Evaluation of a Rapid Enzyme Immunoassay for Diagnosis of Hepatic Amoebiasis

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We compared the capability of rapid enzyme immunoassay (EIA) to detect antiamoebic antibodies during hepatic amoebiasis with those of indirect hemagglutination and latex agglutination. EIA is simple to perform and rapid (20 min) and does not require any special equipment (optical reading is sufficient). EIA of 143 sera (including 43 from patients with proven hepatic amoebic abscesses, 33 from patients with other hepatic disorders and/or parasitic infections, and 67 from healthy individuals) yielded a specificity, a sensitivity, and positive and negative predictive values of 100, 93, 100, and 97.1, respectively. This test could thus be considered another valuable tool for the diagnosis of hepatic amoebiasis.

Extraintestinal amoebiasis is the result of invasive intestinal infection with Entamoeba histolytica (7). Even though the liver is the site most frequently involved, induced abscesses usually form without concurrent colitis. Also, it is difficult to demonstrate the parasite in the contents of the abscesses. Consequently, serological tests have been developed to confirm the diagnosis and usually supplement clinical and radiological data. The diagnostic value of these tests is of great importance, especially in areas where amoebiasis is not endemic, where distinctions between past and acute infections are not frequently a problem. During the last few years, easily performed tests such as indirect hemagglutination (IHA) or latex agglutination (LA) have become available and have yielded values higher than or equal to 0.5 was considered positive.

IHA. For IHA, 1/80, 1/160, and 1/320 dilutions of sera were dispensed into the wells of a sterile microtiter plate and incubated for 2 h at 37°C with sensitized erythrocytes. We used optical reading to determine whether agglutination, which indicated the presence of antiamoebic antibodies in the tested serum, had occurred. Hemagglutination with a serum dilution of 1/320 was considered a positive reaction.

LA. Agglutination of sensitized red latex particles was performed with 1/5-diluted sera. After mixing and after the slide was moved back and forth for 5 min, red agglutinates formed an edge surrounding a green central area, indicating a positive reaction.

EIA. The kit used in this study includes microtiter wells coated with the E. histolytica HK9 antigen. Diluted sera (1/64) were dispensed into the wells and incubated for 10 min at room temperature. After the wells were washed three times, 100 μl of protein A-peroxidase conjugate was added, and the mixture was incubated for 5 min at room temperature. We then washed the wells three times and rinsed them with distilled water. Fifty microliters of hydrogen peroxide (substrate A) and 50 μl of tetramethylbenzidine (substrate B) were then disposed into each well. After a 5-min incubation at room temperature, the reaction was stopped by the addition of 100 μl of 1 M phosphoric acid. For the optical reading, we compared the tested serum with negative, slightly positive, and highly positive controls provided by the manufacturer. Stain intensity higher than that for the slightly positive control indicated a positive result. Each test was read independently by two of us (L.K. and C.H.). Also, a spectrophotometric reading of the plate was performed at a wavelength of 450 nm. After evaluation, and according to the manufacturer’s recommendations, an optical density (OD) higher than or equal to 0.5 was considered positive.

Sera. One hundred forty-four sera were examined by each of the three tests, and the results were compared. The sera were divided into three groups. Group A included 43 sera from patients with clinical and ultrasound examinations consistent with hepatic amoebiasis. Also, the results of previous testing by immunofluorescence assay, counterimmunoelectrophoresis, or IHA for amoebic antibodies had been positive. Group B included 33 sera from patients with a serologically proven hepatic disease other than amoebiasis and/or parasitic infection. The patients included five with hydatidosis, six with toxocariasis, six with acute toxoplasmosis, two with fascioliasis, two with chronic disseminated candidiasis with liver involvement, five with active chronic hepatitis B, five with acute hepatitis A, and two with typhoid fever. Group C included 67 sera obtained from healthy individuals. All serum specimens were kept frozen at −20°C until they were tested.

Statistics. Tests were evaluated by calculating specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), and efficiency (the percentage of individuals correctly identified as having or not having liver amoebic abscess) (5). These values were compared by Fisher’s exact test. A P value below 0.05 was considered to indicate a significant difference.

Cost. Finally, we evaluated the cost of both the screening and semiquantitative determinations for antiamoebic antibodies for the three tests.

RESULTS

The mean OD (± standard deviation) for the 67 sera from healthy individuals by EIA was 0.156 ± 0.089. A cutoff value evaluated by this mean plus 3 standard deviations would be 0.421. We thus considered the OD cutoff proposed by the manufacturer (0.5) appropriate for our population.

Table 1 shows the positive results for the three methods according to serum group. No discrepancies leading to a change in positive versus negative interpretation were noted for the two optical readings. Also, no discrepancies between the visual and the spectrophotometric readings of the EIA were observed (Fig. 1).

The evaluation indices of the three tests are given in Table 2. All three exhibited specificities, sensitivities, PPV, NPV, and efficiencies higher than 88.6%. LA showed slightly lower results, particularly for specificity and PPV. The highest sensitivity was obtained with IHA (97.6%), whereas EIA gave the highest specificity (100%). The combination of IHA and either
EIA or LA led to the identification of 100% of the patients with proven hepatic amoebiasis. In contrast, two of these patients could not be identified by a combination of EIA and LA.

The prices of the screening determinations as performed in this study were $3.78, $4.88, and $5.10 for IHA, LA, and EIA, respectively. In the case of realistic population, the prevalence of positive sera, requiring further tests with higher dilutions (e.g., three more for IHA and two more for LA) for a semi-quantitative determination, might be, for example, 1 or 10%. Then the cost for IHA for prevalences of 1 and 10% would be $3.82 and $4.16, respectively. For LA, it would be $4.98 and $5.89, respectively. In contrast, as EIA results can be directly quantitated by calculating the ratio of the OD of the serum to the OD of the negative control, the price of this test remained unchanged.

### Table 1. Positive results for the detection of antiamoebic antibodies by method and serum group

<table>
<thead>
<tr>
<th>Serum group</th>
<th>No. of sera tested</th>
<th>No. (%) of sera positive by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EIA</td>
</tr>
<tr>
<td>A</td>
<td>43</td>
<td>40 (93)</td>
</tr>
<tr>
<td>B</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>67</td>
<td>1 (1.5)</td>
</tr>
</tbody>
</table>

\(^a\) For details, see Materials and Methods.

\(^b\) One patient with hepatitis A and one with hepatitis B.

\(^c\) One patient with fascioliasis, one with hepatitis B, and one with acute toxoplasmosis.

### DISCUSSION

The detection of amoebic antibodies is an important clue in the diagnosis of extraintestinal amoebiasis. For many parasitic diseases, EIA has been demonstrated to be highly sensitive and specific (6, 12). However, most reports describe homemade techniques with various antigens, leading to a lack of standardization (2, 3, 10, 13). Also, these conventional EIAs are often time-consuming and sometimes laborious. During the past few years, commercial kits for rapid EIA have become available for the diagnosis of parasitic diseases and have been demonstrated to be highly reliable (4, 8, 11). Here, the EIA was easily performed within 20 min and did not require any special equipment, not even for the reading, which can be optical. Whereas visual detection may lead to variability in the determination of the positivity of a serum, the concomitant use of three control sera, i.e., negative, slightly positive, and highly positive, enables the reduction of interreader variability, as assessed in our study. The evaluation indices for this test were all higher than 93%, and some (such as specificity and PPV) were significantly better for EIA than for IHA and LA.

### Table 2. Evaluation indices of tests for diagnosing patients with hepatic amoebiasis

<table>
<thead>
<tr>
<th>Test</th>
<th>Specificity (%)</th>
<th>Sensitivity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA</td>
<td>100</td>
<td>93</td>
<td>100</td>
<td>97.1</td>
<td>97.9</td>
</tr>
<tr>
<td>IHA</td>
<td>97</td>
<td>97.6</td>
<td>93.3</td>
<td>98.9</td>
<td>97.2</td>
</tr>
<tr>
<td>LA</td>
<td>95(^a)</td>
<td>90.7</td>
<td>88.6(^b)</td>
<td>95.9</td>
<td>93.7</td>
</tr>
</tbody>
</table>

\(^a\) P < 0.03 (Fisher’s exact test) in comparison with value for EIA.

\(^b\) P < 0.04 (Fisher’s exact test) in comparison with value for EIA.

**FIG. 1.** Correlation between spectrophotometrical and optical readings of EIA. Sera with a stronger stain intensity than that of the slightly positive control were considered slightly positive, and sera with a stronger stain intensity than that of the highly positive control were considered highly positive. ◦, one serum; ●, five sera.
higher than those for LA. Results obtained for sensitivity were slightly lower than but not significantly different from those of the other tests. Also, the kinetics of the antiamoebic-antibody production detected by this test need to be studied further. Moreover, since the test can be used on an individual basis but can also simultaneously test 93 sera, further studies could evaluate its value in the diagnosis of intestinal amoebiasis in an epidemiological setting.

IHA was the cheapest test, regardless of the prevalence of positive sera in the tested population. The price of EIA tends to decrease with increasing positivity prevalence, but a spectrophotometrical reading is then required. Of course, a semi-quantitative approach with further dilutions of the sera is also applicable to EIA, but this method leads to a higher cost. The difference in price between screening by IHA and screening by EIA (26%) should be considered by low-budget laboratories.

In summary, EIA is a rapid, easily performed, and valuable test and can thus be a useful aid for the diagnosis of amoebic abscess. IHA is the cheapest commercial kit; EIA costs more, depending on the frequency of the antibodies in the sera tested.

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REFERENCES


