

INTRODUCTION . Susceptibility testing, identification by PCR, and DNA fingerprinting of the rapidly growing mycobacteria and other nontuberculous mycobacteria and related aerobic actinomycetes are performed at the UTHSCT Mycobacteria/Nocardia Laboratory. The laboratory is College of American Pathologists (CAP) accredited and holds a CLIA license and a Florida CLIA license.

The Mycobacteria/Nocardia Laboratory has been in operation for approximately 30 years and accepts pure culture isolates (not gross specimens) of the above groups for susceptibility, identification, and DNA fingerprinting. Please note there will be an additional charge and increased turn around time for any isolates that are not pure. If desired, gross specimens should be submitted to the UTHSCT Pathology Laboratory (See Mycobacterium/Mycology Referral; call **903-877-5065** for details).

SUSCEPTIBILITY TESTING . Susceptibility testing is performed using a broth microdilution MIC method for the nontuberculous mycobacteria (NTM), Nocardia, and related aerobic actinomycetes. For rapidly growing mycobacteria (RGM), the panel of drugs* includes clarithromycin, amikacin, imipenem, linezolid, tigecycline, tobramycin, ceftazidime, cefepime, cefoxitin, moxifloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole, and doxycycline or minocycline. Incubation time is 3-5 days at 30°C except for clarithromycin which is held up to 14 days to check for induction of in vitro resistance (erm gene). For Nocardia and other related aerobic actinomycetes, the panel of drugs* includes amoxicillin/clavulanic acid, linezolid, clarithromycin, trimethoprim-sulfamethoxazole or sulfamethoxazole, ciprofloxacin, moxifloxacin, ceftriaxone, imipenem, tobramycin, amikacin, and minocycline. Incubation time is 3-5 days at 35°C. Slowly growing NTM (other than *M. avium* complex) are tested against clarithromycin, ethambutol, amikacin, sulfamethoxazole, rifabutin, ciprofloxacin, moxifloxacin, minocycline, linezolid, and rifampin. In accordance with the American Thoracic Society (ATS) and CLSI recommendations, isolates of *M. kansasii* are reported with rifampin and clarithromycin susceptibility only, if they are rifampin susceptible. Rifampin resistant *M. kansasii* will have a panel of drugs reported. Incubation time is 7-14 days at 35°C.

Current recommendations by the ATS and the CLSI/NCCLS M24-A, 2003 advise testing only clarithromycin against isolates of *Mycobacterium avium* complex (MAC). Clarithromycin is used as a "class drug" in susceptibility testing of azithromycin and other related macrolides. Thus, isolates of MAC are only tested for susceptibility to clarithromycin. Resistance to clarithromycin confers resistance to azithromycin and vice versa. It may be reasonable to test agents other than TB agents such as moxifloxacin and linezolid and these may, in some cases, provide useful adjunctive treatment options. They are not, however, used as treatment substitutions for any of the standard treatment agents (macrolide, ethambutol, rifampin/rifabutin).

The methods and breakpoints for susceptibility testing of RGM and aerobic actinomycetes have been published by the CLSI. Recommendations for breakpoints for these organisms using the broth microdilution MIC method with nine drugs (amikacin, tobramycin, sulfamethoxazole, ceftazidime, imipenem, linezolid, doxycycline, clarithromycin and ciprofloxacin) are shown in Tables 1 and 2.

Generally, turn-around time for susceptibility testing is approximately **7-14 working days** for RGM, **7-14 working days** for Nocardia and related aerobic actinomycetes, and **21-35 working days** for the slowly growing NTM and some other aerobic actinomycetes. These times assume receipt of a viable pure culture isolate. Results can be sent by FAX on request. **FOR SUSCEPTIBILITY RESULTS OR TO CHECK RECEIPT OF ISOLATES PLEASE CALL (903) 877-7682.**

IDENTIFICATION . Identification of isolates by the Mycobacteria/Nocardia Laboratory is currently performed using PCR of the hsp65 gene followed by restriction enzyme analysis (PRA). Identification is available as soon as 48-72 hours on some isolates. Isolates which give indeterminate results may be submitted for additional testing which may include 16S sequence analysis if requested. **FOR PCR RESULTS, PLEASE CALL (903) 877-5947 .**

DNA FINGERPRINTING . Isolates of NTM or other aerobic actinomycetes may also be submitted for DNA fingerprinting by pulsed field gel electrophoresis (PFGE) or random amplified polymorphic DNA PCR (RAPD-PCR) in cases of outbreaks, pseudo-outbreaks, or epidemics. Before sending isolates for RFLP analysis, please call Barbara Brown-Elliott. Turn around time is approximately 6-8 weeks from receipt of the isolate depending on the type and number of organisms submitted for testing. (See [Fees for Laboratory Services](#))

FOR TECHNICAL CONSULTATION CALL BARBARA BROWN-ELLIOTT AT (903) 877-7685.

***Subject to Change (See Table 3 for expanded current panels available)**

Table 1. Suggested broth microdilution breakpoints for rapidly growing mycobacteria^a.

Drug	MIC ($\mu\text{g/mL}$) for category:		
	Susceptible	Intermediate	Resistant
Amikacin	≤ 16	32	≥ 64
Cefoxitin	≤ 16	32-64	≥ 128
Ciprofloxacin	≤ 1	2	≥ 4
Clarithromycin	≤ 2	4	≥ 8
Doxycycline			

Imipenem	≤1	2-8	≥16
Linezolid	≤4	8	≥16
Moxifloxacin	≤8	16	≥32
Sulfamethoxazole	<1	2	≥4
Tobramycin	≤32	-	≥64
	≤4	8	≥16

^aBreakpoints from the CLSI (formerly NCCLS), NCCLS-M24-A, 2003

Table 2. CLSI/NCCLS M24-A, Suggestions for susceptibility testing of *M. abscessus*, *M. chelonae*, and the *M. fortuitum* group by broth microdilution^a.

Drug	Comment
Tobramycin	If the initial MIC is >4 µg/mL, the test should be repeated. If the repeat result is >4 µg/mL, the MIC should be reported with a comment. ^b
Sulfonamides	MIC is read at 80% inhibition of growth.
Doxycycline	Breakpoints are ≤1 µg/mL (susceptible), 2-4 µg/mL (intermediate), and ≥8 µg/mL (resistant).
Cefoxitin	Breakpoints are ≤16 µg/mL (susceptible), 32-64 g/mL (intermediate), and ≥128 µg/mL (resistant).
Imipenem	If MIC for <i>M. fortuitum</i> group is >8 µg/mL, test should be repeated with incubation period of no more than 3 days. If the repeat result is >8 µg/mL, the MIC should be reported with comment.
Amikacin	Isolates of <i>M. abscessus</i> for which MIC is ≥64 µg/mL should be retested. If the repeat result is ≥64 µg/mL, the MIC should be reported with a comment. ^b
Clarithromycin	Isolates of <i>M. fortuitum</i> group with a trailing endpoint should be considered resistant. Results for <i>M. chelonae</i> and <i>M. abscessus</i> should be read optimally at 3 days (no longer than 4 days).

^a For laboratories that infrequently isolate rapidly growing mycobacteria, sending isolates to an experienced reference laboratory is recommended. For laboratories that perform MIC testing, (i) proficiency testing by comparison of test results with those of an experienced reference laboratory is necessary upon initial validation and at regular

intervals thereafter and (ii) identification of isolates to the species level or, at a minimum, differentiation of the *M. fortuitum* group from the *M. chelonae*-*M. abscessus* group is recommended.

^b Comment: (i) the MIC is greater than expected for this species and (ii) if the drug is being considered for therapy, the laboratory should be notified so the isolate can be sent to a reference laboratory for confirmation of resistance.

Table 3. ANTIBIOTIC PANELS AVAILABLE

SLOWLY GROWING MYCOBACTERIA	RAPIDLY GROWING MYCOBACTERIA	NOCARDIA
Amikacin	Amikacin	Amikacin
Ciprofloxacin	Cefoxitin	Amoxicillin-Clavulanic Acid
Clarithromycin ¹	Ciprofloxacin Clarithromycin ¹	(Augmentin)
Doxycycline or Minocycline	Doxycycline or Minocycline	Ceftriaxone
Ethambutol	Ertapenem ²	Ciprofloxacin
Linezolid	Imipenem ³	Clarithromycin ¹
Moxifloxacin	Kanamycin ²	Imipenem
Rifabutin	Linezolid	Kanamycin ²
Rifampin	Meropenem ²	Linezolid
Sulfamethoxazole or TMP/SMX	Moxifloxacin ²	Meropenem ²
	Sulfamethoxazole and or TMP-SMX	Minocycline
	Tigecycline ⁵	Moxifloxacin ⁴
	Tobramycin ⁶	Trimethoprim- Sulfamethoxazole or TMP-SMX
		Tigecycline ⁵
		Tobramycin

¹ Class drug for newer macrolides (i.e., azithromycin)

² KB disk plates, non CLSI (formerly NCCLS) approved method

³ MIC to reported for *M. chelonae* or *M. abscessus* per CLSI recommendations (NCCLS M4-A, 2003). However, because this is a clinically useful drug and the alternative drug

⁴ MIC interpretations based on CLSI M100-S19, 2009, bacterial breakpoints.

⁵ MIC reported without interpretation since no breakpoints yet established.

⁶ MIC only reported for *M. chelonae*.

⁷ MIC reported as per recommendations for modifications to M-24A, 2003

** Isolates of *Mycobacterium avium* complex (MAC) are routinely tested for clarithromycin susceptibility only, and rifampin susceptible *M. kansasii* are tested for susceptibility to rifampin and clarithromycin only.

Upon request, isolates of MAC may be tested for susceptibility to moxifloxacin and linezolid but no first line TB drugs are reported.

Upon request, isolates of rifampin susceptible *M. kansasii* may be tested for susceptibility to other agents.

NOTE: Drugs tested are subject to change without notice!

For susceptibility to agents other than listed, please call (903-) 877-7685

References

1. Brown BA, Wallace RJ Jr: **Broth microdilution MIC test for *Nocardia* spp** . In: Clinical Microbiology Procedures Handbook, Section 5: Antimicrobial Susceptibility Testing, p. 5.12.1. American Society for Microbiology Book Division, Washington, DC. 1992.
2. Wallace RJ Jr, Nash DR, Steele LC, Steingrube VA: **Susceptibility testing of slowly growing mycobacteria utilizing a microdilution MIC method with 7H9 broth** . *Journal of Clinical Microbiology* 24:976-981, 1986.
3. Brown, BA, Swenson JM, Wallace RJ Jr: **Broth microdilution MIC Test for rapidly growing mycobacteria** . In: Clinical Microbiology Procedures Handbook, Section 5: Antimicrobial Susceptibility Testing, p. 5.11.1. American Society for Microbiology Book Division, Washington, DC, 1992 .
4. Wallace RJ Jr., Cook JL, Glassroth J, Griffith De, Olivier KN, Gordin F: **Diagnosis and treatment of disease caused by nontuberculous mycobacteria** . *American Thoracic Society Statement. American Journal of Respiratory and Critical Care Medicine* , 156:S1-S25, August 1997.
5. Steingrube VA, Gibson JL, Brown BA, Zhang Y, Wilson **Endonuclease analysis of a 65-Kilodalton heat shock protein gene sequence for taxonomic separation of rapidly growing mycobacteria** . *Journal of Clinical Microbiology* , 33:149-153, 1995.
6. Steingrube VA, Brown BA, Gibson JL Wilson RW, Brown J, Blacklock Z, Jost K, Locke S, Ulrich RF, Wallace RJ Jr: **DNA amplification and restriction endonuclease analysis for differentiation of 12 species and taxa of *Nocardia*, including recognition of four new taxa within the *Nocardia asteroides* complex** . *Journal of Clinical Microbiology* , 33:3096-3101, 1995.
7. Steingrube VA, Wilson RW, Brown BA, Jost KC Jr., Blacklock Z, Gibson JL, Wallace RJ Jr: **Rapid identification of clinically significant species and taxa of aerobic actinomycetes, including *Actinomadura*, *Gordona*, *Nocardia*, *Rhodococcus*, *Streptomyces*, and *Tsukamurella* isolates, by DNA amplification and restriction endonuclease analysis** . *Journal of Clinical Microbiology* , 35:817-822, April 1997.
8. Telenti A, Marchesi F, Balz M, Bally F, Böttger EC, Bodmer,T: **Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis** . *Journal of Clinical Microbiology* , 31:175-178, 1993.
9. Swenson JM, Wallace RJ Jr, Silcox VA, Thornsberry C: **Antimicrobial susceptibility of 5 subgroups of *Mycobacterium fortuitum* and *Mycobacterium chelonae*** . *Antimicrobial Agents Chemotherapy* 28:807-811, 1985.
10. Woods GL, Bergmann JS, Witebsky FG, Fahle GA, Wanger A, Boulet B, Plaunt M, Brown BA, Wallace RJ Jr: **Multisite reproducibility of results obtained by the broth microdilution method for susceptibility testing of *Mycobacterium abscessus* , *Mycobacterium chelonae* , and**

- Mycobacterium fortuitum* . *Journal of Clinical Microbiology* , 37:1676-1682, 1999.
11. Zhang Y, Rajagopalan M, Brown BA, Wallace RJ Jr: **Randomly amplified polymorphic DNA PCR for comparison of *Mycobacterium abscessus* strains from nosocomial outbreaks** . *Journal of Clinical Microbiology* , 35:3132-3139, 1997.
 12. Hector JSR, Pang Y, Mazurek GH, Zhang Y, Brown Ba, Wallace RJ Jr: **Large restriction fragment patterns of genomic *Mycobacterium fortuitum* DNA as strain-specific markers and their use in epidemiologic investigation of four nosocomial outbreaks** . *Journal of Clinical Microbiology* , 30:1250-1255, 1992.
 13. Wallace RJ Jr, Zhang Y, Brown BA, Fraser V, Mazurek GH, Maloney S: **DNA large restriction fragment patterns of sporadic and epidemic nosocomial strains of *Mycobacterium chelonae* and *Mycobacterium abscessus*** . *Journal of Clinical Microbiology* , 31:2697-2701, 1993.
 14. Villanueva A, Calderon RV, Vargas BA Ruiz F, Aguero S, Zhang Y, Brown BA, Wallace RJ Jr: **Report on an outbreak of postinjection abscesses due to *Mycobacterium abscessus* , including management with surgery and clarithromycin therapy and comparison of strains by random amplified polymorphic DNA polymerase chain reaction** . *Clinical Infectious Diseases* , 24:1147-1153, 1997.
 15. Lai KK, Brown BA, Westerling JA, Fontecchio SA, Zhang Y, Wallace RJ Jr: **Long-term laboratory contamination by *Mycobacterium abscessus* resulting in two pseudo-outbreaks: Recognition with use of random amplified polymorphic DNA (RAPD) polymerase chain reaction**. *Clinical Infectious Diseases*, 27:169-175, 1998.
 16. Meier A, Heifets L, Wallace RJ Jr, Zhang Y, Brown BA, Sander P, Böttger EC. **Molecular mechanisms of clarithromycin resistance in *Mycobacterium avium*: observation of multiple 23S rDNA mutations in a clonal population**. *The Journal of Infectious Diseases*, 174:354-60, 1996.
 17. Woods GL, Brown-Elliott BA, Desmond EP, Hall, GS, Heifets L, Pfyffer GE, Plaunt MR, Ridderhof JC, Wallace RJ Jr., Warren NG, Witebsky FG: **Susceptibility Testing of Mycobacteria, Nocardia, and Other Aerobic Actinomycetes; approved standard**. *NCCLS*, M24-A, 2003.
 18. Brown-Elliott BA, Crist CJ, Mann LB, Wilson RW, Wallace RJ Jr: **In vitro activity of linezolid against slowly growing nontuberculous mycobacteria**. *Antimicrob. Agents Chemother.* 47:1736-1738, May 2003.
 19. Schinsky MF, Morey RE, Steigerwalt AG, Douglas MP, Wilson RW, Floyd MM, Butler WR, Daneshvar MI, Brown-Elliott BA, Wallace RJ Jr, McNeil MM, Brenner DJ, Brown JM: **Taxonomic variation in the *Mycobacterium fortuitum* third biovariant complex: description of *Mycobacterium boenickei* sp. nov., *Mycobacterium houstonense* sp. nov., *Mycobacterium new orleansense* sp. nov and *Mycobacterium brisbanense* sp. nov. and recognition of *Mycobacterium porcinum* from human clinical isolates**. *Int. J. Syst. Evolution. Microbiol.* 54:1653-1667, 2004.
 20. Brown-Elliott BA, Wallace RJ Jr: **Rapidly growing mycobacteria**. In: Tuberculosis & Nontuberculous Mycobacterial Infections, 5th edition, D Schlossberg, ed. McGraw-Hill Companies, Inc. 35:451-462, 2006.
 21. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin, Holland SM, Horsburgh R, Huittt G, Iademarco MF, Iseman M, Olivier K,

Ruoss S, von Reyn FC, Wallace RJ Jr., Winthrop K. **An Official ATS/IDSA Statement: Diagnosis, Treatment, and Prevention of Nontuberculous Mycobacterial Diseases.** *Am. Respir. Crit. Care Med*, 175:367-416, 2007.

22. Nash KA, Brown-Elliott, BA, Wallace JR Jr. **A Novel Gene *erm(41)*, Confers Inducible Macrolide Resistance to Clinical Isolates of *Mycobacterium abscessus* but Is Absent from *Mycobacterium chelonae*.** *Antimicrob. Agents Chemother.*, 53:1367-1376, 2009.