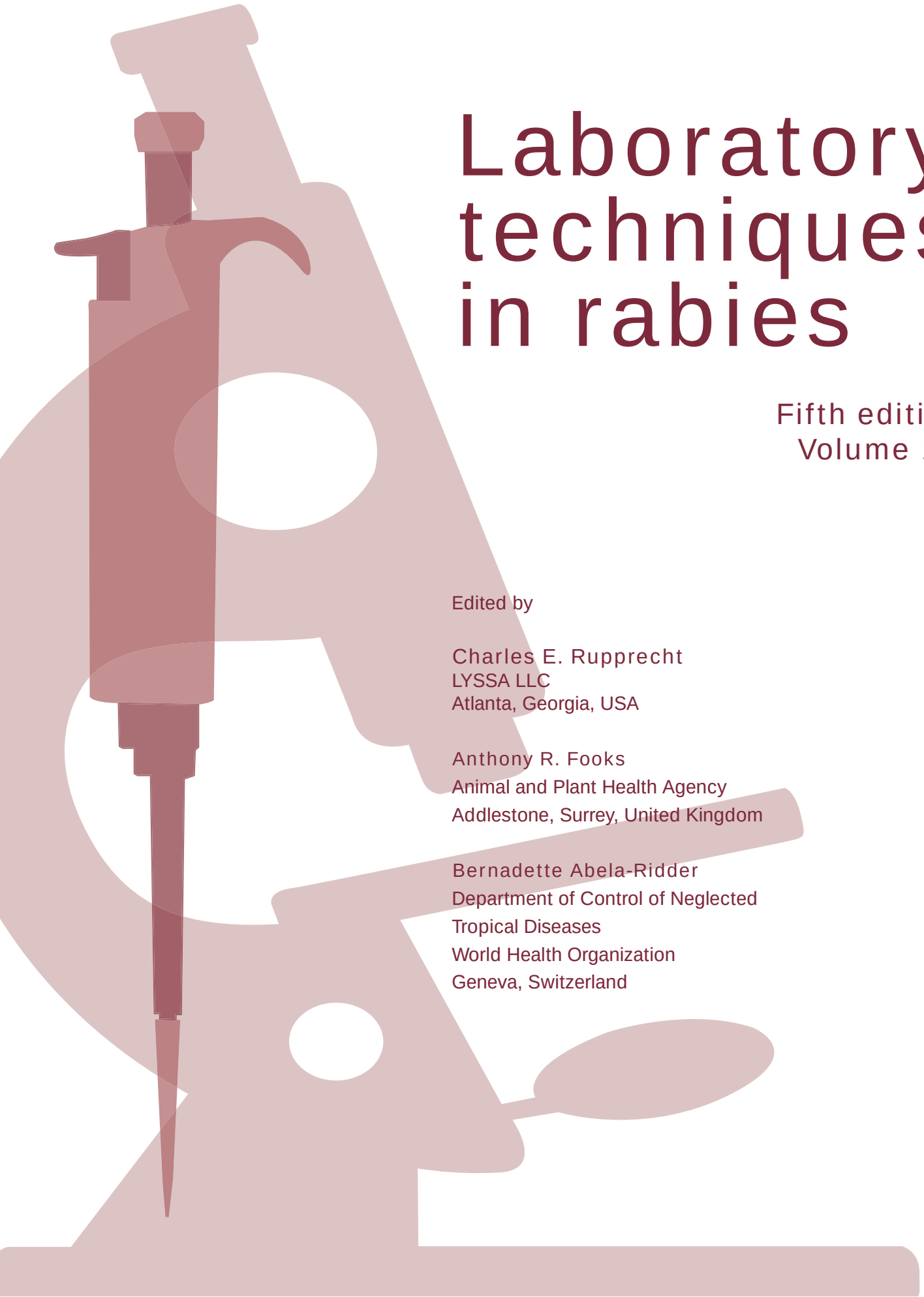

Laboratory techniques in rabies

Fifth edition
Volume 1

Edited by

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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.

Food and Agriculture Organization of the United Nations

WORLD ORGANISATION FOR ANIMAL HEALTH

World Health Organization

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	dodecenyl succinic anhydride
dNTP	deoxynucleosidetriphosphate
DEAE	diethylaminoethyl
Defra	Department for Environment, Food and Rural Affairs
DEPC	diethylpyrocarbonate
DFAT	direct fluorescent antibody test
DH20	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