Use of Serologic Assays in Rabies Product Development

Susan M. Moore, PhD, MS, HCLD(ABB), MT(ASCP)SBB
Rabies Laboratory, KSVDL/College of Veterinary Medicine
Kansas State University, Manhattan, Kansas 66502, USA
Rabies Virus Neutralizing Antibodies

- Proof or evaluation of response to vaccination
- Clinical trials - International
- Diagnostic samples
  - Serum
  - CSF
- Dogs, cats, horses, ferrets, zoo animals, wildlife, etc.
- Pet travel
- Research
  - Product development
  - Field surveys

Kansas State Veterinary Diagnostic Laboratory
Correlation of rabies serology and protection

• Dog/Cat Minimum Acceptable RVNA level based on challenge studies
  – Measurement of RVNA by mouse neutralization test (MNT) or Rapid Fluorescent Focus Inhibition Test (RFFIT)
  – Determination of protection from challenge

• T.O. Bunn and H.D. Ridpath, 1984:
  – Using probit analysis of pre-challenge titers and survival, there was a 1% probability of death for titers at 1:30.9 (MNT)/1:44.4 (RFFIT).

• M.F.A Aubert, 1992:
  – “for this purpose [“protective threshold”], either method of seroneutralisation (RFFIT or MNT) can be employed, provided a correlation between the two methods has been demonstrated in the same laboratory”
  – Effective levels: 0.1 IU/mL in cats and 0.2 IU/mL in dogs by RFFIT.
The Relationship Between Rabies Antibody Titers in Dogs and Protection from Challenge

T. O. Bunn and H. D. Ridpath

Table 2 - Relationship Between Antibody Titers and Projected Death Rate

<table>
<thead>
<tr>
<th>Death rate %</th>
<th>Antibody Titer</th>
<th>MSNT</th>
<th>RFFIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td></td>
<td>.9</td>
<td>1.8</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>1.3</td>
<td>2.6</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>1.9</td>
<td>3.7</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>3.2</td>
<td>5.7</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>6.2</td>
<td>10.5</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>10.9</td>
<td>17.4</td>
</tr>
</tbody>
</table>

MSNT = Mouse Serum Neutralization Test
RFFIT = Rapid Fluorescent Focus Inhibition Test
Practical significance of rabies antibodies in cats and dogs.

Aubert MF1.

1Centre national d’études vétérinaires et alimentaires, Laboratoire d’études sur la rage et la pathologie des animaux sauvages, Malzéville, France.

Abstract

Doubt has sometimes been cast upon the protective effect of rabies antibodies in serum. Animals and humans suffering from fatal rabies often produce high antibody titres, while rabies cases are also observed in vaccinated animals. Cellular immunity is also largely involved in protection. Nevertheless, a large number of laboratory experiments and field observations clearly demonstrate that cats and dogs which develop antibodies after vaccination and before challenge have a very high probability of surviving any challenge, no matter how strong the dose and which virus strain was used. Rabies antibody titration can, therefore, afford a strong additional guarantee to the vaccination certificates accompanying domestic carnivores during transportation between countries. Quarantine rules should also be adapted to the epidemiological features in the exporting country, e.g. statistics of vaccination failure in cats and dogs and host-virus adaptation of the rabies strains circulating in these countries.

Moreover, based on a designated minimum level of neutralising antibodies, and could be proposed as an alternative to quarantine measures. The designated threshold could be based on the results presented in this study. The security of the protection constituted by this threshold would be increased by the extent to which it exceeds the level recognised as effective against experimental challenge in cats and dogs (0.1 IU/ml and 0.2 IU/ml, respectively, measured by RFFIT).
Human Minimum Acceptable RVNA level

• Based on early vaccine clinical trials
  – Measurement of RVNA by mouse neutralization test (MNT) or Rapid Fluorescent Focus Inhibition Test (RFFIT)
  – Determination of adequate vaccine response

• Two guidelines give recommendations:
  – World Health Organization (WHO) – 0.5 IU/mL
  – Advisory Committee on Immunization Practices (ACIP) – complete neutralization of rabies virus at a 1:5 serum dilution in the RFFIT (0.1 IU/mL)
• What level is “significant”?  
  – Protection or seroconversion?  
  – Different exposure levels  
  – Different rabies strains  

• Does the same level apply for all situations?  
  – All vaccination statuses? Age? Health?  
  – All serologic methods? Time since vaccination?  

• What is more important vaccination status or rabies antibody level?
Background – Rabies Serology

• Rabies vaccine clinical trials and Rabies Immune globulin potency
  – Mouse neutralization test
  – RFFIT
    • 1973 published method
    • 1991 QA guidelines given
    • 1996 WHO Methods manual
  – FAVN
    • Reasons for development
    • Standardization and proficiency testing
  – ELISA
    • Types: competitive, blocking, indirect
    • Kits
General Description of the RFFIT and FAVN assays

- The RFFIT first described in 1973 publication by Smith et al.
- The FAVN was developed in the 1990s by Cliquet et al.
- Both test for measuring *functional*, rabies virus neutralizing antibodies
  - correlates with mouse neutralization test
ELISA

- Binding antibodies measured (EU/mL)
- Plasma donor screening for RIG production
- Research projects
- Not recommended for RVNA monitoring
Key Components and RFFIT Setup

1. Five-fold serial dilutions of SERA
2. Transfer sera dilutions to 8-well chamber slides
3. Add VIRUS
   90 min @37°C
   ~50 TCID₅₀/chamber
4. Add BHK-21 cells to serum-virus mixture
   20-24 hours @37°C
5. Wash and Fix
   Wash/Fix in cold 80% Acetone → Air Dry
6. Immunostaining with Abs
   FITC-conjugated Ab (anti-N) → 30 min @37°C
   Wash-1X PBS 1X Water Air Dry
   Count virus positive fields
RFFIT Slide Reading

No virus – complete inhibition

0/20 0/20

Some fields with virus – endpoint dilution

10/20

All fields with virus – no inhibition

20/20

Negative field

Positive field
Controls Included in Each RFFIT Assay Performance

• WHO Reference Standard
  – Reference Standard + virus + cells
  – Contains 2 International Unit (IU)/mL in working stock solution
• Internal antiserum controls (n=4)
  – Internal antiserum control + virus + cells
  – has pre-set acceptance range
• Virus control (back titration)
  – virus + cells only
  – Target 50 TCID$_{50}$/chamber
• Cell control
  – Cells only
  – Monitoring cell monolayer and assay performance
Titer & IU/mL Calculation from the RFFIT Test Data

• End Point Titer determination
  – Number virus positive fields per 20-field count
  – Calculate titer value using Reed and Muench formula

• IU/mL value is calculated by the following formula:
  – Based on the test serum titer in relation to the assigned WHO reference standard

\[
\text{Endpoint titer of test serum} \times \frac{\text{Assigned Reference Serum Concentration (IU/mL)}}{\text{Endpoint titer of Reference Serum}}
\]
Fit for purpose

- Sero-conversion after vaccination or exposure
- Individual or population
- Protection or detection of immune response
- Specificity of response
- Longevity of vaccination response
- Bioequivalence of biologics
- Evaluation of poly/monoclonal antibodies (research, reagent, therapeutic)
- Investigative studies/development of assay/regulated purposes

Validation will determine ‘Fit for Purpose’
Fit for Purpose: Method variations that can be applied to neutralization or antigen binding assays

<table>
<thead>
<tr>
<th>Neutralization Assays</th>
<th>Antigen Binding Assays</th>
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<tbody>
<tr>
<td><strong>Strain of challenge virus</strong></td>
<td><strong>Antigen – virus strain</strong></td>
</tr>
<tr>
<td><strong>Dose of challenge virus</strong></td>
<td><strong>Antigen – virus protein(s)</strong></td>
</tr>
<tr>
<td><strong>Cell type</strong></td>
<td><strong>Whole virus</strong></td>
</tr>
<tr>
<td><strong>Serial dilution scheme</strong></td>
<td><strong>Purified protein</strong></td>
</tr>
<tr>
<td><strong>Detection system</strong></td>
<td><strong>Detection system</strong></td>
</tr>
<tr>
<td>Fluorescent-labeled antibody</td>
<td>Species specific or non-species specific</td>
</tr>
<tr>
<td>Enzyme-labeled antibody</td>
<td>Immunoglobulin specific for class or subclass</td>
</tr>
<tr>
<td>Modified challenge virus</td>
<td>Platform – slides, plates, or beads</td>
</tr>
<tr>
<td>(ex. Green Fluorescent Protein)</td>
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Challenges for rabies monoclonal testing

- Specificity – need to grow and qualify rabies strains and test in equivalent dose for comparison
- Adapting to cell culture, verification of sequence for possible epitope alteration
- Unit of reporting - µg/mL
- For mixture of mabs – need assays to differentiate between the mabs in clinical samples both circulating and potential inhibition of RVNA development
Thank you for your attention. Questions?