Technical method

A rapid method for the detection of antibodies to *Toxoplasma gondii* using a modification of the Toxoreagent latex test

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The recently introduced latex agglutination test kit (Toxoreagent) for the detection of antibodies specific against *Toxoplasma gondii* is convenient and simple to perform but has the drawback of a requirement for overnight settling.¹ The recent occurrence of acute toxoplasmosis in seronegative cardiac transplant recipients at Papworth (to be published) has emphasised the need for a more rapid test. By introducing a centrifugation step, the latex settling pattern can be read within an hour of the test being set up, allowing a rapid assessment of the antibody status of a potential donor. Elimination of the need for overnight settling generally enhances the diagnostic use and value of the test.

Material and methods

Toxoreagent, formerly known as Toxotest-M(TMT), an indirect agglutination test kit produced by the Eiken Chemical Company of Japan, was supplied by Diamed Diagnostics Ltd, Mast House, Derby Road, Bootle, Merseyside, L20 1EA.

STANDARD TOXOREAGENT TEST (STT)

Standard tests were carried out as described by Balfour *et al.*² in U-shaped well microtitre plates, with overnight incubation at room temperature. The end-point was taken as the highest serum dilution showing significant agglutination. In the absence of specific antibody a compact button was obtained.

RAPID TOXOREAGENT TEST (RTT)

The rapid test was carried out in V-shaped well microtitre plates. An initial 1/8 dilution was prepared by

dispensing 10 μ l of serum with 70 μ l of the diluent supplied. Doubling dilutions were made by picking up 25 μ l of diluted serum and delivering this into a further 25 μ l of diluent. A 25 μ l aliquot of sensitised latex was then added to each test well. The positive control serum supplied and an antigen control (25 μ l of latex plus 25 μ l diluent) were included in each test batch. The plates were then shaken for 10 s on a microshaker and left to settle at room temperature for 30 min. The plates were then centrifuged for 4 min at 260 g (1000 rpm) in a Mistral 4L centrifuge (MSE Ltd) fitted with microtitre plate carriers. The plates were then left to stand at room temperature for 10 min before reading against a dark background, using a Titertek plate reader (Flow Laboratories Ltd). In the absence of specific antibody the latex formed an opaque central button surrounded by an area of milky opalescence. In the presence of specific antibody a uniform pattern of agglutination was seen, although the margins of the latex sheet had a tendency to roll towards the centre of the well. End-points were taken as the last dilution to show unequivocal agglutination. In case of doubt due to button formation it can be useful to remove a drop with a pipette, place it on a microscope slide under a cover slip and examine with a $\times 40$ objective. Clumped latex is readily distinguishable from unagglutinated particles.

DYE TEST (DT)

The dye test was performed in flat-bottomed microtitre plates as described by Hunter *et al.*³

INDIRECT HAEMAGGLUTINATION TEST (IHAT)

The indirect haemagglutination was performed in microtitre plates with V-shaped wells as described by Hunter *et al.*⁴

Sera routinely submitted to the PHL (Leeds) for toxoplasma serology were examined using the dye test and the indirect haemagglutination test. Subsequently the sera were sent by post to Cambridge for testing blind in the standard Toxoreagent test (STT) with overnight settling, and the modified rapid Toxoreagent test (RTT) with a shortened settling period.

Results

A total of 100 sera were examined, 75 seropositive and 25 seronegative by DT and IHAT. The qualitative agreement between the rapid and standard

Comparison of the standard Toxoreagent test (overnight agglutination) (STT) and the rapid Toxoreagent test (RTT)

Standard Toxoreagent test	Rapid Toxoreagent test											Total
	<16	16	32	64	128	256	512	1024	2048	4096	>8192	
< 16	27	1	—	1	—	—	—	—	—	—	—	29
16	2	—	—	1	1	—	—	—	—	—	—	4
32	1	—	2	—	7	2	—	—	—	—	—	12
64	—	1	—	1	2	1	1	—	—	—	—	6
128	—	—	1	2	3	3	2	—	—	—	—	11
256	—	—	—	—	2	4	2	3	1	—	—	12
512	—	—	—	—	—	—	1	4	3	1	—	9
1024	—	—	—	—	—	1	3	—	4	—	—	9
2048	—	—	—	—	—	—	—	—	—	2	2	4
4096	—	—	—	—	—	—	—	—	—	—	2	2
>8192	—	—	—	—	—	—	—	—	—	—	2	2
Totals	30	2	3	5	15	11	9	7	8	3	7	100

Toxoreagent tests was good with 98/100 sera giving the same result. The remaining two sera were both positive in the RTT (titres 1/16, 1/64) but negative in the STT overnight test.

The quantitative results also showed good agreement (Table) with 70 sera giving titres of within ± 1 serial dilution in both forms of the test. Only eight sera gave titres outside the range of two serial dilutions. In all cases the higher titre occurred in the RTT indicating that the modified test has an increased sensitivity.

Qualitative agreement between the RTT and the dye test occurred in 92/95 (97%) and between the RTT and IHAT agreement was 94/100.

The RTT and STT were negative with three sera that had a DT of 1/8 and one serum with an IHAT titre of 1/32. The RTT was negative with a single serum that had an IHAT of 1/64 and an STT titre of 1/32. It was positive with four sera that had IHAT titres of $< 1/32$ and the STT was also positive in three of them. The general agreement was therefore very good.

Discussion

Serodiagnosis of *Toxoplasma gondii* infections relies heavily on the use of the Sabin and Feldman dye test which has undergone modification,³ but still requires the use of live parasites, maintained in mice. This has resulted in the use of the test being restricted to specialised centres and in consequence a delay before results are known. The recently introduced latex agglutination test, Toxoreagent, is a simple rapid test showing fairly good agreement with the

dye test² and which can be performed locally, on demand. However, the overnight settling period is unacceptable in certain clinical situations, such as when the antibody status of a cardiac donor needs to be known. We have introduced a short centrifugation step into the test which reduces the settling time and allows the level of antibodies to *Toxoplasma gondii* in a given serum to be determined in under one hour. This modification should allow more laboratories to consider offering the test and using the reference centres when confirmation or further examination of the specimen is required.

References

- ¹ Tsubota N, Hiroaka K, Sawada Y, Watanabe T, Oshima S. Studies on latex agglutination test for toxoplasma. (2) Evaluation of the microtitre test as a serological test for toxoplasmosis in man. *Jpn J Parasitol* 1977;**26**:286–90.
- ² Balfour AH, Fleck DG, Hughes HPA, Sharp D. Comparative study of three tests (dye test, indirect-haemagglutination test, latex agglutination test) for the detection of antibodies to *Toxoplasma gondii* in human sera. *J Clin Pathol* 1982;**35**:228–32.
- ³ Hunter P, Chadwick P, Balfour AH, Bridges JB. Examination of ovine foetal fluid for antibodies to *Toxoplasma gondii* by the dye test and an indirect immunofluorescence test specific for IgM. *Br Vet J* 1982;**138**:29–34.
- ⁴ Hunter P, Chadwick P, Balfour AH, Bridges JB. An assessment of a commercially available haemagglutination test for detecting toxoplasma antibodies in ovine sera. *Br Vet J* 1980;**136**:339–42.

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