Rapid Detection of a *Schistosoma mansoni* Circulating Antigen Excreted in Urine of Infected Individuals by Using a Monoclonal Antibody

ABDELFATTAH M. ATTALLAH,1,2* HISHAM ISMAIL,1 SAMIR A. EL MASRY,2 HASSAN RIZK,3 AIA HANDOUSA,4 MAHMoud EL BENDARY,3 ASHRAF TABLL1 AND FAROUK EZZAT2

Biotechnology Research Center, New Damietta City,1 and Gastro-Enterology Center2 and Internal Medicine3 and Parasitology Departments,4 Faculty of Medicine, Mansoura University, Mansoura, Egypt

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*Schistosoma* circulating antigens were used to indicate the infection intensity and to assess cure. An immunoglobulin G2a (IgG2a) mouse monoclonal antibody was used in a fast dot-enzyme-linked immunosorbent assay (ELISA; FDA) for rapid and simple diagnosis of schistosomiasis in the field. Seven hundred Egyptians were parasitologically examined for *Schistosoma mansoni* and other parasitic infections. A rectal biopsy was done as a “gold standard” for individuals showing no *S. mansoni* eggs in their feces. Egg counts were obtained by the Kato smear method for only 100 of 152 individuals with eggs in their feces. Specific anti-schistosome IgG antibodies were evaluated in sera by ELISA. Urine samples from the 700 individuals were tested by FDA for detection of the circulating antigen. The assay showed a sensitivity of 93% among 433 infected individuals and a specificity of 89% among 267 noninfected individuals. FDA showed the highest efficiency of antigen detection (91%) compared with the efficiency of antibody detection by ELISA (75%) and stool analysis (60%). In addition, FDA detected infected patients with 20 eggs/g of feces. Also, the sensitivity of FDA ranged from 90 to 94% among samples from patients with different clinical stages of schistosomiasis. All the assay steps can be completed within 30 min at room temperature for 96 urine samples. The monoclonal antibody identified a 74-kDa antigen in different antigenic extracts of *S. mansoni* and *Schistosoma haematobium* and in the urine of infected individuals. In addition, a 30-kDa degradation product was identified only in the urine samples. On the basis of these results, FDA should be used as a rapid tool for the sensitive and specific diagnosis of *Schistosoma* infection.

Schistosomiasis, the second major parasitic disease in the world after malaria, affects about 250 million people worldwide. The current method for the diagnosis of schistosomiasis in areas of endemicity is the microscopic detection of eggs in stool and urine samples, but this assay does not give reliable results, and several measurements on different days are necessary for the precise diagnosis of schistosomiasis (14). Rectal biopsy is required to obtain better results, but it is invasive and its performance requires experienced physicians rather than technicians, and so it is not suitable for use in mass screening (1). Several schistosome serodiagnostic assays designed for the detection of specific anti-schistosome antibodies have been developed over the years. However, it seems difficult to believe how that a test based on antibody measurement may overcome the drawbacks intrinsic to such types of assays, namely, discrimination between active infections, old infections, and re-infections (12, 19). Standardization of reagents, expression of results, and correct interpretation of data are also difficult to achieve (22).

Recently, detection of circulating schistosome antigens secreted by live schistosomes in body fluids with specific monoclonal antibodies (MAbs) has been shown to be a promising approach to the detection of active infection and to the assessment of treatment efficacy and the effectiveness of future vaccines (8, 9, 13, 15, 21). The overall high degrees of sensitivity of antigen detection assays have been confirmed by comparing the results obtained by those assays with those obtained by quantitative parasitological techniques. A sensitivity of 80 to 90% was shown for patients excreting at least 100 eggs per gram (epg) of stool, and a sensitivity of 100% was shown for patients excreting more than 400 epg. The specificities of antigen detection assays, which all rely on the use of MAbs, are almost 100% (9–11, 16).

Many of the assays based on antigen detection display both high specificities and high sensitivities (25, 28). However, they require special and highly expensive equipment, and the procedures require long periods of time for their completion such that they cannot be easily adapted for field use. The dot enzyme-linked immunosorbent assay (ELISA) type of immunodiagnostic test is becoming widely used in simple qualitative research applications (23) and has already been reported for use in the detection of schistosomiasis (3). A number of modifications have been described in efforts to produce a more field-applicable assay format.

In the present study we evaluated the sensitivity and specificity of circulating antigen detection in urine by a newly developed fast dot-ELISA assay (FDA) and compared them with those of standard traditional techniques for the rapid and simple diagnosis of human schistosomiasis in the field.

**MATERIALS AND METHODS**

**Study subjects.** A total of 700 Egyptian individuals were included in the present study. They were 542 males and 158 females (age range, 3 to 72 years). A total of 450 individuals were symptomatic, and the remaining 250 individuals were asymptomatic. Stool, urine, and blood were collected from all individuals. Rectal biopsies were done for only 394 individuals (309 males and 85 females) among all individuals showing no *Schistosoma mansoni* eggs in their feces.

**Clinical examinations.** Full clinical examinations were done for all individuals and included a medical history. The general examination included assessment of...
with continuous shaking. The filter was washed with PBS for 3 min. An insoluble purple product was developed after the addition of the BCIP-NBT premixed substrate solution (Sigma) for about 3 min. The reaction was stopped with distilled water, and the filter was dried and kept in the dark. Positive controls for the assay were the affinity-purified antigen either from SWAP or from the urine of infected individuals and neat urine samples from infected individuals. Negative controls were urine samples from noninfected individuals. FDA allows a semiquantitative reading of the resulting colored spot in case of antigen detection (i.e., a positive test result). The purple color that was produced varied in its intensity depending on whether it was washed with water (1 + or 2+) or with strong (3+ or 4+). Positive controls with these different color intensities were used. A colorless spot was produced in the case of a negative test result. The resulting color for the tested sample was then compared and related to the color of one of the positive and negative controls with the naked eye.

RESULTS

A total of 700 individuals were subjected to stool analysis and/or rectal biopsy examination. A total of 433 individuals were parasitologically diagnosed as having *S. mansoni* infection (152 patients by stool analysis and 281 patients by rectal biopsy examination). All the infected individuals were symptomatic. A total of 267 individuals were parasitologically diagnosed as being noninfected (113 of them had no *S. mansoni* eggs in their rectal biopsy specimens). Counting of the eggs was done by the Kato technique for 100 of 152 infected individuals with eggs in their feces. Fifty-four patients had mild infections (10 of them had 20 epg of feces), 37 individuals had moderate infections, and 9 individuals had severe infections.

Serum samples from 433 individuals with schistosomiasis and 267 noninfected individuals were tested by indirect ELISA for detection of human anti-schistosome IgG antibodies. ELISA detected schistosomiasis with a sensitivity of 90% and had false-positive results for 132 of 267 noninfected individuals, with a specificity of 56% (Table 1).

Fresh urine samples from *S. mansoni*-infected patients and noninfected individuals were subjected to FDA. The assay detected the circulating antigen in 401 of 433 infected patients with a sensitivity of 93%. However, FDA could detect the circulating antigen in patients with mild infections (20 epg of feces) as well as in patients with heavy infections (more than 400 epg of feces). The assay gave false-positive results for 29 of the noninfected individuals, and this revealed an 89% specificity (Table 1).

### Table 1. Advantages of FDA as an alternative assay for the detection of human schistosomiasis based on antigen detection with stool analysis and the anti-schistosomal antibody detection test.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Specificity (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Positive predictive value (%)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Negative predictive value (%)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Efficiency (%)&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>93 [401(401 + 32)]</td>
<td>89 [238(238 + 29)]</td>
<td>93 [401(401 + 32)]</td>
<td>88 [238(238 + 32)]</td>
<td>91 [401 + 238(700)</td>
</tr>
<tr>
<td>Stool analysis</td>
<td>35 [152(152 + 28)]</td>
<td>100 [267(267 + 0)]</td>
<td>100 [152(152 + 0)]</td>
<td>49 [267(267 + 28)]</td>
<td>60 [152 + 267(700)</td>
</tr>
<tr>
<td>Antibody test&lt;sup&gt;g&lt;/sup&gt;</td>
<td>90 [390(390 + 43)]</td>
<td>56 [135(135 + 132)]</td>
<td>73 [390(390 + 132)]</td>
<td>80 [135(135 + 43)]</td>
<td>75 [390 + 135(700)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Sensitivity, number true positive/(number true positive + number false negative).

<sup>b</sup>Specificity, number true negative/(number true negative + number false positive).

<sup>c</sup>Positive predictive value, number true positive/(number true positive + number false positive).

<sup>d</sup>Negative predictive value, number true negative/(number true negative + number false negative).

<sup>e</sup>Efficiency, (number true positive + number true negative)/total number.

<sup>f</sup>FDA for detection of *Schistosoma* circulating antigen excreted in urine.

<sup>g</sup>Detection of anti-schistosome IgG antibodies in serum samples against SWAP by indirect ELISA technique.
individuals), *Giardia lamblia* (6 individuals), and *Ascaris* (6 individuals); 93% for stools from individuals infected with *Entamoeba histolytica* (136 individuals); and 82% for stools from individuals with no parasitic infections (107 individuals).

FDA showed the highest efficiency (91%) compared with stool analysis (60%) and ELISA (75%) for the detection of anti-schistosome antibodies. Table 1 summarizes the results obtained by stool analysis, the serological method, and FDA. Figure 1 shows that FDA is an easily applicable assay for the mass screening of schistosomiasis patients. A large number of urine samples (96 samples per plate) could be tested within 30 min.

The target antigen for the IgG2a BRL4 MAb was identified as a single band of 74 kDa and a degradation product of 30 kDa. Figure 2 demonstrates the reactivity of this MAb with the circulating antigen in the urine of *S. mansoni*-infected and non-infected individuals and also in the antigenic extract preparations (SEA, SWAP, and CAP) of *S. mansoni* and *S. haematobium* by Western blotting.

**DISCUSSION**

Several immunodiagnostic assays based on MAbs for the detection of schistosome antigens in the serum and urine of schistosomiasis patients have been described. Deelder et al. (5–7) and De Jonge et al. (9, 10) used a mouse MAb against the gut-associated proteoglycans, the circulating anodic antigen, the circulating cathodic antigen, or the M antigen in several enzyme immunoassays. These assays cannot be easily applied in the field, where the sample must be pretreated with trichloroacetic acid followed by dialysis, and the assay also requires a long time for its completion and special and highly expensive equipment.

So, it is very important that a rapid, simple, and reliable test for the diagnosis of schistosomiasis in the field be developed. Available reagent strip assays for the demonstration of blood in the urine of *S. haematobium*-infected individuals are rapid and simple and have been shown to correlate with the parasitological diagnosis, but they are not specific and remain of no use for the detection of *S. mansoni* or Schistosoma japonicum infections. Recently, a rapid reagent strip assay based on the detection of schistosoma circulating cathodic antigen in urine was developed (26). The assay can be completed in only 75 min and showed more than 95% sensitivity and specificity, and this assay was also applied for the assessment of cure of schistosomiasis patients (27).

In the present study, the FDA developed on the basis of an IgG2a MAb for the detection of *Schistosoma* circulating antigen excreted in urine is a simple, rapid, sensitive, and specific enzyme immunoassay. The assay could therefore be used in the field as part of a mass screening program. The urine sample was used neat, i.e., without any treatment, the assay needs no sophisticated equipment, and 96 urine samples could be run in about 30 min. In addition, all assay steps were done at room temperature.

We evaluated the sensitivity and specificity of our assay for the detection of *S. mansoni* in comparison with those of stool analysis and testing for antibody by ELISA. We found that FDA had a higher sensitivity (93%) than microscopic examination of eggs in stool (35%) and a higher specificity (89%) than anti-schistosomal antibody detection in serum by ELISA (56%). Moreover, FDA had sensitivities ranging from 90 to 94% for the different clinical stages of schistosomiasis. Also, the assay could detect the schistosome antigen by the Kato technique in urine samples from patients with light infections of 20 epg of feces. The circulating antigens were detected in individuals with low egg counts (9, 10).

The target antigen of our BRL4 MAb was identified at 74 kDa in the urine of *S. mansoni*-infected individuals and in three developmental stage antigenic extracts of *S. mansoni*. This antigen has been characterized as a protein in nature, with 56.9% hydrophilic amino acids and 43.1% hydrophobic amino acids (2). Only the 30-kDa degradation product was identified in the urine samples, and this may be due to the unsuitable environment of urine. In addition, the BRL4 MAb identified a 74-kDa antigen in the three developmental stage antigenic extracts of *S. haematobium*. Preliminary data from a diagnostic

**TABLE 2. Detection of Schistosoma circulating antigen by FDA in the four clinical stages of human schistosomiasis**

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>No. of specimens</th>
<th>No. of specimen positive by FDA</th>
<th>% Sensitivity</th>
</tr>
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<tbody>
<tr>
<td>Simple intestinal bilharziasis</td>
<td>241</td>
<td>225</td>
<td>92</td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>100</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>Shrunken liver and splenomegaly</td>
<td>34</td>
<td>32</td>
<td>94</td>
</tr>
<tr>
<td>Ascites</td>
<td>58</td>
<td>52</td>
<td>90</td>
</tr>
<tr>
<td>Total</td>
<td>433</td>
<td>401</td>
<td>93</td>
</tr>
</tbody>
</table>

**FIG. 1.** Random screening of schistosomiasis patients by FDA. The assay allows a semiquantitative reading; i.e., faint spots (1+ or 2+ color intensity) are considered weak positive and dark spots (3+ or 4+ color intensity) are considered strong positive. Wells A1 to C1 represent positive controls, and wells D1 to H1 represent negative controls.

**FIG. 2.** Identification of the Schistosoma antigen recognized by the BRL4 MAb by Western blotting. Lane A, urine from a noninfected individual; lanes B and C, urine from *S. mansoni*-infected patients; lanes D to F, antigenic extracts of *S. mansoni* (lane D, CAP; lane E, SWAP; lane F, SEA); lanes G to I, antigenic extracts of *S. haematobium* (lane G, CAP; lane H, SWAP; lane I, SEA). Molecular size standards (not shown) were triosephosphate isomerase, 34.1 kDa; lactic dehydrogenase, 40.8 kDa; fumarase, 57.8 kDa; pyruvate kinase, 72.7 kDa; fructose-6-phosphate kinase, 91.8 kDa; β-galactosidase, 117.0 kDa; and α-2-macroglobulin, 191.0 kDa.
study performed for the detection of *S. haematobium* infection showed that FDA with BRL4 MAb detected *S. haematobium* eggs in more than 80% of individuals with eggs in their urine samples.

On the basis of these results, FDA has a number of advantages that make it a preferable technique over the other diagnostic assays. It is a simple, rapid, noninvasive, specific, and sensitive assay for the detection of schistosome antigens among humans with all clinical stages of schistosomiasis. This will enhance the application of this assay in the field and for mass screening and control programs of *S. mansoni* and *S. haematobium* schistosomiasis.

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REFERENCES