MAYO CLINIC LABORATORIES

Test Definition: MTBPZ

Mycobacterium tuberculosis Complex, Pyrazinamide Resistance by pncA DNA Sequencing, Varies

Overview

Useful For

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

Reflex Tests

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

Testing Algorithm

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

Special Instructions

- Infectious Specimen Shipping Guidelines

Method Name

DNA Sequencing

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

Specimen source and suspected organism identification are required.

Specimen Required

Specimen Type: Organism

Supplies: Infectious Container, Large (T146)

Container/Tube: Middlebrook 7H10 agar slant

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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

Agar plate	Reject

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."

Wildtype *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

The correlation between *pncA* sequencing results and in vitro broth susceptibility test results was evaluated using 21 reference strains of *Mycobacterium tuberculosis* complex with known broth susceptibility profiles. Nine of 21 isolates were from the American Type Culture Collection (ATCC) and 12 of 21 isolates were from completed and closed Proficiency Testing (PT) testing events from the Center for Disease Control and Prevention (CDC), the College of American Pathologists (CAP), or the New York State Department of Health. Isolates demonstrating a polymorphism by sequencing were resequenced and all isolates had identical results between the first and second sequencing evaluation. Results are presented in Table 1.

Table 1. Accuracy of *pncA* Sequencing for Reference/PT Isolates

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	

(a)SNP=single nucleotide polymorphism; see Table 3 for a description of the silent SNPs detected; a silent SNP does not result in an amino acid change.

pncA sequencing was also compared to a US Food and Drug Administration (FDA)-approved, rapid broth method(VersaTREK, TREK Diagnostic Systems) for 141 *Mycobacterium tuberculosis* complex isolates consisting of 96 clinical isolates and 45 reference strains (ATCC and closed PT). Any discordant results were resolved by additional testing using either the BACTEC 460 or BACTEC MGIT 960 broth methods (Becton Dickinson), which are also FDA-approved. Any isolate that had a polymorphism or that had a sequencing result that did not correlate with the broth susceptibility testing result was resequenced and identical results were found for all isolates between the first and second sequencing run. See Table 2 for *pncA* sequencing versus arbitrated broth susceptibility testing().

Table 2. Accuracy of *pncA* Sequencing vs Arbitrated Broth Susceptibility Testing

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	

(a) for 30 isolates with discrepant VersaTREK broth and *pncA* sequencing results, a second broth method (either BACTEC MGIT 960 or BACTEC 460TB) was performed to determine whether the VersaTREK or sequencing result was correct.

-Sensitivity versus arbitrated broth methods=102/102 x 100=100%
-Specificity vs arbitrated broth methods=39/39 x 100=100%
-Very major error rate=0%
-Major error rate=0%

Table 3 provides a list of the *pncA* polymorphisms found in the validation of this method.

Table 3. *pncA* Nucleotide Polymorphisms Detected In House During Validation

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	Gly-Ala	Resistant
408	GAT-GAC	Asp-Asp	Susceptible
416	GTG-GCG	Val-Ala	Resistant
422	CAG-CCG	Gln-Pro	Resistant
445	7 nt deletion	Deletion	Resistant
484	1 nt deletion	Deletion	Resistant

nt=nucleotide
Silent SNPs were seen at nt positions 195, 222, 306, 408

Interday precision was evaluated by sequencing *Mycobacterium tuberculosis* (ATCC 27294, also known as H37Rv, PZA susceptible), *Mycobacterium bovis* (ATCC 19210, PZA resistant), and water (negative control) 12 times over 10 days. *Mycobacterium tuberculosis* ATCC 27294 gave a 100% match to the wildtype (wt) *pncA* sequence 12 of 12 times with good specimen quality scores (> or =37) and an average consensus length of 682 + /-15 bases. Similarly, *Mycobacterium bovis* ATCC 19210 had a SNP present at *pncA* amino acid position 169, which is consistent with published literature reports for this organism. The 169 SNP was seen 12 of 12 times with good specimen quality scores (> or =40) and an average consensus length of 701 +/-9 bases. Interday precision was done by 2 operators using 2 ABI sequencers (Applied Biosystems) and no interoperator or interinstrument differences in performance were noted.

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(2):595-599
2. Dormandy J, Somoskovi A, Kreiswirth BN, Driscoll JR, Ashkin D, Salfinger M: 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(2):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(3):164-168
4. Bouzouita I, Cabibbe AM, Trovato A, Draoui H, Ghariani A, Midouni B, Essalah L, Mehiri E, Cirillo DM, Slim-Saidi L. Is sequencing better than phenotypic tests for the detection of pyrazinamide resistance? Int J Tuberc Lung Dis. 2018 Jun 1;22(6):661-666. doi:10.5588/ijtld.17.0715

Performance

Method Description

Organisms identified as *Mycobacterium tuberculosis* complex using the *Mycobacterium tuberculosis* AccuProbe (GenProbe) are lysed using the PrepMan Ultra lysis buffer. Using the *pncA* primers described by Shenai and colleagues, an approximately 700 base pair-polymerase chain reaction (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 wildtype *pncA* sequence using MicroSeq Microbial ID software. A custom library of non-wildtype 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, Sukhadia N, Mehta A: Comparison of phenotypic and genotypic methods for pyrazinamide susceptibility testing. Indian J Tuberc. 2009;56(2):82-90)

PDF Report

No

Day(s) Performed

Varies

Report Available
7 to 21 days

Specimen Retention Time
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
87153-Mtb PZA Confirmation, pncA Sequence

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
MTBPZ	Mtb PZA Resistance, pncA Sequencing	46245-7

Result ID	Test Result Name	Result LOINC® Value
MTBPZ	Mtb PZA Resistance, pncA Sequencing	46245-7