Evaluation of the TGS TA system for the detection of anti-Toxoplasma antibodies

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Summary

Background and aims. The aim of the present study was to evaluate the new chemiluminescence TGS TA system of Technogenetics (Milan, Italy) for detecting anti-Toxoplasma IgG and IgM antibodies and IgG avidity. The TGS TA system was compared with our chemiluminescence routinely used system, LIAISON XL, supplied by Diasorin (Saluggia, Italy), for the detection of IgG and IgM antibodies. Only in positive IgM samples (retrospective study) and for the IgG avidity (if existent), TGS TA system was compared to an Enzyme Linked Fluorescent Assay (ELFA) test (VIDAS, BioMérieux, Marcy-l'Étoile, France).

Materials and methods. Three hundred and one sera samples, from women who came to our centre for the routine follow up pregnancy, were examined with the TGS TA system and divided in 3 groups according to IgG and IgM screening LIAISON XL tests: 106 were non-immune women (Group 1), 100 were pregnant with past infection (Group 2) and 95 were pregnant with positive or equivocal IgM (82 with positive IgG and 13 with negative IgG) (Group 3).

Results. The overall concordance of the IgG results between LIAISON XL and TGS TA was 99.3%: 100% in Group 1, 98% in Group 2 and 100% in Group 3. The overall concordance of the IgM results between LIAISON XL and TGS TA was 93.9%: 100% in Group 1, 94% in Group 2 and 82.8% in Group 3. In Group 3, the concordance between the results of the IgG avidity with the ELFA and TGS TA tests was 81.7%. Comparing the clinical diagnosis obtained with our protocol and that of the TGS TA system, the overall concordance was 92.7%: 100% in Group 1, 92.0% in Group 2 and 78.9% in Group 3.

Conclusions. The overall concordance of IgG antibodies is excellent for both protocols while for IgM antibodies is very high in the first group and lower in the third group, due to the presence of non-specific IgM subjects in this group. The TGS TA avidity test seems to predict ealier the maturation of the IgG compared to the ELFA test since many samples with low avidity with the ELFA were seen with moderate avidity with TGS TA and all those with borderline avidity with the ELFA were seen with high avidity with TGS TA. This system shows to be a valuable tool with overall good clinical correlation and able to clearly identify non-specific subjects, those with a non-recent infection.

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Key words: Toxoplasma; Pregnancy; IgM; IgG; Avidity.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 18 January 2017.
Revision received: 13 March 2017.
Accepted for publication: 13 March 2017.

Microbiologia Medica 2017; 32:6583
doi:10.4081/mm.2017.6583

Introduction

Toxoplasma gondii is an obligate intracellular parasite, the cat is the definitive host and many warm-blooded animals, including humans, are intermediate hosts. The infection by *Toxoplasma gondii* is generally benign but can be particularly severe during pregnancy, leading to malformations of the foetus or a series of complications in the newborn (3, 6, 7, 11). This is the reason why the antibody screening campaign in pregnancy was implemented (1, 2, 5, 8, 10) to indicate possible acute infections with the risk of transmission to the foetus (8). Currently, there are several analytical systems for the detection of antibodies, with different level of automation, such as Enzyme-Linked Immunosorbent Assay (ELISA), Chemiluminescence Immunoassay (CLIA) and Enzyme Linked Fluorescent Assay (ELFA) able, depending on the instrument used, to process a large number of samples in a short time. The com-

mercial tests available generally have excellent sensitivity and specificity levels, showing slight differences in the results, depending on different formulations and on the use of natural, recombinant or synthetic antigens. The extent of these differences must be known. We compared the data obtained with the Technogenetics TGS TA system in the detection of anti-toxoplasma IgG and IgM antibodies and IgG avidity with the results we obtained with our current standard protocol, which consists of the LIAISON XL system from DiaSorin for IgG and IgM antibodies detection, in order to assess the differences that may arise using different systems. In our protocol, the samples are confirmed using an ELFA test and the IgG avidity is determined (if existent) with an ELFA test in the event of positivity for IgM.

Materials and methods

Three hundred and one sera samples from women who came to our centre for the routine follow-up in pregnancy were examined and divided into the groups below:
Group 1: 106 samples from non-immune women
Group 2: 100 samples from women with past infection
Group 3: 95 samples from women with positive or equivocal IgM (82 with positive IgG and 13 with negative IgG).

Routinely, samples are tested using chemiluminescence LIAISON XL system for IgG and IgM (LIAISON Toxoplasma IgG, IgM, DiaSorin, Saluggia, Italy). In positive subjects for IgM, samples are tested using an ELFA test (VIDAS Toxo IgM, BioMérieux, Marcy l'Etoile, France). The avidity of the IgG (if present) is determined with an ELFA test (VIDAS TOXO IgG AVIDITY, BioMérieux, France).

All samples are tested with TGS TA system for detecting IgG and IgM and, for Group 3, IgG avidity (TGS TA Toxoplasma IgG, Toxoplasma IgM, Toxoplasma IgG Avidity, Technogenetics, Milan, Italy).

The reference values for the different tests are shown in Tables 1 and 2.

Low avidity is suggestive for a possible infection in the past 4 months preceding the sampling for both systems. High avidity indicates an infection occurred more than 4 months before while for borderline (ELFA)/moderate (TGS TA) avidity, the interpretation is equivocal as a recent infection is not excluded but past infection with partial maturation of the IgG avidity may also be indicated.

This work does not require an ethics statement. We used residual samples after required routine tests have been completed providing that there was no link to the patient's identity.

Toxoplasma IgG and IgM values are compared using the concordance index in the three groups.

Linear regression correlates LIAISON XL and TGS TA Toxoplasma IgG values.

Results

The overall concordance in the different groups arising from a comparison of the results obtained with the TGS TA and LIAISON XL systems for the detection of IgG and IgM is shown in Table 3.

The linear regression obtained from the comparison of all the IgG values of the three groups with the LIAISON XL and TGS TA systems is shown in Figure 1.

The discordant samples for IgM (30) in Group 3 were examined with the ELFA IgM test; 19 were negative, 5 positive and 6 equivocal (Table 4).

Further, in the 82 samples with IgG in Group 3, the avidity was determined with both ELFA and TGS TA (Table 5).

As far as the clinical assessment is concerned, the TGS TA correctly considered all the subjects as non-immune in Group 1 (non-immune women). 100% of clinical concordance.

Table 1. Reference values for the anti-Toxoplasma IgG and IgM tests with the LIAISON XL, ELFA and TGS TA systems.

Reference values	IgG		IgM		
	LIAISON XL, IU/mL	TGS TA, IU/mL	LIAISON XL, IU/mL	ELFA (index)	TGS TA, IU/mL
Negative	<7.2	<1.5	<6	<0.70	<15
Equivocal	7.2-8.8	-	6-8	0.70-0.90	-
Positive	>8.8	≥1.5	>8	>0.90	≥15

The LIAISON XL test for IgG is standardised on the Second WHO International Standard of 1980 while the TGS TA test is calibrated against the First WHO International Standard of 2003.

Table 2. Reference values for the anti-Toxoplasma IgG avidity tests with the LIAISON XL and TGS TA systems.

Reference value	IgG avidity	
	ELFA (index)	TGS TA (index)
Low	<0.200	<0.100
Borderline/moderate	0.200-0.300	0.100-0.150
High	>0.300	≥0.150

In Group 2 (pregnant women with past infection), the TGS TA system showed two cases, which had weakly positive IgG values with LIAISON XL (9.3 and 13.2 IU/mL) as non-immune.

However, in the same group, the TGS TA test showed 6 subjects with positive IgM (negative to the LIAISON XL) and 5 of them were confirmed with the ELFA IgM test. These patients had high avidity with both tests except for one which was insufficient to carry out the TGS TA avidity test. 92% of clinical concordance.

The samples with our protocol were typified as follows in Group 3 (IgM samples positive or equivocal to the LIAISON XL IgM): 17 patients with recent infection (ELFA IgM positive and low ELFA avidity) of whom 4 with negative IgG; 4 with a non-determinable infection (ELFA IgM positive and intermediate avidity to ELFA); 42 patients with past infection (ELFA IgM positive and high ELFA avidity); 23 patients with IgM considered non-specific (IgM not confirmed with the ELFA test and with high ELFA avidity); 9 non-immune patients (IgM supposed non-specific: ELFA IgM negative without IgG).

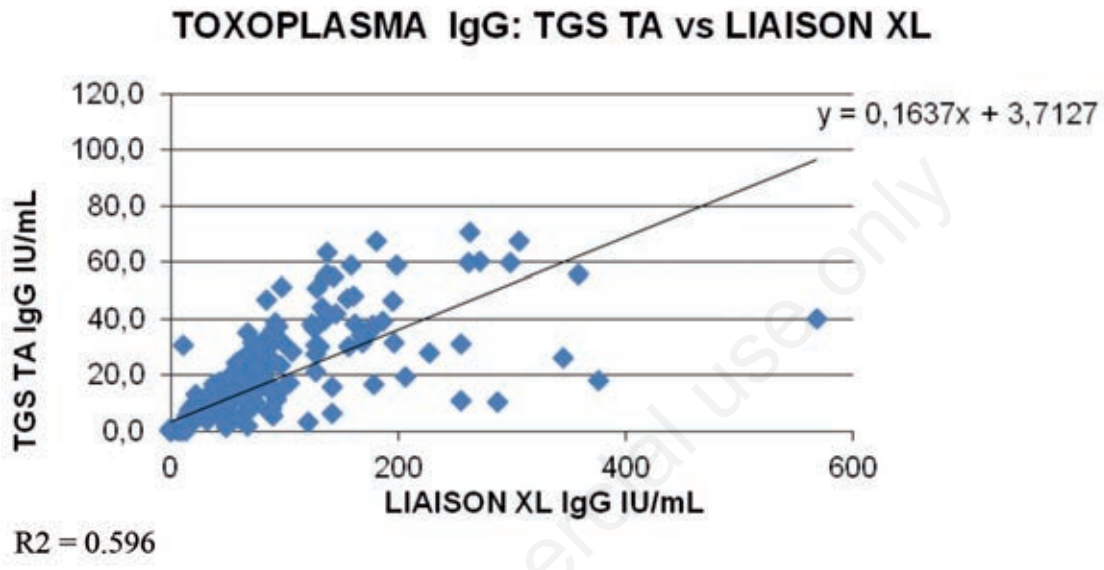

Figure 1. Linear regression between TGS TA and LIAISON XL of the anti-Toxoplasma IgG (IU/mL).

Table 3. Overall concordance of LIAISON XL and TGS TA for the detection of anti-Toxoplasma IgG and IgM in samples from pregnant women.

	IgG		IgM	
	TGS TA neg	TGS TA pos	TGS TA neg	TGS TA pos
Overall concordance (301 samples from pregnant women)*				
LIAISON XL neg	119	0	200	6
LIAISON XL pos	2	180	10	48
LIAISON XL eq	-	-	20	17
Group 1 concordance (106 samples from pregnant non-immune women)**				
LIAISON XL neg	106	0	106	0
LIAISON XL pos	0	0	0	0
Group 2 concordance (100 samples from pregnant women with past infection)***				
LIAISON XL neg	0	0	94	6
LIAISON XL pos	2	98	0	0
Group 3 concordance (95 samples from pregnant women with positive or equivocal IgM to the LIAISON)****				
LIAISON XL neg	13	0	0	0
LIAISON XL pos	0	82	10	48
LIAISON XL eq	-	-	20	17

*Overall concordance (excluding equivocal cases): IgG 99.3%; IgM 93.9%. **Overall concordance: IgG 100%; IgM 100%. ***Overall concordance: IgG 98%; IgM 94%. ****Overall concordance (excluding equivocal cases): IgG 100%; IgM 82.8%.

The typing obtained with the TGS TA system (IgG, IgM and IgG avidity) in these sub-groups is shown in Table 6.

Considering all three groups, the clinical concordance is shown in Table 7.

Discussion and Conclusions

In conclusion, the concordance between LIAISON XL and TGS TA system for IgG is excellent in all groups (99.3%). The results of the IgG with TGS TA correlate well as a qualitative result with the LIAISON XL test but, considering the quantitative values in IU/mL, the linear correlation is low with an R2 of 0.596. This evidence may be due to the fact that the two tests are calibrated towards different international standards.

The concordance of the tests for IgM is excellent in the first group. In the third group, the discordance is greater, also because in this group there were subjects with non-specific IgM.

The discrepancies may also be influenced by different antigen preparation in the two systems, but the majority of the samples scored as non-specific with our current protocol,

Table 4. Results of the ELFA test on 30 samples equivocal or positive to an IgM LIAISON XL test and negative with the TGS TA IgM.

IgM positive or equivocal to the LIAISON XL system	LIAISON XL	ELFA	TGS TA
Negative	0	19	30
Positive	10	5	0
Equivocal	20	6	0

Table 5. The concordance between the ELFA and TGS TA tests for IgG avidity in 82 subjects positive or equivocal (with positive IgG) to the LIAISON XL IgM.

	TGS TA avidity			
	Low	Moderate	High	Total
ELFA avidity				
Low	2	10	1	13
Borderline	0	0	4	4
High	0	0	65	65
Total	2	10	70	82

Concordance 81.7%.

Table 6. Comparison of the clinical interpretations given with the LIAISON/ELFA protocol and TGS TA system.

LIAISON/ELFA protocol	TGS TA system				
	Probable recent infection	Indeterminate infection	Probable non-recent infection	Non immune	Total
Probable recent infection	5	10	1	1	17
Indeterminate infection	0	0	4	0	4
Probable non-recent infection	0	0	65	0	65
Non immune	4	0	0	5	9
Total	9	10	70	6	95

Concordance 78.9%.

Table 7. Clinical concordance between the LIAISON/ELFA protocol and the TGS TA system considering all three groups.

LIAISON/ELFA protocol	TGS TA system				
	Probable recent infection	Indeterminate infection	Probable non-recent infection	Non immune	Total
Probable recent infection	5	10	1	1	17
Indeterminate infection	0	0	4	0	4
Probable non-recent infection*	0	0	162	2	164
Non immune	4	0	0	111	115
TOTAL	9	10	167	114	300

Total concordance 92.7%. *One sample insufficient quantity for TGS TA avidity.

were indicated as negative in the TGS TA system, suggesting good specificity. Furthermore, the TGS TA system does not have a grey area around the cut-off unlike the LIAISON XL and ELFA tests, therefore the calculation of the concordance suffers from this limitation. Another limitation of the study is the lack of cases of recent Toxoplasma infection but this is due to low incidence of acute infection in pregnant women in our area (4) that makes observation of recent infection rare.

Most of the samples with low avidity with ELFA test were seen as moderate avidity to the TGS TA test while all samples borderline to the ELFA test were seen with high avidity to the TGS TA test, suggesting that TGS TA system may predict the maturation of IgG in advance with respect to the ELFA test. The samples analysed came from asymptomatic pregnant women so the clinic was unable to provide additional indications on the status of the infection. Further investigations would be necessary for both avidity tests with an indeterminate result.

Finally, the overall clinical concordance is good; the TGS TA system clearly indicates subjects with probable non-specific IgM or non-recent infection but, while it indicates additional possible recent infections (four) on one hand, on the other, it indicates some less (two) than our protocol.

In conclusion, TGS TA is a useful system for the evaluation of Toxoplasma infection.

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