Rabies NIH test replacement

The BSP148 project
&
the EDQM / BSP activities

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epaa

NIH Potency Test

- Developed by EB Seligmann Jr. and published in Laboratory Techniques in Rabies (2nd Edition, 1966)
- Adopted for use as the potency assay for first licensed rabies virus vaccines
- Immunization of groups of mice (16-20 mice per group) with dilutions of test and reference vaccines on days 0 and 7 followed by intracerebral challenge with live rabies virus on day 14 after the initial immunization
- ED50 is calculated and potency is determined relative to the standard at day 28
- Immediately recognized as a problematic assay

Is it possible to institute non-animal based replacement tests to evaluate product potency ?

The replacement of several animal-based immunogenicity tests by ELISA-based assays

has been successfully approved by regulation authorities

- Neutralizing epitopes were well-defined
- Antibody used in the assay bound to critical conformational epitopes
- Clear correlations could be shown between
  amount of antigen required to induce immune response in animals
  vs. amount of antigen measured using alternative in vitro assays
- Studies successfully conducted as part of clinical development

Can we do this with rabies virus vaccines ?

Introduction : context (1)

- Regulatory requirements for Human rabies vaccines (Ph. Eur. 0216, WHO TRS 941):
  - product potency is to be estimated by the in vivo challenge (NIH) test
  - the test must be performed on each final lot

- Issues with the in vivo challenge (NIH) test:
  - painful in vivo challenge assay, contrary to the Ph. Eur. 3Rs strategy
  - very high variability: 25-400%
  - need for BSL3 containment due to the use of live rabies virus

- The in vitro ELISA, as an alternative to the NIH test, is:
  - in accordance with the Ph. Eur. 3Rs strategy : replacement
  - already used by some manufacturers/Official Control Laboratories for the blending and monitoring of the consistency of production

N.B.: the NIH test is not used to set the vaccine dose

Introduction : context (2)

The global replacement of the NIH test by an in vitro method is hindered by the absence of a common standardized method

- International initiatives
  for the development of an alternative in vitro method

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

  conclude on the feasibility of an ELISA approach for the batch release of non-adjuvanted vaccines

Introduction : context (3)

→ 2012 Workshop (Arcachon-1 meeting)

Based on the availability of ELISAs using well-characterized monoclonal antibodies recognizing only the protective trimeric form of the glycoprotein,

an international Working Group for the replacement of the NIH test by an ELISA was created

- sponsored by EPAA and ECVAM
- made up of international experts in human rabies vaccines from government, industry and academia
- with the mission to define the roadmap and coordinate the replacement of the NIH test by an in vitro glycoprotein assay

- the Working Group set up an international feasibility study to select an appropriate ELISA

Design of the feasibility study

- 3 manufacturers provided samples
- 3 Rabies virus strains : PM, Flury LEP, PV
- 3 sample types : untreated ("normal"), heat-treated ("degraded"),
  mix of normal & degraded ("50% spiked normal")

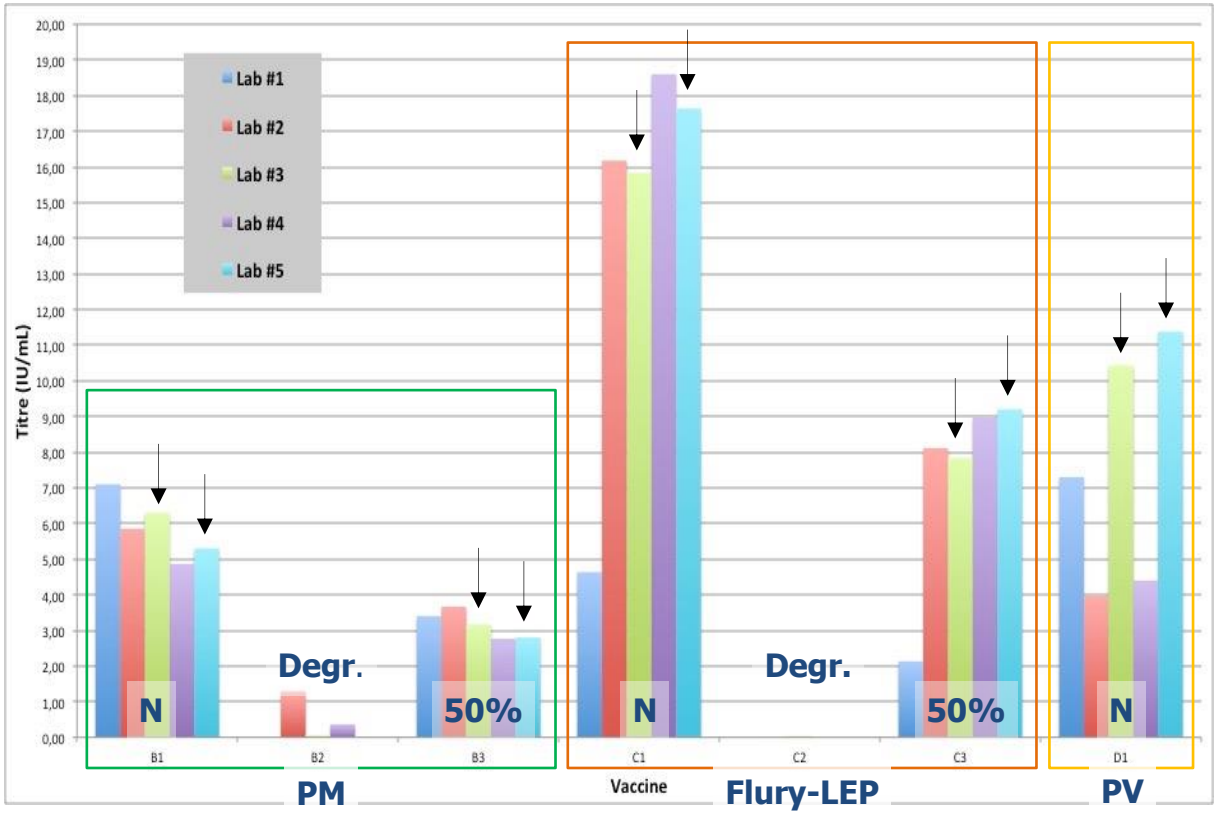
	Source	Rabies strain	Assigned* glycoprotein content (IU/mL)	Assigned NIH potency value (IU/mL)
WHO 6th IS (07/162)	NIBSC	Pitman-Moore	6.6 (reconstituted in 0.5 mL)	8 (reconstituted in 1 mL)
"Normal" (freeze-dried)	Manuf. A	Pitman-Moore	6.6	12.4
	Manuf. B	Flury LEP	13.6	2.7
	Manuf. C	PV	-	5 (reconstituted in 4 mL)
"Degraded" (freeze-dried)	Manuf. A	Pitman-Moore	<0.2	below detection level
	Manuf. B	Flury LEP	0.0	0.0
"50% spiked normal" (reconstituted)	Manuf. A	Pitman-Moore	2.8	3.0
	Manuf. B	Flury LEP	6.4	0.8

* by each manufacturer using own method

Results from the feasibility study

5 laboratories : 2 manufacturers & 3 NCLs

3 ELISA methods : from 2 manufacturers & 1 NCL



Lab	Coating Ab	Detection Ab
1	mAb (D1-25)	mAb (D1-25)
→ 3 → 5	mAb (TJU 1112-1)	mAb (D1-25)
2 4	polyclonal	mAb (TW 17)

Conclusions of the feasibility study

→ 2015 Workshop (Arcachon-2 meeting)

The working group determined that the "Sanofi Pasteur ELISA" method is appropriate for further validation in a larger BSP study

The selected ELISA :

- uses 2 mAbs that bind ✓ conformational epitopes
  ✓ on well-defined antigenic sites
  ✓ inducing protection
- does not recognize the non-immunogenic soluble glycoprotein
- clearly discriminates potent from heat-degraded sub-potent vaccines

→ the study report was published in Vaccine (DOI: 10.1016/j.vaccine.2016.12.039)

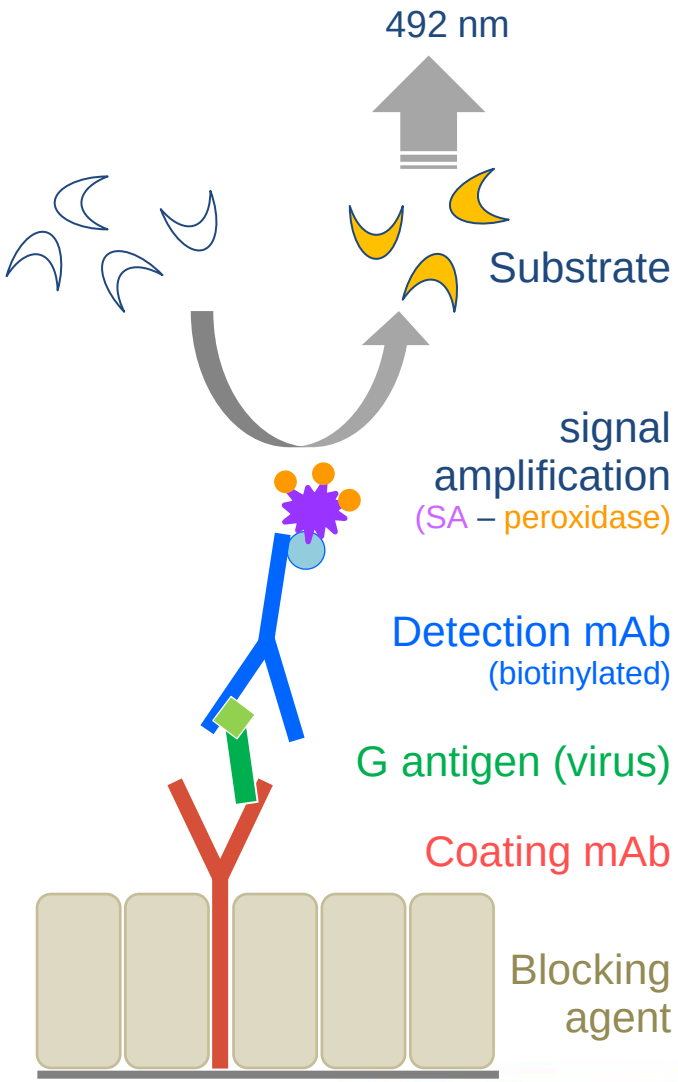
Selected Rabies G protein ELISA : design

a quantitative
direct sandwich ELISA method

using :

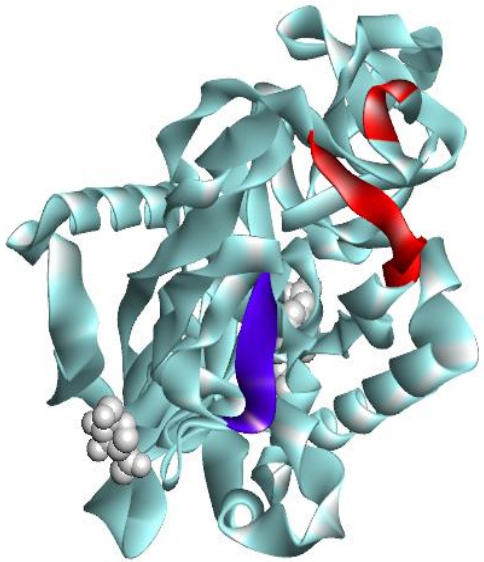
- 2 monoclonal antibodies
  - for coating/capture : TJU 1112-1
  - for detection : D1-25 *biotinylated

- a reference standard (calibrated in IU)
  (in-house reference calibrated vs. WHO 6th IS)



Selected Rabies G protein ELISA : monoclonal antibodies

Coating antibody	Detection antibody
TJU 1112-1 (Wistar Institute, USA)	**D1-25** biotinylated (Pasteur Institute, FR)
Ig G1	IgG1
Antigenic site II (aa 34-42 & 198-200) 2 conformational and discontinuous epitopes linked by a S-S bridge	**Antigenic site III** (aa 330-338) conformational trimeric form of the gp does NOT recognize the soluble gp
recognizes all genotype 1 strains (PV, CVS, PM, Flury LEP)	recognizes genotypes 1 & 6 strains (PV, CVS, PM, Flury LEP & EBL2)
neutralize strains used for the RFFIT on BHK21 cells (CVS-11, PM, Flury LEP)	



Other known antigenic sites

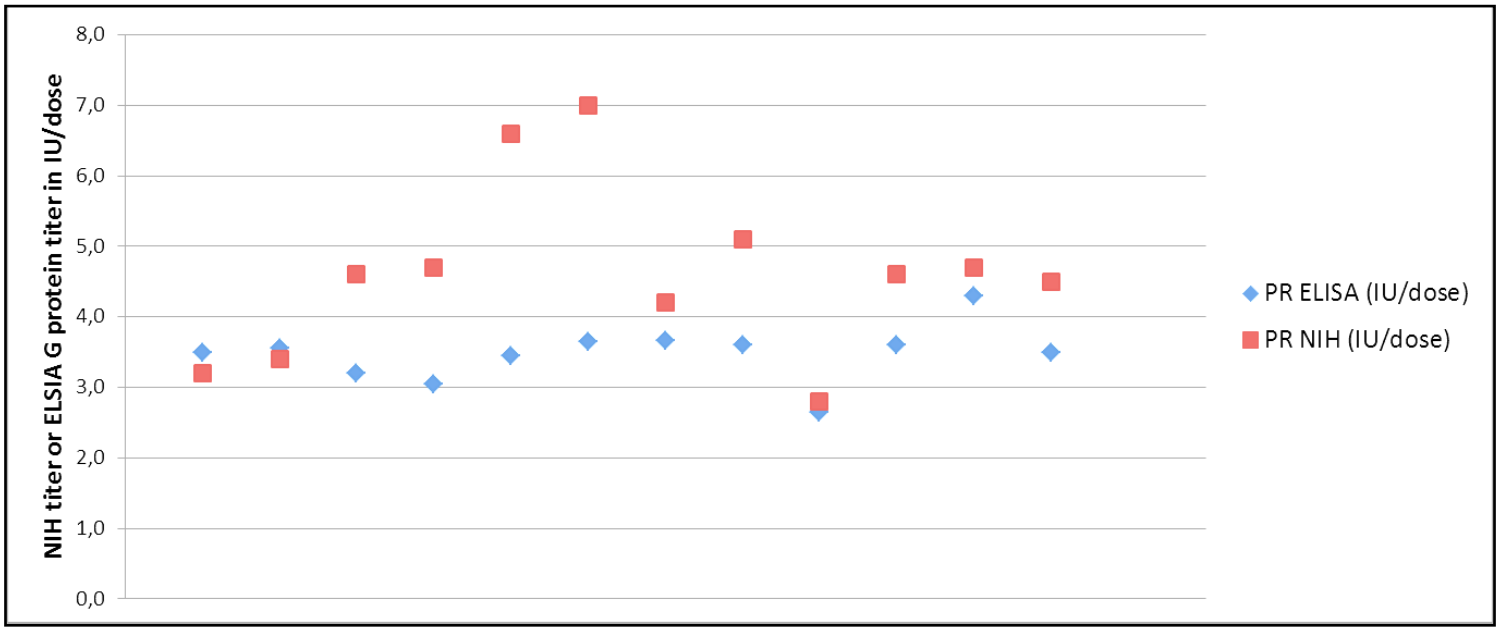
site I : 226-231

site IIIa : 342-343

site IV : 251- 264

Selected Rabies G protein ELISA

- ✓ validated for 1 product according to ICH principles
- ✓ **ELISA** results are more consistent than **NIH** results



Selected Rabies G protein ELISA

can monitor G protein degradation by

- ✓ alkylation/reduction (disulfide bound alteration) (*)
- ✓ heat degradation (**)
- ✓ excess BPL(*)

Sample	BIAcore (RU mAb used)		ELISA (IU/mL)
	D1-25	TJU 1112-1	
initial (no BPL treatment)	418	550	30.7
BPL 1/4000 (= ref. treatment)	372	513	31.4
BPL 1/2000	279	381	26.8
BPL 1/1000	312	419	19.7
BPL 1/500	81	89	6.2

(*) Biologicals.2017.46.124-129

(**) Vaccine.2016.12.039

The proposed ELISA method

- ✓ uses mAbs that are highly characterized
- ✓ uses mAbs that are specific to the conformational trimeric form of the glycoprotein - which is responsible for the protection conferred by the vaccines
- ✓ does not recognize the inactive soluble glycoprotein
- ✓ recognizes most vaccine strains used worldwide for human rabies vaccines
- ✓ discriminates sub-potent vaccines altered by various methods: alkylation/reduction, thermal degradation, BPL inactivation

- ✓ is not based on a commercial kit
- ✓ the mAbs are accessible to all laboratories
- ✓ the preliminary study supports good transferability of the method

→ **Next step : Biological Standardisation programme (BSP)**

The Biological Standardisation Programme (BSP) - 1

✓ Created in 1991

✓ by the Council of Europe and the Commission of the European Union

Aims

Coordinate large collaborative studies to

- establish Ph. Eur. working standards & reagents (BRP, BRR and CRS*)
- standardise pharmacopoeial methods
  including new 3R methods (reduction, refinement, replacement of animal use)
- contribute to international harmonisation
  (collaborations with WHO, US-FDA, other pharmacopoeia,...)

for the quality control of biologicals

* BRP : Biological Reference Preparation
  BRR : Biological Reference Reagent
  CRS : Chemical Reference Standard

The Biological Standardisation Programme (BSP) - 2

Collaborative studies

- aim at
  - calibrating/assigning a unitage to a (candidate) Ph Eur reference standard

  and/or
  - evaluating the transferability and robustness of a method

- are characterised by
  - an international panel of participants : OMCLs, manufacturers, authorities
  - a large number of participants from Europe and other regions
  - common protocol & samples, reagents (as needed) & reporting sheets
  - a central analysis of the datasets

OMCLs: Official Medicines Control Laboratories

The Biological Standardisation Programme (BSP) - 3

The ultimate aim of the collaborative studies is

to provide scientific data to Experts in the field

in order to support the revision of the Ph. Eur. Texts

and encourage global harmonization of test methods

Final reports of BSP studies are published in
Pharmeuropa Bio Scientific Notes
(freely accessible at www.edqm.eu)

Future strategies : Aims of the BSP148 study

International collaborative study
coordinated by the Biological Standardisation Programme (BSP)
of the Council of Europe and the European Union

Project leaders: J-M. Chapsal, S. Morgeaux (ANSM, FR)
EDQM coordinator: E. Terao

Validation of the transferability of the selected Rabies G protein ELISA in view of the

- proposal to the Ph. Eur. Group of Experts 15 on Vaccines to
  - revise the Ph. Eur. texts and
  - include a standardized ELISA
- proposal for a global replacement of the challenge test used for the QC of Human Rabies vaccines by a standardized ELISA

Outlines of the study (1)

- Phase 1
  - Preparatory phase
    - procurement & pre-testing of samples
    - preparation of the study protocol and reporting sheets
    - logistical arrangements (invitations, shipments,...)
- Phase 2
  - Collaborative study
    - transferability & robustness of the method
    - use of the 7th WHO IS
- Phase 3
  - Reporting phase
    - laboratories to test routine batches
    - determination of the potency specifications of the vaccines in view of the revision of the Ph. Eur. monograph 0216

Outlines of the study (2)

- **Participants**
  - OMCLs & manufacturers
  - Europe and other regions (North & South America, India, China,...)
- **Test samples**
  - WHO IS for Rabies vaccines (inactivated, non-absorbed – 7th IS)
  - Panel of marketed vaccines covering various strains
- **Study design**
  - 3 independent assays, duplicate testing of each sample
  - Common ELISA SOP
  - optional, as available : in-house ELISA method
  - Standard reporting sheets
  - Central statistical analysis

Current status of the project

✓ testing of vaccines produced with PM and aGV virus strains

- ❑ preparation of a common SOP and the study protocol
- ❑ arrangements for the commercial availability of the antibodies
- ❑ procurement of vaccine samples from various sources & virus strains
- ❑ preparation of the list of participants

Feasibility study working group

• JM. Chapsal (co-Chair)	• EPAA
• N. Tordo (co-Chair)	• Institut Pasteur, FR
• I. Ragan	• EPAA consultant
• S. Morgeaux	• ANSM, FR
• B. Poirier (Statistician)	• BPSTAT Consulting, FR
• S. Shajhahan, U. Arabin, L. Viviani	• GSK Biologicals, DE
• W. Correa de Moura	• INCQS-FIOCRUZ, BR
• D. Wilkinson	• NIBSC, UK
• H. Meyer	• PEI, D
• F. Guinet-Morlot, P. Riou	• Sanofi Pasteur, FR
• D. Volokhov, R. Levis	• US-FDA, USA
• Y. Kaushik	• Bharat Biotech, IN
• E. Terao	• EDQM
• M. Halder	• EURL-ECVAM
• G. Pulle	• Health Canada – BGTD, CA
• L. Bruckner	• IVI, CH
• C. Jiang	• Jilin University, CN
• L. Yuhua, C. Shouchun	• NIFDC, CN
• M. Gautam, S. Gairola	• Serum Institute of India, IN
• D. Lei	• WHO
• C. Rupprecht	• Wistar Institute, USA

Thank you for your attention

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