

## Overview

### Useful For

Rapid identification to the species level and susceptibility testing for *Mycobacterium* species, *Nocardia* species, and other aerobic actinomycete genera and species from pure culture isolates

### Reflex Tests

| Test Id | Reporting Name                       | Available Separately | Always Performed |
|---------|--------------------------------------|----------------------|------------------|
| RMALM   | Id MALDI-TOF Mass Spec AFB           | No, (Bill Only)      | No               |
| RTBSP   | Id, Mtb Speciation, PCR              | No, (Bill Only)      | No               |
| ISMY    | ID by 16S Sequencing                 | No, (Bill Only)      | No               |
| SRG     | Susceptibility Rapid Grower          | No, (Bill Only)      | No               |
| RSLG    | Susceptibility Slow Grower           | No, (Bill Only)      | No               |
| SSNS    | Susceptibility Nocardia species      | No, (Bill Only)      | No               |
| STV1    | Susceptibility, Mtb Complex, Broth   | No, (Bill Only)      | No               |
| STV2    | Susceptibility, Mtb Cx, 2nd Line     | No, (Bill Only)      | No               |
| STVP    | Susceptibility, Mtb Complex, PZA     | No, (Bill Only)      | No               |
| MTBVP   | Mtb PZA Confirmation, pnc A Sequence | No, (Bill Only)      | No               |
| MIC     | Susceptibility, MIC                  | No, (Bill Only)      | No               |
| LCTB    | Id, MTB complex Rapid PCR            | No, (Bill Only)      | No               |

### Testing Algorithm

When this test is ordered, reflex antimicrobial susceptibility testing will be performed at an additional charge. All mycobacteria and *Nocardia* (including aerobic actinomycetes) submitted will be identified and billed as appropriate.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS) and/or 16S rDNA sequencing is used for identification, when applicable, for slowly and rapidly growing *Mycobacterium* species and aerobic actinomycetes.

*Mycobacterium tuberculosis* complex rapid polymerase chain reaction (PCR) is used to rule out M tuberculosis complex from all broth specimens received with sufficient volume. Testing on solid growth is determined based on growth rate, colony morphology, or specific request by clients.

The *M tuberculosis* complex will be further identified to the species level upon request using rapid PCR testing.

Minimum inhibitory concentration (MIC) determination by either the microtiter broth dilution method or critical concentration testing by broth dilution will be automatically performed as appropriate after species identification.

For more information see [Culture Referred for Identification and Susceptibility for \*Mycobacterium\* and \*Nocardia\* Algorithm](#).

**Special Instructions**

- [Infectious Specimen Shipping Guidelines](#)
- [Culture Referred for Identification and Susceptibility for \*Mycobacterium\* and \*Nocardia\*](#)

**Method Name**

16S rDNA Sequencing/Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI TOF MS)/Rapid Polymerase Chain Reaction (PCR)

**NY State Available**

Yes

**Specimen****Specimen Type**

Varies

**Ordering Guidance**

This test is intended for *Mycobacterium* species, *Nocardia* species, or other aerobic actinomycete genera.

**Shipping Instructions**

1. See [Infectious Specimen Shipping Guidelines](#) for shipping information.
2. Place specimen in a large infectious container (T146) and label as an etiologic agent/infectious substance.

**Necessary Information**

1. Specimen source is required.
2. Isolate description is required: Gram stain reaction, morphology, tests performed.

**Specimen Required**

**Specimen Type:** Organism in pure culture

**Supplies:** Infectious Container, Large (T146)

**Container/Tube:** Middlebrook (7H10 or 7H11) or Lowenstein-Jensen medium slant or in broth (eg, Mycobacteria Growth Indicator Tube [7H9] broth)

**Specimen Volume:** Isolate**Collection Instructions:** Organism must be in pure culture, actively growing. **Do not submit mixed cultures.****Forms**If not ordering electronically, complete, print, and send a [Microbiology Test Request](#) (T244) with the specimen.**Reject Due To**

|            |        |
|------------|--------|
| Agar plate | Reject |
|------------|--------|

**Specimen Stability Information**

| Specimen Type | Temperature         | Time | Special Container |
|---------------|---------------------|------|-------------------|
| Varies        | Ambient (preferred) |      |                   |
|               | Refrigerated        |      |                   |

**Clinical & Interpretive****Clinical Information**

There are approximately 200 recognized species of mycobacteria and more than 100 *Nocardia* species. Many of these species are human pathogens and, therefore, identification to the species level is important to help guide patient care. In addition, there are other aerobic actinomycete genera that can be human pathogens including, but not limited to, *Tsukamurella*, *Rhodococcus*, and *Gordonia* species.

*Mycobacteria* species, *Nocardia* species, and other aerobic actinomycete genera are identified using matrix-assisted laser desorption ionization time-of-flight mass spectrometry or nucleic acid sequencing of a 500-base pair region of the 16S ribosomal RNA gene. *Mycobacterium tuberculosis* complex can also be identified via rapid polymerase chain reaction assay which targets a unique sequence within the *katG* gene, which is present in members of the *M tuberculosis* complex and can also detect genotypic resistance to isoniazid mediated by mutations in the *katG* target, when present.

After identification, antimicrobial susceptibility testing is performed following Clinical and Laboratory Standards Institute M24 guidelines using either broth dilution or critical concentration methods as appropriate for the species.

**Reference Values**

Not applicable

**Interpretation**

Organisms growing in pure culture are identified to the species level whenever possible.

**Cautions**

If the organism is received in mixed culture or contaminated, the report may be delayed, or identification may not be possible.

**Clinical Reference**

1. Martin I, Pfyffer GE, Parrish N. *Mycobacterium*: general characteristics, laboratory detection, and staining procedures. In: Carroll KC, Pfaller MA, eds. *Manual of Clinical Microbiology*. 13th ed. Vol 1. ASM Press; 2023:594-613
2. Clinical and Laboratory Standards Institute. *Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes*. 3rd ed. CLSI document M24-ED3:2018. CLSI; 2018

**Performance****Method Description**

DNA sequence analysis utilizes a 500-base pair region of the 16S rRNA gene as the target for identification of mycobacteria and is performed using the MicroSeq kit from Applied Biosystems. Sequence data generated is compared to several different databases of known mycobacterial and aerobic actinomycete sequences to obtain organism identification. These include MicroSeq, NCBI GenBank, and Mayo Clinic Mycobacteria database. A 100% agreement with a database strain is needed for an acceptable identification to the species level.(Hall L, Doerr KA, Wohlfel SL, Roberts GD: Evaluation of the MicroSeq system for identification of mycobacteria by 16S ribosomal DNA sequencing and its integration into a routine clinical mycobacteriology laboratory. *J Clin Microbiol*. 2003 Apr;41[4]:1447-1453)

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS) analysis is done using the Bruker BioTyper platform and the Bruker BDAL library, Bruker Mycobacterial Library, and the Mayo Clinic Library. A spectral score of 2.0 or greater is required for identification to the species level.

Rapid polymerase chain reaction (PCR) is performed following specimen digestion and decontamination using N-acetyl cysteine and sodium hydroxide. Genomic DNA is extracted using the MagNA Pure (Roche Applied Sciences) extraction platform. The purified genomic DNA is placed on the LightCycler instrument, which amplifies and monitors, by fluorescence, the development of target nucleotide sequences after each PCR cycle. A specific target sequence from a portion of the *katG* gene from *Mycobacterium tuberculosis* complex is amplified and the resulting segment is detected by melt-curve analysis using sequence-specific fluorescence resonance energy transfer hybridization probes. The LightCycler PCR assay is a closed PCR system that greatly reduces the potential for false-positive results due to specimen cross-contamination as compared with traditional open-system PCR or other amplification methods like transcription-mediated amplification.(Buckwalter SP, Connelly BJ, Louison LK, et al. Description, validation, and review of a decade of experience with a laboratory-developed PCR test for detection of *M tuberculosis* complex in pulmonary and extrapulmonary specimens. *J Clin Tuberc Other Mycobact Dis*. 2022;29:100340. doi:10.1016/j.jctube.2022.100340)

The method employed in this assay is broth microtiter dilution using a commercially available plate from Trek Diagnostics. Antimicrobials included in the assay are tested according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.(Clinical and Laboratory Standards Institute: *Susceptibility testing of mycobacteria, nocardia spp., and other aerobic actinomycetes*. 3rd ed. CLSI standard M24. CLSI; 2018)

This test method is based on growth or absence of growth of *M tuberculosis* complex isolates in broth cultures containing critical concentrations of the antimycobacterial agents isoniazid, rifampin, and ethambutol. One of two US

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Mycobacterium and Nocardia with  
Antimicrobial Susceptibility Testing, Varies

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Food and Drug Administration-cleared platforms, the BACTEC MGIT 960 (Becton Dickinson) or the VersaTREK (ThermoFisher) will be used.(Brown-Elliott BA, Cirillo DM, Musser KA, Rowlinson M-C. Susceptibility test methods: Mycobacteria, nocardia, and other actinomycetes. In: Carroll KC, Pfaller MA, eds. Manual of Clinical Microbiology. 13th ed. ASM Press; 2023)

This test utilizes the Sensititre MycoTB broth microtiter dilution plate (Trek/ThermoFisher). Antimicrobials are tested according to CLSI M24 guidelines.(Thermo Scientific Sensititre MIC Susceptibility Plates for *Mycobacterium tuberculosis*. Product Insert. 011-MYCOTB-CID9502. Revision Date: 09/07/2016; Brown-Elliott BA, Cirillo DM, Musser KA, Rowlinson M-C. Susceptibility test methods: Mycobacteria, nocardia, and other actinomycetes. In: Carroll KC, Pfaller MA, eds. Manual of Clinical Microbiology, 13th ed. ASM Press; 2023)

**PDF Report**

No

**Day(s) Performed**

Monday through Sunday

**Report Available**

60 to 70 days

**Specimen Retention Time**

Original specimen: 1 month; Subculture: 1 year

**Performing Laboratory Location**

Mayo Clinic Laboratories - Rochester Main Campus

**Fees & Codes****Fees**

- Authorized users can sign in to [Test Prices](#) for detailed fee information.
- Clients without access to Test Prices can contact [Customer Service](#) 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact [Customer Service](#).

**Test Classification**

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

**CPT Code Information**

Culture Referred for Identification, *Mycobacterium*

87118-Identificaiton of mycobacteria

87158-Identification of mycobacteria by other methods (if appropriate)

87118 -Id MALDI-TOF Mass Spec AFB (if appropriate)

**Test Definition: TBIDS**

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87153-Mycobacteria Identification by Sequencing (if appropriate)  
87150-Id, Mtb Speciation, PCR (if appropriate)  
87186-Susceptibility Rapid Grower (if appropriate)  
87186-Susceptibility Slow Grower (if appropriate)  
87186-Susceptibility Nocardia species (if appropriate)  
87188 x 3-Antimicrobial Susceptibility, Mycobacterium tuberculosis Complex, Broth Method (if appropriate)  
87186-Susceptibility, Mtb Cx, 2nd Line (if appropriate)  
87188-Susceptibility, Mycobacterium tuberculosis Complex, Pyrazinamide (if appropriate)  
87153-Mtb PZA Confirmation, pncA Sequencing (if appropriate)  
87150- Id, MTB complex Rapid PCR (if appropriate)

**LOINC® Information**

| Test ID | Test Order Name                    | Order LOINC® Value |
|---------|------------------------------------|--------------------|
| TBIDS   | Mycobacteria Culture Refer ID+Susc | 543-9              |

| Result ID | Test Result Name                   | Result LOINC® Value |
|-----------|------------------------------------|---------------------|
| TBIDS     | Mycobacteria Culture Refer ID+Susc | In Process          |