M24-A2

Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard—Second Edition

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

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Abstract

This document addresses the susceptibility testing of *Mycobacterium tuberculosis* complex (MTBC), clinically significant slowly and rapidly growing mycobacterial species, *Nocardia* spp., and other aerobic actinomycetes. Included in this standard are recommendations for the selection of agents for primary and secondary testing, organism group-specific methodologies, reporting recommendations, and quality control criteria for the above-listed organisms. Recommendations regarding the selection of agents for testing mycobacteria are based primarily on guidelines from US agencies. For testing MTBC, M24 recognizes agar proportion as the primary methodology on which all other methodologies are essentially based. This document also includes recommendations for use of commercial broth susceptibility methods with shorter incubation times, which are now in widespread use in the susceptibility testing of MTBC.


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Foreword

CLSI document M24 was revised based on thoughtful comments from laboratorians involved with regular testing of mycobacteria and/or aerobic actinomycetes, but further refinements are anticipated as more relevant data become available. The document addresses *Mycobacterium tuberculosis* complex (MTBC), certain nontuberculous mycobacteria (NTM), and *Nocardia* and other aerobic actinomycetes. Currently, sufficient data exist to support recommendations for susceptibility testing of MTBC, *Mycobacterium avium* complex (MAC), *Mycobacterium kansasii*, the rapidly growing mycobacteria, *Nocardia* spp., and certain other aerobic actinomycetes. The breakpoints for *Nocardia* 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.

In tuberculosis (TB), there is generally a mixture of intracellular and extracellular bacilli, as well as actively growing and dormant, metabolically inactive ones. For this reason, pharmacokinetic (PK)/pharmacodynamic (PD) studies have limited ability to predict which drugs will be effective in the treatment of tuberculosis (TB). In fact, it is believed that PK studies would suggest that pyrazinamide should be ineffective against MTBC, when in practice it is a key component of TB treatment.\(^1\) Due to these limitations, experts developed a creative approach to MTBC susceptibility testing, ie, comparing minimal inhibitory concentration (MIC) values of isolates from patients who failed treatment with those of strains never exposed to the anti-TB drug. Clinically resistant strains have higher MIC values, and the wild-type strains, for effective anti-TB drugs, have lower MIC values. The critical concentration is the test concentration that best differentiates these two populations. Follow-up studies showed that testing based on this concept predicted treatment success vs failure for a drug.\(^1\) If, in the future, clinically relevant PK/PD data become available, the information will be included in the document, as appropriate.

Laboratory tests for evaluating the susceptibility of mycobacteria and aerobic actinomycetes can confirm the choice of the initial course of chemotherapy and the emergence of drug resistance when a patient fails to show a satisfactory bacteriological response to treatment. Additionally, they can guide the choice of further treatment with different drugs. Susceptibility testing of MTBC can also be used to estimate the prevalence of primary and acquired drug resistance (defined by the World Health Organization\(^2\) as “drug resistance among new cases” and “drug resistance among previously treated patients,” respectively) in a community. For each of these purposes, use of a reliable technique to perform the test is essential.

Currently, first-line therapy for TB includes isoniazid (INH), rifampin (RMP), ethambutol (EMB), and pyrazinamide (PZA). To ensure that clinicians are provided with comprehensive information regarding this multidrug regimen, initial isolates from all patients should be tested for susceptibility to all four agents. This is a change from the previous edition, which stated that laboratories might consider testing INH, RMP, and EMB only, if their pulmonary and/or infectious disease specialists and TB control officer agree with the reduced panel. Susceptibility testing should be repeated if the patient is culture positive after three months of appropriate therapy or earlier if the patient shows clinical evidence of failure to respond to therapy or is unable to tolerate the initial drug regimen. To ensure the earliest possible detection of resistance, a commercial, shorter incubation system should be used in conjunction with rapid methods for primary culture and identification. In this way, first-line susceptibility test results for most MTBC isolates should be reported within 15 to 30 days of receipt of the specimen in the laboratory.

Multidrug-resistant (MDR) TB, although not a major problem in most developed countries, is a serious threat to TB control globally. Recently, the emergence of extensively drug-resistant TB (isolates resistant to the two best first-line agents—INH and RMP—and the best second-line drugs—the fluoroquinolones and either amikacin, kanamycin, or capreomycin), which is associated with extremely poor outcomes and a high mortality rate in patients with concomitant human immunodeficiency virus (HIV) infection, has heightened the concern of global TB control. Consequently, the importance of susceptibility testing to secondary anti-TB drugs has grown. Moreover, drugs other than the traditional secondary agents—most importantly the newer fluoroquinolones—have been evaluated and shown to be effective additions to the
anti-TB regimen. Given the efficacy and ease of administration, it is anticipated that the use of the newer fluoroquinolones to treat TB will increase in the next several years and, as a result, susceptibility testing of these agents will become more important.

Susceptibility testing of NTM and aerobic actinomycetes should be performed on clinically significant isolates\(^3\) (see the paragraph below) that exhibit variability in susceptibility to clinically useful antimicrobial agents and/or significant risk of acquired mutational resistance to one or more of these agents. Because the latter two criteria are not true for *Mycobacterium marinum*, routine susceptibility testing of this species is not indicated.

To determine clinical significance of NTM recovered from respiratory cultures, the American Thoracic Society (ATS) recommends the following criteria: cultures of at least two positive sputum specimens or one bronchial wash or lavage sample are usually sufficient to confirm clinical significance. Alternatively, a transbronchial or lung biopsy with mycobacterial histopathological features and positive culture for NTM is sufficient to establish clinical significance. In addition, isolates from normally sterile sites (such as blood, cerebrospinal fluid, or tissues) typically are considered clinically significant.

To facilitate further development of CLSI document M24, the subcommittee requests comments and suggestions for improvement with regard to the methods included herein.

All authors of this document donated considerable time to its development; I would like to personally thank all of them for their valuable contributions.

Gail L. Woods, MD  
Chairholder, Subcommittee on Antimycobacterial Susceptibility Testing

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**Key Words**

Antimycobacterial drugs, antituberculous drugs, drug susceptibility
Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard—Second Edition

1 Scope

CLSI document M24-A2 contains protocols for the susceptibility testing of three major categories of mycobacterial species (Mycobacterium tuberculosis complex [MTBC]; the slowly growing nontuberculous mycobacteria [NTM]; and the rapidly growing mycobacteria) and recommendations for susceptibility testing of Nocardia spp. and miscellaneous aerobic actinomycetes. This document also provides guidance on the selection of primary and, for some organisms, secondary agents for testing and reporting; instructions for performing the standard agar proportion method for MTBC and broth microdilution for NTM; and quality control (QC) protocols for each organism category. Testing and reporting recommendations and principles of QC procedures apply to use of commercial shorter-incubation broth systems that have been cleared by the US Food and Drug Administration (FDA) for testing MTBC, as well as the reference methods.

2 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 blood-borne pathogens. Standard and universal precaution guidelines are available from the US Centers for Disease Control and Prevention (CDC). 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

The mycobacteriology laboratory presents a unique set of circumstances in terms of observance of biosafety precautions. For more information, it is suggested that the reader refer to Biological Safety: Principles and Practices by Fleming and Hunt.6 Also, the publication Biosafety in Microbiological and Biomedical Laboratories is now in its fifth edition and is available online at http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.7

3 Terminology

3.1 Definitions

accuracy (measurement) – closeness of agreement between a measured quantity value and a true quantity value of a measurand (ISO/IEC Guide 99).8

antimicrobial susceptibility test interpretive category – a classification based on an in vitro response of an organism to an antimicrobial agent; NOTE 1: For mycobacteria, two different categories, “critical concentration” and “minimum inhibitory concentration,” have been used to categorize the in vitro results; NOTE 2: For members of the MTBC, when tested against the lower concentration of some agents, the “critical concentration” category is applied. Testing of an additional higher concentration may also be recommended for some agents. However, there is no “intermediate” interpretive category when the “critical concentration” category is applied, even when testing is performed both at the critical concentration and the additional higher concentration; NOTE 3: For NTM and for the aerobic actinomycetes, only the “minimum inhibitory concentration” category is applied.
borderline antimicrobial susceptibility test interpretive category – an interpretive category applicable only to certain results obtained with MTBC isolates tested against pyrazinamide by the radiometric instrument method (refer to the manufacturer’s package insert); NOTE: Repeat testing may determine whether the isolate in question is susceptible or resistant.

critical concentration – the “critical concentrations” of antituberculous drugs were adopted by international convention⁹; NOTE: For each drug, the critical concentration is the lowest concentration that inhibits 95% of “wild-type” strains of *M. tuberculosis* that have not been exposed to the drug, but that simultaneously does not inhibit strains of *M. tuberculosis* considered resistant that are isolated from patients who are not responding to therapy.

culture – 1) the intentional growing of microorganisms (such as bacteria or viruses) or tissues, in a controlled environment, for purposes of identification or other scientific study, or for commercial and/or medicinal use; 2) the product resulting from the intentional growth of microorganisms or tissue.

culture medium – a substance or preparation used for the cultivation and growth of microorganisms or tissue.

direct susceptibility test – a procedure based on inoculation of drug-containing media directly with a processed (concentrated after digestion and decontamination) specimen that is smear-positive for acid-fast bacilli (AFB) to determine the proportion or percentage of resistant MTBC in the patient’s bacterial population (see Section 4.2.4).

indirect susceptibility test – a procedure based on inoculation of drug-containing media using organisms grown in culture (see Section 4.2.3).

intermediate antimicrobial susceptibility test interpretive category – for minimal inhibitory concentration, an interpretive category that implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physiologically concentrated or when a high dosage of drug can be used; also indicates a “buffer zone” that should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations.

measurand – quantity intended to be measured (ISO/IEC Guide 99).⁸

minimal inhibitory concentration (MIC) – the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in a broth dilution susceptibility test.

*Mycobacterium avium* complex (MAC) – includes the species *M. avium* (subspecies *avium, colombiense, silvaticum, hominissuis, paratuberculosis*) and *Mycobacterium intracellulare*.

*Mycobacterium tuberculosis* complex (MTBC) – includes the species *M. tuberculosis, Mycobacterium bovis, M. bovis BCG, Mycobacterium caprae, Mycobacterium pinnipedii, Mycobacterium africanum, Mycobacterium microti*, and *Mycobacterium canettii*.

nontuberculous mycobacteria (NTM) – all species of mycobacteria other than those in the MTBC.

resistant antimicrobial susceptibility test interpretive category – 1) For critical concentration, resistance is defined as diminished susceptibility of a strain that differs from wild-type strains from patients who have not been treated with the drug, so that the strain is unlikely to show clinical responsivenes to the drug; 2) For minimal inhibitory concentration, resistant isolates are not inhibited by the usually achievable concentrations of the agent at the site of infection with normal dosage schedules, and/or fall in the range where specific microbial resistance mechanisms are likely (eg, β-lactamases), and clinical efficacy has not been reliable in treatment studies.
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 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 quality system essentials (QSEs) are as follows:

- Documents and Records
- Organization
- Personnel
- Equipment
- Purchasing and Inventory
- Process Control
- Information Management
- Occurrence Management
- Process Improvement
- Customer Service
- Facilities and Safety

M24-A2 addresses the quality system essentials (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.

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Adapted from CLSI 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 document GP26—Application of a Quality Management System Model for Laboratory Services defines a clinical laboratory path of workflow that 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.

M24-A2 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.

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Adapted from CLSI document HS01—A Quality Management System Model for Health Care.
Related CLSI Reference Materials*


**I/LA18-A2** Specifications for Immunological Testing for Infectious Diseases; Approved Guideline—Second Edition (2001). This document addresses specimen collection, handling, and storage, as well as performance criteria for the comparison of immunological test kits and specifications for reference materials.

**M02-A10** Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Tenth Edition (2009). This document contains the current Clinical and Laboratory Standards Institute-recommended methods for disk susceptibility testing, criteria for quality control testing, and updated tables for interpretive zone diameters.

**M07-A8** Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition (2009). This document addresses reference methods for the determination of minimal inhibitory concentrations (MICs) of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.


**M29-A3** Protection of Laboratory Workers From Occupationaly Acquired Infections; Approved Guideline—Third Edition (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.

**M100-S21** Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement (2011). This document provides updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02-A10 and M07-A8.

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