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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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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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

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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.

Combining traditional and alternative methods for mycobacterial isolation and identification offers opportunities to improve the management of patients with mycobacterial disease and to disrupt TB transmission. Despite improvements in sensitivity and time to result, uncertainty remains about the optimal methods and combination of methods that should be used by medical mycobacteriology laboratories.

1.3 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of bloodborne pathogens. Published guidelines are available that discuss the daily operations of diagnostic medicine in humans and animals while encouraging a culture of safety in the laboratory.[4] For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.[5]

1.4 Terminology

1.4.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization whenever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in different countries and regions and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. CLSI recognizes its important role in these efforts, and its consensus process focuses on harmonization of terms to facilitate the global application of standards and guidelines.

It is CLSI practice to spell out the genus name in full each time a new species is introduced. However, because M48 focuses primarily on the genus *Mycobacterium,* all uses of "*M.*" are understood to mean *Mycobacterium* unless otherwise specified. Table 1 is provided to clarify the intended interpretation of other important terms used in this standard.

Table 1. Common Terms or Phrases With Intended Interpretations

Term or Phrase	Intended Interpretation
"Needs to" or "must"	Explains an action directly related to fulfilling a regulatory and/or accreditation requirement or is indicative of a necessary step to ensure patient safety or proper fulfillment of a procedure
"Require"	Represents a statement that directly reflects a regulatory, accreditation, performance, product, or organizational requirement or a requirement or specification identified in an approved documentary standard
"Should"	Describes a recommendation provided in laboratory literature, a statement of good laboratory practice, or a suggestion for how to meet a requirement

Chapter 3:   Clinical Significance of Mycobacteria

This chapter includes:

- Clinical significance of MTBC organisms
- Clinical significance of NTM

Mycobacteria can cause potentially fatal disease in susceptible hosts. The laboratory's role should be to interpret the findings and provide them to the clinician. The clinician is ultimately responsible for determining the mycobacterial isolate's significance and for patient care.

3.1   *Mycobacterium tuberculosis* complex

MTBC organisms do not occur naturally in the environment. Their detection almost always signifies TB and should be reported to clinical providers and public health authorities. Human TB caused by different MTBC organisms is clinically and pathologically indistinguishable. However, identifying MTBC to the species level has implications for public health and for administration of appropriate therapy. From a public health perspective, *Mycobacterium tuberculosis* (MTB) is by far the most clinically significant mycobacterial species, and its detection should be the mycobacteriology laboratory's primary focus, particularly in high-risk areas.

A live-attenuated *M. bovis* vaccine, *M. bovis* bacille Calmette-Guérin (BCG), poses a risk for disseminated disease in HIV-infected and other immunocompromised individuals as well as in patients receiving *M. bovis* BCG as immunomodulatory therapy for bladder carcinoma. Although BCG isolation may signal significant disease when detected in blood or tissue, its recovery may also represent an incidental finding in urine cultures from patients treated with BCG.

M. bovis causes TB in a wide range of animal hosts and is the principal etiological agent responsible for TB in domestic cattle and wild mammals. *M. bovis* infects humans through ingestion of unpasteurized dairy products, through inhalation, and less frequently, through contact with mucous membranes and broken skin. Although the human *M. bovis* disease incidence has been greatly reduced in developed countries since milk pasteurization and widespread testing for infected cattle were introduced, TB caused by *M. bovis* remains an important zoonotic disease as well as a public health problem in certain parts of the world.[28] Both *M. bovis* and BCG are intrinsically resistant to pyrazinamide (as is *M. canettii*).[29,30] Therefore, organism identification has implications for excluding pyrazinamide from treatment regimens.

3.2   Nontuberculous Mycobacteria

Although not commonly communicable from human to human, NTM may cause significant human disease (see Table 2). Therefore, timely detection and identification of clinically relevant NTM is needed.[31,32]

3.2.1   Assessing the Clinical Importance of Nontuberculous Mycobacteria

Any given NTM's clinical importance depends on:

- Each species' pathogenic potential
- Host immune status
- Clinical findings (including imaging and pathology findings)
- Specimen type (eg, sterile vs nonsterile, respiratory vs nonrespiratory)
- Quantity and isolation frequency

4.3 Specimen Processing

Specimens collected from normally sterile body sites (eg, aspirated body fluids, blood, bone marrow, and tissue) do not need a decontamination step and should be inoculated directly onto culture media. Body fluids from normally sterile sites may be concentrated by centrifugation (refrigerated) for 15 minutes at $3000 \times g$ before inoculation onto culture media. Tissues should be ground in sterile nonbacteriostatic saline or 0.2% bovine serum albumin before inoculation onto culture media.[51] However, specimens that may be contaminated with normal flora microorganisms (eg, sputum) need to be digested and decontaminated before inoculation onto culture media. Digestion and decontamination help prevent overgrowth of more rapidly growing microorganisms and break down the mucin that may bind any mycobacteria present and inhibit their recovery. All decontamination reagents are toxic to microorganisms. Therefore, the goal is to inhibit the normal flora but not the hardier mycobacteria, including NTM. The laboratory should monitor the overall specimen contamination rate. The contamination rate should not be reduced to zero, because that would signify that too many mycobacteria are being lost during the decontamination process. Instead, it is expected that, under normal circumstances, 2% to 5% of specimens will be overgrown by normal flora for solid media and 7% to 8% for liquid media containing antimicrobial agents.[52,53] If over time, contamination rates are more than 5% for solid media, the decontamination technique used is likely inadequate. If the rate is less than 2%, it is too harsh.[21,27] Refer to Subchapter 6.2.4 for a comprehensive discussion on contamination rate.

Before increasing the sodium hydroxide (NaOH) concentration during processing, laboratories should first examine and correct problems in clinical and laboratory operations, investigating factors such as:

- Lack of appropriate collection instructions for patients, including when and how to collect the specimen

- Failure to ensure that specimens obtained at different times are not combined

- Failure to refrigerate specimens in a timely manner or lack of refrigeration during hold times and transport

- Similar preexamination processing errors that are major causes of contamination during specimen processing

However, increasing the NaOH concentration might be the only solution in health care systems faced with significant, uncorrectable contamination problems. The appropriate NaOH concentration depends on the observed contamination rate for the individual laboratory. If the contamination rate exceeds 5% to 8% of specimens cultured for MTBC, the NaOH concentration may be increased to 3% or even 4% before being combined with sodium citrate during reagent preparation (see Table 4).

Table 4. NaOH Concentrations

NaOH Amount in 100 mL Water When Reagent Added to Equal Volume	NaOH Concentration	NaOH Concentration When Added to Equal Volume Sodium Citrate	Final Concentration NaOH Specimen
4 g	4%	2%	1%
5 g	5%	2.5%	1.25%
6 g	6%	3%	1.5%
8 g	8%	4%	2%[*]

[*] 2% NaOH (final concentration) can be lethal to mycobacteria, especially in AFB smear–negative patients.
Abbreviation: AFB, acid-fast bacilli.