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# Integrating the Xpert MTB/RIF Assay into a Diagnostic Workflow for Rapid Detection of *Mycobacterium tuberculosis* in a Low-Prevalence Area

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**The Xpert MTB/RIF assay is a rapid and fully automated real-time PCR assay. The performance of the Xpert MTB/RIF assay as a primary screening test for urgent clinical specimens was evaluated during a 2-year period. The results showed that replacing smear microscopy with the Xpert MTB/RIF assay facilitates laboratory handling and improves the sensitivity and specificity of *Mycobacterium tuberculosis* detection.**

Rapid and accurate diagnosis of tuberculosis (TB) is indispensable to adequately manage the disease and control its transmission. Acid-fast bacilli (AFB) smear microscopy is an established low-cost screening procedure to identify patients with tuberculosis. However, the sensitivity of smear microscopy is low, varying between 22 and 80% (1). In general, the routine application of nucleic acid amplification techniques for detection of *Mycobacterium tuberculosis* results in accurate diagnosis of tuberculosis (2, 3, 4, 5, 6) but requires laborious processing time and dedicated biosafety conditions. The recently introduced Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) is a fully automated, walk-away real-time PCR-based assay with a time to result of approximately 2 h. To detect *M. tuberculosis* and mutations associated with resistance to rifampin (RIF), the 81-bp core region of the *rpoB* gene is amplified and probed with five overlapping molecular beacons (7). *M. tuberculosis* is identified when at least 2 probes give a positive signal within a predefined number of cycles. A RIF mutation is detected by lack of or delayed onset of fluorescence of at least one molecular beacon. Disadvantages of the Xpert MTB/RIF are its exceeding costs and a reduced sensitivity in comparison with other PCR *M. tuberculosis* assays (8, 9).

The objective of the present study was to evaluate the feasibility of the Xpert MTB/RIF assay to replace direct smear microscopy as a primary screening test for urgent clinical specimens in a setting of low TB prevalence.

The Institute of Medical Microbiology (University of Zurich) is a tertiary care diagnostic center that receives clinical specimens 7 days/week for AFB microscopy to urgently confirm or rule out TB in newly identified suspect cases. Such samples are marked as urgent cases and are not to be confused with regular samples submitted to the mycobacteriology laboratory. All respiratory and nonrespiratory specimens submitted for urgent smear microscopy between July 2010 and June 2012 were included in this study; ethical approval was not needed. Specimens were adjusted to 5 ml with sterile distilled water when the specimen volume was <5 ml. The Xpert MTB/RIF assay was performed following the manufacturer's instructions for respiratory specimens, using a 1-ml aliquot. Nonrespiratory specimens were tested similarly. The remaining 4 ml of all unprocessed specimen with the exception of cerebrospinal fluid (CSF) was homogenized and decontaminated using an equal volume of *N*-acetyl-L-cysteine (NALC)–2% NaOH for 15 min (10). After neutralization with phosphate buffer (67 mM, pH 6.8) and centrifugation, the resuspended sediment was used to prepare auramine-rhodamine-stained smears and to inoculate MGIT 960 liquid (Becton, Dickinson, Towson, MD) and Middlebrook 7H11 culture media for growth detection. Positive auramine-rhodamine microscopy results were confirmed by Ziehl-Neelsen staining for specificity. AFB growth-positive cultures were identified by 16S rRNA gene sequence analysis as described previously (11, 12, 13). Further differentiation within the *M. tuberculosis* complex (MTC) was achieved using the Genotype MTBC line probe assay (LPA; Hain Lifescience GmbH, Nehren, Germany). Phenotypic drug susceptibility testing (DST) of MTC isolates was performed using the Bactec MGIT 960 system (Becton, Dickinson, Towson, MD) (14). A line probe assay (AID Analytika, Strasburg, Germany) was performed on the processed sediment of a subset of Xpert MTB/RIF-positive specimens to confirm molecular drug resistance results for rifampin (RIF) according to the manufacturer's instructions. Alternative nucleic acid amplification testing for detection of *M. tuberculosis* with the Cobas TaqMan MTB test (Roche Diagnostics, Rotkreuz, Switzerland) was carried out in parallel on specimen sediments.

In total, 71 respiratory and 8 nonrespiratory specimens were tested with the Xpert MTB/RIF assay in a prospective fashion (Tables 1 and 2). The Xpert MTB/RIF assay was positive for *M. tuberculosis* in 17 (21.5%) specimens and negative for *M. tuberculosis* in 60 (76%) specimens. The test was invalid or did not provide a result for *M. tuberculosis* detection in 2/79 (2.5%) specimens (1 sputum and 1 biopsy specimen). Both were smear negative and culture negative (Table 2, specimens 24 and 25). The biopsy specimen gave no Xpert MTB/RIF result due to a negative internal control, suggesting that the specimen contained PCR inhibitors.

Fourteen of the 17 Xpert *M. tuberculosis*-positive specimens were smear positive, and 3/17 were smear negative (Tables 1 and 2). Growth detection confirmed the presence of *M. tuberculosis* in

**Received** 17 January 2013 **Returned for modification** 26 February 2013
**Accepted** 17 April 2013
**Published ahead of print** 24 April 2013
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doi:10.1128/JCM.00151-13

**TABLE 1** Xpert MTB/RIF versus AFB smear microscopy and culture results for 79 clinical specimens[a]

| Xpert MTB/RIF result | Microscopy and culture results | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Smear-positive specimens ($n = 19$) | | | Smear-negative specimens ($n = 60$) | | | All specimens ($n = 79$) | | |
| | No. of cultures positive for MTC | No. of cultures positive for NTM | No. of negative cultures | No. of cultures positive for MTC | No. of cultures positive for NTM | No. of negative cultures | No. of cultures positive for MTC | No. of cultures positive for NTM | No. of negative cultures |
| *M. tuberculosis* detected | 14 | 0 | 0 | 1 | 0 | 2[c] | 15 | 0 | 2 |
| *M. tuberculosis* not detected | 0 | 5 | 0 | 3[b] | 2 | 50 | 3 | 7 | 50 |
| Not interpretable | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 |
| Total | 14 | 5 | 0 | 4 | 2 | 54 | 18 | 7 | 54 |

[a] Among the 79 specimens were 71 respiratory specimens (57 sputa, 10 bronchial aspirates, and 4 bronchoalveolar lavage fluids) and 8 nonrespiratory specimens (3 biopsies, 1 CSF, 1 ascitic fluid, 1 pleural fluid, 1 pus, and 1 pharynx aspirate).
[b] All nonrespiratory specimens.
[c] Both were respiratory specimens.

all 14 smear-positive specimens and in 1 of 3 smear-negative specimens. One of the culture- and smear-negative specimens (Table 2, specimen 8) was a sputum sample from a patient with miliary tuberculosis following intravesical *Mycobacterium bovis* BCG instillation therapy for urine bladder cancer. This sample scored "MTB detected very low" by the Xpert MTB/RIF assay but scored negative by the Cobas TaqMan MTB assay, presumably due to specimen inhomogeneity. The second culture- and smear-nega-

**TABLE 2** Comparison of the Xpert MTB/RIF assay with culture identification and phenotypic RIF susceptibility testing for specimens positive or indeterminate for mycobacteria by culture or Xpert assay ($n = 29$)

| Specimen type and no(s). | Specimen source[a] | Xpert assay[b] | | AFB smear result[c] | Culture identification[d] | Rifampin susceptibility (culture based)[e] | Roche Cobas TaqMan *M. tuberculosis* identification |
|---|---|---|---|---|---|---|---|
| | | *M. tuberculosis* detection | Rifampin resistance | | | | |
| Respiratory | | | | | | | |
| 1 | BAL | Negative | ND | Negative | *M. avium* | ND | Negative |
| 2 | Br asp | Negative | ND | Negative | *M. chimaera* | ND | Negative |
| 3 | Sputum | Negative | ND | Positive | *M. kansasii* | ND | Negative |
| 4, 5, 6 | Sputum | Negative | ND | Positive | *M. vulneris* | ND | Negative |
| 7 | Sputum | Negative | ND | Positive | *M. avium* | ND | ND |
| 8 | Sputum | Positive (vl) | Indeterminate | Negative | Negative | ND | Negative |
| 9 | Sputum | Positive (vl) | Indeterminate | Positive | *M. tuberculosis* | Susceptible | Positive |
| 10 | BAL | Positive (vl) | Positive | Negative | *M. tuberculosis* | Susceptible | Positive |
| 11 | Sputum | Positive (vl) | Positive | Positive | *M. tuberculosis* | Susceptible | Positive |
| 12 | Sputum | Positive (l) | Negative | Negative | Negative | ND | Positive |
| 13 | BAL | Positive (l) | Negative | Positive | *M. tuberculosis* | Susceptible | Positive |
| 14, 15, 16 | Sputum | Positive (l) | Negative | Positive | *M. tuberculosis* | Susceptible | Positive |
| 17 | Sputum | Positive (l) | Negative | Positive | *M. tuberculosis* | Susceptible | ND |
| 18, 19 | Sputum | Positive (m) | Negative | Positive | *M. tuberculosis* | Susceptible | Positive |
| 20 | Sputum | Positive (m) | Negative | Positive | *M. africanum* | Susceptible | Positive |
| 21, 22, 23 | Br asp | Positive (m) | Negative | Positive | *M. tuberculosis* | Susceptible | Positive |
| 24 | Sputum | Invalid result | ND | Negative | Negative | ND | Negative |
| Nonrespiratory | | | | | | | |
| 25 | Biopsy | No result | ND | Negative | Negative | ND | Inhibited |
| 26 | CSF | Negative | ND | Negative | *M. africanum* | Susceptible | ND |
| 27 | Ascitic fluid | Negative | ND | Negative | *M. africanum* | Susceptible | Positive |
| 28 | Pleural fluid | Negative | ND | Negative | *M. tuberculosis* | Susceptible | Negative |
| 29 | Tissue | Positive (m) | Negative | Positive | *M. tuberculosis* | Susceptible | Positive |

[a] BAL, bronchoalveolar lavage fluid; Br asp, bronchial aspirate; CSF, cerebrospinal fluid.
[b] vl, very low; l, low; m, medium; ND, not done.
[c] AFB samples with a positive auramine-rhodamine microscopy result confirmed by positive Ziehl-Neelsen staining were scored smear positive.
[d] Identification of NTM isolates was by 16S rRNA sequence analysis, which when necessary was followed by *rpoB* or *hsp*65 sequence analysis; identification of *M. tuberculosis* isolates by PCR was followed by the MTC line probe assay.
[e] Phenotypic drug susceptibility testing for rifampin using the critical concentration of 1 μg/ml.

tive sputum sample (Table 2, specimen 12) originated from a patient undergoing antituberculosis treatment, which may have limited the detection of viable mycobacteria by growth detection. This sample was confirmed *M. tuberculosis* positive by the Cobas TaqMan MTB assay.

Within the 60 Xpert MTB/RIF-negative specimens, 7 specimens (11.7%) yielded nontuberculous mycobacteria (NTM) by culture (3 *Mycobacterium vulneris*, 2 *Mycobacterium avium*, 1 *Mycobacterium kansasii*, and 1 *Mycobacterium chimaera*; all respiratory specimens). Five of these 7 specimens with NTM were smear positive.

The Xpert MTB/RIF correctly identified *M. tuberculosis* in all 14 smear-positive and culture MTC-positive specimens and ruled out tuberculosis in the 5 smear-positive specimens that grew NTM. These results indicate that the Xpert MTB/RIF is a better prescreening tool than smear microscopy, since it reliably identifies the presence of *M. tuberculosis* in patients with a high bacterial load and differentiates these from patients with NTM. This is especially relevant in our setting, since 26.3% (5/19) of smear-positive specimens in this study contained NTM and approximately 49% of all molecularly identified mycobacterial cultures in our laboratory during 2011 and 2012 contained NTM.

Cultures were MTC positive in 3/60 Xpert MTB/RIF-negative samples (Table 2; 1 cerebrospinal fluid specimen [number 26] and 1 ascitic fluid specimen [number 27], both with *Mycobacterium africanum*, and 1 pleural fluid specimen [number 28] with *M. tuberculosis*). All were smear negative. MGIT liquid culture was positive for all three samples, whereas 7H11 solid culture was only positive for the pleural fluid specimen. Previous studies showed a sensitivity of the Xpert MTB/RIF assay that varied between 46 and 77% in smear-negative and culture-positive specimens (7, 8, 15, 16, 17, 18). In our low-prevalence setting, the number of smear-negative and culture MTC-positive cases was low (1 respiratory and 3 nonrespiratory specimens). The Xpert MTB/RIF system correctly detected *M. tuberculosis* in one respiratory specimen, but it missed all 3 nonrespiratory specimens (ascites, pleural fluid, and CSF specimens). This indicates that nonrespiratory specimens may not be suitable for Xpert MTB/RIF testing, in particular for paucibacillary samples.

Conventional drug susceptibility testing (DST) did not reveal any drug resistance among the 18 cultured MTC isolates, while the Xpert MTB/RIF test indicated the presence of *rpoB* mutations associated with RIF resistance in 2 primary respiratory specimens. Repeated conventional DST and molecular discrepancy analysis by line probe assay and sequencing of the *rpoB* gene did not confirm RIF resistance in these specimens. Consequently, the Xpert MTB/RIF result for RIF resistance was scored as a false positive in these specimens (Table 2, specimens 10 and 11). The performance of the Xpert MTB/RIF assay to detect RIF resistance was not evaluated in this study, due to the absence of RIF-resistant MTC isolates. Nevertheless, as described earlier (19), we identified false RIF resistance by using the Xpert MTB/RIF assay for two respiratory specimens. These results have a significant impact on accurate screening for RIF resistance in a setting of low TB and low multidrug-resistant TB prevalence, such as Switzerland. As a consequence, we strongly recommend that in such settings Xpert MTB/RIF assay results indicating RIF resistance always be confirmed by an alternative molecular assay until conventional DST is available (20).

In conclusion, despite its limitations, e.g., its limited suitability for paucibacillary nonrespiratory samples, our results provide strong evidence that for respiratory samples in a low-prevalence setting, the Xpert MTB/RIF assay improves accurate primary screening and is well suited to replace smear microscopy for urgent specimens. The high costs of the Xpert MTB/RIF assay could be compensated by its fast and walk-away methodology compared to the laborious procedure of AFB microscopy, which involves tedious reading of slides and well-experienced personnel for high-quality results.

## ACKNOWLEDGMENTS

This work was supported in part by the University of Zurich and the BAG (Federal Office of Public Health, Switzerland).

We have no conflicts of interest to disclose.

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