Diagnosis of Amebic Dysentery by Detection of *Entamoeba histolytica* Fecal Antigen by an Invasive Strain-Specific, Monoclonal Antibody-Based Enzyme-Linked Immunosorbent Assay

**ARMANDO GONZALEZ-RUIZ,** 1,2*  **RASHIDUL Haque,** 3 **TAYAB REHMAN,** 1† **AURA AGUIRRE,** 4 **ANDREW HALL,** 3 ‡ **FELIPE GUHL,** 6 **DAVID C. WARHURST,** 1† AND **MICHAEL A. MILES** 1

Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London WC1E 7HT, 1 and Department of Clinical Microbiology, University College London Hospitals, London WC1E 6AU, 2 United Kingdom; International Centre for Diarrhoeal Diseases Research, Bangladesh, Dhaka 1,000, Bangladesh; 3 and Laboratorio de Parasitología y Microbiología, Departamento de Ciencias Biológicas, Universidad de los Andes, Santa Fe de Bogotá, Colombia 4

Received 20 September 1993/Returned for modification 28 November 1993/Accepted 17 January 1994

An invasive strain-specific monoclonal antibody against *Entamoeba histolytica* has been used in a capture enzyme-linked immunosorbent assay (ELISA) for the detection of invasive *E. histolytica* fecal antigen in clinical specimens and for the diagnosis of amebic dysentery in patients from Bangladesh. The fecal antigen capture ELISA (FAC-ELISA) did not cross-react with other parasite species in the clinical specimens or with noninvasive *E. histolytica* present in those specimens and in experimentally seeded stools. The limit of detection of the assay for invasive *E. histolytica* crude antigen diluted in phosphate-buffered saline or in stools was 0.58 and 3.9 μg/ml, respectively, which is the equivalent of approximately 72 and 487 *E. histolytica* trophozoites per well, respectively. The sensitivity, specificity, and efficiency of the FAC-ELISA were 87, 100, and 98%, respectively, for the detection of invasive *E. histolytica* antigens and 100, 100, and 100%, respectively, for the diagnosis of amebic dysentery. The FAC-ELISA is a potential alternative for the field diagnosis of amebic dysentery and for epidemiological studies to define the distribution of invasive *E. histolytica*.

The protozoan parasite *Entamoeba histolytica* is the etiological agent of human amebiasis. Approximately 500 million people are infected worldwide with *E. histolytica*, but only 10% develop invasive amebiasis (38). Like other diarrheal diseases, amebiasis is more prevalent in developing countries and produces between 40,000 and 100,000 deaths per year (40). The discrepancy between the number of carriers and the relatively low percentage of patients developing invasive amebiasis could in part be explained by higher prevalence of infection with noninvasive strains of the parasite, which are morphologically indistinguishable but genotypically distinct from invasive strains (3, 4, 6). Failure to identify nonpathogenic amebae such as *Entamoeba coli* and *Entamoeba hartmanni* may also increase the apparent prevalence of asymptomatic *E. histolytica* infection (17, 22, 29).

Microscopical examination of stools does not differentiate between invasive and noninvasive strains of *E. histolytica*, is time-consuming, and requires a skillful microscopist for the detection and recognition of *E. histolytica* trophozoites with ingested erythrocytes (22, 38). Differential diagnosis of amebic dysentery (AD) at present requires facilities for isolating bacterial agents such as *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., or *Yersinia* spp. or for the recognition of clinical conditions such as rectal infection by *Chlamydia trachomatis*, *Treponema pallidum*, and *Neisseria gonorrhoeae* (19). AD must also be differentiated from idiopathic intestinal inflammatory diseases, namely, Crohn’s disease and ulcerative colitis (28).

The development of fecal antigen detection assays has been designated a priority in amebiasis research by the World Health Organization (40): such assays detect only current infections and can be very useful in epidemiological studies. The adaptation of an invasive strain-specific monoclonal antibody (MAb) to a fecal antigen capture enzyme-linked immunosorbent assay (FAC-ELISA) would enhance the diagnosis of intestinal amebiasis and differentiate that diagnosis from other intestinal infections such as bacillary dysentery in carriers of noninvasive *E. histolytica*.

We have produced an invasive strain-specific MAb (20/7D) against *E. histolytica* which has been used in an indirect immunofluorescence test to characterize *E. histolytica* isolates from several geographical regions (15). Here we report the application of this MAb in a FAC-ELISA for the detection of *E. histolytica* fecal antigen and for the diagnosis of AD in patients from Bangladesh.

**MATERIALS AND METHODS**

Production of *E. histolytica* NP-40 protein extract. *E. histolytica* Nonidet P-40 (NP-40) protein extract was produced from three axenic *E. histolytica* strains, HM-1:IMSS, formerly known as ABM (7); HK9 (13); and NIH:200 (32) (ATCC 30015, ATCC 30458, and ATCC 30459, respectively), which were originally isolated from patients with invasive intestinal amebiasis, and from *E. histolytica* polyxenic isolates cultured from cases of symptomatic and asymptomatic amebiasis. The axenic
strains were cultured in Diamond's TPS-1 medium (9) at 37°C. The polyoxenic isolates were initially isolated and maintained in biphasic Robinson's medium at 37°C, and some were also mass-cultured in liquid Robinson's medium (30) at 37°C. *E. histolytica* trophozoites were harvested from Diamond's and liquid Robinson's media by chilling the culture tubes in ice-water for 15 min, washed three times in cold phosphate-buffered saline (pH 7.2) (PBS), and centrifuged at 250 × g for 5 min at 4°C. The pelleted cells were lysed by the addition of 3 volumes of lysis buffer (20 mM Tris-HCl [pH 8.0], 150 mM NaCl, 0.75% [wt/vol] NP-40 [BDH Chemicals, Poole, Dorset, United Kingdom]) containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride [Sigma Chemical Co., Poole, Dorset, United Kingdom], 1 mM iodoacetamide [BDH]), and 1 μg of tosyl-L-lysine chloromethyl ketone per ml [Sigma]) with continuous stirring on ice for 15 min. The mixture was centrifuged at 16,000 × g for 15 min at 4°C, and the supernatant was harvested and stored at −20°C. The protein content of this supernatant and other preparations was determined with the bicinchoninic acid protein assay reagent (Pierce, Rockford, Ill.) with bovine serum albumin as standard.

**Production of anti-** *E. histolytica** hyperimmune serum.** Two male Half Lop rabbits were immunized with a mixed crude cell extract of trophozoites of three *E. histolytica* axenic strains (HM-1:IMSS, HK9, and NIH-200) as follows: trophozoites were harvested from Diamond’s medium (above), they were disrupted by three sequential cycles of freezing and thawing in liquid N2, and their protein content was determined (above). Rabbits were immunized with 50 μg of extract emulsified 1:1 with Freund’s complete adjuvant, half of the volume being injected subcutaneously and half intramuscularly. Rabbits were boosted every 6 to 8 weeks with extract 1:1 in Freund’s incomplete adjuvant. Antibody response was assayed before each boost by indirect ELISA (36) against NP-40 protein extract of *E. histolytica*. Rabbits were bled after the second boost.

**Affinity purification of rabbit antisera.** A 10-ml affinity-chromatography column was prepared with *E. histolytica* NP-40 protein extract dialyzed at 4°C against two changes of coupling buffer (100 mM sodium bicarbonate–0.5 M sodium chloride [pH 8.3]) and coupled to cyanogen bromide-activated Sepharose 4B at a concentration of 5 mg of protein per ml of gel (Pharmacia Fine Chemicals, Uppsala, Sweden), according to the manufacturer’s instructions. The column was equilibrated with PBS and preeluted with 0.1 M glycine (pH 2.5), and after reequilibration with PBS, 5 ml of ammonium sulfate-precipitated hyperimmune rabbit serum was loaded onto the column and cycled overnight at 4°C. After extensive washing with PBS, bound immunoglobulin was eluted with 0.1 M glycine (pH 2.5), and 3-ml fractions were immediately neutralized with 250 μl of 2 M Tris-HCl (pH 8). Fractions containing the highest antibody activity, as assayed by indirect ELISA (36), were pooled, dialyzed overnight at 4°C against PBS, and concentrated by means of negative-pressure ultrafiltration in an Amicon Ultrafiltration Cell, type YM 10 (10-kDa molecular mass cutoff; Amicon, Danvers, Mass.), and the protein content of the concentrate was assayed. Concentrated antibodies were aliquoted and stored at −20°C.

**Stool samples.** Stool samples were collected at the International Centre for Diarrheal Diseases Research, Bangladesh (ICDDR,B), in Dhaka, from patients of all age groups seen at the hospital and from children less than 14 years old living in a slum in Dhaka. Recently collected stools were examined macroscopically for the presence of blood and mucus, and a smear of feces in 0.9% saline was examined microscopically for the presence of erythrocytes, leukocytes, and *E. histolytica* trophozoites with or without ingested erythrocytes. Feces were concentrated by the formalin-ether concentration technique (23), and the concentrate was examined microscopically for cysts and ova. Feces were also inoculated into Robinson’s medium within 6 h of collection, and *E. histolytica*-positive cultures were subcultured every 48 h.

**Definition of diarrheal disease.** Stool samples were classified into several diagnostic categories according to microscopic examination and detection of *E. histolytica* by Robinson’s culture which was characterized as invasive or noninvasive by zymodeme analyses (37). Invasive disease (DIA) was defined as diarrhea (DIA) with erythropagocytic *E. histolytica* trophozoites seen by microscopy, a finding which is considered the diagnostic hallmark of the disease (37, 39); dysentery of unknown etiology was defined as bloody DIA without evidence of invasive amebic infection; DIA was defined as nondsenteric loose stools with or without invasive *E. histolytica* infection; and asymptomatic carrier (AC) was defined as infection with *E. histolytica* detected in a patient with formed stools.

**Zymodeme identification of *E. histolytica* isolates.** The zymodeme of the *E. histolytica* isolates was determined locally. At the ICDDR,B, this was done by cellulose acetate electrophoresis plates (Zip Zone Chamber and Titan Plus Electrophoresis Power Supply; Helena Laboratories, Beaumont, Tex.) as described previously (20): at Universidad de los Andes (UNIANDES), thin-layer starch gel electrophoresis was used according to the methods of Sargeant and Williams (25) and Sargeant et al. (26) for phosphoglucomutase (EC 2.7.5.1), maltose:NADP+ oxidoreductase (oxaloacetate decarboxylating) (EC 1.1.1.40), and glucose phosphate isomerase (EC 5.3.1.9), and according to Farri et al. (10) for hexokinase (EC 2.7.1.1). Briefly, *E. histolytica* trophozoites were harvested from 2-day-old Robinson’s cultures and washed twice with sterile 0.9% saline, and lysates were prepared by resuspending pelleted cells in distilled water containing enzyme stabilizers (1 mM EDTA, 1 mM diithiothreitol, and 1 mM L-α-aminoacproic acid [Sigma]) and by three sequential cycles of freezing and thawing. Lysates were centrifuged at 14,000 × g for 1 h at 4°C, and supernatants were aliquoted and stored in liquid N2. Isoenzyme profiles were visualized with an agar overlay (10, 27).

**FAC-ELISA.** Three sample sets were assayed for *E. histolytica* antigen. Two were experimentally seeded (spiked) with either *E. histolytica* NP-40 extract (at London School of Hygiene and Tropical Medicine [LSHTM]) or with intact polyoxenic *E. histolytica* trophozoites (at ICDDR,B), and one set consisted of clinical stool samples from patients in Bangladesh. Spiked samples were prepared as follows: *E. histolytica* NP-40 extract from several isolates was diluted at 50 μg/ml in PBS and stored at −20°C. *E. histolytica* isolates were harvested (200 μl at ICDDR,B) from 2-day-old biphasic Robinson’s cultures yielding ~2 × 10⁷ trophozoites (24), mixed with 0.5 ml of parasite-free feces (final protein, ~2.3 μg/ml), frozen, and kept at −20°C until assayed at LSHTM. Estimates of the FAC-ELISA detection limit were made with twofold serial dilutions of *E. histolytica* NIH:200 NP-40 extract in PBS and parasite-free stools at a starting concentration of 50 μg/ml. Aliquots of dilutions (100 μl) were assayed by FAC-ELISA (below). To estimate the number of trophozoites per 100 μl, it was assumed that 10⁶ were equivalent to ~0.8 mg of *E. histolytica* protein (8).

Clinical stool samples collected and examined at ICDDR,B were kept at −20°C and transported frozen to the LSHTM, where they were mixed 1:3 with fecal diluent (PBS–0.02% NaN₃–0.3% Tween 20–50% fecal calci suspension). Fecal supernatants were prepared by freezing and thawing the fecal suspen-
sion three times in liquid N₂ and by centrifugation at 8,500 × g for 30 min and stored at −20°C until assayed blindly at LSHTM.

Alternate rows of flat-bottomed polystyrene microplates (Immulon II; Dynatech Laboratories Ltd., Billingshurst, Sussex, United Kingdom) were coated overnight at 4°C, either with affinity-purified polyclonal rabbit antisera against *E. histolytica* trophozoites or with nonimmune rabbit immunoglobulin G (Jackson Immunoresearch Inc., Philadelphia, Pa.), at 1 μg of protein per well in coating buffer (7.5 mM Na₂CO₃-17.4 mM NaHCO₃-0.02% NaN₃, pH 9.6). All subsequent washes were with PBS-0.05% Tween 20. Working dilutions of reagents were determined by checkerboard titration. After three washings, plates were blocked (coating buffer-2% casein) for 1 h at 37°C and again washed three times, and 100 μl of fetal calf serum was dispensed into all wells, followed by either 100 μl of spiked PBS-parasite-free stools or 100 μl of fecal supernatant dispensed into immune and nonimmune rabbit immunoglobulin G-coated wells. After incubation for 2 h at room temperature, plates were washed five times and 100 μl of a 1/1,500 dilution of mouse ascites containing invasive strain-specific MAb 20/7D (15) was dispensed in PBS-Tween 20-2% casein. Plates were reincubated for 2 h at 37°C and washed five times and 100 μl of 1/10,000 peroxidase-conjugated rabbit anti-mouse immunoglobulins (Jackson Immunoresearch Inc.) in PBS-Tween 20-2% casein added to each well. After further incubation for 1 h at 37°C, plates were washed five times and 100 μl of substrate solution (0.04% orthophenylenediamine [Sigma] in phosphate-citrate buffer, pH 5, with 0.012% H₂O₂) was added to all wells. The chromogenic reaction was stopped after 30 min of incubation in the dark at room temperature with 50 μl of 2.5 M H₂SO₄ per well.

**Interpretation of results and statistical analysis.** Optical densities (ODs) were measured by spectrophotometry (492-nm Dynatech plate reader). To allow for inter-ELISA plate variation, OD values were normalized by using the ODs on each plate of positive controls consisting of *E. histolytica* NP-40 extract at 50 μg/ml in PBS. Negative controls (PBS and stools; conjugate and no second antibody) were also included on each plate. Assay cutoff value was the average of ODs obtained with nonimmune rabbit immunoglobulin G plus three standard deviations. After subtracting the OD of the nonimmune well, which represents nonspecific binding, from the OD of the immune duplicate, samples with ODs of >0.035 were considered positive by FAC-ELISA.

Predictive value of a positive and a negative assay and assay efficiency were calculated according to Vecchio (34) and Galen and Gambino (11), respectively. Statistical analysis of ODs with each group of stool samples was by the unpaired *t* test, and that of demographic characteristics of patients was by the

**TABLE 1.** Specificity of MAb 20/7D in the FAC-ELISA with spiked stool samples

<table>
<thead>
<tr>
<th>FAC-ELISA</th>
<th>No. of isolates and zymodemes</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>

*a* Stools spiked with either 50 μg/ml of *E. histolytica* NP-40 extract per ml at LSHTM or with approximately 400 intact trophozoites (approximately 2.3 μg of protein per ml) at ICDDR,B.

*b* I, noninvasive zymodeme; II and XIV, invasive zymodemes.

**RESULTS**

MAb 20/7D is invasive strain specific in the FAC-ELISA. MAb 20/7D, an invasive strain-specific MAb against *E. histolytica* trophozoites (15), was adapted to the FAC-ELISA. Its specificity in the FAC-ELISA was tested by assaying a series of PBS and parasite-free stools spiked with *E. histolytica* NP-40 extract or *E. histolytica* trophozoites (Table 1). The FAC-ELISA was negative with the 10 samples spiked with noninvasive *E. histolytica* antigen and positive with 12 of 17 samples spiked with invasive *E. histolytica* antigen. Although four of five false-negative samples were in the group of samples spiked with the lowest amount of *E. histolytica* protein, there was a considerable number of true positives in this sample group (Table 2).

**Detection limit of the *E. histolytica* FAC-ELISA.** The detection limit of the *E. histolytica* FAC-ELISA was estimated by assaying serial dilutions of *E. histolytica* protein in PBS and parasite-free stools. The ELISA could detect 0.58 μg of *E. histolytica* protein per ml in PBS (Fig. 1) and 3.9 μg/ml in parasite-free stools, equivalent to approximately 72 and 487 *E. histolytica* trophozoites, respectively, per well (100 μl).

**Detection of *E. histolytica* antigen in clinical stool samples by FAC-ELISA.** Performance of the FAC-ELISA with clinical

**FIG. 1.** Limit of detection of FAC-ELISA with twofold dilutions of invasive *E. histolytica* antigen in PBS and in parasitologically negative stools.
TABLE 3. Intestinal parasites found in stool specimens with or without *E. histolytica* infection

<table>
<thead>
<tr>
<th>Parasite</th>
<th>No. of episodes* (%)</th>
<th><em>E. histolytica</em> present</th>
<th><em>E. histolytica</em> absent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pentatrichomonas hominis</em></td>
<td>5 (10)</td>
<td>5 (8)</td>
<td></td>
</tr>
<tr>
<td><em>Blastocystis hominis</em></td>
<td>3 (6)</td>
<td>7 (11)</td>
<td></td>
</tr>
<tr>
<td><em>Entamoeba coli</em></td>
<td>4 (8)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td><em>Giardia intestinalis</em></td>
<td>0 (0)</td>
<td>5 (8)</td>
<td></td>
</tr>
<tr>
<td><em>Endolimax nana</em></td>
<td>2 (4)</td>
<td>2 (3)</td>
<td></td>
</tr>
<tr>
<td><em>Enteromonas hominis</em></td>
<td>0 (0)</td>
<td>2 (3)</td>
<td></td>
</tr>
<tr>
<td><em>Chilomastix mesnili</em></td>
<td>1 (2)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>12 (25)</td>
<td>22 (36)</td>
<td></td>
</tr>
<tr>
<td><em>Trichuris trichiura</em></td>
<td>9 (19)</td>
<td>12 (19)</td>
<td></td>
</tr>
<tr>
<td>Hookworm sp.</td>
<td>2 (4)</td>
<td>5 (8)</td>
<td></td>
</tr>
<tr>
<td><em>Strongyloides stercoralis</em></td>
<td>3 (6)</td>
<td>2 (3)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>63</td>
<td></td>
</tr>
</tbody>
</table>

* One episode of parasitic infection is the presence of the parasite in a single stool sample.

* Percentages were calculated from 47 stool samples with or without *E. histolytica* infection. In some stool samples, two or more parasites were present simultaneously.

The stool samples were evaluated with stools collected at the ICDDR,B. The stool samples were classified on the basis of results of microscopical examination, Robinson’s culture, and zymodeme analysis. The FAC-ELISA was first tested with three sample groups that differed in the microscopy result: 47 carried *E. histolytica* either alone (25 samples) or with other parasites (22 samples), 33 contained other intestinal parasites but no *E. histolytica* (Table 3), and 28 had no parasitic infection. There were no significant differences in age and sex distribution of patients with and without parasitic infections (not shown).

Among other parasitic protozoa found by microscopy, the most common were the commensal flagellate *Pentatrichomonas hominis* and *Blastocystis hominis*, which is associated with DIA (1). *Ascaris lumbricoides* and *Trichuris trichiura*, both recognized pathogens (2), were the most common helminths. A total of 15 of the *E. histolytica* samples contained invasive strains, and 32 contained noninvasive strains according to zymodeme analysis of cultured isolates. The FAC-ELISA detected *E. histolytica* antigen in 13 of 15 samples containing invasive *E. histolytica* and none of 93 samples from the other three groups (Fig. 2A). Sensitivity and specificity were accordingly 87 and 100%, respectively. The FAC-ELISA was secondly tested with stool samples classified by diagnosis of diarrheal disease (14a). In this case, stool samples were separated into four groups: AD (9 samples), dysentery of unknown etiology (18 samples), DIA (68 samples), and asymptomatic carriers (AC) (7 samples). Samples in the DIA (five samples) and AC (one sample) groups which were included in the first test and known to be from patients infected with invasive *E. histolytica* were excluded from this analysis (see Discussion). The FAC-ELISA detected all AD cases but was negative with dysentery of unknown etiology cases and the remaining 77 samples in the

FIG. 2. Distribution of FAC-ELISA ODs obtained with stool samples infected with invasive *E. histolytica* (open circles), noninvasive *E. histolytica* (closed circles), or other parasites (closed squares) and samples without parasitic infection (closed triangles). (A) Stool samples grouped according to parasitological diagnosis. (B) Stool samples grouped according to diagnosis of diarrheal disease: A, AD; B, dysentery of unknown etiology; C, DIA; D, AC.
TABLE 4. Mean ODs obtained by FAC-ELISA with different types of parasitic infection and diagnosis of diarrheal disease

<table>
<thead>
<tr>
<th>Type of infection or diagnosis</th>
<th>Mean OD*</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive E. histolytica</td>
<td>0.51</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Noninvasive E. histolytica</td>
<td>-0.0009</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Other parasites</td>
<td>-0.0023</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No parasites</td>
<td>-0.002</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AD</td>
<td>0.635</td>
<td></td>
</tr>
<tr>
<td>Dysentery of unknown etiology</td>
<td>-0.0036</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DIA</td>
<td>-0.0009</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AC</td>
<td>-0.001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* ODs from wells coated with nonimmune rabbit antisera were subtracted from ODs obtained with wells coated with rabbit antisera against E. histolytica, resulting in negative values in some categories.

Accordingly, sensitivity and specificity for AD were both 100% in this second test. The differences between the means of the ODs obtained in the assay from the two assessments were statistically significant (Table 4).

On the basis of the observed prevalence of invasive E. histolytica infection (15 cases of 108 individuals; 14%), the positive and negative predictive values of the FAC-ELISA were 100 and 98%, respectively (Table 5). As the population studied was not representative of the general population and since the true prevalence of invasive E. histolytica infection may be close to 1% (12), the effect of different theoretical prevalences on predictive values was calculated (Table 5) (16).

In the study population, efficiencies of FAC-ELISA for detecting fecal antigens of invasive E. histolytica and diagnosing AD were 98 and 100%, respectively.

**DISCUSSION**

We have produced a MAb capable of distinguishing invasive from noninvasive strains of E. histolytica (15) and adapted it to a FAC-ELISA. MAB 20/7D showed a 100% specificity for invasive E. histolytica in the FAC-ELISA with spiked stools and with clinical stool specimens containing other intestinal parasites, including noninvasive E. histolytica. The FAC-ELISA was also 100% sensitive and specific for diagnosis of AD, which suggests a role for specific diagnosis of invasive intestinal amebiasis in the field, where bacillary dysentery may be endemic and there is no access to microbiological facilities. World Health Organization recommendations for treatment of AD (41) rely on empirical treatment after failure to respond to antimicrobial agents against bacillary dysentery.

The sensitivity of FAC-ELISA in this group of clinical samples with invasive E. histolytica was 87% (13 of 15). False negatives may be due to antigen degradation during storage, transportation, freezing, and thawing of the stools (33). The MAB 20/7D epitope might also be masked by local intestinal immune response. Control stools spiked in Bangladesh and in London gave approximately 25% false-negative results by FAC-ELISA (4 of 13 and 1 of 4, respectively). It is evident that there is some degree of antigen degradation during sample processing. False negatives could also result from antigenic variation between invasive E. histolytica strains, although this is unlikely as MAB 20/7D has recognized isolates from at least three diverse regions of endemicity (15).

The limit of detection of the FAC-ELISA was lower with stools than with PBS (Fig. 1). This may be due to background in stools which decreases as stools are diluted (Fig. 1) or by stool interference with the FAC-ELISA. Part of this interference may be the proteolytic activity of feces, which are known to degrade antibodies (35). Although fixation of stool samples with formalin has increased the performance of antigen detection tests for Giardia intestinalis (14, 31), previous antigen detection assays for E. histolytica have produced unsatisfactory results with formalized stool samples (21, 33).

The detection limit of this FAC-ELISA is similar to that of other E. histolytica antigen detection assays, and detection limit in stools even as low as 2.3 μg of soluble E. histolytica protein per ml could be below levels of microscopical detection. Detection of Giardia intestinalis antigen in the absence of microscopically detectable organisms in feces is possible several days after treatment (18).

The cutoff value of the FAC-ELISA was calculated with the OD from all nonimmune wells in the microplate. This avoids the need for a group of parasitologically negative stools which may be difficult to obtain in regions of endemicity.

As a crucial part of our study, we wanted to compare the performance of the FAC-ELISA with a clearly defined group of AD cases and other groups of stool samples without invasive E. histolytica. So, six cases of known invasive E. histolytica infection which did not fulfill the criteria for being classified as dysentery were excluded from the analysis of the FAC-ELISA against diagnosis of diarrheal disease (five in the DIA group and one in the AC group; see Materials and Methods). Should those cases not have been excluded, invasive E. histolytica culture-positive stools diagnostic categories other than AD would have been labelled as false positives or false negatives. Furthermore, all the cases with invasive E. histolytica infection, regardless of diagnosis, were pooled in one group, and the results obtained were compared with groups of samples containing noninvasive E. histolytica, other parasites, and no parasitic infection. Those results showed that with some non-dysenteric stools the FAC-ELISA gave false negatives but maintained a high specificity for invasive E. histolytica.

In conclusion, the FAC-ELISA is a potential alternative to the microscopical diagnosis of AD, although further assay development is required. This assay is more practical than culture and zymode analysis. This rapid FAC-ELISA could improve clinical management of dysentery as the result is available on the same day and for ulcerative colitis and Crohn’s disease (28), which may be clinically indistinguishable from intestinal amebiasis. In regions where E. histolytica is not endemic, FAC-ELISA could distinguish carriers of invasive and noninvasive E. histolytica, particularly for travellers returning from high-risk areas. In 1992, there were approximately 900 cases of amebic infection in the United Kingdom, of which

**TABLE 5. Predictive values of positive and negative FAC-ELISA results with various theoretical prevalences of invasive E. histolytica infection when 1,000 individuals are screened**

<table>
<thead>
<tr>
<th>Prevalence (%)</th>
<th>No. of infected subjects</th>
<th>No. of noninfected subjects</th>
<th>No. of false negatives</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>140</td>
<td>860</td>
<td>18</td>
<td>100</td>
<td>97.1</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>970</td>
<td>4</td>
<td>100</td>
<td>99.8</td>
</tr>
<tr>
<td>1.5</td>
<td>15</td>
<td>985</td>
<td>2</td>
<td>100</td>
<td>99.9</td>
</tr>
</tbody>
</table>

* Prevalence figures estimation. A total of 14% of the total number of samples tested by FAC-ELISA had invasive E. histolytica; the prevalence would be 3% if 1/3 of the estimated 10% worldwide E. histolytica infection prevalence is secondary to invasive strains; the prevalence would be 1.5% if 1/2 of the 10% worldwide E. histolytica infection prevalence is E. hartmanni.

* Estimation based on 87% sensitivity obtained with this FAC-ELISA.
around 60% were acquired abroad (5). An important epidemiological application could be the clarification of the distribution of invasive E. histolytica, with the possibility of targeting antiamebic treatment to areas of high endemicity or carriers of invasive strains (40), such as professional food handlers, and household contacts of index cases of invasive amebiasis.

ACKNOWLEDGMENTS
A.G.-R. was a recipient of a scholarship from Consejo Nacional de Ciencia y Tecnología (CONACYT), México; A.A. is partially supported by a scholarship from the Sir Patrick Manson Bequest; and D.C.W. is supported by the Public Health Laboratory Service, United Kingdom. This work has been funded by the Nestlé Nutrition Research Grant Programme, grant no. 87/3/4; a grant from the Commission of the European Communities, contract no. STD-2 0206-UK; the British Council in Colombia and the United Kingdom; the WHO Programme on Intestinal Parasitic Infections; and Smith Kline Beecham.

We are very grateful to the people who provided us with the following parasite strains: E. histolytica HK9, NIH:200, and HM-1:IMSS, from L. S. Diamond; E. histolytica TE, SI, C29, and 8672, from J. P. Ackers; and E. histolytica SAW 1734, from D. Mirelman.

REFERENCES


