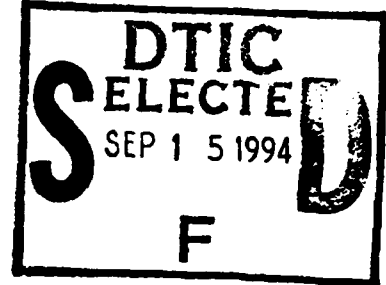
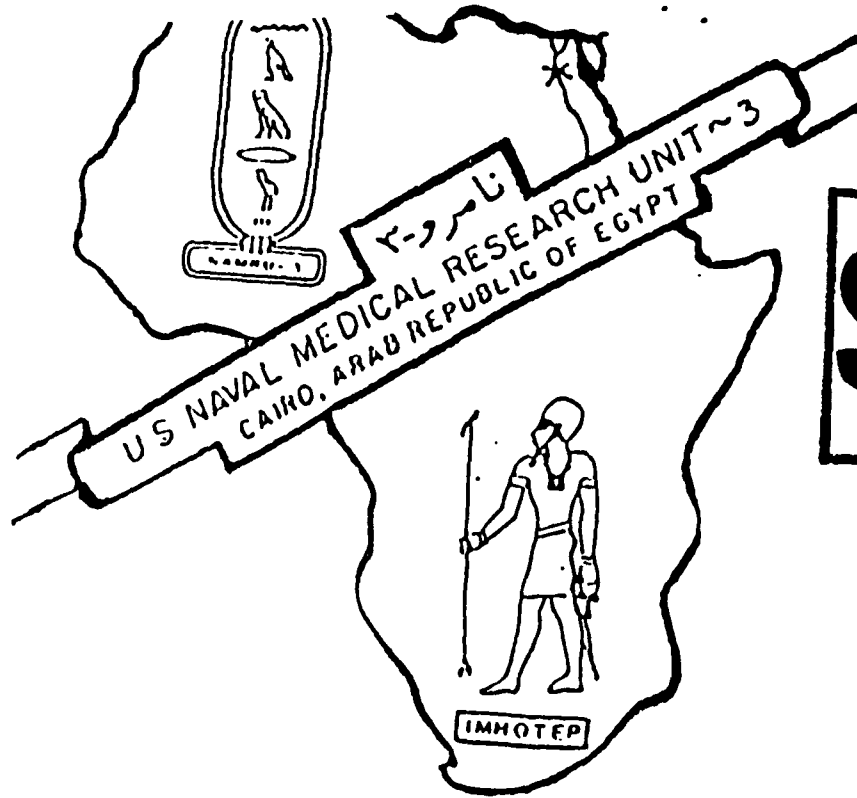


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APPLICABILITY OF ELISA ON BUFFER-ELUATES OF CAPILLARY BLOOD SPOTTED ON FILTER PAPERS FOR THE DIAGNOSIS AND CLINICAL STAGING OF HUMAN SCHISTOSOMIASIS

By

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13. ABSTRACT (Maximum 200 words) The relative concentrations of IgM and IgG antibodies (Ab) to <i>Schistosoma mansoni</i> soluble egg antigen (SEA) were evaluated in paired samples of venous blood sera and buffer-eluates of capillary blood drops dried on filter papers. The samples were obtained from school children at early and chronic stages of schistosomiasis diagnosed on the basis of history, clinical symptomatology and parasitological criteria. Enzyme-linked immunosorbent assay (ELISA), simultaneously performed, revealed paired samples to display comparable Ab levels in all cases. Samples from children with early schistosomiasis had specific IgM:IgG ratios greater than 1 [optical densities (O.D.) in sera and blood eluates of 0.77 ± 0.32 and 0.68 ± 0.30 , respectively for IgM and 0.52 ± 0.25 and 0.50 ± 0.25 for IgG]. This ratio, however, was less than 1 in samples from chronically infected children (O.D. of 0.20 ± 0.11 and 0.20 ± 0.11 for IgM and 0.69 ± 0.33 and 0.73 ± 0.32 for IgG). The specific advantages of this simplified technique are the use of anti-SEA Abs in fingerstick blood eluates, rather than sera of venous blood to serologically diagnose schistosomiasis and to differentiate early from chronic infectious particularly when used for mass screening, such as epidemiologic surveys.				
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saline (PBS; pH 7.2) containing 0.25% bovine serum albumin (BSA) and 0.05% Tween-20 (PBS-BSA-T). The serum dilution in each eluate after removal of the paper disc was estimated to be 1:100. The venous sera were diluted 1:100 when evaluated by ELISA.

Parasitological examination

Merthiolate iodine formaldehyde (MIF) technique was utilized for the detection of *S. mansoni* eggs in faeces. Positive samples were quantitated using the Kato-Katz method.⁹ Two slides from each faecal sample were examined and the mean was considered as the number of *S. mansoni* eggs per gram of faeces. *S. haematobium* eggs were detected in midday urine samples after centrifugal sedimentation. The nucleopore filtration technique¹⁰ was used to quantitate the intensity of infection in those patients found to be positive in the initial screening procedure. The average egg count from two 10 ml samples was considered as the mean.

Children testing negative for schistosome eggs had one to three additional parasitological examinations of faeces and urine collected on subsequent days.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed in 96-well flat-bottomed polystyrene ELISA plates. Volumes of 50 µl of sodium carbonate buffer (0.1 M, pH 9.6) containing 2.5 µg SEA, prepared as described by Boros & Warren¹¹, were used to coat the appropriate wells. After overnight incubation at 4°C, the unbound SEA was washed with 0.01 M PBS with 0.05% Tween-20 at 4°C (PBS-T; washing buffer). The wells were blocked with 100 µl of 0.1% BSA in coating buffer for 60 min. After washing, 50 µl of eluates from filter paper blood spot discs and 50 µl of 1:100 venous serum diluted in 0.25% BSA in PBS-T were simultaneously tested in the same plate for antibodies to SEA. The test samples and appropriate controls were incubated for 2 hours at room temperature, then washed three times. Fifty µl of alkaline phosphatase conjugated anti-human IgM or IgG (Sigma; diluted at 1:1000) were added and the plates incubated for 1 hour at 37°C. After washing, 50 µl of substrate (1 mg para-nitrophenyl phosphate per ml of 0.05 M carbonate buffer, pH 9.8) were added and the plates

incubated at 37°C for 30 minutes. Fifty µl of 1N NaOH were used to stop the reaction. Optical densities (O.D.) were read in a Multiscan Titertek ELISA reader at 405 nm.

RESULTS

Checkerboard titration

For the detection of the assay limits, wells of ELISA plates were sensitized with 50 µl of serially diluted SEA (0.75-100 µg/ml) in coating buffer. Serum pools obtained from parasitologically confirmed schistosomiasis patients and normal healthy individuals were used at serial dilutions of 1:5-1:3200 in PBS to detect anti-SEA IgM and IgG antibodies. The optimum antigen concentration and serum dilution were found to be 50 µg/ml and 1:100, respectively.

Clinical and parasitological characteristics of the study population

Clinical and parasitological examination of the study group revealed 44 children, with known history of disease, to have spleen and/or liver involvement. Urine and/or faeces were positive for schistosome eggs upon the first parasitological examination. Children of this group were defined as chronic schistosomiasis cases (table 1). Repeated parasitological examination of 56 children with neither history nor clinical symptoms of schistosomiasis revealed schistosome eggs in the excreta of 22 children. These characteristics favoured the categorization of this group at the 'early' phase of schistosomiasis. The remaining 34 children were included for control studies.

Table 1 Characteristics of the study groups

	Early infection N=22	Chronic infection N=44	Uninfected N=34
Sex			
Male	3	36	15
Female	19	8	19
Age in years (range)	6-8	8-13	6-12
Mean ± SD	7.1 ± 0.7	11.3 ± 1.5	9.1 ± 2.0
<i>S. haematobium</i>			
Urine positive	n=2	n=12	-
Eggs/10 ml urine (range)	2-5	8-87	
Mean ± SEM	4.0 ± 1.0	29.3 ± 3.2	
<i>S. mansoni</i>			
Stool positive	n=19	n=26	
Eggs/gm stool (range)	20-240	20-600	
Mean ± SEM	60 ± 21	141.8 ± 29.6	
<i>S. haematobium</i> and <i>S. mansoni</i> mixed infections	n=1	n=6	
<i>E. histolytica</i>	n=5	n=7	n=7
<i>G. lamblia</i>	n=1	n=5	n=4
<i>H. nana</i>	n=1	n=4	n=5
Liver involvement		(n=33)	
Liver Spleen involvement		n=11	

Anti-SEA antibody levels in sera and eluates of dry blood spots

A total of 66 paired specimens of venous sera and eluates of whole blood collected on filter papers from infected children were tested in parallel for levels of anti-SEA antibodies in an ELISA format. Thirty-four paired specimens from children with no evidence of schistosomiasis and inhabiting the same endemic area were also tested and reported as controls.

Levels of anti-SEA IgG and IgM antibodies in paired samples of venous sera and filter paper blood eluates were essentially identical (table 2). The 22 paired specimens of sera and blood eluates of the early schistosomiasis children displayed significantly elevated levels of anti-SEA IgM antibodies (0.77 ± 0.32 and 0.68 ± 0.30 O.D. values, respectively) and reduced anti-SEA IgG levels (0.52 ± 0.25 and 0.50 ± 0.25 , respectively). The ratios of anti-SEA IgM:IgG O.D. values were significantly lower ($p < 0.001$) in paired specimens from the chronically infected children than in the early schistosomiasis group without statistically significant differences in values between paired samples within each group.

Regression analyses performed to compare O.D. values obtained from paired samples of sera and blood eluates tested for specific antibodies indicated positive correlations between levels of IgG ($r = 0.92$, $p < 0.001$) and IgM ($r = 0.91$, $p < 0.001$) antibodies in paired specimens of the study population ($n = 66$). Similarly, strong positive correlations were obtained between anti-SEA IgM:IgG ratio in paired specimens from early schistosomiasis children ($r = 0.65$, $p < 0.001$) and chronic disease group ($r = 0.876$, $p < 0.001$), as well as when analysed for the study population taken together ($n = 66$; $r = 0.913$, $p < 0.001$).

DISCUSSION

This investigation was undertaken to determine the reliability of eluates of dried blood drops from finger stick onto filter paper as an alternative to venous serum samples for ELISA detection of schistosome-specific antibodies. Storage of dried blood on filter papers for up to 30 days did not alter the antibody levels as compared to paired venous serum kept at -70°C . Further, anti-SEA IgM antibody levels were consis-

tent in paired samples of blood eluates and sera. These results are in contrast to the results of others indicating that absorption of blood on filter paper may destroy most of the reactivity of IgM antibodies¹², and thus could be detected in eluates only if present at high levels in patient's serum.⁵

In addition, these results indicated a high sensitivity of ELISA when SEA is used, at least for the purpose of seroepidemiological surveys. Similar results, using egg antigens, have been reported previously by several investigators.^{13,14,15}

The possibility of cross-reactions with other parasites, e.g. *Entamoeba histolytica*, *Giardia lamblia* and *Hymenolepis nana*, can be excluded since these parasites were common in 47% of schistosomiasis-free children (table 1) displaying negative anti-SEA ELISA responses (table 2).

The present study also evaluated the use of IgM:IgG antibody ratios to SEA as a means of differentiating early and chronic disease. Besides the clinical and parasitological findings, and in retrospect, two characteristics indicate that this ratio may be valuable in staging children at the early phase of schistosomiasis. First, the majority of these children were found to be females (86%, table 1). Traditionally, girls at this age do not come in frequent contact with canals. Second, schistosome eggs were detected in faeces and/or urine in 12 out of 22 of the serologically positive children in this group on the first parasitological screening, while the remainders became positive upon repeated examinations (data not shown).

It is concluded that the technique is potentially suitable for detection of schistosome-specific IgM and IgG antibodies in eluates of blood spots dried on filter papers. As such, it should be a valuable epidemiological tool for both diagnosis and clinical staging of schistosomiasis.

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Table 2 Levels* of antibodies to *Schistosoma mansoni* egg antigen (SEA)* in paired samples of blood spot eluates and sera of schistosomiasis children

	IgM				IgG				IgM:IgG			
	Serum		Blood eluate		Serum		Blood eluate		Serum		Blood eluate	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Early infection (n=22)	0.77±0.32	(0.30-1.47)	0.68±0.30	(0.32-1.32)	0.52±0.25	(0.14-1.10)	0.50±0.25	(0.13-1.08)	1.58±0.51	(1.01-2.93)	1.55±0.62	(0.73-3.00)
Chronic infection (n=44)	0.20±0.11	(0.03-0.45)	0.20±0.11	(0.03-0.49)	0.69±0.33	(0.30-1.58)	0.73±0.32	(0.37-1.63)	0.35±0.22	(0.02-0.95)	0.29±0.19	(0.04-0.76)
P-value	< 0.001		< 0.001		=0.018		=0.002		< 0.001		< 0.001	
Uninfected (n=34)	0.06±0.04	(0.01-0.11)	0.07±0.03	(0.01-0.11)	0.08±0.04	(0.01-0.14)	0.08±0.03	(0.02-0.12)				

* Represented by ELISA OD values

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