MOLECULAR DIAGNOSTICS

Early identification of *Mycobacterium tuberculosis* complex in BACTEC cultures by ligase chain reaction

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A total of 1431 acid-fast bacilli (AFB) in Bactec® culture vials from 1427 patients was differentiated by the Bactec NAP® method and tested by the LCx *Mycobacterium tuberculosis* ligase chain reaction system. In all, 1321 of 1325 *M. tuberculosis* complex (MTBC) isolates were correctly detected by the LCx assay. All the 106 non-tuberculous mycobacteria (NTM) isolates were negative by the LCx assay. No false MTBC-positive result was obtained from NTM isolates. However, the LCx assay failed to detect four MTBC isolates from one patient. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 99.7, 100%, 100% and 96.4%, respectively. These data suggest that the LCx system can be used to identify MTBC in AFB-positive Bactec broth cultures when the growth index is $>100$. The method gives a 100% PPV and allows a faster turnaround time for MTBC than the NAP test.

Materials and methods

Bactec 12B® cultures showing a minimum growth index (GI) of $>50$ were selected initially for inclusion in the study. An AFB smear was performed and, if positive, a NAP test was performed by standard methods [7]. For those vials in which no AFB were seen and for the LCx procedure, incubation was continued for at least 1 more day to produce a GI of $>100$ with a positive AFB smear. In practice, GIs of cultures used in this study ranged from 100 to 999. Each broth culture that was differentiated as NTM by NAP was subcultured on solid medium and the mycobacterial species was identified by conventional

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biochemical tests and commercial DNA probes (AccuProbe, San Diego, CA, USA). A total of 1431 Bacter 12B broth cultures from 1427 patients with GI of >100 was subjected to the LCx assay following the procedure recommended by the manufacturer for clinical samples. Briefly, 500 μl of each Bactec broth were transferred to a screw-capped microcentrifuge tube containing respiratory specimen buffer. The tube was vortex mixed and centrifuged (1500 g for 10 min) and the resulting pellet was washed and resuspended in buffer. After inactivation at 95°C for 20 min, the suspension was cooled to room temperature and lysed for 10 min in an Abbott Lysor sonicator. After further centrifugation (9000 g for 2 min), 100 μl of supernate were transferred to a tube containing the amplification mixture. Amplification was performed in a thermal cycler (LCx Thermal Cycler) for 37 cycles as follows: 94°C for 1 s, 64°C for 1 s, and 69°C for 40 s with maintenance at 25°C at the end of the last cycle. For each series of tests, the negative control and calibration provided were prepared in duplicate and subjected to the same amplification procedure as the samples. Amplified tubes were pulse-centrifuged (10–15 s) and transferred unopened to the carousel of the LCx analyser, which directly detects amplification products by a microparticles enzyme immunosay, reporting the results as fluorescence rates that are compared to the calibrator rate. Results >30% of the average of the calibrator rate were considered positive.

Results and discussion

Of the 1321 cultures that were positive for MTBC by the LCx assay, 1320 were identified as MTBC by NAP. The remaining sample proved to be a mixture of MTBC and M. kansasi. Of 110 mycobacterial cultures negative for MTBC by the LCx assay, 106 were identified as NTM by NAP (22 M. avium, 21 M. kansasi, 10 M. gordonae, 9 M. chelonaes species group, 6 M. marina, 5 M. fortuitum species group, 2 M. haemophilum and 31 other NTMs). The four MTBC isolates found to be LCx-negative were sub-cultured on solid medium and confirmed as MTBC by conventional biochemical tests plus commercial MTBC DNA probe. These four MTBC isolates were from samples from a single patient. No false-positive results were obtained. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were, therefore, 99.7%, 100%, 100% and 96.4%, respectively.

Several previous studies have evaluated the possibility of rapid detection of MTBC in early-positive Bacter vials. In the majority of these, the commercial Accu- Probe was used [8–10]; the reported sensitivities ranged from 33% to 83%. In only one study were nine of nine positive cultures detected [11]. A commercial amplification assay (Amplicor MTB; Roche Molecular Systems, Somerville, NJ, USA) has also been investigated for the same purpose; the sensitivities obtained ranged from 66% to 100% [12–14]. In one study, an in-house PCR achieved better sensitivity and specificity results than a commercially available PCR product [14]. However, such procedures are only suitable for specialised laboratories, as they require dedicated personnel and facilities. The manufacturer does not recommend the use of the LCx system for the detection of MTBC from positive Bactec culture vials. However, the results of this study have shown that there is a good correlation between NAP and LCx results. The four isolates from a single patient that gave false- negative LCx results could be due to gene mutation or deletion of the LCx target nucleic acid sequence [15, 16]. These data suggest that the LCx system can be used to identify MTBC in AFB-positive Bactec broth cultures when the GI is >100. The method gives a 100% PPV and allows a faster turnaround time for MTBC than the NAP test.

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