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Early Detection of *Mycobacterium tuberculosis* in BACTEC Cultures by Ligase Chain Reaction

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The LCx *Mycobacterium tuberculosis* ligase chain reaction system (Abbott Diagnostic Division, Abbott Park, Ill.) was used to detect *M. tuberculosis* in 150 consecutive BACTEC vials on the day on which a positive growth index (GI) was recorded. By LCx, *M. tuberculosis* DNA was detected in BACTEC vials on average 2.6 days before the presence of acid-fast bacilli could be confirmed by microscopic examination. A total of 106 of 108 *M. tuberculosis* isolates were detected without centrifugation from bottles presenting very low GIs (average, 70; median, 33). No false-positive result was obtained from nontuberculous mycobacteria or from isolates with contaminants.

Rapid diagnosis of tuberculosis is an important component of measures to control the disease (13), which is an objective made more urgent by the appearance of multidrug-resistant strains (7).

Nucleic acid amplification techniques, which allow detection of *Mycobacterium tuberculosis* directly from samples in a matter of hours, apparently represent the ultimate answer to this issue. They lack sensitivity for smear-negative specimens, and their use is generally restricted to selected samples. Culture remains the only mycobacterial investigation performed, along with microscopy, for the majority of clinical specimens.

The radiometric method (BACTEC; Becton Dickinson, Tew- son, Md.) is at present the fastest and most sensitive cultural tool for the diagnosis of mycobacterial infections (9). The sensitivity of the radiometric assay allows a culture to reach a growth index (GI) of ≥10, which is considered the positivity threshold, in a very short time, often within a week. However, a BACTEC vial flagged as positive does not necessarily harbor mycobacteria, since other organisms may overgrow despite the decontamination procedure. Confirmation of the presence of acid-fast bacilli is required. Although microscopic tests are very easy to perform, they are characterized by a very low sensitivity, and examination of smears prepared from BACTEC vials with early positive signals has proven to be so frustrating that a delay in examination until the GI becomes greater than 100 is recommended. Several days of further incubation are often required, thus delaying the detection of mycobacteria. Furthermore, a microscopically confirmed positive culture may be due either to *M. tuberculosis* or to a nontuberculous mycobacterium (MOTT), which poses important dilemmas both for therapy and for the measures to be adopted to prevent spread of the infection.

There is indeed interest in a procedure that would enable early recognition of the *M. tuberculosis* complex as the organism responsible for the positive BACTEC signal; such a method would in fact be consistent with the recommendations of the Centers for Disease Control for a timely identification of isolates to the species level (13).

For this purpose, we investigated the reliability of a commercial ligase chain reaction (LCx *M. tuberculosis*; Abbott Di-
nergistic Division, Abbott Park, Ill.) (14) to detect *M. tubercu-
losis* in BACTEC vials on the same day on which they yield the first positive signal.

A total of 150 consecutive BACTEC 12B (Becton Dickinson) vials with GI scores of ≥10 at the usual reading were subjected to LCx amplification following the procedure recommended for clinical specimens. BACTEC readings were performed according to the recommended schedule, i.e., twice a week in the first 14 days of incubation and weekly in the subsequent 4 weeks.

In short, 500 μl of each BACTEC broth was transferred to a ready-to-use screw-cap microcentrifuge tube containing respiratory specimen buffer; the tube was subsequently vortexed and centrifuged (1,500 × g for 10 min) before the pellet was washed and resuspended with resuspension buffer. After inactivation for 20 min at 95°C in the LCx covered dry bath, the suspension was cooled to room temperature and lysed for 10 min with the Lysor sonicator (Abbott); after further centrifugation (9,000 × g for 2 min), 100 μl of supernatant was transferred to the ready-to-use tube containing the amplification mixture. Amplification was carried out in a separate area for 37 cycles in a thermal cycler (LCx Thermal Cycler) 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 provided negative control and calibrator were prepared in duplicate and subjected to the same amplification procedure as the samples. Amplified tubes were pulse-centrifuged (10 to 15 s) and transferred unopened to the carousel of the LCx analyzer, which directly detects amplification products by a microparticle enzyme immunoassay, reporting the results as fluorescence rates that are compared to the calibrator rate; results greater than 30% of the average of the calibrator rate were considered positive.

BACTEC 12B vials presenting a GI of <100 were further incubated and read daily until a value of ≥100 was achieved, when an acid-fast smear was prepared; the smear was immediately made from vials with initial GI values of >100.

Each broth that was microscopically confirmed as positive for acid-fast bacilli was subcultured on solid medium, and the mycobacteria were identified with commercial DNA probes (AccuProbe, San Diego, Calif.) or by resorting to conventional tests and/or to high-performance liquid chromatographic analysis of cell wall mycolic acids. Overgrowing contaminants were grossly identified as such on the basis of morphology and Gram staining.

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Therefore, we conclude that the LCx can be effectively used to identify M. tuberculosis in BACTEC vials on the same day on which a GI of ≥10 is first detected. Its predictive value must be regarded as absolute for positive results. Some caution is needed in the presence of unusually high negative LCx signals (i.e., those exceeding 50% of the cutoff value), particularly when these are obtained from BACTEC vials having minimal growth as inferred from a decrease in the GI to less than 10 on the following day. The repetition of the test once the GI has increased again to 10, however, is a measure sufficient to obtain a sensitivity of 100%.

LCx results were positive a mean of 2.6 days sooner than microscopic confirmation of positive BACTEC vials. In our laboratory, the average of 17.2 days needed during the past year for detection of acid-fast bacilli which were subsequently identified as M. tuberculosis from more than 300 positive radiometric cultures would thus be shortened to 14.6 days. Moreover, it should be considered that the real gain, which increases from 2.6 to 4 days when only cultures with GIs of <100 are considered, is even greater, since no further time is needed to identify cultures which are detected as positive only by microscopy.

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REFERENCES