M48-P
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Laboratory Detection and Identification of Mycobacteria; Proposed Guideline

PLEASE

This proposed document is published for wide and thorough review in the new, accelerated Clinical and Laboratory Standards Institute (CLSI) consensus-review process. The document will undergo concurrent consensus review, Board review, and delegate voting (i.e., candidate for advancement) for 60 days.

Please send your comments on scope, approach, and technical and editorial content to CLSI.

Comment period ends

16 April 2007

The subcommittee responsible for this document will assess all comments received by the end of the comment period. Based on this assessment, a new version of the document will be issued. Readers are encouraged to send their comments to Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; Fax: +610.688.0700, or to the following e-mail address: customerservice@clsi.org

COMMENT

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®

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Advancing Quality in Healthcare Testing

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In addition to developing and promoting the use of voluntary consensus standards and guidelines, we provide an open and unbiased forum to address critical issues affecting the quality of patient testing and health care.

PUBLICATIONS

A document is published as a standard, guideline, or committee report.

Standard A document developed through the consensus process that clearly identifies specific, essential requirements for materials, methods, or practices for use in an unmodified form. A standard may, in addition, contain discretionary elements, which are clearly identified.

Guideline A document developed through the consensus process describing criteria for a general operating practice, procedure, or material for voluntary use. A guideline may be used as written or modified by the user to fit specific needs.

Report A document that has not been subjected to consensus review and is released by the Board of Directors.

CONSENSUS PROCESS

The CLSI voluntary consensus process is a protocol establishing formal criteria for:

- the authorization of a project
- the development and open review of documents
- the revision of documents in response to comments by users
- the acceptance of a document as a consensus standard or guideline.

Most documents are subject to two levels of consensus—"proposed" and "approved." Depending on the need for field evaluation or data collection, documents may also be made available for review at an intermediate consensus level.

Proposed A consensus document undergoes the first stage of review by the healthcare community as a proposed standard or guideline. The document should receive a wide and thorough technical review, including an overall review of its scope, approach, and utility, and a line-by-line review of its technical and editorial content.

Approved An approved standard or guideline has achieved consensus within the healthcare community. It should be reviewed to assess the utility of the final document, to ensure attainment of consensus (i.e., that comments on earlier versions have been satisfactorily addressed), and to identify the need for additional consensus documents.

Our standards and guidelines represent a consensus opinion on good practices and reflect the substantial agreement by materially affected, competent, and interested parties obtained by following CLSI's established consensus procedures. Provisions in CLSI standards and guidelines may be more or less stringent than applicable regulations. Consequently, conformance to this voluntary consensus document does not relieve the user of responsibility for compliance with applicable regulations.

COMMENTS

The comments of users are essential to the consensus process. Anyone may submit a comment, and all comments are addressed, according to the consensus process, by the committee that wrote the document. All comments, including those that result in a change to the document when published at the next consensus level and those that do not result in a change, are responded to by the committee in an appendix to the document. Readers are strongly encouraged to comment in any form and at any time on any document. Address comments to the Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087, USA.

VOLUNTEER PARTICIPATION

Healthcare professionals in all specialties are urged to volunteer for participation in CLSI projects. Please contact us at customerservice@clsi.org or +610.688.0100 for additional information on committee participation.

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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, *Laboratory Detection and Identification of Mycobacteria; Proposed 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.

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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, are infected with *Mycobacterium tuberculosis*. Coupled to this staggering number are the estimated 2.7 million persons 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 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—*Laboratory Detection and Identification of Mycobacteria* 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 be taken under consideration regarding the isolate's clinical significance are discussed.

Key Words

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

Invitation for Participation in the Consensus Process

An important aspect of the development of this and all Clinical and Laboratory Standards Institute documents should be emphasized, and that is the consensus process. Within the context and operation of Clinical and Laboratory Standards Institute, the term “consensus” means more than agreement. In the context of document development, “consensus” is a process by which Clinical and Laboratory Standards Institute, its members, and interested parties (1) have the opportunity to review and to comment on any Clinical and Laboratory Standards Institute publication; and (2) are assured that their comments will be given serious, competent consideration. Any Clinical and Laboratory Standards Institute document will evolve as will technology affecting laboratory or healthcare procedures, methods, and protocols; and therefore, is expected to undergo cycles of evaluation and modification.

The Area Committee on Microbiology has attempted to engage the broadest possible worldwide representation in committee deliberations. Consequently, it is reasonable to expect that issues remain unresolved at the time of publication at the proposed level. The review and comment process is the mechanism for resolving such issues.

The Clinical and Laboratory Standards Institute voluntary consensus process is dependent upon the expertise of worldwide reviewers whose comments add value to the effort. At the end of a 60-day comment period, each subcommittee is obligated to review all comments and to respond in writing to all which are substantive. Where appropriate, modifications will be made to the document, and all comments along with the subcommittee's responses will be included as an appendix to the document when it is published at the next consensus level.

Laboratory Detection and Identification of Mycobacteria; Proposed 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 will be 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 will be addressed. Mycobacterial susceptibility testing is addressed in the CLSI/NCCLS document M24—*Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes*.

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 (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* 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.[1,2] 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 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, and the National Institutes of Health.[3] In these guidelines, selected agents infectious to humans were coupled with

