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A SLIDE FLUORESCENT ANTIBODY TECHNIQUE WITH ADULT WORM ANTIGEN FOR THE SEROLOGICAL DIAGNOSIS OF SCHISTOSOMIASIS *MANSONI*

Mario E. CAMARGO, Sumie HOSHINO and Luiz Caetano da SILVA

SUMMARY

A new fluorescent antibody technique for the serological diagnosis of schistosomiasis *mansoni* is described. The technique was based on the use of worm particles as antigen fixed on microscope slides. Positive reactions were observed in 65 out of 68 patients (95.6%).

The advantages of the method are discussed.

INTRODUCTION

Simple, economical and reliable tests are required in order to establish the prevalence of schistosomiasis in large population groups, as emphasized by SADUN & BIOCCA [6]. The fluorescent antibody test with preserved cercariae using dried blood smears on filter paper, developed by SADUN et al.[7, 8], and by ANDERSON et al.[1], seems to be a specific and sensible approach to the problem, although still requiring more strict evaluation (SADUN & BIOCCA [6], PELLEGRINO & BIOCCA [5]).

For the performance of the above mentioned test, tubes instead of slides are used, resulting in much added work and large consumption of conjugates. We have tried to simplify such procedures in a new test which employs an adult worm antigen fixed on microscope slides.

MATERIAL AND METHODS

Sera — Were obtained from 68 cases of schistosomiasis, diagnosed by stool examination or by rectal or liver biopsies. Most of these patients were eliminating viable eggs in the stools (Table I). Sera from 100 non-schistosomotic patients were obtained in a non-endemic area. Blood was collected by venipuncture and sera kept frozen at −20°C. In 16 cases blood was also collected in filter paper, as described by SADUN et al.[7]. Sera were inactivated for 30 minutes at 56°C and diluted to 1/10 in buffered saline. Dried blood in filter paper was eluted in buffered saline so as to obtain also about a 1/10 serum dilution.

Antigen — Adult worms, obtained by perfusion of the liver and portal system of mice, were carefully washed in saline and in distilled water and dried under vacuum at −20°C. Dried worms were triturated for five minutes at top speed in a "Virtis 45" in cold absolute ethanol in an ice-salt bath. Sediment was obtained by centrifuging at low temperature (4°C) at 3,000 r.p.m. and resuspended in cold anhydrous ethyl ether, shaken for a few minutes, centrifuged again and dried in a vacuum pump. The sediment was kept for many months at −20°C without impaired antigenic activity. These worm particles were suspended in buffered saline (NaCl 0.15 M, phosphates 0.01 M, pH 7.2, merthiolate to 1/10,000) so as to obtain a few ones per microscopic field in final pre-

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Instituto de Medicina Tropical de São Paulo, São Paulo, Brasil

parations (Fig. 1). This suspension may be kept in the refrigerator for about one month without any loss of antigenic activity.

Microscope slides were prepared as previously described (CAMARGO [3]), with two sets of 5 square areas, each under a 18 × 18 mm coverslip, or with one set of 20 areas under a 24 × 40 mm coverslip. Worm particles suspension was pipetted on each area through an hipodermic needle without bevel and almost completely removed, so as to leave a thin film evenly spread. In order to fix particles, the slides were dried for 30 minutes at 37°C, a sufficient procedure when perfectly clean slides are used.

Conjugate — Rabbit antihuman globulin was obtained by inoculating animals with human globulins incorporated in complete Freund's adjuvant. Rabbit immuneglobulins precipitated from serum with 40% saturated ammonium sulphate, were conjugated to fluorescein isothiocyanate by a dyalisis technique as described by CAMARGO [2]. Resulting conjugate had a F/P ratio of 5.5×10^{-3}, specific staining to 1/100 and unspecific staining at less than 1/5. It was used at a 1/50 dilution containing Evan's blue at a final concentration of 1 mg%, as modified from NICHOLS & MCCOMB [4].

Indirect fluorescent test — About 0.005 ml of each inactivated diluted serum were pipetted on each small area. Slides were incubated for 30 minutes at 37°C in a wet chamber, washed for 10 minutes in three changes of buffered saline and dried by blotting with filter paper. Conjugate was then pipetted on each area and slides incubated again for 30 minutes. After washing again for five minutes each, in three changes of buffered saline, slides were blotted and mounted with coverslip and buffered glycerin (pH 8).

These slides were examined in dark field under oil immersion (obj. 40 ×, oc. 12.5 ×) with HBO200 as the light source, BG 12 as exciting filter and 50 (Zeiss) as barrier filter. An intense green fluorescence of the worm particles was observed with reactive sera (Fig. 2 and 3). A clearcut difference was observed with non-reactive sera, particles showing instead a slight red stain (Fig. 4). The reactions were graded from (+) to (++++), according to the intensity of fluorescence. Dubious readings were considered as negative.

RESULTS AND DISCUSSION

Sixty five positive results were obtained in a group of 68 cases of schistosomiasis (95.6%) tested by the new fluorescent technique as demonstrated in Table I. This table

TABLE I

Results of fluorescent test in 68 sera from schistosomotic patients

Sera no.	Fluorescent test	Clinical forms*	Eggs in stools
1	++	HE	+
2	++++	HE	— (ID)**
3	++++	HE	+
4	+++	HI	+
5	—	HI	+
6	++	HI	+
7	++	HI	+
8	++	?	+
9	+++	HE	+
10	+++	HI	+
11	+++	HI	+
12	+++	HI	+
13	++++	?	+
14	+++	?	+
15	++	HE	+
16	++	HI	+
17	++++	HE	+
18	+	HI	+
19	+++	HI	+
20	++	HI	+
21	+++	HI	+
22	++	?	+
23	++++	HE	+
24	+++	HE	+
25	++	HI	+
26	+++	HE	+
27	++++	HE	+
28	++	HI	+
29	++	HI	+
30	+++	HE	+
31	++++	HE	+
32	+++	HI	+
33	+++	HI	+
34	++	HE	—
35	+++	HI	+
36	+	?	+
37	++++	HI	+
38	+++	HI	+
39	+++	HE	+
40	++	HE	+
41	+++	HE	—
42	—	HI	+
43	+	HE	—

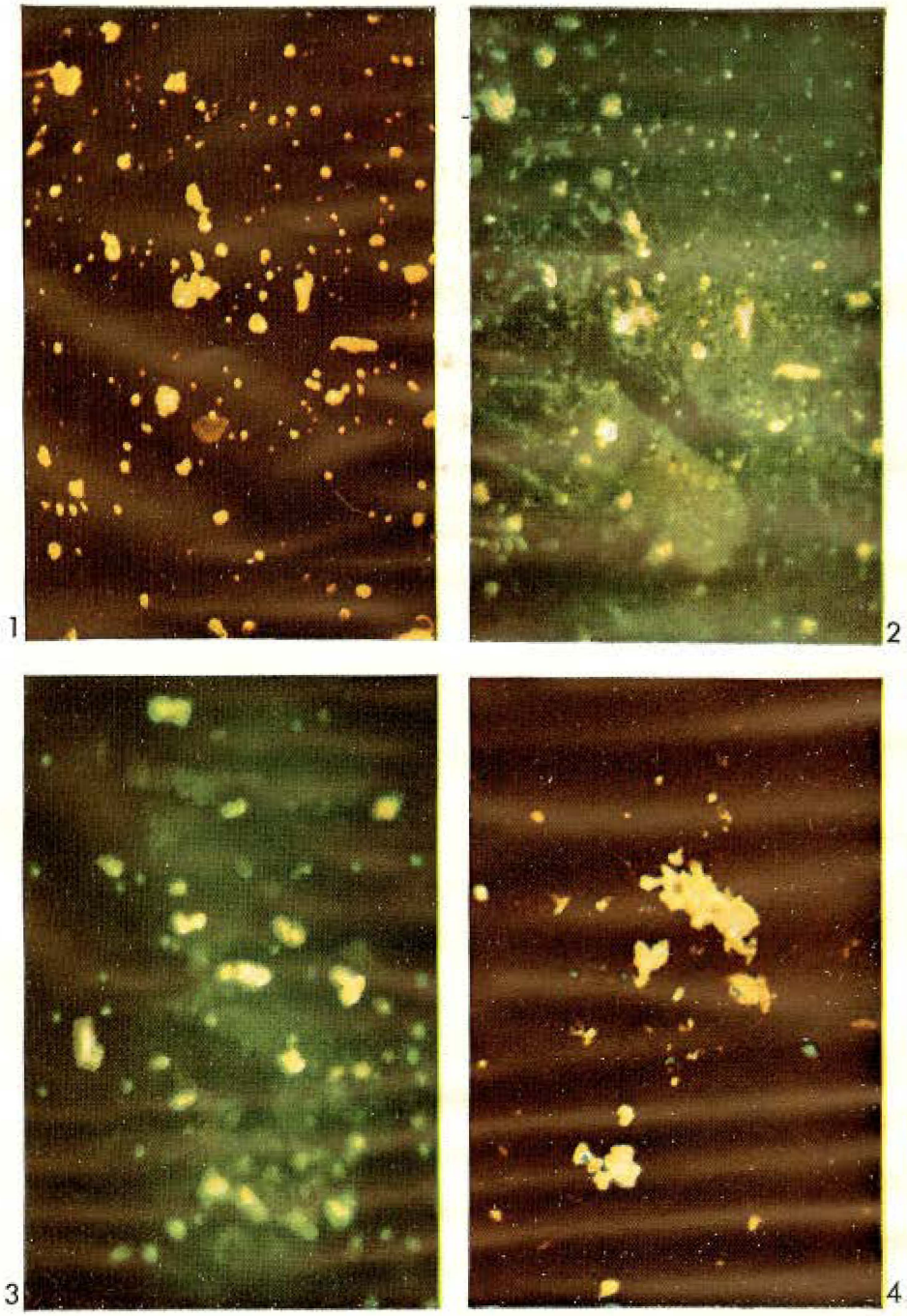

Fig. 1 — Worm particles fixed on slides, as seen in darkfield non-fluorescent microscopy. Fig. 2, 3 — Positive reactions with reactive sera, in fluorescent microscopy. Fig. 4 — Negative reactions with non-reactive serum, in fluorescent microscopy (obj. 40×,oil; Ektachrome High-speed, exp. 1 min).

Sera no.	Fluorescent test	Clinical forms*	Eggs in stools
44	+ + + +	HE	+
45	+ + + +	HE	+
46	+ + + +	HI	+
47	+ + + +	HE	—
48	+ + +	HI	+
49	+ + +	HI	+
50	+ + +	HE	—
51	+ + +	HI	+
52	—	HI	+
53	+ + + +	HE	+
54	+ +	HI	+
55	+ + +	HE	+
56	+ + +	?	+
57	+ + + +	HE	+
58	+ + +	HE	+
59	+ + +	HE	+
60	+ + +	?	+
61	+	HI	+
62	+ + +	?	+
63	+ + +	HI	+
64	+ + +	HI	+
65	+ + + +	?	+
66	+ + + +	HI	+
67	+ + + +	HI	+
68	+ + + +	HI	+

* HE = Hepato-splenic form
HI = Hepato-intestinal form
? = Clinical data not available
** ID = Intradermal test resulted positive

also includes data on clinical forms and presence of eggs in stools. Control sera furnished only negative results. In 16 cases tests were performed in sera and in eluates of filter paper blood smears, with almost identical results, as seen in Table II.

It is clear from what has been shown above that the described method of immunofluorescence on slides with worm antigens is very sensitive, easy to perform and adaptable to the study of a large groups of patients. As compared with complement fixation test, the described fluorescent technique is very economical in relation to antigen consumption, just a few worms being sufficient for a large number of reactions. Despite the negative results in all cases in the control group, the specificity of the method remains to be evaluated. The possibility exists that a similar technique could be employed for the serum diagnosis of other helminthiasis.

TABLE II

Results of fluorescent technique in cases of schistosomiasis with sera and with eluates of filter paper blood smears

Case no.	Sera	Filter paper eluates
35	+ + +	+ + +
31	+ + + +	+ + + +
27	+ + + +	+ + + +
32	+ + +	+ +
54	+ +	+ +
36	+	—
56	+ + +	+ +
60	+ + +	+ + +
61	+	—
62	+ + +	+
63	+ + +	+ +
64	+ + +	+ + +
65	+ + + +	+ +
66	+ + + +	+ +
67	+ + + +	+ +
68	+ + + +	+ + +

RESUMO

Reação de imunofluorescência em lâminas de microscopia, com antígeno de verme adulto, para o diagnóstico sorológico da esquistossome mansônica

Descreve-se nova técnica de imunofluorescência para o diagnóstico sorológico da esquistossomose mansônica.

Como antígeno foram utilizadas partículas de vermes adultos, fixadas sôbre lâminas de microscopia. Reações positivas foram obtidas em 65 de 68 pacientes (95,6%). Discutem-se as vantagens do método descrito.

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