Evaluation of a New Bicolored Latex Agglutination Test for Immunological Diagnosis of Hepatic Amoebiasis

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A new bicolored latex agglutination amoeba test (BLA) for detection of antibodies against *Entamoeba histolytica* was evaluated for its practicability and diagnostic sensitivity and specificity. BLA is rapid (5 min) and simple to perform. It requires only 20 μ l of a 1/3-diluted serum, 17 μ l of reagent, and a glass slide. Reading of the test is easy because a positive result shows a green spot with a red surrounding edge. This bicolored pattern is easily distinguishable from the negative test result, which shows a homogeneous dark-brown spot. By using serum samples from 348 individuals, BLA was compared with immunofluorescence assay, indirect hemagglutination, and counterimmunoelectrophoresis. Sensitivity, specificity, efficiency, and positive and negative predictive values of the four methods were almost identical. The results of this study indicate that BLA could be very useful both as a screening method for the diagnosis of invasive amoebiasis and for epidemiological purposes.

The diagnosis of extraintestinal amoebiasis is based on the presence of symptoms and clinical signs usually supplemented by serological methods. Among these methods, indirect hemagglutination (IHA) (9, 11, 18), complement fixation (11), immunoelectrodifusion or counterimmunoelectrophoresis (CIE) (7, 13, 16), immunofluorescence assay (IFA) (3, 10), and enzyme-linked immunosorbent assay (1, 2, 4, 6, 19-23, 25) are probably the best known. Technically speaking, except for IHA, these techniques are relatively laborious and require expensive equipment and specialized skills. On the other hand, latex agglutination (15) is very easy to perform, needs very little material, and is suitable for laboratory and field applications.

In this report, a new commercial bicolored latex agglutination amoeba test (BLA) (Bichrolatex-Amibe; Fumouze Diagnostic, France) for detection of antibodies against *Entamoeba histolytica* is compared with conventional methods.

MATERIALS AND METHODS

Latex agglutination slide test. BLA (Fumouze Diagnostic) was performed according to the manufacturer's instructions. Sensitized red latex particles were coated with antigen from *E. histolytica* and suspended in 0.15 M phosphate-buffered saline (pH 7.2) (PBS) containing 0.3% bovine serum albumin and a dye giving a green coloration to the buffer. Before use, the reagent was shaken so as to get a homogeneous dark-brown suspension. Twenty microliters of serum 1/3 diluted in the diluent supplied in the kit was added to 17 μ l (a drop) of the sensitized latex suspension and mixed to cover a delineated circle (diameter, 1.5 cm) on a white glass slide. The slide was rocked slowly, manually or mechanically. Five minutes later, the sample was examined visually for agglutination. Pictures obtained by using BLA are as follows. (i) Negative result shows a homogeneous dark-brown spot due to unagglutinated red latex particles which remain suspended in the green buffer. (ii) Positive result shows the agglutinated red particles collected at the edge of the reac-

tion area, contrasting with the central area, which forms a green background. With very weakly positive sera, the colored edge resulting from the collected agglutinate particles is thinner and some of the red agglutinates may remain in the central part of the spot, contrasting with the green background. This pattern strongly differs from the homogeneous brown spot observed with negative sera. Positive and negative controls were tested by using sera delivered by the manufacturer. Six lots of BLA were used.

Maintenance of *E. histolytica* trophozoites. *E. histolytica* HM1 was provided by M. C. Rigother (Faculty of Pharmacy-Chatenay-Malabry, Chatenay-Malabry, France) and was cultured in a screw-cap borosilicate glass tube (16 by 25 mm) by serial passage, at 3- to 4-day intervals, in Diamond medium (5) supplemented with 15% bovine serum. Organisms were obtained by centrifuging tubes at $250 \times g$ for 10 min.

IFA. The organisms were washed three times with 40 volumes of 0.15 M PBS and resuspended in PBS containing 3.7% Formalin (Merck-France) to a concentration of 10^6 cells per ml and then incubated at 37°C for 1 h. The Formalin-fixed organisms were centrifuged at $250 \times g$ for 15 min, washed with 40 volumes of PBS, and resuspended in PBS, to a ratio of 10^6 organisms per ml; 10 μ l of suspension was placed on each well of the slide (10 wells per slide). Assays were performed as described by Jeanes (10). In our

TABLE 1. Positive results of four tests for amoebiasis^a

Serum group (no. of sera tested)	No. of positive results with:			
	BLA	IFA	IHA	CIE
1 (61)	60	56	57	54
2 (22)	3	2	1	0
3 (185)	7	14	6	3
4 (80)	1	0	0	0

^a Positive results obtained with sera from patients with hepatic amoebiasis (group 1), intestinal amoebiasis (group 2), or liver disorders other than amoebiasis (group 3), or from healthy people (group 4), tested comparatively with the bicolored latex agglutination amoeba (BLA) test, immunofluorescence assay (IFA), indirect hemagglutination (IHA), and counterimmunoelectrophoresis (CIE).

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TABLE 2. Values for *E. histolytica* antibodies

Test	Value ^a (% $\pm$ SD)				
	Sensitivity	Specificity	Predictive value		Efficiency
			Positive	Negative	
BLA	98.3 $\pm$ 3.3	96.1 $\pm$ 2.2	84.5 $\pm$ 3.8	99.6 $\pm$ 0.67	96.5 $\pm$ 1.9
IFA	91.8 $\pm$ 7	94.4 $\pm$ 2.7	77.7 $\pm$ 18.1	98.1 $\pm$ 1.4	94 $\pm$ 2.5
IHA	93.4 $\pm$ 6.3	97.5 $\pm$ 1.8	89 $\pm$ 3.3	98.5 $\pm$ 1.3	96.8 $\pm$ 1.8
CIE	88.5 $\pm$ 8.1	98.9 $\pm$ 1.2	94.7 $\pm$ 2.4	97.5 $\pm$ 1.6	97.1 $\pm$ 1.1

^a Value in differentiating patients with hepatic amoebiasis from all control subjects.

laboratory, the test was considered positive when fluorescence was observed at dilutions of 1/80 and above.

CIE. Antigen for CIE was prepared by the following method. *E. histolytica* trophozoites were washed three times with 40 volumes of PBS by centrifugation at $250 \times g$ for 10 min and resuspended to 10^7 cells per ml in 0.05 M Tris-glycine buffer (pH 9). The suspension was sonicated in an ice-water bath for 3 min at 20 kilocycles in an ultrasonic disrupter (Sonifier 450; Branson Sonic Power Co., Danbury, Conn.) and centrifuged at $40,000 \times g$ for 30 min at 4°C. The protein content of the supernatant, corresponding to the soluble antigenic extract, was determined by the method of Lowry et al. (14). The antigen was stored frozen in aliquots of 0.1 ml at -80°C and used at a protein concentration of 4 mg/ml for CIE on cellulose acetate membrane (Sartorius, Paris, France) as described by Pinon et al. (17). In our laboratory, the test was considered positive on the appearance of at least two precipitating bands.

IHA. IHA (Fumouze Diagnostic) was performed according to the manufacturer's instructions. The test was considered positive when hemagglutination was observed at a dilution of 1/320 and above.

Sera. The serum specimens tested came from many sources and were divided into 4 groups. Group 1 included 61 serum specimens obtained from patients with amoebic liver abscess. The diagnosis was made when the following symptoms were present (24): fever, hepatomegaly, elevation of the right diaphragm and right upper quadrant, pain, and tenderness. All cases of amoebic hepatic abscess had a space-occupying liver lesion demonstrated by scintiphoto or ultrasound scan. Laboratory data showed leukocytosis and positive serology by CIE, IHA, and IFA in amoebiasis. Group 2 included 22 serum specimens from patients with intestinal amoebiasis (stools positive for trophozoites or cysts of *E. histolytica*). Group 3 included 185 serum specimens from hospitalized patients with various liver diseases other than amoebiasis. Group 4 included 80 serum specimens from healthy individuals. All serum specimens were collected before treatment and were kept frozen at -20°C until testing.

Statistics. Sensitivity, specificity, efficiency (percentage of individuals correctly identified as having or not having hepatic disorders), and positive and negative predictive values were calculated as described by Kozinn et al. (12). Mean values were compared by using Student's *t* test.

RESULTS

A total of 348 serum specimens were tested. The results are shown in Table 1.

Table 2 shows that BLA was as sensitive and specific as IFA or IHA. The differences were not significant ($P > 0.05$) for all three methods. BLA was more sensitive than CIE (P

> 0.03). The negative and positive predictive values and the efficiencies were high and almost identical for all four methods. Reproducibility studies on the six lots of BLA were performed with the 61 positive serum specimens from group 1 and with the 80 negative serum specimens from group 4. The results were identical for all six lots.

With the dilution used (1/3) no prozone phenomenon was observed.

DISCUSSION

Rapid colored latex tests have been recently developed (8), but as yet few applications are available for the diagnosis of parasitic diseases.

In this study, a new commercial test (BLA) applied to the detection of antibodies against *E. histolytica* was assessed. When it was tested on 348 serum samples, its sensitivity (98%) and its specificity (96%) were as high as those obtained with IFA or IHA. This study shows that BLA is rapid (5 min) and easy to perform. It requires only a small amount of bench space and does not need sophisticated equipment. The test is simpler to read than other latex agglutination tests. This is due to the appearance of two complementary colors (green and red) with positive sera whereas negative sera remain homogeneous and brown, which remarkably differs from the positive pattern.

BLA could be useful as an adjunct to standard methods for routine diagnosis of hepatic amoebiasis or as a screening test for sera to be titrated by a more quantitative technique. In addition, it could be a very convenient method for epidemiological studies of this disease because it is suitable for field application.

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