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EVALUATIONS OF ANTIBODY LEVELS IN TOXOPLASMA INFECTION BY THE IMMUNOFLUORESCENT ANTIBODY TEST AND ELISA TEST

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Abstract. In this study 292 sera were screened by the IFAT. 46 sera with variable IFAT titres were tested with ELISA Reagent Set and microtitration tests. A comparative evaluation of the specificity and reliability of ELISA method with that of IFAT for the detection of *Toxoplasma* infection was done.

Toxoplasmosis is an asymptomatic infection rather than an overt disease. The first isolation of the human toxoplasmosis agent in Egypt was achieved by Rifaat et al. (1973). In a study on its prevalence in different governorates of Egypt, Rifaat et al. (1975) reported a percentage positivity of 16.8 % using the Dye test at the titre 1/16 and above. Osman et al. (1978) examined 61 blind boys from school for blind in Cairo and could demonstrate *Toxoplasma* antibodies among 18 of them by the IFAT. Recently, Fikry et al. (1980) reported a prevalence in women in the child bearing period in Cairo that mounted to 25.0 % positivity by the Dye test and 24 % by the IFAT. A recent review on new advances in the immunodiagnosis of parasitic infection including toxoplasmosis was recorded by Hillyer and Kagan (1979).

MATERIAL AND METHODS

The group under study was composed of 229 cases from various clinics including gynaecological, obstetric, pediatric, ophthalmic, cancer and tropical medicine hospitals.

***Toxoplasma* strain.** The highly virulent RH strain fatal to Swiss albino mice was used for the preparation of antigen for IFAT according to Goldman (1957).

Conjugate. Fluorescein labelled antihuman globin (sheep) was obtained commercially from Wellcome research laboratories Beckenhan, England. Procedure of IFAT was carried out after Kramář (1963). For fluorescence microscopy, Zeiss West Germany photomicroscope III, eguipped with an Osram HBO 200 W/4 lbmp. As primary exciter filter BG12, UG5, UG1 as ocular filters, 41/47/530 were used.

Immunoenzyme *Toxoplasma* Antibody Reagent Set. It was obtained from Warthington Diagnostic/Millipore U.S.A. It is for the qualitative determination of antibodies against *T. gondii* in serum samples. Each set consisted of 4 casettes readily coated with *Toxoplasma* antigen in a soluble form, Tris buffer powder, lyophilised antihuman immunoglobulin linked with horseradish peroxidase enzyme, positive and negative control sera and AP stain powder to be reconstituted in hydrogen peroxidase.

The technique used was a modification of the indirect method described by Voller et al. (1975). It was used for testing 46 serum samples of known positive IFAT value, and a single dilution of sera at 1/16 was performed. A positive serum sample produced a pink red colour in the well compared to appropriate positive and negative controls. In order to double check the system, 19 of the 46 samples were sent to the "Institut für Tropische Parasitologie, Bonn University, Federal Republic of Germany" where they were tested with the ELISA microtitration technique according to Voller et al. (1975).

Table 1. Results of screening of sera with IFAT at different titres

Diagnosis	No. of examined cases	No. of positive cases	Titres								Positivity %
			< 1/16	1/16	1/32	1/64	1/128	1/256	1/512	1/1 024	
Gynaecological and obstetric cases	200	32	168	17	9	5	1				16
Ophthalmic cases	30	12	18	8	3	1					40
Bilharzial hepatosplenomegaly cases	25	13	12	5	4	3	1				52
Acute lymphoblastic leukaemia cases	11	6	5	3	1	1	1				54.2
Generalized lymphadenopathy cases	10	8	2	4	2	1	1				80
Cases of undiagnosed fever	8	—	8								0
Cases presenting with jaundice	5	1	4							1	20
Cases of Hodgkin's disease	2	1	1	1							
Case of systemic lupus erythematosis under corticosteroid therapy	1	1				1					
Total	292	74	218	38	19	12	4			1	25.34

Table 2. Results of serological examination by IFAT at different titres and by ELISA Reagent Set

Diagnosis	No. of tested sera	IFAT	ELISA	IFAT	ELISA	IFAT	ELISA
		1/16	Positive	1/32—1/128	Positive	1/256 to 1/1 024	Positive
Gynaecological and obstetric cases	23	13	12	10	10	—	—
Ophthalmic cases	10	7	5	3	3	—	—
Bilharzial hepatosplenomegaly cases	7	6	6	1	1	—	—
Acute lymphoblastic leukaemia cases	2	2	2	—	—	—	—
Generalized lymphadenopathy cases	2	2	2	—	—	—	—
Hodgkin's disease case	1	—	—	1	1	—	—
Case presenting with jaundice	1	—	—	—	—	1	1
Total	46	30	27	15	15	1	1

RESULTS

In the present study 292 sera were first screened by using IFAT where 74 cases were found to be positive at titres ranging from 1/16—1/1024 (Table 1). Of these, 46 were simultaneously examined by ELISA Reagent Set. Results are presented in Table 2. In addition, 19 positive sera were selected and additionally tested by the ELISA microtitration test; the results are seen in Taole 3.

Table 3. Results of serological examination of selected cases screened by IFAT and tested by ELISA microtitration test and ELISA Reagent Set

Diagnosis and No. cases	Serial number	IFAT	ELISA	ELISA	Comments on IFAT & ELISA microtitration
		end titre	micro-titration test	Reagent set	
Gynaecological and obstetric cases (9)	471	1/32	1/16	N.D	IFAT one fold higher
	492	1/16	1/16	+	Absolute agreement
	371	1/16	1/64	+	ELISA two fold higher
	288	1/128	1/256	+	ELISA one fold higher
	283	1/64	1/64	+	Absolute agreement
	109	1/16	1/16	—	Absolute agreement
	425	1/16	1/64	N.D	ELISA two fold higher
	437	1/16	1/64	+	ELISA two fold higher
	174	—	—	N.D	Absolute agreement
Bilharzial hepatosplenomegaly (5)	48	1/16	1/16	+	Absolute agreement
	46	1/32	1/16	+	IFAT one fold higher
	43	1/16	1/64	N.D	ELISA two fold higher
	34	1/16	1/64	+	ELISA two fold higher
	17	1/32	1/64	+	ELISA one fold higher
Acute lymphoblastic leukaemia (2)	10	1/16	1/16	+	Absolute agreement
	41	—	—	N.D	Absolute agreement
Case presenting with jaundice (1)	13	1/1 024	1/4 000	+	ELISA two fold higher
Case presenting with fever (1)	4	1/16	1/64	+	ELISA two fold higher
Hodgkin's disease case (1)	11	1/16	1/16	+	Absolute agreement

N.D = Not done, + = Positive, — = Negative.

DISCUSSION

Most of the reports published show a satisfactory agreement between IFAT and others including ELISA test. The mean antibody titres as indicated by various authors are, however, rather different. Probably due to individual modifications of the respective reaction as conducted in various laboratories resulting in different sensitivity levels. Numerous articles have been published utilizing ELISA test for

serodiagnosis of toxoplasmosis (Voller et al. 1976, Walls et al. 1977, Camargo et al. 1978). The World Health Organisation (1976) described this test as practical, easy to perform and quite sensitive. Walls et al. (1977) utilizing solubilized tachyzoites found the test 98 % specific, highly reproducible and obtained results equivalent to the IFAT. Camargo et al. (1978) performed the ELISA test utilizing anti IgG and anti IgM antibodies and considered it an acceptable substitute for IFAT.

In this study, 292 sera were collected from cases presenting with different signs, symptoms, results are seen in (Tables 1—3). The total number of positive cases with IFAT was 74 with a percentage of positivity of 25.34 %, at a titre range from 1/16—1/1024. The Centre for Disease Control (1974) established that the Immunozyme *Toxoplasma* Reagent Set is designed to produce a reaction with sera having a titre equal to or greater than 1/64 by Indirect haemagglutination test, while Voller et al. (1976) considered it equivalent to a titre of 1/40 or above with the same test. Even though the number of sera was small, there was a positive correlation between the three tests for all the 19 cases (Table 3). Absolute agreement as regard the titres was found in 8 cases. Two control cases proved negative by IFAT were also negative by ELISA microtitration technique. Unfortunately it was not possible to test them also with the ELISA Reagent Set. The other 6 cases were positive and having the same end titre in IFAT and ELISA microtitration tests. Discrepancy in the end titres obtained by IFAT and ELISA microtitration test occurred in 11 cases belonging to different groups. In 2 cases the IFAT was one fold higher, in 9 cases the ELISA test titre was higher: 2 one fold, 6 two fold 1 nearly three fold higher.

These data clearly demonstrate the specificity and reproducibility of ELISA test as forementioned by different authors. It appears to be as sensitive and specific as IFAT, if not more so, as indicated by one, two and almost three dilutions differences.

ОПРЕДЕЛЕНИЕ УРОВНЯ АНТИТЕЛ ПРИ ТОКСОПЛАЗМОЗЕ ПУТЕМ РЕАКЦИИ ИММУНОФЛУОРЕСЦИРУЮЩИХ АНТИТЕЛ И МЕТОДА ELISA

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Резюме. Изучали 292 сывороток при помощи реакции иммунофлуоресцирующих антител (РИФА). 46 сывороток с разными титрами изучали с помощью метода ELISA и микротитрации. Сравнивали специфичность и чувствительность методов ELISA и РИФА для выявления токсоплазменных антител.

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