



Laboratory techniques in rabies

**Fifth edition
Volume 2**

Edited by

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World Health
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Laboratory techniques in rabies, fifth edition. Volume 2/Charles E Rupprecht, Anthony R Fooks, Bernadette Abela-Ridder, editors.

ISBN 978-92-4-151530-6

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Suggested citation. Rupprecht CE, Fooks AR, Abela-Ridder B, editors. Laboratory techniques in rabies, fifth edition. Volume 2. Geneva: World Health Organization; 2018. Licence: [CC BY-NC-SA 3.0 IGO](https://creativecommons.org/licenses/by-nc-sa/3.0/igo).

Cataloguing-in-Publication (CIP) data. CIP data are available at <http://apps.who.int/iris>.

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Contents

| | |
|---|-----|
| Foreword | iv |
| Preface | v |
| List of abbreviations and acronyms used in this manual | vi |
| Part 5. Demonstration of viral nucleic acids and sequences | |
| Chapter 27. Conventional pan-lyssavirus reverse transcriptase polymerase chain reaction..... | 1 |
| Chapter 28. Rabies real-time reverse transcriptase polymerase chain reaction..... | 17 |
| Chapter 29. Sanger sequencing of lyssaviruses | 35 |
| Chapter 30. The FTA sampling method for collecting, storing brain material and identification of lyssaviruses..... | 44 |
| Chapter 31. Application of next generation sequencing to rabies virus and other lyssaviruses..... | 49 |
| Chapter 32. Reverse transcriptase loop-mediated isothermal amplification system for the detection of rabies virus | 62 |
| Chapter 33. Detection of lyssavirus nucleic acids by in situ hybridization | 71 |
| Chapter 34. Rapid diagnosis and genetic typing of rabies virus and other lyssaviruses using SYBR Green RT-PCR and pyrosequencing assays..... | 80 |
| Part 6. Production of biologicals | |
| Chapter 35. Regulatory perspectives on the design of human rabies biologicals..... | 94 |
| Chapter 36. Regulatory issues in the development of animal biologicals for rabies..... | 107 |
| Chapter 37. Preparation of fluorescent antibody conjugate for the direct fluorescent antibody test | 112 |
| Chapter 38. Anti-rabies monoclonal antibody production using mammalian expression systems | 128 |
| Chapter 39. Generation of anti-rabies single domain antibodies by display technologies..... | 137 |
| Chapter 40. Production of monospecific polyclonal rabies virus antibodies in birds | 150 |
| Chapter 41. Plant production of monoclonal antibodies for rabies | 160 |
| Part 7. Potency determinations | |
| Chapter 42. The NIH test for potency testing of vaccines | 180 |
| Chapter 43. The serological potency assay for batch potency testing of inactivated rabies | 189 |
| Chapter 44. In vitro tests for rabies vaccine potency testing..... | 192 |

Laboratory techniques in rabies

Foreword

For more than 5000 years, humans have lived in fear of a bite from a rabid animal, so much so that the first written account of rabies, in the 23rd century BC, set the penalty for an owner's dog biting another individual at "two-thirds of a mine of silver", or about a half-day's work. Today, our focus is more on preventing rabies and advocating for its elimination, rather than imposing penalties, and our understanding of the virus has greatly improved since the 23rd century BC.

The Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE) and the World Health Organization (WHO) have prioritized action against rabies and, together with Member countries, have set a goal of zero rabies deaths by 2030. Diagnostics are crucial in attaining this goal.

New laboratory techniques and advancements in science have yielded better diagnostic techniques and control strategies to aid the more than 3 billion people, mainly children, in Asia and Africa who are threatened by the virus every day. Rabies is a preventable disease, yet despite the availability of efficacious and affordable vaccines, more than 60 000 people worldwide die agonizing deaths every year from the disease.

No diagnostic tests are available to detect the rabies virus before the onset of clinical disease, and further research on diagnostic techniques in the field of rabies is therefore paramount. The impact of suitable laboratory capacity on surveillance and elimination of the disease worldwide is evident.

The OIE's Manual of diagnostic tests and vaccines for terrestrial animals provides internationally agreed standards for the production and control of validated veterinary diagnostic methods and vaccines for use in animals. The fourth edition of WHO's Laboratory techniques in rabies has been a guiding reference for many rabies laboratories. The first edition (1954) stated that "rabies research is far from static" and, since its publication more than 60 years ago, OIE and WHO have worked to evaluate subsequent advancements in laboratory techniques in rabies. This fifth edition provides insight into validated methods recommended for use in diagnostic laboratories, but it also includes research. While not currently applicable to all settings, these research methods may stimulate the development of improved techniques for diagnosis of rabies in the future. Improved diagnostics will strengthen surveillance of the disease, leading to enhanced control of rabies where it is most needed.



Laboratory techniques in rabies

Preface

Rabies has an enormous impact on both agriculture and conservation biology, but its greatest burden is undeniably on public health. As such, routine methods for rapid risk assessment after human exposures to rabies as well as applications for laboratory-based surveillance, production of biologicals and management of this infectious disease are critical. Given its mandate to improve human health and control disease among its Member States, WHO has led the production of this fifth edition of *Laboratory techniques in rabies*.

During the more than 60 years that have elapsed since the first edition was published, methods of viral diagnosis, characterization of pathogens and production of biologicals have advanced. At that time, only a single etiological agent was recognized as causing rabies. Detection of Negri bodies was the standard for diagnosis. Nerve tissue-based vaccines were the norm. Combination use of vaccines and rabies immunoglobulins in human prophylaxis was not standard. Global elimination of canine rabies was merely a dream. Rabies in wildlife was managed via population reduction. All of that has changed for the better.

In the ensuing decades, further advancements in detection, prevention and control of lyssaviruses have been monitored by regular meetings of WHO experts, international research groups and countries in which rabies is endemic. The second edition of the manual was published in 1966, the third in 1973 and the fourth in 1996. The late Martin Kaplan and Hilary Koprowski were instrumental in editing the previous editions, as was input on the fourth edition by François-Xavier Meslin, now retired from WHO. Initial plans for preparation of this edition were made in 2016 and its contents were discussed at the WHO Expert meeting on rabies (Bangkok, Thailand) and modified in response.

This fifth edition of *Laboratory techniques in rabies* contains 44 detailed chapters written by more than 85 authors from Africa, the Americas and Eurasia. The text was peer reviewed by Dr Matthias Schnell, Head of the WHO Collaborating Centre for Neurovirology; Professor Thiravat Hemachudha, Head of the WHO Collaborating Center for Research and Training on Viral Zoonoses; and Dr Asefa Deressa, Team Leader of Zoonoses Research at the Ethiopian Public Health Institute. The manual focuses on the basic methods for detection of lyssavirus antigens, antibodies and nucleic acids and the relevance of their use under different operating conditions, from the basic to the advanced. The chapters on older, less sensitive techniques used to detect Negri bodies have been removed, as have those chapters on methods of vaccine production given the progress made in the commercial use of tissue culture products in human and veterinary medicine. Recommendations for the preparations of antibodies by homologous or heterologous production have been replaced by newer methods in an effort to promote a next generation of less expensive and more readily available immunoglobulins in the future. Other basic chapters have been retained and updated and more than a dozen added. Each of the protocols described are prescriptive and should be followed point by point in the laboratory.

We gratefully acknowledge the collaboration of the many eminent scholars who contributed to the current volume, and look forward to the publication of the next edition as continued advances in the field are made.

List of abbreviations and acronyms used in this manual

| | |
|-------|--|
| 3Rs | “Replacement, Reduction and Refinement” of laboratory animal testing |
| AALAS | American Association for Laboratory Animal Science |
| Ab | antibody |
| ABLV | Australian bat lyssavirus |
| ACD | acid citrate dextrose |
| ACIP | Advisory Committee on Immunization Practices |
| ACS | American Chemical Society |
| AEC | 3-Amino-9-ethylcarbazole |
| Ag | antigen |
| ANSM | Agence Nationale de Sécurité du Médicament et des produits de santé |
| AMA | African Medicines Agency |
| AP | alkaline phosphatase |
| APS | ammonium persulphate |
| ARAV | Aravan virus |
| ATCC | American Type Culture Collection |
| AVMA | American Veterinary Medical Association |
| BBLV | Bokeloh bat lyssavirus |
| BCIP | 5-bromo-4-chloro-3-indolyl-phosphate |
| BEEM | better equipment for electron microscopy |
| BHK | baby hamster kidney |
| bnAbs | broadly neutralizing antibodies |
| bp | base pair |
| BP | British Pharmacopeia |
| BPL | β-propiolactone |
| BRP | Biological Reference Preparation |
| BSA | bovine serum albumin |
| BSC | biosafety cabinet |
| BSL | biosafety level |
| CCID | cell culture infectious dose |
| CDC | United States Centers for Disease Control and Prevention |
| cDNA | complementary deoxyribonucleic acid |
| CER | chicken embryo-related |
| CFIA | Canadian Food Inspection Agency |

| | |
|--------|--|
| CHAPS | 3-(3-cholamidopropyl) dimethylammonium 1-propanesulfonate |
| CHO | Chinese Hamster Ovary cells |
| CIE | counter immunoelectrophoresis |
| CLRW | clinical laboratory reagent water |
| CNS | central nervous system |
| CPE | cytopathic effect |
| CSF | cerebrospinal fluid |
| Ct | Cycle threshold |
| CVS | challenge virus standard strain |
| ddNTP | dideoxynucleotide |
| DDSA | dodecanyl succinic anhydride |
| dNTP | deoxynucleosidetriphosphate |
| DEAE | diethylaminoethyl |
| Defra | Department for Environment, Food and Rural Affairs |
| DEPC | diethylpyrocarbonate |
| DFAT | direct fluorescent antibody test |
| DH2O | distilled water |
| DIG | digoxigenin |
| DMEM10 | Dulbecco's minimum essential medium with 10% fetal calf serum |
| DMP30 | tris dimethylaminomethyl phenol |
| DMSO | dimethyl sulfoxide |
| DNA | deoxyribonucleic acid |
| dNTP | deoxy-nucleotide-tri phosphate |
| DPX | mixture of distyrene (a polystyrene), a plasticizer (tricresyl phosphate) and xylene |
| DRIT | direct rapid immunohistochemistry test |
| dsDNA | double stranded DNA |
| DSMZ | German Collection of Microorganisms and Cell Cultures |
| DTT | dithiothreitol |
| DUVV | Duvenhage virus |
| EBLV-1 | European bat lyssavirus, type 1 |
| EBLV-2 | European bat lyssavirus, type 2 |

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