Test Definition: TBNGS

Susceptibility, Mtb Complex, NGS

Overview

Useful For

Molecular detection of drug resistance variants in culture isolates of the *Mycobacterium tuberculosis* complex

May provide a more rapid detection of drug resistance than phenotypic, broth-based testing

Aiding in the resolution of discrepant results obtained using phenotypic methods testing for *M tuberculosis* isolates that are not sufficiently viable to allow for culture-based testing

Highlights

This assay may provide results within 1 week as opposed to phenotypic broth-based or agar-based assays that can take, on average, 14 days to produce results. Additionally, this assay can be useful as an aid in the resolution of discrepant phenotypic results or in instances when the isolate does not grow sufficiently well to allow for phenotypic testing.

Testing Algorithm

Whole genome sequencing (WGS) of *Mycobacterium tuberculosis* complex isolates is performed followed by evaluation of selected genes of interest for the presence of well-characterized, drug resistance-conferring variants.

Traditional broth-based, phenotypic drug resistance testing should also be performed, since not all genes associated with resistance within the *M tuberculosis* complex genome have been fully elucidated or are evaluated in this test. If traditional broth-based phenotypic drug resistance testing is desired, add TB1LN / Antimicrobial Susceptibility, *Mycobacterium tuberculosis* Complex, First Line; TB2LN / Susceptibility, *Mycobacterium tuberculosis* Complex, Second Line; and TBPZA / Susceptibility, *Mycobacterium tuberculosis* Complex, Pyrazinamide.

Special Instructions

- Infectious Specimen Shipping Guidelines

Method Name

Whole Genome Sequencing

NY State Available

Yes

Specimen

Specimen Type

Varies

Additional Testing Requirements

If traditional broth-based phenotypic drug resistance testing is desired, add TB1LN / Antimicrobial Susceptibility, *Mycobacterium tuberculosis* Complex, First Line; TB2LN / Susceptibility, *Mycobacterium tuberculosis* Complex, Second Line; and TBPZA / Susceptibility, *Mycobacterium tuberculosis* Complex, Pyrazinamide.

Shipping Instructions

See Infectious Specimen Shipping Guidelines in Special Instructions

Necessary Information

1. Specimen source is required.

2. Organism identification is required; if not provided then organism identification will be reflexed to CTBID / Culture Referred for Identification, *Mycobacterium* and *Nocardia* with additional charges.

3. Method of identification **is required** (eg, nucleic acid hybridization probes, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), DNA sequencing, line probe assay).

Specimen Required

Supplies: Infectious Container, Large (T146)

Specimen Type: *Mycobacterium tuberculosis* complex isolate growing in pure culture. Isolates older than 5 weeks or not in a pure culture may require subculture for fresh, isolated growth so the turnaround time for results may be delayed.

Container/Tube: Middlebrook (7H10 or 7H11) medium slant; growth in broth medium (eg, Mycobacteria Growth Indicator Tube [7H9] broth) or on a Lowenstein-Jensen medium slant can be sent but turnaround time for results may be delayed because subculture to Middlebrook agar medium may be required. Organisms received in mixture may result in additional charges for isolation and identification.

Specimen Volume: Isolate with visible growth on solid media; if broth is sent, > or =3 mL of broth culture required.

Collection Instructions:

1. Organism must be in pure culture, actively growing. **Do not submit mixed cultures.**

2. Place specimen in a large infectious container (T146) and label as an etiologic agent/infectious substance.

Reject Due To

Other	Agar plate Mixed culture

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)	7 days	
	Refrigerated	7 days	

Clinical and Interpretive

Clinical Information

An important component of disease management for patients with tuberculosis is testing of *Mycobacterium tuberculosis* complex isolates for resistance to first- and second-line antituberculous medications. Phenotypic culture-based drug resistance testing is often performed using broth methods since they are more rapid than the gold-standard agar proportion method. However, even the rapid broth methods require approximately 14 days culture and identification of the isolate as *M tuberculosis* complex before susceptibility testing can be performed.

This whole genome sequencing (WGS) testing provides molecular detection of well-characterized drug-resistance variants in *M tuberculosis* complex by sequencing *M tuberculosis* isolates.It is intended to aid in the detection of resistance to first- and second-line antituberculous agents including isoniazid, rifampin, ethambutol, pyrazinamide, the fluoroquinolones (moxifloxacin and ofloxacin) and the aminoglycosides (streptomycin, kanamycin, and amikacin). This testing evaluates selected genes of interest including:

Drug/Drug Class	Gene
Isoniazid	*ahpC*
	fabG1
	inhA
	katG
Rifampin	*rpoB*
Ethambutol	*embB*
Pyrazinamide	*pncA*
Fluoroquinolones	*gyrA*
Aminoglycosides	*eis*
	gidB
	rpsL
	rrs

Reference Values

Results are reported as variant detected or no variant detected.

Interpretation

Variants detected in the queried genes of *Mycobacterium* Â *tuberculosis* complex that are highly associated with drug resistance are reported along with an indication of how often the detected gene variant correlated with phenotypic culture-based drug resistance in a verification study of the whole genome sequencing method. For example, detection of an *rpoB* S450L variant would be reported as "*rpoB* S450L" and a comment would be included on the report stating "probable rifampin resistance; in a study of 173 isolates, 35/35 (100%) of isolates with this variant were resistant to rifampin".

If no variants associated with drug resistance are detected in the *M tuberculosis* complex isolate, a "no variant detected" result is reported along with an indication of how often isolates in the verification study that displayed phenotypic culture-based drug resistance had a variant in the evaluated gene. For example, if no variant was detected in the *gyrA* gene, the report would indicate "No variant detected" and a comment stating "In a study of 173 isolates, 22/23 (95.7%) of fluoroquinolone resistant isolates had a variant in *gyrA*."

Genetic variants of unknown significance are not reported.

Cautions

The Mayo genetic variant database contains more than 380 high-confidence variants in selected genes within the *Mycobacterium tuberculosis* complex that are strongly associated with drug resistance. There may be other genetic variants in the queried genes, or additional genes not examined, that have an undefined correlation with resistance in *M tuberculosis* complex. Therefore, traditional phenotypic antimicrobial resistance testing is required to supplement the genotypic sequencing results.

The absence of a genetic variant in this assay does not indicate that the isolate is susceptible to an antimicrobial agent since not all genes in the *M tuberculosis* complex are queried and since the effect of genetic variant combinations is currently unknown.

The detection of a variant may not imply phenotypic resistance as the gene may not be expressed, may be expressed in low levels, or may be nonfunctional.

Clinical Reference

1. Kozyreva VK, Truong C-L, Greninger AL, et al: Validation and Implementation of a Clinical Laboratory Improvements Act-Compliant Whole Genome Sequencing in the Public Health Microbiology Laboratory. J Clin Microbiol 2017;55:2502-2520

2. Shea J, Halse TA, Lapierre P, et al: Comprehensive Whole-Genome Sequencing and Reporting of Drug Resistance Profiles on Clinical Cases of *Mycobacterium tuberculosis* in New York State. J Clin Microbiol 2017;55:1871-1882

3. Campbell PJ, Morlock GP, Sikes RD, et al: Molecular Detection of Mutations Associated with First- and Second-Line Drug Resistance Compared with Conventional Drug Susceptibility Testing of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2011;55:2032-2041

Performance

Method Description

Mycobacterium tuberculosis complex isolates are lysed and genomic nucleic acid is purified and quantified. Library preparation for whole genome sequencing is performed using 50-100 ng of the purified *M tuberculosis* complex DNA and the Illumina TruSeq Nano DNA Library Preparation Kit. Following library preparation, samples are quantitated and pooled on an Illumina sequencer for multiplexed, paired-end whole genome sequencing. Sequences obtained are compared to the *M tuberculosis* reference genome (strain H37Rv) to identify variants. Variant review and interpretation is completed by comparing the any variants detected with the Mayo Experience Database and any matching variants are reported. A minimum of 40X position coverage, 20X variant coverage, and 15% variant frequency compared to the H37Rv reference genome is required before reporting a variant. Variants of unknown significance are not reported.(Unpublished Mayo method)

PDF Report

No

Day(s) and Time(s) Test Performed

Tuesday, Thursday

Analytic Time

7-14 days

Maximum Laboratory Time

14 days

Specimen Retention Time

1 Year

Performing Laboratory Location

Rochester

Fees and 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 Regional Manager. 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. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

81479

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
TBNGS	Susceptibility, Mtb Complex, NGS	94053-6

Result ID	Test Result Name	Result LOINC Value
TBOR	Organism Identification	9825-1
TBIS	Culture isolate grown from specimen source	31208-2
602791	rpoB	94065-0
603383	rpoB Interpretation	89489-9
603384	katG	94054-4
603385	katG Interpretation	89488-1
603386	inhA	94055-1
603387	inhA Interpretation	89488-1
603388	fabG1	94056-9
603389	fabG1 Interpretation	89488-1
603390	ahpC	94057-7
603391	ahpC Interpretation	89488-1
603392	embB	94058-5
603393	embB Interpretation	89491-5
603394	pncA	94059-3
603395	pncA Interpretation	92242-7
603396	gyrA	94060-1
603397	gyrA Interpretation	89487-3
603398	gidB	94061-9
603399	gidB Interpretation	89490-7
603400	rrs	94062-7

Result ID	Test Result Name	Result LOINC Value
603401	rrs Interpretation	89490-7
603402	rpsL	94063-5
603403	rpsL Interpretation	89490-7
603404	eis	94064-3
603405	eis Interpretation	89490-7
603535	Method	85069-3