

# Evaluation in clinical practice of the fluorescent amoebic antibody test<sup>1</sup>

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**SYNOPSIS** Serum fluorescent antibody levels against *Entamoeba histolytica* were determined in patients with amoebic infection and in control cases. Titres of 1 : 64 or above were obtained only in cases of active or recent amoebic infection, and were recorded in 75·0% of intestinal cases and in 95·2% of extraintestinal cases. Titres given by symptomless carriers of *Entamoeba histolytica*, though not exceeding 1 : 32, were proportionately higher than those obtained in normal subjects or in patients with non-amoebic disorders.

In preliminary reports of the application of the indirect fluorescent antibody technique to the detection of serum antibody to *Entamoeba histolytica* (Jeanes, 1966; Mullan, Ward, Shah, and Jeanes, 1967) it was shown that sera obtained from patients with hepatic amoebiasis were reactive to a titre which clearly distinguished them from the sera of randomly selected patients with diseases or disorders other than amoebiasis. Similar studies have been reported by Goldman (1966) and by Boonpucknavig and Nairn (1967). The purpose of the present paper is to report the titres obtained in a larger series of cases of intestinal and extraintestinal amoebic infection, and to compare them with the titres obtained in normal subjects, patients with diseases other than amoebiasis, and symptomless carriers of *Entamoeba histolytica*.

## MATERIALS AND METHODS

**THE SERA TESTED** Samples of serum were obtained during or soon after the acute stage of the disease, from 40 cases of intestinal and 61 cases of extraintestinal amoebic infection. In all cases, the diagnosis was based on the clinical evidence, the results of other investigations, and the response of the patients to antiamoebic therapy.

Control sera were obtained from 128 blood donors living in the London area, 116 donors of African race from the Durban area, and 270 patients attending or admitted to Guy's Hospital, London, with diseases or disorders other than amoebiasis. The latter group included 38 cases of non-amoebic liver disease and four

cases of non-specific ulcerative colitis. In addition, sera were obtained from 37 patients who had no symptoms attributable to amoebic infection, but whose stools were found on routine examination to contain cysts or trophozoites with the characteristics of *Entamoeba histolytica*.

Finally, further samples of serum were obtained from each of three patients with hepatic amoebiasis, at intervals of 20 months, 16 months, and 11 months respectively, following the commencement of anti-amoebic therapy.

**TITRATION OF SERA** Serial dilutions of each sample of serum were tested against *Entamoeba histolytica* by the indirect fluorescent antibody technique, and the titre was expressed as the highest dilution of serum giving definite (+ +) fluorescent staining of the amoebae. Details of the method used have been described previously (Jeanes, 1966).

## RESULTS

The Table shows the distribution of serum fluorescent antibody titres against *Entamoeba histolytica* in patients with amoebic infection and in the control cases.

**AMOEBIC INFECTION** In cases of intestinal amoebic infection, the titres ranged from 1 : 8 to 1 : 1,024. Thirty-eight (95·0%) of the 40 cases gave a titre of 1 : 32 or above and 30 (75·0%) gave a titre of 1 : 64 or above. Proportionately higher titres, ranging from 1 : 8 to 1 : 16,000, were given by cases of extraintestinal amoebic infection: in 59 (96·8%) of the 61 cases, the titre was 1 : 32 or above, and in 58 cases (95·2%) it was 1 : 64 or above.

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TABLE

SERUM FLUORESCENT ANTIBODY TITRES IN PATIENTS WITH AMOEBIC INFECTION AND IN CONTROL CASES

Fluorescent Antibody Titre	Amoebic Infection		Normal Subjects		Non-amoebic Disorders	Symptomless Carriers
	Intestinal	Extraintestinal	British	African		
1:16,000	0	3	0	0	0	0
1:8,000	0	0	0	0	0	0
1:4,000	0	1	0	0	0	0
1:2,048	0	7	0	0	0	0
1:1,024	2	6	0	0	0	0
1:512	2	7	0	0	0	0
1:256	4	12	0	0	0	0
1:128	11	15	0	0	0	0
1:64	11	7	0	0	0	0
1:32	8	1	0	0	2	5
1:16	0	1	2	2	10	11
1:8	2	1	20	14	92	8
Less than 1:8	0	0	106	100	166	13
Total cases	40	61	128	116	270	37

In four cases of amoebic infection, the titre was less than 1 : 32. In one of these, clinically diagnosed as hepatic amoebiasis, serological tests for amoebiasis, including complement-fixation, gel-diffusion, and haemagglutination tests, were reported as negative by all other laboratories participating in an international trial (Dr I. G. Kagan, personal communication, 1966). In the second case, diagnosed as amoebic empyema, the amoebic gel-diffusion precipitin test was reported to be positive (Dr S. J. Powell, personal communication, 1966). In the remaining two cases, diagnosed as amoebic dysentery, no other serological tests were performed.

**NORMAL SUBJECTS: BRITISH** One hundred and twenty-six (98.5%) of the 128 subjects in this group had a titre of 1 : 8 or less, and none had a titre higher than 1 : 16.

**NORMAL SUBJECTS: AFRICAN** One hundred and fourteen (98.3%) of the normal African subjects had a titre of 1 : 8 or less, and in no case was the titre higher than 1 : 16. The range and distribution of titres was therefore similar to that observed in the normal British subjects.

**NON-AMOEBIC DISORDERS** Of the 270 patients in this group, 258 (95.6%) had a titre of 1 : 8 or less, 10 (3.7%) had a titre of 1 : 16, and two (0.7%) had a titre of 1 : 32. There was thus a slight 'upward shift' of titres compared with those obtained in normal subjects.

Of the two sera with a titre of 1 : 32, one was from a patient with carcinoma of the caecum and the other from a patient with carcinoma of the breast metastasizing to the liver. In 18 other cases of malignant disease, including seven with metastases in the liver, the titre did not exceed 1 : 16.

In 33 of the total of 38 cases of nonamoebic liver disease, the titre was 1 : 8 or less. In the four cases of nonspecific ulcerative colitis, the titre was less than 1 : 8.

**SYMPTOMLESS CARRIERS** In 21 (56.8%) of the 37 symptomless carriers of *Entamoeba histolytica* the titre was 1 : 8 or less, but in 11 (29.7%) it was 1 : 16 and in five (13.5%) it was 1 : 32. Thus, although the titre did not exceed 1 : 32 in any of the carriers, the titres were proportionately higher than those observed either in normal subjects or in patients with non-amoebic disorders.

**FOLLOWED-UP CASES** Of the three patients with hepatic amoebiasis in whom follow-up studies were possible, initial titres during the acute stage of the illness were respectively 1 : 1,024, 1 : 2,048, and 1 : 1,024. At intervals of 20 months, 16 months, and 11 months respectively following the commencement of antiamoebic therapy, the three patients were clinically well, and their titres had fallen to 1 : 32, 1 : 32, and 1 : 8.

#### DISCUSSION

Following the early observations of Izar (1914) and of Craig (1927) on the application of complement-fixation tests to the diagnosis of amoebiasis, many of the further studies in the serology of the disease have been handicapped by the difficulty of preparing amoebic antigen of standard potency, free from non-amoebic protein. For this reason, the results of serological tests for amoebiasis have often lacked uniformity and reproducibility, and the diagnostic value of such tests has, in the past, been open to question. In recent years, however, the development of antigen preparations of a higher degree of purity,

and the introduction of new immunological techniques, have stimulated further interest in the application of serological methods to the diagnosis of this important disease.

While it is not intended to discuss here technical details at length, it may be said that the outstanding advantage of the fluorescent antibody method is that the tests are read by direct visualization of intact amoebic cells, thus eliminating the risk of false positive results arising from chance reactions of the test serum with impurities in the antigen preparation. The tests are simple to perform, only a single 'drop' of serum is required for a full titration, and the result of a titration may be given less than two hours after receiving the sample in the laboratory.

A disadvantage of the method, as illustrated by the results presented here, is that low-titre reactions are given by a small proportion of normal subjects, and by a rather higher proportion of patients with non-amoebic disorders. It is possible that in some cases, particularly in those from endemic areas, such reactions are due to previous amoebic infection or to the undetected carriage of amoebae in the intestine. The relative distribution of titres in normal British subjects was almost identical, however, with that observed in normal African subjects residing in the Durban area, where amoebiasis is prevalent. It therefore seems likely that the majority of low-titre reactions given by 'non-amoebic' sera do not result from previous or subclinical amoebic infection.

From the results obtained in this study it is seen that a titre of 1 : 32 is suggestive of active amoebic infection, but may also occur in symptomless carriers of *Entamoeba histolytica*, patients with a history of previous amoebic infection, and, very occasionally, in patients with non-amoebic disease. Titres of 1 : 64 or above, however, were obtained only in cases of active or recent amoebic infection and could therefore be regarded as 'diagnostic'. Such titres were recorded in 75.0% of cases of intestinal amoebic infection and in 95.2% of cases of extraintestinal infection.

The laboratory diagnosis of intestinal amoebiasis depends primarily on the identification of the causative organism in the stools, and to a much lesser extent on serological studies. Since 'diagnostic' titres were obtained in a substantial proportion of cases, however, the fluorescent antibody test might well be of confirmatory value in doubtful cases, and

a changing titre could serve as a guide to the effectiveness of therapy.

The incidence of generally higher titres in extra-intestinal cases has been noted in many previous studies in the serology of amoebiasis, and suggests that the antibody response varies with the extent of tissue invasion by *Entamoeba histolytica*. The observation is of more than academic interest, since it is in the diagnosis of extraintestinal amoebiasis, so often characterized by an absence of amoebae from the stools, that serological tests are likely to be of most value.

Although patients rarely become infected in Britain, the incidence of amoebiasis among immigrants and returning overseas travellers is sufficiently high to emphasize the need even in Britain for reliable serological tests, if only to exclude the diagnosis. The evidence so far accumulated suggests that the 'fluorescent amoebic antibody test' is suitable for routine use by those with experience of immunofluorescence techniques. For the routine screening of large numbers of sera, each serum may be tested at a single dilution of 1 : 32, and titrated only if reactive at that dilution.

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