

# AN EVALUATION OF THE INDIRECT HAEMAGGLUTINATION TEST USING AXENICALLY-GROWN *ENTAMOEBIA HISTOLYTICA* AS ANTIGEN

By E. H. Yap, Mulkit Singh, B. C. Ho and E. H. Sng

## SYNOPSIS

The indirect haemagglutination (HA) test using as antigen *Entamoeba histolytica* trophozoite grown in bacteria-free cultures was evaluated in the diagnosis of amoebiasis. 89.1% (81/91) of patients with intestinal and extraintestinal amoebiasis showed positive HA titres ranging from 1:32 to dilutions greater than 1:16384. Sera in 4.3% (2/46) of apparently healthy blood donors were positive for anti-amoebic antibodies. In contrast, 70.3% (64/91) of the same amoebic sera were positive by the indirect fluorescent antibody technique. The merits of the HA technique are discussed.

## INTRODUCTION

Several serological techniques have been devised for the detection of antibodies to *Entamoeba histolytica*. Amongst the more reliable of these techniques are the indirect haemagglutination (HA) (Thompson *et al.*, 1968), indirect fluorescent antibody (Ambroise-Thomas and Truong, 1969) and intradermal (Savanat *et al.*, 1973) tests. Earlier tests utilized antigens prepared from *E. histolytica* trophozoites grown in cultures containing one or more species of bacteria. Soluble antigens prepared from these cultures may give rise to false positive reactions as a result of the patients' sera cross-reacting with the bacterial associate (Maddison and Elsdon-Dew, 1961). Diamond (1968) first described a technique for axenic cultivation of *E. histolytica* trophozoites. Although the ingredients for preparation of this culture medium are expensive, it can be used as a commercial preparation of antigen. Alternatively, satisfactory antigen can be prepared at little cost from bacteria-free cysts of *E. invadens* which cross-reacts with *E. histolytica* (Yap, Zaman and Aw, 1970). We report here the results of

the HA tests of amoebic patients' sera using the axenically-grown *E. histolytica* trophozoites as antigens. Comparison is also made of this test with the indirect fluorescent antibody (FA) technique.

## MATERIALS AND METHODS

### Serological Tests

#### 1. Indirect HA test

The indirect HA test was performed using the trial test kit from Hoechst (Behringwerke AG, Marburg-Lahn). The antigen was a soluble purified *E. histolytica* from axenically cultivated trophozoites bound onto stabilized erythrocytes. These sensitized red cells were then lyophilized and rehydrated when ready for testing. Serum dilutions were made with Tris-HCl-buffer pH 8.0. Microtitration using the Takatski apparatus was used throughout. Control serum for the test was immune serum prepared from goat anti-amoeba sera of known titre. The titre of a serum is the greatest dilution in which a smooth peppery mat of cells was seen in the well of the microtitre tray and no indication of a button is seen. Titres up to 1:16 dilution of sera were considered as negative, at 1:32 as doubtful and titres exceeding dilutions of 1:32 were considered as positive.

2. The indirect FA technique was performed using as antigen *E. histolytica* trophozoite isolated from a local patient with amoebic dysentery. The procedure was essentially according to that described by Jeanes (1966) and used routinely for laboratory diagnosis of amoebiasis in Singapore.

---

Department of Parasitology, Faculty of Medicine, University of Singapore.

E.H. YAP, Ph.D., Lecturer in Parasitology.

MULKIT SINGH, M.Sc., Ph.D., Senior Lecturer in Parasitology.

B.C. HO, M.A., Ph.D., Senior Lecturer in Parasitology.

Department of Pathology, Singapore.

E.H. SNG, M.B., B.S., Dip. Bact., Consultant Immunologist.

---

### Sera

Sera were collected over a period of two years from patients attending hospitals in Singapore. The sera were stored at  $-70^{\circ}\text{C}$  until ready for testing. The patients were classified into the following categories:

#### Intestinal amoebiasis

Acute intestinal amoebiasis—patients suffering from severe or mild diarrhoea, with bloody stools containing trophozoites of *E. histolytica*.

Chronic intestinal amoebiasis—patients showing tenderness of the large bowel, with a previous history of amoebic dysentery and passing *E. histolytica* cysts in stools.

Amoeboma—diagnosed in a single patient who developed pain in a tumour localized in the caecal area.

#### Hepatic amoebiasis

Amoebic liver abscess—patients with fever and enlarged tender livers in whom amoebic liver abscess was confirmed by aspiration of typical bacteriologically sterile 'anchovy-sauce' pus.

Amoebic hepatitis—patients with fever and enlarged tender livers in whom no pus was obtained on aspiration but who showed a favourable response to anti-amoebic therapy.

Amoebic lung abscess—patients showing abscess in the lungs as revealed by radiological and other findings along with hepatic involvement suggestive of amoebiasis.

Amoebic empyema—diagnosed in two patients who developed this condition after rupture of an amoebic abscess into the right pleura.

### RESULTS

Results of the indirect HA test are shown in Table I. 89.1% (81/91) of all the amoebic sera had antibodies detectable by the HA technique. Only 17.5% (7/40) of patients with acute intestinal amoebiasis did not appear to have antibodies detectable by this technique. Even lower percentage of positive HA results were obtained from patients with chronic intestinal amoebiasis (33.4%). However, 97.7% (43/44) of sera from patients with extraintestinal amoebiasis were found to be positive. Generally high HA titres (of greater than 1:16384) were seen in 56.8% (25/44) of patients with hepatic and lung involvement. Of the 46 blood donor sera tested 95.7% (44/46) were found to be negative. Only 1 showed HA titre of more than 1:16384 while one other had a borderline positive HA titre of 1:32. It is likely that both these patients had prior exposure to amoebic antigen.

Table II shows the overall results of the HA tests in comparison with the results obtained by the indirect FA technique. 82.5% of the sera

TABLE I

RESULTS OF THE INDIRECT HAEMAGGLUTINATION (HA) TEST IN AMOEBIC PATIENTS AND APPARENTLY HEALTHY BLOOD DONORS

Diagnosis	HA TITRE				No. of Cases
	Neat to 1:16	1:32	1:64 to 1:8192	1:16832 and above	
Acute intestinal amoebiasis	7	2	11	20	40
Chronic intestinal amoebiasis	2	0	0	1	3
Amoeboma	0	0	1	0	1
Amoebic hepatitis	1	2	4	9	16
Amoebic liver abscess	0	0	12	14	26
Amoebic lung abscess	0	0	0	2	2
Intestinal and hepatic amoebiasis	0	0	3	0	3
Blood donors	44	1	0	1	46

TABLE II  
COMPARISON OF THE INDIRECT HA AND THE INDIRECT FA TECHNIQUES FOR DETECTION OF ANTI-AMOEBIC ANTIBODY

Diagnosis	No. of Cases	Percentage Positive	
		*Indirect Haemagglutination Test	Indirect Fluorescent Antibody Test
Acute intestinal amoebiasis	40	82.5	75.0
Chronic intestinal amoebiasis	3	33.4	33.4
Amoeboma	1	100.0	100.0
Amoebic hepatitis	16	93.8	56.3
Amoebic liver abscess	26	96.2	73.1
Amoebic lung abscess	2	100.0	50.0
Intestinal and hepatic amoebiasis	3	100.0	100.0
Blood donors	46	4.3	5†

\*HA titre of 1:32 was considered as positive.

†Ref. Yu and Lim, Sing. Med. J. 1970, 11, 59-61.

from acute intestinal amoebiasis cases were detectable by the HA test whilst 75% (30/40) were positive by the FA test. In patients with extraintestinal amoebiasis, 65.9% (29/44) had antibodies detectable by the FA test. Negative control value of 5.0% of apparently healthy individuals were found to be positive by the FA technique in a previous study (Yu and Lim, 1970).

## DISCUSSION

The results which we have obtained with the indirect HA test compare favourably with the published results of similar serologic testing for amoebiasis in which positive results ranged from 47% to 100% in the case of extraintestinal amoebiasis (Lewis and Kessel, 1961; Kessel *et al*, 1965; Thompson *et al*, 1968; Juniper *et al*, 1972). The rather lower seropositivity rate of 82.5% for various types of intestinal amoebiasis still compares well with the range of 33-100% reported by other workers (Juniper *et al*, 1972). The generally higher titres seen in the extraintestinal involvement when compared to those seen in intestinal cases can be anticipated primarily because of the invasiveness of the trophozoites and also the longer time course and subsequent production of high levels of antibodies expected in patients with hepatic or lung abscesses. Though there were only 3 cases of chronic intestinal amoebiasis tested (too few in number to make any definite interpretation),

only one case was found positive by both the HA and the FA tests. This is not surprising because the presence of cysts may not elicit sufficient antibodies detectable by either of these techniques. In this context, it is pertinent to remark that it had previously been found that HA titre was not helpful in differentiating extraintestinal from intestinal, nor even symptomatic from asymptomatic amoebiasis. However, antibodies detectable by HA tests tend to remain positive after cure, occasionally for 1 to 3 years (Juniper, 1972).

The lower percentage of positive results obtained with the indirect FA test can be expected when compared with the higher sensitivity of the HA tests. Besides, the inherent problem with this technique is the difficulty of discriminating positive fluorescence, especially in low titred sera.

It can be concluded that the combination of a highly sensitive, reproducible technique and the utilization of an antigenic material derived from the aetiologic agent, *E. histolytica* trophozoites grown in the absence of bacterial contaminants warrants the application of the indirect HA test in the routine laboratory diagnosis of amoebiasis.

## ACKNOWLEDGEMENT

We thank Hoechst (Behringwerke) for the indirect haemagglutination test kits for amoebiasis and Messrs Goh Ting Kiam and Lim Ah Loo

for their excellent technical assistance. We are grateful to Dr. Ong Yong Wan, Singapore Blood Transfusion Service, for sera from blood donors.

#### REFERENCES

1. Ambroise-Thomas, P. and Truong, T.K.: "Le diagnostic serologique de l'amibiase humaine par la technique des anticorps fluorescents." Bull. Wld. Hlth. Organ., 40, 103, 1969.
2. Diamond, L.S.: "Techniques of axenic cultivation of *Entamoeba histolytica* Schaudinn, 1903 and *E. histolytica*-like amoebae." J. Parasit., 54, 1047, 1968.
3. Jeanes, A.L.: "Indirect fluorescent antibody test in diagnosis of hepatic amoebiasis." Br. Med. J., 1, 1464, 1966.
4. Juniper, K. Jr., Cynthia, L., Worrell, M., Minshew, C., Roth, L.S., Cypert, H. and Lloyd, R.E.: "Serologic diagnosis of amoebiasis." Am. J. Trop. Med. Hyg., 21, 157, 1972.
5. Kessel, J.F., Lewis, W.P., Pasquel, C. and Turner, J.: "Indirect haemagglutination and complement fixation tests in amoebiasis." Am. J. Trop. Med. Hyg., 14, 540, 1965.
6. Lewis, W.P. and Kessel, J.F.: "Haemagglutination in the diagnosis of toxoplasmosis and amoebiasis." Arch. Ophthalmol., 66, 471, 1961.
7. Maddison, S.E. and Elsdon-Dew, R.: "Non-specific antibodies in amoebiasis." Exp. Parasit., 11, 90, 1961.
8. Thompson, P.E., Graedel, S.K., Schneider, C.R., Stucki, W.P. and Gordon, R.M.: "Preparation and evaluation of standardized amoeba antigen from axenic cultures of *Entamoeba histolytica*." Bull. Wld. Hlth. Organ., 39, 349, 1968.
9. Savanat, T., Bunnag, D., Chongsuphajaisiddhi, T. and Viriyanond, P.: "Skin test for amoebiasis: An appraisal." Am. J. Trop. Med. Hyg., 22, 168, 1973.
10. Yap, E.H., Zaman, V. and Aw, S.E.: "The use of cyst antigen in the serodiagnosis of amoebiasis." Bull. Wld. Hlth. Organ., 42, 553, 1970.
11. Yu, M. and Lim, A.L.: "A study of fluorescent antibodies against *Entamoeba histolytica*." Sing. Med. J., 11, 59, 1970.