MAYO CLINIC LABORATORIES

Test Definition: CTBID

Culture Referred for Identification, Mycobacterium and Nocardia, Varies

Overview

Useful For

Rapid identification to the species level 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
LCTB	Id, MTB complex Rapid PCR	No, (Bill Only)	No

Testing Algorithm

Reflex tests may be performed at an additional charge. All mycobacteria and *Nocardia* (including aerobic actinomycetes) submitted will be identified and billed as appropriate.

See Culture Referred for Identification *Mycobacterium* and *Nocardia* Algorithm.

Special Instructions

- Culture Referred for Identification Mycobacterium and Nocardia Algorithm
- Infectious Specimen Shipping Guidelines

Highlights

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

Mycobacterium tuberculosis (Mtb) complex rapid polymerase chain reaction is used to rule out Mtb 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 Mtb complex will be further identified to the species level upon request using rapid PCR testing.

Method Name

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

NY State Available

Yes

Specimen

Specimen Type
Varies

Shipping Instructions
1. See Infectious Specimen Shipping Guidelines.
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: Mycobacterium species or aerobic actinomycetes 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:
Solid media: Visible growth of isolate
Isolate in broth media: > or =3 mL
Collection Instructions: Organism must be in pure culture, actively growing. **Do not submit mixed cultures.**
Additional Information: A minimum volume of 3 mL is recommended to perform all initial testing, this may include: stains, sub-culture media, nucleic acid probes, and any additional testing that may be required to determine the identification. If the broth sample volume is less than 3 mL, initial testing may be limited, and increased turnaround time is likely.

Forms
If not ordering electronically, complete, print, and send a Microbiology Test Request (T244) with the specimen.

Specimen Minimum Volume
See Specimen Required

Reject Due To

Agar plate	Reject
Isolates other than Mycobacterium species or aerobic actinomycetes	Reject

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

Approximately 200 recognized species of mycobacteria and more than 100 *Nocardia* species exist. Many of these species are human pathogens and, therefore, identification to the species level is important to help guide patient care. In addition, other aerobic actinomycete genera 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.

Reference Values

Not applicable

Interpretation

Organisms growing in pure culture are identified to the species level where indicated.

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, Landry ML, et al, eds. Manual of Clinical Microbiology. 12th ed. Vol 1. ASM Press; 2019:558-575
2. Warshauer DM, Salfinger M, Desmond E, Lin SYG. Mycobacterium tuberculosis complex. In: Carroll KC, Pfaller MA, Landry ML, et al, eds. Manual of Clinical Microbiology. 12th ed. Vol 1. ASM Press; 2019:576-594
3. Caulfield AJ, Richter E, Brown-Elliott BA, Wallace RJ Jr, Wengenack NL. Mycobacterium: Laboratory characteristics of slowly growing mycobacteria other than Mycobacterium tuberculosis. In: Carroll KC, Pfaller MA, Landry ML, et al, eds. Manual of Clinical Microbiology. 12th ed. Vol 1. ASM Press; 2019:595-611
4. Brown-Elliott BA, Wallace RJ Jr. Mycobacterium: Clinical and laboratory characteristics of rapidly growing mycobacteria. In: Carroll KC, Pfaller MA, Landry ML, et al, eds. Manual of Clinical Microbiology. 12th ed. Vol 1. ASM Press; 2019:612-629
5. Conville PS, Brown-Elliott BA, Witebsky FG. Nocarida, rhodococcus, gordonia, actinomadura, streptomyces and other aerobic actinomycetes. In: Carroll KC, Pfaller MA, Landry ML, et al, eds. Manual of Clinical Microbiology. 12th ed. Vol 1. ASM Press; 2019:525-557

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% or greater agreement with a database strain is needed for an acceptable identification to the species level.(Hall L, Doerr KA, Wohlfiel 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;41[4]:1447-1453)

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry 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 more is required for identification to the species level.(Buckwalter SP, Olson SL, Connelly BJ, et al. Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of Mycobacterium species, Nocardia species, and other aerobic actinomycetes. J Clin Microbiol. 2016;54[2]:376-384)

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 *kat*G 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 *Mycobacterium tuberculosis* complex in pulmonary and extrapulmonary specimens. J Clin Tuberc Other Mycobact Dis. 2022;29:100340. doi:10.1016/j.jctube.2022.100340)

PDF Report

No

Day(s) Performed

Monday through Sunday

Report Available

60 to 70 days

Specimen Retention Time

2 years

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 has been cleared, approved, or is exempt by the US Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA requirements.

CPT Code Information

Culture Referred for Identification, *Mycobacterium*
87118-Identification of mycobacteria
87158-Identification of mycobacteria by other methods (if appropriate)
87118 -Id MALDI-TOF Mass Spec AFB (if appropriate)
87153-Mycobacteria Identification by Sequencing (if appropriate)
87150-Id, Mtb Speciation, PCR (if appropriate)
87150- Id, MTB complex Rapid PCR (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
CTBID	Culture Refer for ID, Mycobacterium	543-9

Result ID	Test Result Name	Result LOINC® Value
CTBID	Culture Refer for ID, Mycobacterium	In Process