

SpringerBriefs in Microbiology

More information about this series at <http://www.springer.com/series/8911>

Guadalupe García-Elorriaga
Guillermo del Rey-Pineda

Practical and Laboratory Diagnosis of Tuberculosis

From Sputum Smear to Molecular Biology



Springer

Guadalupe García-Elorriaga
National Medical Center La Raza, CMNR
Mexican Social Security Institute, IMSS
Mexico City, Mexico

Guillermo del Rey-Pineda
Department of Infectology
Federico Gomez Children's Hospital
Mexico City, Mexico

ISSN 2191-5385
SpringerBriefs in Microbiology
ISBN 978-3-319-20477-2
DOI 10.1007/978-3-319-20478-9

ISSN 2191-5393 (electronic)
ISBN 978-3-319-20478-9 (eBook)

Library of Congress Control Number: 2015944099

Springer Cham Heidelberg New York Dordrecht London
© Springer International Publishing Switzerland 2015

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media
(www.springer.com)

Preface

This book is the result of a joint effort acknowledging the challenge of writing and publishing a book on the diagnosis of tuberculosis (TB). It is particularly appealing due to its advantage over other books, since it specifically focuses on the diagnosis of TB, encompassing the elemental diagnostic methods up to cutting-edge technology-based tests, including the diagnosis of TB infection (latent TB infection, LTBI).

This treaty is exclusively centered on the diagnosis of TB, including the spectrum of clinical diagnosis through the microbiological and molecular gold standard, the most practical, due to its celerity and high sensitivity. The diagnosis of LTBI, key to TB control, is also addressed.

Since TB diagnostic methods are still evolving, training must also be continuous. Great advances in this dynamic and ever-changing field have developed in the past few years, particularly resulting from the introduction of Molecular Biology. But unfortunately, this has led to increased costs and hence great disadvantages, leaving many patients without a timely diagnosis and appropriate treatment, particularly in highly endemic countries.

With comprehensive mastery, a change in the paradigm on TB diagnosis could well revitalize the required technology, making it more efficient, faster, predictable, and at a more accessible cost.

A century after Robert Koch discovered the bacillus causing TB, a great number of countries still depend on bacilloscopy as the only means of disease detection. We build on the past and we are all a product of our parents, professors, and colleagues as well as of our God-given talents and challenges.

I have had the privilege of working on the routine microbiological diagnosis of TB at the *Laboratorio de Microbiología de la Unidad Médica de Alta Especialidad “Dr. Gaudencio González Garza,”* all the way to the Molecular Biology techniques in the Immunology and Infectious Disease Research Unit of the Infectious Disease Hospital at the *Centro Médico Nacional “La Raza,”* affiliated with the *Instituto Mexicano del Seguro Social.*

We believe that particularly in countries with high TB endemicity, a quick and handy reference book on the diagnosis of TB is useful for Clinicians, Microbiologists, teachers and students of Medicine and Microbiology.

I wish to express my gratitude to many colleagues and physicians for their support and close collaboration, particularly during those fruitful meetings of the Center for National Epidemiological Surveillance and Disease Control (*Centro Nacional de Vigilancia Epidemiológica y Control de Enfermedades*). Also, to the *Dirección General de Epidemiología. SS*, primarily for the “Modification of the Mexican Official Policy NOM-006-SSA2-1993,” for the prevention and control of tuberculosis at the primary health care level, published in the *Diario Oficial* on September 27, 2005; and secondly, for the elaboration of the “Practical guide to the care of tuberculosis in children and adolescents,” in association with the National Tuberculosis Program, ISBN 970-721-334-5. December 2006.

I must also especially acknowledge all those silent heroes that have been of great assistance in the preparation of this manuscript: Gabriel Natan Pires, the Clinical Medicine Associate Editor that wholly believed in the Project; the always patient and kind Associate Editor at Life Sciences and Biomedicine at Springer Brazil, Roberta Gazzarolle Del Rossi, and our attentive project coordinator, Susan Westendorf.

My coauthor, Dr. Guillermo del Rey-Pineda, an expert Immunologist, and I hope that our initiative will motivate the interest of our readers not only in the solution of TB clinical diagnostic dilemmas but to prompt them to present new questions on routine and basic diagnosis, fostering a continuous bidirectional exchange between the realms of health care and those of clinical and basic research.

Mexico DF, Distrito Federal, Mexico

Guadalupe García-Elorriaga

Contents

1	Introduction.....	1
2	Clinical Diagnosis.....	7
2.1	A Complete Medical Evaluation for Tuberculosis (TB) Includes the Following Five Components	8
2.1.1	Medical History	8
2.1.2	Physical Examination.....	8
2.1.3	Test for TB Infection.....	9
2.1.4	Chest X-Ray.....	9
2.1.5	Bacteriologic Examination of Clinical Specimens	11
2.2	Other Tests	11
2.2.1	Adenosine Deaminase (ADA)	11
2.2.2	Histopathology	12
2.3	Evaluation of Diagnostic Methods in EPTB.....	14
2.3.1	Diagnosis of Miliary TB	14
2.3.2	Diagnosis of Pleural TB	16
2.3.3	Diagnosis of Meningeal TB	16
2.3.4	Diagnosis of Pericardial TB	16
2.3.5	Diagnosis of Lymph Node TB	16
2.3.6	Diagnosis of Abdominal TB	17
2.3.7	Diagnosis of Resistance to Anti-TB Drugs.....	17
	References.....	17
3	Bacteriological Diagnosis.....	19
3.1	Sampling Methods	19
3.1.1	Importance of Sample Collection and Processing in Pulmonary TB	20
3.1.2	Specimen Collection Methods in Extrapulmonary TB	20
3.1.3	Acid-fast Bacilli Smear Classification and Results	21
3.1.4	Evolution of the Microbiological Techniques Used to Diagnose Tuberculosis.....	21

3.1.5	Conventional Microbiological Techniques in the Diagnosis of Tuberculosis.....	22
3.1.6	Smear Microscopy	22
3.1.7	Auramine–Rhodamine Fluorescent Staining	23
3.1.8	Light-Emitting Diode Microscopy.....	24
3.2	Culture Methods.....	24
3.2.1	LJ Culture.....	24
3.2.2	Liquid Culture, DST	26
3.2.3	MB/BacT System.....	26
3.2.4	The MGIT	26
3.2.5	Non-commercial Culture Methods	27
3.2.6	Newer Solid Cultures	27
3.3	Identification of Mycobacteria.....	27
3.3.1	Reporting Results.....	28
3.3.2	In Vitro MTB Drug Susceptibility Testing.....	29
3.3.3	Diagnosis of Active TB	30
3.3.4	Volatile Organic Compounds	30
3.3.5	Breath Sample Collection	30
3.3.6	Breath Sample Analysis	31
3.3.7	Active Non-PTB	31
	References.....	33
4	Molecular Diagnosis.....	35
4.1	Introduction.....	35
4.2	Nucleic Acid Amplification Test	37
4.2.1	PCR	38
4.2.2	Line Probe Assays (LPAs) (INNO-LiPA Rif TB Assay, MTBDRsI).....	38
4.2.3	GeneXpert	38
4.2.4	Policy Updates	39
4.2.5	Implementation of Existing Technologies	40
4.2.6	Planned Technology Refinements of GeneXpert	41
4.3	Other Isothermal NAATs	42
4.3.1	Transcription Mediated Amplification/Nucleic Acid Sequence Based Amplification	42
4.3.2	Simple Method for Amplifying RNA Targets.....	43
4.3.3	Recombinase Polymerase Amplification	44
4.3.4	Helicase-Dependent Amplification	44
4.3.5	Rolling Circle Amplification.....	45
4.3.6	Ramification-Extension Amplification.....	46
4.3.7	Loop-Mediated Isothermal Amplification.....	47
4.3.8	Cross-Priming Amplification	48
4.3.9	Smart Amplification Process (SmartAmp).....	49

4.3.10	Strand Displacement Amplification	49
4.3.11	Nicking Enzyme Amplification Reaction.....	50
4.3.12	Nicking Enzyme-Mediated Amplification (NEMA).....	50
4.3.13	Isothermal Chain Amplification	50
4.3.14	Exponential Amplification Reaction	51
4.3.15	Limitations of Amplification Tests.....	51
4.3.16	Future Perspectives	51
4.4	Conclusions.....	51
	References.....	53
5	TB Infection.....	55
5.1	Introduction.....	55
5.2	Tuberculin Skin Test	57
5.2.1	Tuberculin	58
5.2.2	Pathogenic Basis of TST	58
5.2.3	Immunological Bases of the Tuberculin Reaction	58
5.2.4	Factors Influencing the Test Result	59
5.2.5	False Negative Readings	63
5.2.6	False Positive Readings.....	63
5.2.7	Boosted Reaction and Serial Tuberculin Skin Testing	64
5.2.8	Previous BCG Vaccination.....	64
5.2.9	Definition of TST Conversions	64
5.2.10	Anergy Test in HIV-Infected Individuals	65
5.2.11	TST Indications	65
5.3	IFN γ Detection (IGRA)	65
5.3.1	Immune Response to TB Infection	66
5.3.2	General Recommendations for the Use of IGRA.....	66
5.3.3	Types of IGRA	68
5.3.4	Test Performance and Interpretation	68
5.3.5	Advantages of IGRA Over TST	68
5.3.6	Sensitivity and Specificity.....	69
5.3.7	Clinical Performance of IGRA.....	70
5.3.8	IGRA in Immunosuppressed Patients	70
5.3.9	Cost-Effectiveness.....	71
5.3.10	International Guidelines on IGRA Use	71
	References.....	72
	Index.....	73

Abbreviations

ADA	Adenosine deaminase
AFB	Acid fast bacteria
ATB	Active tuberculosis
ATD/GC/MS	Automated thermal desorption, gas chromatography, and mass spectroscopy
BAL	Bronchoalveolar lavage
BCA	Breath collection apparatus
BCG	Bacilo de Calmette-Guérin
CD1	Cluster of differentiation 1
CDC	Centers for Disease Control and Prevention (USA)
CFP-10	Culture filtrate protein 10
CPA	Cross priming amplification
CRI	Colorimetric redox indicator
CT	Computed tomography
CXR	Chest X-ray
DNA	Deoxyribonucleic acid
DST	Drug susceptibility testing
DTH	Delayed-type hypersensitivity
EPTB	Extrapulmonary tuberculosis
ESAT-6	Early secretory antigenic target 6
FDA	Food and Drug Administration (USA)
FIND	Foundation for Innovative New Diagnostics
HDA	Helicase-dependent amplification
HIV	Human immunodeficiency virus
HRCT	High resolution computed tomography
ICT	Immunochemical test
IGRA	Interferon gamma release assay
IFN γ	Interferon gamma
IL-1b	Interleukin-1 beta
IL-12	Interleukin-12
IL-15	Interleukin-15

IL-18	Interleukin-18
ISTC	International Standards for Tuberculosis Care
LAMP	Loop-mediated amplification
LED	Light-emitting diode
LJ	Lowenstein–Jensen media. A solid culture media used for tuberculosis diagnosis
LPA	Line probe assay
LRP	Luciferase reporter phage
LTBI	Latent TB infection
MDCT	Multidetector-computed tomography
MDR-TB	Multidrug-resistant tuberculosis
MGIT	Mycobacteria Growth Indicator Tube
MODS	Microscopic-observation drug-susceptibility
MRI	Magnetic resonance imaging
MTB	<i>Mycobacterium tuberculosis</i>
MTBC	<i>Mycobacterium tuberculosis</i> complex. A genetically related group of <i>Mycobacterium</i> that cause tuberculosis
MVL	Mercury vapor lamp
NAAT	Nucleic acid amplification test
NK	Natural killer
NEAR	Nicking enzyme amplification reaction
NRA	Nitrate reductase assay
NTM	Non-TB mycobacteria
PCR	Polymerase chain reaction
PEPFAR	United States President's Emergency Plan for AIDS Relief
PET	Positron emission tomography
POCT	Point-of-care testing
PPD	Purified protein derivative
PTB	Pulmonary tuberculosis
RAM	Ramification-extension amplification
RCA	Rolling circle amplification
RD1	Region of difference
RNA	Ribonucleic acid
RPA	Recombinase polymerase amplification
SDA	Strand displacement amplification
SMART	Simple method for amplifying RNA targets
SmartAmp	Smart amplification process
TB	Tuberculosis
TBLB	Transbronchial lung biopsy
TLA	Thin layer agar
TMA	Transcription mediated amplification
TNF α	Tumor necrosis factor alpha
TPE	Tuberculous pleural effusion
TST	Tuberculin skin test
USAID	United States Agency for International Development

VOC	Volatile organic compounds
WHO	World Health Organization
XDR-TB	Extensively drug-resistant tuberculosis
ZN	Ziehl–Neelsen staining