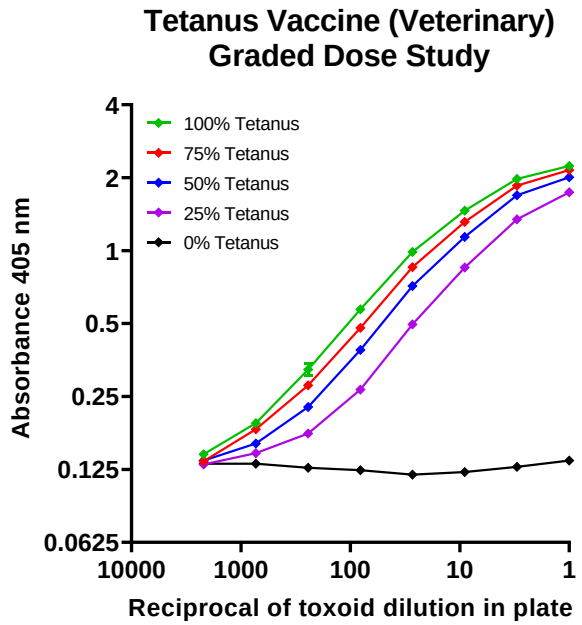
Evaluated 20 different sample types including drug product and drug substance from 2 human manufacturers and 4 vet manufacturers
Adjuvant types, including..
Aluminium adjuvants: AlOOH / $Al(OH)_3$ / $AlPO_4$ / $Al(OH)_3$ + $AlPO_4$ / $KAl(SO_4)_2$
Aluminium adjuvant + non-aluminium adjuvant: $AlPO_4$ + ISCOMS
Non-aluminium adjuvants: Carbomer / Saponin



Note: all examples shown here are for diluted whole vaccine
for some products containing an aluminium adjuvant a desorption step may be needed

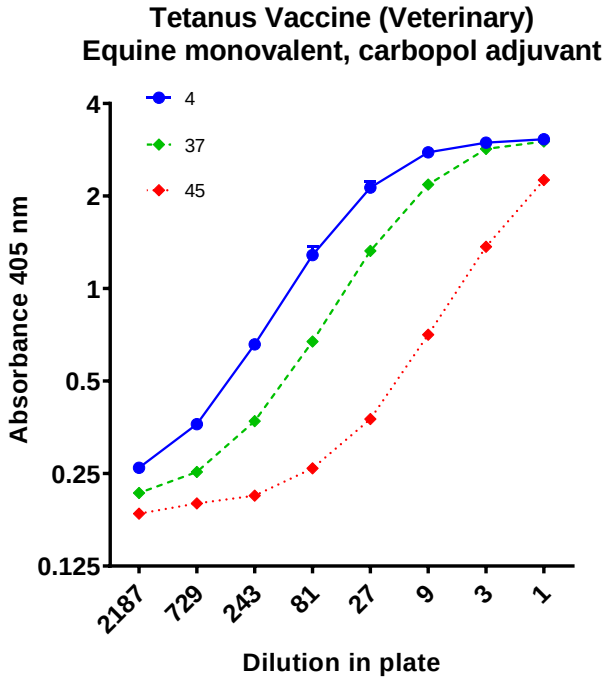
Monoclonal antibody ELISA – discriminative power

The mAb ELISA is sensitive to changes in **antigen content**



Sample	Antigen content relative to 100% tetanus sample (%)
0% Tetanus	< LOD
25% Tetanus	24.3
50% Tetanus	49.1
75% Tetanus	73.3

The mAb ELISA is sensitive to changes in **antigen quality**



Drug product was incubated for 8 weeks at different temperatures (+4°C, +37°C and +45°C)

Monoclonal antibody ELISA – Assay performance

In-house qualification Study at NIBSC

2 batches of each product were tested with one batch in each case being assigned as a “reference” for the purposes of relative antigen content calculation

- *4 independent runs (different days)*
- *2 (duplicate) plates per run*
- *2 operators performing 2 runs each*

ELISA	Vaccine (all $AlOH_3$)	n (plates)	n (assays)	CV%
Tet	DTaP (HuB)	9*	5	**4.6**
Tet	Ruminant multivalent (VetB)	8	4	**5.9**

Intermediate precision of the mAb ELISAs is acceptable

Transfer Study

A transfer study protocol was designed with success criteria defined based on performance of the assay during in-house qualification at NIBSC (including validity criteria applied to each plate/assay); NIBSC and receiving lab performed **3 assays each**, **2 plates per run** (total of 6 plates per lab)

Study details	Product	Intermediate precision GCV%	
		Partner	NIBSC
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

Successful transfer of the tetanus ELISA has been demonstrated (to multiple laboratories)

**One run repeated due to very high variability on 1 plate (excluded); results from plate 2 included in overall summary giving n = 9*

Conclusions and next steps

- **Proof of concept has been demonstrated** for the T ELISA, including evidence that the assay may be stability indicating
- Assays are robust and **successful transfer to other laboratories has been achieved**
- **A desorption step is likely to be necessary for some, but not all, vaccines** – this will increase complexity for validation (*NB: will not necessarily increase variability*)
- **A suitable reference vaccine/antigen will need to be identified for each vaccine** – one reference may be suitable for multiple products (but unlikely to be the case across *all* products)
- **Purified monoclonal antibodies for T ELISA are available from NIBSC (www.nibsc.org) for laboratories who want to establish and validate these methods**
- Discussions to be held with EDQM about potential future BSP study

Acknowledgements

NIBSC
UK

Intravacc
The Netherlands

Sciensano
Belgium

+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@nibsc.org