matography on cellulose plates using water in one direction and n-butanol methanol:water:ammonium hydroxide (60.20.20 l, v/v) in the second direction. After eluting the zones corresponding to standard 6 MA and 5 MC, the extracts were analysed separately HPLC. The retention time and the 280/254 ratio of these compound coincided with that of the authentic compounds. This method is very efficient, sensitive and reproducible for detecting the bases in the complex biological extracts.

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A SIMPLE AND RELIABLE TECHNIQUE FOR MASS SCALE SERODIAGNOSIS OF HUMAN AMOEBIASIS USING DROP OF BLOOD ON FILTER PAPER

SANJAY KUMAR, P. DAS, A. MALIK, S. K. KATIYAR, P. ANAND AND S. R. DAS
Central Drug Research Institute, Lucknow 226 001, India.

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 Kagan[1]. 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 Krupp[6] who used gluteraldehyde fixed sheep RBC's sensitized with antigen and the IFA method[4]. The AI test followed was same as described by Prakash *et al*[8]. 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 eluted 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 (iii) 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 catagories 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 respectivity 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 1 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 (47.1%), trophozoites in 33 (31.7%), and cysts and trophozoites in 3 (2.9%). 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 seroepidemiological 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 amoebiasis[11].

Table 1

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

*No. of cases showing reciprocal IHA titres.**

≥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%)

*No. of cases showing reciprocal IFA titres***

≥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).

IHA positive samples				Stool examination/criteria % positive
Total	Male	Female	Total	
104 (66.35%)	69 →	35 (33.65%)	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%)

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 / μm in diam; ** Trophozoite = with RBC in the cytoplasm; +ve = positive; −ve = negative.

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THE USE OF G-BANDING TECHNIQUE IN THE CHROMOSOME STUDIES OF A MILLIPEDE SPECIES—*SPIROSTREPTUS ASTHENES*

K. P. ACHAR

Department of Zoology, Sri Bhuvanendra College, Karkala 574 104, India.

ABSTRACT

The chromosomes of *Spirostreptus asthenes*, a millipede from Southern India, has been studied by air-drying and banding techniques. In this cytologically difficult material it was possible to get well-spread and banded metaphases by pretreatment with hypotonic Na citrate (0.016 to 0.021 M) followed by ASG staining. Both mitotic and meiotic tissues were studied.

INTRODUCTION

INTEREST in the chromosome cytology of Diplopoda has been renewed in recent years and several species of Indian Diplopoda are cytologically known at present[1,2]. An attempt is made here to introduce the combined use of different cytological techniques including intrahaemocoelic injection of colchicine, hypotonic pretreatment[3], air drying[4], and