

Pyrosequencing (PSQ) for XDR TB Screening

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PSQ is a rapid screening technique for molecular detection of drug resistance. For confirmation of PSQ results, culture-based drug susceptibility testing should be performed.

Intended use	Pyrosequencing (PSQ) provides: <ul style="list-style-type: none">• Identification of <i>M. tuberculosis</i> complex (MTBC).• Screening for resistance to INH, RIF, quinolones and injectable drugs.					
Date of implementation	3-26-2012					
Testing schedule	The assay is performed 3-4 times a week. If urgent, additional runs can be scheduled. Turnaround time: 1-3 days.					
Principle	The test involves two steps: <ol style="list-style-type: none">1. Use PCR to amplify the target sequences.2. Use pyrosequencing technology to perform realtime sequencing. The sequencer, PyroMark Q96ID, dispenses one kind of dNTP at a time according to the order specified by the assay. If the dNTP being dispensed is complementary to the first available base in the DNA template, the dNTP will anneal to the template and pyrophosphate (ppi) will be generated. The ppi will trigger a cascade of chemical reactions and result in the emission of light. The light generated is proportional to the dNTP incorporated. The identity of dNTP incorporated represents the base(s) sequenced. The sequence grows when the incorporation of dNTP complementary to the DNA template occurs until the end of the dispensation of dNTPs.					
Specimens	Sediments: NALC-NaOH processed specimens, at least 0.5 ml, and AFB-smear positive (1+ or greater). Ship with cold packs. Cultures: solid media or broth (0.5-1 ml). Ship at room temperature or with cold packs.					
Molecular targets	INH	<i>katG</i> (codon 312-316), <i>inhA</i> promoter and <i>ahpC-oxvR</i> intergenic region				
	RIF	<i>rpoB</i> core region from codons 507 to 533.				
	Quinolones	<i>gyrA</i> from codons 88 to 95.				
	Injectable drugs	<i>rrs</i> , 1397 to 1406				
Performance characterization (130 isolates + 115 sediment specimens from CA)	DST results by MGIT 960 (KAN: by agar proportion)					
	INH (n =245) 0.1 µg/ml	RIF (n = 239) 1.0 µg/ml	Quinolones (n=125) MOX 0.25 µg/ml or LEV 1.5 ug/ml	AMK (n =120) 1.5 µg/ml	CAP (n=119) 3.0 µg/ml	KAN (n=55) 5 µg/ml
Overall agreement	94.3%	98.7%	97.6%	99.2%	99.2%	96.4%
Sensitivity	87.6%	96.3%	87%	100%	100%	85.7%
Specificity	100%	100%*	100%	99%	99%	100%
Limitations	<ul style="list-style-type: none">• Insufficient DNA, or presence of inhibitory substance in sediments will yield invalid results due to no amplification.• Heavily contaminated specimens may decrease the sensitivity due to relatively reduced MTB organisms.					
* For RIF, we have tested RIF MIC on strains with various mutations in the <i>rpoB</i> core region, and identified several mutations that do not confer resistance. When those mutations are detected, we will specify they are not associated with RIF resistance. If a new mutation is identified, we will report that its association with rifampin resistance is unknown.						