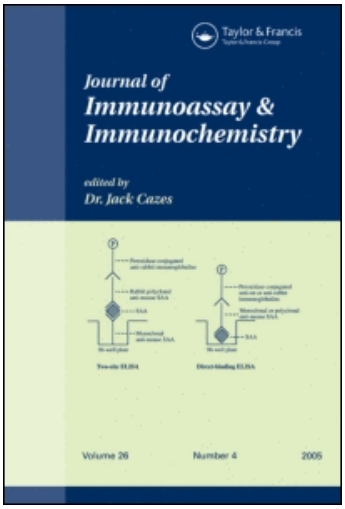

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A Standardized Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection of Antibodies to *Toxoplasma Gondii*

Roy R. Mondesire[a]; David E. Charlton[a]; Ian R. Tizard[b]
[a] Dept. of Immunobiology Research, Ortho Diagnostics, Don Mills, Ontario [b] Ontario Veterinary College Dept. of Veterinary Microbiology & Immunology, University of Guelph,

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A STANDARDIZED ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DETECTION OF ANTIBODIES TO TOXOPLASMA GONDII

Roy R. Mondesire*[1], David E. Charlton[1]
and Ian R. Tizard[2]

[1]Ortho Diagnostics, Dept. of Immunobiology Research,
19 Green Belt Drive, Don Mills, Ontario
[2]Ontario Veterinary College
Dept. of Veterinary Microbiology & Immunology
University of Guelph

ABSTRACT

An enzyme-linked immunosorbent assay (ELISA) for the serodiagnosis of Toxoplasma gondii infections on single serum dilutions was developed.

This test system is a standardized kit designed to detect circulating specific antibodies to Toxoplasma gondii in human sera. It consists of Toxoplasma gondii soluble antigen-coated microtitration multiwell plates, specific immunoglobulin-enzyme conjugate and other required reagents.

In a clinical trial performed on sera from 1,035 clinically suspected toxoplasmosis cases, the Sabin Feldman Dye Test (SFDT) and this ELISA system agreed closely. Relative to the SFDT, the sensitivity and specificity of the latter was 98.0% and 97.6% respectively with a correlation coefficient of 0.97. In a further study of 121 sera, the Indirect Fluorescent Antibody Test (IFAT), the Indirect Haemagglutination Test (IHAT) and this ELISA procedure showed over 90% agreement, with correlation coefficients of 0.98 and 0.95 respectively. Within the working concentration of specific antibody to T. gondii in human serum, there was a linear relationship between the ELISA values and the WHO international standard for human anti-Toxoplasma serum.

INTRODUCTION

Human toxoplasmosis is a multisystem disease caused by the obligate intracellular protozoan parasite, T. gondii. Most toxoplasmic infections are subclinical and the associated symptoms are frequently nonspecific. The diagnosis of toxoplasmosis is very dependent on serological methods for the detection of antibodies to T. gondii. The most widely-used tests are the Sabin Feldman Dye Test (SFDT) (10); the Indirect Fluorescent Antibody Test (IFAT) (4, 7); the Indirect Haemagglutination Test (IHAT) (5) and the Complement Fixation Test (CFT) (9, 18).

Since the first description of ELISA by Engvall and Perlmann (1971), numerous articles have been published describing its application in clinical diagnosis (11, 13, 15 & 19). In particular, its adaptation for the serodiagnosis of toxoplasmosis has been successfully worked out (1, 2, 16 & 17). This standardized ELISA system overcomes the problems associated with the two most widely-used tests for toxoplasmosis (the SFDT and the IFAT). It does not require serial titration and serum pretreatment and the results are easily read visually or with a simple spectrophotometer. In this report, we present the results of a comparative clinical evaluation of the ELISA, SFDT, IHAT and IFAT systems for the detection of antibodies to T. gondii.

MATERIALS AND METHODS

Serum Samples

The human serum samples were obtained from Dr. I. Tizard, Toxoplasmosis Laboratory, Dept. Veterinary Microbiology and

Immunology, University of Guelph, Guelph, Ontario. A total of 1,035 serum samples were tested by the SFDT and ELISA. A further 121 serum samples were tested by IFAT, IHAT and ELISA. All testing was performed in duplicate by ELISA. The samples which had been tested by the SFDT and the IHAT had been stored at -20°C for periods ranging from a few weeks to three years before the ELISA was performed. IFAT was performed within a few weeks of ELISA.

Serial dilutions of sera ranging from 1/16 - 1/1024 were used in the SFDT, IFAT and IHAT procedures. A single 1/50 dilution of serum was used in the ELISA procedure.

Serial twofold dilutions of the WHO standard for anti-T. gondii serum (human) containing 1000 IU/ml was used to establish a standard curve. The range covered 1/50 to 1/6400. These dilutions covered the values of IU/ml which would be found in an actual ELISA procedure utilizing sera containing anti-toxoplasma activity ranging from 1000 IU/ml to 7.5 IU/ml.

T. gondii Antigen

T. gondii tachyzoites (RH strain), originally purchased from the National Institute of Health (NIH), Washington, in 1959, were obtained from the Ontario Veterinary College, Guelph, Ontario, with the permission of Dr. I.R. Tizard. The organism was maintained by intraperitoneal passage in Swiss Webster mice. Upon recovery from the peritoneal cavity, the tachyzoites were harvested by differential centrifugation to separate them from the mouse leukocytes. Following three phosphate buffered saline

(PBS) washings (2,000 x g, 10 min.), the tachyzoites were then suspended in distilled water and sonicated at 20 KHz for 5 minutes in an ice bath. The supernatant was mixed 1:1 with PBS, stored at 4°C and used the next day. Polystyrene microtitre plates (Micro-ELISA substrate plates, Greiner, Germany) were coated at 4°C for 18 hrs. with optimally-diluted T. gondii antigen in 0.01M $Na_2CO_3/NaHCO_3$ buffer, pH 9.6. They were then washed, dried and packaged in moisture-free pouches.

Sheep Anti-Human-Globulin Horeseradish Peroxidase Conjugate

The sheep anti-human globulin horseradish peroxidase (SAHG HRP) conjugate was prepared by the method of Nakane and Kawoi (8) using anti-human immunoblogulin G (γ-chain specific) and HRP (Sigma Chemical Co., St. Louis, Mo.). The optimal conjugate dilution was determined and a 100x concentrate was prepared in a conjugate buffer. The working dilution was prepared in 0.05% PBS Tween-20.

Optimization of Reagents

Numerous in-process and finished product tests were performed on the reagents and rigid limits were established for the reactivity and quality of each reagent. The reagents tested included T. gondii tachyzoites, T. gondii-soluble antigen, sheep anti-human immunoglobulin G, sheep anti-human immunoglobulin G horseradish peroxidase, polystyrene substrate plates, T. gondii antigen-sensitized polystyrene substrate plates, O-phenylene-diamine (OPD) 3% hydrogen peroxide (H_2O_2) PBST and other packaging components.

The ELISA Procedure

The ELISA procedure performed was a modification of that described by Voller et al, (14). Briefly, 200 µl of PBST was added to the wells of a freshly opened T. gondii antigen-sensitized microtitration plate. Two 4 µl volumes of each serum (including high, low and negative control sera) were added to duplicate wells of the plate. Two 4 µl volumes of each serum (including high, low and negative control sera) were added to duplicate wells of the plate. The plates were mixed vigorously with a mechanical mixer, covered and incubated for 0.5 hr. at 37°C in a humidified incubator. The wells were then washed 3x with PBST using the ELISA 8 port wash bottle (Ortho Diagnostics, Canada Ltd.). 200 µl of optimally-diluted SAHG HRP was added to each well. The plate was covered and incubated for 0.5 hr. at 37°C in a humidified incubator. The wells were again washed 3x with PBST. Freshly-prepared OPD/H_2O_2 substrate solution was added to all of the wells of the plate and the latter was incubated in the dark for 0.5 hr. at room temperature. The substrate hydrolysis was stopped by the addition of 60 µl 4N H_2SO_4 to all wells and the colour development was read at 492 nm with an ELISA spectrophotometer (Titertek* Multiskan; Flow Laboratories, Rockville, Maryland, or MicroELISA* Minireader; Dynatech Laboratories, Alexandria, Virginia). The mean of duplicate absorbance results was reported for each serum specimen.

A World Health Organization International Standard for anti-toxoplasma serum, human, reconstituted to 1,000 IU/ml with distilled

TABLE 1

Cross tabulation of SFDT titres and ELISA values obtained on 1,035 serum sample specimens tested for antibodies to T. gondii.

SFDT TITRE	ELISA VALUE 0.15	0.2	0.25	0.3	0.6	0.8	1.0	1.2		TOTALS
< 16	540	80	10	5						635
16	2	2	3	2	2					11
32										
64	1	1	6	7	32	1				48
128			3	4	22	1				30
256	2		1	3	78	40	13	4		141
512					7	12	7	1		27
1024					9	35	44	23	22	133
4096								3	7	10
TOTALS	545	83	23	21	150	89	64	31	29	1035

water, was used as a reference serum. It was also serially-diluted in 0.05% PBST to determine the relationship of the antibody activity to the absorbance value.

RESULTS

Comparison of SFDT and ELISA

A total of 1,035 sera from patients clinically suspected of having toxoplasmosis was tested by both methods. The number of agreements and disagreements is shown in Table 1 as a function

of a cut-off value of 0.2. At a cut-off value of 0.2, the relative sensitivity and specificity of the ELISA test system based on the SFDT was 98% and 97.6% respectively and the percent agreement was 97.8%. Table 1 shows the distribution of ELISA for each SFDT titre. A schematic of the regression line and the 95% confidence limits for the line and the ELISA means is shown in Fig. 1. The correlation coefficient for the linear regression was 0.966.

Comparison of IFAT, IHAT and ELISA

A total of 121 serum samples was investigated. The agreements between the systems were 92.6% and 90.1% respectively for the IFAT/ELISA and the IHAT/ELISA comparisons. When the cut-off values are increased from 0.20 to 0.25 and 0.30 respectively for the IFAT/ELISA and the IHAT/ELISA comparisons, the percent agreement climbs to 94.2% for each.

A linear regression between the mean values of ELISA and the logarithm of the corresponding test dilution gave a coefficient of correlation of 0.982 ($p<0.05$) and 0.945 ($p<0.05$) respectively for IFAT vs ELISA and IHAT vs ELISA.

Reproducibility of the ELISA System

The use of standardized reagents and control sera has resulted in a highly reproducible ELISA system for the detection of T. gondii antibodies as shown in Table 2 for example. The coefficient of variation (CV) for the within run assay ranged from 3.78% to 12.03% for four batches of ELISA kits.

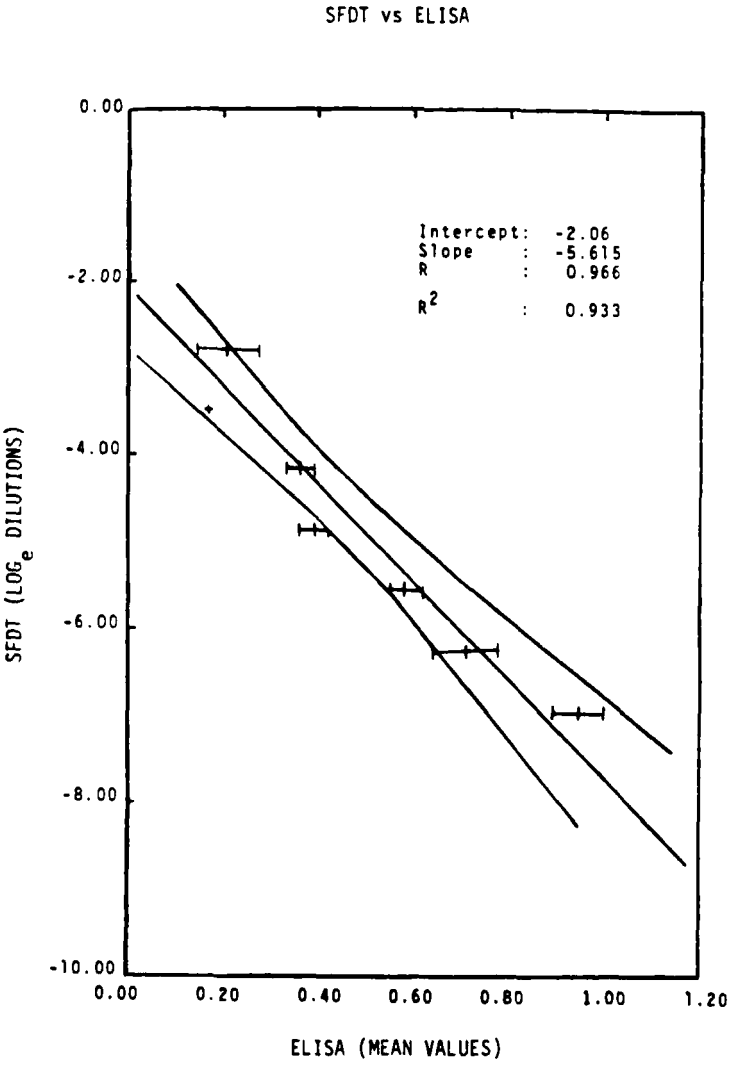

FIGURE 1. Schematic of the regression line, the 95% confidence limits for the line and the ELISA means from a linear regression analysis between the mean ELISA values and the logarithm of the corresponding SFDT dilutions.

TABLE 2

Reproducibility studies performed on four lots (A-D) of ELISA kits for T. gondii antibody detection. The values are from random sampling of plates from each lot.

Lot No.	Plate No.	N	Mean	Var.	SD	% C.V.
A	1	48	1.0585	0.005	0.0683	6.45
	2	48	0.9583	0.001	0.0362	3.78
B	3	48	1.0453	0.016	0.1258	12.03
	4	48	1.0698	0.009	0.0995	9.30
	5	48	1.1572	0.014	0.1199	10.36
C	6	48	1.1390	0.009	0.0945	8.30
	7	48	1.1026	0.015	0.1221	11.07
	8	48	1.1236	0.011	0.1060	9.43
	9	48	1.0962	0.008	0.0940	8.58
	10	48	1.0746	0.013	0.1158	10.78
D	11	48	1.0218	0.007	0.0930	9.05
	12	41	0.9920	0.009	0.0950	9.58
	13	48	0.9512	0.005	0.0588	6.18
	14	48	1.0756	0.107	0.1292	12.01
	15	48	1.0716	0.015	0.1215	11.30
	16	48	1.0526	0.003	0.0964	9.16
	17	48	1.0122	0.008	0.0895	8.84

N = Number of Replicates

SD = Standard Deviation

CV = Coefficient of Variation

DISCUSSION

These results indicate that the 1/50 single serum dilution method in this indirect ELISA gives comparable results with the established serological methods for toxoplasmosis which rely on the serial dilution method. The ELISA absorbance values increase proportionally with the titres obtained on the various sera. The wide range of plus or minus fourfold dilution allowed for the SFDT and the IFAT procedures (12) does not apply to this system of objective evaluation. This limitation, characteristic of serial dilution methods, is reflected in the distribution of ELISA absorbance values obtained for each group of sera (see Table 2). We have found that the % CV of 3.78 - 12.03 obtained for intraplate and plate-to-plate reproducibility is reduced as the worker becomes more familiar with the technique.

The system was generally shown to be comparable to the three conventional tests in sensitivity and specificity. In the SFDT, IHAT, IFAT and ELISA comparisons, three sera which were negative by IHAT (probably reflecting recent infections) (6) were positive by SFDT, IFAT and ELISA. These findings and the high level of agreement with ELISA suggest that this ELISA system detects antibodies to both the cell membrane and the cytoplasmic antigens of T. gondii. The"false positive"and"false negative"results obtained may be a reflection of the cut-off value which was used to maximize agreement between two systems.

The linearity between IU/ml and A_{492}(Fig. 2) means that the ELISA values may be interpolated from a standard curve and reported to clinicians in a more intelligible form.

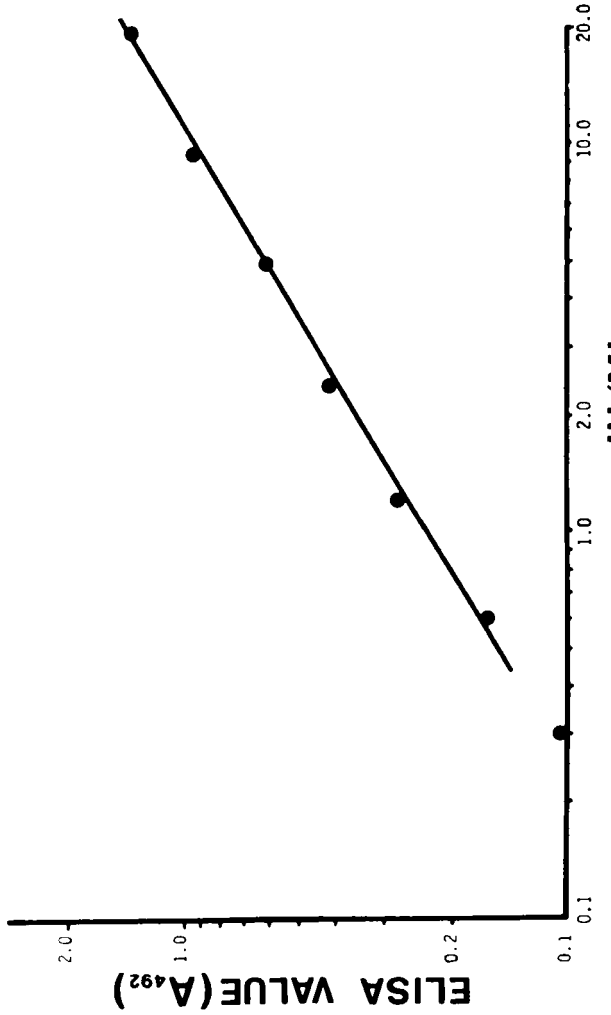

FIGURE 2. Example of a standard curve obtained with the WHO standard, demonstrating the linear relationship between the absorbance values and IU/ml, in working range of the ELISA system for T. gondii antibodies.

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Address requests for reprints to: Roy R. Mondesire, Ortho Diagnostics, Dept. of Immunobiology Research, 19 Green Belt Drive, Don Mills, Ontario M3C 1L9.

REFERENCES

(1) Bout, D., Dugimont, J.C., Farag, H. et Capron A. (1975). Le Diagnostic Immunoenzymologique des Affections Parasitaires. II - Immunoenzymologie Quantitative (E.L.I.S.A.) Lille Med. 20:561-655.

(2) Camargo, M.E., Ferreira, A.W., Mineo, J.R., Takiguti, C.K. and Nakahara, O.S. (1978). Immunoglobulin G and Immunoglobulin M Enzyme-Linked Immunosorbent Assays and Defined Toxoplasmosis Serological Patterns. Infect. Immunity, 21: 55-58.

(3) Engvall, E. and Perlmann, P. (1971). Enzyme-Linked Immunosorbent Assay (ELISA) Quantitative Assay Immunoglobulin G. Immunochem. 8:871-874.

(4) Goldman, M. (1957). Staining Toxoplasma gondii with Fluorscein-Labelled Antibody. II. A new serologic test for antibodies to Toxoplasma based upon inhibition of specific staining. J. Exp. Med. 105:557-573.

(5) Jacobs, L. and Lunde, M.N. (1957). A Haemagglutination Test for Toxoplasmosis. J. Parasit. 43:308-314.

(6) Karim, K.A. and Ludlam, G.B. (1974). The Relationship and Significance of Antibody Titres as Determined by Various Serological Methods in Gladular and Ocular Toxoplasmosis. J. Clin. Path. 28:42-49.

(7) Kelen, A.E., Allyon-Leindle, L. and Labzoffsky, M.A. (1962). Indirect Fluorescent Antibody Method in Serodiagnosis of Toxoplasmosis. Can. J. Microbiol. 8:545-554.

(8) Nakane, P.K. and Kawaoi, A. (1974). Peroxidase-Labelled Antibody. A new method of conjugation. J. Histochem. 22: no. 12, 1084-1091.

(9) Sabin, A.B. (1949). Complement Fixation Test in Toxoplasmosis and Persistence of Antibody in Human Beings. Pediatrics, 4:443-453.

(10) Sabin, A.B. and Feldman, H.A. (1948). Dyes as Microchemical Indicators of a New Immunity Phenomenon Affecting a Protozoan Parasite (Toxoplasma). Science, 108:660-663.

(11) Saunders, G.C. and Clinard, E.H. (1976). Rapid Micromethod of Screening for Antibodies to Disease Agents Using the Indirect Enzyme-Labelled Antibody Test. J. Clin. Microbiol. 3:no. 6, 604-608.

(12) Sulzer, A.J. and Hall, E.C. (1966). Indirect Fluorescent Antibody Tests for Parasitic Diseases. iv. Statistical study of variation in the indirect fluorescent antibody (IFA) test for toxoplasmosis. Amer. J. Epi. 86:401-407.

(13) Voller, A., Bartlett, A. and Bidwell, D.E. (1978). Enzyme Immunoassays with Special Reference to ELISA Techniques. J. Clin. Path. 31:507-520.

(14) Voller, A. and Bidwell, D.E. (1975). A Simple Method for Detecting Antibodies to Rubella. Br. J. Exp. Path. 56: 338-339.

(15) Voller, A. and Bidwell, D.E. (1976)[a]. Enzyme Immunoassays for Antibodies in Measles Cytomegalovirus Infections and After Rubella Vaccination. Br. J. Exp. Path. 57:243-247.

(16) Voller, A., Bidwell, D.E. and Bartlett, A. (1976)[b]. Enzyme Immunoassays in Diagnostic Medicine. (Theory and Practice). Bull. Wld. Hlth. Org. 53:55-65.

(17) Walls, K.W., Bullock, S.L. and English, D.K. (1977). Use of the Enzyme-Linked Immunosorbent Assay (ELISA) and its Microadaptation for the Serodiagnosis of Toxoplasmosis. J. Clin. Microbiol. 5:273-277.

(18) Warren, J., and Russ, S.B. (1948). Cultivation of Toxoplasma in Embryonated Egg: And antigen derived from chorioallantoic membrane. Proc. Soc. Exper. Biol. and Med. 67:85-89.

(19) Wisdom, G.B. (1976). Enzyme Immunoassay. Clin. Chem. 22: 1243-1255.