Health for Animals
global animal health association

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Clostridium tetani ELISA – applicability in veterinary industries

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Critical Points for ELISA applicability in Veterinary Industries

1. Applicability in Quality Control (GMP) environment

- Analytical Target Profile/Quality Attribute
- Applicability : Matrix effect, Equipment, Software,…
- Controls/Reference-Standard in place and stability indicator
- Feasibility/Qualification prior Validation according to VICH guidelines
- Specifications (minimum and maximum titers)
- Transferability and Robust method for long term use in Quality Control labs

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- Previous studies run during Vac2Vac project had promising results
- Additional studies confirm the quality of reagents-antibodies

Critical Points for ELISA applicability in Veterinary Industries

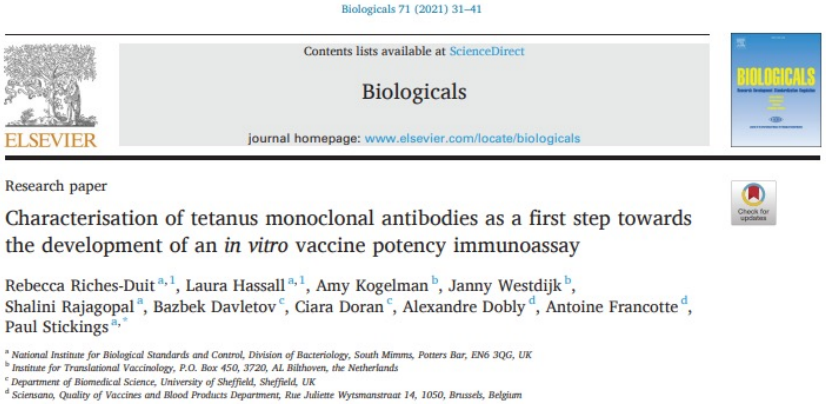
2. Quality of reagents (e.g. antibodies)

- Characterization of Reagents (e.g. antibodies)

✓Affinity study e.g. Biosensor
✓Neutralisation study
✓Epitope study e.g. Epitope Competition study

- Testing of Reagents (e.g. antibodies)

✓SDS-PAGE/SE-HPLC
✓Dilution/dose response curve
✓Stability after reconstitution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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,*]

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- Full characterization of antibodies completed during Vac2Vac project
- Hybridomas-central and global distribution of purified antibodies
- Supply from catalogue - MTA to fill in

3-5, 32-1, 629E1, FHADETOX/6, PS21C2.2.1, G10F8C3, 1-7, 69K/16
https://nibsc.org/products/brm_product_catalogue/sub_category_listing.aspx?category=Vaccines&SubCategory=Pertussis

DT05 & DIM9
https://nibsc.org/products/brm_product_catalogue/sub_category_listing.aspx?category=Vaccines&SubCategory=Diphtheria

TT010 & 8E1-1H1.2.1
https://nibsc.org/products/brm_product_catalogue/sub_category_listing.aspx?category=Vaccines&SubCategory=Tetanus

3. Sustainability of reagents (e.g. antibodies)

- Long term supply
- Production's consistency
- Minimization of batch-to-batch variability

Next steps for substitution

1. Analytical target profile/quality attribute

- Assessed during development within Vac2vac
- Can substitute *in vivo* potency assay as quantitative and specific test targeting relevant immunological epitope and stability indicator

Previous studies done during Vac2Vac project have promising results based on

- Multiple products tested
- Altered products tested

Robust results even if changing some conditions (eg shaking, duration of incubation...)

2. Applicability

- Equipment/software (different requirements depending on countries);
- controls for validity of the test and standard/reference might be needed for each product
- To optimize analytical test with each new matrix : check if presence of interferences by testing different formulations; Might need or not antigen extraction process (depending on each product tested)

3. Feasibility/Qualification prior going to validation

Next steps for substitution

4. Validation according to VICH guidelines

VICH Topic GL2 (validation-methodology)

- *Specificity*
- *Linearity*
- *Range*
- *Accuracy*
- *Precision (repeatability, intermediate precision, reproducibility)*
- *LOD/LOQ*
- *Robustness*

Previous studies done during Vac2Vac project have promising results based on

- multiple products tested to date
- good specificity, linearity, accuracy, precision observed (see Laura Hassall presentation)

5. Specifications

Each vaccine type needs specifications set up/reevaluation; link to flocculation (Lf) might be difficult to establish

Next steps for substitution

6. Transferability in GMP environment

- For release and stability testing
- Routine use
- Multiple operators
- Multiple products
- Multiple sites/labs
- During decades

Previous studies done during Vac2Vac have promising results based on Involvement of multiple labs at different locations

Having appropriate equipment in all labs is critical

Pipetting remain a critical step for accurate results

Conclusions

Veterinary industries are working towards company specific ELISA approach to substitute current *in vivo C. tetani* potency test.

Each vaccine matrix needs specific assessment (consistent antigen recovery, validation, specifications) but having reliable reagents and robust antigen ELISA helps to move forward.