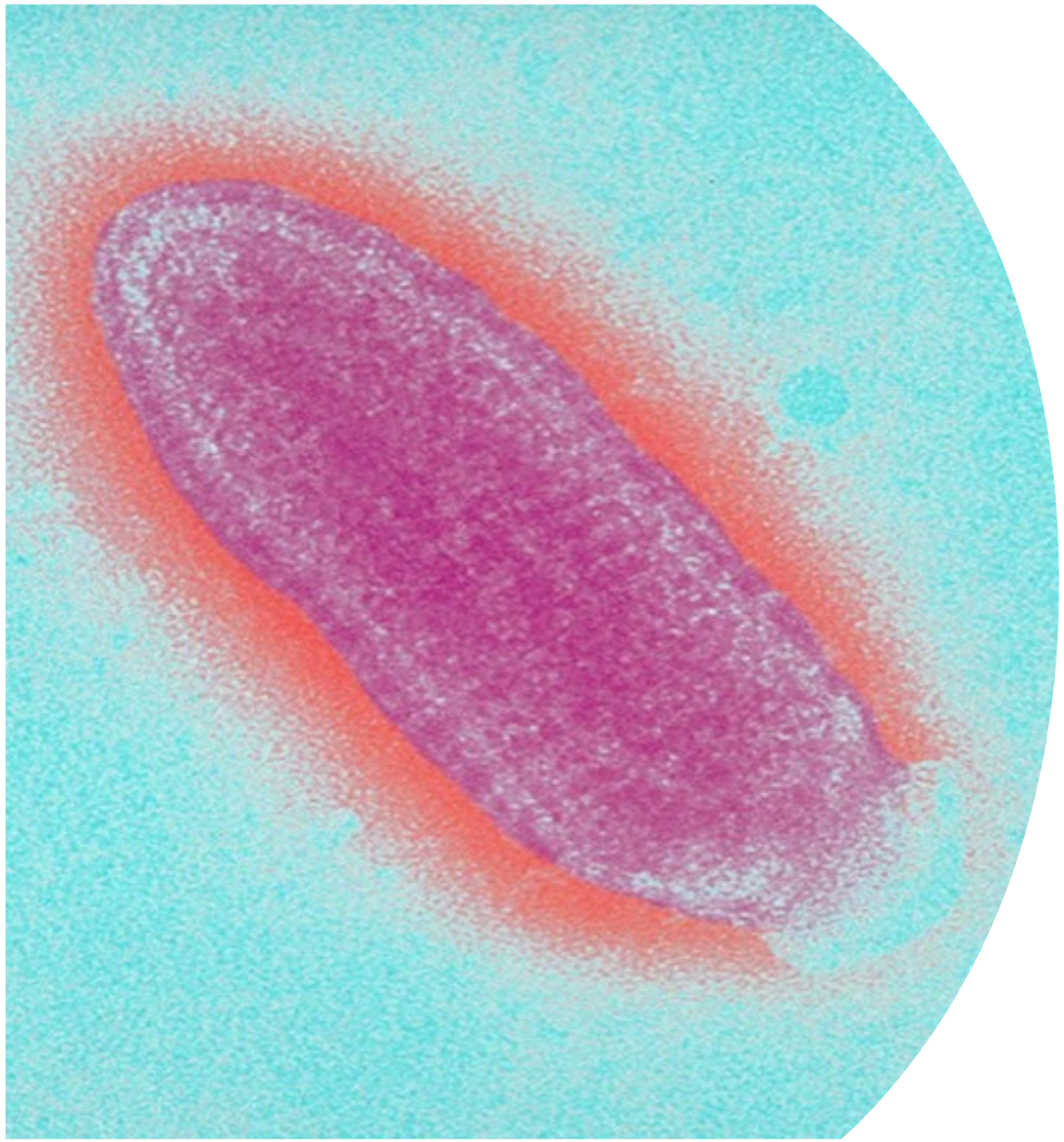

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Human Rabies Vaccines Part I
Switching from *in vivo* to *in vitro* potency testing

Patrice Riou

Global Analytical Strategy and Regulatory Compliance
R&D
Sanofi Vaccines

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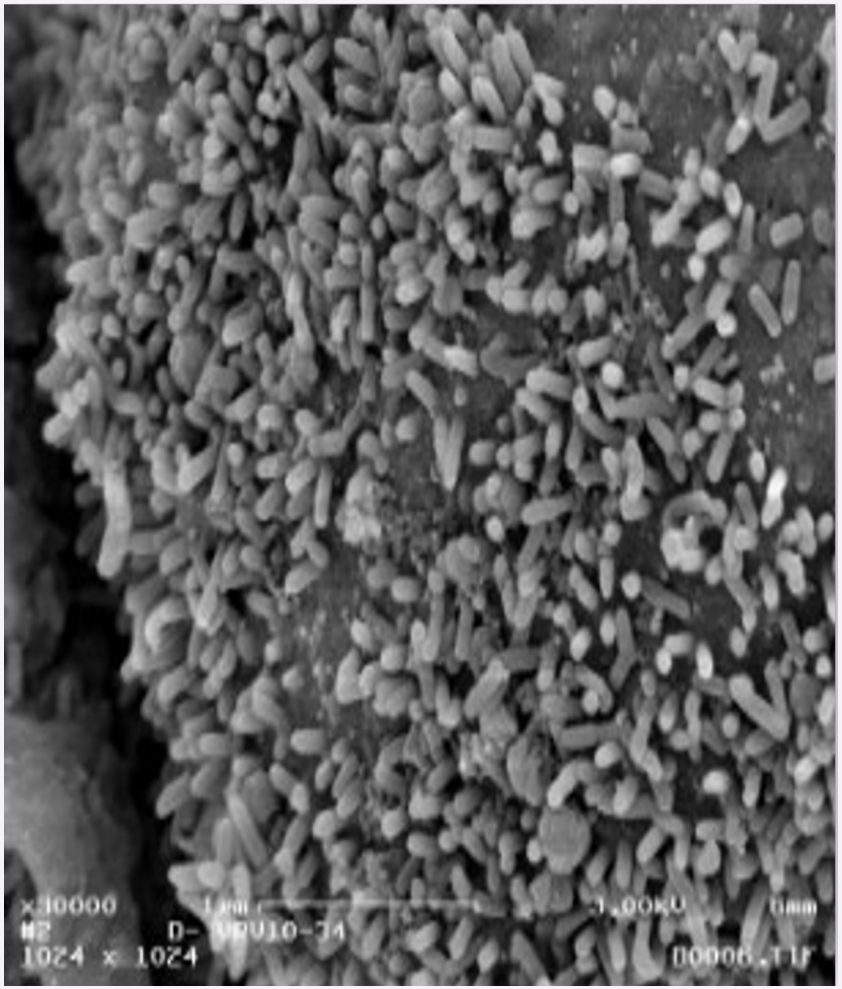

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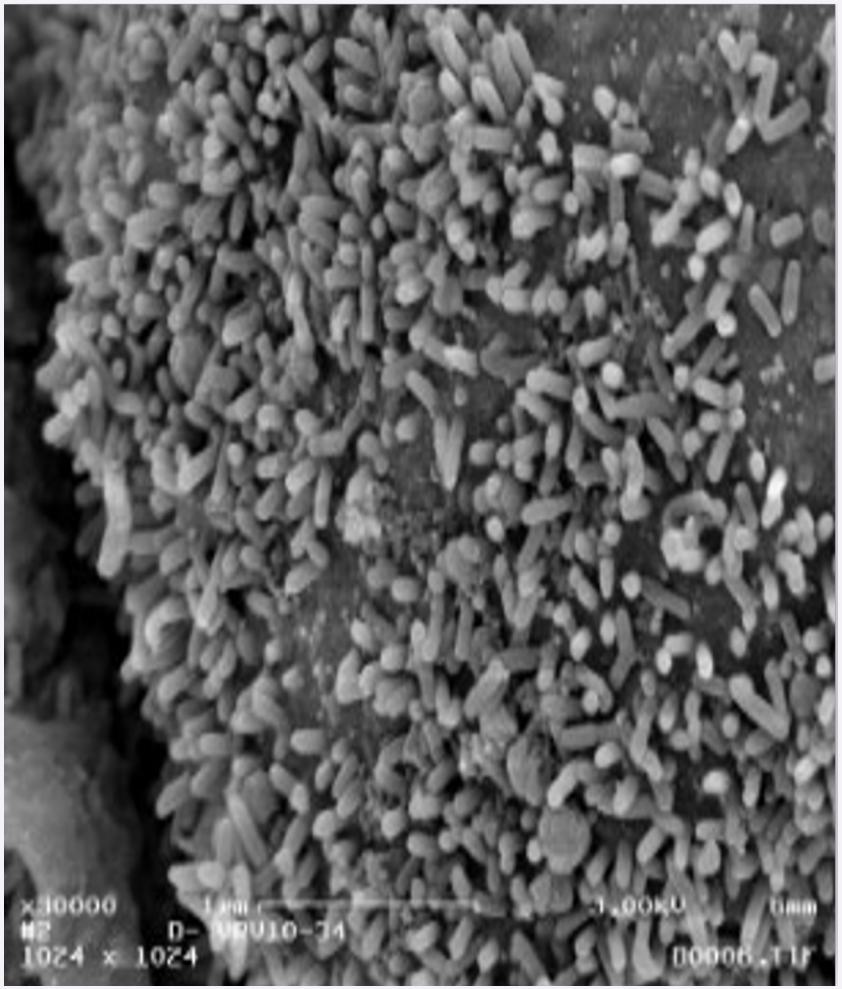
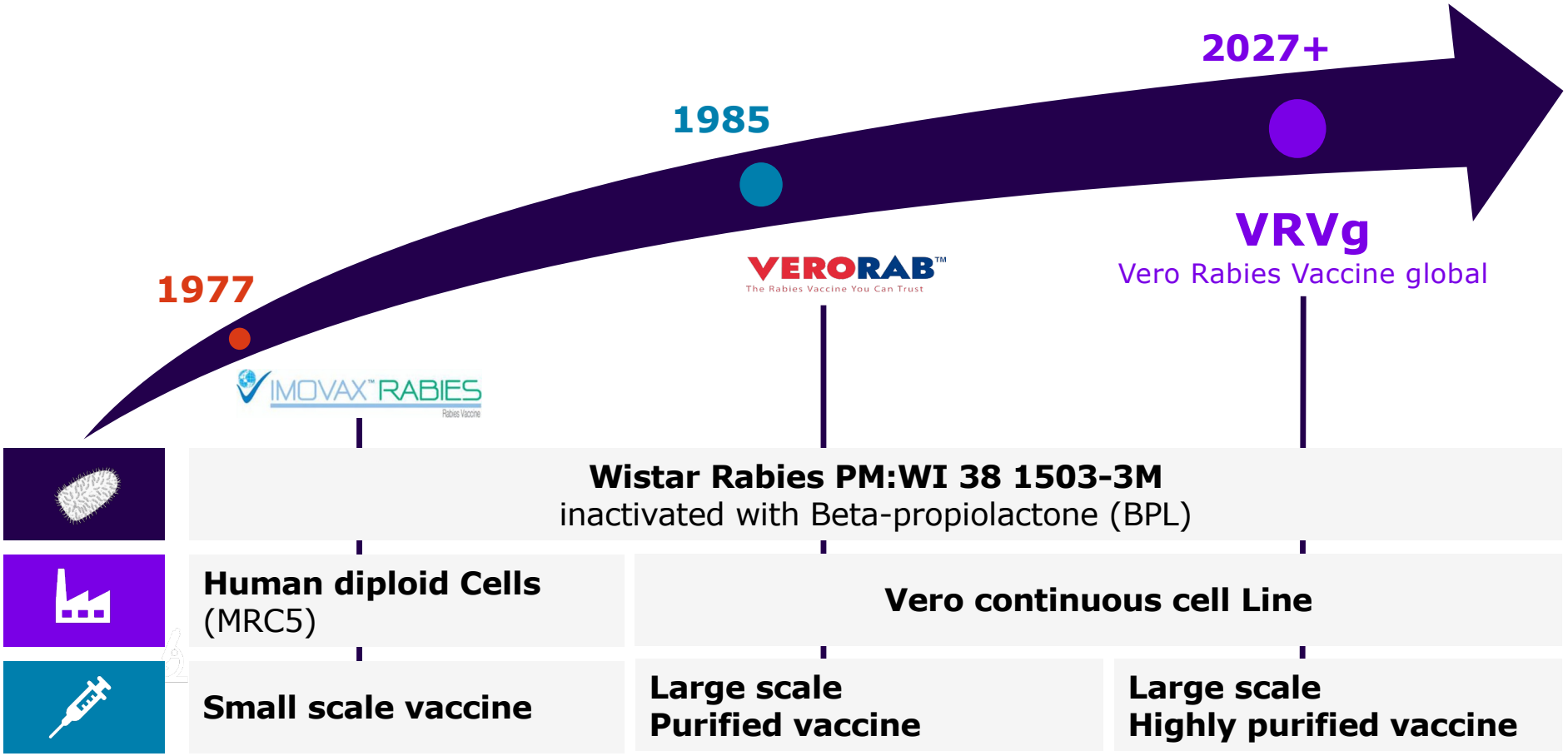


Sanofi Human Rabies Vaccine Portfolio



Rabies Vaccine Potency Assays

NIH *in vivo* potency assay

- Immunization followed by lethal challenge in mice with IC injection of virulent rabies suspension (CVS strain)
- Developed in 1966(1) and used for more than 50 years to release rabies vaccines
- Compendial test described in WHO TRS 941 & Ph. Eur. 0216
- High variability observed & use of a large number of animals per test

Objective → REPLACEMENT of animal model

3Rs

Current Method	Regulatory context	New method
 **NIH *in vivo* Potency test**	• **DIRECTIVE 2010/63/EU on the protection of animals used for scientific purposes** • **Ph. Eur. 0216 – Rabies vaccine for human use**	 **ELISA *in vitro* Potency test**

(1) Seligmann EB Jr. Laboratory techniques in rabies. Potency-test requirements of the United States National Institutes of Health (NIH). Monogr Ser World Health Organ. 1966;23:145-51

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G protein ELISA – Good surrogate of potency !?

Major correlate of protection is due to glycoprotein G neutralizing antibody	≈ 83% of human rabies neutralizing Abs are against G protein domain III	The protection mainly depends on the preservation of its three-dimensional structure	Denatured glycoproteins are shown to be poorly immunogenic	Initial studies indicate good agreement between NIH test and the ELISA antigen content
•*Wiktor T et al ; J Immunol 1973;110:269–76*	•*Kramer et al, Eur J Immunol. 2005 Jul;35(7):2131-45*	•*Bunschoten et al, J Gen Virol. 1989 Jun;70 (Pt 6):1513-21* •*Bunschoten et al, J Gen Virol. 1989 Feb ;70 (Pt 2):291-8*	•*Gamoh et al, Biologicals 1996;24:95–101* •*Dietzschold et al, Virology 1983;124:330–7*	•*Lafon et al, J. Biol. Standard., 13 (1985), pp. 295–301* •*Thraenhart et al, J. Biol. Standard., 17 (1989), pp. 291–309* •*Perrin et al, Biologicals, 18 (1990), pp. 321–330* •*Rooijakkers et al, J. Virol. Methods, 58 (1996), pp. 111–119* •*Rooijakkers et al, Dev. Biol. Stand; 1996; 137–145.* •*Gibert et al, Vaccine. 2013 Dec 5;31(50):6022-6029*

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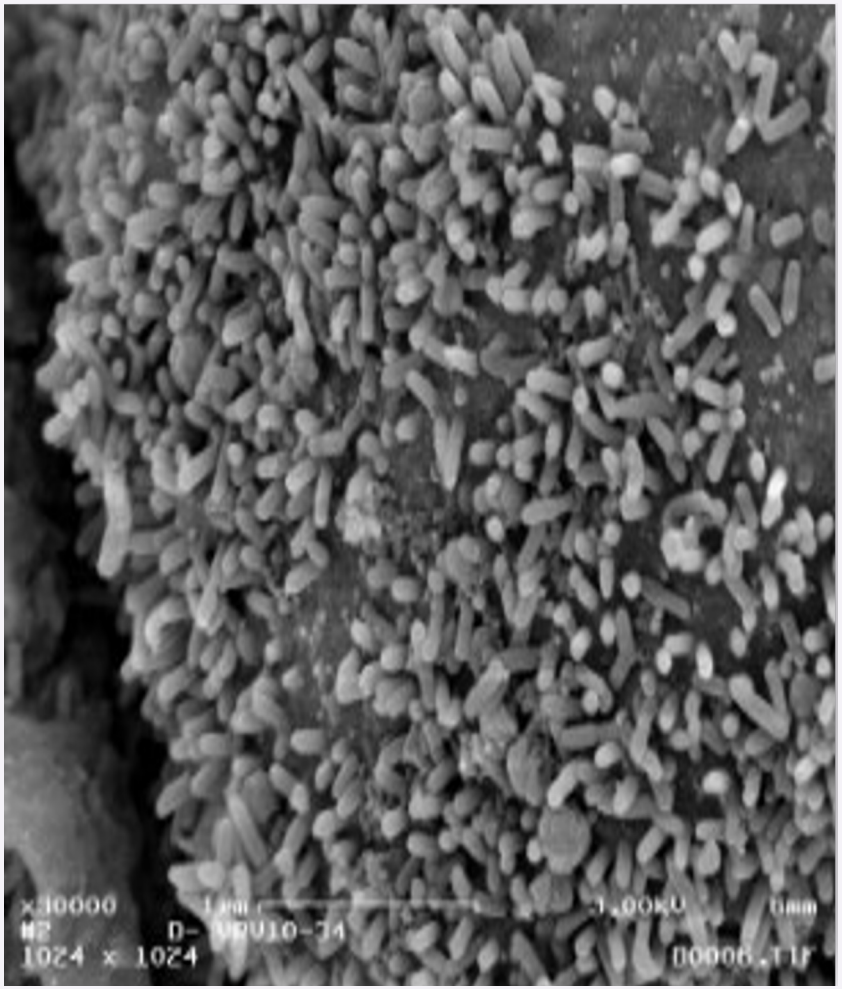


Rabies G protein ELISA – monoclonal Antibodies

Quantitative sandwich direct ELISA method using two monoclonal antibodies against specific rabies G protein epitopes

Capture mAb **1112-1**
(Wistar Institute, Philadelphia, PA, USA)

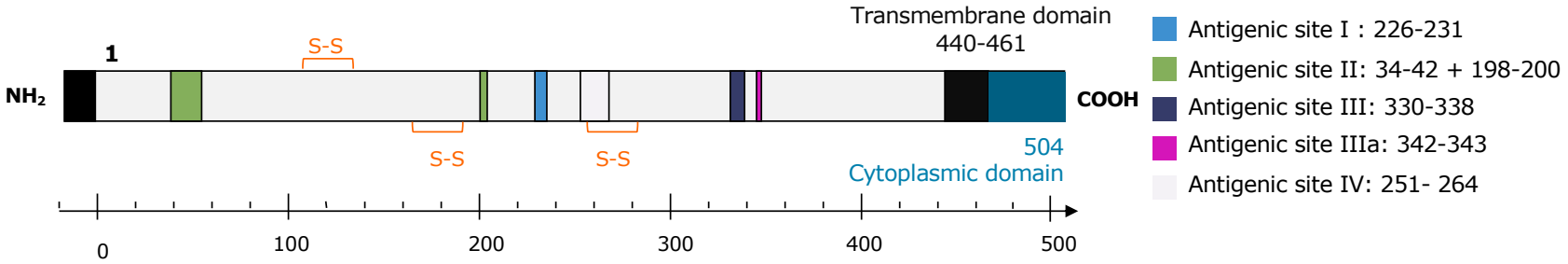
- IgG1 isotype : neutralizes all genotype 1 strains
- Against the **antigenic site II** of the glycoprotein
- Recognizes **conformational and discontinuous epitopes** (aa 34-42 and aa 198-200 associated by S-S bridge)

Dietzschold et. al. (1992) PNAS 89(15):7252-7256

Detection Biotinylated mAb **D1-25**
(Pasteur Institute, Paris)

- IgG1 isotype : neutralizes genotype 1 (PV, CVS, PM and Flury LEP strains) and genotype 6 (EBL2 strain)
- Against the **antigenic site III** of the glycoprotein
- Recognizes **conformational epitope** of the glycoprotein (aa 330- 343)

J. Fournier-Caruana et al. (2003) Biologicals 31:9-16



Bakker et al. (2005) J. Virol., 79: p9062

Rabies G Protein ELISA – Functional Monoclonal Antibodies

Neutralizing activity using Rapid Focus Fluorescent Inhibition Test (RFFIT)

mAb	Neutralizing activity (IU/µg mAb)		
	CVS11 strain	PM strain	Flury LEP strain
D1-25	0.079	0.16	0.10
1112-1	3.22	2.66	2.72

Chabaud-Riou M et al, Biologicals 46 (2017) 124-129

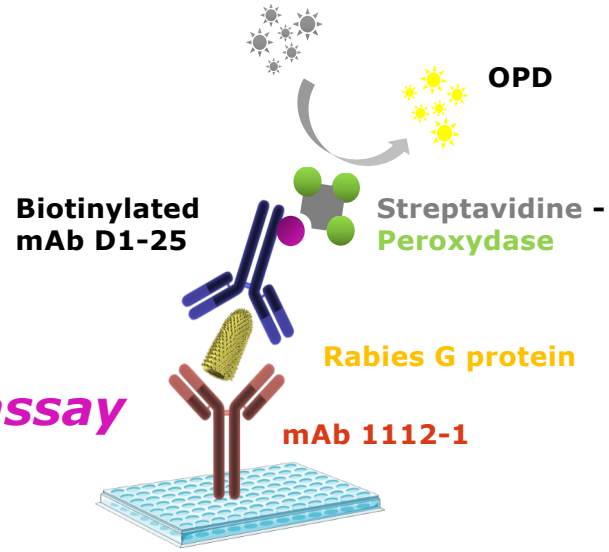
- Both D1-25 and 1112-1 mAbs show similar neutralizing activity against the 3 rabies strains CVS11, Pitman More and Flury LEP
- 1112-1 mAb has superior neutralizing activity compared to D1-25 mAb

1112-1 and D1-25 are both neutralizing antibodies

Rabies G protein ELISA*

- Develop ***in vitro* ELISA potency** test for the detection of **Rabies G protein**
- Generate data to support the NIH test replacement on the next generation of rabies vaccine VRVg

- Quantitative sandwich direct ELISA method
- Use of two neutralizing monoclonal antibodies against specific rabies G protein epitopes
- Titration relative to an internal reference calibrated in IU against the 6th WHO IS



Implementation of the in vitro ELISA potency assay

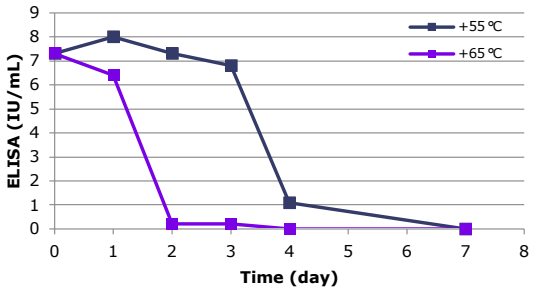
1. *Next generation of rabies vaccine **VRVg***
2. *Commercialized rabies vaccine* VERORAB™ The Rabies Vaccine You Can Trust

** Chabaud-Riou M, et al G-protein based ELISA as a potency test for rabies vaccines. Biologicals. 2017 Mar;46:124-129*

Sanofi Rabies G Protein ELISA : Stability Indicating Assay

- **Strategy**
  - Set up experimental conditions to produce altered / degraded rabies virus
    (Chabaud-Riou M et al, Biologicals 46 (2017) 124-129)

Heat degradation



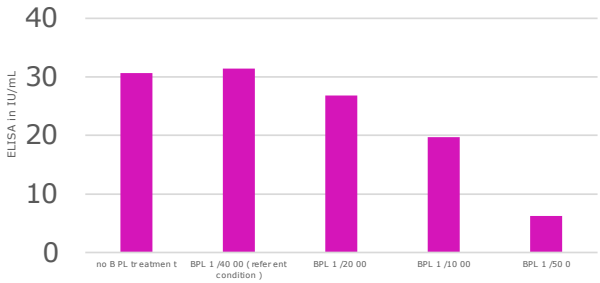
Loss in ELISA signal after several days of heating

Reduction / alkylation degradation

Sample	ELISA results (IU/mL)
Initial	28,0
Control (non reduced / alkylated)	21,1
Reduced & alkylated	< LLOQ

Loss of ELISA signal after Reduction & Alkylation : Antigenic site conformation of G protein is S-S bridge associated (1)

Hyperinactivation with BPL degradation



Loss of ELISA signal after Inactivation with an excess of BPL : Alteration of G protein antigenic sites (2)

The Sanofi Pasteur rabies G protein ELISA detects the alteration of the G protein and is a stability indicating assay

(1) Jallet et al, (1999), J Virol 73:225–33 ; Bakker et al. (2005) J. Virol., 79: p9062
(2) Morgeaux et al. (1993), Vaccine 11-1:82-90

Rabies G protein ELISA agreement with NIH …

…but more discriminative

G protein ELISA is more discriminant than *in vivo* NIH test

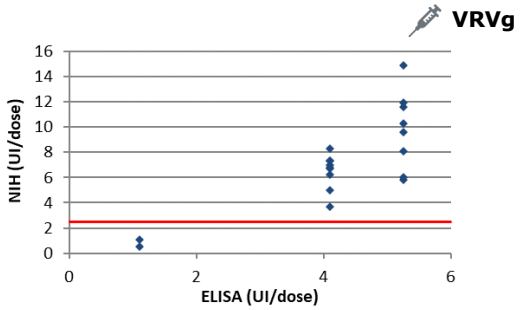
Based on **Ph. Eur. 5.2.14**: Substitution of *in vivo* method(s) by in vitro method(s) for the quality control of vaccines

Sub-potent lots by thermodegradation (1)

Assay	VRVg			VERORAB		
	Intact vaccine	Vaccine degraded	50/50 Spiked vaccine	Intact vaccine	Vaccine degraded	50/50 Spiked vaccine
ELISA - UI/dose	3.3	< LLOQ	1.4	3,3	< LLOQ	1.6
NIH - UI/dose (IC95 interv)	6.2 (2.7 – 13.9)	< LLOQ	1.5 (0.7 – 3.0)	8,1 (3,6 - 19,9)	< LLOQ	7,5 (2,8 - 21,9)

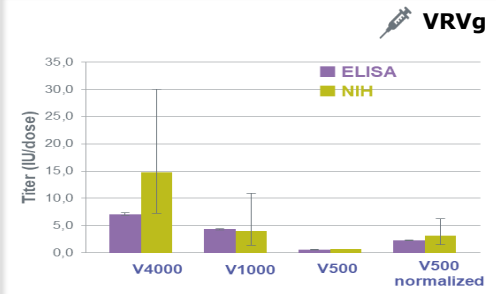
ELISA is able to discriminate intact vaccine, heat-treated vaccine and mixed sub-potent lots

Sub-potent lots by sub-formulation (1)



Agreement between G protein content by ELISA & NIH results

Sub-potent lots by hyperinactivation (2)



ELISA can discriminate sub-potent lots obtained after hyperinactivation

Rabies G protein ELISA is a good candidate to replace NIH potency test

(1) Chabaud-Riou M et al, Biologicals 46 (2017) 124-129
(2) Toinon A et al, Biologicals 60 (2019) 49-54

Sanofi Rabies G protein ELISA - ICH Validation

The method is validated at the Drug Substance (DS) and Drug Product (DP) stages for both vaccines according to ICH principles

VRVg

Specificity
- Vaccine matrix

Linearity range
- DS : [9 – 270 UI/mL]
- DP : [2.4 – 46.4 UI/mL]

Accuracy
- DS : [96% - 104%]
- DP : [95% - 108%]

Intermediate precision
(95% CI for 1 run with 1 measurement)
- DS : x/÷ 1.11
- DP : x/÷ 1.07 to 1.15 depending on formulation level

VERORAB™

The Rabies Vaccine You Can Trust

Specificity
- Vaccine matrix

Linearity range
- DS : [1.0 – 323.9] IU/mL
- DP : [0.62 – 11.15] IU/dose

Accuracy
- DS : [95% - 102%]
- DP : [93% - 104%]

Intermediate precision
(95% CI for 1 run with 1 measurement)
- DS : x/÷ 1.08
- DP : x/÷ 1.12

Critical parameters identified and evaluated during robustness studies

ICH

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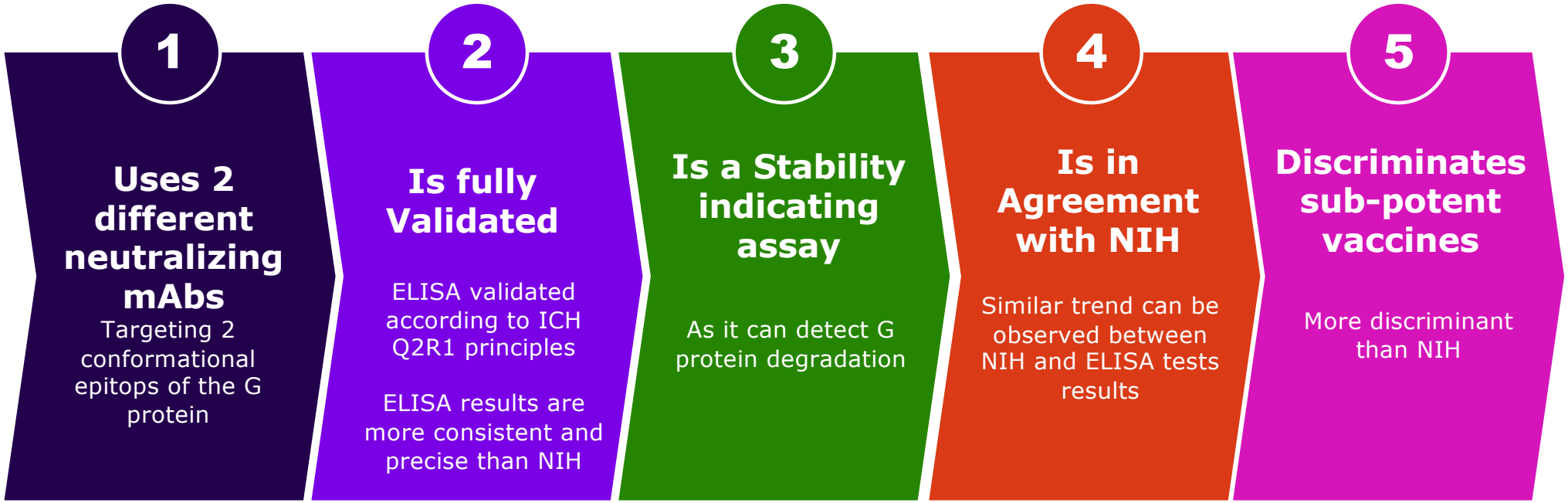
Sanofi Rabies G protein ELISA

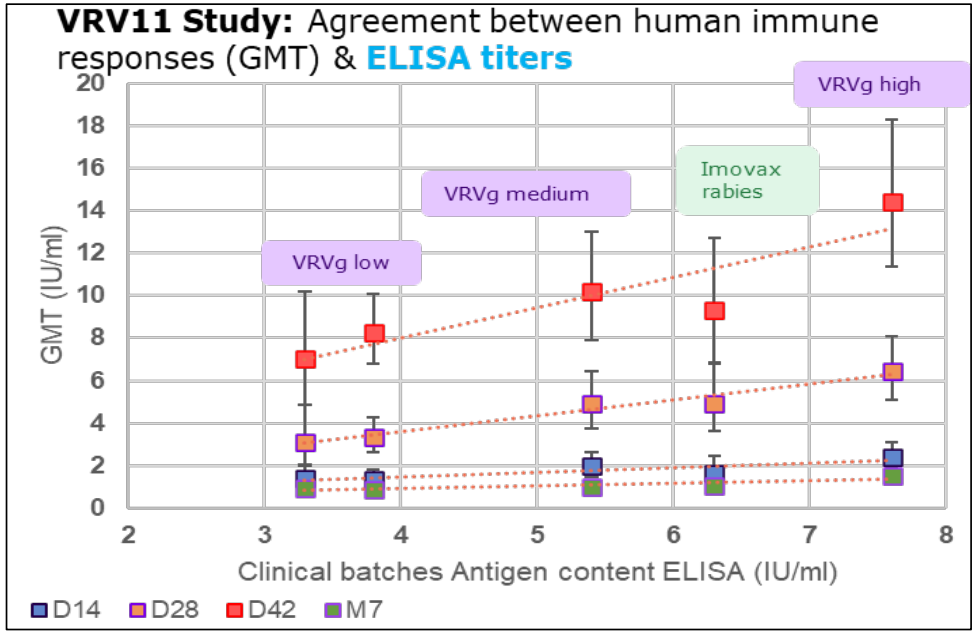
Sanofi Rabies G protein ELISA is a good candidate to replace NIH potency test

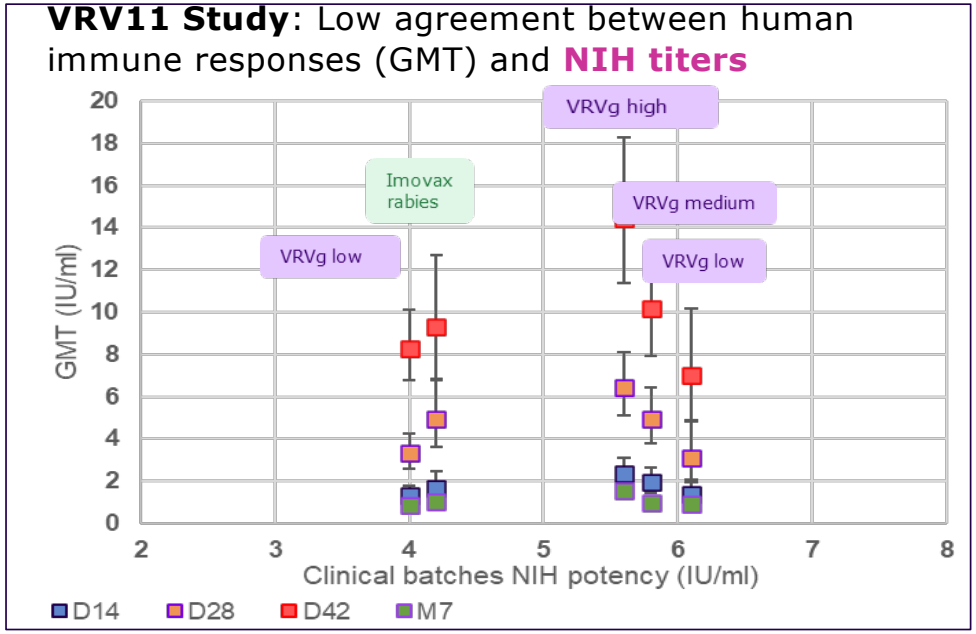


Dose dependent relationship between Rabies G Protein Content (by ELISA) & Human immune response (GMT)

VRV11 Phase II dose-ranging clinical study

Pichon S, Moureau A, Petit C, et al. Safety and immunogenicity of a serum-free purified Vero rabies vaccine in healthy adults: A randomised phase II pre-exposure prophylaxis study. Vaccine. 2022;40(33):4780-4787. doi:10.1016/j.vaccine.2022.06.040





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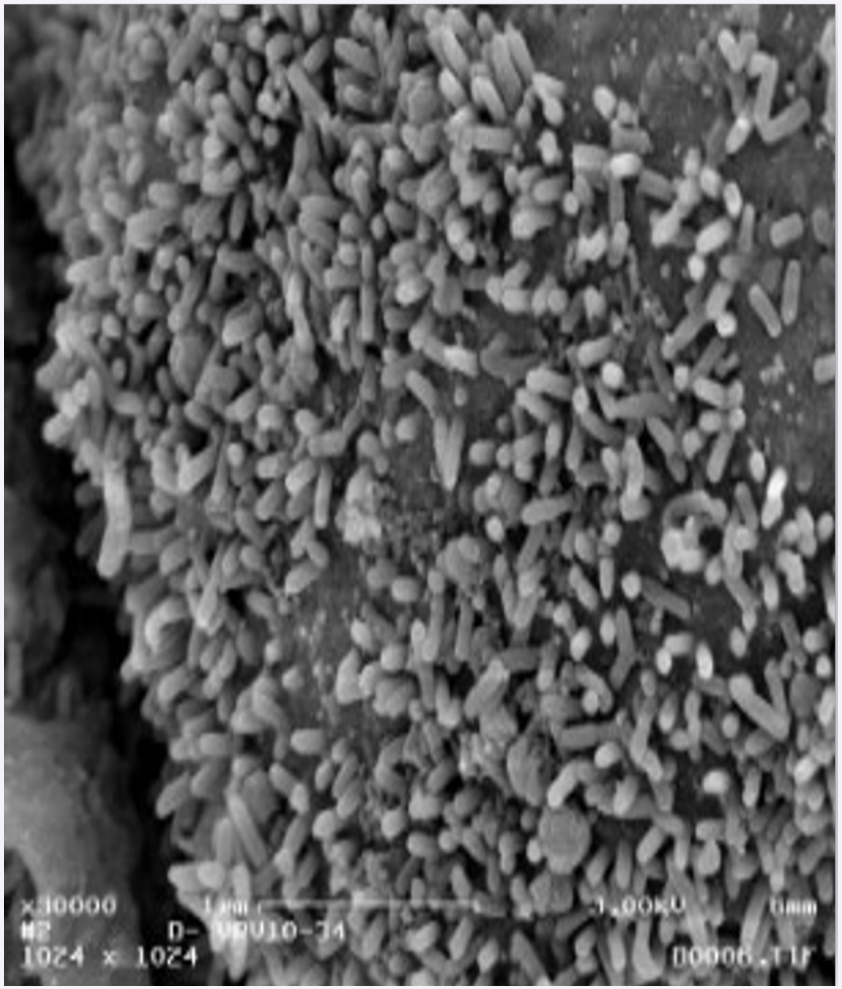


Sanofi Rabies G Protein ELISA
Support to VRVg process development

- Rabies G protein ELISA implemented on DS process intermediates:
  - To monitor process yields/losses
  - To ensure consistent quality along DS process

- Rabies G protein ELISA used to **formulate** VRVg FBP

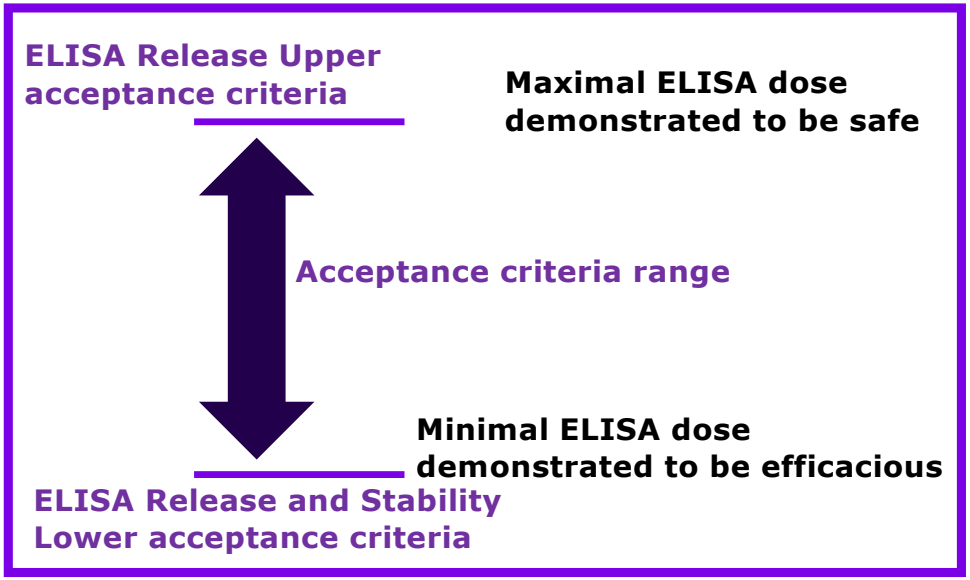
- Rabies G protein ELISA used to monitor VRVg DS and DP **stability**

- VRVg DP **Clinical dose**(s) expressed in ELISA units since phase 1 and all along clinical development
  - NIH test performed on DP as a specification test in parallel to ELISA on all clinical DP batches

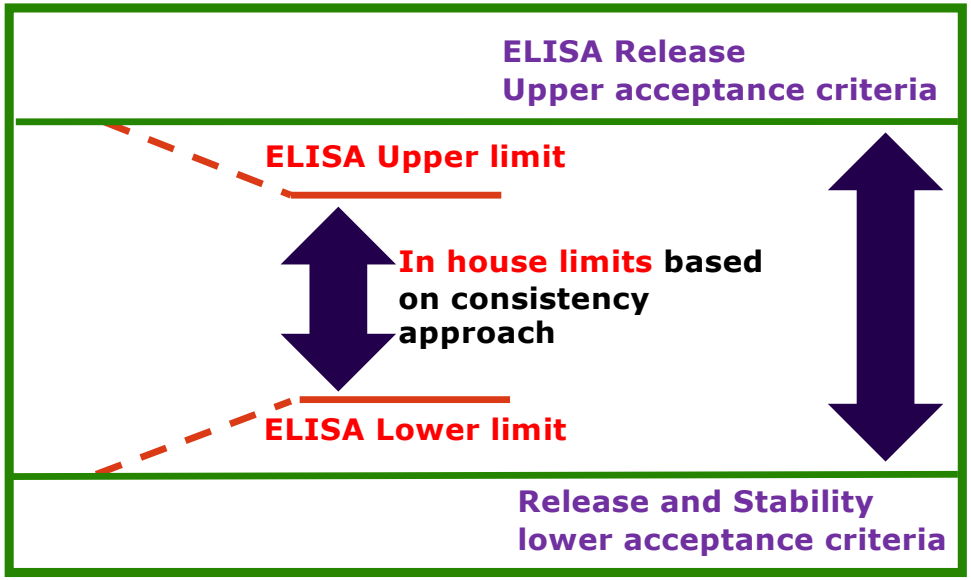
Strategy for New Vaccine (e.g. VRVg)
DP in vitro potency (ELISA) Potency acceptance criteria

- For CTD submission: To define acceptance criteria supported by clinical data
- For life-cycle management: to define in-house action limits based on process consistency

CTD submission strategy



Life-Cycle Management



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ELISA Potency acceptance criteria for VERORAB™ DP

Strategy

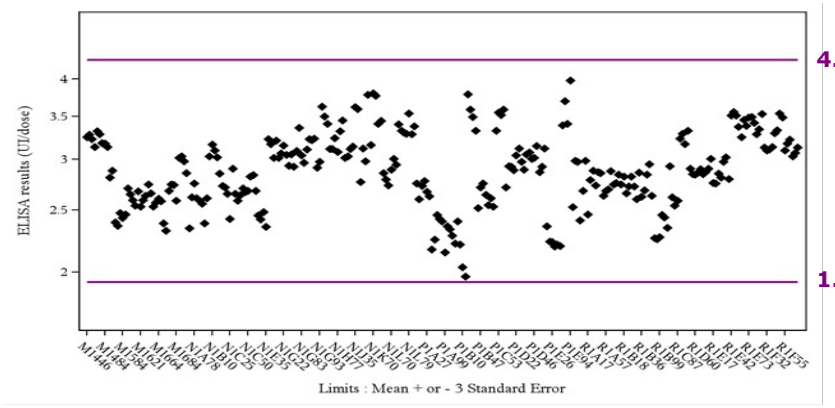
Consistency approach of the **VERORAB™** product with the *in vitro* ELISA

Calculation of the new acceptance criteria : mean ± 3 standard deviations

Results

Representative batches of VERORAB™ vaccine production
(*i.e.,* well-established safety and efficacy profile, with consistent manufacturing)

279 batches covering 3 years of manufacturing



VERORAB™ The Rabies Vaccine You Can Trust

4.3 UI/dose

1.9 UI/dose

Agreement Study

Assess the proposed acceptance criteria with sub-potents batches tested in both NIH & ELISA

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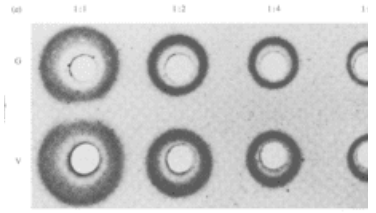
G protein ELISA for DP formulation and DS monitoring

New ELISA potency on DP is associated with:



- New DP ELISA formulation target (replacement of SRID)
  - To match DP acceptance criteria and taking into consideration F&F and shelf-life losses
- Implementation of ELISA on VeroRab™ DS (replacement of SRID)
  - To monitor DS stability

Fergusson et al 1982
DOI: 10.1099/0022-1317-59-1-197

- Implementation Of G rabies ELISA on VeroRab™ DS intermediates
  - ELISA has a wider linearity range and is less sensitive to matrix interference
  - Better monitors process yields/losses to ensure consistency

VERORAB™
The Rabies Vaccine You Can Trust

EMA

2022

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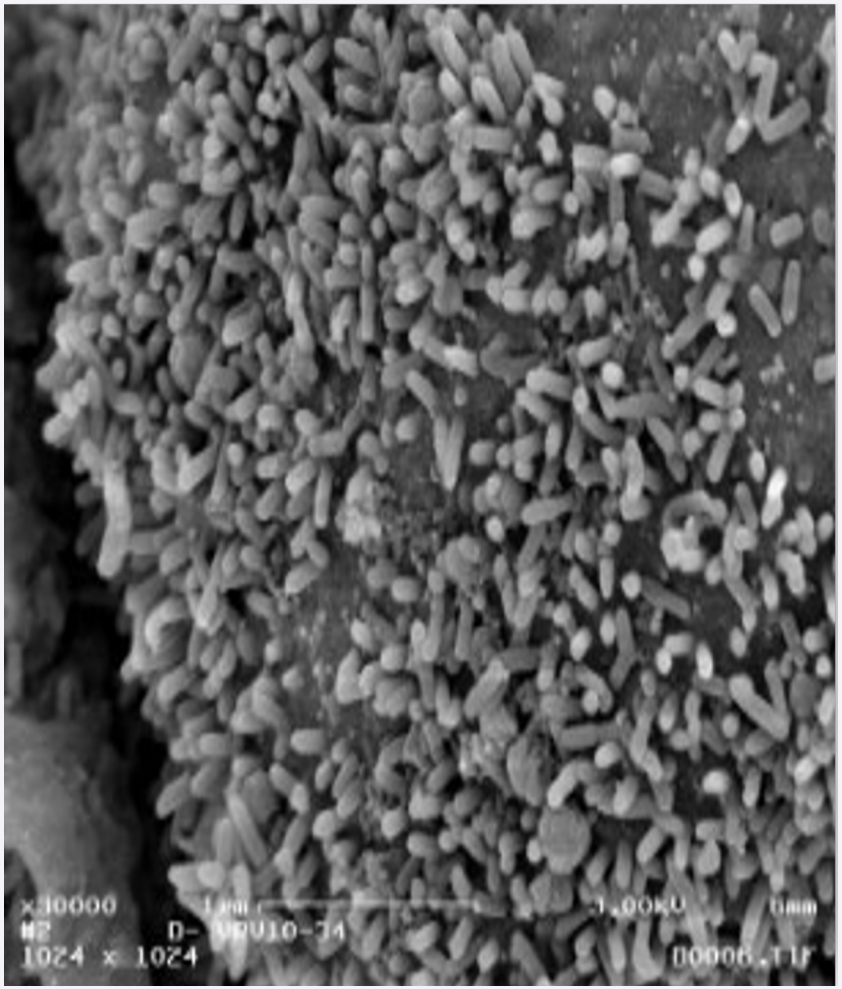

Conclusions

Replacement of *in vivo* method by *in vitro* method and setting specifications

- *In vitro* method suitability & validation package is key
- Consider implementing in vitro method not only at DP stage but also in upstream intermediates (DS intermediates, DS, FBP, Filled Product) and for stability studies

- **For new products**
  - Clinical trial design is critical in order to have clinical data supporting potency acceptance criteria
  - Defining the DP dose for phase 1/2 dose ranging and for phase 3 efficacy studies is important
  - F&F product losses and product stability should also be taken into consideration

- **On already commercialized product**
  - Consistency approach requires to set product specific criteria calculated using a set of batches representative of manufacturing variability
  - Implementation of *in vitro* method not only on DP but also in intermediates

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Thank you

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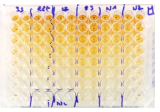
THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)



Case study: Human rabies vaccines

Switching from *in vivo* to *in vitro* potency testing

Part II : towards a global harmonised change

Eriko TERAO

Council of Europe

European Directorate for the Quality of Medicines & HealthCare (EDQM)

Biological Standardisation Programme

Rabies vaccines – from *in vivo* to *in vitro* potency testing

- *European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS No. 123, Council of Europe, 1986)*
- *European Directive on the use of animals for scientific purposes (Directive 86/609/EEC, replaced by 2010/63/EU)*

International initiatives for the development of an alternative *in vitro* method
for the potency control of human rabies vaccines
conclude on the feasibility of an ELISA approach

2010 : workshop on the consistency control of vaccines (Strasbourg, FR)
2011 : workshop on alternate rabies virus vaccine potency test development (Ames, USA)

→ Despite the development of various alternative approaches
the global acceptance for the replacement of the NIH test by an *in vitro* method
is hindered by the absence of a common standardised method

Establishing a common standardised replacement method

Advantages

- acceptance at large (global) level → international initiative
- no need to maintain multiple validated methods for lot release testing
- increased proficiency of operators
- higher precision & shorter lead times of an ELISA approach
- optimised resources
- cost effective

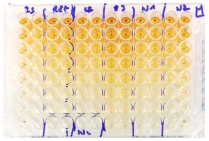
Pre-requisites of the method

- no proprietary rights on method
- accessible reagents and equipments
- applicable to most products
- transferable and robust method

→ international collaborative project

Rabies vaccines – from *in vivo* to *in vitro* potency testing

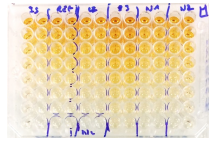
Step 3
International
agreement & implementation



Step 2
Is the selected method suitable for global use?
- transferability
- applicability to routine release testing

Step 1
- what methods are available ?
- which to select?

Rabies vaccines – from *in vivo* to *in vitro* potency testing



Step 1
- what methods are available ?
- which to select?

EPAA project

European Partnership for Alternative Approaches to Animal Testing (EPAA)

Vision Replacement, reduction and refinement (3Rs) of animal use for meeting regulatory requirements through better & more predictive science

a collaboration between

- **European Commission** **5 Directorate General** : DG GROW, DG ENV, DG SANTE, DG JRC, DG RTD including Partner Agencies : ECHA, EFSA, EMA
- **Industry stakeholders** **39 companies & 9 associations from 8 industrial sectors**

* Steering Committee
* Advisory body (Mirror Group) representatives of civil society, including academia, animal welfare and 3Rs centres, acting as a consultation forum in an advisory capacity to the steering committee
* Secretariat GROW-EPAA@ec.europa.eu

Step 1: selection of a candidate method: EPAA project

✓ **2012 EPAA Workshop 1 (Arcachon-1 meeting)**

- creation of an international Working Group including
  - public laboratories & manufacturers
  - from Europe, Americas, Asia
  - academia, WHO, EDQM
- Scientific coordinator: JM Chapsal (independent, EPAA)

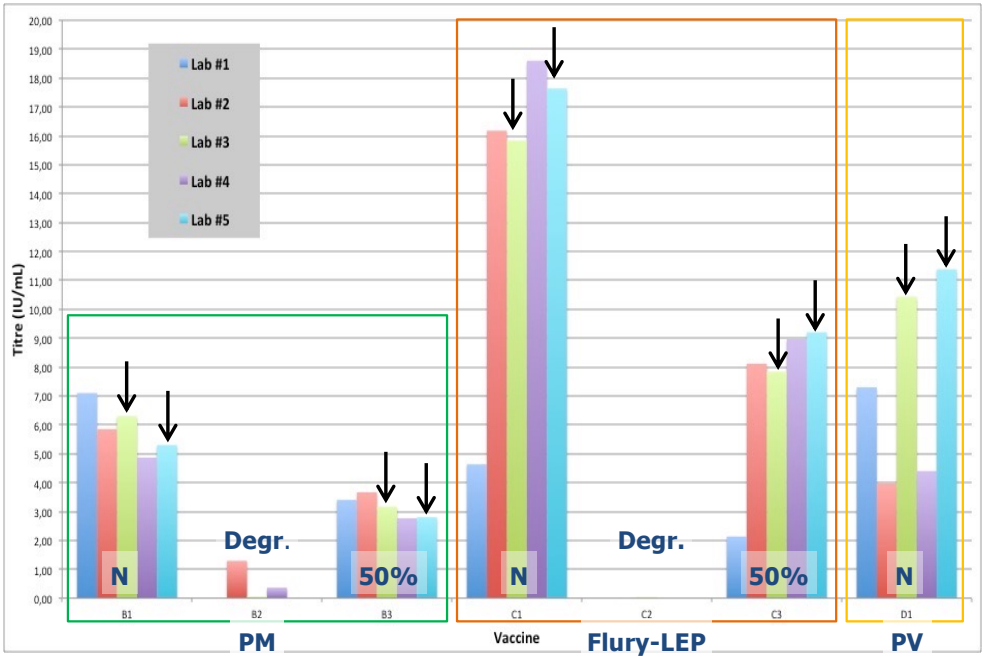
→ inventory of available sandwich ELISA methods
using well-characterised monoclonal antibodies
recognising the protective trimeric form of the rabies glycoprotein

→ launch of an inter-laboratory study to select an appropriate ELISA method

Step 1: selection of a candidate method: EPAA project

- ✓ 5 laboratories: 2 manufacturers & 3 NCLs
- ✓ 3 ELISA methods: from 2 manufacturers & 1 NCL
- ✓ 3 products, 3 virus strains (PM, Flury-LEP, PV)
- ✓ 7 samples: intact (N), heat degraded (Degr), mix of 50% intact-spiked degraded (50%)
- ✓ WHO Rabies vaccine IS as reference standard to express results in IU

Method	Lab	Coating Ab (clone)	Detection Ab (clone)
A	1	mAb (D1)	mAb (D1)
B	3 5	mAb (1112-1)	mAb (D1)
C	2 4	polyclonal -	mAb (TW 17)

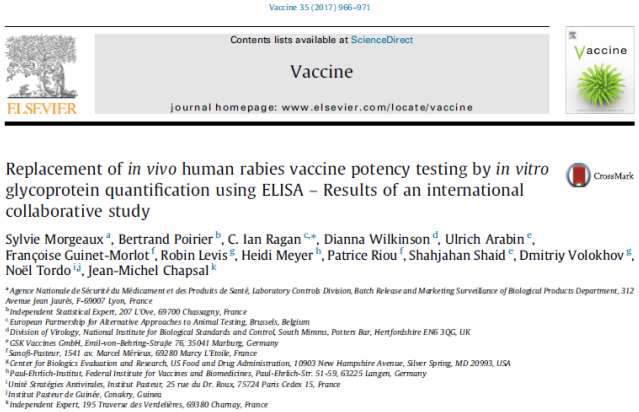


Step 1: selection of a candidate method: EPAA project

✓ **2015 EPAA Workshop 2 (Arcachon-2 meeting)**

The Working Group determined that the GP ELISA (method B, Sanofi Vaccines) is the most promising method for further evaluation in a wider collaborative study

- ✓ no proprietary rights by the developer of the selected method
- ✓ highly characterised specific monoclonal antibodies owned by public laboratories
- ✓ recognises at least 3 virus strains used for vaccine production (data from 2015; at least 6 strains by 2022)
- ✓ preliminary data support good transferability of the method



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Replacement of *in vivo* human rabies vaccine potency testing by *in vitro* glycoprotein quantification using ELISA – Results of an international collaborative study

Sylvie Morgeaux [a], Bertrand Poirier [b], C. Ian Ragan [c,*], Dianna Wilkinson [d], Ulrich Arabin [e], Françoise Guinet-Morlot [f], Robin Levis [g], Heidi Meyer [h], Patrice Riou [f], Shahjahan Shaid [e], Dmitriy Volokhov [g], Noël Tordo [i,j], Jean-Michel Chapsal [k]

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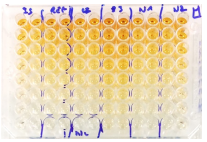
Vaccine 2017;35(6):966-71

→ Step 2

Step 3
International
agreement & implementation



Step 2

Is the selected method suitable for global use?

- transferability
- applicability to routine release testing

BSP148 project

Step 1

- what methods are available?
- which to select?

EPAA project (2012–2016)
→ selection of the GP ELISA

Biological Standardisation Programme (BSP)

Co-funded by the European Union — EUROPEAN UNION — COUNCIL OF EUROPE — CONSEIL DE L'EUROPE — Co-funded and implemented by the Council of Europe

A programme co-funded by the
- **Council of Europe/EDQM**
- **Commission of the European Union**

✓ organises international collaborative studies for the
- establishment of common reference materials and critical reagents
- evaluation of the transferability and robustness of common (new/improved) testing methods

* Steering Committee — Chairs of the Ph. Eur. Groups of Experts for biological products (human & vet.)
EU Commission, European Medicines Agency & WHO representatives
ad hoc specialists from public institutions

* coordinated by a technical secretariat based at the EDQM/Council of Europe

- is independent : no financial interest, neutral focal point for open discussions
- holds discussions with all interested parties worldwide (NCLs, manufacturers, WHO, WOAH, pharmacopeia,…)
- works for the improvement of international harmonisation (e.g. joint studies with other organisations)
- ensures a link to the Ph. Eur. texts (e.g. via Ph. Eur. Groups of Experts and Ph. Eur. Commission)

2016 : Launch of the joint EDQM/BSP – EPAA project : BSP148

Project Leaders *S. Morgeaux (ANSM, FR) & JM Chapsal (Independent, EPAA)*

Scientific coordinator *E. Terao (EDQM/BSP, Council of Europe)*

→ Is the selected method suitable for global use?

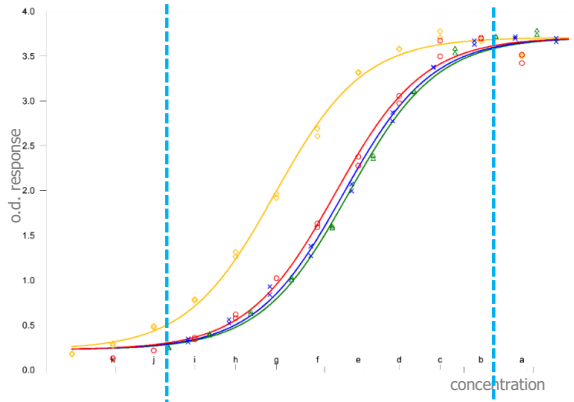
- transferability
- applicability to routine release testing

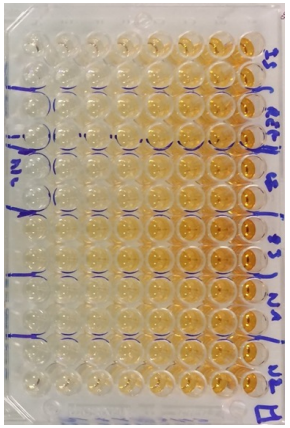
- Phase 1 . preparatory phase
- Phase 2 . collaborative study
- Phase 3 . reporting study

Phase 1 . preparatory phase (project management team)

- ✓ Licensing agreements established by the owner institutes of the antibodies (Wistar Institute, Institut Pasteur) with 2 commercial suppliers (2016-2019)
- ✓ Procurement of test samples
  - 7 manufacturers worldwide, 11 samples
  - 5 virus strains (PM, PV, Flury-LEP, aGV, CTN)
  - various potencies (low, medium, high)
- ✓ Pre-testing by 2 laboratories
  - determination of the pre-dilutions of the samples
  - qualification of lots of critical reagents
- ✓ Determination of the statistical data analysis models
- ✓ Elaboration of a detailed SOP, study design & study protocol

Step 2: evaluation of the selected method for global use (BSP148)





Determination of the statistical analysis models for data analysis

- ✓ full dose-response curves (12 dilution points)
- ✓ fitting of 2 statistical models to the data
  - → 5 parameter logistic (5PL) model (asymetrical sigmoid curve)
  - → parallel line (PL) model (linear part of the dose-response curve)

Study & assay design

- ✓ Selection of 8 dilution points - covering the linear range + lower/upper points
- ✓ optimised pre-dilutions of samples & standard
- ✓ duplicate testing (using independent predilutions)
- ✓ WHO 7th IS for rabies vaccine in each plate to express results in IU/mL
- ✓ blank wells for assessment of assay quality
- ✓ 3 independent assays, balanced plate layout

Phase 2 . Collaborative study outline

- Participants
  - 31 laboratories : public/NCLs & manufacturers
  - Europe, North & South Africa, North & South America, Asia
- Test samples & ref. standard
  - set of 11 marketed vaccines covering 5 virus strains and various potencies
  - WHO IS for Rabies vaccines (inactivated, non-absorbed – 7th IS)
- Study protocol
  - Common ELISA SOP with standardised critical reagents (antibodies & detection conjugate)
  - optional, as available : *in-house* ELISA method
  - Standard reporting sheets
  - Central statistical analysis

Phase 2 . Collaborative study outline

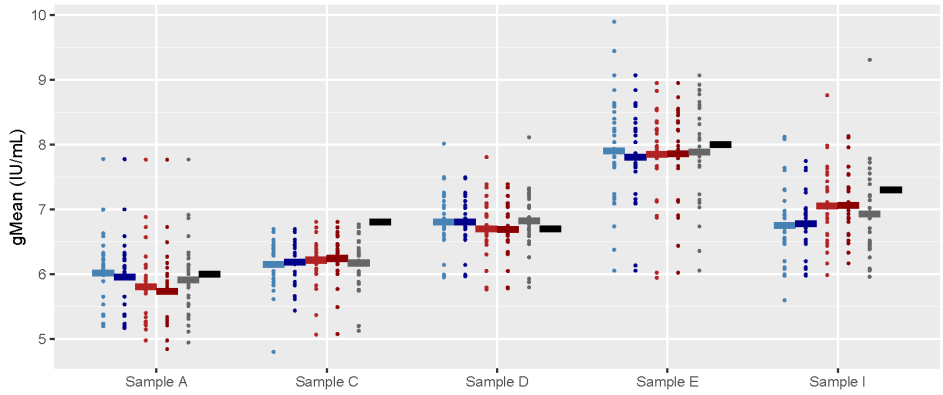
2020/12-2021
- dispatch of samples to participants
- technical support for method transfer (trouble-shooting and adjustment of testing conditions)

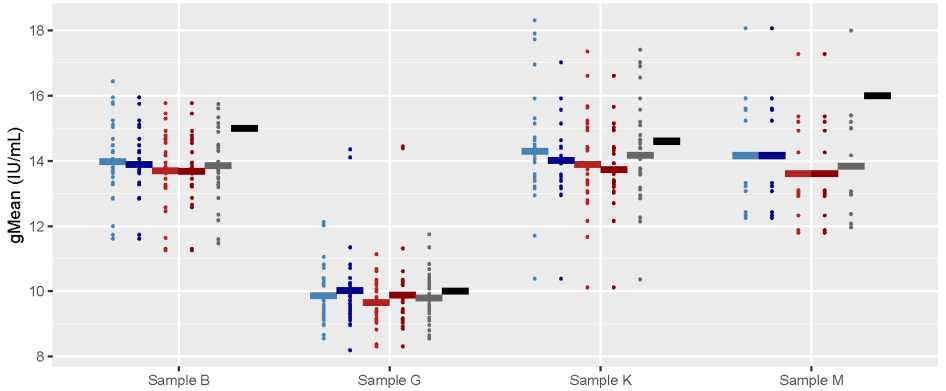
2022/02
- 25/31 laboratories reported results for 10 samples
- 10 laboratories reported results for an additional 11th sample (procured in 2021)

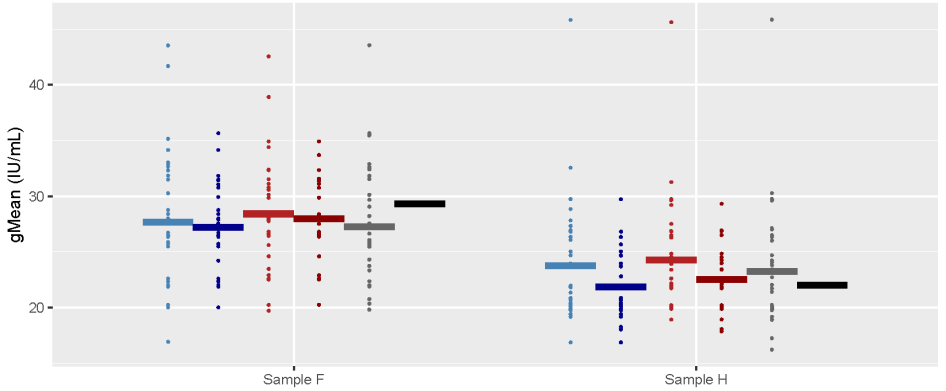
2023/04
- central data analyses & Phase 2 report
  - 2 analysis models : all datapoints (5PL), linear part of dose-respone curves (PL)
  - all datatsets & subset of datasets from assays complying to the SOP
  - evaluation of possible assay suitability criteria (slope, inflection point, OD_{50},...)

NOTE: due to the limited availability of the samples, the study timeline and the pandemic context, some reported data were generated from sub-optimal assays

Phase 2 . Collaborative study
overview of results







Model
5PL
5PL (following scientific review)
Parallel line
Parallel line (following scientific review)
Participant
Ph1 results (2 laboratories, rounded values)

Step 2: evaluation of the selected method for global use (BSP148) . Phase 2 conclusions

✓ Applicability	• to all tested strains : PM, PV, Flury-LEP, aGV, CTN (and at least 1 additional strain)
✓ Potency estimates	• similar between participants' and centrally calculated values
✓ Assay precision	• all confidence limits within 80-125% → satisfactory despite sub-optimal method transfer
✓ Assay repeatability	• Mean variation (gCV) <15% for most laboratories* <1-2% in some laboratories * largest variation for laboratories optimising testing conditions in-between reported assays → satisfactory intra-laboratory variation despite limited proficiency in method
✓ Assay reproducibility	• inter-laboratory variation of gMeans : 5.9-12.9%* depending on sample * higher variation with some samples requiring higher pre-dilutions → linked to the efficiency of method transfer & proficiency → satisfactory inter-laboratory variation despite sub-optimal method transfer

Phase 3 . Reporting phase : applicability to routine batch testing

Launched 2023/12

'simulation of a real life situation'

→ testing of as many batches of different products as possible with the standardised GP ELISA to generate data supporting the discussions on future specifications & assay validity criteria

Participants	• 19-25* laboratories : public/NCLs & manufacturers, in all regions with - access to routine batches of marketed vaccines - fully transferred GP ELISA * including 4 new study participants
Method	• GP ELISA SOP used in routine (no imposed lot of critical reagents) • WHO 7th IS as standard to express results in IU
Test samples	• non-expired lots from routine production (no sample provided by EDQM)

- data reporting by 12/2024
- central data analysis at EDQM & study report elaboration

Step 2: BSP148 project timeline

- ✓ Phase 1 (preparatory phase) — 2016-2020
- ✓ Phase 2 (collaborative study) — 2021-2023
  - ✓ Technical workshop (study participants) — 2021-2023

- ➢ *Phase 3 (reporting phase)* — *2024 -2025*
  - *Publication of the BSP148 study outcomes*

- *Symposium - for discussions on method implementation* — *2025*
- *Proposal for the global replacement of the in vivo potency test by a standardised ELISA (revision of compendial texts & WHO guidelines)*

BSP148 study participants

19 official control & public laboratories and 12 manufacturers
additional 4 laboratories joining after Phase 2

Thank you for your attention

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