Human Rabies vaccine potency testing,
The test for G-protein : Report of the pre-collaborative study and future strategies

3Rs and the consistency Approach in vaccine Lot Release Testing
IABS meeting Egmond aan Zee
September 16-18 Sept 2015
Human Rabies vaccine potency testing

The test for G-protein

Towards an international consensus

Positive and encouraging results

What is still missing?
NIH potency assay in the field

- NIH potency tests used and relied upon for more than 30 years but

  ~ 50 to 70% of the total production cycle

  ~ 20 % of the mice use for release control
Towards the replacement of the NIH test

- NIH potency tests used and relied upon for more than 30 years but
  - **High variability** (C.V: ~ 25-400% versus 2-10% for in vitro test in general), poor reproducibility
  - **Invalidity** requiring investigations & re-tests
  - Animal strain selection-Genetic backgrounds
  - Animals availability and health status
  - Animal housing capacity
  - **Retesting** by National Control Authorities
  - Challenge dose:
    » USA : 5-50 DL50
    » Out USA : no less than 10 DL50
Towards an international consensus
Towards the replacement of the NIH test

EPAA project  Application of the 3Rs and the Consistency Approach for Improved Vaccine Quality Control

2005, the European Partnership for Alternatives Approaches to Animal Testing (EPAA)

European Commission, trade federations and companies
Towards the replacement of the NIH test

A specific workshop on the consistency approach for the quality control of vaccines

- **Technical platform creation**
  - general strategies and policies
    - to introduce the consistency approach
    - to define minimal acceptance criteria for the consistency tests (in vitro and analytical)
    - to set up specific technical task forces to address specific vaccines, tests, and their validation
PROJECT PRIORITIES

• for human vaccines: DTaP and Rabies vaccines.

• for veterinary vaccines: Rabies and Clostridial vaccines
Replacement of the NIH test

Shift in paradigm

- The major correlate of protection of rabies vaccine is the induction of neutralizing antibody against the unique transmembrane glycoprotein G (Wiktor TJ, 1973)
- This property mainly depends on the preservation of its three-dimensional structure (Bunschoten H, 1989)
- A set of immunological and biochemical characterization assays have been set up focusing on the G protein.
Regulators and manufacturers stressed that

- The NIH test should be replaced as soon as possible

- The current in vivo immunization-challenge assay should not be used for correlation, since it is highly variable and therefore a ranking strategy should be followed.

- The ELISA methods should correlate with the protective titers in the target species (historical or newly generated clinical data) and should be able to discriminate between potent and sub-potent batches: Ranking study

Stokes et al Biologicals 40 (2012) 369-381
Towards the replacement of the NIH test
The Best: ELISA approach

- A sandwich direct ELISA method

- Titration versus an internal reference calibrated in IU: the 6th WHO standard that included a potency determination by ELISA (3 IU/ml)

- Selection of the most appropriate ELISA: to be determined by a pre-collaborative study

Creation of an International Working Group for ELISA replacement of NIH test
Scope of the International Working Group for ELISA replacement of NIH test Preliminary Study design

Three ELISA methods, Five Laboratories:

- Novartis ELISA (proprietary mAb, polyclonal)
- PEI ELISA (same as above)
- ANSM ELISA: involving D1-25 mAb (Institut Pasteur)
- Sanofi Pasteur ELISA: involving D1-25 and TJU 11-12 (Wistar Institute) mAbs
- FDA (same as above)
Scope of the International Working Group for ELISA replacement of NIH test Preliminary Study design

Design

– The 6th WHO standard (potency determination by ELISA (3 IU/ml)
– 3 Vaccines batches including 2 sub-potents generated by heat treatment, agreement between ELISA and NIH Tests

• Results statistically analysed by an independent expert (BP stat)

• This strategy has already been used for the replacement of in vivo test by in vitro alternatives for different vaccines (IPV, HBs, Hep A)
International Working Group for ELISA replacement of In vivo Rabies method: Pre-validation study

- Novartis and Sanofi Pasteur have prepared subpotents lots by heat treatment

- ELISA Protocol

  - normal vaccine(T0): INCQS, Sanofi Pasteur and Novartis
  - subpotents batches: Sanofi Pasteur and Novartis
  - 50% spike: 50/50 mix of normal and thermo degraded subpotents batches
International Working Group for ELISA replacement of In vivo Rabies: Pre-validation study

- Collect/generate collaborative study materials-test vaccines, Sub-potent lots, reference, dispatch of test materials, reagents, finalised methodologies and data capture sheets to participants
- Material Transfer Agreement
- Laboratory tests
- Statistical evaluation

Roche et al, Science, 2006
2nd Joint EPAA-ECVAM Workshop on

Replacement of In Vivo Human Rabies Vaccine Potency Testing by “in vitro” Glycoprotein Quantification Methods

Results from the International Collaborative Study and Implementation Strategy
May 2015
Define the best ELISA suitable for the replacement of the NIH test

Review and Discuss the results of the Pre-collaborative study

Select the most appropriate ELISA
Positive and encouraging Results

Towards the replacement of the NIH test
## Results Rabies vaccines Potency test

Pre collaborative study

<table>
<thead>
<tr>
<th>Sample N°</th>
<th>Rabies strain</th>
<th>Assigned glycoprotein content (IU/ml)</th>
<th>NIH potency value (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO 6th IS 07/162</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>Pitman-Moore</td>
<td>6.6 (reconstituted in 0.5 ml of H₂O)</td>
</tr>
<tr>
<td>Freeze-dried “Normal”</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>Pitman-Moore</td>
<td>6.6</td>
<td>12.4</td>
</tr>
<tr>
<td>C1</td>
<td>Flury LEP</td>
<td>13.6</td>
<td>2.7</td>
</tr>
<tr>
<td>D1</td>
<td>PV</td>
<td>NP*</td>
<td>5 (reconstituted in 4 ml of H₂O)</td>
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<tr>
<td>Freeze-dried “Degraded”</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>Pitman-Moore</td>
<td>&lt;0.2</td>
<td>Under detection level</td>
</tr>
<tr>
<td>C2</td>
<td>Flury LEP</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Reconstituted “50% spiked normal”</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>Pitman-Moore</td>
<td>2.8</td>
<td>3.0</td>
</tr>
<tr>
<td>C3</td>
<td>Flury LEP</td>
<td>6.4</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*NP: Not Performed

Towards the replacement of the NIH test
Results Rabies ELISA Potency
Pre collaborative study

Towards the replacement of the NIH test
Results Rabies ELISA Potency
Pre collaborative study

Thermodegraded lots ➔ all ELISAs negative
Except Lab 1 ➔ No significantly different results

ELISA Lab 1 recognise less Flury LEP strain
Identical mAb for detection and capture

PV lot ➔ Differences are observed

Freeze-Dried Reconstituted lot : no stability data
## Results Rabies ELISA Potency

**Pre collaborative study**

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Vaccine sample</th>
<th>Test labs</th>
<th>$s^2_{WL}$</th>
<th>$s^2_{BL}$</th>
<th>$s^2_{R}$</th>
<th>Precision (log$_{10}$ IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM</td>
<td>Normal</td>
<td>#1-5</td>
<td>0.0017</td>
<td>0.0033</td>
<td>0.0051</td>
<td>±0.15</td>
</tr>
<tr>
<td></td>
<td>50% spiked</td>
<td>#1-5</td>
<td>0.0021</td>
<td>0.0022</td>
<td>0.0043</td>
<td>±0.14</td>
</tr>
<tr>
<td></td>
<td>normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flury LEP</td>
<td>Normal</td>
<td>#1-5</td>
<td>0.0010</td>
<td>0.0567</td>
<td>0.0577</td>
<td>±0.50</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>#2-5</td>
<td>0.0011</td>
<td>0.0006</td>
<td>0.0017</td>
<td>±0.09</td>
</tr>
<tr>
<td></td>
<td>50% spiked</td>
<td>#1-4</td>
<td>0.0001</td>
<td>0.0876</td>
<td>0.0877</td>
<td>±0.65</td>
</tr>
<tr>
<td></td>
<td>normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50% spiked</td>
<td>#2-4</td>
<td>0.0001</td>
<td>0.0009</td>
<td>0.0010</td>
<td>±0.07</td>
</tr>
<tr>
<td>PV</td>
<td>Normal</td>
<td>#1-5</td>
<td>0.0018</td>
<td>0.0460</td>
<td>0.0478</td>
<td>±0.46</td>
</tr>
</tbody>
</table>
Results Rabies ELISA Potency
Pre collaborative study

Select the most appropriate ELISA

Capture and detection : 2 different Monoclonal antibodies

Neutralizing against the unique trans-membrane trimeric form of Rabies glycoprotein G
G glycoprotein ELISA detection method

- A quantitative sandwich direct ELISA method
- Use of different monoclonal antibodies
  - For coating and/or detection (ex: mAb TJU 1112)
  - For detection (ex: biotinylated mAb D1-25)
- Titration versus an internal reference calibrated in IU (6th WHO IS)
Coating monoclonal antibodies: TJU 1112
(Wistar Institute, Philadelphia, PA, USA)
IgG1 isotype: Neutralize all genotype 1 strains (PV, CVS, PM and Flury LEP strains)

Against the antigenic site II of the glycoprotein

Recognizes conformational and discontinuous epitopes: aa 34 to 42 and aa 198 to 200 associated by S-S bridge

Müller et al. (2009) Plos, 3(11):e542

Towards the replacement of the NIH test
Detection monoclonal antibody: Biotinylated D1-25 (Instituts Pasteur, Paris)

IgG1 isotype: Neutralizes genotype 1 (PV, CVS, PM and Flury LEP strains) and genotype 6 (EBL2 strain))

- Recognizes the conformational trimeric form of the glycoprotein and not the soluble G protein
- Against the site III of the glycoprotein: aa 330 to 338


Towards the replacement of the NIH test
What is still missing?
Pre-validation Study: next steps

Study on different vaccines strains
   Mostly from Chinese, Russian and Indian manufacturers
   Results to be presented June 2016

Set up of a BSP collaborative study
Integration in the new WHO reference project
Presentation to Group 15
Our roadmap?
From
To a clear roadmap
Thanks to The team and you

**Participants**

E. Terao  
E. Meyer  
S. Morgeaux  
D. Wilkinson  
D. Lei, U. Rosskopf  
G. Pulle  
W. Chizhikov, D. Volokhov, R. Levis  
L. Yuhua, C. Shouchun  
S. Shajhahan, L. Viviani  
JM. Chapsal (co-Chair)  
F. Guinet-Morlot, P. Riou  
M. Halder  
W. Correa de Moura  
B. Poirier (Statistician)  
N. Tordo (co-Chair)  
C. Rupprecht  
Y. Kaushik  
M. Gautam, S. Gairola  
C. Jiang

**Organizations**

EDQM  
PEI  
ANSM  
NIBSC  
WHO  
BGTD  
FDA  
NIFDC  
GSK  
EPAA  
Sanofi Pasteur  
EURL-ECVAM  
INCQS-FIOCRUZ  
BPSTAT Consulting  
Institut Pasteur  
Wistar Institute  
Bharat Biotech  
Serum Institute of India  
Jilin University

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