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# Comparison of Two Molecular Methods for Rapid Diagnosis of Extrapulmonary Tuberculosis[▽]

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**Application of real-time PCR for the detection of *Mycobacterium tuberculosis* enables results to be obtained in about 2 h. A total of 340 nonrespiratory samples were processed using two real-time PCR assay kits: Xpert MTB/RIF and Cobas TaqMan MTB. The sensitivity and specificity of the Xpert assay were 95% and 100%, respectively, compared to 78% and 98% for the Cobas assay.**

*Mycobacterium tuberculosis* is a major public health concern. Because the bacterium spreads from person to person, effective and, above all, rapid diagnosis is a key objective of worldwide tuberculosis control strategies.

Conventional *M. tuberculosis* detection techniques, based on microscopic examination of Ziehl-Neelsen or auramine-stained specimens, are still in widespread use for diagnostic purposes, even though they fail to provide the required sensitivity and specificity.

Since the discovery of the PCR in the mid-1980s, a number of molecular techniques have been developed which yield a high degree of sensitivity and, above all, specificity for *M. tuberculosis*.

Although various supports have been tested both for direct specimen-based detection and for identification on culture media, growth in a solid or liquid medium is still considered the reference method for the diagnosis of *M. tuberculosis* infection. In 2009, nevertheless, the Centers for Disease Control and Prevention recommended the use of at least one molecular technique per patient for *M. tuberculosis* detection (5).

One of the molecular techniques most widely used for the detection of *M. tuberculosis* in respiratory samples is the commercial PCR kit Cobas Amplicor MTB (Roche Diagnostics, Indianapolis, IN), which has been available since the 1990s (4, 7). Problems have been reported using this kit, particularly with nonrespiratory samples, due to the presence of inhibitor enzymes and contamination (9). Even so, this is still generally considered the molecular reference technique (11, 15).

Although the development of real-time PCR assays has improved the speeds, sensitivities, and specificities of these molecular techniques, the new real-time methods have still not been widely adopted. A number of noncommercial techniques are available for *M. tuberculosis* detection (6, 14).

Cobas TaqMan MTB (Roche Molecular Systems, Branchburg, NJ) is a real-time PCR-based kit using TaqMan hydrolysis probes and primers that bind to a specific, highly conserved region of the *Mycobacterium* genome containing the gene for 16S rRNA. Specimens are prepared manually for the extraction of *M. tuberculosis* DNA, and the TaqMan kit is then used for amplification and detection in batches of 10 samples with two controls, in order to optimize reagent use. This technique is routinely used in modern molecular microbiology laboratories for the diagnosis of tuberculosis in respiratory specimens.

The new Xpert MTB/RIF (Cepheid AB, Bromma, Sweden) assay technique is based on heminested PCR technology and uses 5 molecular probes to confirm *M. tuberculosis* detection; the assay targets the *rpoB* gene of wild-type *M. tuberculosis* strains. The real-time PCR assay consists of a single-use multichambered cartridge preloaded with the buffers and reagents required for sample processing, amplification, and detection. A barcode on each cartridge enables test details to be completed automatically by the software.

This paper reports on a comparison of these two widely used and intensively marketed real-time PCR test kits for the detection of *M. tuberculosis* in extrapulmonary samples, using a standard culture system as the reference method.

A total of 340 consecutive extrapulmonary samples were taken from 289 patients ages 5 to 83 years (mean, 45) between May 2009 and December 2010; males outnumbered females (63.8% versus 30.9%). Sample sources were as follows: 50 cerebrospinal fluid (CSF) samples, 34 pleural fluid samples, 58 articular fluid samples, 20 ascitic fluid samples, 98 biopsy specimens (80 lymph node, 10 lung, 4 stomach, and 4 bone samples), 54 gastric aspirates, 12 pericardial fluid samples, and 14 purulent exudates.

Samples deemed nonsterile (all except CSF and pleural fluid) were decontaminated using the *N*-acetyl-L-cysteine–sodium hydroxide (NALC-NaOH) method. All samples were then centrifuged for 20 min at 3,000 rpm, stained with auramine, and visualized by fluorescent microscopy. They were then inoculated into pyruvate-enriched Lowenstein-Jensen medium and Middlebrook 7H9 broth.

Decontaminated samples were used for the manual extraction of *M. tuberculosis* DNA, as required by the Cobas TaqMan MTB assay protocol, and for the preparation of samples using the Xpert MTB/RIF assay kit.

DNA extractions were prepared using the Amplicor respiratory specimen preparation kit, adding 500 μl of wash solu-

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TABLE 1. Statistical results for the performances of the two molecular assay techniques evaluated, relative to reference culture results[a]

| Assay | No. of positive tests/no. of positive samples (% sensitivity; 95% CI) | No. of negative tests/no. of negative samples (% specificity; 95% CI) | % PPV (95% CI) | % NPV (95% CI) |
|---|---|---|---|---|
| Xpert MTB/RIF | 39/41 (95; 87–100) | 299/299 (100; 99–100) | 100 (98–100) | 99 (98–100) |
| Cobas TaqMan MTB | 32/41 (78; 64–91) | 294/299 (98; 96–99) | 86 (74–98) | 97 (94–99) |

[a] Total of 340 samples tested.

tion to 100 μl of sample. The mixture was shaken and then centrifuged at 12,500 × *g* for 10 min. The supernatant was aspirated with a fine-tip pipette, and 100 μl of lysis reagent was added to the pellet. Tubes were vortexed for 5 s, incubated in a dry-heat block at 60°C for 45 min, pulse centrifuged for 5 s, and neutralized by the addition of 100 μl of neutralizing reagent; finally, tubes were shaken for a further 5 s. Sample eluate was used for the real-time PCR Cobas TaqMan MTB assay (in a TaqMan 48 analyzer) once the appropriate master mix had been prepared. The positive and negative controls supplied with the two kits were used.

For the Xpert MTB/RIF test, 1 ml of decontaminated sample was diluted in 2 ml of the sample buffer included in the assay kit. The solution was vortexed for 15 s and then left to settle for 15 min, with vortexing for 15 s halfway through. A specific volume was collected using the calibrated pipette supplied with the kit and transferred to the cartridge. The software was then programmed, and the cartridge was inserted in the GeneXpert instrument.

The results obtained using these two assay protocols were compared with those of cultures in solid and liquid media and with acid-fast bacillus (AFB) smear results.

Statistical analysis was carried out using EpiInfo version 6.04d.

The culture method was used for reference purposes. AFB smear-positive, culture-negative samples were deemed to be culture positive, for statistical purposes, wherever chart review indicated that a patient with earlier culture-positive specimens had become culture negative following anti-TB (tuberculosis) drug therapy, since PCR assays can detect nonviable mycobacteria.

Of the 340 samples, 41 grew in solid or liquid medium, taking a median time of 14 days. Of these, 39 were identified as positive by Xpert MTB/RIF (the two false negatives—one CSF specimen and one biopsy specimen—were both AFB smear negative). Thirty-two of the 41 were identified as positive using the Cobas TaqMan MTB assay kit. The nine false negatives (3 biopsy specimens, 2 CSF specimens, 2 gastric aspirates, 1 purulent exudate, and 1 pleural fluid specimen) were all AFB smear negative.

The culture-positive specimens included 18 biopsy specimens (16 lymph node and 2 bronchial), 6 cerebrospinal fluid specimens (2 from the same patient), 8 gastric aspirates, 4 pleural fluid specimens, and 5 purulent exudates. AFB smear results were negative in 38 cases and positive in 3 cases.

For the 299 culture-negative samples, Xpert MTB/RIF yielded no positive PCR result, while the Cobas TaqMan MTB assay identified 5 culture-negative specimens as PCR positive (2 biopsy specimens, 1 gastric aspirate, 1 CSF fluid sample, and 1 pleural fluid sample). These possible false positives were attributed to PCR contamination, since there was no sign of *M. tuberculosis* infection in the other specimens from these patients tested using conventional TB detection techniques. Institutional review board (IRB) approval was granted for a chart review, including all five apparently false-positive cases: none of the patients concerned had received anti-TB therapy, and none exhibited signs or symptoms suggestive of tuberculosis. The findings were thus classed as false positives.

Sensitivity and specificity were 95% and 100%, respectively, for the Xpert MTB/RIF assay kit, compared with 78% and 98% for the Cobas TaqMan MTB kit. Positive predictive values (PPV) and negative predictive values (NPV) were 100% and 99%, respectively, for the Xpert MTB/RIF kit, compared with 86% and 97% for the Cobas TaqMan MTB kit.

A 79.9% match was achieved between the two molecular techniques, with a kappa index of 0.8 (95% confidence interval [CI] of 0.7 to 0.9).

Statistical data for performance are shown in Table 1.

Xpert MTB/RIF appears to be as effective in nonrespiratory samples as it has proved to be in preliminary studies using respiratory samples (2, 3, 10). Results for sensitivity were similar to those reported elsewhere (ranging from 90% in AB smear-negative samples to 99% in AFB smear-positive specimens; overall sensitivity, 97%). Findings for specificity (98%) were also similar to those reported by other authors (2, 3, 10).

Specificity and negative predictive values were similar to those recorded for other molecular techniques used in nonrespiratory samples, while sensitivity and positive predictive values in our study were considerably better than the 57% and 78%, respectively, reported for the Cobas Amplicor MTB test in nonrespiratory samples (12) and closer to the 83 to 88% sensitivity reported for the GenProbe amplified *M. tuberculosis* direct (AMTD) assay, also in extrapulmonary samples (1, 8).

In general terms, the Xpert/MTB assay performed better than the Cobas TaqMan MTB; sensitivity, in particular, was substantially greater (87 to 100% versus 64 to 91%). Statistical performance data reported elsewhere for the Cobas TaqMan MTB assay are similar to those indicated here: sensitivity of 79 to 91%, specificity of 98%, PPV of 73%, and NPV of 98% (13).

The high sensitivity recorded for the Xpert MTB test in the present study (95%) may be due to the use of heminested real-time PCR technology rather than simple real-time PCR. A similar sensitivity of 90.2% has been reported elsewhere for AFB smear-negative respiratory samples (most of the nonrespiratory specimens tested in this study were also smear negative) using the Xpert MTB assay kit (3). Moreover, as other authors have suggested, the Xpert MTB/RIF assay system appears to be less susceptible than other PCR-based methods to cross-contamination by amplicons generated by other methods that might give rise to false positives (2).

Both assay techniques tested here were rapid, providing results in less than 2 h, and required little handling by labora-

tory staff. This is particularly true of the Xpert MTB/RIF kit, in which extraction, amplification, and detection take place within a single-use multichambered cartridge, thus ensuring minimal sample contamination. Since manual extraction is recommended when using the Cobas TaqMan MTB assay kit, there is clearly a greater scope for contamination. The Xpert MTB/RIF system allows sample processing to be carried out on demand, sample by sample, rather than having to set up a set of samples (usually at least 10 samples and two controls) in order not to waste reagents, as generally happens with the Cobas TaqMan MTB kit.

Both molecular techniques represent an important contribution to the detection of *M. tuberculosis*, since they can provide results in a matter of hours, whereas the reference culture method takes days. Real-time PCR techniques afford greater sensitivity and specificity and a much-reduced response time, as well as enabling visualization of amplification curves.

One limitation of these techniques is that, in detecting *M. tuberculosis* DNA, they cannot distinguish between viable and nonviable microorganisms. For that reason, although these assays are semiquantitative, they should not be used for monitoring patient progress or treatment efficacy.

Molecular techniques, of course, are considerably more expensive than traditional culture methods using either liquid or solid medium, but they represent a major contribution to the modern-day detection of tuberculosis.

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