Contribution of the "Recomline *Toxoplasma* IgM" Kit in the Distinction between Toxoplasmic IgM and Natural IgM

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ABSTRACT

Diagnosis of toxoplasmosis is based essentially on serology by systematically searching for anti-toxoplasmic IgG and IgM. One of the difficulties encountered in the interpretation of *Toxoplasma* serology in pregnant women is the distinction between anti-*Toxoplasma* IgM and non-specific IgM called "natural". A total of 58 sera from pregnant women have been tested. All these sera were positive in ELISA-IgM and negative in ELISA-IgG and IFI-IgG. These sera are divided into 2 groups: Group 1: 30 sera with natural IgM confirmed after 3 weeks checkups for a period up to 3 months. Group 2: 28 sera with specific anti-toxoplasmic IgM. The 28 sera were tested by the immunoblot kit using the recomLine *Toxoplasma* IgM assay. This test is based on 8 recombinant toxoplasmic antigens which are: ROP1c, MIC3, GRA7, GRA8, p30, MAG1, GRA1, and rSAG1. Among the 28 confirmed IgM-specific sera, 11 sera were positive in recomLine *Toxoplasma* IgM, that is, a sensitivity of 39.3% [22.1-59.3]. Among the 30 confirmed natural IgM sera, 28 sera were negative in recomLine *Toxoplasma* IgM, that is, a specificity of 93.33% [76.5-98.8]. Positive predictive value and negative predictive value were 84.6% [53.7-97.3] and 62.2% [46.5-75.8], respectively. In conclusion, the recomLine *Toxoplasma* IgM test can be recommended in situations where IgM is positive and IgG is negative. The positivity of this test makes the diagnosis of toxoplasma seroconversion very likely. However, its negativity does not rule out the diagnosis.

Key Words: *Toxoplasmosis, Diagnosis, IgM natural, RecomLine Toxoplasma IgM, Specific IgM, Pregnant women*

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INTRODUCTION

Toxoplasmosis is a widespread parasitosis caused by an obligate intracellular protozoan, *Toxoplasma* (*T.*) *gondii*. It is benign in immune-competent individuals. However, it becomes serious in immune-compromised individuals and pregnant women due to the risk of congenital toxoplasmosis through infection of the fetus [1]. Diagnosis of toxoplasmosis is based essentially on serology by systematically searching for anti-toxoplasmic IgG and IgM [1, 2]. One of the difficulties encountered in the interpretation of *Toxoplasma* serology in pregnant women is the distinction between anti-*Toxoplasma* IgM and non-specific IgM called "natural" [3-5].

In the case of *Toxoplasma* seroconversion, IgM is the first to appear. However, the detection of IgM alone in the serum is not a reliable criterion for the diagnosis of recent toxoplasmosis. The presence of IgM without IgG in the first serum may correspond to toxoplasmic seroconversion in the early stages before the appearance of IgG, or it may be non-specific natural IgM detecting ubiquitous antigens [6-8].

Until now, for a serum where IgG is negative and IgM is positive, the only recommendation is to follow the serology until the appearance of IgG. This is the only argument that makes it possible to consider the specificity of IgM and to confirm toxoplasmic seroconversion. This follow-up should cover an interval of 2 to 3 months, which

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will delay the diagnosis and consequently the therapeutic management [9].

Several serodiagnostic kits are available on the market. These kits use different antigenic preparations extracted from tachyzoites. The diagnostic performances of these tests are overall satisfactory. However, they sometimes raise the problem of non-standardization, which requires the use of other more sensitive kits. Recently, recombinant antigens are increasingly used in serodiagnostic kits [10].

The objective of this work was to evaluate the diagnostic performance of an immunoblot kit "recomLine *Toxoplasma* IgM" (Mikrogen, Diagnostik) in the distinction between specific anti-*Toxoplasma* IgM and natural IgM.

MATERIALS AND METHODS

Material

A total of 58 sera from pregnant women referred to the Parasitology-Mycology laboratory for *Toxoplasma* serodiagnosis have been tested. All these sera were positive in ELISA-IgM (Platelia Toxo IgM®, BioRad) and negative in ELISA-IgG (Platelia Toxo IgG®, BioRad) and in IFI-IgG (Toxo Spot IF®, BioMérieux) according to the suppliers' instructions.

These sera are divided into 2 groups:

Group 1: 30 sera with natural IgM confirmed after 3 weeks checkups for a period up to 3 months.

Group 2: 28 sera with specific anti-toxoplasmic IgM.

Methods

The 28 sera were tested by the immunoblot kit using the recomLine *Toxoplasma* IgM assay. This is qualitative in vitro test for the detection of IgM versus *T. gondii* in human serum.

This test is based on 8 recombinant toxoplasmic antigens which are: ROP1c, MIC3, GRA7, GRA8, p30, MAG1, GRA1, and rSAG1. These antigens are fixed on nitrocellulose strips **(Figure 1)**. The test is performed according to the manufacturer's recommendations. Interpretation of the profiles is performed according to the manufacturer's recommendations.

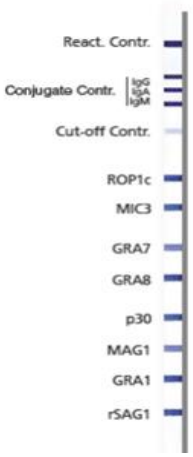

Figure 1. Nitrocellulose strip fixed with 8 recombinant *T. gondii* antigens with a reaction control and a conjugate control (IgG, IgA, and IgM).

For the evaluation of the test, scores were assigned to each antigen. The result is obtained by adding the corresponding scores of each strip. The positive, negative and doubtful evaluation is indicated by the supplier **(Tables 1 and 2)**.

Table 1. Evaluation of *T. gondii* antigen scores in the '*Toxoplasma* recomLine'.

Antigen	IgG Points	IgM Points	IgA Points
ROP1c	1	6	4
MIC3	0	2	4
GRA7	4	4	4
GRA8	4	4	4
P30	6	0	4
MAG1	2	1	2
GRA1	4	0	4
rSAG1	4	0	4

Table 2. Evaluation of test results in the *Toxoplasma* recomLine.

Score	Interpretation
≤3	Negative
4-5	Doubtful
≥6	Positive

RESULTS AND DISCUSSION

The results of the 58 sera tested in our work by the recomLine Toxoplasma IgM kit are shown in **Tables 3 and 4**.

Table 3. Results of sera with specific IgM obtained with recomLine *Toxoplasma* IgM.

N°	ROP1c	MIC3	GRA7	GRA8	P30	MAG1	GRA1	rSAG1	SCORE	Interpretation		
	6	2	4	4	0	1	0	0		Negative ≤3	Doubtful 4-5	Positive ≥6
1	6	0	4	4	0	0	0	0	14		Positive	
2	6	0	0	0	0	0	0	0	6		Positive	
3		0	4	4	0	0	0	0	8		Positive	

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4	0	0	0	0	0	0	0	0	0	Negative
5	0	0	0	0	0	0	0	0	0	Negative
6	6	0	0	0	0	0	0	0	0	Positive
7	0	0	0	0	0	0	0	0	0	Negative
8	6	0	4	0	0	0	0	0	10	Positive
9	6	0	0	4	0	0	0	0	10	Positive
10	0	0	4	0	0	0	0	0	4	Negative
11	0	0	0	0	0	0	0	0	0	Negative
12	0	0	0	0	0	0	0	0	0	Negative
13	0	0	0	0	0	0	0	0	0	Negative
14	0	0	0	0	0	0	0	0	0	Negative
15	0	0	0	4	0	0	0	0	4	Negative
16	6	2	0	4	0	0	0	0	12	Positive
17	6	0	0	4	0	0	0	0	10	Positive
18	0	0	0	0	0	0	0	0	0	Negative
19	0	0	0	0	0	0	0	0	0	Negative
20	0	0	0	0	0	0	0	0	0	Negative
21	6	0	0	0	0	0	0	0	6	Positive
22	0	0	0	0	0	0	0	0	0	Negative
23	0	0	0	0	0	0	0	0	0	Negative
24	0	0	0	0	0	0	0	0	0	Negative
25	6	0	0	0	0	0	0	0	6	Positive
26	6	0	0	0	0	0	0	0	6	Positive
27	0	0	4	0	0	0	0	0	4	Negative
28	0	0	0	0	0	0	0	0	0	Negative

Table 4. Results of sera with natural IgM obtained with recomLine *Toxoplasma* IgM.

Serum N°	**ROP1c**	**MIC3**	**GRA7**	**GRA8**	**P30**	**MAG1**	**GRA1**	**rSAG1**	**SCORE**	**Interpretation**		
	6	**2**	**4**	**4**	**0**	**1**	**0**	**0**		**Negative ≤3**	**Doubtful 4-5**	**Positive ≥6**
1	0	0	0	0	0	0	0	0	0		Negative	
2	0	0	0	0	0	0	0	0	0		Negative	
3	0	0	0	0	0	0	0	0	0		Negative	
4	0	0	0	0	0	0	0	0	0		Negative	
5	0	0	0	0	0	0	0	0	0		Negative	
6	0	0	0	0	0	0	0	0	0		Negative	
7	0	0	0	0	0	0	0	0	0		Negative	
8	0	0	0	0	0	0	0	0	0		Negative	
9	0	0	0	0	0	0	0	0	0		Negative	
10	0	0	0	0	0	0	0	0	0		Negative	
11	0	0	0	0	0	0	0	0	0		Negative	
12	0	0	0	0	0	0	0	0	0		Negative	
13	0	0	0	0	0	0	0	0	0		Negative	
14	0	0	0	0	0	0	0	0	0		Negative	
15	0	0	0	0	0	0	0	0	0		Negative	
16	6	0	0	4	0	0	0	0	10		Positive	
17	0	2	0	0	0	0	0	0	2		Negative	
18	0	0	0	0	0	0	0	0	0		Negative	
19	0	0	0	0	0	0	0	0	0		Negative	
20	0	2	0	0	0	0	0	0	2		Negative	
21	0	0	0	0	0	0	0	0	0		Negative	
22	0	0	0	0	0	0	0	0	0		Negative	
23	0	0	0	0	0	0	0	0	0		Negative	
24	0	0	0	0	0	0	0	0	0		Negative	
25	0	0	0	0	0	0	0	0	0		Negative	
26	0	0	4	4	0	0	0	0	14		Positive	

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27	0	0	0	0	0	0	0	0	0	Negative
28	0	0	0	0	0	0	0	0	0	Negative
29	0	2	0	0	0	0	0	0	0	Negative
30	0	2	0	0	0	0	0	0	0	Negative

Among the 28 confirmed IgM-specific sera, 11 sera were positive in recomLine *Toxoplasma* IgM, that is, a sensitivity of 39.3% [22.1-59.3]. Among the 30 confirmed natural IgM sera, 28 sera were negative in recomLine *Toxoplasma* IgM, that is, a specificity of 93.33% [76.5-98.8]. Positive predictive value and negative predictive value were 84.6% [53.7-97.3] and 62.2% [46.5-75.8], respectively. **Figure 2** shows some examples of recomLine *Toxoplasma* IgM positive and negative profiles.

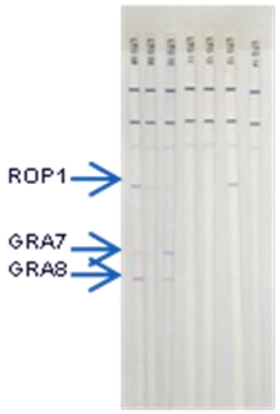

Figure 2. Examples of obtained profiles.
Strips 8-10 and 13: positive. Strips 11-12 and 14: negative.

Although quantitative serological techniques are globally satisfactory in terms of sensitivity and specificity, they often pose the problem of discordance between results [11].

IgG testing allows the definition of the population at risk (non-immune patients). This research is conditioned by the quality of the IgG detection test [12].

Numerous comparative studies have been performed between the different commercialized serodiagnostic kits and have shown discrepancies between them. These discrepancies are mainly due to the lack of standardization and the differences between the used antigens [13].

Staging of infection is important for pregnant women since per gravid infection may result in congenital toxoplasmosis [14].

For these reasons, a seroconversion can only be considered when specific IgG appears, which occurs within a variable period depending on the used technique and the implementation of treatment [15].

Generally, a seroconversion can be excluded only after 3 serology tests showing the presence of IgM and the absence of IgG. This means that there is a considerable delay before concluding the non-specificity of IgM.

Indeed, if the result of the 2nd serum is identical to the 1st, the hypothesis of non-specific IgM tends to be confirmed. Therefore, performing 3 serology tests spaced one to two weeks apart is necessary to retain the hypothesis of natural IgM. And the woman will be considered seronegative vis-à-vis toxoplasma.

One approach to improving serological diagnosis is to replace native antigens with recombinant antigens [10].

The advantages of recombinant antigens are the precise and known antigenic composition of the kit, the use of more than one antigen, the standardization, and the ease of the technique. These antigens are selected according to the acute or chronic phase and can be used for dating the infection [10]. Contrary to native antigens whose composition is generally not provided by the supplier, some antigens are characterized by a strong reactivity during the acute phase, others during the chronic phase, subsequently; the use of a single recombinant protein can identify the phase of the infection from a single serum which allows the dating of the infection and the study of avidity.

In recent years, several dozen genes encoding T. gondii proteins have been cloned. These antigens are mainly the surface antigens for example SAG1 (p30), SAG2 (P22), SAG3 (P43), and P35, the dense granule antigens for example GRA1 (P24), GRA2 (P28), GRA4, GRA5 (P32), GRA7 (P29) and the rhoptries antigens, for example, ROP1 (P66), ROP2 (P54), B10 (P41), MAG1, MIC1. These antigens have been used in their recombinant form to detect T. gondii specific antibodies in serum [10, 16].

Recently commercialized, the "recomLine Toxoplasma IgM" immunoblot test is based on 8 recombinant antigens which are: ROP1c, MIC3, GRA7, GRA8, p30, MAG1, GRA1, and rSAG1. These antigens have been described in the literature as good markers of toxoplasma infection. In our work, we applied this immunoblot assay for the discrimination between natural and specific IgM.

ROP1: this protein has a score of 6 in the "recomLine Toxoplasma IgM". It alone may be sufficient to confirm the positivity of the test and therefore the specificity of IgM. The ROP1 protein is used as a good marker of recent infection. It has been detected in pregnant women with recent infections. However, this protein was weakly present in pregnant women with old infections [17, 18].

MIC3: this protein has a score of 2. Beghetto *et al*. have shown that MIC3 protein can be used as a good marker of recent infection [10, 19].

GRA7: is a good marker of recent infection according to Pfrepper *et al*. It has a score of 4 on the "recomLine

Toxoplasma IgM". Another study showed that GRA7 protein can be expressed during the different stages of toxoplasma infection including tachyzoites and bradyzoites since dense granule proteins can be partially present at the membrane and cytoplasmic level and can be used to detect antibodies during the acute and chronic phase of the infection [20-22].

GRA8: this protein, which has the same score as GRA7, showed 100% avidity during the acute phase of infection according to the study by Sickinger *et al*. [10, 23].

P30: It is the major surface protein of *T. gondii* and is expressed only in tachyzoites. P30 is the most immunogenic protein and plays an important role in host cell attachment, invasion, and modulation of the immune response. It represents 3 to 5% of total proteins. This protein is widely used in its purified, synthetic, or recombinant form in serological tests. Indeed, P30 is the first protein recognized by anti-toxoplasmic IgM and therefore constitutes a good marker of recent infection [24, 25].

MAG1: this protein is a marker of a recent infection. It was well present in 97.3% of patients with acute infections and only 7.5% of pregnant women with chronic infections. Therefore, this protein is also a good marker of acute infection [26].

GRA1: This dense granule protein is secreted by both tachyzoites and bradyzoites. The overall sensitivity of ELISA using the recombinant form of the protein in the detection of anti-Toxoplasma IgG ranges from 60% to 98% [27, 28]. It has been described as a good marker of the chronic phase of toxoplasmosis. For this reason, it is not incriminated in the detection of IgM by the recomLine Toxoplasma IgM test (score = zero). Ferrandiz *et al*. reported a sensitivity of 78.2% in chronic infections versus 34% in acute infections [28].

rSAG1: the usefulness of this protein in the serodiagnosis of toxoplasmosis has been evaluated in numerous studies. It is very sensitive especially in the detection of IgG [29].

According to our results, among the 28 confirmed IgM-specific sera, 11 sera were positive for recomLine *Toxoplasma* IgM, i. e. a sensitivity of 39.28%. Among the 30 confirmed natural IgM sera, 28 sera were negative in recomLine *Toxoplasma* IgM, i. e. a specificity of 93.33%.

CONCLUSION

In conclusion, the recomLine *Toxoplamsa* IgM test can be recommended in situations where IgM is positive and IgG is negative. The positivity of this test makes the diagnosis of toxoplasma seroconversion very likely. However, its negativity does not rule out the diagnosis.

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Conflict of interest: None

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Ethics statement: This research was approved by REPUBLIQUE TUNISIENNT Ministére de la Santé HOPITAL UNIVERSITAIRE FARHAT HACHED – SOUSSE.

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