CLINICAL AND LABORATORY STANDARDS INSTITUTE®

May 2008

M48-A

Laboratory Detection and Identification of Mycobacteria; Approved Guideline

This document provides guidance to clinical mycobacteriology laboratories on the most optimum approach for the diagnosis of mycobacterial infections.

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

Clinical and Laboratory Standards Institute

Setting the standard for quality in clinical 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 clinical 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 advancements 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 addressed according to the consensus process by a committee of experts.

Appeals Process

If it is believed that an objection has not been adequately addressed, the process for appeals is documented in the CLSI Standards Development Policies and Process document.

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

Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: 610.688.0100
F: 610.688.0700
www.clsi.org
standard@clsi.org

M48-A
Vol. 28 No. 17
ISBN 1-56238-669-7 Replaces M48-P
ISSN 0273-3099 Vol. 27 No. 5

Laboratory Detection and Identification of Mycobacteria; Approved Guideline

Volume 28 Number 17

Betty (Betz) A. Forbes, PhD, D(ABMM)
Niaz Banaiee, MD
Kathleen G. Beavis, MD
Barbara A. Brown-Elliott, MS, MT(ASCP) SM
Phyllis Della Latta, PhD, MSc
L. Bruce Elliott, DrPH
Geraldine S. Hall, PhD
Bruce Hanna, PhD
Mark D. Perkins, MD
Salman H. Siddiqi, PhD
Richard J. Wallace, Jr., MD
Nancy G. Warren, PhD

Abstract

The enormous global problem with tuberculosis with roughly one-third of the world's population infected with *Mycobacterium tuberculosis*, coupled with an increasing incidence of infections caused by nontuberculous mycobacteria, present unique challenges for the laboratory diagnosis of mycobacterial infections. Not only must the diagnosis of *M. tuberculosis* be optimized and expedited for good patient management and institution of appropriate control measures to prevent transmission of tuberculosis, but similar demands for accurate identification of the ever-increasing numbers of species of nontuberculous mycobacteria are also pressing for the laboratory. In light of these issues, the Clinical and Laboratory Standards Institute document M48-A—*Laboratory Detection and Identification of Mycobacteria; Approved Guideline* addresses topics related to the laboratory diagnosis of mycobacterial infections including safety and related issues, levels of service and referrals, clinical significance of mycobacteria, acceptable specimen types and their collection, transport and storage, specimen processing methods, methods for the direct detection of mycobacteria in clinical specimens, culture methods including contamination issues, reporting and quality control, and phenotypic and genotypic identification procedures.

Clinical and Laboratory Standards Institute (CLSI). *Laboratory Detection and Identification of Mycobacteria; Approved Guideline*. CLSI document M48-A (ISBN 1-56238-669-7). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2008.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Suggested Citation

CLSI. *Laboratory Detection and Identification of Mycobacteria; Approved Guideline.* CLSI document M48-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

Proposed Guideline
February 2007

Approved Guideline
May 2008

ISBN 1-56238-669-7
ISSN 0273-3099

Contents

Foreword

From a global perspective, the magnitude of the tuberculosis problem is enormous, with estimates that about one-third of the world's population, or roughly 1.7 billion people, is infected with *Mycobacterium tuberculosis*. Coupled to this staggering number are the estimated 2.7 million people who die each year from tuberculosis. During the last decade, much progress has been made with implementation of tuberculosis control programs together with directly observed treatment, short course. Nevertheless, the World Health Organization (WHO) estimates that nearly one billion people will be newly infected in the next 20 years if measures to control the disease are not implemented. In addition to the significant worldwide problem with tuberculosis, the last decade or so has witnessed an increasing incidence of infections caused by the nontuberculous mycobacteria. In 1975, the genus *Mycobacterium* comprised some 30 species; now, 30 years later, it comprises more than 120. This plethora of species poses an additional challenge for the clinical mycobacteriology laboratory to provide timely diagnoses, because newer phenotypic and genotypic laboratory methods for identification of mycobacteria have recognized many new species that are not identified by the traditional phenotypic features found in the Runyon classification scheme.

The clinical microbiology laboratory plays an important role in primary care and public health. Of significance, the laboratory diagnosis of mycobacterial infections—in particular, *M. tuberculosis*—must be optimized and expedited for better patient management and appropriate implementation of infection control and public health measures to control the transmission of tuberculosis. Recognizing that these laboratory methods are increasingly complex, as well as the other significant demands upon the laboratory such as turnaround time for reporting, M48 was developed to provide a consensus guideline for clinical mycobacteriology laboratories such that, depending on their unique set of circumstances, the most optimum approach for the diagnosis of mycobacterial infections can be employed. Essential aspects of safety are addressed in this document, with an emphasis on those practices specific for the mycobacteriology laboratory. Levels of laboratory services are reviewed as well as referral services, recognizing that many laboratories do not possess the appropriate technologies and resources for optimal laboratory diagnosis of mycobacterial infections. Of great importance to successful isolation of mycobacteria from clinical specimens are the appropriate collection, transport, and storage of various specimen types; a table detailing these aspects is included in this document. Optimum methods for specimen processing, direct detection, and culture of mycobacteria are also delineated; important laboratory issues and concerns such as contamination and quality control are also addressed. Finally, both phenotypic and genotypic methods for the identification of mycobacteria are provided. Although this document's primary focus is on the diagnosis of *M. tuberculosis* infections, the nontuberculous mycobacteria are also addressed both in terms of their clinical significance and optimal laboratory methods for direct detection, culture, and identification. Because the relative clinical importance of any given nontuberculous mycobacteria isolated from patient specimens depends both upon the pathogenic potential of the mycobacterial species and the clinical setting in which it is isolated, the issues as well as factors to consider regarding the isolate's clinical significance are discussed.

Key Words

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

Laboratory Detection and Identification of Mycobacteria; Approved Guideline

1 Scope

The combination of traditional and newer alternative methods for the isolation and identification of mycobacteria offers opportunities to significantly impact the management of patients with mycobacterial disease and to disrupt the transmission of tuberculosis. Despite the advantages of improved sensitivity and rapidity of testing, there remain questions regarding the optimal methods and combination of methods that should be employed by clinical mycobacteriology laboratories. As a practical working document, this document is intended to provide guidance to laboratories on the total testing process for patients with suspected mycobacterial infections. Recommendations are offered for the collection, preservation, and transport of clinical specimens. Procedures for the direct detection of mycobacteria by microscopy and amplification techniques, the optimal recovery of mycobacteria from clinical specimens, and the identification of mycobacterial species by traditional (phenotypic) and alternative (phenotypic and genotypic) laboratory methods are addressed. Mycobacterial susceptibility testing is addressed in CLSI/NCCLS document M24.[1]

Many sections of this document, especially those related to identification methods, are tailored to full-service mycobacteriology laboratories in industrialized countries. It is recognized, however, that provision of various laboratory services is contingent upon existing local conditions and resources. For many laboratories in disease-endemic countries, implementing quality-assured direct sputum smear microscopy may be a higher priority than many of the more equipment- and reagent-dependent methods described here. Additional information for such laboratories can be found on the websites of the World Health Organization (WHO) (www.who.int) and the International Union Against Tuberculosis and Lung Disease (www.tbrieder.org). These guidelines, however, should provide useful information for the many international laboratories providing, or planning to provide, services beyond microscopy, such as solid media culture or rapid methods for *M. tuberculosis* complex (MTBC) detection.

2 Safety and Standard Precautions

2.1 Risk Assessment

To determine the type of laboratory practices to employ in the mycobacteriology laboratory, a risk-based assessment should first be performed by the laboratory director in consultation with the infection control staff for the clinical setting, as well as the state tuberculosis laboratory. Factors to be taken into account to minimize the risk for exposure to *M. tuberculosis* include the volume of tests; level of diagnostic tuberculosis services offered; laboratory design; the prevalence of tuberculosis; the rate of multidrug-resistant *M. tuberculosis;* and whether or not aerosol-generating procedures are performed, as well as their respective frequency.[2,3] Although mycobacterial infections can result from direct inoculation of broken skin, inhalation of infectious aerosols poses the greater risk to the laboratorian. Besides evaluating the risks of aerosolization for the services performed, laboratory directors need to provide the necessary training in safe work practices, engineering controls, and personal protective equipment (PPE) to minimize the risk of aerosols and laboratory-acquired infection.

2.2 Biosafety Levels—General

Guidelines to prevent most laboratory-acquired infections were set forth in the United States by the US Department of Health and Human Services, the Centers for Disease Control and Prevention (CDC), and the National Institutes of Health. [4] In these guidelines, selected agents infectious to humans were coupled

with microbiological practices, laboratory facilities, and safety equipment and categorized into four biosafety levels of laboratory operation.

Biosafety level 2 (BSL-2) practices and procedures, primary barriers, safety and containment equipment, and facilities (secondary barriers) are required for aerosol-producing manipulations of clinical specimens for the detection of mycobacteria. Essentially, BSL-2 builds upon BSL-1 criteria. If BSL-2 practices are applied, basic requirements include standard microbiological practices, in addition to training staff in biosafety (eg, safety precautions, exposure prevention, "sharps" precautions) and handling pathogenic agents; policy/procedures whereby only persons meeting entry/training requirements may enter the laboratory; a biohazard sign with appropriate information displayed (ie, name of the agent(s), name/phone number of the supervisor, biosafety level, required PPE, and any procedures for exiting the laboratory); a biosafety manual defining infectious-waste handling and decontamination policies for work surfaces, spills, and contaminated equipment; annual tuberculin skin or gamma interferon testing; and a policy for managing accidental or overt exposures to infectious materials that require immediate reporting to the laboratory director and appropriate follow-up.[4] Primary barriers and safety equipment are needed for BSL-2 level laboratories; these include properly maintained Class I or Class II biological safety cabinets (BSC) for any manipulative procedure that might involve splashes or aerosols of infectious agents, and PPE (eg, disposable protective gloves, gowns, and face protection). Finally, secondary barriers are also required, including benches and sinks (BSL-1), as well as the availability of an autoclave, lockable doors, separation of the mycobacteriology laboratory from general traffic patterns and public areas, availability of an eyewash station, appropriate laboratory furniture and design for easy cleaning and decontamination, and a method for decontaminating all laboratory wastes in the facility.

BSL-3 practices and facilities are requirements for laboratories assessed as being associated with higher risk. These laboratories process specimens for mycobacterial culture, as well as propagate and manipulate cultures of MTBC. If BSL-3 practices are applied, these would include all BSL-2 practices as well as controlled access, decontamination of all waste, and decontamination of laboratory clothing before laundering. In addition, BSL-3 primary barriers and safety equipment require the use of BSCs or other physical containment devices for all manipulations of agents and PPE delineated in the BSL-2 criteria (laboratory coats, gloves, and face protection as needed) and respiratory protection as necessary. Finally, BSL-3 secondary barriers include BSL-2 criteria plus physical separation of the mycobacterial laboratory from access corridors; self-closing, double-door access; and no recirculation of exhausted air and negative airflow into the laboratory.[4]

In addition to the previously discussed CDC document, a number of other resources address biosafety. Many of the general issues are addressed in CLSI document M29.[5] Some additional resources include Canada's Material Safety Data Sheets for Infectious Microorganisms (www.phac-aspc.gc.ca/msda-ftss/index.html), Canada's Laboratory Biosafety Guidelines,[6] the WHO Biosafety Manual (http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/index.html), American Biological Safety Association (www.absa.org/resriskgroup.html), and the European Biosafety Association (http://www.ebsaweb.eu/Links.html). Of note, because many countries and jurisdictions have different biosafety requirements and designations, laboratories must be aware of these and implement such requirements into practice where appropriate.

2.3 Additional Aspects of Biosafety Pertinent to Mycobacteriology

For the most part, laboratory behavior and practices have been matched to the risk level associated with expected hazards. However, recently, microbiologists have begun to rely less on a rigid classification of the organism when designing laboratory practices and mix different levels of containment and safety practices to match the risk posed by the organism.[7] Not all mycobacteriology laboratories have BSL-3 facilities and thus, some laboratories might consider using BSL-2 containment with a higher level of safety practice. However, in light of the three times higher incidence of tuberculosis in laboratory personnel working with MTBC than that of those not working with the agent, the low infective dose of *M.*

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in the most current edition of CLSI/NCCLS document HS01—*A Quality Management System Model for Health Care.* The quality management system approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any health care service's path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The QSEs are:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Customer Service
Personnel	Process Control	Assessments—External & Internal	Facilities & Safety

M48-A addresses the QSEs indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessments—External & Internal	Process Improvement	Customer Service	Facilities & Safety
					X M22 M24 M29 MM03			MM03			X GP17 M29

Adapted from CLSI/NCCLS document HS01—*A Quality Management System Model for Health Care*.

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI/NCCLS document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow, which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

M48-A addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Preexamination				**Examination**			**Postexamination**	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
	X MM03	X MM03	X M24 MM03	X M24 MM03	X M24 MM03	X M24	X M24 MM03	X M24

Adapted from CLSI/NCCLS document HS01—*A Quality Management System Model for Health Care*.

Related CLSI Reference Materials*

GP17-A2 **Laboratory Detection and Identification of Mycobacteria; Approved Guideline (2004).** This document contains general recommendations for implementing a high-quality laboratory safety program, which are provided in a framework that is adaptable within any laboratory.

M22-A3 **Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard—Third Edition (2004).** This standard contains quality assurance procedures for manufacturers and users of prepared, ready-to-use microbiological culture media.

M24-A **Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard (2003).** This standard provides protocols and related quality control parameters and interpretive criteria for the susceptibility testing of mycobacteria, *Nocardia* spp., and other aerobic actinomycetes.

M29-A3 **Laboratory Detection and Identification of Mycobacteria; Approved Guideline (2005).** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

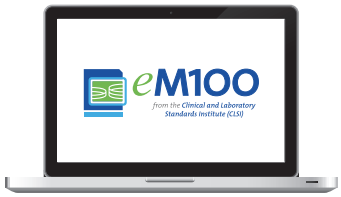
MM03-A2 **Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline—Second Edition (2006).** This guideline addresses topics relating to clinical applications, amplified and nonamplified nucleic acid methods, selection and qualification of nucleic acid sequences, establishment and evaluation of test performance characteristics, inhibitors, and interfering substances, controlling false-positive reactions, reporting and interpretation of results, quality assurance, regulatory issues, and recommendations for manufacturers and clinical laboratories.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Explore the Latest Offerings from CLSI!

As we continue to set the global standard for quality in laboratory testing, we're adding initiatives to bring even more value to our members and customers.



Shop Our Online Products

Including *e*M100, the interactive searchable database for drug selection, interpretation, and quality control procedures within M100-S23.

CLSI U
Laboratory Standards Education Center

Visit the CLSI U Education Center

Where we provide the convenient and cost-effective education resources that laboratories need to put CLSI standards into practice, including webinars, workshops, and more.

Shop Our Online Products

Including eCLIPSE Ultimate Access™, CLSI's cloud-based, online portal that makes it easy to access our standards and guidelines—*anytime, anywhere.*

Find Membership Opportunities

See the options that make it even easier for your organization to take full advantage of CLSI benefits and our unique membership value.

For more information, visit www.clsi.org today.

CLINICAL AND
LABORATORY
STANDARDS
INSTITUTE®

950 West Valley Road, Suite 2500, Wayne, PA 19087 USA

P: 610.688.0100 **Toll Free (US):** 877.447.1888 **F:** 610.688.0700

E: customerservice@clsi.org **www.clsi.org**

ISBN 1-56238-669-7