Test Definition: TBIDS

Mycobacteria Culture Refer ID+Susc

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

Testing Algorithm

When this test is ordered, the reflex antimicrobial susceptibility test will be performed at an additional charge. All mycobacteria and *Nocardia* (including aerobic actinomycetes) submitted will be identified and billed as appropriate. Nucleic acid probes used for identification, when applicable, include those for *Mycobacterium avium*-intracellulare complex, *Mycobacterium gordonae*, and *Mycobacterium tuberculosis* complex. 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 (including *Nocardia* species and *Streptomyces* species). The *M tuberculosis* complex can be further identified to the species level upon request, using a separate rapid PCR test. 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.

See Culture Referred for Identification and Susceptibility for *Mycobacterium* and *Nocardia* Algorithm in Special Instructions.

Special Instructions

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

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
RMALM	Id MALDI-TOF Mass Spec AFB	No	No
RTBSP	Id, Mtb Speciation, PCR	No	No
TBMP	Mycobacteria Probe Ident	No	No
TBPB	Mycobacteria Probe Ident Broth	No	No
ISMY	ID by 16S Sequencing	No	No
SRG	Susceptibility Rapid Grower	No	No
RSLG	Susceptibility Slow Grower	No	No
SSNS	Susceptibility Nocardia species	No	No
STV1	Susceptibility, Mtb Complex, Broth	No	No
STV2	Susceptibility, Mtb Cx, 2nd Line	No	No
STVP	Susceptibility, Mtb Complex, PZA	No	No

MTBVP	Mtb PZA Confirmation, pnc A Sequence	No	No
MIC	Susceptibility, MIC	No	No
LCTB	Id, MTB complex Rapid PCR	No	No

Method Name

Nucleic Acid Probe/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

Shipping Instructions

1. See Infectious Specimen Shipping Guidelines in Special Instructions 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 nearly 200 recognized species of mycobacteria and more than 100 *Nocardia* species. Many 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.

Nucleic acid hybridization probes are utilized that identify specific ribosomal RNA sequences of *Mycobacterium tuberculosis* complex, *Mycobacterium avium* complex, and *Mycobacterium gordonae.* Other *Mycobacteria* species, *Nocardia* species, and other aerobic actinomycete genera are identified using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS) or nucleic acid sequencing of a 500-base pair region of the 16S ribosomal RNA gene.

After identification, antimicrobial susceptibility testing is performed following Clinical and Laboratory Standards Institute (CLSI) 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. Pfyffer GE: *Mycobacterium:* general characteristics, laboratory detection, and staining procedures. In: Jorgensen JH, Pfaller MA, eds. Manual of Clinical Microbiology. 11th ed. Vol 1. ASM Press; 2015:536-569

2. Clinical and Laboratory Standards Institute: Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes. 2nd ed. CLSI document M24-A2. Clinical and Laboratory Standards Institute; 2011

Performance

Method Description

The Gen-Probe AccuProbe system uses an acridinium-labeled, single-stranded DNA probe that is complementary to the ribosomal RNA of the target organism. After the ribosomal RNA is released from the organism, the labeled DNA probe combines with the target organism's ribosomal RNA to form a stable DNA:RNA hybrid. Chemiluminescence is used as an indicator to detect specific hybrids.(Musial CE, Tice LS, Stockman L, Roberts GD: Identification of *Mycobacteria* from culture by using Gen-Probe rapid diagnostic system for *Mycobacterium avium* complex and *Mycobacterium tuberculosis* complex. J Clin Microbiol. 1988 Oct;26[10]:2120-2123)

The 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, 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 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.
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.(CLSI: Susceptibility Testing of *Mycobacteria*, *Nocardiae*, and Other Aerobic *Actinomycetes.* 2nd ed. CLSI document M24-A2. CLSI; 2011; Woods GL, Lin SYG, Desmond EP: Susceptibility test methods: *Mycobacteria*, *Nocardia*, and Other Actinomycetes. In: Jorgensen JH, Pfaller MA, Carroll KC, et al, eds. Manual of Clinical Microbiology. Vol 1. 11th ed. ASM Press; 2015:1356-1378)
M tuberculosis first-line susceptibilities are tested with the presence of critical concentrations of the antimycobacterial drugs isoniazid, rifampin, and ethambutol. One of 2 FDA-cleared platforms may be used. Second-line susceptibilities utilize the MycoTB broth microtiter dilution plate.(Trek Diagnostic Systems Inc.)
Antimicrobials included in the assay are tested according to the CLSI guidelines. The plate contains lyophilized antimicrobials, which are rehydrated prior to testing. A standardized suspension of the *M tuberculosis* isolate is added to the plate wells and the plate is incubated at 36 degrees C in 5% to 10% CO2 for up to 14 days. The first drug-containing well with no visible growth is determined to be the endpoint.(CLSI: Susceptibility Testing of *Mycobacteria*, *Nocardiae*, and Other *Actinomycetes*; Approved Standard. CLSI document M24-A2, 2011; Hall L, Jude KP, Clark SL, et al: Evaluation of the Sensititre MycoTB plate for susceptibility testing of the *Mycobacterium tuberculosis* complex against first- and second-line agents. J Clin Microbiol. 2012;50:3732-3734)

PDF Report
No

Performing Laboratory Location
Rochester

Fees & Codes

Test Classification
This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test 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)
87153-Mycobacteria Identification by Sequencing (if appropriate)
87150-Mycobacteria Probe Ident, Solid (if appropriate)
87150-Mycobacteria Probe Ident, Broth (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-Suscetpbility, 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)