Early Detection of Negative BACTEC MGIT 960 Cultures by PCR–Reverse Cross-Blot Hybridization Assay

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Received 12 April 2002/Returned for modification 10 May 2002/Accepted 15 May 2002

We evaluated the efficacy of a PCR–reverse cross-blot hybridization assay, a test which permits identification of mycobacteria by means of species-specific probes and a Mycobacterium-specific probe, for early detection of negative BACTEC MGIT 960 mycobacterial cultures. Aliquots of 549 cultures were collected 7 days after the culture media were inoculated with various clinical specimens and tested with the molecular assay. PCR results were compared to those obtained at the end times with the BACTEC MGIT 960 system. Of the 549 specimens analyzed, 484 were found to be negative and 64 were found positive by both methods; one specimen, found to be positive by the BACTEC MGIT 960 system, was identified as negative by the molecular assay. In view of its high negative predictive value (99.8%), the PCR–reverse cross-blot hybridization assay appears to be a valid tool for early detection of negative BACTEC MGIT 960 cultures.

Rapid, accurate identification of mycobacterial species in clinical specimens is an essential step in any strategy aimed at limiting the diffusion of infections caused by these slow-growing organisms. Conventional methods for recovery of mycobacteria have been supported by other techniques, such as liquid culture- and molecular biology-based systems, which can be used alone or in combination to obtain accurate results in a much shorter period of time (11). With the new BACTEC MGIT 960 (Becton Dickinson) liquid culture detection system, the presence of mycobacteria in a clinical specimen is detected, on average, 2 weeks after inoculation (2, 5–7, 13, 15, 17). Unfortunately, 6 weeks must pass before a specimen can be reliably declared to be negative for mycobacteria, and this delay may lead to inappropriate empirical treatment with antimycobacterial drugs.

We evaluated a PCR–reverse cross-blot hybridization assay, previously developed for direct detection and identification of mycobacteria in clinical specimens (8–9, 12), as a potential predictor of negativity in BACTEC MGIT 960 cultures, and we used this molecular assay on aliquots of BACTEC MGIT 960 cultures 7 days after the culture media were inoculated with various clinical specimens. Results were compared to those furnished by the BACTEC MGIT 960 instrument at the end of the 6-week incubation period.

Specimen processing and culture by BACTEC MGIT 960. A total of 549 respiratory and nonrespiratory, consecutively enrolled clinical specimens (sputum, 354 samples; bronchoalveolar lavage fluid, 92 samples; urine, 79 samples; others [cerebrospinal fluid, blood, stool, ascitic fluid, abscess fluid, and biopsy specimen], 24 samples [total]) were collected from 549 patients suspected of having mycobacterial infections. Most of these patients were hospitalized in wards reserved for hematology and the treatment of infectious diseases. Specimens from sterile body sites were used directly to prepare smears for Ziehl-Neelsen acid-fast staining and BACTEC MGIT 960 cultures. Those from nonsterile sites were digested and decontaminated by standard procedures (11). The supernatant was discarded, the pellet was suspended in a sterile phosphate buffer (final volume, 2 ml), and the mixture was used for smear and culture preparation.

For each specimen, we prepared two BACTEC MGIT 960 tubes (designated A and B), each containing 0.8 ml of MGIT oleic acid-albumin-dextrose-citrate enrichment (Becton Dickinson) and 0.1 ml of antibiotic mixture MGIT PANTA (polymyxin B, nalidixic acid, trimethoprim, and azlocillin). The inoculum was added (0.5 ml/tube), and the tubes were incubated inside the device at 37°C and automatically monitored for 6 weeks or until an alarm signal indicated mycobacterial growth. In addition, two Löwenstein-Jensen slants were inoculated with 0.25 ml of each suspension, incubated at 37°C for 8 weeks, and inspected weekly. When the BACTEC MGIT 960 system indicated positivity, the sample was removed and subjected to microscopic confirmation and species identification of mycobacteria by the AccuProbe confirmation test (Gen-Probe, Inc., San Diego, Calif.), hsp65 PCR-restriction enzyme analysis (14), or conventional procedures (11). The mycobacterial colonies grown on the Löwenstein-Jensen slants were identified as described above.

Testing of BACTEC MGIT 960 cultures by PCR–reverse cross-blot hybridization assay. On the seventh day of incubation, a 1-ml aliquot from each A-tube culture was aseptically collected for testing with the PCR–reverse cross-blot hybridization assay (see below) and the remainder of each culture was reincubated for 5 more weeks (total incubation, 6 weeks [MGIT end time]). The B tubes remained undisturbed for 6 weeks after inoculation (or until mycobacterial growth was detected by the BACTEC MGIT 960 system).
The PCR–reverse cross-blot hybridization assay was carried out as previously described (12). The PCR products were then analyzed in a reverse cross-blot hybridization assay with probes specific for the genus *Mycobacterium* and the following mycobacterial species: *M. tuberculosis* complex, *M. avium*, *M. intracellularare*, *M. smegmatis*, *M. marinum* (or *M. ulcersans*), *M. kansasii*, *M. xenopi*, *M. triplex*, *M. malmoense* (or *M. szulgai*), *M. gordonae*, *M. genavense*, *M. fortuitum*, and *M. chelonae* (4, 8, 12). The hybridized PCR products were detected by incubation with streptavidin-alkaline phosphatase and a color substrate.

As shown in Table 1, the BACTEC MGIT 960 system detected mycobacterial growth in 65 of 549 (11.8%) specimens (mean time to detection, 11.8 days; range, 8 to 27 days). The same recovery rate was obtained on Löwenstein-Jensen solid medium (data not shown). At the end of the 6-week incubation, the remaining 484 specimens (88.2%) were negative. In the PCR–reverse cross-blot hybridization assay (performed on 7-day MGIT cultures), 485 specimens (88.4%) were negative and 64 (11.6%) were positive. For the 64 specimens found to be positive by both methods, the PCR products hybridized with the probe specific for *M. tuberculosis* (58 specimens) or with the probe specific for *M. avium* (6 specimens), and these results were fully concordant with the identification data obtained with the other methods used. The sensitivity, specificity, and positive and negative predictive values of the PCR–reverse cross-blot hybridization assay, compared to those for the BACTEC MGIT 960 system, were 100% for respiratory tract specimens and only slightly lower for the other specimens tested (Table 1). Overall, the assay was characterized by high sensitivity (98.4%) and a negative predictive value of 99.8%.

The only case of discordant results was that of a urine specimen that proved to be negative in the molecular assay. Despite clinical signs and symptoms of mycobacterial infection, the number of bacilli eliminated can be quite low, and this factor is often responsible for false negativity, even with highly sensitive detection methods (1, 3, 10, 16). The fact that the PCR–reverse cross-blot hybridization assay incorrectly identified this urine specimen as negative for mycobacteria is not a reflection of a lack of sensitivity—the assay’s specificity is, on the contrary, known to be quite high (9)—but rather an artifact of the specimen’s having been divided and cultured in duplicate for control purposes. Another possible explanation for this disagreement between the methods is that *M. gordonae* was a contaminant in this case, not a clinically significant isolate, and the PCR-reverse cross-blot hybridization assay failed to detect it because the A culture either was not contaminated or was not sufficiently contaminated with the organism for it to be detected.

The BACTEC MGIT 960 automated detection system can be a great time-saver in laboratories that process a high number of specimens for detection of mycobacterial species. Our objective in the present study was to evaluate the efficacy of a PCR–reverse cross-blot hybridization assay as an adjunct to the BACTEC MGIT 960 culture detection system for more rapid identification of negative clinical specimens. Performed on MGIT cultures after only 7 days of incubation, the PCR-based assay correctly revealed the absence of mycobacteria in all 484 specimens that were reported to be negative by the automated detection system after the full 6-week period of incubation. The assay was equally effective in identifying positive specimens, and species identification data were 100% concordant with the results of the AccuProbe culture confirmation test, *hsp65* PCR-restriction enzyme analysis, and conventional procedures for mycobacterial identification.

PCR–reverse cross-blot hybridization is economical enough to be used routinely ($1.50/specimen), and in our opinion, its cost is more than offset by the potential savings allowed by earlier reporting (e.g., the cost of unnecessary antimycobacterial therapy). Naturally, our data need to be confirmed by additional studies, but use of the PCR–reverse cross-blot hybridization assay with the BACTEC MGIT 960 system seems to be a reliable approach to accelerating the assessment and, in particular, the exclusion of suspected mycobacterial infections.

This work was supported by a grant from the Ministero dell’Università, Ricerca Scientifica e Tecnologica (2000–2001; ex-MURST, 60%).

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**TABLE 1. Results of the PCR–reverse cross-blot hybridization assay and the BACTEC MGIT 960 system for 549 clinical specimens from patients suspected of having mycobacterial infections**

<table>
<thead>
<tr>
<th>Specimens (n)</th>
<th>No. of negative MGIT cultures that were PCR</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>NPV&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>PPV&lt;sup&gt;b&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>Positive 0</td>
<td>64</td>
<td>98.4</td>
<td>100</td>
<td>99.8</td>
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<tr>
<td></td>
<td>Negative 484</td>
<td>1</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Others (103)</td>
<td>Positive 0</td>
<td>58</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Negative 388</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup> NPV, negative predictive value.

<sup>b</sup> PPV, positive predictive value.
REFERENCES


