

Molecular Beacon Testing

At MDL, CA Department of Public Health

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Intended use	Molecular beacon (MB) test provides: <ul style="list-style-type: none"> • Identification of <i>M. tuberculosis</i> complex (MTBC). • Screening for INH and rifampin (RIF) resistance. 					
Schedule	The assay is performed on Tuesdays & Fridays.					
Principle	<p>The test employs realtime PCR technology—using PCR to amplify the target sequences with simultaneous detection of mutations by molecular beacons. MB is a short oligonucleotide probe with a loop-and-stem structure. One stem is labeled with a fluorophore and the other with a quencher.</p> <ul style="list-style-type: none"> • The loop contains a sequence complementary to the wildtype sequence of MTBC. When a strain of MTBC contains no mutations, the MB will anneal to the amplicon and fluoresce. If a strain of MTBC contains mutations within the MB's target sequence, the MB will not anneal to the amplicon and no fluorescence will be generated. • Due to primer specificity, NTM will not be amplified. 					
Specimens*	<p>Sputum sediments (at least 0.5 mL) with positive AFB-smear (1+ or greater). Ship with cold packs.</p> <p>Growth from solid media or broth (1 ml). Ship at room temp or with cold packs.</p>					
INH resistance	2 MBs are used to detect mutations in the <i>katG</i> gene and the promoter region of the <i>inhA</i> gene where most prevalent mutations have been found.					
Rifampin resistance	3 MBs are used to detect mutations in the core region of the <i>rpoB</i> gene where most prevalent mutations have been found.					
Performance	Initial Study				Overall agreement with phenotypic drug susceptibility testing (3/26/03-4/12/05)	
	Tested 196 archived cultures in 2001-2002					
	Sensitivity	Specificity	PPV	NPV		
	INH	82.7%	100%	100%	98.1% * ¹	95.6%
	RIF	97.5%	100%	100%	99.95% * ²	96.7%
* ¹ Calculated for a prevalence of 10% resistance.						
* ² Calculated for a prevalence of 2% resistance.						
Limitations	<ul style="list-style-type: none"> • Insufficient DNA, Non-TB, or presence of inhibitory substance in sediments will yield invalid results due to no amplification. • Mixed susceptible and resistant populations may be interpreted as susceptible. • Silent mutations (rare) do not confer resistance, but are interpreted as resistant. • Some mutations (rare) in <i>rpoB</i> not conferring resistance to RIF have been detected. • Heavily contaminated specimens may decrease the sensitivity due to relatively reduced target organisms. 					
References	<p>Lin, S-Y G. JCM. 42: 4204-4208, 2004. (Sep. 2004).</p> <p>Lin, S-Y G. Abstract. ICAAC 2005.</p> <p>Piatek, A.S. Antimicrob. Agents Chemother. 44:103-110, 2000.</p>					

* Subsequent testing will be accepted if dates of collection are at least 2 months apart from initial testing and development of drug resistance is strongly suspected.