

Comparison of the Toxogen® rapid test with ELISA immunoenzymatic technique for toxoplasmosis serology in pregnant women

*Francine Isabelle
Rakotonindrina*

Laboratory of the Professor Zafisaona Gabriel (PZaGa)
University Hospital Center- Faculty of Medicine, University
of Mahajanga, Madagascar.

Rakotomalala Rivo S

Laboratory of the Professor Zafisaona Gabriel (PZaGa)
University Hospital Center- Faculty of Medicine, University
of Mahajanga, Madagascar.

Tsatoromila Fenosoa A

Directorate of Blood Transfusion - Faculty of Medicine,
University of Antananarivo.

Rabenandrianina Tahirimalala

Laboratory of the Professor Zafisaona Gabriel (PZaGa)
University Hospital Center- Faculty of Medicine, University
of Mahajanga, Madagascar.

Rajaonatahina Davidra

Paraclinical Training and Research Unit in Immunology -
Faculty of Medicine,
University of Antananarivo.

Rakotovao Luc A

CHU / JRB Laboratory - Faculty of Medicine, University of
Antananarivo.

Rakoto Alson Olivat A

CHU / JRB Laboratory - Faculty of Medicine, University of
Antananarivo.

Rasamindrakotroka Andry

Laboratory for Training and Research in Medical Biology,
Faculty of Medicine, University of Antananarivo.

Randriamanantany Zely

Directorate of Blood Transfusion - Faculty of Medicine,
University of Antananarivo.

Razanokolona Lala R S

National Reference Laboratory for HIV / AIDS and Sexually
Transmitted Infections - Ministry of Public Health - Faculty
of Medicine, University of Antananarivo.

The aim of this study was to compare the technical performance of the Toxogen® rapid test against the ELISA technique for serological screening and/or serological monitoring of toxoplasmosis in pregnant women.

Methods and material: A prospective, descriptive and analytical study was conducted over a period of 7 months from January to July 2018. One hundred and eighty-two toxoplasmosis serologies were carried out on 144 pregnant women followed in two hospitals in Mahajanga and in a private laboratory in Antananarivo. Two serologies were performed for each case, the first by the rapid test Toxogen®, a latex agglutination test, and the second by the IgG-ELISA and IgM-ELISA technique.

Results: The Toxogen® rapid test had a sensitivity of 62.5%, a specificity of 97.1%, a Positive Predictive Value of 94.3%, a Negative Predictive Value of 76.7% compared to the IgG-ELISA technique. The correlation between the dilution titer by the Toxogen® rapid test and the optical density (OD) of anti-toxoplasma IgG by the ELISA technique was very low ($r^2 = 0.17$, $p < 0.001$). The crude prevalence of toxoplasmosis was 30.6% ($n=44$) by the Toxogen® rapid test and 46.5% ($n=67$) by the IgG-ELISA technique, the difference was significant ($p < 0.001$). We had 2 cases (0.01%) positives by the IgM-ELISA which were not detected by the Toxogen® rapid test.

Conclusion: The Toxogen® rapid test cannot substitute the ELISA technique for the serological screening and/or serological monitoring of toxoplasmosis in pregnant women.

Keywords: ELISA; pregnant women; latex agglutination test; serology; toxoplasmosis.

Introduction

Toxoplasmosis is a zoonanthroponosis due to *Toxoplasma gondii*. It's a ubiquitous parasitosis affecting about 10% to 90% of the world's population [1]. This pathology is normally asymptomatic in healthy subjects. Its severity lies in cases where it invades immunocompromised individuals (AIDS patients, transplant recipients or cancer patients) and pregnant women [2]. In the latter case, it can be transmitted to the fetus by transplacental route during a primary infection during pregnancy due to a lack of protective immunity. The clinical manifestations and the risk of transmission of the parasite to the fetus are variable depending on the time of the primary maternal infection [3]; early infection can cause severe fetopathy such as hypotonia, microcephaly, and microphthalmia [1]; late infection can increase the risk of fetal transmission of the parasite, ranging from 6% to 13% at 13 weeks to 72% at 36 weeks [4][5]. Hence the importance of early screening and regular monitoring of pregnant women to detect possible seroconversion at an early stage and thus adapt management according to the risk incurred by the fetus.

Screening and serological monitoring of toxoplasmosis in pregnant women is part of durable objective development No. 3.1 to 3.3, which aim to reduce maternal, neonatal and infant morbidity and to reduce mortality from communicable diseases. In Madagascar, there is no systematic screening program for toxoplasmosis in pregnant women and access to toxoplasmosis serology is still limited. Only a few laboratories in large cities can perform good assay techniques such as ELISA. Peripheral health center, which represent a large proportion of health structures, cannot perform this analysis. Moreover, the high cost of this analysis does not allow all Malagasy pregnant women to perform it, thus limiting access to diagnosis and possible follow-up in case of negative serology, especially since the follow-up should be done monthly. To overcome this problem, we looked at the Toxogen® rapid test, which is less expensive and easier to perform. The objective of this study was to compare the technical performance of the Toxogen® rapid test against the ELISA technique, which serves as a reference for the determination of IgG and IgM antitoxoplasma antibodies, in order to validate or not its use for the screening and/or serological monitoring of toxoplasmosis in pregnant women.

Materials and methods

Type, Duration, Setting and Study Population

A prospective, descriptive and analytical study was carried out over a period of 7 months from January to July 2018. Most of the biological study was carried out in the laboratory of the University Hospital Center Professor Zafisaona Gabriel in Mahajanga, Madagascar.

Blood samples were taken from pregnant women followed in two hospital centers in Mahajanga, the laboratory of CHU PZaGa and the Integrated Health Center (CSI) Mahabibo, and in a private laboratory in Antananarivo, the laboratory Excellence.

After informed consent, during prenatal consultation or pregnancy check-ups, pregnant women who agreed to participate in our study were given one or two serologies of toxoplasmosis one month apart, according to their choice, free of charge.

In total, 144 pregnant women were included and 182 toxoplasmosis serologies were performed, including 144 screenings and 38 serological follow-ups.

Serological analysis

After centrifugation at 2500 g/min for 10 minutes of 5mL of venous blood taken from a dry tube, the sera were collected in numbered aliquots. For each sample, 50 to 100 µL of serum were used to perform toxoplasmosis serology using the Toxogen® rapid test, and 10 µL of which 5 µL was used for the determination of IgG anti-toxoplasmic and 5 µL for the determination of IgM using the ELISA technique. The different reagents were used according to the manufacturers' recommendations. The internal controls of the kits were run with the samples for each test series.

- **Toxogen® Reagent** (Tulip Diagnostics Ltd, Goa, India, Lot No. 208802, expiry date January 2019)

The Toxogen® kit is a rapid glass slide test for the qualitative and semi-quantitative detection of total antibodies to *Toxoplasma gondii* in human serum and plasma by the latex agglutination method. The Toxogen® reagent is made of a suspension of polystyrene latex particles coated with soluble *T. gondii* antigen. It has been standardized to detect an antibody titer of 10-15 IU/mL or greater.

Toxoplasmosis serology is positive if agglutination of the latex particles was observed when they came into contact with the patient's serum. Agglutination indicates the presence of a significant level of antibodies to *T. gondii* (dilution titer $\geq 1/16$). Absence of agglutination is a negative result and indicates the absence of a significant level of antibodies to *T. gondii* (dilution titer $< 1/16$).

If the result is positive, a semi-quantitative titration by cascade dilution will be performed using the serum pre-diluted to 1/16. The highest dilution of serum showing agglutination corresponds to the titer of antibodies against *T. gondii* (1/16, 1/32, 1/64, ...).

IgG-IgM differentiation will be applied to all positive sera using 2-mercaptoethanol, which destroys IgM. A semi-quantitative titration will be performed after pretreatment of the serum sample with the reducing agent. The sample is considered positive for IgM when there is a decrease in agglutination reactivity and/or a decrease in antibody titer.

The serologies of pregnant women with stable titers of total anti-toxoplasmic antibodies on two samples spaced one month apart have been considered «toxoplasmosis old infection».

The serologies of pregnant women with anti-toxoplasmic IgM antibodies, after differentiation of immunoglobulins, and / or an elevation of at least the double of the titer in total anti-toxoplasmic antibodies on two samples spaced one month apart have been considered "toxoplasmosis recent infection".

- ELISA kit

EIA Toxo-IgG kits (Rapid Labs Ltd, Colchester, United Kingdom; Lot N ° 1712218, expiry date 07/22/2019), the principle of which is based on the indirect method, and EIA Toxo-IgM (Rapid Labs Ltd, Colchester, United Kingdom; Lot N ° 1804001, expiry date 15/09/2019) whose technique is based on the principle of immunocapture, served as a reference in this study. Each of these kits have a relative sensitivity of more than 99.9% and a relative specificity of 99% compared to a reference technique, according to the manufacturer's technical sheet.

The serology of pregnant women with stable IgG (IgG +) antibodies was considered to be "old toxoplasmosis infection" on two samples spaced one month apart. Have been considered "toxoplasmosis recent infection" serologies of pregnant women with:

- Either anti-toxoplasmic IgM antibodies (IgM +) alone at the first serology followed by an appearance of IgG during the follow-up after one month,
- Either an appearance of IgM and / or anti-toxoplasmic IgG during serological monitoring at one month intervals,
- Either a significant increase in the OD of anti-toxoplasmic IgG (twice the 95% confidence interval), or > 0.84 after calculation, on two samples spaced one month apart.

Data analysis

The data were entered on Epi info 7.0 software, standardized on Excel 2013 and analyzed on R software French version 3.5.2 (developed by Center for Disease Control and Prevention, Atlanta, USA). We used nonparametric tests including the Fisher test and the Spearman correlation. Obtaining a probability p less than or equal to 0.05 was in favor of the existence of a significant link between the variables studied.

Limit of the study

We were unable to perform the anti-toxoplasmic IgG avidity test for more precise dating of infections due to lack of resources. The reduced size of pregnant women who have had serological monitoring is also a limit in this study.

Results

A total of 182 toxoplasmosis serologies, including 144 screenings and 38 follow-ups, were performed on pregnant women during the study period. The ages of pregnant women varied between 15 and 42 years with an average of 26.7 ± 6.4 years and a median of 26 years.

Toxoplasmosis seroprevalence in pregnant women

Toxogen® rapid test

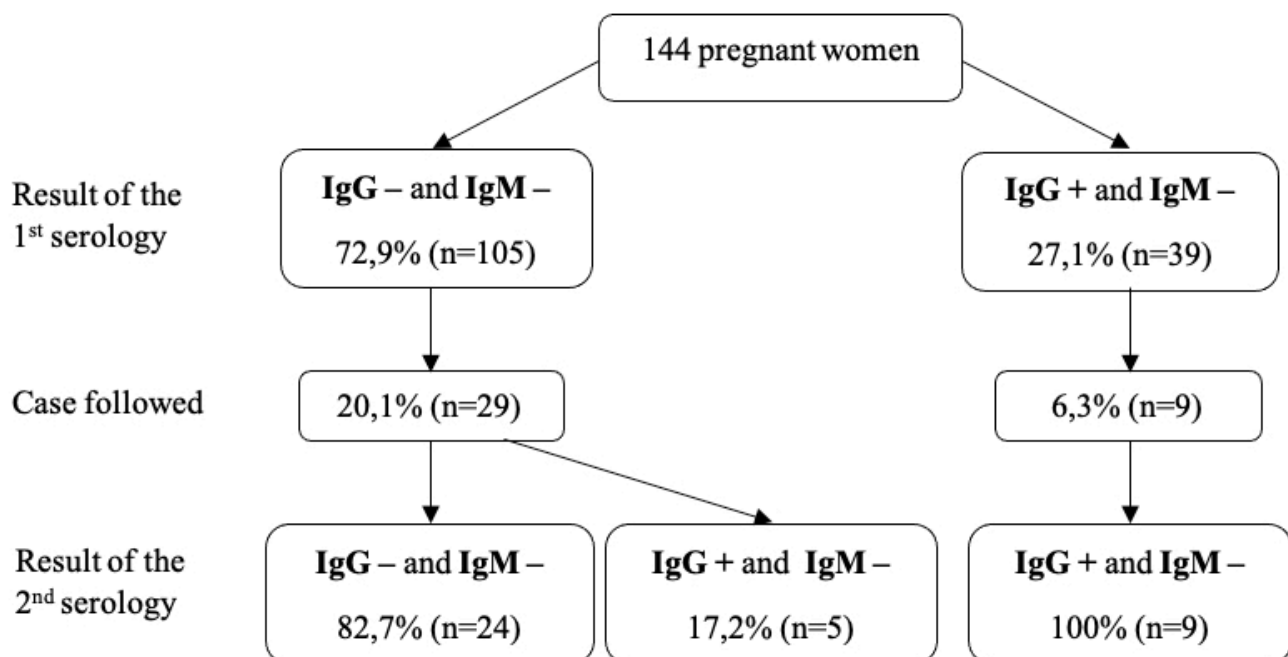


Figure 1. Results of toxoplasmosis serology in pregnant women by the Toxogen® rapid test

Among the 38 pregnant women who carried out a serological follow-up, we noted 31.6% (n=12) of seroconversion of which 5 cases were negative at the first serology and were found to be positive at the second serology, and 7 cases in which the antibody titer increased by at least double at the second serology.

Dilution titer at the 1 st serology	Dilution titer at the 2 nd serology							Total (n=38)
	< 1/16(n=24)	1/16 (n=6)	1/32 (n=1)	1/64 (n=1)	1/128 (n=2)	1/256 (n=2)	1/512 (n=2)	
< 1 /16	24	5						29
1/16		1			1	1		3
1/32			1	1				2
1/64					1			1
1/128						1		1
1/256							2	2

Table 1. Comparison of the dilution titer at the first and at the second serology for the de termination of toxoplasmosis recent infection by the Toxogen® rapid test in pregnant women who have done serological follow-up

No presence of anti-toxoplasmic IgM has been found, either during screening or during serological follow-up, among the 53 sera positive by the Toxogen® rapid test after their treatment with 2-mercaptoethanol.

In total, the seropositivity of toxoplasmosis by the Toxogen® rapid test was 30.6% (n = 44).

ELISA technique

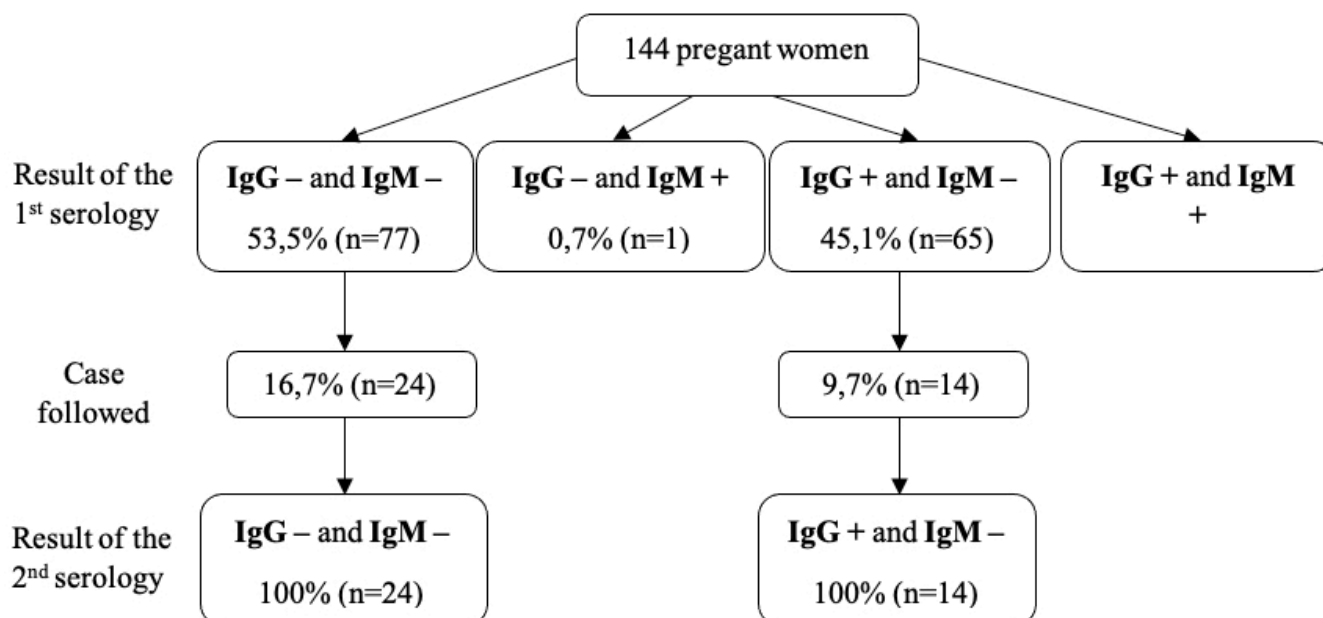


Figure 2. Results of toxoplasmosis serology in pregnant women by the ELISA technique

Among the 38 pregnant women who did serological follow-up, we have found 13.2% (n=5) which presented a significant increase in the OD of anti-toxoplasmic IgG among seropositive pregnant women, raising suspicion of a recent infection.

In summary, the seropositivity of toxoplasmosis in pregnant women was 46.5% (n = 67) by the ELISA technique.

Performance of the Toxogen® rapid test compared to the ELISA technique

Toxogen® rapid test	ELISA-IgG		Total n=182 (100%)
	Negative n=102 (56%)	Positive n=80 (44%)	
Negative	99 (54.4%)	30 (16.5%)	129 (70.9%)
Positive	3 (1.6%)	50 (27.5%)	53 (29.1%)

Table 2. Comparison of Toxogen® Rapid Test versus ELISA Technique for the Qualitative Determination of Anti-toxoplasmic IgG in Pregnant Women

	Percentage (%)
Sensitivity	62.5
Specificity	97.1
Positive Predictive Value	94.3
Negative Predictive Value	76.7

Table 3. Performance of the Toxogen® Rapid Test compared to ELISA for the determination of anti-toxoplasmic IgG in pregnant women (n=182)

Table 2 and 3 summarize the comparison of the Toxogen® rapid test and its performance compared to the ELISA technique for the qualitative determination of anti-toxoplasmic IgG in pregnant women. The difference between the two techniques was significant ($p < 0.001$).

For the quantitative determination of anti-toxoplasmic IgG, the correlation line between the dilution titer by the Toxogen® rapid test and the OD of anti-toxoplasmic IgG by the ELISA technique is shown in Figure 3 with its equation and its coefficient of determination r^2 ($p < 0.001$).

Figure 3. Correlation between the dilution titer by the Toxogen® rapid test and the optical density of anti-toxoplasmic IgG antibodies by the ELISA technique (n = 182)

We had 2 cases of positive IgM by the ELISA technique, one of which with an IgM-OD of 1.02 (IgG-OD = 0.109) was not detected at all by the Toxogen® rapid test; and the other with an IgM-OD greater than 3 combined with positive IgG (IgG-OD = 2.14) was not found by Toxogen® after treatment of the sample with 2-mercaptoethanol.

Discussion

The Toxogen® rapid test had a sensitivity of 62.5%; a specificity of 97.1%, a positive predictive value of 94.3%, a negative predictive value of 76.7% compared to the ELISA technique for the qualitative determination of anti-toxoplasmic IgG antibodies (table 3).

In Utah in 1988, a study on the technical evaluation of a latex agglutination test (LAT) versus IgG ELISA found a sensitivity of 86%, specificity of 100%, PPV of 100%, and NPV of 91% (n=125) for the detection of anti-*T. gondii* antibodies [6]. In South Korea, a more recent study of a rapid test using the immunochromatographic technique showed a sensitivity of 97.1% and a specificity of 100% compared to the IgG ELISA for the detection of antibodies to *T. gondii* [7].

The performance of the Toxogen® rapid test was poorer compared to the IgG-ELISA and in comparison to other rapid tests of the same or different technique evaluated with the same methods, i.e. compared to the enzyme immunoassay method. The results of the latex agglutination tests are not superimposable from one reagent to another, nor with the other techniques, and the nature of the antigens and the way they are revealed may explain these differences [6]. Some LAT do not detect the antibody response in acute *T. gondii* infection for several weeks because there is a predominance of cytoplasmic antigens in their assays [8]. In contrast, techniques involving recombinants of *T. gondii* surface antigens, such as the rapid test used in Korea, could detect early antibodies [7].

There may be other reasons why LATs are less sensitive than IgG ELISA for the determination of anti-toxoplasmic antibodies. LATs are easy to handle but interpretation of the results is operator-dependent. The observation of agglutination could be subjective. Reading and interpreting the results requires a well-lit area and an operator with good vision, especially when the agglutinations are less obvious. In addition, LATs may be subject to the prozone effect, i.e. the result is negative in the presence of high titers [4]. We had 2 cases that could correspond to this phenomenon, because despite a pre-dilution of the sera at 1/16, the result was negative by the Toxogen® rapid test while it was positive with a high OD above 5 by the IgG-ELISA technique (Figure 1).

The difference in test sensitivity could also be due to the detection limit of the reagents. Note that the Toxogen® reagent has been standardized to detect an antibody titer of 10-15 IU/mL or higher, such as some ELISA techniques [9][10], while some tests have a lower detection limit of 4 IU/mL [11]. As a result, Toxogen® has a lower sensitivity compared to other tests with a lower detection threshold.

We found 1.6% false positives which could be due to either cross-reactions, misinterpretation, or inter-serial contamination from dried positive samples that may remain on the plate, improperly washed, or previously used.

Some studies have shown that LATs have been sensitive for the detection of anti-toxoplasmic antibodies, but it is their specificity that has been problematic [8]. In contrast, we found a specificity of 97.1% with the Toxogen® rapid test, and the LAT used in Utah had a specificity of 100% compared to the IgG ELISA. This confirms that the LAT results are not overlapping from one reagent to another [6], as some tests are more specific than others. Again, the nature of the antigens could explain this difference.

The positive predictive value (PPV) of Toxogen® compared to the IgG-ELISA was 94.3%. A qualitative screening test for anti-toxoplasmic antibodies should have a high PPV while maintaining

high levels of specificity [6]. An acceptable PPV should be above 95%. The Toxogen® rapid test did not meet this criterion.

Toxogen® NPV was also low (76.7%) for anti-toxoplasmic IgG. Therefore, there is a higher risk of having a false negative result with the test, we had 16.5% cases in our study. Thus, a negative result obtained with the test does not really exclude an infection with *T. gondii*. Screening and prevention programs for congenital toxoplasmosis are mainly based on the serological identification of seronegative pregnant women (IgG-, IgM-) at the beginning of pregnancy, who must adopt preventive attitudes, and their follow-up [12]. However, the use of the Toxogen® rapid test could pose a problem of interpretation of serological status during subsequent follow-ups and could also delay the management of pregnant women to limit transplacental transmission of the parasite to the fetus in the event of a negative result. Thus, the prevention of congenital toxoplasmosis is not optimal.

Comparison of the antibody dilution titer of the Toxogen® rapid test with the optical density of anti-toxoplasmic IgG by the IgG-ELISA technique showed that there was a mean positive linear correlation between the two variables (correlation coefficient r between 0.3 and 0.5), based on an interpretation according to Cohen's 1988 beacons. But considering the coefficient of determination r^2 which is 0.176, meaning that only 17.6% of the variance of the dilution titer by the rapid Toxogen® test was explained by the correlation line, we can say that the correlation between the two techniques was poor. The false negatives generated by the Toxogen® rapid test corresponded to OD ranging from 0.25 to 6.30 (Figure 1). From this we can deduce that the Toxogen® rapid test is not at all reliable compared to the ELISA-IgG technique for the semi-quantitative determination of anti-toxoplasmic IgG. Moreover, some authors have already concluded that latex agglutination tests cannot replace the reference test, the dye test, for the serological diagnosis of toxoplasmosis because of the variability in the concentration of antibodies detected [13].

The Toxogen® rapid test found 31.6% seroconversion compared to 13.2% using the IgG-ELISA technique, a difference of 18.4%. The Toxogen® rapid test would then have led to an 18.4% over-treatment rate for suspected cases of recent infection. Therefore, it could also lead to a significant economic loss since the cost of treatment, which would have to be continued until the end of the pregnancy, is considerable even for a single pregnant woman.

About the performance of the Toxogen® rapid test compared to ELISA-IgM, we had 2 cases positive for anti-toxoplasmic IgM by the ELISA technique which were not found by the Toxogen® rapid test. IgM-ELISA techniques using the immunocapture method have been developed to minimize false positive results by cross-reactions with rheumatoid factors and anti-nuclear antibodies [8]. So, it would be the sensitivity of the Toxogen® rapid test for the determination of anti-toxoplasmic IgM that would be questioned. According to studies, the performance of techniques detecting anti-toxoplasmic IgM is variable, especially in terms of early detection [14][15]. Other studies with a higher number of positive IgM samples could allow better visibility of the sensitivity of this test towards early IgM, but with the poor performance for the detection of IgG, this would not be really interesting. We first suggest technical developments followed by a re-evaluation of the test according to the criteria of the World Health Organization for the selection of diagnostic tests [16].

Conclusion

At the end of our study, the performance of the Toxogen® rapid test was much worse compared to the ELISA technique. This test cannot substitute the ELISA technique for serological screening and / or monitoring of toxoplasmosis in pregnant women. All tests provided by distributors should not be accepted and used without being reassessed and technically validated by a medical biologist.

Conflict of interest: None.

References

1. Torgerson P, Mastroiacovo P. The global burden of congenital toxoplasmosis : a systematic review. Bull World Health Organ. 2013;91(7):501-8.
2. Pfister P, Dromigny J. Avidity of IgG anti-*Toxoplasma gondii*. Study to establish a new decision tree in screening for the disease. Arch Inst Pasteur Madagascar. 2001 ; 67 (1-2) : 57-60.
3. Yeraa H, Parisc L, Bastiene P, Candolfi E. Biological diagnosis of congenital toxoplasmosis. Revue Francophone des Laboratoires. 2015 ; 470 : 65-72.
4. Wallon M, Peyron F. Toxoplasmosis. Encycl Med Chir Biologie medicale. 2014;9(4) :90-40-0190-A.
5. Nizard J. Toxoplasmosis and pregnancy. Journal de Gynécologie Obstétrique et Biologie de la Reproduction. 2008 (37) ; Hors-série 1 :F4-F9.
6. Mazumder P, Chuang H, Wentz M, Wiedbrauk D. Latex agglutination test for detection of antibodies to *Toxoplasma gondii*. J Clin Microbiol. 1988 ; 26(11): 2444-6.
7. Kim Y, Lee J, Ahn S, Kim T-S, Hong S-J et al. Seroprevalence of Toxoplasmosis with ELISA and Rapid Diagnostic Test among Residents in Gyodong-do, Incheon city, Korea: A Four-Year Follow-up. Korean J Parasitol. 2017 ; 55(3) : 247-54.
8. Saadatnia G, Golkar M. A review on human toxoplasmosis. Scand J Infect Dis. 2012 ; 44(11) : 805-14.
9. Naghili B, Abbasalizadeh S, Tabrizi S, Rajaii M, Akramiyan M et al. Comparison of IIF, ELISA and IgG avidity tests for the detection of anti-*Toxoplasma* antibodies in single serum sample from pregnant women. Infez Med. 2017;25(1):50-6.
10. Drogmigny J, Pecarrere J, Leroy F, Ollivier G, Boisier P. Prevalence of toxoplasmosis in Tananarive. Study conducted at the Pasteur Institute of Madagascar (PIM) on a sample of 2354 subjects. Bull Soc Path Exot. 1996; 89(3) : 212-6.
11. Khalil MK, Elrayah IE. Seroprevalence of *Toxoplasma gondii* antibodies in farm animals (camels, cattle, and sheep) in Sudan. Journal of Medicine and Animal Health. 2011 ; 3(3) : 36-9.
12. Flori P, Chene G, Varlet M, Tran Manh Sung R. Serology of toxoplasmosis in pregnant women: characteristics and pitfalls. Ann Biol Clin. 2009 ; 67(2) : 125-33.
13. Payne Ra, Francis JM, Kwantes W. Comparison of a latex agglutination test with other serological tests for the measurement of antibodies to *Toxoplasma gondii*. J Clin Pathol. 1984;37:1293-7.
14. Villard O, Jung-Etienne J, Cimon B, Franck J, Fricker-Hidalgo H et al. Toxoplasmosis serodiagnosis in 2010: procedure to be followed and interpretation according to the serological profiles obtained by screening methods. Feuillet de Biologie. 2011 ; 52(298) :43-9.
15. Hounto A, Chokki F, De Tove Y, Bankole B, Adinsi De Souza V et al. Evaluation of a rapid diagnostic test in the diagnosis of toxoplasmosis in pregnant women in Cotonou (Bénin). Bull Soc Pathol Exot. 2014 ; 107(2) : 85-9.
16. Kosack C, Page A-L, Klatser P. A guide to aid the selection of diagnostic tests. Bull World Health Organ. 2017;95:639-45.

References

1. Torgerson P, Mastroiacovo P. The global burden of congenital toxoplasmosis : a systematic review. Bull World Health Organ. 2013;91(7):501-8.
2. Pfister P, Dromigny J. Avidity of IgG anti-*Toxoplasma gondii*. Study to establish a new decision tree in screening for the disease. Arch Inst Pasteur Madagascar. 2001 ; 67 (1-2) : 57-60.
3. Yeraa H, Parisc L, Bastiene P, Candolfi E. Biological diagnosis of congenital toxoplasmosis. Revue Francophone des Laboratoires. 2015 ; 470 : 65-72.
4. Wallon M, Peyron F. Toxoplasmosis. Encycl Med Chir Biologie medicale. 2014;9(4)

:90-40-0190-A.

5. Nizard J. Toxoplasmosis and pregnancy. *Journal de Gynécologie Obstétrique et Biologie de la Reproduction*. 2008 (37) ; Hors-série 1 :F4-F9.
6. Mazumder P, Chuang H, Wentz M, Wiedbrauk D. Latex agglutination test for detection of antibodies to *Toxoplasma gondii*. *J Clin Microbiol*. 1988 ; 26(11): 2444–6.
7. Kim Y, Lee J, Ahn S, Kim T-S, Hong S-J et al. Seroprevalence of Toxoplasmosis with ELISA and Rapid Diagnostic Test among Residents in Gyodong-do, Incheon city, Korea: A Four-Year Follow-up. *Korean J Parasitol*. 2017 ; 55(3) : 247-54.
8. Saadatnia G, Golkar M. A review on human toxoplasmosis. *Scand J Infect Dis*. 2012 ; 44(11) : 805-14.
9. Naghili B, Abbasalizadeh S, Tabrizi S, Rajaii M, Akramiyan M et al. Comparison of IIF, ELISA and IgG avidity tests for the detection of anti-*Toxoplasma* antibodies in single serum sample from pregnant women. *Infez Med*. 2017;25(1):50-6.
10. Drogmigny J, Pecarrere J, Leroy F, Ollivier G, Boisier P. Prevalence of toxoplasmosis in Tananarive. Study conducted at the Pasteur Institute of Madagascar (PIM) on a sample of 2354 subjects. *Bull Soc Path Exot*. 1996; 89(3) : 212-6.
11. Khalil MK, Elrayah IE. Seroprevalence of *Toxoplasma gondii* antibodies in farm animals (camels, cattle, and sheep) in Sudan. *Journal of Medicine and Animal Health*. 2011 ; 3(3) : 36-9.
12. Flori P, Chene G, Varlet M, Tran Manh Sung R. Serology of toxoplasmosis in pregnant women: characteristics and pitfalls. *Ann Biol Clin*. 2009 ; 67(2) : 125-33.
13. Payne Ra, Francis JM, Kwantes W. Comparison of a latex agglutination test with other serological tests for the measurement of antibodies to *Toxoplasma gondii*. *J Clin Pathol*. 1984;37:1293-7.
14. Villard O, Jung-Etienne J, Cimon B, Franck J, Fricker-Hidalgo H et al. Toxoplasmosis serodiagnosis in 2010: procedure to be followed and interpretation according to the serological profiles obtained by screening methods. *Feuillets de Biologie*. 2011 ; 52(298) :43-9.
15. Hounto A, Chokki F, De Tove Y, Bankole B, Adinsi De Souza V et al. Evaluation of a rapid diagnostic test in the diagnosis of toxoplasmosis in pregnant women in Cotonou (Bénin). *Bull Soc Pathol Exot*. 2014 ; 107(2) : 85-9.
16. Kosack C, Page A-L, Klatser P. A guide to aid the selection of diagnostic tests. *Bull World Health Organ*. 2017;95:639-45.