SHORT REPORT

Limits of commercial molecular tests for diagnosis of pulmonary tuberculosis

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ABSTRACT: Several studies report high specificity, but variable sensitivity, of Amplified *Mycobacterium tuberculosis* Direct Test (AMTDT, Gen-Probe) based on ribosomal ribonucleic acid (rRNA) amplification and Amplicor *Mycobacterium tuberculosis* test (Amplicor, Roche) based on deoxyribonucleic acid (DNA) amplification for diagnosis of pulmonary tuberculosis.

We have retrospectively evaluated these assays on selected acid-fast bacilli (AFB)-positive and -negative smear specimens and compared the results obtained from nucleic acid amplification with those of AFB staining of semi-quantitative cultures as determined by radiometric Bactec and Löwenstein-Jensen cultures.

In comparison to cultures, Amplicor and AMTDT assays exhibited identical overall sensitivities of 80%, while the staining had a lower sensitivity of 62%. The sensitivities of Amplicor and AMTDT were 98% and 100%, respectively, for the AFB-positive specimens, and 50 and 46%, respectively for the AFB-negative specimens in comparison to cultures.

The sensitivities of both assays appeared similar, and were directly related to the number of bacilli in the specimens studied. The low sensitivity (50%) for smear-negative specimens showed that current amplification assays may be unsuitable to replace cultures for diagnosis of tuberculosis. The decision to perform these molecular techniques should result from close co-operation between clinicians and microbiologists, taking into account the sensitivity results reported here, as well as the expense of the assays.

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Diagnosis of mycobacterial infections traditionally requires a long period of culturing. It may, therefore, benefit from a rapid and reliable diagnostic test. Culture, being the current gold standard, permits drug susceptibility testing but its performance may require up to 6–8 weeks. Direct examination of smears for acid-fast bacilli (AFB) is the most rapid method for detection of mycobacteria. However, it has a low sensitivity (the limit of detection being around 10^4 bacteria·mL⁻¹), and does not differentiate between species of *Mycobacterium* [1]. Novel methods, such as the radiometric Bactec culture technique and the commercially available deoxyribonucleic acid (DNA) probes have considerably reduced the time needed for culture and identification of mycobacteria. However, these procedures still require up to 1–3 weeks to be completed.

Several investigators have reported successful results of detection with specific sequences for *Mycobacterium tuberculosis* (MTB) in clinical specimens by the Amplified *M. tuberculosis* Direct Test (AMTDT) (Gen Probe Inc., San Diego, CA, USA) [1–9] and the Amplicor *M. tuberculosis* test (Amplicor) (Roche Diagnostic Systems, Basel, Switzerland) [1, 10–17]. The AMTDT test is based on amplification of ribosomal ribonucleic acid (rRNA) via DNA intermediates, followed by a chemiluminescent detection of amplicon with an acridinium-ester labelled DNA probe. The Amplicor assay uses the polymerase

chain reaction (PCR) to amplify a specific DNA region corresponding to the 16S rRNA genes, followed by an enzyme-linked immunosorbent assay (ELISA) detection. A high variation in sensitivity (53–100%) is reported for both these assays, mainly when smear-negative samples are included in the reported studies [5, 17].

Methods

We have performed a retrospective evaluation of these two commercially available assays for MTB detection in a selected range of 95 AFB-positive and AFB-negative smear specimens representative of those found in our laboratory in clinical practice.

Specimens were decontaminated by the N-acetyl-L-cysteine NaOH method [18]. One aliquot of sediments was used for auramine-fluorochrome staining, the second for cultures, and two further aliquots were stored at -20°C until the amplification tests were performed. For culture, 0.5 and 0.2 mL of sediments, were inoculated into Bactec Middlebrook vials (Becton Dickinson Diagnostic, Mannes-la-Coquette, France) and onto a Löwenstein-Jensen (LJ) slant (Institut Pasteur Diagnostics, Le Pont de Clair, France), respectively, and incubated at 37°C for 6 and 12 weeks, respectively. Mycobacteria were identified as described previously

[19]. Fifty and 100 µL of sediments were used for the AMTDT and the Amplicor assays, respectively, which were performed according to the manufacturer's instructions. The results obtained were compared with those of AFB staining of semiquantitative culture as determined by the growth index value in radiometric Bactec medium and the number of colony-forming units (cfu) of MTB complex obtained on the LJ slants.

Results and discussion

Of the 95 specimens studied, 69 were positive in cultures for MTB complex. Of these, 43 were AFB-positive smears and 26 were AFB-negative. All MTB culture-positive specimens included in the study were from patients with a confirmed clinical diagnosis of tuberculosis infection. Twenty six specimens were positive in cultures for atypical mycobacteria: *Mycobacterium avium complex* (MAC) in 21 cases, with 10 AFB-positive smears; *Mycobacterium xenopi* and *Mycobacterium kansasii* in two cases, of which one was an AFB-positive smear in each case; and *Mycobacterium gordonae* in one case, with AFB-negative smear.

Of the 69 specimens that were culture-positive for MTB, 55 were detected by the Amplicor and the AMTDT assays. Among the 43 AFB-positive smears showing a time delay for the first positive Bactec reading of 8–15 days and producing ≥ 200 cfu on LJ slants, 42 and 43 specimens were detected by Amplicor and AMTDT, respectively. Of the 26 AFB-negative smears, 9 and 10 specimens were detected by Amplicor and AMTDT respectively, among the 10 specimens showing a time delay for the first positive Bactec reading ≥ 20 days and producing 30–200 cfu on LJ slants. Only four and two specimens were detected by Amplicor and AMTDT, respectively, among the 16 paucibacillary specimens producing only 1–9 cfu on LJ slants and a time delay for the first positive Bactec reading >30 days. No positive results for MTB complex detection were obtained either by Amplicor or AMTDT assays for the 26 specimens which gave atypical mycobacteria in cultures, even in those (12 out of 26 specimens (46%)) which possessed high levels of bacilli as noted by fluorochrome staining.

In comparison to combined liquid and solid medium cultures, which are currently considered as the gold standard [20], Amplicor and AMTDT tests exhibited an overall sensitivity of 80%, while the staining had a lower sensitivity of 62%. Data on amplification assay results for specimens with low numbers of organisms are sparse. The results of most previous studies, therefore, have only limited clinical usefulness since they included preselected samples with a high percentage of smear-positive specimens. The overall reported sensitivities of these tests ranged 65–98% for AMTDT [1–9], and 67–95% for Amplicor [1, 10–17]. Sensitivities have appeared lower (46–74%) as a function of the number of AFB-smear negative specimens included in the studies either for the AMTDT [3, 5, 6] or for the Amplicor [11–13, 16, 17] assays.

In the present study, we have correlated the sensitivity of these two commercially available assays with the MTB cfu number. The sensitivity of approximately 50% for the two amplification assays for AFB-smear nega-

tive specimens was similar to that found by some other investigators [6, 16]. The low sensitivity of both these assays may also be explained by the type of culture used as gold standard [20]. Indeed, when liquid and solid media cultures were combined, a total of 0.7 mL of the sediment was used for culture, although 0.1 and 0.05 mL of the same sediment were used for Amplicor and AMTDT assays, respectively.

No false-positive results were obtained in the present study for specimens with atypical mycobacteria. Our results permit the use of these tests for rapid diagnosis of tuberculosis in patients whose specimens are AFB-smear positive. However, if the result is negative diagnosis of tuberculosis cannot be ruled out without the use of an internal amplification control to detect presence of potential inhibiting factors. In fact, false-negative Amplicor results were obtained in this study for two specimens showing a high degree of positivity on LJ slants, of which one was an AFB-positive smear.

In summary, both of these novel amplification assays are similar in terms of their sensitivity and specificity. The sensitivity of both assays is greater than that of acid-fast bacilli smear staining, and lower than that of combined radiometric Bactec and Löwenstein-Jensen slant cultures. Although these amplification assays were not sensitive for smear-negative and culture-positive specimens, as recently published by CATANZARO [21], they provided rapid results within 1 day in 50% of the cases studied. Data from our study may indicate that, at the present time, these molecular techniques cannot replace mycobacterial culture for diagnosis of tuberculosis. They must be regarded as an additional tool in limited clinical situations as determined by close co-operation between clinicians and microbiologists.

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