Diagnostic Value of the Strand Displacement Amplification Method Compared to Those of Roche Amplicor PCR and Culture for Detecting Mycobacteria in Sputum Samples

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We compared the ability of the semiautomated BDProbeTec-SDA system, which uses the strand displacement amplification (SDA) method, with that of the Roche Amplicor-PCR system and the Septi-Chek AFB culture system to directly detect Mycobacterium tuberculosis complex (MTB) and other mycobacteria in sputum samples. A total of 530 sputum samples from 299 patients were examined in this study. Of the 530 samples, 129 were culture positive for acid-fast bacilli with the Septi-Chek AFB system; 95 for MTB, 29 for M. avium-M. intracellulare complex (MAC), and 5 for other mycobacteria. The BDProbeTec-SDA system detected 90 of the 95 samples culture positive for MTB (sensitivity, 94.7%), and the Amplicor-PCR system detected 85 of the 95 samples culture positive for MTB (sensitivity, 89.5%). The specificity of each system, based on the clinical diagnosis, was 99.8% for SDA and 100% for PCR, respectively. Among the 29 samples culture positive for MAC, the BDProbeTec-SDA system detected MAC in 24 samples (sensitivity, 82.8%), whereas the Amplicor-PCR system detected MAC in 23 samples (sensitivity, 79.3%).

Various amplification methods for the direct detection and identification of mycobacteria in clinical samples have been developed. PCR is one of the most important features of these methods. The sensitivities and specificities of in-house PCRs have varied significantly, reflecting not only the operational skills of each laboratory but also the target regions of nucleic acids and the amplified products detected by each system (1, 7, 16, 20–22, 24). In attempts to standardize the laboratory application of such methods, several amplification kits for detecting mycobacteria have been developed. The PCR-based Roche Amplicor Mycobacterium system (Roche, Basel, Switzerland) for detecting Mycobacterium tuberculosis complex (MTB), M. avium, and M. intracellulare (2–4, 6, 8, 9, 13, 17, 22, 24, 30, 32) and the rRNA amplification-based Gen-Probe Amplicor Mycobacterium Tuberculosis Direct Test system (MTD; Gen-Probe Inc., San Diego, Calif.) for detecting MTB (1, 13, 15, 16, 18, 22, 23, 29, 30) are now commercially available. However, the reported sensitivities of these kits are insufficient for smear-negative samples (1, 3, 4, 8, 16, 17, 32). Other amplification methods, such as ligase chain reaction (Abbott Laboratories, Chicago, Ill.) (14), Q-beta replicase-amplified assay (GENE-TRAK, Framingham, Mass.) (25), and a nucleic acid sequence-based amplification assay (Organon Teknika, Amsterdam, The Netherlands) (28) are now being clinically evaluated for their efficacies at detecting mycobacteria.

A new amplification method for detecting mycobacteria, strand displacement amplification (SDA; Becton Dickinson Microbiology Systems, Sparks, Md.) (11, 12, 26, 31), has recently been developed. SDA is a novel method in which DNA segments of the IS6110 repetitive element specific to MTB (27), together with a segment of the 16S rRNA gene common to all members of genus Mycobacterium (5), are amplified isothermally. The BDProbeTec-SDA system (Becton Dickinson), which uses this method, is capable of automatically performing rapid detection of mycobacteria. In the present study, we compared the ability of the BDProbeTec-SDA system to directly detect MTB and other mycobacteria in sputum samples with those of the Amplicor-PCR system and a liquid culture method, the Septi-Chek AFB system (Becton Dickinson).

MATERIALS AND METHODS

Sample processing and culture. Clinical samples were obtained from patients being followed during antituberculosis chemotherapy and from those suspected of having mycobacterial infection or other pulmonary diseases. A total of 530 sputum samples from 299 patients were examined in this study. 265 samples from Chubu National Hospital, Aichi Prefecture, Japan, collected between October 1995 and February 1996; 99 samples from Nagoya University Hospital, Nagoya, Japan, collected between September and December 1996; and 166 samples from the BML Research Laboratories, Tokyo, Japan, collected between March and May 1996. All samples were decontaminated by treatment with an equal volume of N-acetyl-L-cysteine (NALC)-NaOH (final concentration, 2%) for 15 min at room temperature and were neutralized with sterile 0.067 M phosphate buffer (pH 6.8). After centrifugation at 3,000 g for 15 min, each pellet was resuspended in 2.0 ml of sterile 0.067 M phosphate buffer (pH 6.8). The 265 specimens from Chubu National Hospital were subjected to auramine-rhodamine staining for the detection of acid-fast bacilli. Culture for mycobacteria was performed by inoculation of 0.5 ml of the decontaminated material into a Septi-Chek AFB vial at 35°C, and the vials were monitored for growth of mycobacteria for 8 weeks. Growth-positive cultures were confirmed to contain acid-fast bacilli by Ziel-HNelsen staining. The remaining 265 specimens from Nagoya University Hospital and the BML laboratory were screened, and growth-positive cultures were confirmed to contain acid-fast bacilli by Ziel-HNelsen staining. Mycobacterial cul-
TABLE 1. Detection of MTB in sputum samples with BDProbeTec-SDA and Amplicor-PCR systems

<table>
<thead>
<tr>
<th>Culture result (no. of samples)</th>
<th>BDProbeTec-SDA system</th>
<th>Amplicor-PCR system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>90</td>
<td>85</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

* Samples yielding initially discrepant results between the BDProbeTec-SDA and Amplicor-PCR systems were retested with each system to confirm the results. However, the test results from assays with one or the other system for 19 of these samples could not be reproduced. Due to our limited sample volumes, the results of the initial or repeat test could not be confirmed, and, hence, the results for these 19 samples were excluded from the analyses in Tables 1 and 2. Samples from which mycobacteria other than MTB had been cultured were excluded from this analysis. These samples included spuva from patients during or after antituberculosis chemotherapy, patients with tuberculosis before chemotherapy, and patients who did not have tuberculosis.

Amplification-positive but culture-negative samples might have had those results because of the decontamination of nonviable organisms by PCR or SDA and to the lack of use of solid media for culture for the 265 samples from Chubu National Hospital. The agreement between the results obtained with the BDProbeTec-SDA system and the Amplicor-PCR system for the detection of MTB was 96.4% (478 of 496 samples). There were 4 SDA-negative but PCR-positive samples and 14 SDA-positive but PCR-negative samples. Two of the 4 SDA-negative but PCR-positive samples and 7 of the 14 SDA-positive but PCR-negative samples were positive for MTB by culture.

Table 2 presents the results of detection of MAC by the two systems in 430 samples, excluding 100 culture-positive samples for mycobacteria other than MAC. Among the 29 culture-positive samples for MAC, the BDProbeTec-SDA system detected MAC in 24 samples (sensitivity, 82.8%), whereas the Amplicor-PCR system detected MAC in 23 samples (sensitivity, 79.3%). The specificities of the systems were 98.3% (394 of 401 samples) and 100% (401 of 401 samples), respectively. Since the BDProbeTec-SDA system amplifies a segment of the 16S rRNA gene common to all members of the genus Mycobacterium, some of the seven SDA-positive but culture-negative samples might have had those results because of the detection of mycobacteria other than MTB or MAC. The level of agreement between these systems for the detection of MAC was 96.7% (416 of 430 samples). There were 3 SDA-negative but PCR-positive samples and 11 SDA-positive but PCR-negative samples. All 3 SDA-negative but PCR-positive samples and 4 of the 11 SDA-positive but PCR-negative samples were culture positive for MAC. Of the five samples which yielded positive results by culture for mycobacteria other than MTB or MAC, only one sample culture positive for M. kansasii was detected with the BDProbeTec-SDA system.

The application of amplification assays to the diagnosis of tuberculosis has been widely reported in the literature. The rRNA-based MTD system (Gen-Probe) and the Amplicor-PCR system (Roche) are commercially available in the United States, Europe, and Japan. The U.S. Food and Drug Admin-

TABLE 2. Detection of MAC in sputum samples with the BDProbeTec-SDA and Amplicor PCR systems

<table>
<thead>
<tr>
<th>Culture result (no. of samples)</th>
<th>BDProbeTec-SDA system</th>
<th>Amplicor-PCR system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

* Samples from which mycobacteria other than MAC had been cultured were excluded from this analysis.
recommendation has illustrated the clinical use of the MTD system only with smear-positive samples because of the lack of sufficient sensitivity with smear-negative samples and the high cost of this system. The cost implications of using amplification test kits are still an issue in clinical laboratories (10).

For the detection of MTB, we found that the BDProbeTec-SDA system had an excellent sensitivity (94.7%). This system also showed a sensitivity of 75%, as an initial detection test, for the eight smear-negative, culture-positive samples from patients with no history of antituberculosis chemotherapy. The high sensitivity of this system might be due in part to the mechanical disruption of organisms in the processing steps, but it is more likely to be principally due to the amplification of sequences in the repetitive IS6110 element in MTB. Differences in the volumes of the original specimen do not account for the differences in sensitivity, since the BDProbeTec-SDA system actually tests less (13.75 μl) of the original NALC-NaOH-treated specimen than the Amplicor-PCR system does (25 μl). For the detection of MAC, in which both systems amplify a single copy of the 16S rRNA gene sequence, the sensitivities of the two systems were similar, 82.8% for the BDProbeTec-SDA system and 79.3% for the Amplicor-PCR system. With the exception of one sample, no false-positive result due to amplicon contamination was obtained with the BDProbeTec-SDA or the Amplicor-PCR system. In both systems, a single-tube method is used for lysis of the organisms and decontamination with uraic DNA glycosylase. These methods for protection against cross-contamination might contribute to the extremely low rates of false-positive results.

The BDProbeTec-SDA system is semi-automated and can process up to 48 samples in a single run in under 6 h (for decontamination, amplification, and detection). Because of its high sensitivity and specificity, the BDProbeTec-SDA system can be recommended for use in clinical laboratories for the rapid detection of mycobacteria from sputum samples.

ACKNOWLEDGMENT

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