MAYO CLINIC LABORATORIES

Test Definition: MTBPZ

Mtb PZA Resistance, pncA Sequencing

Overview

Useful For

Detection of genotypic resistance to pyrazinamide by *Mycobacterium tuberculosis* complex isolates

Testing Algorithm

When this test is ordered, the reflex test may be performed and charged.

Special Instructions

- Infectious Specimen Shipping Guidelines

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
MTBVP	Mtb PZA Confirmation, pncA Sequence	No	No

Method Name

DNASequencing

NY State Available

Yes

Specimen

Specimen Type

Varies

Shipping Instructions

1. See Infectious Specimen Shipping Guidelines in Special Instructions.
2. Place specimen in a large infectious container (T146) and label as an etiologic agent/infectious substance.

Necessary Information

Specimen source and suspected organism identification are required.

Specimen Required

Specimen Type: Organism

Supplies: Infectious Container, Large (T146)

Container/Tube: Middlebrook 7H10 agar slant

Specimen Volume: Isolate

Collection Instructions: Organism must be in pure culture, actively growing.

Forms

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

Reject Due To

Other Agar plate

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

The protein product of the *Mycobacterium tuberculosis* complex *pncA* gene is an enzyme that is responsible for activation of the prodrug pyrazinamide (PZA). DNA sequencing of the *Mycobacterium tuberculosis* complex *pncA* gene can be used to detect mutations that correlate with in vitro PZA resistance.(1,2) The sequencing result can be available in as little as 1 day after the *Mycobacterium tuberculosis* complex isolate grows in culture, thereby providing a more rapid susceptibility result than the average 10 to 14 days required by phenotypic broth methods.

Reference Values

Pyrazinamide resistance not detected

Interpretation

Polymorphisms in the *pncA* gene that have been previously correlated in our laboratory with pyrazinamide (PZA) resistance will be reported as "Mutation was detected in *pncA* suggesting resistance to pyrazinamide."
Wild-type *pncA* or a silent *pncA* gene polymorphism (ie, no change in the amino acid translation) will be reported as "No mutation was detected in *pncA*."
New polymorphisms in the *pncA* gene that have not previously been seen in our laboratory will require additional testing using a reference broth method to determine their correlation with PZA resistance.

Cautions

According to the literature,(3) 72% to 97% of pyrazinamide (PZA)-resistant clinical isolates carry mutations in the *pncA* gene or promoter region. However, other resistance mechanisms (eg, changes in PZA uptake or increased PZA efflux) will not be detected by this method.
Correlation of the in vitro sequencing result with clinical presentation is strongly recommended.

Supportive Data

Sequencing Result	ATCC or PT Isolate Broth Susceptibility Result	% Categorical Agreement
Susceptible	Resistant	pncA wild-type or silent SNP(a)
15	0	100%
pncA polymorphisms	0	6
Sequencing Result	Arbitrated(a) Broth Susceptibility Testing Result	% Categorical Agreement
Susceptible	Resistant	pncA wild-type or a silent SNP
102	0	100%

pncA polymorphisms	0	39
Nucleotide Position(S) in pncA Coding Region	Codon Change	Amino Acid Change
Pyrazinamide Broth Susceptibility Result	35	GAC-GCG
Asp-Ala	resistant	106 and 107
GC insertion	insertion	resistant
151	CAC-GAC	His-Asp
resistant	152	CAC-CGC
His-Arg	resistant	153
CAC-CAA	His-Gln	resistant
169	CAC-GAC	His-Asp
resistant	195	TCC-TCT
Ser-Ser	susceptible	202
TGG-CGG	Trp-Arg	resistant
222	AGC-AGT	Ser-Ser
susceptible	249	1 nt deletion
deletion	resistant	289
GGT-AGT	Gly-Ser	resistant
290	1 nt deletion	deletion
resistant	306	GCG-GCA
Ala-Ala	susceptible	322
GGA-TGA	Gly-Stop	resistant
374	GTC-GGC	Val-Gly
resistant	395	GGT-GCT

Clinical Reference

1. Somoskovi A, Dormandy J, Parson LM, et al: Sequencing of the *pncA* Gene in members of the *Mycobacterium tuberculosis* complex has important diagnostic applications: identification of a species-specific *pncA* mutation in "*Mycobacterium canettii*" and the reliable and rapid predictor of pyrazinamide resistance. J Clin Microbiol 2007;45:595-599

2. Dormandy J, Somoskovi A, Kreiswirth BN, et al: Discrepant results between pyrazinamide susceptibility testing by the reference BACTEC 460TB method and *pncA* DNA sequencing in patients infected with multi-drug resistant W-Beijing *Mycobacterium tuberculosis* strains. Chest 2007;131:497-501

3. Somoskovi A, Parson LM, Salfinger M: The molecular basis of resistance to isoniazid, rifampin, and pyrazinamide in *Mycobacterium tuberculosis*. Respir Res 2001;2:164-168

Performance

Method Description

Organisms identified as *Mycobacterium tuberculosis* complex using the *Mycobacterium tuberculosis* AccuProbe(GenProbe, San Diego, CA) are lysed using the PrepMan Ultra lysis buffer. Using the *pncA* primers described by Shenai and colleagues, an approximately 700 bp-PCR product is generated that flanks the entire *pncA* gene and the upstream promoter region. The PCR product is cleaned and sequenced using the Big Dye terminator v 1.1 Cycle

Sequencing reagents(Applied Biosystems). Results are analyzed versus the wild-type *pncA* sequence using MicroSeq Microbial ID software (v2.0). A custom library of non-wild-type sequences was constructed in MicroSeq. An exact match to the custom nucleotide library is required to report the result.(Shenai S, Rodrigues C, Sadani M, et al: Comparison of phenotypic and genotypic methods for pyrazinamide susceptibility testing. Indian J Tuberc 2009;56:82-90)

PDF Report

No

Specimen Retention Time

1 year

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

87153-Mtb PZA Confirmation, pncA Sequence