

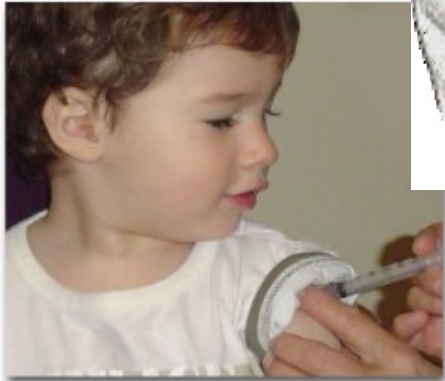


Susan M. Moore, PhD, MS, HCLD(ABB), MT(ASCP)SBB  
Rabies Laboratory, KSVDL/College of Veterinary Medicine  
Kansas State University, Manhattan, Kansas 66502, USA

# Use of Serologic Assays in Rabies Product Development

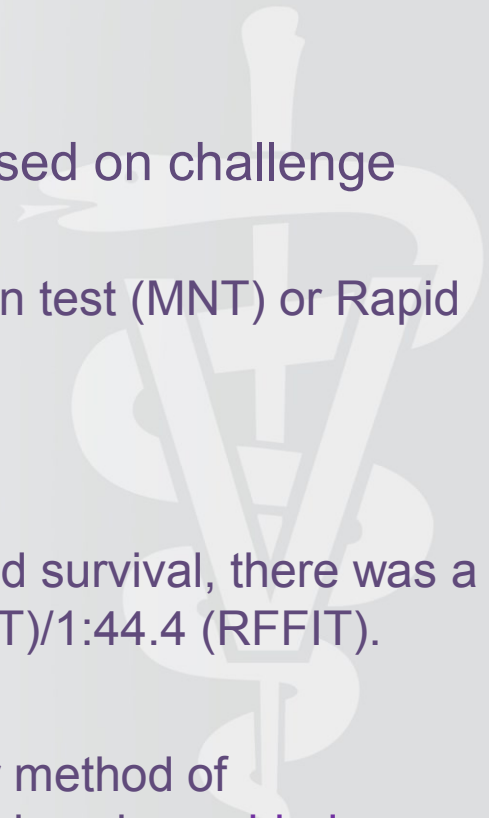
# Rabies Virus Neutralizing Antibodies

- 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



# 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

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

MSNT = Mouse Serum Neutralization Test

RFFIT = Rapid Fluorescent Focus Inhibition Test

**KANSAS STATE**

Veterinary Diagnostic Laboratory

# 0.5 IU/mL – what does it mean?

[Rev Sci Tech.](#) 1992 Sep;11(3):735-60.

## **Practical significance of rabies antibodies in cats and dogs.**

[Aubert MF](#)<sup>1</sup>.

<sup>1</sup>Centre national d'études vétérinaires et alimentaires, Laboratoire d'études sur la rage et la pathologie des animaux sauvages, Malzéville, France.

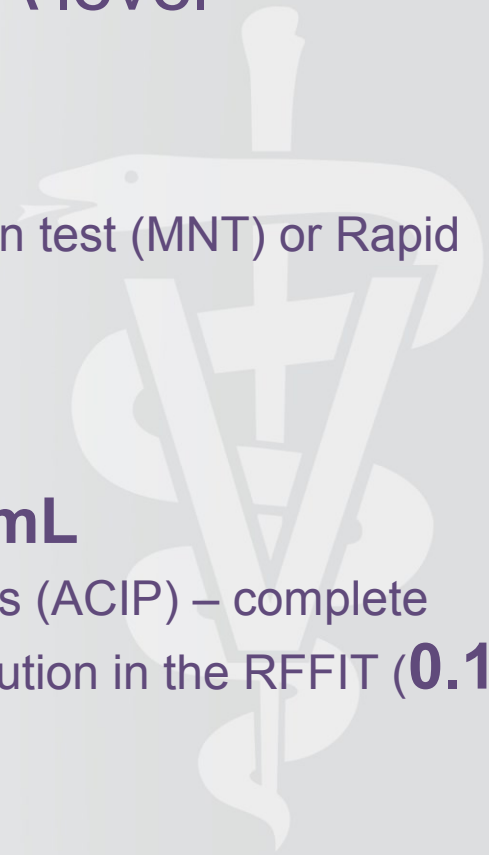
### **Abstract**

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.

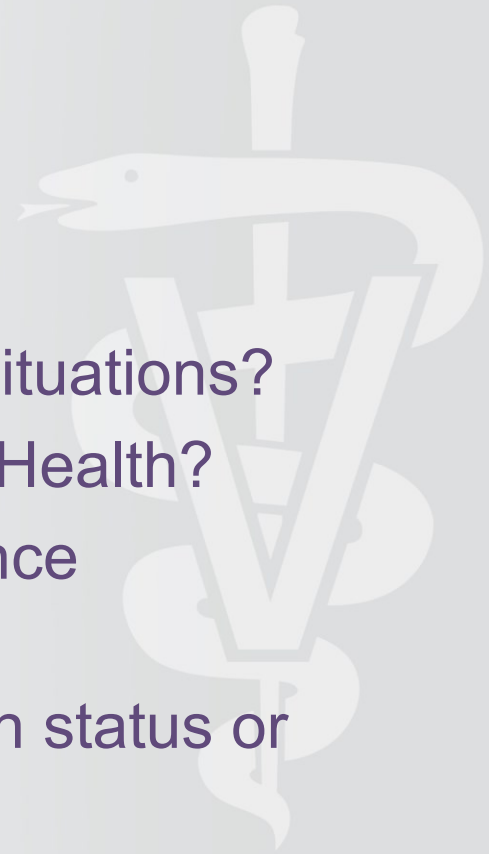
Therefore, a designated minimum level of neutralising antibodies, and could be proposed as an alternative to quarantine measures. The designated threshold could be based on the results presented in this study. The security of the protection constituted by this threshold would be increased by the extent 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

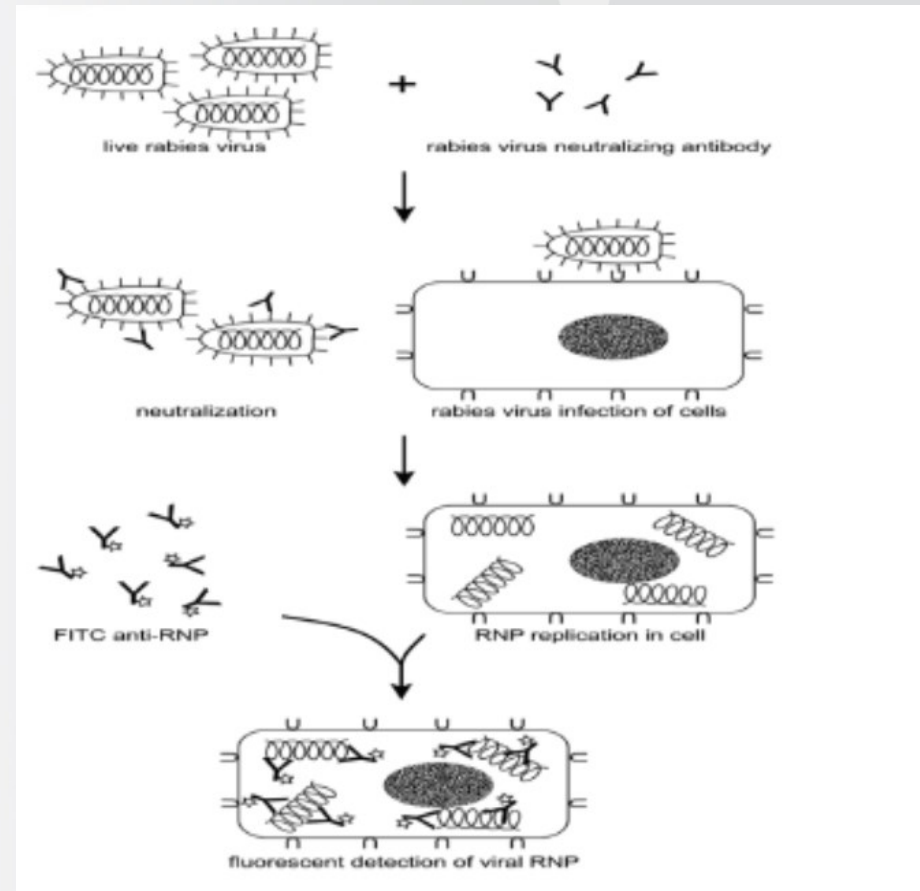
- Rabies vaccine clinical trials and Rabies Immune globulin potency
  - Mouse neutralization test
  - RFFIT
    - 1973 published method
    - 1991 QA guidelines given
    - 1996 WHO Methods manual
  - FAVN
    - Reasons for development
    - Standardization and proficiency testing
  - ELISA
    - Types: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



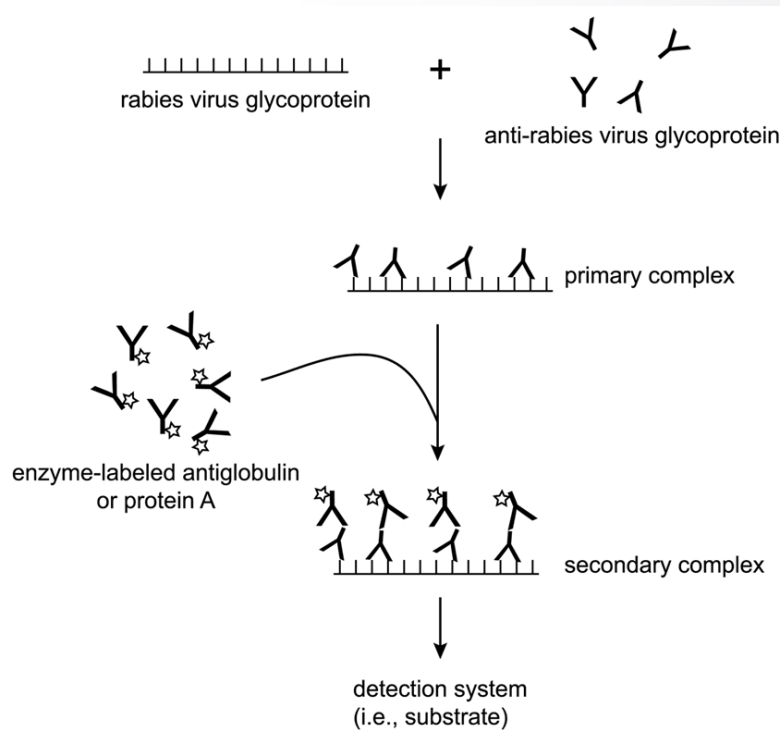


# General Description of the RFFIT and FAVN assays

- 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

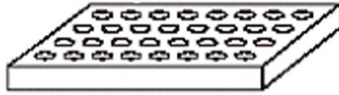


- Binding antibodies measured (EU/mL)
- Plasma donor screening for RIG production
- Research projects
- Not recommended for RVNA monitoring

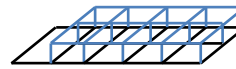
# Key Components and RFFIT Setup

1. Five-fold serial dilutions of **SERA**

96-well plate



2. Transfer sera dilutions to 8-well chamber slides

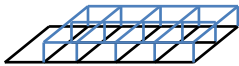


3. Add **VIRUS**

90 min @37°C

~50 TCID<sub>50</sub>/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

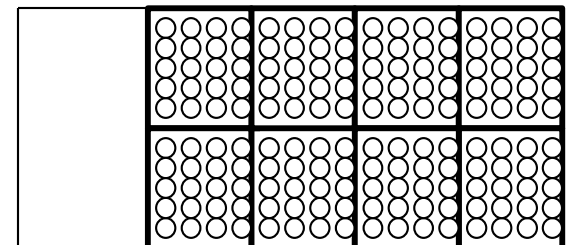
6. Immunostaining with **Abs**

FITC-  
conjugated  
Ab (anti-N)

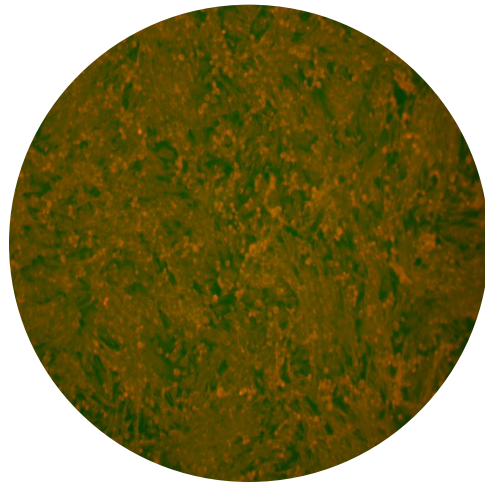
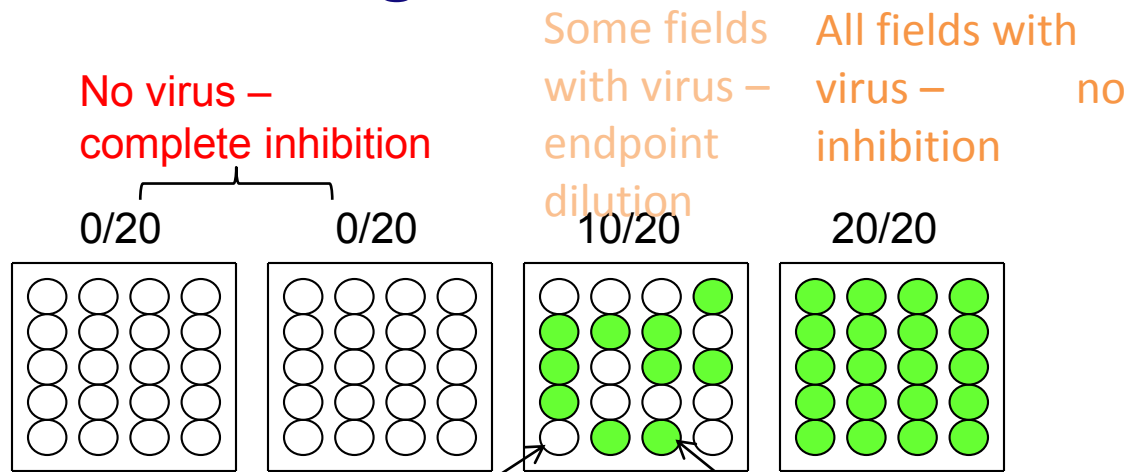
30 min  
@37°C

Wash-  
1X PBS  
1X Water  
Air Dry

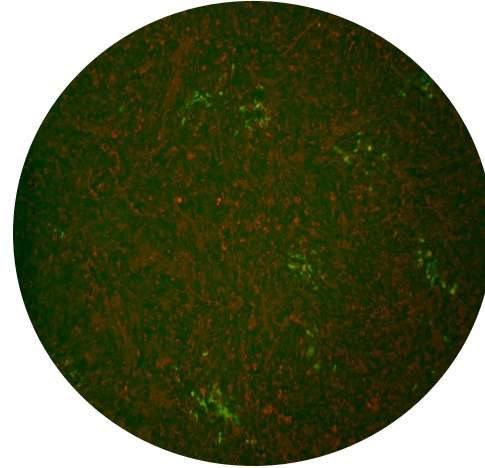
Count virus positive fields



# RFFIT Slide Reading



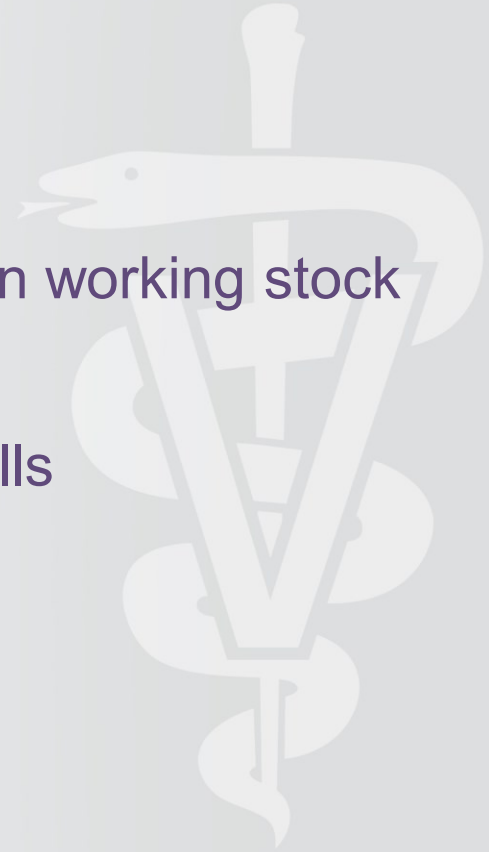
Negative field



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<sub>50</sub>/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

$$\frac{\text{Endpoint titer of test serum}}{\text{Endpoint titer of Reference Serum}} \times \text{Assigned Reference Serum Concentration (IU/mL)}$$

# 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

## **Neutralization Assays**

---

**Strain of challenge virus**

**Dose of challenge virus**

Cell type

Serial dilution scheme

Detection system

Fluorescent-labeled antibody

Enzyme-labeled antibody

Modified challenge virus  
(ex. Green Fluorescent Protein

## **Antigen Binding Assays**

---

**Antigen – virus strain**

**Antigen – virus protein(s)**

**Whole virus**

**Purified protein**

Detection system

Species specific or non-species specific

Immunoglobulin specific for class or subclass

Platform – slides, plates, or beads

insert)

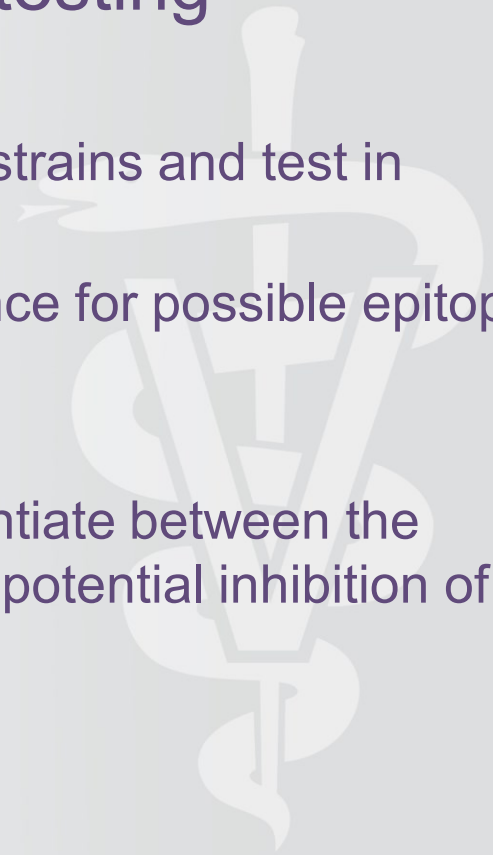
**KANSAS STATE**

**Veterinary Diagnostic Laboratory**



# 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 -  $\mu\text{g/mL}$
- 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?

