

sanofi

Transition from *in vivo* to *in vitro* potency testing for Diphtheria, Tetanus and Acellular Pertussis (DTaP) combination vaccines

Emmanuelle Coppens, Global Analytical Expert, Global Analytical Sciences, Sanofi, France

AFSA/IABS

October 7, 2026

AGENDA

1 Global Situation for Vaccines Potency Testing at Sanofi

2 VAC2VAC Collaboration

3 Sanofi Vaccines Antigenicity Assays for DTaP Antigens

4 Next Steps

Global situation for vaccines potency testing at sanofi



Potency Tests: Transition from *in vivo* to *in vitro* – Current situation

Antigen	*In vivo* assay	*In vitro* assay	Curent status of replacement
Hepatitis A	Mouse serology	Antigenicity assay (ELISA)	Full implementation
Rabies	Mouse challenge (NIH test)	G protein ELISA	Ongoing implementation
Polio	Rat/Guinea-pig/chicken serology	D antigen assay (ELISA)	Full implementation
Hepatitis B	Mouse serology	Antigenicity assay (ELISA)	Full implementation
Haemophilus	Mouse serology	Polysaccharide content & structure (physico-chemical methods)	Full implementation
Diphtheria, Tetanus	Mouse/Guinea-pig challenge Guinea-pig serology	Antigenicity assay (ELISA)	Ongoing implementation
Acellular Pertussis	Mouse/Guinea-Pig serology	Antigenicity assay (ELISA)	Ongoing implementation

From *in vivo* to *in vitro* for DTaP potency testing (only remaining *in vivo* potency test)

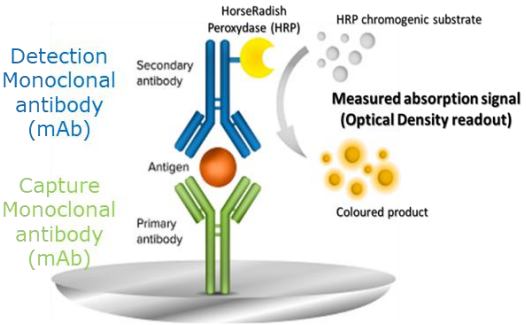
CURRENT

- different antigen and product-specific tests

	US	EU/ International		
D	G.Pig serum neutralization (USPHS)	G.Pig Serological	G.Pig intradermal challenge	G. Pig Single Immunogenicity Assay*
T	G.Pig serum neutralization (USPHS)	G.Pig Serological	Mouse paralytic challenge	
aP	Mouse Serological			

FUTURE

Sandwich ELISAs for all antigens and all markets



Moving to *in vitro* is the opportunity to move away from market specific testing and complex testing profiles

D: Diphtheria antigen
T: Tetanus antigen
aP: pertussis antigens

sanofi

VAC2VAC Collaboration



IMI Vac2Vac

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

- **Demonstrate proof of concept of novel physicochemical, immunochemical or biological *in vitro* tests for vaccines**
- **Facilitate regulatory acceptance**

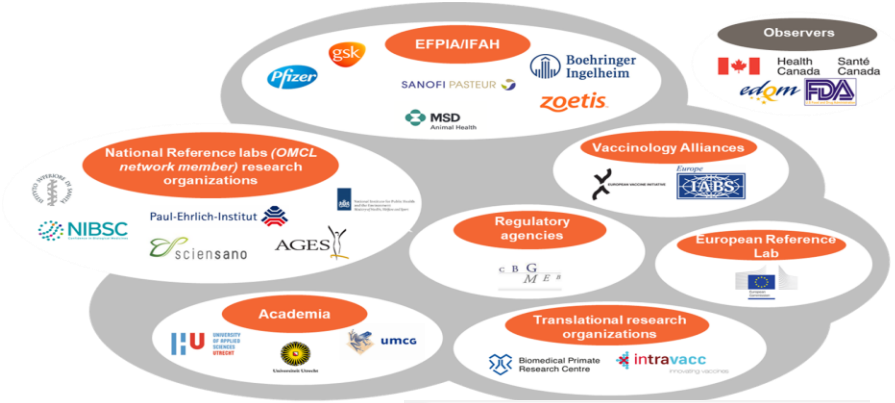
23 European Partners

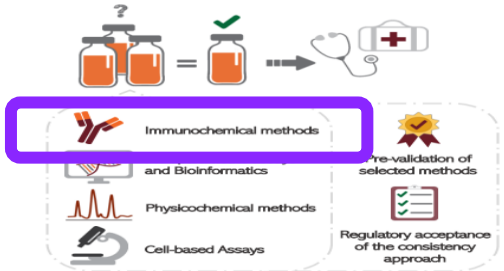
5 years
2016 –2022

16 M€

7 Vaccines
5 veterinary, 2 human & 1 adjuvant

33 tasks
4 technical work packages





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

VAC2VAC Outputs : Immunoassays for DTaP vaccines & available reagents

Immunoassays



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[1], Alexandre Dobly[1], 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[1], Alexandre Dobly[1], 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

Journal of Immunological Methods 517 (2023) 113483

Contents lists available at ScienceDirect

Journal of Immunological Methods

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

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 [c], Lorenzo Tesolin [a], Wim Van Molle [a], Romain Pizzato [a], Thierry Laurent [d], Charline Hoebreck [a], Paul Stickings [c], Alexandre Dobly [a]

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,*]

Sustainability plan



VAC2VAC

NEWSLETTER Vol. VI May 2022

Vaccine batch to vaccine batch comparison by consistency testing

VAC2VAC SUSTAINABILITY PLAN

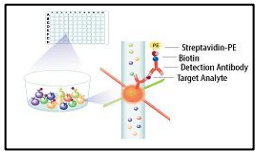
Implementation of the sustainability plan

NIBSC

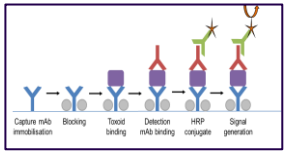
Monoclonal antibodies available at the NIBSC catalogue (www.nibsc.org)

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.

MULTIPLEX



ELISA



- **Proof of concept for DTaP immunoassays**
- **A pair of monoclonal antibodies (mAbs) was selected for each antigen**
- **A model was created for sustainable supply of these critical reagents through MHRA (Medicines and Health care products Regulatory Agency)**

VAC2VAC Outputs : Demonstration of assay suitability included assessment of several options for reference standards

- **Different Diphtheria and Tetanus antigen containing materials**
- **Drug products against other drug products of the same manufacturer**

(dose response curve shapes)

TETANUS

Material evaluated :
WHO 16/302 (non-adsorbed toxoid)
WHO 08/218 (adsorbed toxoid)
manufacturer drug substance (pre-adsorbed or adsorbed toxoid) against a range of manufacturer drug products.

Conclusion: manufacturer drug substances had more comparable curve shapes to final drug products compared to the ISs*

DIPHTHERIA

Material evaluated :
WHO 13/212 (non-adsorbed toxoid)
WHO 07/216 (adsorbed toxoid),
manufacturer drug substance (pre-adsorbed or adsorbed toxoid) against a range of manufacturer drug products.

Conclusion : adsorbed material showed slightly better parallelism to drug products than non-adsorbed material

- **Different options** are available for the **choice of reference standard** to use
- **Specific drug products** were suitable to act as a reference preparation **for other products** produced by the same manufacturer

sanofi

*IS : International Standard

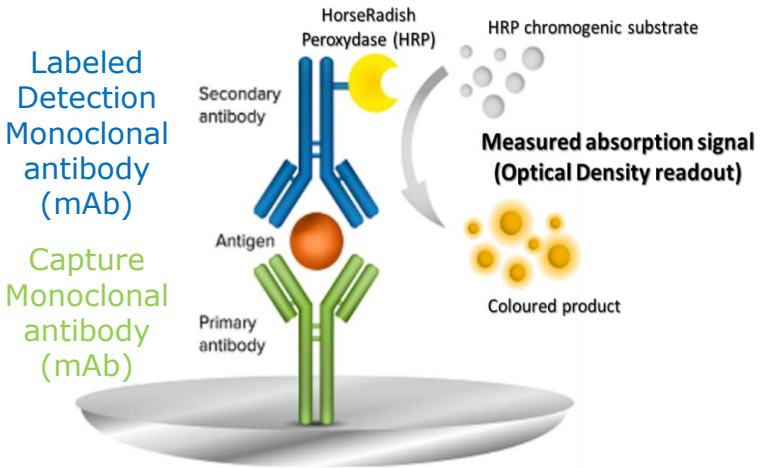
Hassall and al, ALTEX 41(4), 2024

Hassall and al, ALTEX 41 (1), 2024

Sanofi Vaccines Antigenicity Assays

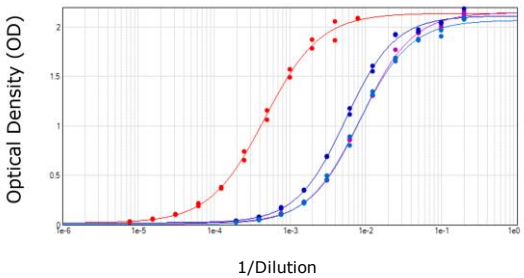


Antigenicity ELISA assays_One Assay principle for all DTaP combinations



ELISAs ID CARD	
ELISA format	Sandwich ELISA (96well plate)
Readout	Colorimetric (HRP)
In-house Reference standard (Ref Std)	**Non-homologous (non adsorbed DS)**
Unitage	**Arbitrary Antigenicity Units/mL (AU/mL)**
Curve-fitting model	4PL
Parallelism stat model	Equivalence
Acquisition and analytical Software	SoftmaxPro

Antigenicity ELISA assays_Rational for selection of in-house reference standards



Reference Standard (DS)
Batch 1 (DP)
Batch 2 (DP)
Batch 3 (DP)

Example of dose response curves

- **No available international or EDQM reference standard** for DTaP antigenicity
  - **Applicability of such a standard?**
- Consideration of **existing in-house references** used for antigenicity ELISAs
  - **Practice in place** and **Prior knowledge**
- **Representative materials** with ability to be used over
  - Different **products**
  - Different **manufacturing steps**
- Ensure **sustainable assay** for **product consistency**

DS : Drug substance
DP : Drug product

Antigenicity ELISA assays_ choice of in-house reference standards and characterization

Diphtheria & Tetanus

Acellular pertussis

FIM · PRN · FHA · PT

CASE 1

- Pool of drug substances (non adsorbed)
- Characterization: meeting all specifications
- Storage: +5°C ± 3°C

- 1 batch of drug substance (non adsorbed)
- Characterization: meeting all specifications
- Storage: +5°C ± 3°C or ≤20°C or ≤60°C

CASE 2

"Ultra-Stable" reference standard :

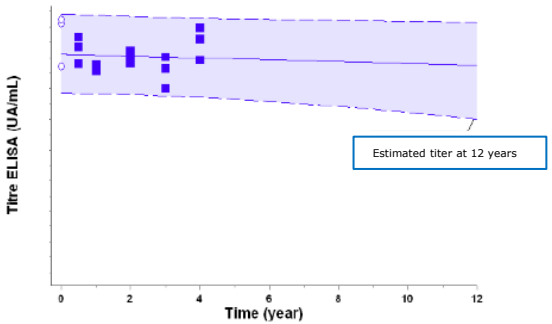
- 1 batch of drug substance (non adsorbed) Stabilized with trehalose Lyophilized
- **Specific characterization and stability protocol**
- Storage: +5°C ± 3°C

BATCH SELECTION

- **Routinely manufactured batches**
- **Meeting all licensed requirements**
- **Choice based on production availability**

ALL CASES — **Aliquots for single use are manufactured**

Antigenicity ELISA assays_Unitage and expiry date assignment of in-house reference standards



Example of prediction bands (5°C)

- **Arbitrary unitage assignment (AU):**
  - Not necessarily based on same numerical value as protein or Lf content
- **Expiry date assignment based on:**
  - **Case 1 : Prior knowledge**
    - previous similar material used as Ref Std in other ELISAs or on established expiry assigned to routine lots
  - **Case 2 : Advanced kinetic modelling (AKM)**
    - antigenicity experimental data obtained under recommended storage conditions (5°C) and accelerated stability (25°C to 55°C)

Antigenicity ELISA assays_Stability monitoring and Life Cycle Management



- **Common practices for all vaccine reference standards**:
  - **Continuous control chart monitoring** (eg slope, EC50, internal control result)
- **Specific stability study for stabilized and lyophilized Ref Std**:
  - **Real time** and **accelerated** study
  - **Physico-chemical attributes** (eg appearance, residual moisture, pH, dissolution time,…)
- **Qualification of new vaccine reference standards**:
  - **Pairwise assays** candidate vs current reference standard
  - **Calibration** (Assignment of a titer relatively to current reference titer) or **Comparison** (check for nondifference/equivalence between candidate and current ref standard)
  - **Impact assessment** on product consistency/potency evaluation

Next Steps



Current status and next steps

Current status	• **All methods validated for all products and antigens** • **Pre-submission consultations and submissions engaged in 2020** • **Approval granted by different health authorities (eg FDA & EMA) for several antigens and products**
Next steps	• **Evaluation of International Reference Standards or other BRPs** **-> Monitor assay system with regular testing of IRS/BRP?** • **Transfer of assays to OMCLs**

Acknowledgements : Sandrine Guichard, Annabelle Lebas, Meili Li, Juthika Menon, Romain Pizzato & scientists from Analytical Sciences and Quality Control Laboratories

Thank You

This work was funded by Sanofi.
Emmanuelle Coppens is a Sanofi employees and may hold shares and/or stock options in the company.

sanofi