Medicines & Healthcare products Regulatory Agency

Development of an immunoassay for potency testing of tetanus vaccines

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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

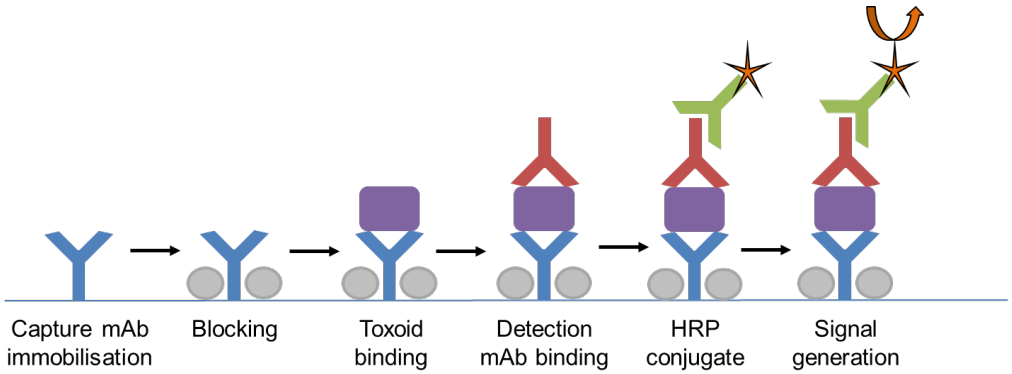
Current situation for potency testing of tetanus vaccines

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

- Although refined animal models are available, and reduction schemes can be implemented with reduction of total animal number, the animal models have significant limitations:
  - Ethical concerns
  - High cost
  - Prolonged testing period
  - High variability / poor discriminative power

A new approach for testing legacy vaccines

- In the VAC2VAC project, we developed an ELISA that is intended to provide a quantitative (in relative terms) estimate of T **antigen content**
- The ELISA uses well characterised monoclonal antibodies (mAbs), directed against functionally relevant epitopes on the target antigen, that are sensitive to changes in the **quality/integrity** of the antigen



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

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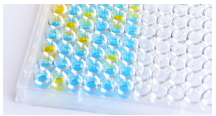
Biologicals, Volume 71, 2021
https://doi.org/10.1016/j.biologicals.2021.04.002

ELISA assay performance

- Assay performance characteristics of the ELISA have been evaluated, and the assay has been shown to have many advantages over the *in vivo* potency tests

	Animal potency test 	**VAC2VAC ELISA** 
Time required	4-6 weeks	2 days
No. animals per assay (for 2 lots)	Assay dependent Can be >200	0
Precision of potency estimate*	Assay dependent Typically 70 – 130%	~90 – 110%
Variability of assay	~16-36%*	~10%
Discriminative power	Poor	Good

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

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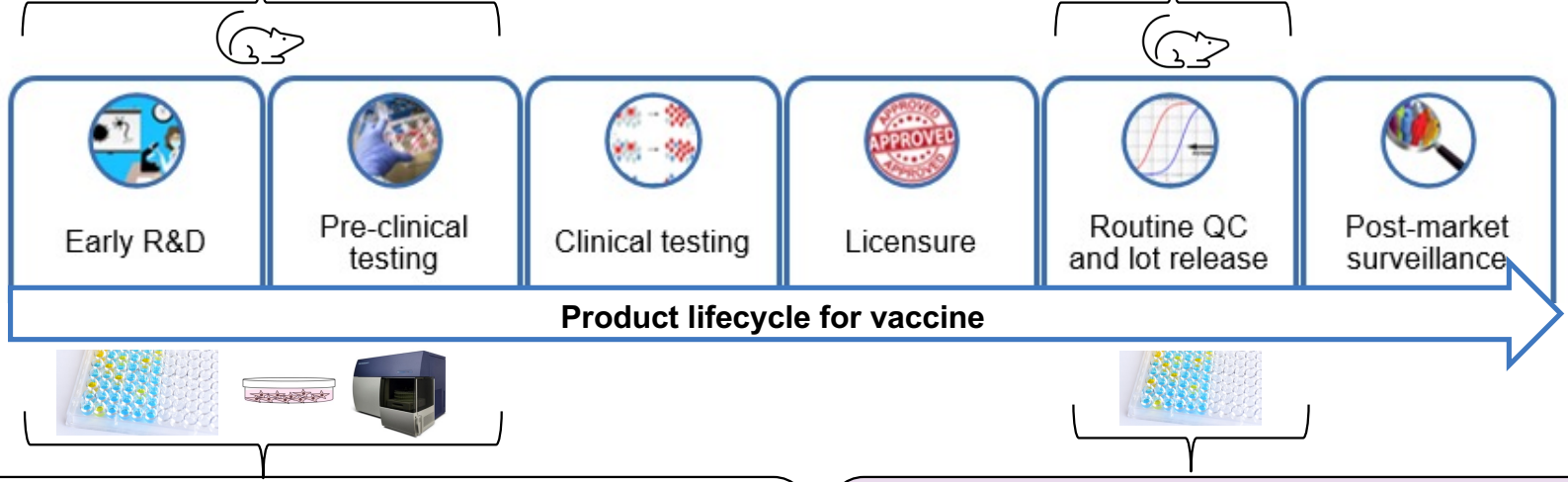
Altex, accepted manuscript
https://doi.org/10.14573/altex.2401171

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

VAC2VAC ELISA for T Vaccines – proposed use

1. *In vivo* assays have an important role in early R&D and non-clinical development

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



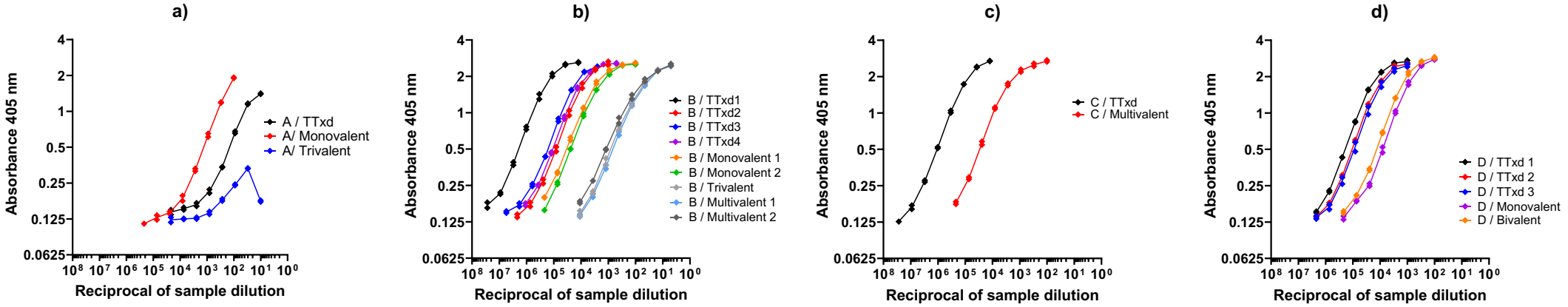
2. For new vaccines, *in vitro* assays can be developed alongside *in vivo* assays used in early R&D and non-clinical development such that they can be taken forward for use in the routine control strategy post-licensure

4. VAC2VAC ELISAs were developed with the aim of substituting existing animal potency tests –either alone or in combination with other in vitro assays as part of a consistency approach for batch testing of existing vaccines

ELISA – Applicability

- The ELISA is suitable for testing a range of vaccine products and drug substances from different human and veterinary manufacturers

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)



Note: all samples shown here are **diluted whole vaccine**. For some products containing an aluminium adjuvant a desorption step may be needed (as with Veterinary company A trivalent product).

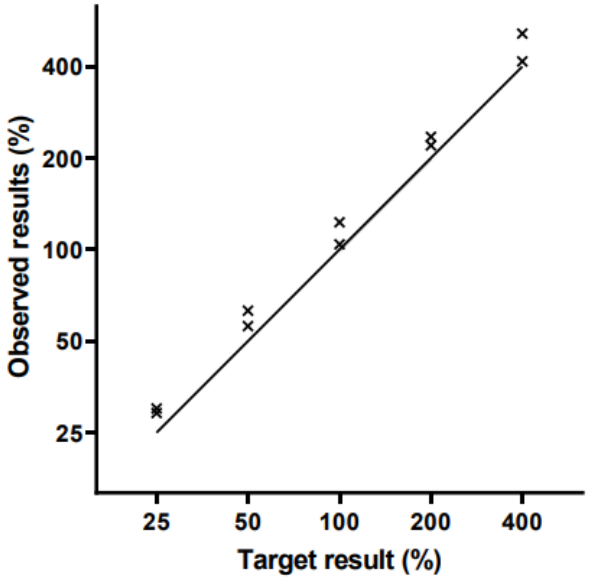
ELISA – validation

- **Specificity**
  - Assessed by testing drop out samples for a range of representative vaccine products that contained all components of the vaccine except the T toxoid antigen
  - ✓ No response was observed for any of the drop out samples except the one containing a Hib component conjugated to T toxoid as a carrier protein

- **Intermediate precision**
  - Determined by testing a human and a veterinary vaccine product (one reference batch and one test batch for each) across duplicate plates on several days.
  - ✓ GCV of 4.8% for human vaccine product and 6.1% for the veterinary vaccine product

ELISA – validation continued

- **Dilutional linearity**
  - A “drop out” vaccine consisting of diphtheria and acellular pertussis antigens (plus adjuvant) **but NO T antigen** was spiked with increasing amounts of T toxoid
  - Estimates for the spiked samples were calculated relative to the normal drug product vaccine sample

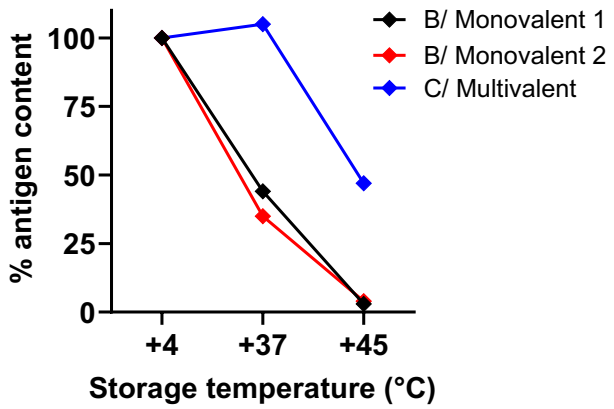


- ✓ Fitted slope not significantly different to 1.0, and intercept not significantly different to 0
- ✓ ELISA is sensitive to changes in antigen content

ELISAs are sensitive to changes in antigen quality

Heat stress

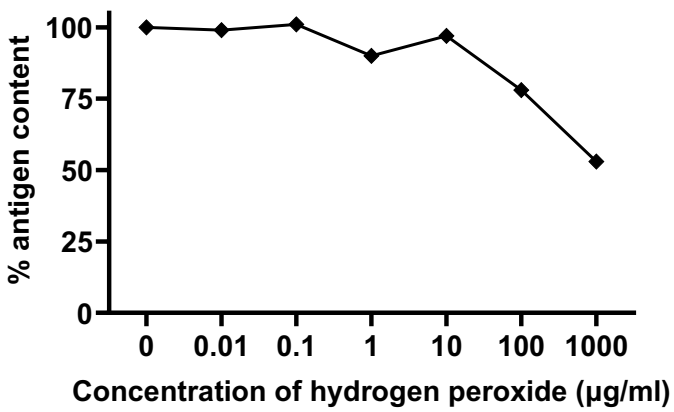
Non-aluminium adjuvanted vaccine products



Samples incubated for 8 weeks at elevated temperatures, with control samples held at the normal storage temperature of +4°C

Oxidative stress

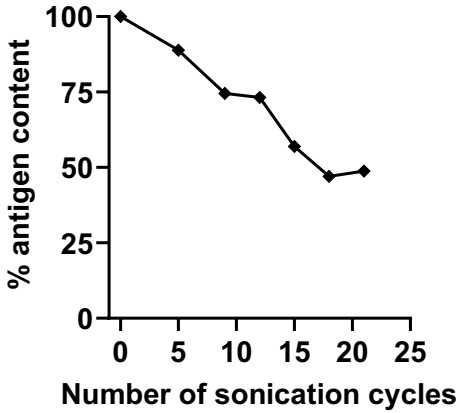
Non-aluminium adjuvanted vaccine product



Sample incubated with increasing concentrations of H_2O_2 for 1 week at 37°C

Shear stress

Tetanus Toxoid



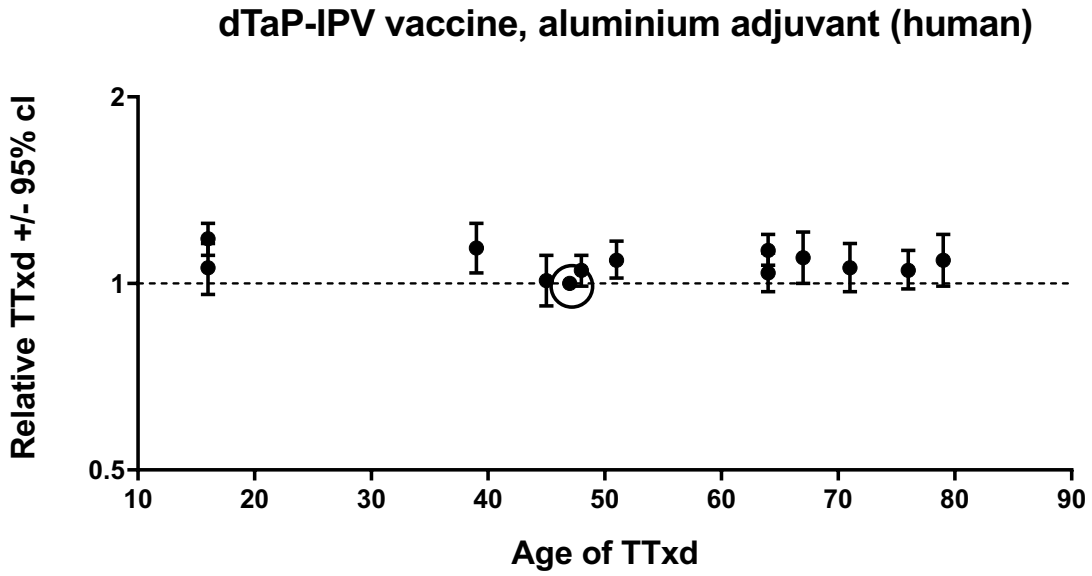
Sample diluted to final vaccine concentrations and subjected to increasing no. of sonication cycles (15s with 15s interval)

Desorption step

- Adsorption onto an aluminium adjuvant can mask the epitopes on the T toxoid either preventing or reducing binding of the mAbs to their target epitopes
  - Antigen detection for a veterinary trivalent vaccine from manufacturer A was significantly improved when desorbed compared to the whole vaccine
  - Desorption also increased the amount of T antigen available for antibody detection in the other 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
- Desorption step may not be 100% efficient, and effect of the desorption process on the antigen is not fully understood.
- In terms of monitoring consistency of production, it may not be necessary for 100% of the antigen to be detected

ELISA performance – consistency with real world samples

- Suitability of the ELISA for monitoring batch-to-batch consistency investigated by testing multiple lots of the same product covering a range of different ‘ages’ (from the date of manufacture of the final bulk to the date of testing)



- Estimates are relative to the batch circled
- Between batch variability only slightly higher than the within batch variability
- No evidence of toxoid-age dependent impact on estimates of relative antigenicity

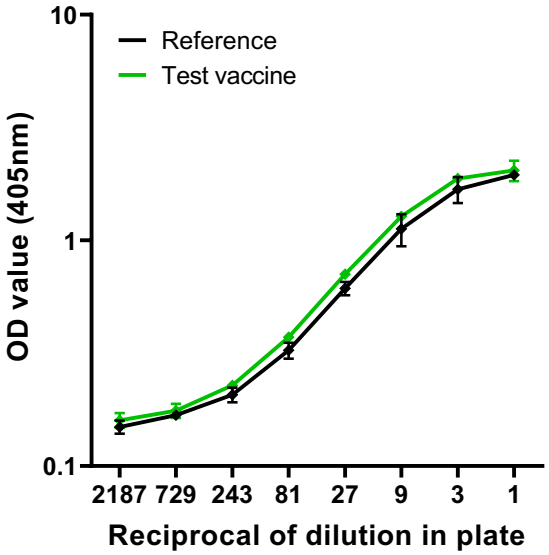
ELISA – curve analysis

- Suitability of different materials to act as a reference preparation in the ELISA was assessed for different products by examining the **similarity of the dose-response curve shapes**.
  - Materials evaluated: WHO standards **16/302** (non-adsorbed toxoid) and **08/218** (adsorbed toxoid), manufacturer matched drug substance (T Toxoid or adsorbed T Toxoid), and a range of manufacturer matched drug products

- Sample and Reference curves were considered to be similar if the **difference** between the upper or lower asymptotes fell within -0.05 and 0.05 and if the **ratio** of the slopes was between 0.90 and 1.11.

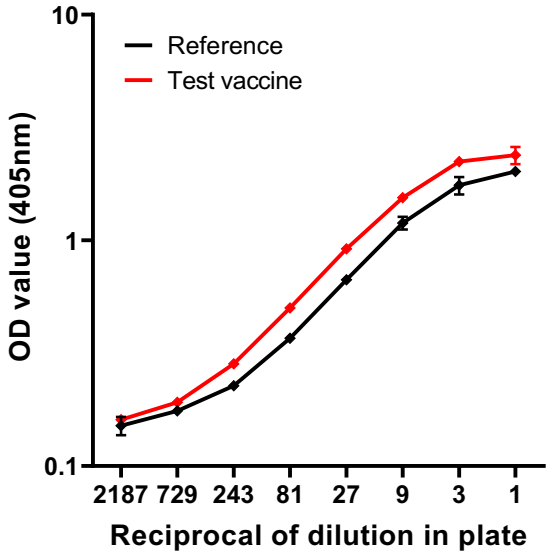
ELISA – curve analysis examples

Example 1
No differences in slope or asymptotes



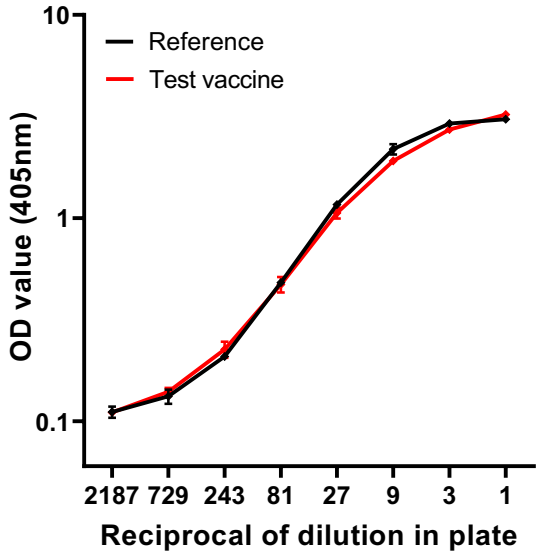
Slope ratio = 1.0347
Upper asymptote difference = 0.013
Lower asymptote difference = 0.024

Example 2
Different upper asymptote



Slope ratio = 0.960
Upper asymptote difference = 0.069
Lower asymptote difference = -0.006

Example 3
Different slopes



Slope ratio = 0.812
Upper asymptote difference = 0.042
Lower asymptote difference = -0.042

ELISA – curve analysis conclusions

- Small differences observed in the upper asymptotes and slopes for *some* of the products compared to WHO standards, but generally not against the drug substances from the same manufacturer

- Results suggest that, for many products, there will be different options available for the choice of reference standard to use in the assay

Transfer of T ELISA to other laboratories

- Successful transfer of the ELISA has been demonstrated to multiple laboratories
  - Each lab performed **3 assays**, **2 plates per run** (total of 6 plates per lab)

Study	Product + adjuvant type	Geomean relative estimate difference (%)	Intermediate precision GCV%	
			Partner	MHRA
Lab 1 (H)	Tdap + $AlPO_4$	1.6	4.5	3.8
Lab 2 (H)	DTaP-IPV-HepB-Hib + $Al(OH)_3$	14.2*	3.4	4.4
Lab 3 (H)	DTaP + $Al(OH)_3$	5.5	2.7	3.9
	dTaP + $Al(OH)_3$	4.4	7.2	3.3
Lab 4 (V)	Ruminant multivalent + Alum	4.8	12.3	13.5
	Ruminant multivalent + $Al(OH)_3$	9.6	5.3	7.0
Lab 5 (V)	Ruminant multivalent + Alum	4.6	1.8	1.9
	Equine bivalent + $AlPO_4$ / ISCOMS	3.6	4.6	6.6

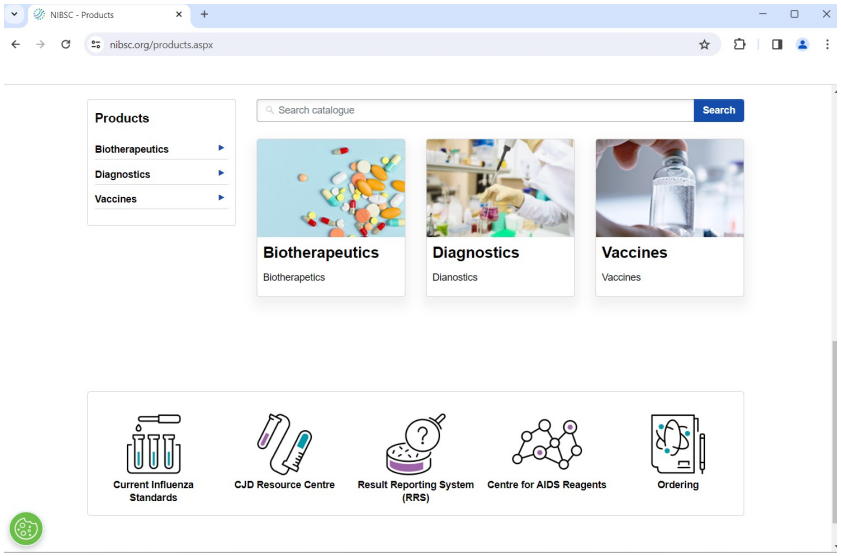
- Intermediate precision <15% for all products tested and GCV obtained by the partner lab was within 5% of that obtained by MHRA
- Geomean relative estimate difference within 10% for all products except one*

Conclusions

- Proof of concept has been demonstrated for the T ELISA, including evidence that the assay may be stability indicating
- T 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

Availability of purified mAbs

- The mAb pairs used in the T ELISA shown in this presentation are available from www.nibsc.org for laboratories who want to establish and validate these methods
- Removes one of the most common barriers to development of alternative methods – restrictions on the availability and use of critical reagents



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

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

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