CLINICAL AND LABORATORY STANDARDS INSTITUTE®

2nd Edition

M24S

Performance Standards for Susceptibility Testing of Mycobacteria, *Nocardia* spp., and Other Aerobic Actinomycetes

This document includes updated breakpoint and quality control tables for the Clinical and Laboratory Standards Institute susceptibility testing standard M24.

A CLSI supplement for global application.

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Performance Standards for Susceptibility Testing of Mycobacteria, *Nocardia* spp., and Other Aerobic Actinomycetes

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Abstract

Clinical and Laboratory Standards Institute document M24S—*Performance Standards for Susceptibility Testing of Mycobacteria,* Nocardia *spp., and Other Aerobic Actinomycetes* includes the minimal inhibitory concentrations and QC ranges developed following the standards described in CLSI document M24.[1] The data in the tables are valid only when the methodology in CLSI document M24[1] is followed.

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Overview of Changes

This document replaces the previous edition of the approved document, M62-Ed1, published in 2018. **This supplement contains the most current information and serves as a means to notify laboratories of important changes that occur between published editions of CLSI document M24.**[1] Several changes were made in this edition, including:

Section/Table	Action	Change to:	Reason/Specific Change
Key Words	Added	Key words	*Mycobacterium avium* complex
General	Revised	Footnotes	Footnotes were reorganized as general and specific comments.
Table 1. Broth Microdilution Breakpoints and Interpretive Categories for MTBC Tested in Middlebrook 7H9 Broth Supplemented With OADC Using MIC Panels	Added	Comments and associated references	• Regarding important changes between this document and CLSI document M24[1] • Regarding establishment of breakpoints using lyophilized MIC panels, their incubation requirements, and importance of following manufacturer's media formulations • Regarding testing for rifampin susceptibility using automated broth-based systems
	Revised	Comment (5)	NOTE regarding repeat testing clarified
		MIC breakpoints for rifampin	Updated WHO standard concentration
	Deleted	Title	The term "commercially available"
Table 2. Susceptibility Testing of MTBC to Second-Line Drugs Using the Fluorescence-based Commercial Shorter-Incubation Liquid Media System	Revised	Comment	Clarified amikacin and kanamycin guidance
Table 3. Antimicrobial Agents and Breakpoints for Testing MTBC	Added	Alternate routine QC strains and comment	For clarithromycin, linezolid, and moxifloxacin

Overview of Changes (Continued)

Section/Table	Action	Change to:	Reason/Specific Change
Table 4. Antimicrobial Agents and Breakpoints for Testing *Mycobacterium kansasii*	Added	Alternate routine QC strains and comment	For clarithromycin, linezolid, and moxifloxacin
Table 5. Antimicrobial Agents and Breakpoints for Testing Slowly Growing Nontuberculous Mycobacteria Other Than MAC and *M. kansasii*	Added	Alternate routine QC strains and comment	For clarithromycin, linezolid, and moxifloxacin
Table 6. Antimicrobial Agents and Breakpoints for Testing Rapidly Growing Mycobacteria	Revised	Comment	• Included newly described species: – *Mycobacterium conceptionense* – *Mycobacterium farcinogenes* – *Mycobacterium syngnathidarum*
Table 8. Expected Antimicrobial Susceptibility Patterns of the Most Commonly Isolated *Nocardia* spp.	Added	Comments	• General comment regarding *Nocardia* susceptibility patterns • Interpretive comment for *Nocardia brasiliensis*
		References	Regarding susceptibility testing and profiling of *Nocardia* spp.
	Revised	Expected patterns	• Various agents with: – *N. brasiliensis* – *Nocardia cyriacigeorgica* – *Nocardia farcinica* – *Nocardia nova* complex – *Nocardia otitidiscaviarum* – *Nocardia pseudobrasiliensis*
Table 10. MIC QC Ranges When Testing MTBC Using *Mycobacterium tuberculosis* ATCC®[a] 27294 (H37Rv) Tested in Middlebrook 7H9 Medium Supplemented With OADC	Added	Comment	Regarding the establishment of breakpoints using lyophilized or frozen MIC panels, incubation requirements, and importance of following manufacturer's media formulations
	Deleted	General	The term "commercially available"

Overview of Changes (Continued)

Section/Table	Action	Change to:	Reason/Specific Change
Table 12. Broth Microdilution QC Ranges When Testing Slowly Growing Nontuberculous Mycobacteria in Cation-Adjusted Mueller-Hinton Broth With OADC	Added	Title	Text specifying broth used
		MIC QC ranges, comments, and associated reference	Fobrepodacin tested with routine and supplemental QC strains
		Comment	Regarding alternate QC strains for clarithromycin, linezolid, and moxifloxacin
		Comment	Regarding morphological variations observed with *M. avium* ATCC® 700898
		QC strain	*M. avium* ATCC® 700898 as an alternate QC organism for MAC testing for amikacin, clarithromycin, linezolid, and moxifloxacin
	Revised	MIC QC ranges	• *Staphylococcus aureus* ATCC® 29213 – Moxifloxacin – Rifampin
Table 13. Broth Microdilution QC Ranges When Testing Rapidly Growing Mycobacteria in Cation-Adjusted Mueller-Hinton Broth Without OADC	Added	Title	Text specifying broth used
		MIC QC ranges, comments, and associated reference	Fobrepodacin tested with routine and supplemental QC strains
		MIC QC ranges	Omadacycline tested with routine and supplemental QC strains
	Revised	MIC QC ranges	• *Pseudomonas aeruginosa* ATCC® 27853 – Ciprofloxacin – Meropenem • *S. aureus* ATCC® 29213 – Imipenem
Table 14. Broth Microdilution QC Ranges When Testing *Nocardia* spp. and Other Aerobic Actinomycetes in Cation-Adjusted Mueller-Hinton Broth Without OADC	Added	Title	Text specifying broth used
	Revised	MIC QC range	*S. aureus* ATCC® 29213 and imipenem

Abbreviations. ATCC®, American Type Culture Collection; MAC, *Mycobacterium avium* complex; MIC, minimal inhibitory concentration; MTBC, *Mycobacterium tuberculosis* complex; OADC, oleic acid-albumin-dextrose-catalase; QC, quality control; WHO, World Health Organization.

Overview of Changes (Continued)

Footnote

a. ATCC® is a trademark of the American Type Culture Collection.

NOTE: The content of this document is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

Key Words

aerobic actinomycetes, antimicrobial agents, antimicrobial susceptibility testing, antituberculous drugs, ***Mycobacterium avium*** **complex,** *Mycobacterium tuberculosis* complex, *Nocardia* spp., nontuberculous mycobacteria

Abbreviations and Acronyms

ATCC®[a]	American Type Culture Collection
CO_2	carbon dioxide
HPLC	high-performance liquid chromatography
I	intermediate
IV	intravenous
MAC	*Mycobacterium avium* complex
MIC	minimal inhibitory concentration
MTBC	*Mycobacterium tuberculosis* complex
OADC	oleic acid-albumin-dextrose-catalase
QC	quality control
QMS	quality management system
QSE	quality system essential
R	resistant
S	susceptible
V	variable
WHO	World Health Organization

[a] ATCC® is a registered trademark of the American Type Culture Collection.

Reference

[1] CLSI. *Susceptibility Testing of Mycobacteria*, Nocardia *spp., and Other Aerobic Actinomycetes*. 3rd ed. CLSI standard M24. Clinical and Laboratory Standards Institute; 2018.

Table 1. Broth Microdilution Breakpoints and Interpretive Categories for MTBC Tested in Middlebrook 7H9 Broth Supplemented With OADC Using MIC Panels[1-3]

QC Recommendation (see Table 10 for acceptable QC ranges)

Routine QC strain:

- *Mycobacterium tuberculosis* ATCC® 27294 (H37Rv)

General Comments

(1) ATCC® is a registered trademark of the American Type Culture Collection.

(2) This table contains updated information and notifies laboratories of important changes among published versions of CLSI document M24.[4]

(3) The breakpoints in Table 1 were established using lyophilized MIC panels incubated in 5% to 10% CO_2. Incubating tests in ambient air may result in erroneous MICs because MTBC grow more slowly in ambient air. If preparing in-house MIC panels, care should be taken to match the manufacturer's formulation because the use of polysorbate or glycerol in the broth may affect the MIC values obtained.

(4) This antimicrobial susceptibility testing system is not regulatory organization cleared.

NOTE: Information in black boldface type is new or modified since the previous edition.

Antimicrobial Agent	MIC Breakpoints, µg/mL			Comments
	S	Inconclusive[3]	R	
Ethambutol	≤ 2	4	≥ 8	(5) Inconclusive MIC for ethambutol. An MIC of 4 µg/mL obtained by broth microdilution using **lyophilized panels** does not correlate with either a susceptible or resistant result in commercial automated, short-incubation broth systems, and there are no clinical data correlating such a result with ethambutol treatment response. **NOTE:** Repeat testing **using an alternative broth method (eg, critical concentration) or genotypic method** may determine whether the isolate in question is susceptible or resistant.