J Clin Pathol 1984;**37**:1293–1297

Comparison of a latex agglutination test with other serological tests for the measurement of antibodies to *Toxoplasma gondii*

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SUMMARY One hundred sera from 49 patients with glandular toxoplasmosis were examined by a latex agglutination test, the dye test, an indirect haemagglutination test, and a double antibody sandwich enzyme linked immunosorbent assay (ELISA) for antitoxoplasma IgM. The results support previous findings that the dye test, indirect haemagglutination test, and latex agglutination test measure different antibodies to *Toxoplasma gondii*. In early glandular toxoplasmosis, when specific IgM was detected, the titres of both the latex agglutination test and the indirect haemagglutination test were lower than the dye test. Repeat specimens from 11 of the patients showed four cases in which the latex agglutination test titres never exceeded 1/256, whereas both the dye test and the indirect haemagglutination test showed significant titres and specific IgM was detected in every case.

We conclude that the latex agglutination test should not be used as a substitute for the dye test in the serological diagnosis of glandular toxoplasmosis. All sera giving a positive latex agglutination test result should be referred for further tests. A combination of the dye test and double antibody sandwich ELISA gives the most reliable serological diagnosis of early glandular toxoplasmosis.

The Swansea Public Health Laboratory is one of three in England and Wales to which specimens are referred from other laboratories for serological evidence of toxoplasmosis. The tests routinely used are the dye test[1] and a test for toxoplasma specific IgM antibody. Since 1982 a double antibody sandwich enzyme linked immunosorbent assay (ELISA) has been used for the latter.[2]

In recent years some laboratories have been testing sera by a latex agglutination test (Toxotest-MT, Eiken, Tokyo, Japan) before submitting them to the reference laboratories and many discrepancies between latex agglutination test and dye test titres have been noted. This study shows the results obtained with 100 serum samples from 49 cases of glandular toxoplasmosis tested by a latex agglutination test, the dye test, an indirect haemagglutination test, and double antibody sandwich ELISA.

Material and methods

LATEX AGGLUTINATION TEST

The Toxotest MT, an indirect latex agglutination test, was carried out as recommended in the manufacturer's instructions. Four kits were purchased, each enabling 50 samples to be tested; all were from the same batch (lot no 37046) and were used before the stated expiry date.

DYE TEST

The dye tests were performed in flat bottomed microtitre plates and read directly with an inverted microscope. Details of the method are given in PHLS Monograph 13.[3]

INDIRECT HAEMAGGLUTINATION TEST

The reagents were prepared by the method of Thorburn and Williams[4] and the test performed as described in PHLS Monograph 13.[3]

DOUBLE ANTIBODY SANDWICH ELISA FOR IgM

The method of detecting antitoxoplasma IgM by double antibody sandwich ELISA has been described elsewhere.[2] The method has been modified in that the conjugate is prepared from human serum with a high dye test titre. Dye test negative human serum is used for the blocking procedure. The results are expressed as test to negative (T:N) ratios where N is the mean absorbance of six IgM

Accepted for publication 15 August 1984

Table 1 *Results of four serological tests on sera from 49 cases of toxoplasma lymphadenopathy related to time of onset of symptoms*

Patient's serum no	*Dye test*	*Indirect haemagglutination test*	*Latex agglutination test*	*T:N ratio ELISA IgM*	*Time after onset (mo)*
1A	16000	2048	1024	13	1
2A	4096	1024	512	13	1
3A	4096	512	256	10	1
4A	32000	2048	1024	9	1
5A	2048	512	512	9	1
6A	512	256	128	8	1
7A	4096	4096	2048	7	1
8A	128	64	256	7	1
9A	8192	256	128	6	1
10A	16000	1024	1024	5	1
11A	1024	2048	256	5	1
12A	16000	32000	2048	5	1
13A	16000	2048	1024	5	1
14A	16000	2048	1024	5	1
15A	2048	1024	1024	4	1
16A	4096	4096	256	3	1
2B	4096	2048	2048	15	2
17A	512	64	128	13	2
18A	4096	1024	256	11	2
19A	32000	16000	2048	10	2
3B	8192	2048	256	8	2
20A	256	128	1024	8	2
6B	4096	2048	256	7	2
21A	4096	2048	1024	6	2
22A	4096	2048	1024	6	2
23A	4096	1024	1024	5	2
24A	4096	1024	512	5	2
25A	4096	2048	256	5	2
15B	16000	4096	1024	4	2
26A	8192	16000	2048	4	2
27A	2048	1024	128	3	2
28A	2048	1024	64	15	3
1B	16000	4096	1024	12	3
29A	4096	4096	256	7	3
3C	4096	1024	256	7	3
30A	4096	1024	128	7	3
24B	4096	4096	512	6	3
31A	2048	512	128	6	3
32A	4096	2048	128	5	3
30B	2048	1024	128	5	3
33A	16000	1024	512	4	3
34A	8192	2048	512	4	3
35A	4096	16000	512	4	3
27B	2048	512	64	4	3
23B	4096	4096	512	3	3
36A	4096	1024	128	3	3
11B	1024	4096	512	3	3
22B	32000	16000	4096	1	3
16B	1024	2048	128	1	3
37A	4096	4096	256	9	4

negative sera tested at the same dilution as the test sera. A ratio of 2 or more is considered to be a positive result. The toxoplasma national control serum (PHL, St George's Hospital, London) was included as a positive control with each batch of tests. This serum has a dye test titre of 1/2048 (1000 IU/ml), indirect haemagglutination test titre of 1/1024, and an immunofluorescent IgM antibody titre of 1/8. The double antibody sandwich ELISA consistently gave a T:N ratio of between 4 and 6.

SPECIMENS

One hundred sera from 49 patients with glandular toxoplasmosis were selected from specimens submitted to the laboratory for examination. The initial specimen from each patient showed the presence of specific antitoxoplasma IgM. Three or more specimens were sent from 11 of the patients. The interval between the date of collection and the onset of lymphadenopathy was established to within one month in each case.

Results

The results obtained with the four serological tests and the interval between specimen collection and the onset of lymphadenopathy are shown in Table 1. Although toxoplasma antibody could be detected by the dye test, indirect haemagglutination test, and latex agglutination test in all specimens, the titres

Table 1—*continued*

Patient's serum no.	*Dye test*	*Indirect haemagglutination test*	*Latex agglutination test*	*T:N ratio ELISA IgM*	*Time after onset (mo)*
38A	1024	1024	128	7	4
39A	16000	4096	512	5	4
40A	512	4096	512	5	4
41A	32000	4096	1024	4	4
42A	16000	32000	4096	4	4
43A	16000	16000	1024	2	4
18B	4096	2048	128	8	5
44A	4096	8192	1024	6	5
45A	16000	2048	512	5	5
39B	8192	2048	512	5	5
8B	512	256	256	5	5
19B	32000	64000	8192	4	5
24C	2048	2048	128	4	5
46A	16000	2048	512	3	5
17B	4096	4096	512	2	5
9B	4096	2048	256	1	5
2C	4096	16000	1024	9	6
28B	2048	2048	64	8	6
10B	8192	4096	1024	3	6
47A	8192	16000	1024	2	6
45B	8192	8192	512	6	7
19C	16000	64000	8192	4	7
30C	2048	4096	256	4	7
38B	1024	1024	128	1	7
28C	2048	512	64	6	8
42B	4096	64000	2048	4	8
10C	2048	2048	512	3	8
18C	2048	2048	128	3	8
48A	8192	64000	2048	2	8
12B	2048	4096	256	1	8
6C	4096	8192	512	4	9
39C	1024	1024	128	4	9
17C	2048	2048	512	2	9
26B	8192	64000	2048	1	9
25B	1024	8192	512	1	9
6D	4096	4096	1024	4	10
49A	8192	64000	4096	3	10
30D	1024	2048	64	2	10
35B	4096	32000	512	1	10
24D	128	1024	64	1	10
28D	2048	512	64	3	11
43B	2048	16000	1024	1	12
10D	1024	2048	128	1	12
13B	512	1024	64	1	12
17D	4096	8192	512	1	13
14B	512	4096	64	1	13
4B	1024	2048	256	1	14
6E	2048	4096	1024	1	15
49B	2048	64000	2048	1	16
19D	1024	4096	512	1	20

Table 2 *Comparison of latex agglutination test and dye test titres of sera from cases of glandular toxoplasmosis*

Dye test titre	*Latex agglutination test titre*								*Total*
	64	*128*	*256*	*512*	*1024*	*2048*	*4000*	*8000*	
<512	1		1		1				3
512	2	2	1	1					6
1024	1	5	2	3					11
2048	5	5	2	3	3	1			19
4000		4	9	9	5	3			30
8000		1	1	3	3	3	1		12
16000				4	7	1	1	1	14
32000					2	1	1	1	5
Total	9	17	16	23	21	9	3	2	100

varied greatly. In specimens taken early in the disease the dye test titres were usually higher than the indirect haemagglutination test titres, whereas specimens taken late in the disease showed higher indirect haemagglutination test titres than dye test titres. Dye test titres were higher than indirect

Table 3 *Comparison of latex agglutination test and indirect haemagglutination test titres of sera from cases of glandular toxoplasmosis*

Indirect haemagglutination test titre	*Latex agglutination test titre*								*Total*
	64	*128*	*256*	*512*	*1024*	*2048*	*4000*	*8000*	
<512		3	2		1				6
512	3	1	1	1					6
1024	3	7	2	3	3				18
2048	2	6	6	6	6	1			27
4000	1		5	7	6	1			20
8000				4	1	2			7
16000				1	4		1		6
32000				1		5	2	2	10
Total	9	17	16	23	21	9	3	2	100

Table 4 *Results of four serological tests on sequential sera from 11 cases of glandular toxoplasmosis related to time of onset of symptoms*

Patient's serum no	*Dye test*	*Indirect haemagglutination test*	*Latex agglutination test*	*T:N ratio ELISA IgM*	*Time after onset (mo)*
2A	4096	1024	512	13	1
B	4096	2048	2048	15	2
C	4096	16000	1024	9	6
3A	4096	512	256	10	1
B	8192	2048	256	8	2
C	4096	1024	256	7	3
6A	512	256	128	8	1
B	4096	2048	256	7	2
C	4096	8192	512	4	9
D	4096	4096	1024	4	10
E	2048	4096	1024	1	15
10A	16000	1024	1024	5	1
B	8192	4096	1024	3	6
C	2048	2048	512	3	8
D	1024	2048	128	1	12
17A	512	64	128	13	2
B	4096	4096	512	2	5
C	2048	2048	512	2	9
D	4096	8192	512	1	13
18A	4096	1024	256	11	2
B	4096	2048	128	8	5
C	2048	2048	128	3	8
19A	32000	16000	2048	10	2
B	32000	64000	8192	4	5
C	16000	64000	8192	4	7
D	1024	4096	512	1	20
24A	4096	1024	512	5	2
B	4096	4096	512	6	3
C	2048	2048	128	4	5
D	128	1024	64	1	10
28A	2048	1024	64	15	3
B	2048	2048	64	8	6
C	2048	512	64	6	8
D	2048	512	64	3	11
30A	4096	1024	128	7	3
B	2048	1024	128	5	3
C	2048	4096	256	4	7
D	1024	2048	64	2	10
39A	16000	4096	512	5	4
B	8192	2048	512	5	5
C	1024	1024	128	4	9

haemagglutination test titres, with 38 of 49 sera taken during the first 3 months after the onset of lymphadenopathy and with nine of 21 sera taken 4 to 6 months after onset. Only two of 30 sera taken more than 6 months after onset gave dye test titres higher than indirect haemagglutination test titres. This confirms the results of Karim and Ludlam.[5] There was no such correlation between the latex agglutination test and dye test, or latex agglutination test and indirect haemagglutination test; high and low latex agglutination test titres occurred both early and late in the disease. Antitoxoplasma IgM antibody could be detected by double antibody sandwich ELISA in most cases for about nine months after the onset of lymphadenopathy with higher concentrations occurring early in the disease (Table 1).

Table 2 compares latex agglutination test with dye test results and Table 3 compares latex agglutination test with indirect haemagglutination test results. Only 33% of the sera gave latex agglutination test titres within a fourfold dilution of the dye test and 42% within fourfold of the indirect haemagglutination test.

Three or more sera were obtained from 11 of the patients and the results of the tests are shown in Table 4. In four cases the latex agglutination test titre never exceeded 1/256, whereas both the dye test and indirect haemagglutination test showed much higher titres and specific antitoxoplasma IgM was detected in every case. In one case (patient 28) four sera collected over an 8 month period gave constant latex agglutination test titres of 1/64, whereas both the dye test and indirect haemagglutination test titres reached 1/2048 and specific antitoxoplasma IgM was detected in each of the four specimens.

Discussion

The sera examined were from a selected group of patients with glandular toxoplasmosis and this study was not designed to determine whether the latex agglutination test could be used to screen those sera containing toxoplasma antibody from negative sera. Other workers have found closer agreement between quantitative estimations with the latex agglutination test, dye test, and indirect haemagglutination test than those reported here. Balfour *et al*[6] found 78% of titres within a fourfold dilution with 339 sera titrated in latex agglutination test and dye test. Tsubota *et al*[7] compared the results obtained by the latex agglutination test and indirect haemagglutination test and reported a 90·7% agreement, and high agreement was shown by Kobayashi *et al*[8] between the latex agglutination test and indirect haemagglutination test and also between the latex agglutination test and dye test. The sera examined by these workers were not related to clinical findings or the onset of illness and the differences in quantitative estimations reported here may therefore be due to the selection of specimens examined. Of the 100 specimens examined, 70% were collected within 6 months of onset of lymphadenopathy and 82% contained toxoplasma specific IgM antibody. Balfour *et al*[6] did conclude, however, that the pattern of antigenic determinants to which antibody concentrations are measured are different in these three test systems and the results reported here support their findings.

Our conclusions from this study are as follows. Firstly, the latex agglutination test should not be used as a substitute for the dye test in the serological diagnosis of glandular toxoplasmosis because of the variability in the antibody concentrations detected. Secondly, if the latex agglutination test is used as a screening test for glandular toxoplasmosis all sera giving a positive result, whatever the titre, should be sent to a reference laboratory for further studies. Thirdly, early glandular toxoplasmosis may be most reliably diagnosed serologically by a combination of the dye test and a test which detects specific antitoxoplasma IgM antibody. The double antibody sandwich ELISA is a most suitable test for the latter as it is sensitive, specific, and reproducible.

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