



# M24

## Susceptibility Testing of Mycobacteria, *Nocardia* spp., and Other Aerobic Actinomycetes

This standard provides protocols and related quality control parameters for antimicrobial susceptibility testing of mycobacteria, *Nocardia* spp., and other aerobic actinomycetes.

A standard for global application developed through the Clinical and Laboratory Standards Institute consensus process.

# Clinical and Laboratory Standards Institute

*Setting the standard for quality in medical laboratory testing around the world.*

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing medical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

## Consensus Process

Consensus—the substantial agreement by materially affected, competent, and interested parties—is core to the development of all CLSI documents. It does not always connote unanimous agreement but does mean that the participants in the development of a consensus document have considered and resolved all relevant objections and accept the resulting agreement.

## Commenting on Documents

CLSI documents undergo periodic evaluation and modification to keep pace with advances in technologies, procedures, methods, and protocols affecting the laboratory or health care.

CLSI's consensus process depends on experts who volunteer to serve as contributing authors and/or as participants in the reviewing and commenting process. At the end of each comment period, the committee that developed the document is obligated to review all comments, respond in writing to all substantive comments, and revise the draft document as appropriate.

Comments on published CLSI documents are equally essential and may be submitted by anyone, at any time, on any document. All comments are managed according to the consensus process by a committee of experts.

## Appeal Process

When it is believed that an objection has not been adequately considered and responded to, the process for appeal, documented in the CLSI *Standards Development Policies and Processes*, is followed.

All comments and responses submitted on draft and published documents are retained on file at CLSI and are available upon request.

## Get Involved—Volunteer!

Do you use CLSI documents in your workplace? Do you see room for improvement? Would you like to get involved in the revision process? Or maybe you see a need to develop a new document for an emerging technology? CLSI wants to hear from you. We are always looking for volunteers. By donating your time and talents to improve the standards that affect your own work, you will play an active role in improving public health across the globe.

For additional information on committee participation or to submit comments, contact CLSI.

Clinical and Laboratory Standards Institute

500 West Valley Road, Suite 2500

Wayne, PA 19087 USA

T: +1.610.688.0100

F: +1.610.688.0700

[www.clsi.org](http://www.clsi.org)

[standard@clsi.org](mailto:standard@clsi.org)

---

# Susceptibility Testing of Mycobacteria, *Nocardia* spp., and Other Aerobic Actinomycetes

Gail L. Woods, MD  
Nancy L. Wengenack, PhD, D(ABMM)  
Grace Lin, MS  
Barbara A. Brown-Elliott, MS, MT(ASCP)SM  
Daniela Maria Cirillo, MD, PhD  
Patricia S. Conville, MS, MT(ASCP)  
Edward P. Desmond, PhD, D(ABMM)  
Scott B. Killian, BS  
Nicole M. Parrish, PhD, MHS, D(ABMM)  
Richard Pfeltz, PhD  
Elvira Richter, PhD  
John D. Turnidge, MD, BS, FRACP, FRCPA, FASM

## Abstract

Clinical and Laboratory Standards Institute standard M24—*Susceptibility Testing of Mycobacteria, Nocardia spp., and Other Aerobic Actinomycetes* includes susceptibility testing procedures for *Mycobacterium tuberculosis* complex (MTBC), clinically significant slowly and rapidly growing mycobacterial species, *Nocardia* spp., and other aerobic actinomycetes. Also included in this standard are recommendations for selecting agent for first-line and second-line drug testing, organism group-specific methodologies, reporting recommendations, and external quality control criteria. Recommendations regarding agent selection for testing mycobacteria are based primarily on published guidelines. For testing MTBC, M24 recognizes agar proportion as the reference methodology on which all other methodologies are based. In addition, this standard includes recommendations for using commercial broth susceptibility methods with shorter incubation times, which are now in widespread use for MTBC susceptibility testing, and information on molecular methods for detecting drug resistance and their integration with culture-based methods.

Clinical and Laboratory Standards Institute (CLSI). *Susceptibility Testing of Mycobacteria, Nocardia spp., and Other Aerobic Actinomycetes*. 3rd ed. CLSI standard M24 (ISBN 978-1-68440-025-6 [Print]; 978-1-68440-026-3 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2018.

The Clinical and Laboratory Standards Institute consensus process, which is the mechanism for moving a document through two or more levels of review by the health care community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI documents. Current editions are listed in the CLSI catalog and posted on our website at [www.clsi.org](http://www.clsi.org). If you or your organization is not a member and would like to become one, or to request a copy of the catalog, contact us at: Telephone: +1.610.688.0100; Fax: +1.610.688.0700; E-Mail: [custserv@clsi.org](mailto:custserv@clsi.org); Website: [www.clsi.org](http://www.clsi.org).

Copyright ©2018 Clinical and Laboratory Standards Institute. Except as stated below, any reproduction of content from a CLSI copyrighted standard, guideline, derivative product, or other material requires express written consent from CLSI. All rights reserved. Interested parties may send permission requests to [permissions@clsi.org](mailto:permissions@clsi.org).

CLSI hereby grants permission to each individual member or purchaser to make a single reproduction of this publication for use in its laboratory procedures manual at a single site. To request permission to use this publication in any other manner, e-mail [permissions@clsi.org](mailto:permissions@clsi.org).

### **Suggested Citation**

CLSI. *Susceptibility Testing of Mycobacteria, Nocardia spp., and Other Aerobic Actinomycetes*. 3rd ed. CLSI standard M24. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

### **Previous Editions:**

July 1990, December 1995, December 2000, April 2003, March 2011

ISBN 978-1-68440-025-6 (Print)  
ISBN 978-1-68440-026-3 (Electronic)  
ISSN 1558-6502 (Print)  
ISSN 2162-2914 (Electronic)

Volume 38, Number 21

## Committee Membership

### Consensus Council

**Dennis J. Ernst, MT(ASCP),  
NCPT(NCCT)  
Chairholder  
Center for Phlebotomy Education  
USA**

**Mary Lou Gantzer, PhD, FACB  
Vice-Chairholder  
USA**

J. Rex Astles, PhD, FACB, DABCC  
Centers for Disease Control and  
Prevention  
USA

Lucia M. Berte, MA, MT(ASCP)SBB,  
DLM, CQA(ASQ)CMQ/OE  
Laboratories Made Better!  
USA

Karen W. Dyer, MT(ASCP), DLM  
Centers for Medicare & Medicaid Services  
USA

Thomas R. Fritsche, MD, PhD, FCAP,  
FIDSA  
Marshfield Clinic  
USA

Loralie J. Langman, PhD, DABCC, FACB,  
F-ABFT  
Mayo Clinic  
USA

James R. Petisce, PhD  
BD Diagnostic Systems  
USA

Andrew Quintenz  
Bio-Rad Laboratories, Inc.  
USA

Robert Rej, PhD  
New York State Department of  
Health – Wadsworth Center  
USA

Zivana Tezak, PhD  
FDA Center for Devices and  
Radiological Health  
USA

### Document Development Committee on Antimycobacterial Susceptibility Testing

**Gail L. Woods, MD  
Chairholder  
Arkansas Children's Hospital  
USA**

**Nancy L. Wengenack, PhD, D(ABMM)  
Vice-Chairholder  
Mayo Clinic  
USA**

**Grace Lin, MS  
Committee Secretary  
California Department of Public  
Health  
USA**

Lynette Y. Berkeley, PhD, MT(ASCP)  
FDA Center for Drug Evaluation and  
Research  
USA

Barbara A. Brown-Elliott, MS,  
MT(ASCP)SM  
University of Texas Health Science  
Center at Tyler  
USA

Daniela Maria Cirillo, MD, PhD  
WHO Collaborating Centre and  
TB Supranational Reference Laboratory  
Italy

Patricia J. Conville, MS, MT(ASCP)  
FDA Center for Devices and Radiological  
Health  
USA

Edward P. Desmond, PhD, D(ABMM)  
California Department of Public Health  
USA

Scott B. Killian, BS  
Thermo Fisher Scientific  
USA

Beverly Metchock, DrPH,  
D(ABMM)  
Centers for Disease Control and  
Prevention  
USA

Nicole M. Parrish, PhD, MHS,  
D(ABMM)  
Johns Hopkins Hospital - Pathology  
USA

Richard Pfeltz, PhD  
BD Life Sciences  
USA

Elvira Richter, PhD  
Laboratory Limbach  
Germany

John D. Turnidge, MD, BS,  
FRACP, FRCPA, FASM  
University of Adelaide  
Australia

### Staff

Clinical and Laboratory Standards  
Institute  
USA

Marcy L. Hackenbrack, MCM,  
M(ASCP)  
*Project Manager*

Megan L. Tertel, MA, ELS  
*Editorial Manager*

Catherine E.M. Jenkins  
*Editor*

Kristy L. Leirer, MS  
*Editor*

Laura Martin  
*Editor*

## Acknowledgment for the Expert Panel on Microbiology

CLSI, the Consensus Council, and the Document Development Committee on Antimycobacterial Susceptibility Testing gratefully acknowledge the Expert Panel on Microbiology for serving as technical advisors and subject matter experts during the development of this standard.

### Expert Panel on Microbiology

**Richard B. Thomson, Jr., PhD,  
D(ABMM), FAAM**  
**Chairholder**  
Evanston Hospital, NorthShore  
University HealthSystem  
USA

**Jean B. Patel, PhD, D(ABMM)**  
**Vice-Chairholder**  
Centers for Disease Control and  
Prevention  
USA

Kevin Alby, PhD, D(ABMM)  
University of Pennsylvania Health  
System  
USA

Lynette Y. Berkeley, PhD, MT(ASCP)  
FDA Center for Drug Evaluation and  
Research  
USA

Carey-Ann Burnham, PhD, D(ABMM)  
Washington University School of Medicine  
USA

Karissa Culbreath, PhD, D(ABMM)  
University of New Mexico Department of  
Pathology  
USA

German Esparza, BSc  
Proasecal SAS  
Colombia

Margie Morgan, PhD, D(ABMM)  
Cedars-Sinai Medical Center  
USA

Mark G. Papich, DVM, MS  
College of Veterinary Medicine,  
North Carolina State University  
USA

David H. Pincus, MS,  
RM/SM(NRCM), SM(ASCP)  
bioMérieux, Inc.  
USA

Audrey N. Schuetz, MD, MPH,  
D(ABMM)  
Mayo Clinic  
USA

Ribhi M. Shetty, PhD, D(ABMM)  
FDA Center for Devices and  
Radiological Health  
USA

### Acknowledgment

CLSI, the Consensus Council, and the Document Development Committee on Antimycobacterial Susceptibility Testing gratefully acknowledge the following volunteers for their important contributions to the development of this standard:

Joan Miquel Balada-Llasat, PharmD,  
PhD, D(ABMM)  
Ohio State University Wexler Medical  
Center  
USA

Pennan Barakat, M.D., MPH  
California Department of Public Health  
USA

Janice Washington, MT(ASCP),  
MPH  
FDA Center for Devices and  
Radiological Health  
USA

Gene Raniga, PhD  
Janssen Research and Development  
USA

## Contents

Abstract.....	i
Committee Membership.....	iii
Foreword.....	vii
Chapter 1: Introduction.....	1
1.1 Scope.....	1
1.2 Background.....	2
1.3 Standard Precautions.....	3
1.4 Terminology.....	3
Chapter 2: Antimicrobial Susceptibility Testing Process.....	9
Chapter 3: Antimicrobial Susceptibility Testing of <i>M. tuberculosis</i> complex.....	11
3.1 Agar Proportion Method.....	14
3.2 Commercial Broth Systems With Shorter Incubation Times.....	21
3.3 Broth Microdilution Method for Determining <i>M. tuberculosis</i> complex Minimal Inhibitory Concentrations.....	29
3.4 Molecular Detection of Drug Resistance.....	30
Chapter 4: Nontuberculous Mycobacteria.....	37
4.1 Antimycobacterial Susceptibility Testing of Slowly Growing Nontuberculous Mycobacteria.....	38
4.2 Antimycobacterial Susceptibility Testing of Rapidly Growing Mycobacteria.....	40
4.3 Broth Microdilution Testing Procedure.....	40
Chapter 5: <i>Nocardia</i> spp. and Other Aerobic Actinomyces.....	45
5.1 Preparing the Inoculum.....	47
5.2 Inoculating and Incubating the Panels.....	48
5.3 Confirming Sulfonamide Results and Verifying Appropriate Organism Concentration for <i>Nocardia</i> spp.....	49
5.4 Reading and Interpreting Broth Microdilution Panels.....	49
5.5 Reading and Interpreting Disk Diffusion Test for <i>Nocardia</i> spp.....	51
5.6 Reporting Results.....	52
Chapter 6: Process Management: Quality Control and Quality Assurance.....	53
6.1 Quality Control.....	53
6.2 New Method Implementation.....	60
Chapter 7: Conclusion.....	62
Chapter 8: Supplemental Information.....	62
References.....	63
Additional Resources.....	70
Appendix A. Role of Pharmacokinetics-Pharmacodynamics in Tuberculosis Management.....	71
Appendix B. Antituberculous Drugs and Their Recommended Concentrations in Middlebrook 7H10 and 7H11 Agar Media and LJ Medium.....	78

**Contents (Continued)**

Appendix C. Drugs Available for <i>Mycobacterium tuberculosis</i> complex Susceptibility Testing Using Regulatory Organization–Cleared or –Approved Commercial Short-Incubation Liquid Media Systems and Their Equivalence in the Agar Proportion Method .....	80
Appendix D. Suggested Approach to <i>Mycobacterium tuberculosis</i> complex Susceptibility Testing in Resource-Limited Countries .....	81
Appendix E. Example Illustrating Drug Calculation for Meropenem Trihydrate .....	82
Appendix F. Stock, Working, and Final Concentrations of Antituberculous Drug Solutions for Agar Proportion.....	83
Appendix G. Preparing and Plating Middlebrook 7H10 and 7H11 Agar Media .....	85
Appendix H. Preparing Middlebrook 7H10 and 7H11 Agar Media With Antituberculous Agent–Containing Disks .....	86
Appendix I. Preparing Middlebrook 7H10 and 7H11 Agar Media With Liquid Drug .....	87
Appendix J. 0.5 McFarland Barium Sulfate Turbidity Standard .....	88
Appendix K. Determining Percentage of Resistance.....	89
Appendix L. Procedure for Verifying the Inoculum Density for Broth Microdilution Susceptibility Testing of Mycobacteria .....	91
Appendix M. Agar Disk Elution Method for <i>Mycobacterium haemophilum</i> .....	92
Appendix N. Broth Microdilution Inoculation Procedure for Testing Nontuberculous Mycobacteria, <i>Nocardia</i> spp., and Other Aerobic Actinomyces Using Frozen Panels .....	94
Appendix O. Clarithromycin Susceptibility or Resistance Reporting in Select Rapidly Growing Mycobacteria Based on <i>erm</i> Gene Type .....	95
The Quality Management System Approach .....	96
Related CLSI Reference Materials .....	97

## Foreword

This standard includes recommendations for testing *Mycobacterium tuberculosis* complex (MTBC), certain nontuberculous mycobacteria (NTM), *Nocardia* spp., and other aerobic actinomycetes. Currently, sufficient data exist to support recommendations for antimicrobial susceptibility testing (AST) of MTBC, *Mycobacterium avium* complex (MAC), *M. kansasii*, *M. marinum*, the rapidly growing mycobacteria (RGM), *Nocardia* spp., and certain other aerobic actinomycetes. Breakpoints for some NTMs, *Nocardia* spp., and other aerobic actinomycetes are based on organism population distributions, clinical data, breakpoints used for other organisms, and the experience of experts in the field. M24 was revised in response to new developments in mycobacterial susceptibility testing and comments from laboratorians who perform routine mycobacterial and/or aerobic actinomycete testing. Additional revisions are anticipated as more relevant data become available.

## Overview of Changes

This standard replaces the previous edition of the approved standard, M24-A2, published in 2011. Several changes were made in this edition, including:

- Removed information related to the short-incubation, liquid-radiometric testing system, because this system is no longer available
- Expanded the description of molecular testing for both MTBC and NTM to determine antimicrobial susceptibility or resistance
  - For MTBC, Table 3 (Considerations for Molecular or Repeat Testing After Initial Testing on MTBC Using a Commercial Short-Incubation Broth System) and text are included to describe the integration of molecular and culture-based test results for the best possible prediction of the expected drug efficacy.
  - For NTM, text is included to describe integration of molecular techniques to assist in determining efficacy of macrolides and amikacin in the treatment of infections caused by MAC and various RGM.
- Added a description of recently discovered challenges to MTBC AST accuracy with use of rapid broth systems and/or the agar proportion method, particularly limited sensitivity in detection of low-level resistance to rifampin and ethambutol
- Added information in Appendix A regarding the relationship of pharmacokinetics and pharmacodynamics to determining breakpoints and interpretive criteria
- Updated all breakpoint and quality control tables and moved them to a newly created informational supplement, CLSI document M62<sup>1</sup>

**NOTE:** The content of this standard is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

## Key words

Aerobic actinomycetes, antimicrobial susceptibility testing, antimycobacterial drugs, antituberculous drugs, *Mycobacterium tuberculosis* complex, *Nocardia* spp., nontuberculous mycobacteria

Currently in preview, click buy full version

# Susceptibility Testing of Mycobacteria, *Nocardia* spp., and Other Aerobic Actinomycetes

## Chapter 1: Introduction

This chapter includes:

- Standard's scope and applicable exclusions
- Background information pertinent to the standard's content
- Standard precautions information
- "Note on Terminology" that highlights particular use and/or variation in use of terms and/or definitions
- Terms and definitions used in the standard
- Abbreviations and acronyms used in the standard

### 1.1 Scope

M24 includes antimicrobial susceptibility testing (AST) protocols for three major categories of mycobacterial species:

- *Mycobacterium tuberculosis* complex (MTBC)
- The slowly growing nontuberculous mycobacteria (SGM)
- The rapidly growing mycobacteria (RGM)

Also provided are:

- AST recommendations for *Nocardia* spp. and other aerobic actinomycetes
- Guidance on selecting first-line and, for some organisms, second-line antimicrobial agents for testing and reporting
- Instructions for performing the standard agar proportion (AP) method for MTBC and broth microdilution for mycobacteria and aerobic actinomycetes
- Molecular methods for detecting mutations associated with MTBC drug resistance
- QC protocols for each organism category

Testing and reporting recommendations and QC procedures apply to both reference methods and commercial shorter-incubation broth systems that have been regulatory organization cleared or approved for testing MTBC. This standard does not cover identification methods, nor does it provide an in-depth discussion of molecular test procedures. This standard is intended for use by hospital, public health, and referral laboratories that perform AST on MTBC, nontuberculous mycobacteria (NTM), *Nocardia* spp., and/or other aerobic actinomycetes.