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How Industry and Regulatory innovation and collaboration can promote the transition from *in vivo* to *in vitro* potency testing for human legacy vaccines

WCBP

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AGENDA

1 From *in vivo* to *in vitro* quality control (QC) testing for vaccines

2 European Pharmacopoeia 5.2.14 Guidance on the Substitution of *in vivo* method(s) by *in vitro* method(s) for the quality control of vaccines

3 Vac2Vac collaboration for ensuring potency through alternative *in vitro* methods for DTaP (Diphtheria, Tetanus, Acellular pertussis) vaccines

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From *in vivo* to *in vitro* QC Testing of vaccines

Sanofi's strategy for vaccines : Quality Control with scientifically relevant non-animal-based analytical testing

Ongoing

Test in **animals only for preclinical** translational package

Innovative **state of the art *in vitro/in silico*** technologies applied for **clinical batch testing** (eg High Throughput Sequencing) and **product characterization**

New Vaccines

Established Vaccines

Legacy or redundant safety tests removal eg Abnormal toxicity test (ATT) & specific toxicity tests

Development of tetanus residual toxicity *in vitro* functional assay (BINACLE)

Rabbit Pyrogen test replacement by *in vitro* functional assays (MAT/BET)

***In vitro* potency development for DTaP (Diphtheria, Tetanus & acellular pertussis)**

Achieved

In vitro CHO cells clustering assay (residual acellular pertussis toxin)

VERO cell assay (residual Diphtheria toxin)

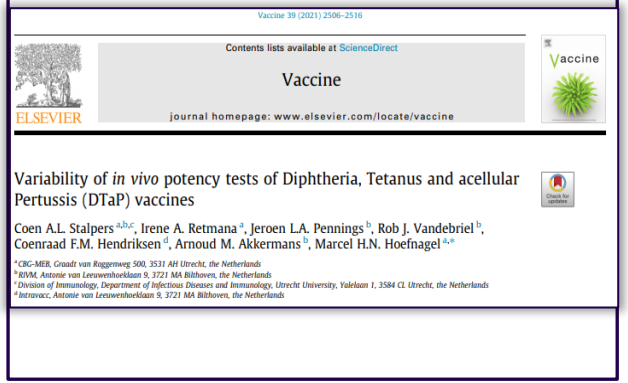
Full in vitro Adventitious agents testing

In vitro potency for viral and polysaccharide vaccines

Specific animal based testing requirements (National/Regional Pharmacopoeias, Importation)

Current in vivo potency testing for DTaP vaccines : Are they scientifically appropriate?

High variability of in vivo potency testing of DTaP vaccines



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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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- These ***in vivo*** assays (on guinea pigs or mice) :
  - are **labor intensive**, **costly, lengthy**
  - remain **an ethical concern**
  - have **high inherent variability**
  - show **poor discriminative power**
  - show high **invalidity rate**
  - can lead to **false out of specification** results

Their use in routine batch release testing is questionable versus more scientifically relevant *in vitro* methods

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5.2.14 Guidance on Substitution of in vivo method(s) by in vitro method(s) for the quality control of vaccines

European Pharmacopoeia

edqm

Chapter 5.2.14 : what is it ?

5.2.14. SUBSTITUTION OF *IN VIVO* METHOD(S) BY *IN VITRO* METHOD(S) FOR THE QUALITY CONTROL OF VACCINES

PURPOSE

The purpose of this general chapter is to provide guidance to facilitate the implementation of *in vitro* methods as substitutes for existing *in vivo* methods, in cases where a typical one-to-one assay comparison is not appropriate for reasons unrelated to the suitability of one or more *in vitro* methods. This general chapter will not discuss the details of assay validation as such, since those principles are described elsewhere.

The general chapter applies primarily to vaccines for human or veterinary use, however the principles described may also apply to other biologicals such as sera.

- This guidance (implemented in 2018) :
  - supports the deployment of the **consistency approach** for quality control of **legacy products**
  - aims to facilitate **the acceptance at international** level of the transition to **more scientific** and **less animal-centric** testing approaches
  - introduces a new concept of **substitution** : where one-to-one comparisons are not feasible or scientifically justified

"The consistency approach is a concept which includes the strict application of GMP rules and guidelines, process validation and in process and final product tests and is aimed at verifying if a manufacturing process produces final batches which are consistent with one that fulfils all the criteria of Quality, Safety and Efficacy as defined in the marketing authorization, ultimately resulting in replacement of routinely used in vivo tests."

De Mattia et al, *Biologicals* 39:59-65, 2011

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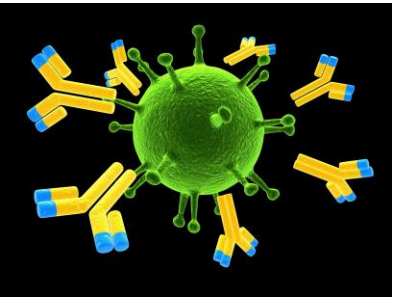
Chapter 5.2.14 : General Considerations



- Tests methods used for **QC** are intended to monitor **production consistency**
  → the inherent variability of ***in vivo* assays** can make them **less suitable** than appropriately designed *in vitro* assays for that purpose

- ***In vitro* bioassays** can mimic **specific elements** of complex *in vivo* responses :
  - The **quality attribute** of the product will likely be **assessed differently**
  - with generally **lower variability** and **higher sensitivity**

  → a typical one-to-one assay comparison may not be not appropriate for reasons unrelated to the suitability the *in vitro* method(s) used

- Assays must be :
  - **fit for purpose** (including stability indicating capacity)
  - **properly validated-**Not necessarily validated through collaborative multicentric studies and widely applicable to a range of products

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Chapter 5.2.14 : Potency Tests

- Design of the assay needs to reflect antigen **content** and **functionality**

- Assay **evaluation** :
  with samples at **different concentrations**
  with samples submitted to **stress conditions** (stability indicating potential)

- **Agreement** with ***in vivo*** **assay** :
  Not necessarily possible as *in vitro* assay will have a superior discriminative power

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VAC2VAC collaboration: Ensuring potency through alternative in vitro methods for DTaP

IMI Vac2Vac

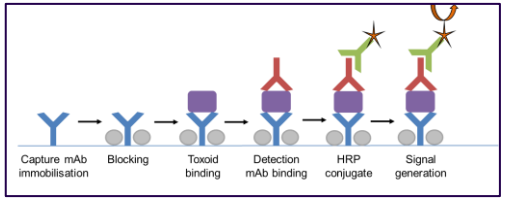
Vac2Vac stands for Vaccine batch to Vaccine batch comparison by consistency testing*

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

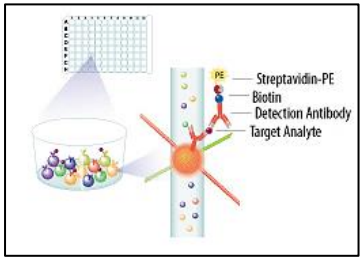
- http://www.vac2vac.eu/
- **23 European Partners**
- **5 years** project (Mar 2016 – Feb 2022)
- **16 M€** total budget
- Products: **7 Vaccine Franchise**
  5 veterinary + 2 human + 1 adjuvant
- **33 tasks** organised in **4 technical work packages** to replace animal assays in Quality Control

VAC2VAC Outputs (1) : Immunoassays for DTaP vaccines

ELISA



MULTIPLEX



ALTEX, accepted manuscript
published August 23, 2024
doi:10.14573/altex.2401171

Research Article

Development of a Monoclonal Antibody Sandwich ELISA for the Quality Control of Human and Animal Tetanus Vaccines

Laura Hassall[1], Daniel Alejandro Yara[1], Rebecca Riches-Duit[2], Peter Rigsby[3], Alexandre Dobly[3], Maxime Vermeulen[3], Antoine Francotte[4], Bart Faber[5] and Paul Stickings[1]

[1]Medicines and Healthcare products Regulatory Agency, South Mimms, UK; [2]Medicines and Healthcare products Regulatory Agency, London, UK; [3]Sciensano, Quality of Vaccines and Blood Products, Brussels, Belgium; [4]Sciensano, Human Infectious Diseases, Brussels, Belgium; [5]Department of Parasitology, Biomedical Primate Research Center, Rijswijk, The Netherlands

Research Article

Development of a Monoclonal Antibody Sandwich ELISA for the Determination of Antigen Content and Quality in Diphtheria Vaccines

Laura Hassall[1], Daniel Alejandro Yara[1], Rebecca Riches-Duit[2], Peter Rigsby[3], Alexandre Dobly[3], Maxime Vermeulen[3], Antoine Francotte[4] and Paul Stickings[1]

[1]Medicines and Healthcare products Regulatory Agency, National Institute for Biological Standards and Control, South Mimms, UK; [2]Medicines and Healthcare products Regulatory Agency, Canary Wharf, London, UK; [3]Sciensano, Quality of Vaccines and Blood Products, Brussels, Belgium; [4]Sciensano, Human Infectious Diseases, Brussels, Belgium

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journal homepage: www.elsevier.com/locate/jim

ELSEVIER

Development of a multiplex-based immunoassay for the characterization of diphtheria, tetanus and acellular pertussis antigens in human combined DTaP vaccines

Maxime Vermeulen [a,*], Isabelle Feck [a], Antoine Francotte [b], Laura Hassall [f], Lorenzo Tesolin [a], Wim Van Molle [a], Romain Pizzato [a], Thierry Laurent [d], Charline Hoebreck [c], Paul Stickings [f], Alexandre Dobly [a]

VAC2VAC demonstrated **proof of concept** for **DTaP** immunoassays

- Wide applicability to different vaccines
- Excellent precision
- Ability to detect small changes in antigen *content* and *quality*
- Successful transfer to different labs

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VAC2VAC Outputs (2): Available reagents

Characterization of mAbs

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Research paper

Characterisation of diphtheria 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], Alexandre Dobly [c], Antoine Francotte [c], Paul Stickings [a,*]

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

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,*]

A **pair of monoclonal antibodies (mAbs)** was selected for **each antigen :**

- directed against **relevant epitopes** on the target antigen
- able to bind **native** and **detoxified** antigen
- able to recognise **heat-altered** antigen
- **well characterized**

Sustainability plan

VAC2VAC

Vaccine batch to vaccine batch comparison by consistency testing

NEWSLETTER Vol. VI May 2022

VAC2VAC Sustainability Plan

NIBSC

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.

A model was created for **sustainable supply** of these **critical reagents** through MHRA (Medicines and Health care products Regulatory Agency)

VAC2VAC Outputs (3): Open letter

Open Research Europe

Open Research Europe 2022, 2:116 Last updated: 06 MAR 2023

Check for updates

OPEN LETTER

REVISED **The consistency approach for the substitution of *in vivo* testing for the quality control of established vaccines: practical considerations and progressive vision [version 2; peer review: 2 approved]**

Jean-Francois Dierick[1], Marlies Halder[2], Carmen Jungbaeck[3], Julie Lorenz[4], Jean-Marie Préaud[3], Patrice Riou[5], Lorenzo Tesolin[6], Sylvie Uhlrich[5], Wim Van Molle[6], Joris Vandeputte[3]

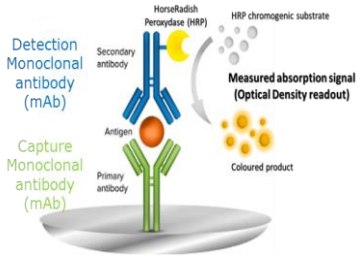
« Recently produced batches can be considered comparable to the original clinical batches »

« The introduction of a new analytical method neither changes the variability of the manufacturing process nor the quality of the poduct »

« If results generated with in vivo substitution method show decrease/increase over time an End of Shelf Life acceptance criterion can be defined »

« Robust science and early interaction between manufacturers and competent authorities are key elements to success »

Remaining activities for manufacturers



- Further **development** and **optimization** of assays to **specific products,** including the potential selection of alternative mAbs as analytical tool, will allow for **optimal assay performance.**

- Demonstration of **suitability** further to V2V deliverables, including:
  - mAbs screening and characterization
  - Studies demonstrating assays' capacity to :
    - detect changes in antigen quantity and quality
    - serve as stability indicators (ability to detect product degradation)
  - Comparison to *in vivo* assays.

- Full **method validation.**

- **Publication** of additional data (e.g.: pertussis mAbs characterisation and assay development).

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Emmanuelle Coppens is a Sanofi employee and may hold shares and/or stock options in the company.

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