



Laboratory techniques in rabies

Fifth edition
Volume 1

Edited by

Charles E. Rupprecht
LYSSA LLC
Atlanta, Georgia, USA

Anthony R. Fooks
Animal and Plant Health Agency
Addlestone, Surrey, United Kingdom

Bernadette Abela-Ridder
Department of Control of Neglected
Tropical Diseases
World Health Organization
Geneva, Switzerland

Laboratory techniques in rabies

Fifth edition

Volume 1

Edited by

Charles E. Rupprecht

LYSSA LLC

Atlanta, Georgia, USA

Anthony R. Fooks

Animal and Plant Health Agency

Addlestone, Surrey, United Kingdom

Bernadette Abela-Ridder

Department of Control of Neglected Tropical Diseases

World Health Organization

Geneva, Switzerland



Laboratory techniques in rabies, fifth edition. Volume 1/Charles E Rupprecht, Anthony R Fooks, Bernadette Abela-Ridder, editors.

ISBN 978-92-4-151515-3

© World Health Organization 2018

Some rights reserved. This work is available under the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 IGO licence (CC BY-NC-SA 3.0 IGO; <https://creativecommons.org/licenses/by-nc-sa/3.0/igo>).

Under the terms of this licence, you may copy, redistribute and adapt the work for non-commercial purposes, provided the work is appropriately cited, as indicated below. In any use of this work, there should be no suggestion that WHO endorses any specific organization, products or services. The use of the WHO logo is not permitted. If you adapt the work, then you must license your work under the same or equivalent Creative Commons licence. If you create a translation of this work, you should add the following disclaimer along with the suggested citation: "This translation was not created by the World Health Organization (WHO). WHO is not responsible for the content or accuracy of this translation. The original English edition shall be the binding and authentic edition".

Any mediation relating to disputes arising under the licence shall be conducted in accordance with the mediation rules of the World Intellectual Property Organization.

Suggested citation. Rupprecht CE, Fooks AR, Abela-Ridder B, editors. Laboratory techniques in rabies, fifth edition. Volume 1. 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>.

Sales, rights and licensing. To purchase WHO publications, see <http://apps.who.int/bookorders>. To submit requests for commercial use and queries on rights and licensing, see <http://www.who.int/about/licensing>.

Third-party materials. If you wish to reuse material from this work that is attributed to a third party, such as tables, figures or images, it is your responsibility to determine whether permission is needed for that reuse and to obtain permission from the copyright holder. The risk of claims resulting from infringement of any third-party-owned component in the work rests solely with the user.

General disclaimers. The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of WHO concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by WHO in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by WHO to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall WHO be liable for damages arising from its use.

The named editors alone are responsible for the views expressed in this publication.

Contents

| | |
|--|-----|
| Foreword | iv |
| Preface | v |
| List of abbreviations and acronyms used in this manual | vi |
| Part 1. General considerations | |
| Chapter 1. Introduction | 1 |
| Chapter 2. Lyssaviruses | 7 |
| Chapter 3. Biosafety | 26 |
| Chapter 4. The role of diagnostics in surveillance | 35 |
| Chapter 5. An overview of antemortem and postmortem tests for diagnosis of human rabies | 43 |
| Chapter 6. Histopathological techniques in the laboratory diagnosis of human rabies | 55 |
| Chapter 7. Brain removal | 67 |
| Part 2. Detection of virus | |
| Chapter 8. Virus isolation in animals: the mouse inoculation test | 74 |
| Chapter 9. Virus isolation in cell culture: the rabies tissue culture infection test | 85 |
| Chapter 10. Transmission electron microscopy in rabies diagnosis, ultrastructural studies and research | 96 |
| Part 3. Demonstration of antigens | |
| Chapter 11. The direct fluorescent antibody test | 108 |
| Chapter 12. The direct rapid immunohistochemistry test for the detection of lyssavirus antigens | 130 |
| Chapter 13. Immunohistochemistry | 136 |
| Chapter 14. Antigenic typing of lyssaviruses by monoclonal antibodies | 142 |
| Chapter 15. Use of a rapid skin biopsy technique for human rabies antemortem diagnosis | 155 |
| Chapter 16. Demonstration of lyssavirus antigens by flow cytometry | 169 |
| Chapter 17. Rapid immunochromatographic tests for the detection of rabies virus antigens in brain material | 176 |
| Chapter 18. Mass spectrometry-based proteomic approaches for the detection of rabies virus peptides | 183 |
| Part 4. Demonstration of viral antibodies | |
| Chapter 19. The rapid fluorescent focus inhibition test | 196 |
| Chapter 20. The fluorescent antibody virus neutralization test | 219 |
| Chapter 21. An indirect fluorescent antibody test for the detection of rabies virus immunoglobulin G and immunoglobulin M antibodies | 232 |
| Chapter 22. The mouse neutralization test | 246 |
| Chapter 23. Demonstration of lyssavirus antibodies by pseudotype virus micro-neutralization assays | 252 |
| Chapter 24. A simplified fluorescence inhibition microtest for the determination of rabies virus neutralizing antibodies | 259 |
| Chapter 25. The immunoperoxidase inhibition assay | 266 |
| Chapter 26. Demonstration of rabies virus antibodies by the counter immunoelectrophoresis test | 271 |
| Appendix 1 List of contributors | 283 |
| Appendix 2 WHO collaborating centres on rabies, neurovirology, viral zoonoses and zoonoses control | 288 |

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 |

预览已结束，完整报告链接和二维码如下：

https://www.yunbaogao.cn/report/index/report?reportId=5_25633

