Use of Rapid Dipstick and Latex Agglutination Tests and Enzyme-Linked Immunosorbent Assay for Serodiagnosis of Amebic Liver Abscess, Amebic Colitis, and Entamoeba histolytica Cyst Passage

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A homemade enzyme-linked immunosorbent assay (ELISA) and a dipstick assay (Dipstick) for the detection of anti-Entamoeba histolytica antibodies in serum were developed and evaluated together with a commercially available latex agglutination test (LAT; Laboratoires Fumouze) for their use in serodiagnosis of amebiasis. The sensitivity of these assays was evaluated with sera from 27 patients with radiologically proven, cellulose acetate precipitation (CAP) test-positive amebic liver abscess, 7 patients with parasitologically and PCR-proven amebic colitis, and 11 patients with parasitologically and PCR-proven E. histolytica cyst passage. The sensitivities of the ELISA, Dipstick, and LAT were all 93.3% (42/45). With a combination of Dipstick and LAT, all abscess and colitis patients had at least one positive result. The specificity was assessed with 238 sera from patients with various parasitic, bacterial, viral, and fungal infectious diseases, sera containing autoimmune antibodies, and sera from healthy blood donors. The specificities of the ELISA, Dipstick, and LAT were 97.1%, 98.1%, and 99.5%, respectively. Of 61 sera from patients with PCR-proven E. dispar infection, 60 (98.4%) were negative in both Dipstick and LAT and 59 (96.7%) were negative in ELISA. Our data suggest that all three assays are sensitive serological tests. The rapid LAT and Dipstick provide fast results and can easily be applied in routine laboratories in order to facilitate the diagnosis of amebiasis.

With an estimated 40,000 to 100,000 infected people dying annually, amebiasis remains one of the most important parasitic infections worldwide (34). The causative agent of amebiasis, the protozoan Entamoeba histolytica, has a worldwide distribution and is a serious health threat, especially in places where fecal-oral hygiene is low.

Among the 500 million people that pass cysts of Entamoeba spp. in their feces, most (90%) are infected with Entamoeba dispar, a commensal protozoan which causes no disease in humans but which is microscopically indistinguishable from E. histolytica (5). Differentiation of the two species from stool can be performed with specific antigen detection assays or PCR (28). Alternatively, detection of specific antibodies to E. histolytica in blood could be a useful indicator for infection with E. histolytica or E. dispar, assuming specific antibody production in E. histolytica cyst passers and the absence of such production in E. dispar cyst passers. Until now, however, little has been known about antibody production in these two different groups.

Although most infections with E. histolytica are also asymptomatic, 4 to 10% of infected patients develop amebic disease within a year (27), with amebic abscess and colitis being the most important clinical entities. Colitis arises subacutely when trophozoites of E. histolytica invade the colonic epithelium and cause symptoms such as abdominal pain, tenderness, (bloody) diarrhea, and weight loss. The presence of erythrocytes in hemoglobinophagous trophozoites of E. histolytica in freshly passed stools is pathognomonic for amebic colitis. An antibody response against E. histolytica arises in a large proportion of these patients (21).

Amebic liver abscess is caused by hematogenous spread of the invasive trophozoites. This complication is seen mainly in young males between 18 and 50 years of age (1). Diagnosis depends on clinical findings, ultrasound or radiographic imaging techniques, and, especially, also on serological studies. Because an amebic liver abscess is potentially life threatening, rapid diagnosis is mandatory. However, there are few serological tests for amebiasis which are both rapid and easy to perform and are well evaluated (28). In general, amebic serology is performed in reference laboratories, with results being available after days to weeks. In patients with known diagnosis of (uncomplicated) amebic abscess, prompt treatment with, e.g., metronidazole or tinidazole will result in rapid clinical recovery (21). However, without precise diagnosis, in clinical practice, broad-spectrum antibiotics are sometimes added to cover a potential bacterial etiology of the abscess. Therefore, rapid serodiagnosis in patients suspected of amebic abscess is often an important tool in clinical decision making and can be of help in the reduction of the costs of additional treatment and prolonged hospital stay.

In this study, we evaluated a rapid and easy-to-perform homemade dipstick assay (Dipstick) and a commercially available latex agglutination test (LAT) for serodiagnosis of amebi-
bias. In addition, an enzyme-linked immunosorbent assay (ELISA) was studied for which only low quantities of *E. histolytica* antigen were needed for coating and which provided quantitative information about antibody titers.

All tests were extensively evaluated for sensitivity and specificity in patients with amebic abscesses and, in addition, in patients with amebic colitis and *E. histolytica* and *E. dispar* cyst passage.

### MATERIALS AND METHODS

**Patients and sera.** Sera incorporated in this study were collected from patients in the Academic Medical Center (Amsterdam, The Netherlands), the Prince Leopold Institute of Tropical Medicine (Antwerp, Belgium), and The Harbor Hospital and Institute of Tropical Diseases (Rotterdam, The Netherlands). A total of 196 sera from patients returning from the tropics infected with *E. histolytica* or *E. dispers* were used to evaluate all three tests: 27 with amebic liver abscess, 7 with amebic colitis, 11 cyst passers with *E. histolytica*, and 61 cyst passers with *E. dispar*. A patient was regarded as having amebic liver abscess when there was a clinical history suggestive of the disease, a space-occupying lesion in the liver in radiographic or ultrasound imaging, and a positive cellulose acetate precipitation test (CAP) result (see below) on the day of admission or in a later stage of the disease.

Patients with amebic colitis had dysenteric stools with trophozoites of *E. histolytica* containing erythrocytes. Cyst passers had cysts of *E. dispar* spp. in stools which were detected with microscopy which were identified as *E. histolytica* or *E. dispers* cysts by PCR and stool antigen ELISA. These tests were also used to confirm the presence of *E. histolytica* in patients with amebic colitis (see below). All tested sera were drawn at the first moment of clinical suspicion. Sera were stored at −20°C until use. Totals of 238, 209, and 213 sera from patients with or without an infectious disease were used for specificity testing of the ELISA, Dipstick, and LAT, respectively (Table 1).

**Triple feces test.** Parasitological diagnosis of *E. histolytica* or *E. dispers* in stools was performed with the triple feces test as described before (31). In short, patients’ stools were collected in three tubes, two tubes with sodium acetate acetate acid formalin fixative and one tube without a fixative, on three consecutive days by the patient (day 1 and 3 stools in a fixative, day 2 stool unpreserved) and sent afterwards to the laboratory. The preserved samples were screened with iodine staining for cysts and trophozoites of protozoa and, in case of positive findings, examined by permanent staining with Chlorazol black stain. The unpreserved samples (day 2) were treated with the formalin-ether concentration method according to Ridley (24a) and examined for cysts of protozoa and eggs of helminths. In cases of amebic colitis, stools recently passed (within 1 h after production) were also examined directly for the presence of motile erythrocyte-containing trophozoites of *E. histolytica*.

**Cellulose acetate precipitation test.** This test was described before and is regarded a reference test for amebic serology (26, 29). The test is based on the principle of counterimmunoelectrophoresis. On Sephract III cellulose-acetate strips (Gelman Sciences, Ann Arbor, Michigan), two parallel lines of wells were made. One line was filled with *E. histolytica* antigen (1.5 mg/ml) in Veronal buffer (Beckman-Catalin KG, Basum, Germany), pH 8.75; in the opposite line, serum was added. Electrophoresis was performed for 30 min at 200 V. Antigen moves toward the anode and antibodies toward the cathode. In case of a positive reaction, a precipitate forms at the place where antibody and antigen meet, which can be visualized by staining with nigrosine (Merck).

**Stool antigen ELISA.** A TechLab *E. histolytica* II kit (TechLab, Blacksburg, Virginia) was used for the detection of specific antigen of *E. histolytica* in stool samples. The ELISA was performed according to the manufacturer’s instructions. Briefly, diluted stool samples were added to microtiter wells coated with polyclonal anti-*E. histolytica* adhesion antibodies. Bound activity is detected with horseradish peroxidase (HRP)-conjugated monoclonal mouse anti-*E. histolytica* antibodies and subsequent incubation with tetramethylbenzidine and H₂O₂.

**E. histolytica and *E. dispers* PCR.** PCR and subsequent polyacrylamide gel electrophoresis were performed as described previously by Gomes et al. (8).

**Antigen.** Antigen was obtained by culturing *E. histolytica* HM1:IMSS (ATCC 30459; American Type Culture Collection, Rockville, Maryland) on TYI medium with bovine serum (Sigma-Aldrich, St. Louis, Missouri). The trophozoites were collected in phosphate-buffered saline (PBS), sonicated, and pelleted by centrifugation. The amount of antigen was determined by the Lowry method (20). Antigen was stored at −20°C until use.

**Specific-antibody ELISA.** Flat-bottom polystyrene high-binding microplates with small wells (Coming, Inc., Acton, Massachusetts) were used. Wells were coated overnight at 4°C with *E. histolytica* antigen in 50 mM carbonate buffer, pH 9.6. Subsequently, the wells were treated with PBS containing 1% (wt/vol) chicken egg white (PBS Egg) at 37°C for 30 min. After three washings with PBS, 0.05% Tween 20, serum samples, diluted in PBS Egg, were added for 30 min at 37°C. After five washings, detection of bound antibodies was done with a 1:1000 dilution of HRP-conjugated goat anti-human immunoglobulin G (IgG) (Nordic Immunological Laboratories, Tilburg, The Netherlands) in PBS Egg for 30 min at 37°C. Subsequently, the wells were washed three times and substrate solution (0.1% 5-amino salicylic acid in phosphate buffer, pH 5.95, with 0.03% hydrogen peroxide).
RESULTS

Cutoff values and specificity and sensitivity of the tests. The specificity of the ELISA was assessed with a total of 238 sera from patients suffering from different parasitological, bacterial, and viral infections, from patients with high titers of antinuclear antibodies and rheumatoid factor in their blood (sera known to cause aspecific reactions in serological tests), and from healthy blood donors. The cutoff value in the ELISA (164) was calculated from the mean OD of the 238 samples plus twice the standard deviation. The specificities of ELISA, Dipstick, and LAT were 97.1% (231/238), 98.1% (205/209), and 99.5% (212/213), respectively. Sera used for the assessment of the specificity for all three tests are depicted in Table 1; results are shown in Fig. 2 and Table 2.

For assessment of the sensitivities of the three assays, 45 sera were used: sera from patients with amebic liver abscess (27 sera), amebic colitis (7 sera), and E. histolytica cyst passage (11 sera) (Table 1). The sensitivities of ELISA, Dipstick, and LAT for amebic liver abscess, amebic colitis, and cyst passage are depicted in Table 2. For the whole group, including all 45 cases, the sensitivity of all three tests was 93.3%. Figure 2 shows the results of the ELISA. The concordance between the ELISA and the Dipstick assay was 100% (45/45) among the samples from patients with E. histolytica disease or cyst passage and 98.5% (266/270) among the negative controls. In total, 308 of 315 results were concordant (97.8%).

Results with sera of E. dispar cyst passers. Among 61 sera from PCR-proven E. dispar cyst passers, the numbers of sera showing seronegativity with ELISA, Dipstick, and LAT were 60 (98.4%), 59 (96.7%), and 60 (98.4%), respectively.

DISCUSSION

In this study we evaluated a home-made Dipstick, an ELISA, and a commercial rapid latex agglutination test (LAT) for serodiagnosis of E. histolytica disease in a western European hospital setting. All three tests proved to be highly sensitive (93.3%) and specific (97.1 to 99.5%). Furthermore, most (96.7 to 98.4%) E. dispar cyst passers were seronegative with the three serological tests, whereas most (81.8 to 90.9%) E. histolytica cysts passers proved positive.

In patients with a clinical history of a hepatic abscess and space-occupying lesions as revealed by ultrasound or radiographic imaging techniques, the presence of specific antibodies to E. histolytica provides strong evidence for an amebic abscess (21). Several tests, including indirect fluorescent antibody tests (6, 12, 36), ELISA, and agglutination assays (4, 15), have proven useful for this purpose. For this study we defined patients as harboring an amebic liver abscess in cases with a clinically suspect history and with radiological proof of one or more space-occupying lesions in the liver, in combination with a positive cellulose acetate precipitation test result on the day of presentation. The CAP test is a highly sensitive and specific test for serodiagnosis of amebic liver abscess which becomes negative relatively quickly after successful treatment (29). For all 27 patients with amebic liver abscess in this study, both the ELISA and Dipstick were positive in serum samples taken at

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of sera</th>
<th>ELISA</th>
<th>Dipstick</th>
<th>LAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amebic colitis</td>
<td>7</td>
<td>6 (85.7)</td>
<td>6 (85.7)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>Amebic liver abscess</td>
<td>27</td>
<td>27 (100)</td>
<td>27 (100)</td>
<td>25 (92.6)</td>
</tr>
<tr>
<td>E. histolytica passage</td>
<td>11</td>
<td>9 (81.8)</td>
<td>9 (81.8)</td>
<td>10 (90.9)</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>42 (93.3)</td>
<td>42 (93.3)</td>
<td>42 (93.3)</td>
</tr>
</tbody>
</table>
the first time of clinical suspicion. Two samples were negative in LAT initially but became positive 3 to 6 days later (Table 3). Seronegativity in an early phase of clinical disease is a well-known important pitfall in (sero)diagnosis of hepatic amebiasis (15).

For amebic colitis, demonstration of erythrocyte containing amebal trophozoites in freshly passed stools is regarded the gold standard for diagnosis (21). This microscopic technique is rapid, results being available within minutes, and cheap. Although often satisfactory, detecting trophozoites of *E. histolytica* with ingested erythrocytes can be difficult, as can be the differentiation from motile erythrocyte-containing macrophages (2). When microscopic studies of unpreserved stools are delayed for a prolonged period (>1 h), false-negative results can occur (22). As a result, patients with amebic colitis have been misdiagnosed as suffering from inflammatory bowel disease, often with dramatic consequences after initiating corticosteroid treatment (23, 30). In earlier studies among patients with amebic colitis, specific antibodies were observed with a variety of tests in 70 to 90% of cases (10, 17, 18, 24). In this study, specific antibodies were demonstrated with LAT in seven out of seven cases and with Dipstick and ELISA in six out of seven cases. These findings suggest that, as an additional tool to microscopic examinations, serodiagnosis could be useful for supporting or rejecting diagnosis of *E. histolytica* infection as the cause of colitis.

Cysts of *E. dispar* or *E. histolytica* are frequently observed in stools of travelers returning from the tropics. Recent studies demonstrated that up to 90% of these patients are infected with the nonpathogenic species *E. dispar* and not *E. histolytica* (7, 11, 14, 35). Because cysts of *E. histolytica* are morphologically similar to those of *E. dispar*, other techniques (e.g., PCR and ELISA for detection of specific antigens) are in use for differentiation of species from stools (8, 32). Although often satisfactory, antigen detection can lack sensitivity, and PCR requires advanced laboratory infrastructure and specific knowledge, especially because commercial PCR tests for ame-

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**TABLE 3.** Results of four different assays of serial sera from two patients with amebic liver abscess

<table>
<thead>
<tr>
<th>Patient</th>
<th>No. of days after diagnosis</th>
<th>Test result&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAP</td>
<td>ELISA&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Pos</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>Pos</td>
</tr>
</tbody>
</table>

<sup>a</sup> Pos, positive; Neg, negative.

<sup>b</sup> Results of *E. histolytica* ELISA; ELISA is considered positive when this value is above 164.
bias are not available. Therefore, reliable differentiation of E. histolytica from E. dispar infection often remains difficult in routine practice, especially in smaller laboratories. In the present study results for >95% of cyst passers of E. dispar were negative in all three serological tests, which was in sharp contrast to 9/11 to 10/11 (~80 to ~90%) positive findings in E. histolytica cyst passers. Earlier studies in which sera of PCR-confirmed cyst passers of E. histolytica/E. dispar were examined demonstrated 91% seropositivity with E. histolytica and 84% negative test results in patients with E. dispar, data which are in agreement with our findings (33). Although erosive bowel lesions have been described, E. dispar is regarded as noninvasive and relatively harmless in contrast to E. histolytica, which could explain the much lower frequency of detectable antibodies in the serological tests (21). Although (indirect) serological tests are no replacement for (direct) PCR or antigen-detecting assays, results of this and previous studies suggest that travelers from Western Europe with cyst passage and positive amebic serology might be infected with E. histolytica.

Positive serological test results can be of limited clinical significance for the diagnosis of amebic abscess, amebic colitis, and cyst passage of E. histolytica in patients originating from countries with high endemicity of E. histolytica infection, especially when titers are low. In these cases positive test results can indicate past infection with E. histolytica without being related to the present clinical status. Specific amebic antibodies can persist up to 4 years after infection with E. histolytica (23). In this respect the CAP test, used in this study as a reference test, is valuable for confirmation of positive test results, because after successful treatment of amebic abscess, results of this test in general become negative after 6 months to 1 year (12). The clinical importance of positive findings with amebic serology in general is high in Western countries where the background titer of these antibodies is low or even absent (35).

The specificity of the LAT in this study was 99.5%, and sensitivities for patients with amebic abscess and amebic colitis were 92.6% and 100%, respectively. These findings are in agreement with earlier findings in which, among patients with amebic abscess or colitis, sensitivities of 91 to 98% and specificities of 95 to 96% were observed (16, 25). Use of other agglutination assays in patients with invasive amebiasis demonstrated sensitivities in the range of ~90% and specificities of ~95% (15, 19).

ELISA is among the most popular serological methods used in diagnostic laboratories worldwide because of its high sensitivity and specificity and the possibility of quantifying the antibody reaction (21). We developed an ELISA in which microtiter wells with a smaller volume (30 µl as opposed to 300 µl) are employed in an effort to minimize the use of amebic antigen, which, in general, is difficult to obtain. In this set up, our ELISA had a specificity of 97.1%. Sensitivities for patients with amebic abscess or amebic colitis and for E. histolytica cyst passers were 100% or 85.7% and 81.8%, respectively. A number of homemade and commercially available ELISAs for demonstration of antibodies against E. histolytica have been described with sensitivities of ~80 to 100% and specificities of ~95 to 100% in cases with amebic abscess (28). After successful treatment ELISA values decline slowly (21).

Recently, dipstick assays have been introduced for various infectious and noninfectious diseases (3, 9, 13, 37, 39). Dipsticks are easy to use, do not require skilled personnel, and can be read with the naked eye. Our Dipstick could be performed in 45 min and resulted in the unambiguous absence or presence of a line at the site where the amebic antigen was incubated. The agreement between Dipstick and ELISA from this study was very good (κ = 0.951). Yamaura et al. described a dot-ELISA with a principle similar to that of our Dipstick (38). In this study with 37 cases with amebic abscess or colitis and 68 negative controls, a sensitivity of 97.3% and specificity of 100% was found, which is comparable to our results. Cyst passers were not studied.

In conclusion, all three tests studied proved to be highly reliable diagnostic tools. Both Dipstick and LAT are rapid and easy to use with a high sensitivity and specificity, and both can easily be applied in routine clinical laboratories. Improvement of sensitivity can be obtained by combining the tests. The ELISA is not as rapid as the other two tests but may provide an excellent tool for follow-up of treated patients, since quantitative results are obtained. Moreover, only a small amount amebic antigen is required for the ELISA compared to the Dipstick (0.12 versus 0.75 µg per patient). For western travelers with cyst passage of E. histolytica or E. dispar in stools and positive amebic serology, infection with E. histolytica is likely and treatment with luminal amebicidal drug should be considered.

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REFERENCES


