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2nd Edition

M48

Laboratory Detection and Identification of Mycobacteria

This guideline provides recommendations for medical mycobacteriology laboratories on the optimal approach for diagnosis of mycobacterial infections.

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

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Laboratory Detection and Identification of Mycobacteria

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Abstract

Clinical and Laboratory Standards Institute guideline M48—*Laboratory Detection and Identification of Mycobacteria* covers topics related to laboratory diagnosis of mycobacterial infections, including safety and risk assessment, referrals, clinical significance, acceptable specimen types, specimen collection, transport, and storage, specimen processing methods, microscopy for direct detection, molecular methods for directly detecting mycobacteria in patient specimens, culture methods, contamination issues, reporting, quality control, and conventional identification methods as they relate to mass spectrometry and genotypic procedures. Recommendations for managing the unique challenges associated with the increasing incidence of *Mycobacterium tuberculosis* and nontuberculous mycobacteria infections are included.

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Contents

Foreword

Tuberculosis (TB) is a serious global disease, with approximately 9.6 million cases worldwide. In 2014, it was estimated that 1.5 million deaths from TB occur each year.[1] During the past decade, much progress has been made in controlling spread through implementation of TB control programs along with directly observed, short-course treatment.

Since M48 was last published in 2008, an increase in nontuberculous mycobacteria (NTM) infections has added to the significant worldwide TB problem. In 1975, the genus *Mycobacterium* encompassed approximately 30 known species. As of 2018, it includes more than 170.[2] Newer laboratory methods for mycobacteria identification have uncovered many new species not previously identified by traditional phenotypic and biochemical methods, and the abundance of species poses an additional challenge to the mycobacteriology laboratory for providing timely diagnoses.

The medical microbiology laboratory plays an important role in patient care and public health. To reduce TB transmission, laboratory TB diagnosis must be optimized and accelerated for better patient management and implementation of appropriate infection control and public health measures. The document development committee recognizes the increasing complexity of these laboratory methods, as well as other significant demands on the laboratory (eg, turnaround time for reporting). Therefore, this edition of M48 focuses particular attention on providing a consensus guideline for medical laboratories to achieve the optimal approach for diagnosing mycobacterial infections, regardless of available resources.

Essential safety aspects are discussed in this guideline, with an emphasis on specific practices for the mycobacteriology laboratory. Because many laboratories do not have the appropriate technologies and resources for optimal diagnosis of mycobacterial infections, referral services are recommended. A table describing appropriate collection, transport, and storage conditions for various specimen types is included, because these aspects are important for successfully isolating mycobacteria from patient specimens. Optimal methods for specimen processing, direct detection, and mycobacterial culture are also provided. Important laboratory issues and concerns, such as contamination, QC, and quality assurance, are discussed. Finally, current methods for mycobacterial identification are provided. Although this guideline's primary focus is the diagnosis of active *Mycobacterium tuberculosis* infections, the NTM are also discussed in terms of clinical significance and optimal laboratory methods for detection, culture, and identification. The relative clinical importance of any given NTM and the considerations regarding the isolate's clinical significance are discussed.

Overview of Changes

This guideline replaces the previous edition of the approved guideline, M48-A, published in 2008. Several changes were made in this edition, including:

- Reorganized to fit the CLSI quality management system and path of workflow format
- Removed information on service levels for mycobacteriology laboratory services
- Revised the safety and risk assessment chapter
- Expanded the review of the clinically significant NTM isolates from various patient specimens to reflect the significant increase in the number of different species and emerging roles in various clinical settings
- Expanded the discussion and review of the role of nucleic acid amplification tests for detecting *Mycobacterium tuberculosis* complex directly in patient specimens

- Described the method for determining contamination rates for mycobacterial culture

- Removed information on conventional biochemical tests and high-performance liquid chromatography, because these methods are no longer recommended for mycobacterial identification
  - Other conventional phenotypic tests such as growth rate on subculture to solid mycobacterial media, pigment production, and colony morphology were retained, because these tests remain essential for accurate identification of some mycobacterial species.

- Added guidance for using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for mycobacterial identification

- Expanded the discussion of molecular sequencing methods for mycobacterial identification

- Developed an algorithm for mycobacterial identification using different methods

- Added a subchapter discussing the collection of performance parameters for monitoring internal QC indicators

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

Key Words

Acid-fast bacilli, mycobacteria, *Mycobacterium tuberculosis,* nontuberculous (or non–*M. tuberculosis*) mycobacteria, tuberculosis

Laboratory Detection and Identification of Mycobacteria

Chapter 1: Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Background information pertinent to the guideline'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 guideline
- Abbreviations and acronyms used in the guideline

1.1 Scope

This guideline provides recommendations for laboratories on the total testing process for patients with suspected mycobacterial infections. Recommendations are provided for patient specimen collection, preservation, and transport. Procedures for detecting mycobacteria directly in specimens using microscopy and amplification techniques, optimal recovery of mycobacteria from patient specimens, and identification of mycobacterial species by traditional (phenotypic) and alternative (phenotypic and genotypic) laboratory methods are discussed. Mycobacterial susceptibility testing is not included in this guideline and is covered in CLSI document M24.[3]

This guideline is intended for medical and public health laboratories performing procedures for identifying mycobacteria from patient specimens. However, many chapters of this guideline, especially those related to identification methods, are intended for full-service mycobacteriology laboratories in industrialized countries. It is recognized that providing various laboratory services depends on existing local conditions and resources. For many laboratories in tuberculosis (TB)-endemic countries, implementing quality-assured, direct sputum smear microscopy may be a higher priority than the more equipment- and reagent-dependent methods described. Additional information for such laboratories is publicly available from the World Health Organization (WHO) and other scientific and public health organizations. However, these guidelines should provide useful information for international laboratories providing or planning to provide services beyond microscopy, such as solid media culture or rapid methods for *Mycobacterium tuberculosis* complex (MTBC) detection.

1.2 Background

Since M48 was last published in 2008, laboratory diagnosis of TB and nontuberculous mycobacterial (NTM) infections has drastically changed. Numerous laboratory assays have been introduced to rapidly and more accurately diagnose these infections. Strategies optimizing the evaluation and implementation of these new assays have also undergone numerous changes that affect various laboratory settings and are no longer "one size fits all." These new developments, including new assays or laboratory practice strategies, were considered when revising this guideline.