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AN IFAT CERCARIAL SLIDE ANTIGEN PREPARATION FOR SCHISTOSOMIASIS

M. E. AZAB, E. H. SAFER* and F. M. A. GHAFFAR

Department of Parasitology, Faculty of Medicine, Ain Shams University, and *Institute of Ophthalmology, National Research Center, Dokki, Cairo

Abstract. A modified technique for the preparation of a cercarial microscopic slide antigen for use in IFAT, that could be stored at —20 °C for 6 months is described. Cercariae were deposited by cooling, fixed in 0.5 % buffered formaline, mixed with 7 % egg albumin, dropped on slides and dried. Before use, antigen was post-fixed in absolute methyl alcohol. Evans blue was used as a counter-stain. The antigen was evaluated with known human positive and negative sera, also with sera from normal individuals and others residing in endemic areas and exposed to infections with schistosomiasis; as well as with sera of patients with other helminthic infections (strongyloidiasis, ankylostomiasis, ascariasis and microfilariasis). It indicated a sensitivity of 83 % and a specificity of 61.5 %.

The immunofluorescent antibody test (IFAT) for schistosomiasis with *S. mansoni* cercariae as antigen was first introduced by Sadun et al. (1960). At that time cercariae freshly emerged were used in the tube method. Later attempts at the preparation of cercarial slide antigens were reported (Wilson et al. 1974, Saathoff and Dogba 1974, Wolstenholme and Fripp 1981).

In this investigation another modification of a microscopic slide cercarial antigen that can be stored for up to 6 months is described. The antigen was evaluated with human sera from active schistosomiasis cases; uninfected individuals residing in a known non endemic area (Siwa Oasis); sera from normal individuals exposed to schistosomal infection in endemic areas. Determination of possible cross reaction with other helminthic infections (strongyloidiasis, ascariasis, ankylostomiasis and microfilariasis) was attempted using sera from patients with those infections.

MATERIAL AND METHODS

Antigen. A fresh suspension of *S. mansoni* cercariae was collected from laboratory infected *Biomphalaria alexandrina* snails and allowed to stand undisturbed until snail faeces had settled to the bottom. The supernatant of suspended cercariae was gently transferred to 10 ml, preferably siliconised, centrifuge tubes that were cooled until the cercariae became inactive and started to sediment. A compact deposit was then obtained by centrifugation at 1000—2000 rpm for 10 minutes. The discarded supernatant was replaced by 0.5 % formaldehyde in phosphate buffered saline (PBS) at a pH 7.2, in which the cercariae were suspended for 30 minutes at room temperature. They were washed once by centrifugation with PBS (7.2), resuspended in egg albumin 7 % in distilled water and their number adjusted to about 20—40 cercariae per 0.05 ml drop. One row of 4 wells was dropped on clean grease free slides, allowed to dry at room temperature, and then in an incubator (37 °C) for 1/2 an hour. The slides could then be used immediately or stored at —20 °C.

Sera. Serum samples used for evaluation of antigen were as follows; 20 serum samples taken from patients actively infected with *S. mansoni;* 133 from individuals residing in an endemic area; 20 from residents of Siwa Oasis; 39 from patients with other helminthic infections (13 strongyloidiasis, 6 ascariasis, 6 ankylostomiasis and 14 filariasis) and 20 from individuals with no detectable helminthic infection.

Conjugate. Fluorescein-labelled antihuman globulin (goat), obtained from bioMerieux, Charbonnieres — les Bains — France, was suitably diluted with 0.02 % Evans-blue counter-stain.

Microscope. Zeiss West Germany Photomicroscope III, equipped with an Osram HBO 200 W/4 lamp. As primary exciter filter BG12, UG5, UG1 as ocular filters 41/47/530 were used.
Test procedure. Frozen slides allowed to dry at room temperature were fixed in absolute methyl alcohol for 10 minutes, followed by one wash in PBS (7.2) for another 10 minutes. The slides were drained up right. Serial dilutions were prepared in PBS from 1/8—1/512, the sera were all first screened at 1/8 and those found positive examined at higher dilutions. A drop (0.05 ml) of the diluted serum was added to the antigen and incubated at 37 °C for 30 minutes. The slides were then washed once in PBS for 10 minutes without shaking and then drained dry, 0.05 ml of the conjugate — Evans-blue mixture was added and the slides reincubated at 37 °C for another 30 minutes, rewashed once in PBS and dried. A drop of buffered glycerol (pH 8) was added and covered by a cover slip.

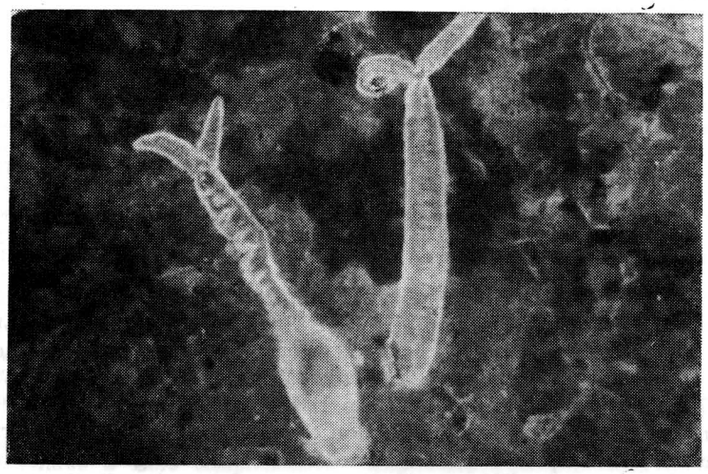

Fig. 1. Cercaria - positive reaction by IFAT.

Table 1. Results of the total number of cases examined by IFAT using cercarial antigen

Human sera	Total no. tested	% positive	% positive titres						
			1 : 8	1 : 16	1 : 32	1 : 64	1:128	1:256	1:512
I. Schistosomiasis									
— Sera from endemic area	133	83.5	25.5	6	15	15	6	6	10
— Sera from cases with active infection	20	100.0	30	5	20	20	10	—	15
— Sera from Siwa Oasis	20	0	—	—	—	—	—	—	—
II. Other helminthic infections									
— Strongyloidiasis	13	61.5	—	61.5	—	—	—	—	—
— Ascariasis	6	16.7	—	16.7	—	—	—	—	—
— Ankylostomiasis	6	33.3	—	33.3	—	—	—	—	—
— Microfilariasis	14	28.6	—	28.6	—	—	—	—	—
III. Sera from normal individuals with no detectable helminthic infections	20	0	—	—	—	—	—	—	—

RESULTS

With positive sera a specific yellow green precipitate outlined the cuticle of the cercariae (Fig. 1) contrasting vividly with the bright red coloration inside the bodies. With negative sera the cercariae were totally stained bright red. The black back ground showed minimal non-specific fluorescence. The same reaction could be produced with slides stored for 6 months at —20 °C. The results of the IFAT employing this cercarial antigen are presented in Table 1.

DISCUSSION

In an attempt toward the development of a simple method for preserving cercariae on slides for IFAT for diagnosis of schistosomiasis, Wilson et al. (1974) prepared frozen sections of the cercariae, but found them unsatisfactory. Saathoff and Dogba (1974), used cercariae fixed in 0.5 % buffered formaline and then to slides soaked in 5—10 % albumin, and no counter-stain. Wolstenholme and Fripp (1981), fixed cercariae strained through a fine mesh vitally stained with Rhodamine bovine albumin, in 5 % formaldehyde, followed by their fixation, after deposition and drying on slides in ice cold acetone.

In this investigation cooling of cercariae resulted in the deposition and collection of larger numbers. Fixation in 0.5 % buffered formaline followed by their mixture with 7 % egg albumin before dropping on the slides, complete dryness and post fixation in methyl alcohol prevented loss of cercariae during handling of slides and washing. The Evans-blue counterstained the cercariae underneath the cuticle so that the fluorescing cercariae stood out clearly, and it obliterated any non specific background fluorescence. The cercarial antigen preparation as described was sensitive having reacted with 133 sera from an endemic area with 83.3 % positivity and titrations up to 1/512 and 100 % positivity with active schistosomiasis cases. It reacted negatively with sera from Siwa Oasis, a known non endemic area (Rifaat et al. 1965, Khalil et al. 1979).

Specificity of the antigen was tested against sera from patients with other helminthic infections and found to be 61.5 % where the highest titre recorded was 1/16. Nairn (1976) and a report by C.D.C. (1978) stated that when high concentrations of patients sera are used, numerous other serum proteins including globulins not belonging to the antibody fraction may also be bound unspecifically to the preparation in addition to the specific proteins. It was concluded that no great importance should be attached to positive results with sera not diluted at least 1/10. Also the patients were all from rural areas and may have been exposed to schistosomal infection or to infection with non human cercariae, which could account for this cross reaction at this low titre. Cross-reactions with non human and non-pathogenic schistosomes were reported by Amin et al. (1969), also with other helminthic infections e.g. trichinellosis, echinococcosis and fascialiasis (Sadun et al. 1960, 1961, Thomas et al. 1979).

ПРИГОТОВЛЕНИЕ АНТИГЕНА НА МИКРОСКОПИЧЕСКИХ ПРЕПАРАТАХ ЦЕРКАРИЙ ДЛЯ РЕАКЦИИ ИММУНОФЛУОРЕСЦИРУЮЩИХ АНТИТЕЛ ПРИ ШИСТОЗОМОЗЕ

М. Е. Азаб, Е. Х. Сейфер и Ф. М. А. Чаффар

Резюме. Описана модифицированная техника приготовления антигена из микроскопических препаратов церкарий для использования при реакции иммунофлуоресцирующих антител, который можно хранить при температуре —20 °С во время 6 месяцев. Церкарии

охлаждали, фиксировали в 0,5 % формалине, смешивали с 7 % овальбумином, накапливали на микроскопические стекла и высушивали. Перед дальнейшей обработкой антиген вновь фиксировали в абсолютном метиловом спирте. Синий Эванса применяли в качестве противоокраски. Антиген тестировали с положительными и отрицательными сыворотками человека, с сыворотками нормальных и других особей, живущих в эндемических областях и выставленных заражению шистозомами и с сыворотками больных другими гельминтозами (стронгилоидозом, анкилостомозом, аскаридозом и микрофиляриозом). Выявлена чувствительность 83 % и специфичность 61,5 %.

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M. E. A., Department of Parasitology, Faculty of Medicine, Ain Shams University, Dokki, Cairo, Egypt