

An evaluation of the ToxHA test for the detection of antibodies to *Toxoplasma gondii* in human serum

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SUMMARY The titre of antibodies to *Toxoplasma gondii* was compared in 1985 sera by use of an indirect haemagglutination test (ToxHA test—Wellcome Reagents Ltd) and the dye test. Sera from 42 patients with clinical or serological indications of recent toxoplasmosis were also examined to determine if the ToxHA test would be of value in detecting early stages of the disease. Nine recent cases of lymphadenopathy and four cases of eye infection were not detected by the test. In addition, a large number of sera found negative in the dye test gave a positive result in the haemagglutination test.

The method of choice in diagnosing an infection due to *Toxoplasma gondii* is by the detection of specific antibodies against the parasite in the patient's serum.¹ The dye test of Sabin and Feldman,² subsequently modified,^{3,4} is still regarded as the definitive test in the diagnosis of toxoplasmosis.

Although specific, sensitive, and reproducible,⁵ the dye test is technically difficult and slow, requiring the use of live parasites and suitable human serum as a source of 'accessory factor'. This has led to a search for less exacting but equally effective techniques.

The indirect haemagglutination test (IHAT) of Jacobs and Lunde⁶ and its modifications⁷⁻⁹ is a potential alternative to the dye test. A test kit is now commercially available which detects antibodies against *T. gondii* by the agglutination of sensitised turkey cells (ToxHA test—Wellcome Reagents Ltd).

In order to evaluate this test for routine use, we have compared results in the ToxHA test and dye test using 1985 sera routinely submitted to us. In addition, we have examined 42 sera chosen from patients where the available serological and clinical evidence suggested a toxoplasma infection of recent origin, to determine the performance of the ToxHA test in detection of these early cases.

Material and methods

A total of 1985 sera, submitted to the Regional Public Health Laboratory at Leeds for routine toxoplasma serology, were initially examined. On

receipt they were heat inactivated (56°C for 30 minutes) and stored at 4°C for up to two days with any further storage at -20°C. A further 42 sera selected from cases with a strong indication of recent infection, including (i) a fourfold or greater rise in titre in the dye test, (ii) a specific IgM titre $\geq 1/16$, or (iii) a dye test titre $\geq 1/128$ with a haemagglutination test titre $\leq 1/256$,⁹ were examined to determine the value of the ToxHA test in detecting such cases.

METHYLENE-BLUE DYE TEST

The dye test was performed using the method of Beverley and Beattie,³ a modification of the original Sabin and Feldman method.²

TOXHA TEST

The Wellcome ToxHA test is an indirect haemagglutination test utilising turkey erythrocytes sensitised with an ultrasonicate of *T. gondii*. In the presence of specific antibody, agglutination of the red cells is produced. The test is supplied freeze dried, in kit form, together with control cells, positive and negative control sera, and buffered diluent.

Reagents were rehydrated according to the manufacturers' instructions and the test was carried out in V-shaped microtitre plates. Serum samples of 25 μ l were picked up using a Compu-pet 100 diluter/dispenser (William R Warner & Co Ltd, Eastleigh, SO5 3ZQ) and delivered to the appropriate well in column 1 of the plate together with 75 μ l of diluent to give an initial 1/4 dilution. After column 1 had been loaded with sera, further dilutions were prepared across the plate using an 8-channel micro-Compu-pet multidiluter (WR Warner & Co) set to

pick up 25 µl and deliver a total volume of 100 µl at each step, leaving a final volume of 75 µl in each well. Dilutions of 1/64, 1/256, 1/1024, and 1/4096 were prepared as tests for each serum, and 25 µl of the sensitised turkey red cells was added to each using a Micro-re/pettor (Alpha Laboratories, 45 Ruislip Road, Greenford, Middlesex UB6 9QD). A further pickup of 25 µl was made from the 1/16 column and used to deliver a 1/64 serum dilution for use as controls, a 25 µl aliquot of unsensitised cells being added to each. Positive and negative sera as well as antigen controls were included with each batch of sera. After shaking, the plates were left for 1-2 hours at room temperature and the agglutination pattern was determined. The last well showing complete agglutination was taken as the endpoint; whenever non-specific agglutination was observed, the serum was absorbed with unsensitised turkey red cells and re-titrated. Test cell batch K5661/1 was used throughout this study.

OTHER SEROLOGICAL TESTS

The indirect haemagglutination test (IHAT) and the indirect immunofluorescence test for IgM antibodies (IgM-IFAT) were performed using the methods described by Karim and Ludlam.⁹ All anomalous results were checked by retesting.

STANDARDISATION

The *Toxoplasma* National Control serum, produced at the Public Health Laboratory, St George's Hospital, Blackshaw Road, London SW17 0QT, was used as a reference serum. This serum has been standardised against the WHO International Control Serum, issued by the State Serum Institute, DK-2300, Copenhagen S, Denmark.

Results

A total of 1985 sera, submitted for routine toxoplasma serology, were examined in the initial study, and titres in the ToxHA test and dye test were compared. The results are shown in Table 1. Non-

Table 1 Examination of routinely submitted human sera by the ToxHA and dye tests

Dye test	ToxHA test					Totals
	<64	64	256	1024	≥4096	
<16	900	319	130	25	12	1386
16	23	118	76	5	3	225
64	4	60	104	16	1	185
256	0	8	67	32	4	111
1 024	1	4	18	11	6	40
4 096	2	2	4	10	9	27
16 000	0	1	3	2	1	7
≥ 16 000	0	0	1	0	1	2
Totals	930	512	403	101	37	1983

specific reactions with unsensitised red cells were found with 38 sera (1.9%). Two of these sera, with dye test titres of 1/64 and 1/16, still showed non-specific reactions to a titre of 1/64 after absorption and have been excluded from Table 1. Taking a titre of ≥ 1/64 as indicating a positive result in both the dye test and the ToxHA test, the relative distribution of positive and negative sera between the two tests was as shown in Table 2. The ToxHA test detected 98.1% (365/372) of the dye test positive sera but also gave positive results for 42.7% (688/1611) of the sera found negative by the dye test. A total of 1072/1983 (54%) of the sera gave the same titre in both tests.

A total of seven sera were positive in the dye test but negative in the ToxHA test (Table 3). Lymphadenopathy associated with a high dye test titre and suggesting a recent infection occurred with three of these sera. A low dye test titre (1/64) was associated with a fourth case of lymphadenopathy, suggesting a previous rather than current *T. gondii* infection.

Table 2 Distribution of positive and negative sera detected by the dye test and ToxHA test (1983 sera)

Dye test	ToxHA test		Total
	Positive	Negative	
Positive	365	7	372
Negative	688	923	1611

Table 3 Dye test positive sera not detected by the ToxHA test (< 1/64)

Dye test titre	No. sera	Clinical symptoms	Comments
1/64	1	Lymphadenopathy	Significance doubtful Evidence of a previous infection which may have caused the eye damage
1/128	1	Uveitis	
	1	Uveitis	
	1	Choroiditis	
1/1024	1	Lymphadenopathy (7 mth)	Consistent with an active infection
1/4096	1	Lymphadenopathy (5-6 wk)	
1/8192	1	Lymphadenopathy (5-6 wk)	

There were three eye infections in this group of sera. Eye damage is often not apparent until long after the initial infection and is associated with low levels of residual antibody.

Further evidence on the value of the ToxHA test in detecting early infections was sought by examining sera from 42 selected patients meeting the serological criteria listed in Table 4, together with clinical symptoms suggestive of a recent infection. The ToxHA test failed to detect specific antibody in

seven cases (16.7%) giving a titre of $< 1/64$. Of these sera, six were from patients presenting with lymphadenopathy of not more than three months' duration, while the remaining serum was from a patient later found to have a long-standing choroiditis.

Table 4 Examination by the ToxHA test of sera from 42 patients with a recent toxoplasma infection

Criteria for a 'recent infection'	No. of patients	ToxHA $< 1/64$	Clinical symptoms in ToxHA -ve sera patients
Rising dye test titre	5	2	Lymphadenopathy
Dye test $\geq 1/128$, plus IgM-IFAT $\geq 1/16$	21	3	Lymphadenopathy
Dye test $\geq 1/128$, IHAT $\leq 1/256$	16	1	Lymphadenopathy
		1	Choroiditis
Total	42	7 (16.7%)	

Discussion

Haemagglutination tests for *T. gondii* are normally prepared using soluble antigenic components of the parasite, while in the dye test viable intact organisms are used. In the course of an infection, exposure of the host's immune system to intact parasites will precede exposure to cellular contents. Previous workers have shown that the antibody response, measured in these two tests, differs.^{6 10 11} Karim and Ludlam⁹ were able to demonstrate that in glandular toxoplasmosis antibody levels, measured in their haemagglutination system, rose more slowly than those measured by the dye test. In the present study, nine cases diagnosed as glandular toxoplasmosis and all showing evidence of being recent infections, were not detected by the ToxHA test commercially supplied.

The ToxHA test also gave titres of $< 1/64$ in four cases of eye infection in which significant levels of antibody were detected by the dye test. In the IHA test used by Karim and Ludlam⁹ antibody levels were shown to persist at similar, or slightly higher, levels than those detected by the dye test in long-standing infections.

A problem with the ToxHA test demonstrated in this study is the large number of sera showing positive and even high titres in this test but low or negative titres in the reference test, the dye test. The ToxHA test demonstrated in 167 sera (8.4%) a titre of $\geq 1/256$ and in a further 319 sera (16.1%) a titre of $1/64$, all associated with a dye test titre of $< 1/16$. The total number of sera with a dye test $< 1/64$ and a positive ToxHA result was 688 (34.7%).

Complete agglutination was used to determine end

points in this study, rather than a 50% end point as recommended by the kit manufacturers, but if this end point had been used even higher titres would have been expected in the ToxHA test. While this might have helped in detecting some of the earlier cases it would also have resulted in more sera showing positive ToxHA titres and a negative dye test.

The ToxHA titres are all specific in that reaction with unsensitised cells has been excluded but high ToxHA titres and negative dye test titres suggest that at least some of this specificity must be directed against non-toxoplasma constituents of the antigenic material used in the red cell sensitisation. As the parasite is grown in the mouse, the non-toxoplasma antigenic constituents could possibly be of mouse origin. The detection of antibodies against this material can be of only doubtful diagnostic significance.

The use of paired sera to test for a fourfold rise in titre should remove some of the uncertainty in interpretation of ToxHA test results, but further clarification of immunity of *T. gondii* requires the use of a second test, preferably one measuring antibody against cell wall component.⁹ A single production batch of sensitised cells was used throughout this study, and variations in the level of false positives and undetected early infections between batches could not be determined. Standardisation, with good reproducibility between batches, is claimed by the manufacturers. The ToxHA test, an indirect haemagglutination test for the detection of specific antibodies to *T. gondii*, is relatively simple to perform, and end points can be readily determined. The test is designed for use in the microtitre system, lending itself to mechanisation. The rehydrated test cells have a limited storage life which may be a disadvantage for the smaller laboratory where only occasional sera are examined. The indirect haemagglutination test system has many advantages for the smaller laboratory with limited equipment and the larger laboratory because of the possibilities of mechanisation. However, the ToxHA test needs modification to reduce the level of false positive results, but, at the same time, the sensitivity for the detection of early cases needs to be improved.

Identification and isolation of the antigenic components of *T. gondii*, eliciting the earlier immune response as measured in the dye test, should allow the development of an indirect haemagglutination test more closely aligned to the dye test.

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