Replacing the in vivo potency test for human rabies vaccine: a global collaborative initiative

BSP148 project

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AFSA-IABS-Humane World for Animals Global conference, Bangkok, 2-4 Dec 2025

Context

Human rabies vaccines prevent a 100% lethal disease

- the potency control of these vaccines is a particularly sensitive issue
- an *in vivo* challenge test in mice *(NIH potency test)* is used since 1953 for lot release (WHO TRS 941, Ph. Eur. monograph 0216,...)

The NIH test has proved to be useful however it has many issues:

- disputable scientific rationale
- high (intra-/inter-laboratory) variability
- serious ethical concerns
- time-consuming procedure
- requires large numbers of animals
- occupational safety concerns due to the use of live rabies virus
- high costs

Context

There is a **long-standing global scientific agreement*** that:

- the *in vivo* test for rabies vaccines **needs to be replaced** by an *in vitro* method
- the ideal replacement method is **an ELISA using well-characterised mAbs** because the only antigen conferring protection is the native trimeric form of the virus surface GP
- a single method is requested by several parties to avoid multiplicity of methods that - cause technical & regulatory hurdles and
  - increase cost of routine lot release

However, despite the development of various alternative approaches,
the lack of a standardised common method hinders the global change

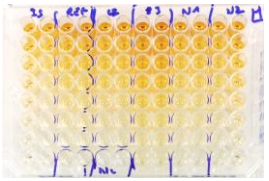
→ **there is a need for an international initiative**

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

Context : from *in vivo* to *in vitro* potency testing - an international initiative

Step 1

- methods available ?
- which one to select ?



European Partnership for Alternative Approaches to Animal testing

a European Commission & Industry partnership

EPAA Human rabies study (2012 – 2015)

Project Leader : J-M Chapsal

- ✓ 5 testing laboratories (Europe, N&S America)
- ✓ 3 ELISA methods
- ✓ 3 virus strains, 7 compliant & degraded lots

→ **selection of the Rabies GP ELISA (2015)**

** Arcachon meetings 2012 & 2015*

Vaccine 35 (2017) 966–971

Contents lists available at ScienceDirect

Vaccine

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

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

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Morgeaux et al Vaccine 2017;35(6):966-71

Context : from *in vivo* to *in vitro* potency testing - an international initiative

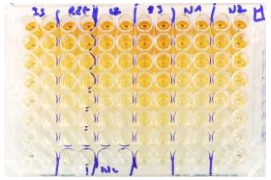
Step 1 - EPAA project selected method : Rabies GP ELISA (2015)

- uses 2 monoclonal antibodies with high affinity to conformational epitopes of the rabies virus glycoprotein in its native trimeric form (antigenic sites II & III)
- recognises at least 3 virus strains used in human rabies vaccine production (PM, PV, Flury-LEP)
- discriminates potent and subpotent vaccines generated by various degradation methods
- is stability indicator
- **shows concordance of results with the immune response in humans**

  Low-medium-high dose clinical lots were discriminated by ELISA -but not by NIH

  Pichon S et al, Hum Vaccin Immunother 2023;19(3):2275453

Context : from *in vivo* to *in vitro* potency testing - an international initiative



Step 2

Is the selected method suitable for global use?

- transferability
- applicability to routine release testing

EDQM/BSP-EPAA project (2016-)

Project Leaders:
S. Morgeaux & J-M. Chapsal

BSP148

- Phase 1: preparatory work
- Phase 2: collaborative study
- Phase 3: reporting study (routine batches testing)

BSP148–Phase 1 : preparatory work (2016-2019)

- Critical reagents
  - ✓ both monoclonal antibodies made globally available from 2 commercial suppliers
    - *licensing agreements established between*
      - 2 public institutions (Institut Pasteur Paris, Wistar Institute USA) and
      - 2 companies (RD Biotech & Millipore Sigma)
- Method
  - ✓ elaboration of a detailled standardised procedure
  - ✓ including 2 biostatistical models for the data analysis (PLA, 5PL)
- Study specifics
  - ✓ production/procurement and qualification of specific batches of critical reagents (2 testing laboratories)
  - ✓ procurement of commercial vaccines to be used as common study test samples
    - panel of various virus strains (PM, PV, Flury-LEP, CTN, aGV) & formulations
    - from manufacturers worldwide
  - ✓ preparatory testing (determination of assay conditions by 2 laboratories)

→ move to the next study phase

BSP148-Phase 2 : collaborative study

(12/2019 – 03/2023)

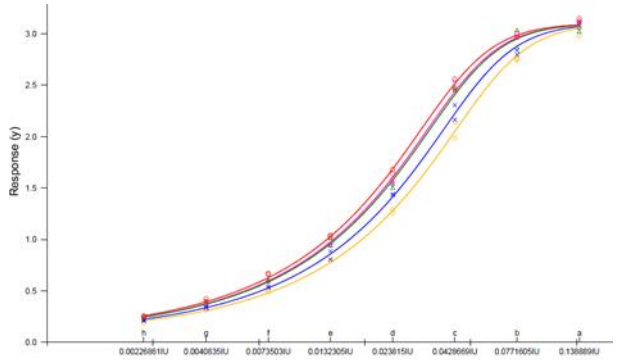
- 31 participants
  - from Europe, Americas, Africa, Asia
  - public & manufacturers laboratories
- 11 vaccines to be tested
  - with the WHO 7th IS for rabies vaccine (16/204, 2.5IU/amp.) as reference standard
  - using common lots of reagents & detailled SOP

➢ *Technical support provided by the coordination team for the method transfer*
(preliminary assays & trouble-shooting)

- ✓ 25 participants reported data by the deadline (04/2022)
- ✓ data were centrally analysed & the Phase 2 report was issued (03/2023)
- ✓ a technical debriefing meeting was organised with all study participants (06/2023, 2 sessions)

BSP148-Phase 2 conclusions

Despite cases of sub-optimal method transfer
limited proficiency/experience with the method
contrains due to the COVID-19 pandemic,



the study data showed that:

the GP ELISA
- ✓ is transferable to laboratories worldwide
- ✓ is applicable to all tested vaccines & virus strains (5 strains + 1 additional by a participant)
  - ❖ vaccines potency expressed in IU/mL vs. the WHO 7th IS (2 statistical models: 5PL, PLA)
- ✓ has very satisfactory
  - precision (all confidence limits: 80-125%)
  - repeatability (intra-laboratory variation: gCV <1-2% to 15%)
  - reproducibility (inter-laboratory variation of gMeans : 5.9-12.9%)
- ✓ is robust

→ move to the next study phase

BSP148-Phase 3: reporting study

Aims
- test the applicability of the rabies GP ELISA to routine QC testing
- determine - adequate specification strategies
  - assay suitability criteria

- ✓ 19 participants (manufacturers & national control laboratories)
- ✓ testing routine batches of products by GP ELISA (no samples/reagents provided by EDQM)
- ✓ data returned by 08/2025
- data are centrally analysed at EDQM
- final study report in preparation

❖ *27 Feb. 2026 BSP148 Technical debriefing meeting (Brussels, BE)*

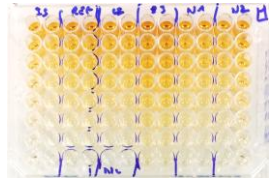
incl. round table discussions on assay suitability criteria, method implementation strategies, and key regulatory issues of the replacement of the NIH test

with study participants, interested regulators & parties
Project leaders, study biostatistician & coordinator

❖ new additional laboratories expressed interest in the project and are transferring the rabies GP ELISA

Rabies vaccines – from in vivo to in vitro potency testing : next steps

Step 3
International
agreement & implementation



Step 2
Transferability
Applicability to routine use

Texts
Revisions
(2026 ? -)

❖ *Ph. Eur. 0216, WHO TRS,…*

BSP148 project
Phases 1-2-3
(2016-2026)

➢ *Technical debriefing meeting*
(27/2/2026, Brussels, BE)

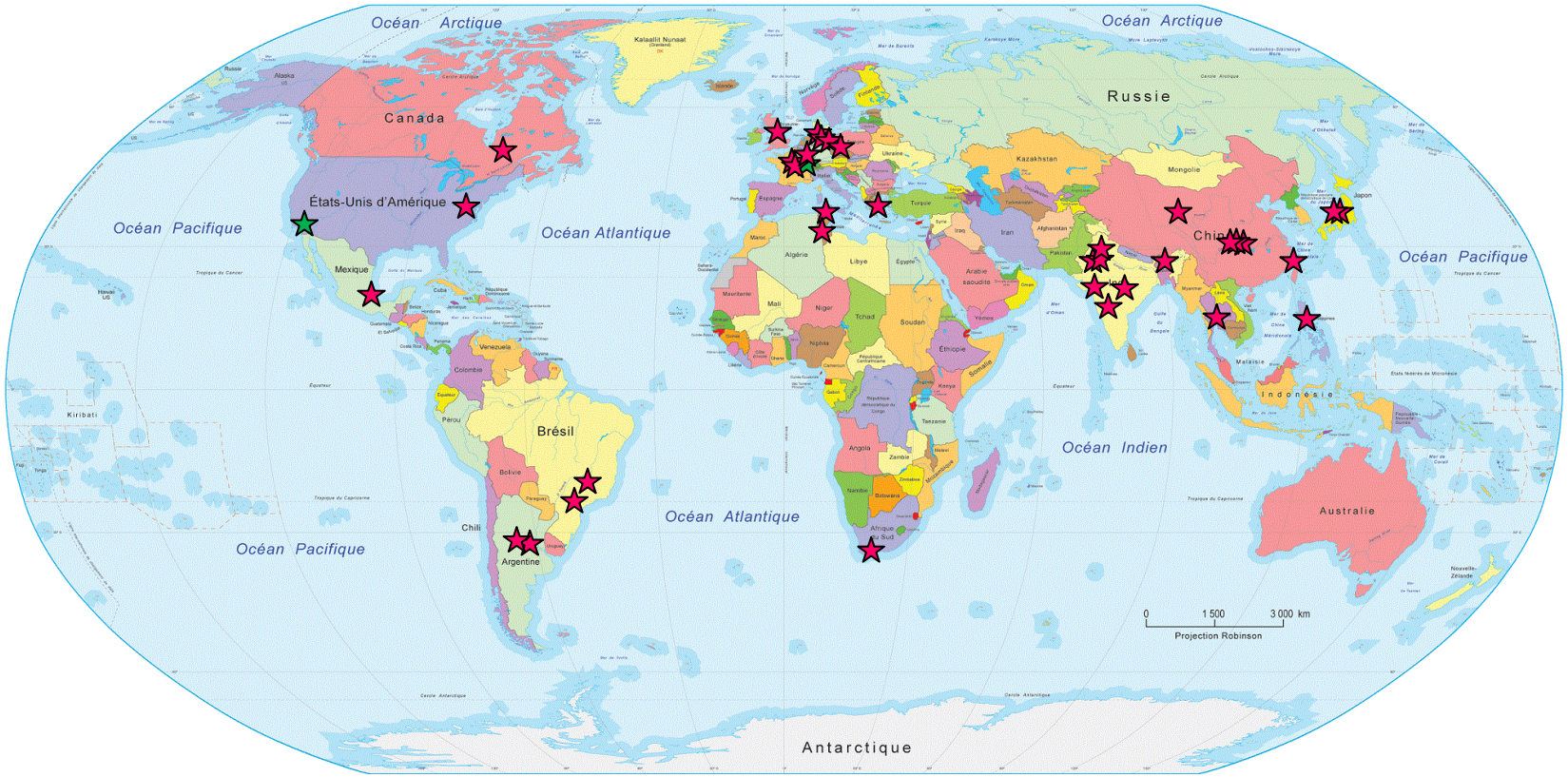
❖ *data to support proposals for Step 3*

Step 1
Selection of method

EPAA project
(2012-2016)

❖ Rabies GP ELISA
Vaccine 2017;35(6):966-71

BSP148 study participants



★ 19 official control and public laboratories & 12 manufacturers

additional 2 public laboratories & 2 manufacturers joined in 2023-24

★ 2 suppliers of laboratory reagents

Thank you for your attention

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