Rabies NIH test replacement

The BSP148 project

&

the EDQM / BSP activities

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NIH Potency Test

- 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
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")

<table>
<thead>
<tr>
<th>Source</th>
<th>Rabies strain</th>
<th>Assigned* glycoprotein content (IU/mL)</th>
<th>Assigned NIH potency value (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO 6th IS (07/162)</td>
<td>NIBSC</td>
<td>Pitman-Moore</td>
<td>6.6 (reconstituted in 0.5 mL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8 (reconstituted in 1 mL)</td>
</tr>
<tr>
<td>&quot;Normal&quot; (freeze-dried)</td>
<td>Manuf. A</td>
<td>Pitman-Moore</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>Manuf. B</td>
<td>Flury LEP</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>Manuf. C</td>
<td>PV</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 (reconstituted in 4 mL)</td>
</tr>
<tr>
<td>&quot;Degraded&quot; (freeze-dried)</td>
<td>Manuf. A</td>
<td>Pitman-Moore</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td></td>
<td>Manuf. B</td>
<td>Flury LEP</td>
<td>0.0</td>
</tr>
<tr>
<td>&quot;50% spiked normal&quot; (reconstituted)</td>
<td>Manuf. A</td>
<td>Pitman-Moore</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Manuf. B</td>
<td>Flury LEP</td>
<td>6.4</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Lab</th>
<th>Coating Ab</th>
<th>Detection Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>mAb (D1-25)</td>
<td>mAb (D1-25)</td>
</tr>
<tr>
<td>3</td>
<td>mAb (TJU 1112-1)</td>
<td>mAb (D1-25)</td>
</tr>
<tr>
<td>2</td>
<td>polyclonal</td>
<td>mAb (TW 17)</td>
</tr>
<tr>
<td>4</td>
<td>mAb (D1-25)</td>
<td></td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Coating antibody</th>
<th>Detection antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TJU 1112-1</strong> (Wistar Institute, USA)</td>
<td><strong>D1-25</strong> biotinylated (Pasteur Institute, FR)</td>
</tr>
<tr>
<td>Ig G1</td>
<td>IgG1</td>
</tr>
</tbody>
</table>

**Antigenic site II**  
(aa 34-42 & 198-200)  
2 conformational and discontinuous epitopes linked by a S-S bridge  
recognizes all genotype 1 strains (PV, CVS, PM, Flury LEP)  
neutralize strains used for the RFFIT on BHK21 cells (CVS-11, PM, Flury LEP)

**Antigenic site III**  
(aa 330-338)  
conformational trimeric form of the gp does NOT recognize the soluble gp  
recognizes genotypes 1 & 6 strains (PV, CVS, PM, Flury LEP & EBL2)

## 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(*)

<table>
<thead>
<tr>
<th>Sample</th>
<th>BIAcore (RU mAb used)</th>
<th>ELISA (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D1-25</td>
<td>TJU 1112-1</td>
</tr>
<tr>
<td>initial (no BPL treatment)</td>
<td>418</td>
<td>550</td>
</tr>
<tr>
<td>BPL 1/4000 (= ref. treatment)</td>
<td>372</td>
<td>513</td>
</tr>
<tr>
<td>BPL 1/2000</td>
<td>279</td>
<td>381</td>
</tr>
<tr>
<td>BPL 1/1000</td>
<td>312</td>
<td>419</td>
</tr>
<tr>
<td>BPL 1/500</td>
<td>81</td>
<td>89</td>
</tr>
</tbody>
</table>

(*) 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:
  alklylation/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 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)
- N. Tordo (co-Chair)
- I. Ragan
- S. Morgeaux
- B. Poirier (Statistician)
- S. Shajhahan, U. Arabin, L. Viviani
- W. Correa de Moura
- D. Wilkinson
- H. Meyer
- F. Guinet-Morlot, P. Riou
- D. Volokhov, R. Levis

- Y. Kaushik
- E. Terao
- M. Halder
- G. Pulle
- L. Bruckner
- C. Jiang
- L. Yuhua, C. Shouchun
- M. Gautam, S. Gairola
- D. Lei
- C. Rupprecht

- EPAA
- Institut Pasteur, FR
- EPAA consultant
- ANSM, FR
- BPSTAT Consulting, FR
- GSK Biologicals, DE
- INCQS-FIOCRUZ, BR
- NIBSC, UK
- PEI, D
- Sanofi Pasteur, FR
- US-FDA, USA

- Bharat Biotech, IN
- EDQM
- EURL-ECVAM
- Health Canada – BGTD, CA
- IVI, CH
- Jilin University, CN
- NIFDC, CN
- Serum Institute of India, IN
- WHO
- Wistar Institute, USA
Thank you for your attention

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