Pyrosequencing (PSQ) for XDR TB Screening

At MDL, CA Department of Public Health

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

<table>
<thead>
<tr>
<th>Intended use</th>
<th>Pyrosequencing (PSQ) provides:</th>
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<tbody>
<tr>
<td></td>
<td>Identification of <em>M. tuberculosis</em> complex (MTBC).</td>
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<td></td>
<td>Screening for resistance to INH, RIF, quinolones and injectable drugs.</td>
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<tr>
<th>Date of implementation</th>
<th>3-26-2012</th>
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<tr>
<th>Testing schedule</th>
<th>The assay is performed daily excluding weekend. Turnaround time: 1-3 days, median: 1 day. Results are usually available next day following specimen receipt. If retesting is necessary, a preliminary report will be issued.</th>
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<th>Principle</th>
<th>The test involves two steps:</th>
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<td>1. Use PCR to amplify the target sequences.</td>
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<td></td>
<td>2. Use pyrosequencing technology to perform realtime sequencing.</td>
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<tr>
<td></td>
<td>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.</td>
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<tr>
<th>Specimens</th>
<th>Sediments: NALC-NaOH processed specimens with positive AFB-smear (1+ or greater). Volume: at least 0.5 ml, more is preferred. Ship with cold packs. Cultures: solid media or broth (0.5-1 ml with biomass). Ship at room temperature or with cold packs.</th>
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<tr>
<th>Molecular targets</th>
<th>INH</th>
<th><em>katG</em> (codon 312-316), promoter of <em>inhA</em> and <em>ahpC</em>, and fabG1.</th>
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<td></td>
<td>RIF</td>
<td><em>rpoB</em> (codons 507 to 533, and 176).</td>
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<td></td>
<td>Quinolones</td>
<td><em>gyrA</em> (codons 88 to 95).</td>
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<td></td>
<td>Injectable drugs</td>
<td><em>rrs</em> (positions 1397 to 1406).</td>
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| Testing algorithm | 1. Primary panel: loci associated with INH or RIF resistance are tested. |
|                  | 2. Reflexive panel: loci associated with quinolone or injectable drug resistance are tested, when any mutation is detected in the primary panel. |
|                  | *Clinicians may request both panels to be tested at the same time if desired.* |

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<tr>
<th>Performance characterization (130 isolates + 115 sediment specimens from CA)</th>
<th>DST results by MGIT 960 (KAN: by agar proportion)</th>
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<tbody>
<tr>
<td>INH (n=245) 0.1 µg/ml</td>
<td>RIF (n=239) 1.0 µg/ml</td>
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<tr>
<td>Overall agreement</td>
<td>94.3%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>87.6%&lt;sup&gt;1&lt;/sup&gt;</td>
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<tr>
<td>Specificity</td>
<td>100%</td>
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| Limitations | *Insufficient DNA, or presence of inhibitory substance in sediments will yield invalid results.* |
|            | *Heavily contaminated specimens may decrease the sensitivity due to relatively reduced MTB organisms.* |

<sup>1</sup> Addition of fabG1 and rpoB-176 increases the sensitivity for detecting INH resistance to 88.5%, and that for RIF to 96.5%.<sup>2</sup> For RIF, we have tested RIF MIC on strains with various mutations in the rpoB core region, and identified several mutations that do not confer resistance by grow-based DST. We will specify those mutations in the report.

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

PSQ factsheet 1/8/16