AN EVALUATION OF THE INDIRECT HAEMAGGLUTINATION TEST USING AXENICALLY-GROWN ENTAMOEBA 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 antiamoebic 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 (Ambrose-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.

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Sera

Sera were collected over a period of two years from patients attending hospitals in Singapore. The sera were stored at -70°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 antiamoebic 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>
<thead>
<tr>
<th>Diagnosis</th>
<th>HA Titre</th>
<th>No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neat to 1:16</td>
<td>1:32</td>
</tr>
<tr>
<td>Acute intestinal amoebiasis</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Chronic intestinal amoebiasis</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Amoeboma</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amoebic hepatitis</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Amoebic liver abscess</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amoebic lung abscess</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intestinal and hepatic amoebias</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blood donors</td>
<td>44</td>
<td>1</td>
</tr>
</tbody>
</table>
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 anticipat-
ed 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 be-
cause the presence of cysts may not elicit suf-
cient 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 extra-
testinal from intestinal, nor even symptomatic
from asymptomatic amoebiasis. However, anti-
bodies 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 ex-
pected when compared with the higher sensi-
tivity of the HA tests. Besides, the inherent pro-
blem with this technique is the difficulty of
discriminating positive fluorescence, especially
in low titre 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

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REFERENCES