

M62

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.

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

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Abstract

Clinical and Laboratory Standards Institute document M62—*Performance Standards for Susceptibility Testing of Mycobacteria, Nocardia spp., and Other Aerobic Actinomycetes* includes the minimal inhibitory concentrations and quality control 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 M24¹ is followed.

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Overview of Changes

This document incorporates and updates the tabular information from CLSI document M24-A2, published in 2011. Several changes were made in this edition, including:

- **General:**
 - Removed all QC and interpretive information (eg, critical concentrations, breakpoints, interpretive categories) from CLSI document M24¹ and used the information to create M62, this supplement
 - Removed former Table 6 in M24-A2, Antimycobacterial Agents and Minimal Inhibitory Concentration (MIC) Values Indicating Resistance for Susceptibility Testing of *M. marinum* (Routine Testing Is Not Recommended)
- **Table 1. Broth Microdilution Breakpoints and Interpretive Categories for MTBC Tested in Middlebrook 7H9 Broth Supplemented With OADC Using Commercially Available MIC Plates:**
 - Added new table
- **Table 2. Susceptibility Testing of MTBC to Second-Line Drugs Using the Fluorescence-based Commercial Shorter-Incubation Liquid Media System:**
 - Added new table
- **Table 3. Antimycobacterial Agents and Breakpoints for Testing MAC (Table 4 in M24-A2):**
 - Revised routine and supplemental QC strains recommended for QC testing
 - Reformatted and revised footnotes as general comments
 - Added breakpoints and interpretive categories for amikacin (intravenous and liposomal inhaled)
- **Table 4. Antimycobacterial Agents and Breakpoints for Testing *M. kansasii* (Table 5 in M24-A2):**
 - Added recommended QC strain information
 - Added breakpoints and interpretive categories for:
 - Minocycline
 - Removed:
 - Ethambutol
 - Isoniazid
 - Streptomycin
- **Table 5. Antimycobacterial Agents and Breakpoints for Testing Slowly Growing Nontuberculous Mycobacteria Other Than MAC and *M. kansasii*:**
 - Added new table
- **Table 6. Antimycobacterial Agents and Breakpoints for Testing Rapidly Growing Mycobacteria (Table 7 in M24-A2):**
 - Revised QC strains for supplemental QC testing
 - Revised and reformatted comments
- **Table 7. Antimycobacterial Agents and Breakpoints for Testing *Nocardia* spp. and Other Aerobic Actinomycetes:**
 - Added new table

- **Table 8. Expected Antimicrobial Susceptibility Patterns of the Most Commonly Isolated *Nocardia* spp.:**
 - Added new table
- **Table 9. Expected Results for Routine and Supplemental QC Strains When Testing MTBC by a Culture-based Method:**
 - Added new table
- **Table 10. MIC QC Ranges When Testing MTBC Using *M. tuberculosis* ATCC® 27294 (H37Rv) Tested in Middlebrook 7H9 Media Supplemented With OADC:**
 - Added new table
- **Table 11. QC Ranges for Susceptibility Testing of *M. tuberculosis* ATCC® 27294 (H37Rv) to Second-Line Drugs Using the Fluorescence-based Commercial Shorter-Incubation Liquid Media System:**
 - Added new table
- **Table 12. Broth Microdilution QC Ranges When Testing Slowly Growing Nontuberculous Mycobacteria:**
 - Added new table
- **Table 13. Broth Microdilution QC Ranges When Testing Rapidly Growing Mycobacteria (Table 8 in M24-A2):**
 - Added QC ranges for tigecycline
- **Table 14. Broth Microdilution QC Ranges When Testing *Nocardia* spp. and Other Aerobic Actinomycetes:**
 - Added new table

Key Words

Aerobic actinomycetes, antimicrobial susceptibility testing, antimycobacterial drugs, antituberculous drugs, *Mycobacterium tuberculosis* complex, *Nocardia* spp., nontuberculous mycobacteria

Abbreviations and Acronyms

ATCC® ^a	American Type Culture Collection
HPLC	high-performance liquid chromatography
I	intermediate
IV	intravenous
MAC	<i>Mycobacterium avium</i> complex
MIC	minimal inhibitory concentration
MTBC	<i>Mycobacterium tuberculosis</i> complex
OADC	oleic acid–albumin–dextrose–catalase
QC	quality control
R	resistant
S	susceptible
V	variable

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

Table 1. Broth Microdilution Breakpoints and Interpretive Categories for MTBC Tested in Middlebrook 7H9 Broth Supplemented With OADC Using Commercially Available MIC Plates¹⁻³

QC recommendations (see Table 10 for acceptable QC ranges):	
Routine QC strain:	
<ul style="list-style-type: none"> <i>M. tuberculosis</i> ATCC^{®*} 27294 (H37Rv) 	

Antimicrobial Agent	Breakpoints, µg/mL [†]		
	S	Inconclusive ³	R
Ethambutol	≤2	4 [‡]	≥8
Isoniazid	≤0.12	–	≥0.25
Rifampin	≤1	–	≥2

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

[†] This antimycobacterial susceptibility testing system is not regulatory organization cleared/approved.

[‡] Inconclusive MIC for ethambutol. An MIC of 4 µg/mL obtained by broth microdilution using commercially available plates 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 may determine whether the isolate in question is susceptible or resistant.

Abbreviations: ATCC[®], American Type Culture Collection; MIC, minimal inhibitory concentration; MTBC, *Mycobacterium tuberculosis* complex; OADC, oleic acid–albumin–dextrose–catalase; QC, quality control; R, resistant; S, susceptible.

References for Table 1

- Nosova EY, Zimenkov DV, Khakhalina AA, et al. A comparison of the Sensititre MycoTB plate, the BACTEC MGIT 960, and a microarray-based molecular assay for the detection of drug resistance in clinical *Mycobacterium tuberculosis* isolates in Moscow, Russia. *PLoS One*. 2016;11(11):e0167093.
- Heysell SK, Pholwat S, Mpagama SG, et al. Sensititre MycoTB plate compared to BACTEC MGIT 960 for first- and second-line antituberculosis drug susceptibility testing in Tanzania: a call to operationalize MICs. *Antimicrob Agents Chemother*. 2015;59(11):7104-7108.
- Martin IW, Dionne K, Deml SM, Wengenack NL, Parrish NM. Automated broth-based systems versus the MYCOTB plate for antimicrobial susceptibility testing of the *Mycobacterium tuberculosis* complex: challenges in interpretation. *Diagn Microbiol Infect Dis*. 2018;91(1):38-41.

Table 2. Susceptibility Testing of MTBC to Second-Line Drugs Using the Fluorescence-based Commercial Shorter-Incubation Liquid Media System

QC recommendations (see Table 11 for acceptable QC ranges):

Routine QC strain:

- *M. tuberculosis* ATCC®* 27294 (H37Rv)

Antituberculous Agent	Critical Concentration, µg/mL [†]
Amikacin [‡]	1.0 ^{1-3,§}
Capreomycin	2.5 ¹⁻³
Ethionamide	5.0 ^{1,3,4}
Kanamycin [‡]	2.5 ³
Levofloxacin [¶]	1.5 ^{4,5,§}
Linezolid	1.0 ¹
Moxifloxacin ^{¶, #}	0.25 ^{6,7,§}
<i>p</i> -Aminosalicylic acid	4.0 [§]
Rifabutin	0.5 ^{1,8}

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

[†] Most concentrations listed are based on multicenter studies. The systems are not regulatory organization cleared for testing second-line antituberculous drugs.

[‡] Amikacin and kanamycin are aminoglycosides, but kanamycin resistance may not indicate amikacin resistance. It may be desirable to test both aminoglycosides. **NOTE:** As of this document's publication, kanamycin is not available in the United States.

[§] For the following drugs listed in the table, the critical concentrations deviate from the technical report on critical concentrations released by WHO in 2018⁶:

- For levofloxacin, the WHO-recommended critical concentration is 1.0 µg/mL.
- For moxifloxacin, the WHO-recommended higher concentration (clinical breakpoint) is 1.0 µg/mL.
- For *p*-aminosalicylic acid, the WHO has withdrawn the critical concentration.

[¶] Two fluoroquinolone drugs are listed. Laboratories should test at least one fluoroquinolone drug. It is not necessary to test both fluoroquinolones listed.

[#] Moxifloxacin susceptibility at 0.25 µg/mL by the fluorescence-based shorter-incubation liquid media system predicts levofloxacin susceptibility. Because moxifloxacin is more potent than levofloxacin and older quinolones, moxifloxacin may have clinical efficacy when it demonstrates resistance at this concentration but susceptibility at a higher concentration. However, clinical data are not available at this time. It may be advisable to implement moxifloxacin reflex testing at 2 µg/mL for quantitative determination of the resistance level. Future clinical studies are warranted to fully investigate moxifloxacin's clinical efficacy for strains with MIC between 0.5 and 4 µg/mL.

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; MTBC, *Mycobacterium tuberculosis* complex; QC, quality control; WHO, World Health Organization.

References for Table 2

- ¹ Rüsç-Gerdes S, Pfyffer GE, Casal M, Chadwick M, Siddiqi S. Multicenter laboratory validation of the BACTEC MGIT 960 technique for testing susceptibilities of *Mycobacterium tuberculosis* to classical second-line drugs and newer antimicrobials. *J Clin Microbiol.* 2006;44(3):688-692.
- ² Krüüner A, Yates MD, Drobniowski FA. Evaluation of MGIT 960-based antimicrobial testing and determination of critical concentrations of first- and second-line antimicrobial drugs with drug-resistant clinical strains of *Mycobacterium tuberculosis*. *J Clin Microbiol.* 2006;44(3):811-818.

Table 6. Antimycobacterial Agents and Breakpoints for Testing Rapidly Growing Mycobacteria

<p>QC recommendations (see Table 13 for acceptable QC ranges):</p> <p>Routine QC strain:</p> <ul style="list-style-type: none"> <i>M. peregrinum</i> ATCC®* 700686 <p>Supplemental QC strains:</p> <ul style="list-style-type: none"> <i>Staphylococcus aureus</i> ATCC® 29213 <i>Pseudomonas aeruginosa</i> ATCC® 27853 and/or <i>Enterococcus faecalis</i> ATCC® 29212 may also be used, if desired.
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Antimicrobial Agent	MIC, µg/mL			Comments
	S	I	R	
Amikacin (IV)	≤16	32	≥64	(1) <i>M. abscessus</i> complex isolates with MICs of ≥64 µg/mL should be retested, or the <i>rrs</i> gene for the amikacin mutation may be sequenced. If the repeat result is ≥64 µg/mL, the MIC should be reported with the comment: “The MIC is greater than expected for this species; if the drug is being considered for therapy, the laboratory should be notified so the isolate can be sent to a referral laboratory for confirmation of resistance.”
Cefoxitin	≤16	32–64	≥128	(2) Ciprofloxacin and levofloxacin are interchangeable. Both are less active <i>in vitro</i> than the newer 8-methoxy fluoroquinolones.
Ciprofloxacin	≤1	2	≥4	
Clarithromycin	≤2	4	≥8	(3) See CLSI document M24 ¹ for guidance regarding incubation period, molecular testing, and interpretation of clarithromycin results for rapidly growing mycobacteria. Clarithromycin is the class representative for the newer macrolides (ie, azithromycin and roxithromycin).
Doxycycline	≤1	2–4	≥8	

Table 13. Broth Microdilution QC Ranges When Testing Rapidly Growing Mycobacteria

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	<i>M. peregrinum</i> ATCC®* 700686† (routine organism)	<i>S. aureus</i> ATCC® 29213 (supplemental organism)	<i>P. aeruginosa</i> ATCC® 27853 (supplemental organism)	<i>E. faecalis</i> ATCC® 29212 (supplemental organism)
Amikacin	≤1–4	1–4	1–4	64–256
Cefoxitin	4–32	1–4	–‡	–‡
Ciprofloxacin	≤0.12–0.5	0.12–0.5	0.25–1	0.25–2
Clarithromycin	≤0.06–0.5	0.12–0.5	–‡	–‡
Doxycycline	0.12–0.5	0.12–0.5	–‡	2–8
Imipenem	2–16	0.015–0.06	1–4	0.5–2
Linezolid	1–8	1–4	–‡	1–4
Meropenem	2–16	0.03–0.12	0.25–1	2–8
Minocycline	0.12–0.5	0.06–0.5	–‡	1–4
Moxifloxacin	≤0.06–0.25	0.016–0.12	1–8	0.06–0.5
Tigecycline	0.03–0.25	0.03–0.25	–‡	0.03–0.12
Tobramycin	2–8	0.12–1	0.25–1	8–32
Trimethoprim-sulfamethoxazole§	≤0.25/4.8–2/38	≤0.5/9.5	8/152–32/608	≤0.5/9.5

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

† *M. peregrinum* should be incubated at 30°C±2°C.

‡ A dash indicates no studies have been performed by current recommended methods.

§ MIC is indicated by 80% growth inhibition.

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC, quality control.