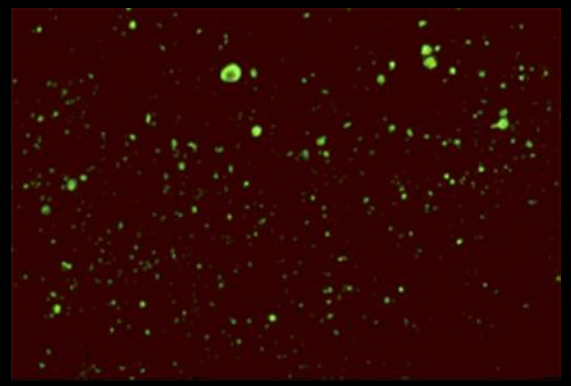
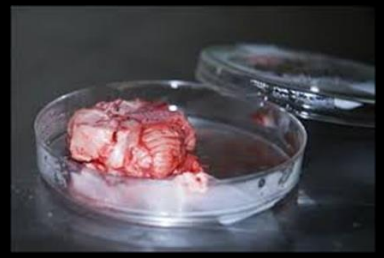
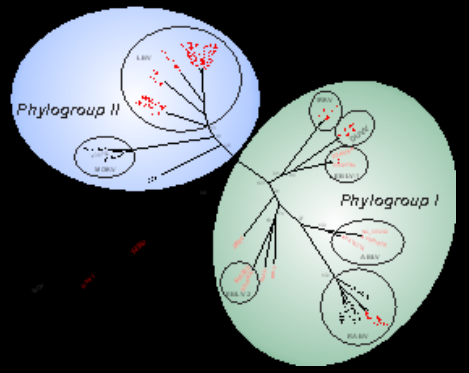
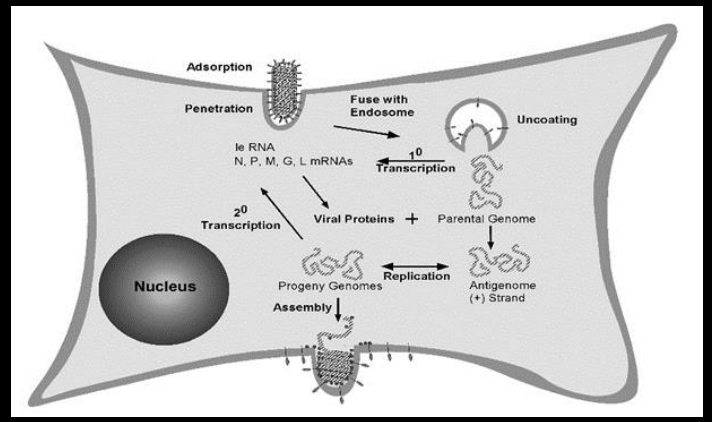
# PROGRESS TOWARDS PUBLICATION OF A NEW EDITION OF THE WHO LABORATORY TECHNIQUES IN RABIES MANUAL

**CE RUPPRECHT, VMD, MS, PhD**
**WHO EXPERT TECHNICAL ADVISOR ON RABIES**







# HISTORICAL PARADIGM SHIFTS IN RABIES SUSPICION

**Concept of animal observation for millennia**

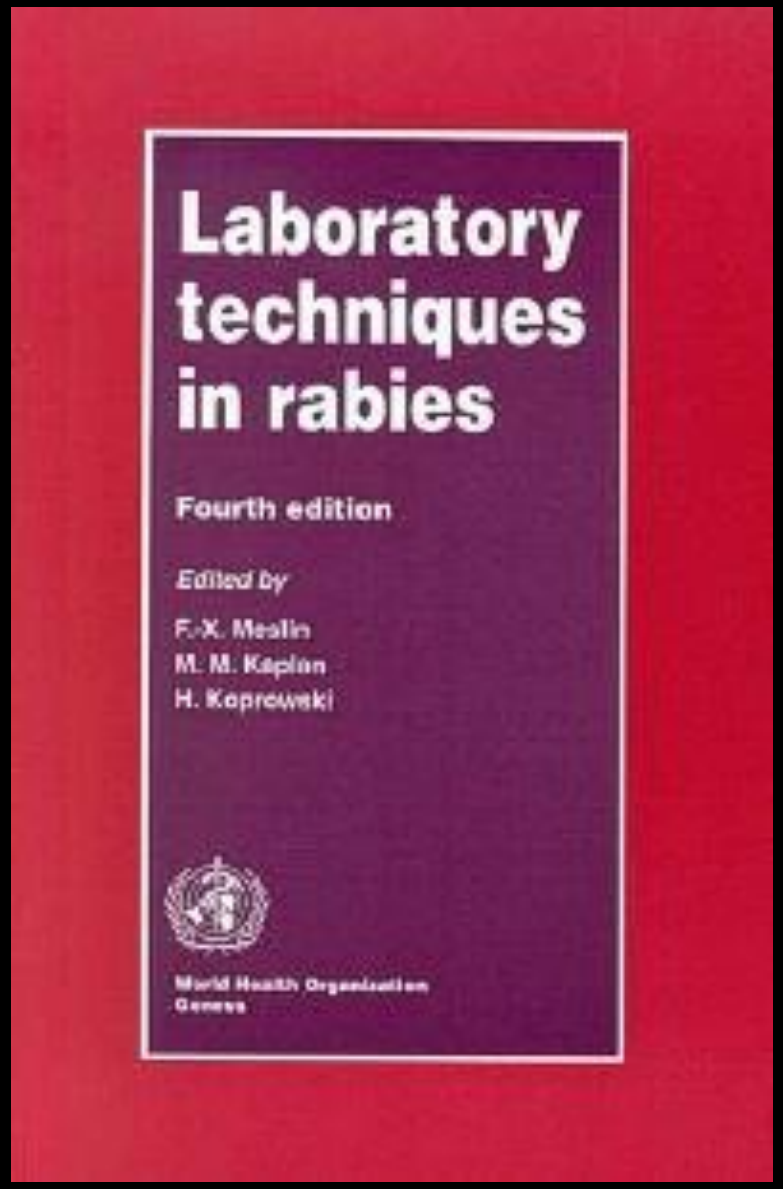
**Improved clinical understanding of pathogenesis in 19th century**

**Research on experimental inoculation and animal models (early 1800s)**

**Microscopy and non-specific histological lesions (~late 1800s)**

**20th century improvements for laboratory-based surveillance**

**21ST century applications towards canine-mediated rabies elimination**




- More than 20 years since publication of the previous 4th edition

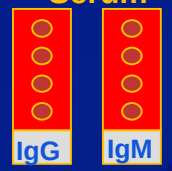
- 5th edition consists of > 40 chapters

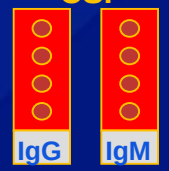
- Authored by global experts in academia, government and industry

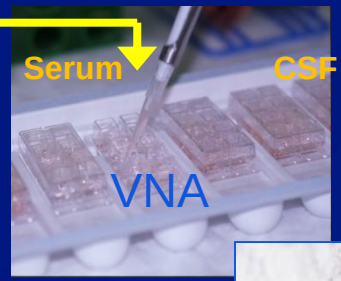
- Focused on global program for canine rabies elimination

# A Focus on Basic Laboratory Techniques in Rabies

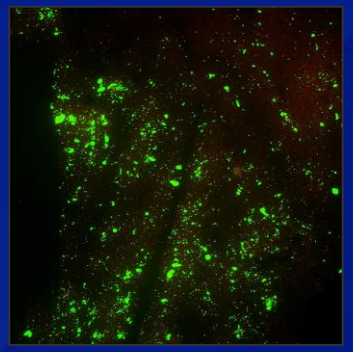
## Serology

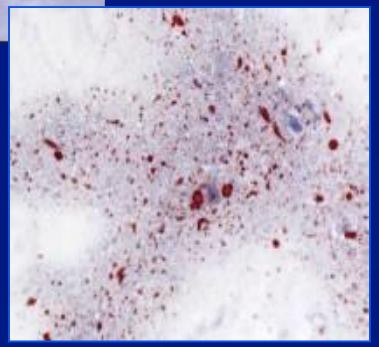



## Antigen Detection





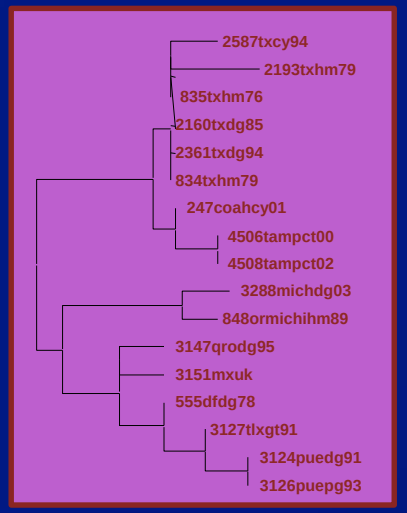
## Virus Isolation

### Mouse Inoculation

### Cell Culture

## Molecular Techniques

### RT-PCR Sequencing



# WHO LABORATORY TECHNIQUES IN RABIES, 5TH EDITION, 2018

## I. GENERAL CONSIDERATIONS:

Introduction

Lyssaviruses

Biosafety

The role of diagnostics in surveillance

An overview of antemortem and postmortem tests for diagnosis of human rabies

Histopathological techniques in the laboratory diagnosis of human rabies

Brain removal

Use of a rapid skin biopsy technique for human rabies antemortem diagnosis

The FTA sampling method for collecting, storing brain material and identification of lyssaviruses

Regulatory perspectives on the design of human rabies biologics

Regulatory issues in the development of animal biologics for rabies

# WHO LABORATORY TECHNIQUES IN RABIES, 5TH EDITION

II. Detection of virus:

Transmission electron microscopy (TEM) in rabies diagnosis, ultrastructural studies and research

Virus isolation in animals: the mouse inoculation test (MIT)

Virus isolation in cell culture: the rabies tissue culture infection test (RTCIT)

# WHO LABORATORY TECHNIQUES IN RABIES, 5TH EDITION

**III. Demonstration of antigens:**

**The direct fluorescent antibody test (DFAT)**

**The direct rapid immunohistochemistry test (DRIT) for the detection of lyssavirus antigens**

**Immunohistochemistry**

**Antigenic typing of lyssaviruses by monoclonal antibodies**

**Demonstration of lyssavirus antigens by flow cytometry**

**Rapid immunochromatographic tests for the detection of rabies virus antigens in brain material**

**Mass spectrometry-based proteomic approaches for the detection of rabies virus peptides**

# PROGRESS IN DETECTION OF ANTIGENIC VARIANTS OF RABIES VIRUS


Wiktor TJ, Koprowski H. Monoclonal antibodies against rabies virus produced by somatic cell hybridization: detection of antigenic variants. Proc Natl Acad Sci USA. 1978;75:3938-42.

Dietzschold B, et al. Characterization of an antigenic determinant of the glycoprotein that correlates with pathogenicity of rabies virus. Proc Natl Acad Sci U S A. 1983;80:70-4.

Germano PM, et al. Antigenic variants of rabies virus isolated in the northeast and southeast of **Brazil**. Preliminary study. Bol Oficina Sanit Panam. 1990;108:39-45.

Delpietro HA, et al. Monoclonal antibody characterization of rabies virus strains isolated in the River Plate Basin, **Argentina**. Zentralbl Veterinarmed B. 1997;44:477-83.

de Mattos CA, et al. Genetic characterization of rabies field isolates from **Venezuela**. J Clin Microbiol. 1996;34:1553-8.

Diaz AM, et al. Antigenic analysis of rabies-virus isolates from **Latin America** and the Caribbean. Zentralbl Veterinarmed B. 1994;41:153-60.

Loza-Rubio E, et al. Investigation of rabies virus strains in **Mexico** with a panel of monoclonal antibodies used to classify Lyssavirus. Bull Pan Am Health Organ. 1996;30:31-5.

Dyer JL, et al. Evaluation of an indirect rapid immunohistochemistry test for the differentiation of rabies virus variants. J Virol Methods. 2013;190:29-33.

Guarino H, et al. Antigenic and genetic characterization of rabies virus isolates from **Uruguay**. Virus Res. 2013;173:415-20.

Yung V, et al. Typing of the rabies virus in **Chile**, 2002-2008. Epidemiol Infect. 2012;140:2157-62.

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**IV. Demonstration of Viral Antibodies:**

**The rapid fluorescent focus inhibition test (RFFIT)**

**The fluorescent antibody virus neutralization (FAVN) test**

**Demonstration of lyssavirus antibodies by pseudotype virus micro-neutralization assays**

**A simplified fluorescence inhibition microtest for the determination of rabies virus neutralizing antibodies**

**An indirect fluorescent antibody (IFA) test for the detection of rabies virus Immune globulin**

**The immunoperoxidase inhibition assay (IIA)**

**Demonstration of rabies virus antibodies by the Counter immunoelectrophoresis test**

**The mouse neutralization test**

## V. Determination of Viral Nucleic Acids & Sequences:

**Conventional pan-lyssavirus reverse transcription polymerase chain reaction (RT-PCR)**

**Rabies real time reverse transcription polymerase chain reaction (Real-time RT-PCR)**

**Sanger sequencing of lyssaviruses**

**Application of next generation sequencing to rabies virus and other lyssaviruses**

**Reverse transcription loop-mediated isothermal amplification (RT-LAMP) system for the detection of rabies virus**

**Detection of lyssavirus nucleic acids by in situ hybridization**

**Rapid diagnosis and genetic typing of rabies virus and other lyssaviruses using SYBR Green RT-PCR and pyrosequencing assays**

# LIMITATIONS TO CLASSICAL RT-PCR

- Poor Precision
- Low sensitivity
- Short dynamic range < 2 logs
- Low resolution
- Non - automated
- Size-based discrimination only
- Results are not expressed as numbers
- Ethidium bromide for staining is not very quantitative
- Post PCR processing

# USE OF REAL-TIME PCR

- Real-time PCR differs from conventional PCR with regards to the manner in which the amplicon is detected – oligoprobes with fluorescent potential are monitored during the amplification, versus conventional post-amplification detection using UV-irradiated agarose gel electrophoresis with a DNA intercalating dye.

## VI. Production of Biologics:

**Preparation of fluorescent antibody conjugate for the direct fluorescent antibody test (DFAT)**

**Anti-rabies monoclonal antibody production using mammalian expression systems**

**Generation of immune globulin single variable domains by display technologies**

**Production of monospecific polyclonal rabies virus antibodies in birds**

**Plant production of monoclonal antibodies for rabies**

WHO LABORATORY TECHNIQUES IN RABIES, 5TH EDITION

**VII. Potency Determinations:**

The NIH test for potency testing of vaccines

The serological potency assay for rabies inactivated vaccines for veterinary use

In vitro tests for rabies vaccine potency testing

**APPENDICES** (e.g., WHO CC address, etc.)

**INDEX**

| TEST | TARGET | SAMPLE | OBJECTIVE | LEVEL | ADVANTAGES | DISADVANTAGES | COMMENTS |
|---|---|---|---|---|---|---|---|
| **Direct Rapid Immuno-histo-chemistry Test (DRIT)** | Viral protein (nucleo-protein) | Brain | Primary post-mortem diagnosis; confirmatory testing; enhanced surveillance | Central and local lab network | High sensitivity and specificity; uses light microscopy upon CNS impressions collected from suspect mammals; rapid; suitable for surveillance under field conditions; uses biotin-labeled monoclonal or polyclonal antibodies either from OIE/WHO reference laboratories or self-produced | Requires basic laboratory equipment, reagents and training for application | Under consideration as a recommended OIE diagnostic test; broad spectrum choice of antibodies allows detection of all known lyssaviruses; in routine use in North America for support of oral wildlife rabies vaccination programs |

Coetzer A, et al. Comparison of biotinylated monoclonal and polyclonal antibodies in an evaluation of a direct rapid immunohistochemical test for the routine diagnosis of rabies in southern Africa. PLoS Negl Trop Dis. 2014 Sep 25;8(9):e3189.

| ASSAY | TARGET | TISSUE | USE | FOCUS | UTILITY | CHALLENGE | COMMENT |
|---|---|---|---|---|---|---|---|
| Indirect Rapid Immuno-histo-chemistry Test (IRIT) | Viral protein | Brain | Antigenic typing of confirmed cases | Central refer-ence and local lab net-work | Provides confirmation of canine rabies virus identity via monoclonal antibody typing by light microscopy; such panels are widely available from the WHO CCs | Same as DRIT | Typing of antigenic variants has been widespread throughout Latin America in support of canine rabies elimination programs |

Dyer JL, et al. Evaluation of an indirect rapid immunohistochemistry test for the differentiation of rabies virus variants. J Virol Methods. 2013 Jun;190(1-2):29-33.

| ASSAY | TARGET | TISSUE | USE | FOCUS | UTILITY | CHALLENGE | COMMENT |
|---|---|---|---|---|---|---|---|
| RT-PCR (convention-al and real time) | Viral RNA | Ante-mortem (saliva, nuchal skin, csf, tears, corneal wash, etc.) and post-mortem tissues (e.g., CNS) | Primary diagnosis; viral variant typing | Central refer-ence lab | High sensitivity and specificity; antemortem diagnosis in human rabies aids in confirmation of clinical diagnosis and patient management, institution of barrier nursing and PEP to close contacts; can also be used for post-mortem confirmation on brain tissue (human or animal); amplified material can be sequenced for further virus characterization | High technological lab requirement; sensitivity depends on the type of specimen collected; ~ 100% with nuchal skin biopsy and at least 3 saliva samples; if such requirements are not fulfilled, then negative test result do NOT rule out a diagnosis of rabies; need for Stringent Quality Assurance and ideal preservation of the sample | Obtaining brain tissue continues to be a challenge in human rabies diagnosis; therefore, such tests may be the only feasible techniques for human rabies diagnosis, especially for antemortem testing |

Fischer M, et al. A step forward in molecular diagnostics of lyssaviruses--results of a ring trial among European laboratories. PLoS One. 2013;8(3):e58372.

| ASSAY | TARGET | TISSUE | USE | FOCUS | UTILITY | CHALLENGE | COMMENT |
|---|---|---|---|---|---|---|---|
| Competi-tive ELISA (kits) | Host antibody | Serum; sera adsorbed on filter paper; muscle extract | Screening for rabies virus antibodies; post-vaccination monitoring; sero-surveillance | Central and local lab network | Good repeatability between laboratories; controlled suppliers; internal controls; non-species specific; easy and rapid to collect directly in the field without the use of needles, syringes, or vacutainer tubes by using filter paper strips | Requires some basic laboratory equipment; correlation with neutralizing antibody dependent on homology between the kit antigens and the rabies virus vaccine strain, as well as the host genetics | Useful in wildlife oral rabies and canine vaccination monitoring; not recommended for routine human rabies vaccination response monitoring |

Wasniewski M, et al. First international collaborative study to evaluate rabies antibody detection method for use in monitoring the effectiveness of oral vaccination programmes in fox and raccoon dog in Europe. J Virol Methods. 2016 Dec;238:77-85.

| ASSAY | TARGET | TISSUE | USE | FOCUS | UTILITY | CHALLENGE | COMMENT |
|---|---|---|---|---|---|---|---|
| Indirect ELISA (kits) | Host antibody | Serum, plasma | Screening for rabies virus antibodies; post-vaccination monitoring; sero-surveillance | Central and local lab network | Good repeatability between laboratories; controlled suppliers; internal controls | Requires some basic laboratory equipment; may not be useful for all species; may be most relevant for humans; detects only IgG rabies virus antibodies; general correlation with neutralizing antibody levels may be variable in some individuals | Not suitable for routine rabies diagnosis in humans, as antibodies are typically present in sera and csf only during the late clinical phase; may be useful for monitoring antibody titers in exposed personnel or during PEP in immune-compromised patients or when major deviations from recommended PEP schedules occur; can be used for serosurveys under certain conditions |

| ASSAY | TARGET | TISSUE | USE | FOCUS | UTILITY | CHALLENGE | COMMENT |
|---|---|---|---|---|---|---|---|
| **Immuno-chromato-graphic test for rabies virus detection** | **Viral protein (e.g., nucleo-protein)** | **Brain** | **Screening for animal rabies virus (domestic and wild animals)** | **Central and local lab net-work** | **Low technological requirement; low containment requirement; can be used at point of sampling; suitable for surveillance under field conditions** | **Need for much better standardization, and quality control of the kits** | **Cannot substitute for currently recommende d reference techniques, but may be helpful in developing countries where surveillance is lacking** |

Léchenne M, et al. Validation of a Rapid Rabies Diagnostic Tool for Field Surveillance in Developing Countries. PLoS Negl Trop Dis. 2016 Oct 5;10(10):e0005010.

# Utility of Lateral Flow Diagnostic Tests for Rabies?

- Potential Limitations:
  - *Sensitivity?*
  - *Specificity?*
  - *Cost?*
  - *Flexibility?*

***"…The high number of false negative results reiterates the necessity to perform a proper test validation before being marketed and used in the field…"***

Eggerbauer E, et al. Evaluation of 6 Commercially Available Rapid Immunochromatographic Tests for the Diagnosis of Rabies in Brain Material. PLoS Negl Trop Dis. 2016 Jun 23;10(6):e0004776.

AUGMENTATION OF BASIC QA/QC IN VALIDATION OF RABIES LABORATORY METHODS:

PRE-ANALYTICAL (e.g., equipment and supplies)

ANALYTICAL (e.g. written standard protocols)

POST-ANALYTICAL (e.g., proficiency testing)

INFRASTRUCTURE (long-term administrative support to break the cycle of neglect)

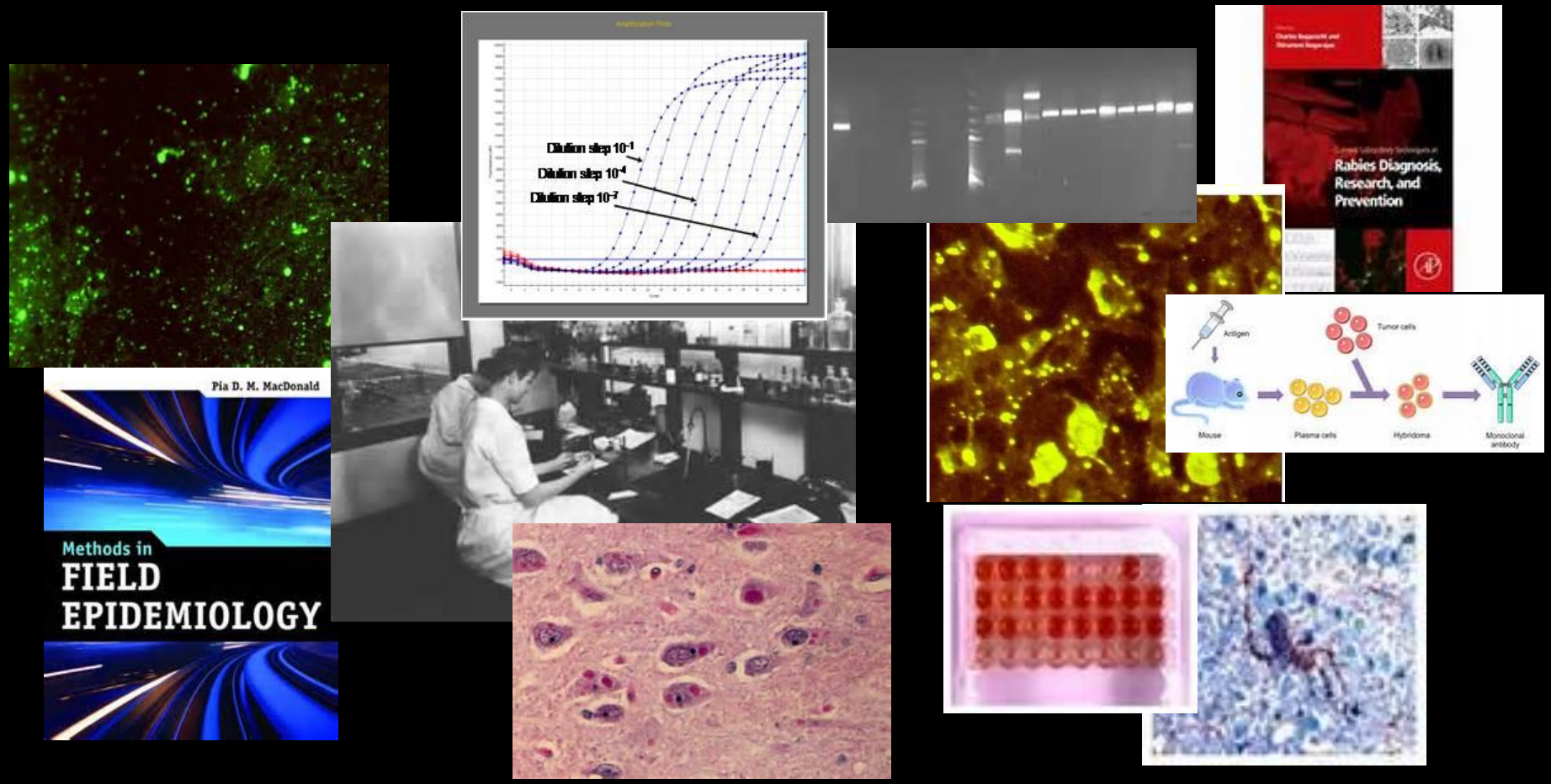
PERSONNEL (e.g., including professional growth)

BUDGET (e.g., for multiple years and estimated growth)

TRAINING (e.g., with routine continuing education)

Modern laboratory methods have revolutionized the identification of lyssavirus species and provided new insights to the evolution and epidemiology of rabies viruses as relevant to public health, veterinary medicine and conservation biology and are critical to the global program for the elimination of canine-mediated rabies in the decades ahead

**DECADES OF METHODOLOGICAL IMPROVEMENTS IN DETECTION OF LYSSAVIRUS ANTIGENS, ANTIBODIES, AMPLICONS EMPHASIZED IN THE NEXT EDITION…**



# QUESTIONS?

SURVEILLANCE


RESPONSE

charleseruprechtii@gmail.com