Development of an alternative, in vitro potency assay for rabies virus vaccines.

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Disclaimer

“*My comments are an informal communication and represent my own best judgment. These comments do not bind or obligate FDA.*”
Regulations Related to Potency for Human Vaccines

- No specific tests are defined in the CFR for potency of vaccines.
  - CFR 610.10 states: “Tests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in 600.3(s) of this chapter.

- Assigned potency must be shown to correlate with clinical efficacy.
Introduction

- Rabies vaccines
  - Potency assignment
  - NIH potency test

- History of alternate test development
  - WHO collaborative study using SRID – 1980s
  - FDA/NIBSC working group – 2000s
  - EDQM/EMA collaborative working group - 2012

- Current work on alternate test development
  - ELISA assay development
  - Regulatory pathway for licensure
Licensed Rabies Vaccines – US

- sanofi pasteur - Human Diploid Cell Vaccine
  - IMOVA X rabies - licensed in 1999
  - Pitman-Moore Strain
  - grown in MRC-5 cells
- Novartis Vaccines and Diagnostics - Purified Chick Embryo Cell Vaccine
  - RabAvert – licensed in 1989
  - Fixed Rabies Virus Strain - Flury LEP
  - grown in primary chicken fibroblasts
Rabies virus vaccine potency

- Rabies virus vaccine efficacy was originally defined as protection from death by rabies disease
  - based on a field study
    - Iranian wolf study (published 1976)
      - Potency assignment based on survival – 1 IU/dose
      - WHO recommends vaccines have a potency of > 2.5 IU/mL

- Current rabies vaccines are licensed with a potency specification of > 2.5 IU/mL (as determined using the NIH potency test)

- Efficacy of currently licensed vaccines has been demonstrated in controlled clinical trials

- No vaccine failures* using current post-exposure treatment regimen
Successful protection of humans exposed to rabies infection. Postexposure treatment with the new human diploid cell rabies vaccine and antirabies serum.

Bahmanyar M, Fayaz A, Nour-Salehi S, Mohammadi M, Koprowski H.

Abstract
Forty-five persons severely bitten by rabid dogs and wolves in Iran were treated after exposure with a new rabies vaccine produced in cultures of human diploid cells. All except one also received one injection of rabies immune serum. This treatment, in contrast to past experience with other vaccines, resulted in protection of all individuals against rabies. Thus, almost a century after the postexposure treatment of humans was initiated, an effective tool for protecting man against rabies has finally been developed.
Current potency test:

- Originally developed for neural tissue based vaccines
- Animal-based immune challenge assay:
  - Immunize mice at day 0 and 7 (5 groups/5-fold dilutions)
  - I.C. Challenge on day 14
  - Observe for rabies disease*
- Potency is calculated based on survival relative to a reference vaccine.
- Assay is currently used as release test and as a stability indicating test
Why do we need a replacement test?

- NIH potency test has never been considered a great assay.
  - Test uses ~600 mice.
  - High degree of variability: 25 – 400%
  - Takes up to 6 weeks to complete.
  - Pass on potency is the geometric mean of two valid tests in the US and Canada.
    - Single test used everywhere else.
- Ongoing discussions on test replacement for several decades
  - Collaborative studies to establish an alternative test started in the early 1980s
A collaborative study on the use of single radial immunodiffusion for the assay of rabies virus glycoprotein.

Ferguson M, Seagroatt V, Schild GC.

Abstract
The single radial immunodiffusion (SRD) technique has been applied to the assay of the glycoprotein content of rabies vaccines produced in cell cultures. Fourteen laboratories in seven countries participated in a collaborative study to evaluate the reproducibility of the SRD technique; some laboratories also examined vaccines in the mouse protection (NIH) test and by enzyme immunoassay. Good agreement was found between potency estimates using the SRD technique: the geometric coefficients of variation for combined potency estimates of all laboratories were about 10%. SRD assays appear to have a role for the in vitro assay of antigen content of vaccine and could complement results obtained in in vivo assays which are subject to wide variability.
Use of the single radial immunodiffusion test as a replacement for the NIH mouse potency test for rabies vaccine.

Fitzgerald EA, Needy CF.

Abstract
The method currently recommended by the World Health Organization (WHO) for the potency assay of rabies vaccine is the NIH mouse potency test, a highly variable test requiring large numbers of animals. The Single Radial Immunodiffusion (SRID) test, an in vitro test, has been used successfully for the quantitation of hemagglutinin in inactivated influenza vaccine and is being evaluated for its utility as an assay for the rabies virus glycoprotein, considered to be the major protective antigen, of rabies vaccine. Potency values calculated using the SRID test were compared with those calculated using the NIH test for rabies vaccines produced in cell culture. The within-test variability was significantly lower with the SRID test but the potency values were generally higher than those from the NIH test. *Vaccines which assay below the minimum acceptable potency value (2.5 International Units/ml) in the NIH test generally gave values above that level in the SRID test. The implications of these results on rabies vaccine control testing are discussed.*
Alternatives to the NIH Rabies Vaccine Potency Test

- Working group re-convened
  - FDA sponsored workshop - September 2000
- Representatives from industry (Chiron Behring and Aventis Pasteur), CDC, Thomas Jefferson University, Kansas State University, NIBSC, AFSSAPS, PEI, EDQM
- Collaborative study between CBER, NIBSC, and two industry sponsors
  - Goal of study was to develop an ELISA assay that would potentially serve as an alternative potency assay
Overview of Study (2000)

- Development of an in vitro ELISA to test for antigen content in rabies vaccines
- ELISA to replace NIH test as a release test for potency
  - No requirement for correlation with NIH test
  - Consistency of manufacturing
- ELISA to replace NIH test to test shelf life and stability
  - Show that ELISA will identify sub-potent lots
Initial ideas for replacement potency test (2000)

- Development of uniform protocol
- Development of uniform reagents
- Establishment of standard values as compared to a reference standard
- Mathematical determination of potency from ELISA results
  - Mass measurement vs. protection in animals
- Lots to test:
  - normal production lots
  - sub-potent lots
Lessons learned from collaboration (2000)

- Developed ELISA assay with available reagents
  - Common reagents were difficult to obtain
    - Even published ones
- Vaccine strain differences matter
  - Potency relative to a reference standard were different based on vaccine strain and reagents used
- Able to identify sub-potent lots
  - Data correlated with NIH test results
- Industry sponsors continued with ELISA testing for information purposes.
Regulatory Catch 22

What is the approval pathway for an alternate test?

- Sponsors want to know NRAs will approve alternate test before expending resources to develop and validate test.
- NRAs (in this case – FDA) would like to see data prior to confirming adequacy of test as a replacement.
Is it possible to institute a replacement test for the rabies potency assay?

- We have successfully approved the replacement of several animal-based immunogenicity assays with ELISA-based assays for the measurement of viral vaccine potency
  - Neutralizing epitopes were well defined – or –
  - 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 \textit{in vitro} assays vs immune response in human vaccinees
  - Can we do this with rabies vaccines?
Replacement of NIH Test

- Currently potency is defined by protection against challenge in animals –
  - By virtue of survival after challenge, the NIH potency assay measures a protective response (animals are doing the work for us)
  - Potency/dose should correlate with clinical efficacy
    - Correlation between protection against disease in animals and potency in humans was established in clinical trials -
  - If neutralizing epitopes are well defined then it should be possible to correlate the amount of antigen with immune response measured in animal. Then,
  - It should be possible to define potency based on the amount of antigen in a dose.
Replacement of NIH Test

- Attributes of alternate assay:
  - Neutralizing epitopes are well defined for rabies
    - Reagents are defined that recognize these epitopes and distinguish appropriate conformation of virus
  - **Protective immune response has been defined** – well accepted for human vaccines
  - Clear correlations **HAVE NOT BEEN shown** between amount of antigen required to induce protective immune response in animals vs amount of antigen measured using alternative *in vitro* assays (how does this translate to vaccine efficacy)
    - How do we show correlation between potency defined by protection in animals vs potency defined by alternate methods?
    - Is there a necessity for clinical studies?
EPAA meeting
(The European Partnership for Alternative Approaches to Animal Testing)

- Archachon, France - October 2012
- Re-initiate collaborative discussion on alternate test development
- Establish timeline for reagent and assay development
- Two phase development approach
  - Phase 1 - reagent selection - labs are testing reagents with individual, in house assays.
  - Phase 2 – collaborative study to define assay
Development of an alternative potency test

- Development of study protocol
- Development/availability of reagents
  - Difficult step*
  - Development of reagents at CBER
- Standard values based on International reference(s)
- Definition of potency from test results
  - Establishment of test specifications
  - Requirement for clinical data
- Lots to test:
  - Normal production lots
  - Lots on stability
Considerations for Approval

- Should there be a requirement to show comparability/equivalence to current mouse potency test?
- If tests are not comparable, then the new test must be well qualified.
  - Clear definition of potency: antigen units vs. international units per dose.
  - Antibodies used for detection must correlate with protection.
  - Antibody binding affinity and vaccine strain differences must be well defined (common reference)
    - Due to strain differences, it may be necessary to utilize different reagents for each vaccine
  - Depending on the reagents used, the level of free G protein vs. virus associated G protein must be determined.
  - Test must be able to distinguish potent vs. non-potent lots.
Considerations for Approval

- Is there a necessity for clinical data to support the new potency assay?
  - For human vaccines – immunogenicity trial to show antibody response to vaccines with potency measured using alternative assay
  - Should this be required for the currently licensed vaccines?
    - History of manufacturing consistency
    - History of clinical efficacy
Summary

- Renewed global effort by both human and veterinary vaccine manufacturers and control authorities to establish alternative rabies vaccine potency tests.
- FDA has licensed non-animal based replacement tests for several vaccine products.
- FDA is working with sponsors and other regulatory authorities in this endeavor regarding the replacement of the NIH potency test for rabies virus vaccines.