

Mass Scale Serodiagnosis of Amoebiasis

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ABSTRACT

A simple method for the collection, preservation, shipment and testing of minute amounts of dried blood for the diagnosis of amoebiasis is described. A drop of blood obtained from finger puncture and collected on filter paper was extracted in buffered saline. The extracted blood was tested by the indirect haemagglutination (IHA), indirect-fluorescent antibody (IFA) and amoeba immobilization (AI) techniques employing axenic *Entamoeba histolytica* antigen prepared in this Institute. The dried filter paper blood specimens were preserved at room temperature and at 4°C for more than 3 months without detectable changes in antibody response. This technique was evaluated for seroepidemiological survey for amoebiasis among 648 staff members of CDRI classified into 3 different socio-economic groups.

INTRODUCTION

SEROLOGICAL methods for laboratory diagnosis of amoebiasis have been reviewed by Kagan¹. The test methods used for sero-diagnosis of amoebiasis cases by collecting a drop of blood on filter paper, are fluorescent antibody (FA)²⁻⁴, indirect haemagglutination (IHA)⁵⁻⁶, and amoeba immobilization (AI)⁷⁻⁸. Stool examination for extra-intestinal amoebiasis does not always give positive results. The present study was designed to develop a simple, sensitive and reliable technique, using minute amounts of dried blood from the finger tips which could be used in the detection of *E. histolytica* antibody and, thereby, survey the cases of amoebiasis in selected populations in CDRI.

MATERIALS AND METHODS

Staff members (648) working in Central Drug Research Institute, Lucknow, were surveyed for specific *E. histolytica* antibody in the sera. A drop of blood from the finger on a strip of filter paper (chromatographic 3 mm) was assayed by IHA test method of Krupp⁹ who used glutaraldehyde fixed sheep KBC's sensitized with antigen and the IFA method*. The AI test followed was same as described by Prakash et al. The lyophilized axenic *E. histolytica* antigen (CENTIGEN) prepared in CDRI from axenically grown *E. histolytica* (NIH-200) was used in these tests.

Filter paper strip containing 0.05 ml of blood was cluted in 0.4 ml phosphate-buffer saline (PBS) pH 7.2 and a final serum dilution of 1:16 was obtained. The diluted serum sample was inactivated

at 56°C for 30 min. The three serological tests IHA, IFA and AI were conducted of all the samples following standard techniques. The present and past clinical history, age, sex, habit. treatment, etc., were recorded for each person. Serologically positive cases were examined for the presence of cyst and trophozoites in the faecal samples.

The persons from whom the blood drops were collected were divided into three socio-economic groups (i) low income group whose monthly income falls between Rs 200 to Rs 500 p.m. (ii) middle income group whose monthly income falls between Rs 501 to Rs 1000, and (ii) high income group whose monthly income falls between Rs 1001 to Rs 3000 p.m.

RESULTS

The results of IHA, IFA and AI tests conducted for sera among different categories of staff members of CDRI showed more or less similar results by IHA and IFA and slightly lower in AI test. In the low income group, the sera positive for *E. histolytica* antibody were 20% by IHA, 20.5% by IFA and 15.5% by AI tests. In middle income groups about 17, 18 and 13% were positive by IHA, IFA and AI tests respectively whereas in the high income groups the positivity was about 14, 14 and 12% respectively. The incidence of positivity of antibodies was highest in the low income group (20%) and lowest (14%) in the high income group. The overall incidence of positivity of *E. histolytica* antibodies in the sera is about 16% among 648 staff screened.

Table I compares the IHA and IFA tests to detect specific *E. histolytica* antibodies in the blood samples collected. Titer 1 in 128 in IHA and titer 1 in 32 in the case of IFA were considered as lowest positive titers. At titer 1 in 128 IHA test revealed 63 sera out of 648. (9.7%) positive and IFA test at 1 in 32 showed 50 out of 648 sera (7.7%) positive. Total positive cases out of 648 sera tested, 2, 4, 15, 20 and 63 were positive at 2048, 1024, 512, 256 and 128 titres respectively by IHA and 2, 3, 17, 36 and 50 were positive at 512, 256, 128, 64 and 32 titres respectively by IFA. Not much difference was noticed by the comparative tests.

Table 2 shows the results of stool examination of serologically positive cases of staff. Out of the 648 persons, 406 were male and 242 were female and belonged to the age group 18 to 58 years; 17% of the males and 14.5% of the females were serologically positive. Stool examination revealed that out of 104 serologically positive cases, the presence of cysts was noticed only in 49 (47.19%), trophozoites in 33 (31.7%). and cysts and trophozoites in 3 (2.97%). Absence of cysts and trophozoites was noticed in 19 (18.3%) persons. Detailed studies are in progress.

DISCUSSION

The present technique seems to be sound and can be used for large scale seroepidemiological survey of amoebiasis patients in endemic areas. It is economical, samples for mass screening are easy to obtain, less costly, samples can be stored and mailed and the field study

can be easily conducted. It is a simple, rapid and reproducible technique for serodiagnosis and sero epidemiological survey of amoebiasis in a country like India, where the disease is endemic.

This technique has been successfully used for the diagnosis of Schistosomiasis.⁹⁻¹⁰, and amoebiasis¹¹.

TABLE – 1

Secological comparison of results obtained on 648 persons in IHA and IFA tests with dried blood on filter paper.

<u>No. of cases showing reciprocal IHA titres.*</u>								
≥2048	≥1024	≥512	≥256	≥128	≥64	≥32	≥16	≥<16
2	4	15	20	63	32	71	62	379
(0.31%)	(0.62%)	(2.31%)	(3.09%)	(9.72%)	(4.54%)	(10.56%)	(9.56%)	(58.49%)
<u>No. of cases showing reciprocal IHA titres.*</u>								
≥512	≥256	≥128	≥64	≥32	≥16	≥<16		
2	3	17	36	50	35	505		
(0.31%)	(0.46%)	(2.62%)	(5.56%)	(7.72%)	(5.40%)	(77.93%)		

*≥128 is the lowest positive titer, **≥ 32 is the lowest positive titer.

TABLE – 2

Comparison of results obtained by serological test (IHA) with dried blood on filter paper and stool samples under microscope (only from those persons found serologically positive).

Total	IHA positive Sample		Total	Stool examination criteria % positive
	Male	Female		
104	69	35	104	Male 58 (55.77%) + ve cysts' = 34 (49.28%) + ve trophoz." = 22(31.88%) + ve cyst and the trophoz. = 2 (2.9%) - ve cyst and the -- ve trophoz. = 11 (15.94%) Female 27 (25.96%) + ve cyst = 15 (42.86%) + ve trophoz** = 11 (31.43%) + ve cyst and the +ve trophoz. = 1 (2.86%) -ve cyst and -ve trophoz. = 8 (22.86%)
(66.35%)	→	(33.65)		

Total samples: 648 (males 406, females 242) *Cyst = 4 nucleated, chromatoid bars, and double walled, with glycogen mass under Lugol's iodine preparation, measuring above 10 /um in diam; ** Trophozoite=with RBC in the cytoplasm; + ve = positive; -ve=negative.

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