Rapid latex agglutination test for extraluminal amoebiasis

A J Cummins, A H Moody, K Lalloo, P L Chiodini

Abstract

***Aims*—To develop a rapid latex agglutination screening test for invasive amoebiasis.**

***Methods*—The performance of an in-house latex agglutination test was compared with three standard serological techniques—the immunofluorescent antibody test (IFAT), the indirect haemagglutination test (IHA), and the cellulose acetate precipitin (CAP) test. Forty six sera were screened; 12 from negative controls; 10 sera from infections other than amoebiasis, and 24 sera from patients with luminal or extraluminal infection with *Entamoeba histolytica*.**

***Results*—Strong positive latex agglutination reactions were observed, with 12 of 12 sera giving combined CAP positive, IFAT positive, and IHA positive results. These results are indicative of invasive amoebiasis. Twelve CAP negative, IFAT positive sera, and 10 of 12 IHA negative gave weak or negative agglutination reactions. One of 12 CAP negative, IFAT positive, and IHA positive sera gave a strong positive latex agglutination result; one with CAP negative, IFAT positive, and IHA positive sera gave a weak latex agglutination reaction. These results correlate with either treated amoebiasis or with the early stages of invasive amoebiasis for which the CAP test is known to have a lower sensitivity than the IFAT, but a higher specificity. No reactions were observed with 12 out of 12 CAP negative, IFAT negative, and IHA negative control sera and all 10 sera from other infections (two giardiasis, three schistosomiasis, three malaria, one filariasis).**

***Conclusions*—The latex agglutination test was a useful indicator test, paralleling the results obtained with standard serological techniques. It could also be a useful screening tool in the field.**

(J Clin Pathol 1994;47:647–648)

Department of Clinical Parasitology Hospital for Tropical Diseases, London NW1 0P6
A J Cummins
A H Moody
K Lalloo
P L Chiodini

Correspondence to:
Dr P L Chiodini.

Accepted for publication
9 February 1994

Serological investigations are currently the mainstay for the laboratory diagnosis of extraintestinal amoebiasis. There is a need for rapid, specific confirmation of the diagnosis. Current methods include the indirect fluorescent antibody test (IFAT), the cellulose acetate precipitin test (CAP), and the indirect haemagglutination test (IHA).

Methods

Sera were selected from 46 patients belonging to three amoebic serological categories and two control groups: 22 sera IFAT negative, CAP negative (negative controls); 12 sera IFAT positive, CAP negative; 12 sera IFAT positive, CAP positive.

All tests were performed using a strain of HK9 amoebae cultured in TTY-1 medium.

The indirect fluorescent antibody test (IFAT) was performed using the technique described by Ambroise-Thomas and Truong,[1] using IgG, anti-human globulin. Amoebic suspension (20 μl) at a concentration of 20 000 amoebae per μl was applied to wells of polytetrafluoroethane (l) multispot slides, air-dried, and fixed in methanol for 15 minutes.

Freeze-dried amoebic antigen was reconstituted with distilled water to a protein concentration of 5 mg/ml for the cellulose acetate precipitin (CAP) test. This test was performed using the technique described by Stamm and Phillips,[2] using Sephraphore 111 cellulose acetate paper.

The indirect haemagglutination (IHA) test was performed using the Cellognost Amoebiasis Kit (Behringwerke AG, Marburg, Germany).

Soluble antigen was prepared using the HK9 strain of *Entamoeba histolytica* cultured in Diamond's medium, harvested, and washed in phosphate buffered saline (PBS) (pH 7·2). The pellet was frozen at −20°C to lyse the amoebae. The protein content was measured according to the method of Lowry *et al*[3] and adjusted to a concentration of 5 mg/ml before freeze-drying in aliquots in an Edwards high vacuum freeze dryer (Edwards High Vacuum Ltd, Crawley, Sussex).

For sensitisation of latex, uniform dyed particles (1·02 μm in diameter) (Bangs Laboratories Inc, Carmel, California, USA) were washed three times with distilled water by spinning at 13 000 rpm for five minutes in a Microcentaur centrifuge (Fisons Instruments) and resuspended to a 5% w/v solid concentration. They were then allowed to react with an equal volume of HK9 antigen equivalent to 5 mg protein/ml on a roller mixer at room temperature for 30 minutes. Excess antigen was removed by three further washing cycles at 13 000 rpm before resuspension of the latex to the original 5% w/v concentration. The agglutination test was performed by combining 4 μl volumes of serum and sensitised latex on a single depression cavity slide and rotating

Results of the latex agglutination tests compared with conventional tests for invasive amoebiasis

Sample	IFAT	CAP	IHA	Latex
1–12	<20	–	<1/8	–
13	160	–	<1/8	+
14	80	–	<1/8	–
15	80	–	<1/8	–
16	320	–	<1/8	+
17	160	–	1/16	+
18	80	–	<1/8	–
19	160	–	<1/8	+
20	320	–	<1/8	–
21	640	–	>1/516	+ + +
22	160	–	>1/516	+
23	160	–	<1/8	–
24	160	–	>1/516	–
25	320	+	>1/516	+ + +
26	320	+	>1/516	+ + +
27	640	+	>1/516	+ + +
28	640	+	>1/516	+ + +
29	320	+	>1/516	+ + +
30	320	+	>1/516	+ + +
31	160	+	>1/516	+ + +
32	160	+	>1/516	+ + +
33	160	+	>1/516	+ + +
34	320	+	>1/516	+ + +
35	640	+	>1/516	+ + +
36	320	+	>1/516	+ + +
37–46	<20	–	<1/8	–

for up to two minutes to observe agglutination.

Discussion

Amoebiasis (infection with *Entamoeba histolytica*) may present as a non-invasive colonic infection or as an invasive colitis with or without extraluminal signs. Complications include spread via the portal vein, resulting in liver abscesses and possible rupture of these into the peritoneum, lung, pericardium, kidney and adjacent structures. Spread via the blood to the brain, usually secondary to liver disease, has also been reported.

Intestinal amoebiasis is reliably diagnosed by microscopic examination of stool samples, rectal scrapes, and biopsy. Diagnosis of extra-intestinal amoebiasis is more difficult without recourse to invasive sampling and is reliant on serology.

The use of standard serological techniques has been the mainstay in the diagnosis of invasive amoebiasis at the Public Health Laboratory Service Amoebiasis Unit, Hospital for Tropical Diseases (HTD), London.

The CAP test and the IFAT are generally performed only in reference centres with access to purified antigen and standardised antisera. The IHA is widely used in the United States,[4 5] with sensitivities of 90–100% on sera from cases of amoebic liver abscess and 75–90% on sera from cases of symptomatic intestinal amoebiasis being reported.

Experience at the Hospital for Tropical Diseases has shown that a combined positive CAP and IFAT result correlates, in 100% of cases, with a diagnosis of invasive amoebic disease.[6] Positive CAP results have never been seen in the absence of a positive IFAT. Negative CAP results may be seen in the first seven to 10 days of illness and within one week of the start of appropriate treatment.

At a titre of 1/80, the IFAT has a false positive rate of 20% on sera from patients with diarrhoea, indicating previous infection or non-specific reaction. At titres higher than this the false positive rate is greatly reduced. In this study the results of the IHA paralleled those of the CAP in 22 of 24 cases. Positive IFAT tests may persist for more than six months after treatment and IHA titres for more than two years, limiting their predictive value for active infection when used alone.

We noted that all sera giving CAP positive, IFAT positive, and IHA positive results also gave strong positive latex agglutination results (table). This indicates the value of this test as a reliable screening tool for extraluminal disease.

Weakly positive latex agglutination reactions were observed in 5 CAP negative sera, suggesting that the test is more sensitive in detecting lower levels of internal antigen[7] than is the CAP.

We observed a clear distinction between strong and weakly positive agglutination results and were able to differentiate these into two diagnostic groups. No false positive results were observed with the negative control sera.

Further work is required to determine the long term stability of the latex reagent and the persistence of the latex agglutination reaction in comparison with that of the CAP.

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