

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, pnc A Sequence	No, (Bill Only)	No

Method Name

DNA Sequencing

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

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 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	
<i>pncA</i> wild-type or silent SNP(a)	15	0	100%
<i>pncA</i> 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 FDA-approved, rapid broth method(VersaTREK, TREK Diagnostic Systems, Cleveland, OH) 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, Sparks, MD), 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. The 2 x 2 table for *pncA* sequencing versus arbitrated broth susceptibility testing is shown below in Table 2.

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

Sequencing Result	Arbitrated(a) Broth Susceptibility Testing Result		% Categorical Agreement
	Susceptible	Resistant	
<i>pncA</i> wild-type or a silent SNP	102	0	100%
<i>pncA</i> 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= $102/102 \times 100=100\%$

-Specificity vs arbitrated broth methods= $39/39 \times 100=100\%$

-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 <i>pncA</i> 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 wild-type (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 single nucleotide polymorphism (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, Foster City, CA) 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;45:595-599
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

LOINC® Information

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

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