


Detection of Mycobacterial Antigens in Sputum by an Enzyme Immunoassay

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An enzyme immunoassay (EIA) for the detection of mycobacterial antigens in sputum was evaluated. The system utilizes commercially available anti-BCG immunoglobulin. BCG protein standard was used as positive control. Thirty-nine patients with culture-proven pulmonary tuberculosis were tested. The EIA was positive in 24 of 29 patients with positive smears and cultures, giving a sensitivity of 86.2%. It was also positive in six of ten patients with smear-negative culture-positive disease, resulting in a sensitivity of 60% in this group. In another 176 patients with different nontuberculous pulmonary infections, only nine were positive by EIA, giving a specificity of 94.9%. The high sensitivity and specificity of the assay make it a useful tool for the early diagnosis of pulmonary tuberculosis.

The diagnosis of pulmonary tuberculosis depends on the identification of *Mycobacterium tuberculosis* in sputum smears and cultures. Demonstration of acid-fast bacilli by direct smear of sputum is the only quick diagnostic test in general use. However, positive results are obtained in only about 25% of newly diagnosed patients with pulmonary tuberculosis (1). The need for a rapid and sensitive diagnostic test is obvious. Enzyme immunoassay (EIA) is a simple and rapid test that has proved useful for antigen detection in several infectious diseases (2). A number of studies have shown that EIAs utilizing anti-BCG antibodies could detect mycobacterial antigens in CSF specimens of patients with tuberculous meningitis (3–5). There are very few reports on the detection of mycobacterial antigens in sputum (6, 7). The present study evaluated an EIA system that detects mycobacterial antigens in sputum samples of patients with pulmonary tuberculosis.

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Materials and Methods. Sputum samples were obtained from patients referred to the Chest Diseases Center and King Khalid University Hospital in Riyadh, Saudi Arabia. These included 39 samples that were culture positive for Mycobacterium tuberculosis. Of these, 29 were also positive by smear. In addition, 176 sputum samples were taken from patients with nontuberculous pulmonary infections such as upper respiratory tract infection, pneumonia, bronchiectasis and chronic bronchitis. These samples were examined by smear and culture for Mycobacterium tuberculosis and were found to be negative.

Sputum samples were digested with N-acetyl-L-cysteine and concentrated for smear and culture on Lowenstein-Jensen medium. Samples were then inactivated by heating to 62 °C for 30 min and stored at -70 °C until tested by EIA, usually within four weeks of collection.

For EIA, the method of Engvall and Perlman (8) was used with some modifications. Polyvinyl U-bottomed microtitre plates were coated with 100 μl of a 1:400 dilution of rabbit IgG antibody to Bacille Calmette Guerin (Dakopatts, Denmark) in carbonate-bicarbonate buffer, pH 9.6. After overnight incubation at 37 °C, the plates were washed three times with phosphate-buffered saline (PBS) containing 2 % Tween 20, blocked with diluent buffer (0.01 M phosphate buffer solution, pH 7.4) containing 3 % bovine serum albumin for 1 h at 37 °C, and washed four times with PBS containing Tween 20.

One hundred microlitres of a 1:1000 dilution of rabbit anti-BCG antibody conjugated to horseradish peroxidase (Dakopatts) was subsequently added to each well and incubated at 37 °C for 1 h. The plates were washed four times as before. The substrate consisted of 40 mg of O-phenylenediamine in 100 ml of citrate phosphate buffer and 40 μl of 30 % hydrogen peroxide. One hundred microlitres of substrate was added to each well and after incubation at 37 °C for 30 min, the reaction was stopped by adding 50 μl of 4M H2SO4. Optical density at 492 nm was read by an EIA reader (Dynatech, USA). A control group containing buffer but no antigen was run in triplicate in each plate. The mean optical density of this negative control indicated non-specific binding of the conjugated enzyme antibody. Optical density for each sample was taken as the average of the three wells after subtraction of nonspecific activity. In each plate 1, 5, 10 and 100 ng/ml of BCG protein standard (Calbiochem, USA) were run in triplicate as positive controls.

The average optical density of each sample was considered in relation to the optical densities of BCG protein standard within the test plate. A sample having an optical density greater than or equal to that of 5 ng/ml of BCG protein was considered positive for the presence of mycobacterial antigens. The relative optical densities of test samples were calculated in relation to the optical density of 5 ng/ml of BCG protein standard.

Results and Discussion. The mean optical densities and standard deviations obtained with 215 sputum samples were as follows: smear-positive culture-positive for Mycobacterium tuberculosis, 0.211 ± 0.148; smear-negative culture-positive samples 0.153 ± 0.184; and smear-negative culture-negative samples 0.009 ± 0.040 (Table 1). The mean optical densities and standard deviations of BCG protein standards are shown in Figure 1. These were higher than the mean optical density for controls by more than two standard deviations. Sputum samples having an optical density greater than or equal to that of 5 ng/ml of BCG protein standard were considered positive for the presence of mycobacterial antigens. Figure 2 shows the results of testing 39 infected sputum samples and 176 control samples in relation to standard BCG protein concentrations. Of 29 smear-positive culture-positive samples, 25

<table>
<thead>
<tr>
<th>Group</th>
<th>Total no.</th>
<th>Optical density</th>
<th>No. (%) EIA positive</th>
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<tbody>
<tr>
<td>Smear positive, culture positive</td>
<td>29</td>
<td>0.211 ± 0.148</td>
<td>25 (86.2)</td>
</tr>
<tr>
<td>Smear negative, culture positive</td>
<td>10</td>
<td>0.153 ± 0.184</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Smear negative, culture negative</td>
<td>176</td>
<td>0.009 ± 0.040</td>
<td>9 (5.1)</td>
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