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

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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 U.S. agencies. For testing MTBC, M24-A recognizes the method of agar proportion as the primary methodology upon which all other methodologies are essentially based; there are also recommendations for use of commercial broth susceptibility methods with shorter incubation times, which are now in widespread use in the susceptibility testing of this significant group of microorganisms.

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

A standard for global application developed through the NCCLS consensus process.



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Foreword

NCCLS document M24-A addresses *Mycobacterium tuberculosis* complex (MTBC), certain nontuberculous mycobacteria (NTM), and in this version of the document, information regarding *Nocardia* and other aerobic actinomycetes is presented for the first time. The breakpoints for *Nocardia* and other aerobic actinomycetes are based on PK/PD data, organism population distributions, clinical data, breakpoints used for other organisms, and the experience of experts in the field. Currently, sufficient data exist to support susceptibility testing recommendations for MTBC and tentative recommendations for *Mycobacterium avium* complex, *Mycobacterium kansasii*, the rapidly growing mycobacteria (*Mycobacterium fortuitum* group, *Mycobacterium abscessus*, and *Mycobacterium chelonae*), and aerobic actinomycetes.

Laboratory tests for evaluating the susceptibility of mycobacteria and aerobic actinomycetes can confirm the choice of the initial course of chemotherapy, and they can confirm the emergence of drug resistance when a patient fails to show a satisfactory bacteriologic response to treatment, as well as 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 as "drug resistance among new cases" and "drug resistance among previously treated patients"¹) in a community. For each of these purposes, use of a reliable technique to perform the test is essential.

For MTBC, susceptibility testing should be performed on the initial isolate from all patients. Susceptibility testing should be repeated if the patient is culture-positive after three months of appropriate therapy or shows clinical evidence of failure to respond to therapy. To assure 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, susceptibility test results for most isolates should be reported within 15 to 30 days of receipt of the specimen in the laboratory.

In contrast to MTBC, susceptibility testing of NTM and aerobic actinomycetes should be performed on the initial isolate only for clinically significant isolates 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 recommended.

To determine clinical significance of NTM recovered from respiratory cultures, the American Thoracic Society currently recommends the following criteria: three positive cultures with negative smears for acid-fast bacilli (AFB) or two positive cultures and one positive smear are usually sufficient to confirm clinical significance. Alternatively, if only one bronchial wash is available and it is culture-positive or the AFB smear is $\geq 2+$, this 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.

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

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Chairholder, Subcommittee on Antimycobacterial Susceptibility Testing

In an effort to increase the clinical utility of the M24 standard, the Subcommittee on Antimycobacterial Susceptibility Testing opted not only to revise the recommendations for testing of MTBC, but also to present susceptibility testing methodologies for nontuberculous mycobacteria and the aerobic actinomycetes.

Standard Precautions

Because it is often impossible to know what might be infectious, all human specimens are to be treated as infectious and handled according to “standard precautions.” Standard precautions are new guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals*. Infection Control and Hospital Epidemiology. CDC. 1996;Vol 17;1:53-80), (MMWR 1987;36[suppl 2S]2S-18S), and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials and for recommendations for the management of blood-borne exposure, refer to the most current edition of NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections*.

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: *Clinics in Laboratory Medicine, 1996: Biosafety in the Clinical Mycobacteriology Laboratory*² and the 1999 U.S. government publication *Biosafety in Microbiological and Biomedical Laboratories*.³

A Note on Terminology

NCCLS, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. NCCLS recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in NCCLS, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all obstacles to harmonization. In light of this, NCCLS recognizes that harmonization of terms facilitates the global application of standards and is an area of immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

Of particular note in M24-A, are two terms whereby NCCLS intends to eliminate confusion, over time, through its commitment to harmonization. For the most part, in this guideline, the term “accuracy” is used correctly in its metrological sense, to refer to the closeness of the agreement between the result of a (single) measurement and a true value of a measurand, thus comprising both random and systematic effects. But there are several instances in this document, where accuracy is defined the way ISO defines “trueness,” i.e., the closeness of the agreement between the average value from a large series of measurements and to a true value of a measurand. To facilitate understanding, when used this way, “trueness” has been inserted parenthetically. Also, the terms are defined in the guideline’s Definitions section along with explanatory notes. During the next scheduled revision of this document, they will be reviewed for consistency with international use, and revised appropriately.

Key Words

Antimycobacterial drugs, antituberculous drugs, drug susceptibility

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1 Scope

This document contains protocols for the susceptibility testing of three major categories of mycobacterial species: 1) *Mycobacterium tuberculosis* complex (MTBC), 2) the slowly growing, nontuberculous mycobacteria (NTM), and 3) the rapidly growing mycobacteria. Additionally, recommendations for susceptibility testing of *Nocardia* spp. and miscellaneous aerobic actinomycetes are included in this document. M24-A contains guidance on the selection of primary and secondary agents for testing and reporting; instructions for performing the standard agar proportion method and other reference methods (e.g., broth micro- and macrodilution for *Mycobacterium avium* complex and broth microdilution for rapidly growing mycobacteria); and quality control protocols for each organism category. Testing and reporting recommendations and principles of quality control procedures apply to using commercial FDA-cleared systems as well as the reference methods. It is anticipated that further refinement of these protocols will occur as laboratories involved in the regular testing of these organism types gain experience with the use of this document. To facilitate further development of M24, the subcommittee requests comments and suggestions for improvement with regard to the methods included herein.

2 Definitions

Accuracy - Closeness of the agreement between the result of a measurement and a true value of the measurand (VIM93-3.5).⁴ **NOTE:** There are several occasions in this document where accuracy is defined the way ISO defines **Trueness**, defined below. When used in this way, the term “trueness” has been inserted parenthetically.

Antimicrobial Susceptibility Test Interpretive Category – A classification based on an *in vitro* response of an organism to an antimicrobial agent; **NOTES:** a) For mycobacteria two different categories, “critical concentration” and “minimum inhibitory concentration” have been used to categorize the *in vitro* results; b) 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; c) 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; **NOTE:** Repeat testing may resolve whether the isolate in question is susceptible or resistant.

Critical concentration – The “critical concentrations” of antituberculous drugs were adopted by international convention;⁵ **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, 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.