Diagnostic Implications of Inconsistent Results Obtained with the Xpert MTB/Rif Assay in Detection of Mycobacterium tuberculosis Isolates with an rpoB Mutation Associated with Low-Level Rifampin Resistance

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Xpert-MTB/Rif is one of the most frequently used molecular screening tests for multidrug-resistant tuberculosis worldwide. We report false-negative assay results in the presence of rpoB Leu533Pro, which is associated with low-level phenotypic rifampin resistance. Accurate and timely confirmation of rifampin susceptibility results obtained with Xpert-MTB/Rif is imperative.

The accurate diagnosis of drug-resistant and multidrug-resistant (MDR) tuberculosis (TB) is imperative to initiate adequate treatment, to avoid transmission of the disease, and to prevent the development of further drug resistance. Because of its worldwide rollout and rapid implementation, the Xpert-MTB/Rif assay (Cepheid) has become one of the most frequently used molecular screening tests for TB and MDR TB in both resource-poor and resource-rich countries (1). Recently, a 67-year-old Swiss-born male patient was admitted to a secondary-care hospital in Switzerland with clinical and radiologic suspicion of pulmonary TB. Rapid testing by Xpert-MTB/Rif showed the presence of Mycobacterium tuberculosis complex in two sputum samples (Table 1; samples 1 and 2). In addition, these specimens showed indeterminate and definite rifampin (RMP) resistance, respectively (Table 1, samples 1 and 2). Since the patient had no history of TB and was from Switzerland (a low MDR TB incidence setting), based on previous reports of false RMP resistance assay results (2, 3), the clinician doubted the accuracy of the Xpert-MTB/Rif RMP results. Therefore, an additional four sputum samples were submitted to the Swiss National Reference Center for Mycobacteria for rapid confirmation before initiation of MDR TB therapy. Further patient testing revealed HIV positivity.

The four additional sputum specimens (Table 1, samples 3 to 6) were acid-fast smear positive by the Ziehl-Neelsen method (4), and Xpert-MTB/Rif testing detected the presence of M. tuberculosis complex. Surprisingly, all of the samples were scored RMP susceptible by the molecular assay (Table 1). In order to resolve the discrepant Xpert-MTB/Rif results and to rapidly confirm or rule out MDR TB, direct rpoB sequencing of the 81-bp core region and additional molecular screening for isoniazid (INH) resistance were performed as described previously (3, 5) and revealed rpoB Leu(CTG)533Pro(CCG) and katG Ser(AGC)315Thr(ACC) mutations, respectively, in all four specimens. Growth detection and quantitative phenotypic drug susceptibility testing (DST) for first- and second-line antituberculosis drugs with the MGIT 960 system and EpiCenter software with the TB eXIST module (Becton, Dickinson Microbiology Systems, Sparks, MD) were performed as described earlier (6). Quantitative DST identified resistance to RMP at 0.5 µg/ml and susceptibility at 1.0, 4.0, and 20 µg/ml, and resistance to rifabutin at 0.1 µg/ml and susceptibility at 0.4 and 2.0 µg/ml. DST for INH showed resistance at 0.1 and 1.0 µg/ml and intermediate resistance at 3.0 and 10.0 µg/ml. No drug resistance was identified by conventional DST for other first- and second-line drugs. Direct molecular results were confirmed by DNA sequencing of the rpoB and katG genes of the culture isolates and gave concordant results.

Previously, in vitro experiments indicated that Xpert-MTB/Rif cannot detect Leu533Pro unless 100% of the DNA population was mutant (7). PCR followed by DNA sequencing directly from the sputum samples or cultures did not show double peaks (potential signs of heteroresistance) at the corresponding Leu533Pro mutant position in the sequence electropherograms. Therefore, we have no indications from molecular testing that a heteroresistant population was present in the patient. Moreover, the clinical information also argues against heteroresistance since resistance in patients with newly diagnosed TB (such as our case) is usually associated with drug resistance in a large proportion of the population compared to patients with acquired drug resistance who were previously treated for tuberculosis (8).

Detailed analysis of the Xpert-MTB/Rif assay parameters revealed that rpoB probe E (encompassing codon 533) was hybridizing significantly less than the other four probes and showed ΔCₜ max values markedly lower than 5 (the Xpert-MTB/Rif software uses a ΔCₜ max cutoff of >5 for the automated detection of RMP resistance) (9) but that did not result in RMP resistance identification by the Xpert software (version 4.3) (Table 1). The bacterial loads of the different specimens were variable, which may have resulted in variable probe E hybridization and lack of detection of mutation Leu533Pro (Table 1). These findings may warrant a thorough revision of the probe E sequence length and its hybridization and software detection parameters. Our report clearly shows that Xpert-MTB/Rif can produce false RMP susceptibility results in the presence of mutation Leu533Pro in clinical speci-
TABLE 1: Xpert-MTB/Rif results and assay parameters of six sputum specimens tested at the primary laboratory (samples 1 and 2, gray shading) and at the Swiss National Reference Center for Mycobacteria (samples 3 to 6).

<table>
<thead>
<tr>
<th>Sample</th>
<th>RMP resistance detection/bacterial load</th>
<th>Cartridge</th>
<th>RMP resistance</th>
<th>Endpoint</th>
<th>$\Delta C_t$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes/very low</td>
<td>G4</td>
<td>Indeterminate</td>
<td>Probe A</td>
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<tr>
<td>2</td>
<td>Yes/low</td>
<td>G4</td>
<td>Indeterminate</td>
<td>Probe B</td>
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<tr>
<td>3</td>
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<td>Probe C</td>
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<tr>
<td>4</td>
<td>Yes/medium</td>
<td>G3</td>
<td>No</td>
<td>Probe D</td>
<td>25.5</td>
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<tr>
<td>5</td>
<td>Yes/medium</td>
<td>G3</td>
<td>No</td>
<td>Probe E</td>
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</tr>
<tr>
<td>6</td>
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<td>G3</td>
<td>No</td>
<td>Probe F</td>
<td>23.0</td>
</tr>
</tbody>
</table>

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**M. tuberculosis complex**

**RMP resistance**

**endpoint**

**$\Delta C_t$ value**

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**Probe E**

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**REFERENCES**


