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Parasitology

Validation of IgG, IgM multiplex plasmonic gold platform in French clinical cohorts for the serodiagnosis and follow-up of *Toxoplasma gondii* infection

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ABSTRACT

We report the use of the multiplexed *T. gondii* IgG, IgM test on plasmonic gold (pGOLD) platform in the setting of *T. gondii* infection by analyzing 244 sera from Nice, France (seroconversion, chronically infected, non-infected and newborns serum samples). Results were compared with commercial tests for the detection of IgG and IgM and their overall clinical final interpretation of a complete serological profile. The IgG and IgM test results on the platform were in agreement in, respectively, 95% and 93% with the commercial kits. When comparing with the overall clinical interpretation of the serological profile, the agreement reached 99.5% and 97.7% for IgG and IgM, respectively. This innovative pGOLD platform allows detection of both IgG and IgM simultaneously with only ~1 microliter of serum. The multiplexed IgG/IgM test on pGOLD platform is a strong candidate for its use in the massive screening programs for toxoplasmosis during pregnancy.

1. Introduction

Toxoplasma gondii is a protozoan parasite potentially infecting all warm-blooded animals. In humans infection can occur by ingestion of oocysts present in the environment, or food ingestion of cysts present in undercooked or more rarely through transplanted organs from an infected donor (Tenter et al., 2000). Though usually asymptomatic in immunocompetent individuals, the infection can be a life-threatening in immunocompromised patients and congenitally infected fetus and newborns. In some countries (Austria, Belgium, France, Norway, Uruguay, and some regions in Italy and Brazil), there are evidence-based guidelines, recommendations and socio-economical analysis that support the monthly follow-up of pregnant women (Cortés et al., 2012). In these countries, screening and treatment programs during pregnancy have shown to be effective in decreasing vertical transmission and severe sequelae of infected offspring (Cortina-Borja et al., 2010; Hotop et al., 2012; Kieffer et al., 2008; Prusa et al., 2015; SYROCOT (Systematic Review on Congenital Toxoplasmosis) study group et al., 2007; Wallon et al., 2013). However, most of the countries worldwide do not screen for *T. gondii* during pregnancy (Pomares and Montoya, 2016). With 213 million pregnancies worldwide in 2012 and the global burden of congenital toxoplasmosis estimated at 190,100 new cases and 1.20 million disability-adjusted life years, screening strategy should be re-considered due to the potential risk of *T. gondii* infection (Sedgh et al., 2014; Torgerson and Mastroiacovo, 2013). Currently, the serological diagnosis is primarily performed through ELISA or chemiluminescence based methods and very few innovative platforms have been used since those assays were introduced to clinical practice (Augustine, 2016; Guigue et al., 2014; Salmona et al., 2014). In order to implement a serological screening at massive scales, serological assays should be more cost effective with multiplexing capacity and use lower amounts of serum (Augustine, 2016; Li et al., 2016; Sahai and Onyett, 1996; Scallan et al., 2015; Stillwaggon et al., 2011). We previously reported the use of plasmonic gold (pGOLD) in a cross-sectional

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study from patients from the United States (USA) (Li et al., 2016). The aim of the study of Li et al. was to compare the data obtained on Toxo-pGOLD to the data of the dye test and IgM ELISA performed at the Palo Alto Medical Foundation *Toxoplasma* Serology Laboratory (PAMF-TSL, Reference laboratory for Toxoplasmosis in the USA). No clinical information and no sequential sera were analyzed in this study. In the present study we described the use of the pGOLD platform in the setting of *T. gondii* infection in several patients cohort from Nice, France, including those with seroconversion, chronic infection, no infection and in newborns and infants born from mothers who seroconverted during pregnancy (Augustine, 2016; Li et al., 2016).

2. Materials and methods

2.1. Study design

A total of 244 consecutive sera were selected over a 4 year period (2012–2016) from the routine outpatients practice at the Clinical Laboratory of Parasitology-Mycology, Nice University Hospital, France. We primarily compared the results from the *T. gondii* IgG, IgM multiplexed pGOLD platform (Toxo-pGOLD) to those obtained from Architect IgG and IgM tests. In addition we compared the performance of the Toxo-pGOLD with the overall and final serological interpretation as provided by the Nice Laboratory using additional serological tests.

2.2. Serological tests

Conventional serologies were performed using: Architect Toxo IgG, Toxo IgM and Toxo IgG Avidity assay (Abbott Laboratories, Wiesbaden, Germany), Toxo-Screen DA, Vidas Toxo IgGII and Toxo IgM assays (bioMérieux, Marcy l'Étoile, France), Platelia Toxo IgA (BIO-RAD, Marnes La Coquette, France), Western blot *Toxoplasma* WB IgG IgM (LDBIO Diagnostics, Lyon, France), Toxo-ISAGA IgM (BioMerieux, Mercy l'Etoile, France). The cutoff values used are those recommended by the manufacturers (Table S1).

2.3. Patients and sera

On a routine basis, in adults, the *T. gondii* serological diagnosis is performed by Architect Toxo IgG, Toxo-Screen DA and Architect Toxo IgM. Depending on the results of these first tests, other assays were performed in order to define whether the serological profile was in favor of an acute, chronic infection or no infection. According to the serological profile and the patient history, several groups were defined (Table 1).

2.4. Criteria for the comparison of Toxo-pGOLD platform test results to the clinical interpretation

For this comparison, the adult population was the sole taken into account and the gray zone IgG and IgM test results obtained on the Toxo-pGOLD platform were considered as positive and negative, respectively. The results of the serology performed on Toxo-pGOLD were classified as true-positive, true-negative, false-positive and false-negative according to the final serological clinical interpretation. The serological clinical interpretation was based on the serological profile of the first line tests results performed on Architect Toxo IgG, bioMérieux Toxo-Screen DA and Architect Toxo IgM. Additional tests results as Architect Toxo IgG Avidity assay, bioMérieux Toxo IgGII, bioMérieux Toxo IgM assays and Platelia Toxo IgA were also performed when the first line tests results were not sufficient to set up a serological clinical interpretation. In the *T. gondii* seroconversion group one Toxo-pGOLD IgG test result (Patient 3, second serum) was classified as false-negative due to its negativity and 2 Toxo-pGOLD IgM test results were considered false-negative (Patient 9, third serum and patient 12, first serum) due to negative Toxo-pGold IgM test results. In the group *T. gondii* chronic infection, 3 IgM test results on Toxo-pGOLD were classified as false-positive due to their positivity. All the results of the comparison of Toxo-pGOLD platform test results to the clinical interpretation were detailed in Table 5.

2.5. Ethical Aspects

This study was approved by the local ethical committee (Comité de protection des personnes CHU Nice, France),

2.6. Multiplex IgG, IgM Plasmonic Gold Platform

The pGOLD™ slides are from Nirmidas Biotech, Inc. based on initial work from Stanford University (Koh et al., 2016; Li et al., 2016; Tabakman et al., 2011; Zhang et al., 2014, 2013). The *T. gondii* antigen from the PAMF-TSL was printed in triplicate and was used to capture *T. gondii* IgG and IgM antibodies. The assay was performed as previously described with the exception that the incubation times were shortened and the sample dilution factor was 1/400 (Li et al., 2016). Briefly, the

Table 1
Details of patients and sera selected for our study.

Groups defined according to the serological profile and the patient history	Number of patients	Number of samples	Comments
T. gondii seroconversion (Patients 1–13)	13	60	For each patient there was at least one negative IgG serum followed by between one to 5 positive samples. Seroconversion was confirmed by the appearance of IgG with positive IgM after an initial negative IgG sample and low avidity index. For some patients, positive IgA (Platelia BIO-RAD) were also present.
T. gondii chronic infection	72	72	Sera were randomly selected from our routine practice with positive Architect IgG and Toxo-Screen direct agglutination and negative Architect IgM. Three Architect IgG tests results were in gray zone. For these samples, the IgG Vidas and Toxo-Screen direct agglutination were positive and no IgM were detected leading to the conclusion of chronic infection. For some patients, Architect IgM tests results were positive or in gray zone. In these patients, the chronic infection was confirmed by a high Architect avidity index.
T. gondii non-infected patient	86	88	Sera were negative for Architect IgG, Toxo-Screen direct agglutination and Architect IgM. For one patient 3 samples were collected at different times. On the first sample, test results were negative for both IgG and IgM but on the 2 follow-up samples, IgM test results were in gray zone and then positive. The non-infected status was confirmed on the next follow-up sample (not tested in this study) with Architect IgG and IgM negative confirming the transitory false-positive IgM.
Newborn and infant samples (Patients 14–20)	7	24	All the samples were from newborns and infants whom mother seroconverted during pregnancy. Diagnosis of congenital toxoplasmosis was made by the presence of Architect IgG and ISAGA IgM in addition to the presences of neosynthesis IgG and/or IgM in newborns samples when performing immunoprofiles comparison between mother and newborn sera (*Toxoplasma* WB IgG IgM). Four newborns were congenitally infected. Exclusion of diagnosis of congenital toxoplasmosis was made by following the serology until the decrease of IgG titers and its disappearance before 12 months of age in the absence of treatment and with negative ISAGA IgM and IgA platelia. Three infants were considered non-infected.

slides were blocked with 5% bovine serum albumin (BSA, Sigma-Aldrich) in phosphate buffered saline (1× PBS, GE healthcare life sciences) for 10 min. Patients serum incubations lasted for 40 min and incubation with the mixture of IR680-labeled anti-human IgG secondary antibody and IR800–labeled anti-human IgM secondary antibody lasted for 15 min. A standard sample with positive *T. gondii* IgG and IgM (Biocheck Inc., CA) was used in each slide as a calibrator. The results, expressed in arbitrary unit, corresponded to the mean fluorescence intensity (MFI) value of the 3 spots minus the blank MFI value divided by the value of the calibrator minus the blank MFI value. In order to express the value in arbitrary unit, the result thus calculated were multiplied by 100 and 10 for IgG and IgM, respectively.

2.7. Statistical Analysis

Statistical analysis was made using the VassarStats website. Sensitivity, specificity, predictive positive value, negative predictive value were calculated in 3 conditions: Gray zone values excluded, gray zone values considered as positive and gray zone value considered as negative.

The agreement among both tests was measured by calculating the Cohen's kappa coefficient. A perfect agreement would equate to a kappa of 1, and agreement by chance would equate to 0. Kappa coefficient between 0.81 and 0.99 is almost perfect, between 0.61 and 0.80 is a substantial agreement and between 0.41 and 0.60 is a moderate agreement (Dai and Jin, 2005; Vlaspolder et al., 2001).

3. Results

3.1. Comparison of Toxo-pGOLD Platform Results with those from Architect IgG and IgM

The IgG, IgM values from the Toxo-pGOLD platform were compared with Architect IgG and IgM values. Because newborn and infant 1 under one year of age test results have different cutoff values, we present the data in the adult population. The qualitative results are summarized in supplementary material (Table S2). Sensitivity, specificity predictive positive value and negative predictive value were calculated in 3 different conditions: Gray zone data excluded, gray zone data considered as positive and gray zone data considered as negative (Fig. S1). For Toxo-pGOLD platform, the best sensitivity (IgG: 98.2%, IgM: 93.8%), specificity (IgG: 96.4%, IgM: 96.5%), PPV (IgG: 96.4%, IgM: 88.2%) and NPV (IgG: 98.1%, IgM: 98.2%) were obtained when gray zone was considered for IgG and IgM, as positive and negative, respectively.

3.2. Agreement between Toxo-pGOLD Results with those from Architect IgG and IgM According to the Defined Groups

The agreement between Architect and Toxo-pGOLD results varied from 90% to 97.7% (Table 2). In the whole population of patients, the agreement measured by Kappa coefficient was 0.97 and 0.89 for IgG and IgM, respectively, measured on the IgG, IgM Toxo-pGOLD platform. These data highlight an almost perfect agreement between the pGOLD platform and the Architect IgG and IgM test results.

3.3. Comparison between Toxo-pGOLD Platform Results with those from Architect IgG and IgM for T. Gondii Seroconversion Samples

Sixty consecutive sera from 13 pregnant women who seroconverted during pregnancy were followed (Table 3). The Toxo-pGOLD platform IgG, IgM results were similar to those found with IgG and IgM Architect with few exceptions. For Patient 3 the IgG from the Toxo-pGOLD platform appeared late comparing to the Architect IgG whereas it is the opposite for Patient 13. Interestingly, for Patient 9, IgM were negative and only detected once in gray zone with Architect IgM whereas with the Toxo-pGOLD platform, the first sample was negative and the following were positive (or in gray zone) as it is expected during seroconversion. For patient 12, the first sample was Architect IgG negative and Architect IgM positive. On this sample, the positive IgM was not detected on Toxo-pGOLD. For this patient, the follow-up sample was tested 195 days later. At that time, both assays were positive for IgG and negative for IgM. Thus, even when IgM were not detected on Toxo-pGOLD, both platforms were able to highlight seroconversion.

3.4. Comparison between Toxo-pGOLD Platform with Architect IgG and IgM for Newborn and Infants Samples

Twenty-four samples of newborns and infants from mothers who seroconverted during pregnancy were tested for IgG and IgM on the Toxo-pGOLD platform (Table 4). For these samples, it appeared clearly that the adult cutoff value for IgM cannot be used. As for the Toxo-ISAGA (bioMérieux) 2 different cutoff should be used depending on the studied population. Thus, by using the positivity cutoff ≥0.50, the sensitivity and specificity for IgM were 100% (CI 95%: 51.7% - 100%) and 94.4% (CI 95%:71% - 99.7%), respectively. The kappa coefficient and the agreement were 0.89 (CI 95%: 0.73–1) and 95.8% (CI 95%: 76.9% - 99.8%), respectively. For the IgG, the kappa coefficient and the agreement were 0.69 (CI 95%: 0.45–0.93) and 79.2% (CI 95%: 57.3% - 92.1%), respectively. When following the kinetic of the IgG on the Toxo-pGOLD platform, the results were very similar to the IgG Architect ones (Table 4).

3.5. Comparison of IgG, IgM Test Results from Toxo-pGOLD Platform with the Clinical Interpretation Provided by Nice Laboratory, France

For some patients, Architect IgG and IgM results do not match the clinical interpretation. Indeed, in chronic infection group, 3 Architect IgG samples were in gray zone, 3 Architect IgM samples were positive and 4 in gray zone. In addition, in *T. gondii* non-infected group, 2 Architect IgM results were false-positive results. When taking into account only the Architect test results, some data of the Toxo-pGOLD platform have been misclassified as false-positive or false-negative whereas the clinical interpretation of serological profile was in agreement with the serological test results of the Toxo-pGOLD platform. Thus, according to the serological profile, the IgG and IgM results of the platform were re-interpreted (Table 5). The gray zone IgG and IgM test results obtained on the Toxo-pGOLD platform were considered as positive and negative, respectively (Table 5). The agreement of IgG and IgM with the clinical interpretation on the multiplex platform were 99.5% and 97.7%, respectively, along with very good sensitivity (IgG: 99.1%, IgM:

Table 2
Kappa coefficient and agreement between the Toxo-pGOLD platform and the Architect IgG and IgM test results.

	Kappa coefficient ([95%CI])		% agreement (%[95%CI])	
	IgG	IgM	IgG	IgM
Adult population	0.97 (NC)	0.89 (0.85–0.94)	95% (90.1–97.4)	93% (88.8–96)
T. gondii seroconversion	0.92 (0.89–0.95)	0.82 (0.70–0.95)	90% (78.9–95.9)	90% (78.8–95.9)
T. gondii chronic infection	NC	0.65 (0.39–0.92)	95.8% (87.5–98.9)	91.7% (82.1–96.6)
T. gondii non-infected patient	NC	NC	97.7% (91.3–99.6)	96.9% (89.7–99.1)

NC = the kappa coefficient was not calculable.

Table 3
Antibody kinetic of the 13 patients (60 samples) with proven seroconversion. Positive (P) and gray zone (GZ) results are in bold and gray, respectively. Negative results (N) are plain.

	Number of days	IgG Architect	IgG Toxo-pGOLD	IgM Architect	IgM Toxo-pGOLD
Patient 1	0	0 (N)	1.51 (N)	0 (N)	0.16 (N)
	39	**12.5 (P)**	**14.16 (P)**	**10.15 (P)**	**12.70 (P)**
	47	**50.8 (P)**	**32.04 (P)**	**8.91 (P)**	**17.18 (P)**
	153	**126.5 (P)**	**85.59 (P)**	**3.66 (P)**	**9.95 (P)**
Patient 2	0	0.8 (N)	0.83 (N)	0.2 (N)	-0.04 (N)
	48	**579.6 (P)**	**102.32 (P)**	**9.68 (P)**	**12.99 (P)**
	62	**1131.7 (P)**	**153.17 (P)**	**7.31 (P)**	**10.75 (P)**
Patient 3	0	0.1 (N)	1.41 (N)	0.08 (N)	1.31 (N)
	54	**9.9 (P)**	2.59 (N)	**11.04 (P)**	**24.82 (P)**
	69	**16.2 (P)**	**7.74 (P)**	**11.64 (P)**	**27.23 (P)**
	89	**47.4 (P)**	**25.33 (P)**	**11.66 (P)**	**20.67 (P)**
	139	**157.9 (P)**	**100.81 (P)**	**8.29 (P)**	**15.70 (P)**
Patient 4	0	0.1 (N)	0.79 (N)	0.34 (N)	2.41 (N)
	30	**4.1 (P)**	**11.39 (P)**	**8.14 (P)**	**15.08 (P)**
	37	**11.7 (P)**	**12.26 (P)**	**8.48 (P)**	**9.87 (P)**
	66	**102.4 (P)**	**63.18 (P)**	**7.98 (P)**	**6.40 (P)**
	83	**139.8 (P)**	**78.85 (P)**	**7.24 (P)**	**7.20 (P)**
	260	**110.9 (P)**	**68.73 (P)**	**4.12 (P)**	**6.80 (P)**
Patient 5	0	0.1 (N)	0.46 (N)	0.26 (N)	0.23 (N)
	34	**6.8 (P)**	**9.48 (P)**	**1.28 (P)**	**5.43 (P)**
	41	**21.1 (P)**	**22.65 (P)**	**1.26 (P)**	**5.96 (P)**
	53	**48.2 (P)**	**37.92 (P)**	**1.06 (P)**	**7.35 (P)**
	124	**40.4 (P)**	**35.57 (P)**	0.58 (GZ)	3.40 (N)
Patient 6	0	0.4 (N)	1.82 (N)	**1.14 (P)**	**6.38 (P)**
	10	**5.7 (P)**	**10.17 (P)**	**1.41 (P)**	**8.64 (P)**
	69	**70.4 (P)**	**45.91 (P)**	**1.2 (P)**	**8.05 (P)**
Patient 7	0	0.1 (N)	1.33 (N)	**4.52 (P)**	**4.87 (P)**
	12	0.2 (N)	1.80 (N)	**16.34 (P)**	**11.57 (P)**
	22	0.7 (N)	0.95 (N)	**12.56 (P)**	**21.18 (P)**
	58	0.9 (N)	3.43 (N)	**6.43 (P)**	**5.19 (P)**
	86	1.3 (N)	4.20 (N)	**5.33 (P)**	**8.16 (P)**
	113	**40.8 (P)**	**40.32 (P)**	**4.8 (P)**	**8.88 (P)**
	124	**72.6 (P)**	**52.54 (P)**	**4.28 (P)**	**7.10 (P)**
	203	**67.6 (P)**	**57.66 (P)**	**3.92 (P)**	**8.59 (P)**
Patient 8	0	0.1 (N)	0.34 (N)	0.09 (N)	1.83 (N)
	29	0.1 (N)	0.98 (N)	**3.3 (P)**	**4.33 (P)**
	29	0.1 (N)	0.86 (N)	**3.41 (P)**	**4.88 (P)**
	39	0.2 (N)	2.12 (N)	**7.48 (P)**	**7.73 (P)**
	52	**5.9 (P)**	**7.44 (P)**	**6.89 (P)**	**8.36 (P)**
Patient 9	0	0.1 (N)	0.80 (N)	0.48 (N)	3.58 (N)
	23	2.2 (GZ)	3.43 (N)	0.52 (GZ)	**5.17 (P)**
	61	**14.2 (P)**	**19.09 (P)**	0.31 (N)	3.91 (GZ)
	81	**18.5 (P)**	**29.30 (P)**	0.32 (N)	**5.38 (P)**
	117	**16.2 (P)**	**27.68 (P)**	0.25 (N)	**5.17 (P)**
Patient 10	0	0 (N)	1.01 (N)	0.07 (N)	2.45 (N)
	66	0 (N)	1.21 (N)	0.25 (N)	3.27 (N)
	89	**4.7 (P)**	**11.81 (P)**	**4.17 (P)**	**20.52 (P)**
	90	**5.4 (P)**	**12.60 (P)**	**4.49 (P)**	**21.46 (P)**
	115	**33.2 (P)**	**48.11 (P)**	**3.23 (P)**	**16.39 (P)**
	204	**11.4 (P)**	**13.94 (P)**	**0.77 (P)**	**4.96 (P)**
Patient 11	0	0.1 (N)	0.75 (N)	0.09 (N)	1.87 (N)
	21	**3 (P)**	4.88 (GZ)	**10.35 (P)**	**15.05 (P)**
	31	**17.8 (P)**	**7.61 (P)**	**14.81 (P)**	**25.10 (P)**
Patient 12	0	0.1 (N)	1.93 (N)	**1.21 (P)**	3.43 (N)
	195	**13.8 (P)**	**26.88 (P)**	0.4 (N)	2.56 (N)
Patient 13	0	0.3 (N)	5.57 (GZ)	**3.84 (P)**	**6.78 (P)**
	3	0.7 (N)	6.60 (GZ)	**6.51 (P)**	**14.47 (P)**
	11	2.1 (GZ)	**8.04 (P)**	**7.02 (P)**	**18.82 (P)**
	24	**7.3 (P)**	**13.37 (P)**	**7.04 (P)**	**19.39 (P)**
	39	**18.9 (P)**	**22.63 (P)**	**6.22 (P)**	**13.91 (P)**

The following titers were considered positive, negative, or equivocal, respectively, in the various tests: IgG Toxo-pGOLD, ≥6.7, <4.5, 4.5–6.6; IgM Toxo-pGOLD (Adult cutoff), ≥4.3, <3.7, 3.7–4.2; IgG Architect Abbott (IU/ml), ≥3, <1.6, 1.6–2.9; IgM Architect Abbott (index), ≥0.60, <0.50, 0.50–0.59.

96%) and specificity (IgG: 100%, IgM: 98.2%) (Table 5). The best sensitivity when compared with Architect data was 98.2% and 93.8% and the best specificity was 96.4% and 96.5% for IgG and IgM, respectively. This data highlight the excellent performance of the Toxo-pGOLD platform in clinical conditions.

4. Discussion

The *T. gondii* IgG/IgM test on pGOLD platform was evaluated in sera from acutely, chronically and non-infected patients samples obtained at Nice Laboratory, France. In addition, 24 sera from newborns and infants whom mothers seroconverted during pregnancy were tested. The Toxo-pGOLD platform demonstrated an overall excellent performance in the different patient cohorts. Owing to its broad assay dynamic range (over 6–7 logs of concentration of biomarkers including IgG and IgM) based on nanoscience (Koh et al., 2016; Li et al., 2016; Tabakman et al., 2011; Zhang et al., 2014, 2013), this innovative technology allows the performance of simultaneous IgG and IgM detection by detecting 2 different near-infrared colors in only ~1 microliter of serum, achieving a very good agreement with clinical interpretation of the serological profiles that often require several serological tests. To our knowledge, pGOLD is the only platform to date capable of simultaneous IgG and IgM detection in a single assay reaching high degrees of agreement with clinical diagnostic results. Indeed, IgG sensitivity and specificity on Toxo-pGOLD platform, when gray zone was considered as positive, were 98.2% and 96.4%, respectively. For IgM assay performed simultaneously, when gray zone is considered as negative, the sensitivity and specificity were 93.8% and 96.5%, respectively. In our study, the sensitivity and specificity of the IgG, IgM assay on the Toxo-pGOLD platform compared to the clinical interpretation was obviously improved by several percent as Architect data were not always accurate. Indeed, when comparing with the clinical interpretation, the Toxo-pGold IgG sensitivity and specificity became 99.1% and 100%, respectively and the IgM sensitivity and specificity was enhanced up to 96% and 98.2%, respectively.

During acute infection, a very good performance of IgG detection is important as only positive IgG confirms the diagnosis of seroconversion. In seroconversion samples, the Architect IgG assay appeared to be the most capable of detecting early IgG in pregnant women with acute infection compared to Vidas (bioMérieux) and Liason (DiaSorin, Italy) (Murat et al., 2013). The IgG detection on the Toxo-pGOLD platform seemed to be as early as the Architect IgG assay. Indeed, in most seroconversion samples, kinetic of IgG detection on both assays was very similar. For patient 3, the IgG detection on pGOLD platform was delayed compared to the Architect IgG whereas for patient 13, the IgG detection on the pGOLD platform occurred earlier. In case of acute infection, positive IgM usually appear first and are followed by positive IgG. However, positive IgM can also be due to false-positive IgM or persistent *T. gondii* IgM during chronic phase of infection (Dhakal et al., 2015). Thus, in presence of positive IgM, only the appearance of positive IgG confirms the seroconversion. In seroconversion samples the detection of IgM on the Toxo-pGOLD platform showed a very good correlation with the Architect IgM assay (Kappa coefficient: 0.82). For patient 9, the IgM detection on the Toxo-pGOLD platform was even better with presence of positive IgM whereas IgM were only detected once with gray zone values and negative on all the other samples with Architect. For patient 12, the IgM detection on the Toxo-pGOLD platform was negative for the first positive Architect IgM sample. However, the follow-up sample tested late (195 days after the first sample) in this patient was IgM negative for both Architect and Toxo-pGOLD. Because of the time period between the 2 samples, it was impossible to determine if the detection of IgM on the Toxo-pGOLD platform was delayed or not detected at all for this patient. Taking together the IgG and IgM detection on the Toxo-pGOLD platform, all of the patients acutely infected (seroconversion samples) were correctly detected in timely manner by this platform. Thus the Toxo-pGOLD platform exhibits excellent performance in term of sensitivity, specificity and identification of seroconversion samples in adult population.

In newborns and infants population whom mother seroconverted during pregnancy, the Toxo-pGOLD platform detected 3 (Patients 14, 15, 16) out of 4 patients congenitally infected (Table 4). For one patient (patient 15), the negative IgG on the platform could have led to the false consideration that the newborn was non-infected but the positive IgM on the same sample confirmed the congenital infection. For patient 17

Table 4
Detailed results of IgG and IgM in newborns and infants population (24 samples) whom mothers seroconverted during pregnancy. Patients 14–17 were congenitally infected whereas patients 18–20 were not. Positive (P) and gray zone (GZ) results are in bold and gray, respectively. Negative results (N) are plain.

	Months	IgG Architect	IgG Toxo-pGOLD	IgM Architect	IgM Toxo-pGOLD	IgA	ISAGA IgM	WB IgG	WB IgM
Patient 14	0	**125.2 (P)**	**98.12 (P)**	**3.01 (P)**	**0.53 (P)**	**1.55 (P)**			
	0.5	**859 (P)**	**90.23 (P)**	**3.98 (P)**	**0.82 (P)**	**2 (P)**	**12 (P)**	Absence	**Presence**
Patient 15	0	**5.1 (P)**	4.57 (GZ)	0.24 (N)	0.20 (N)	0.22 (N)			
	0.5	2.2 (GZ)	2.98 (N)	**1.14 (P)**	**4.35 (P)**	0.36 (N)	**12 (P)**	Absence	**Presence**
	7	**9.6 (P)**	**9.86 (P)**	0.23 (N)	0.32 (N)	0.21 (N)			
Patient 16	0	**3.7 (P)**	5.20 (GZ)	**13.23 (P)**	**7.87 (P)**	**1.46 (P)**		Absence	**Presence**
	1	**146.3 (P)**	**74.71 (P)**	**11.13 (P)**	**4.43 (P)**	**2.45 (P)**	**12 (P)**	**Presence**	**Presence**
	2	**114.8 (P)**	**65.48 (P)**	**5.23 (P)**	**1.99 (P)**	0.94 (N)			
	6	**39.7 (P)**	**46.29 (P)**	0.18 (N)	**0.74 (P)**	0.21 (N)			
Patient 17	0	**30.7 (P)**	**30.45 (P)**	**0.76 (P)**	0.35 (N)	0.3 (N)			
	0.5	**21.5 (P)**	**20.74 (P)**	0.55 (GZ)	0.22 (N)	0.16 (N)	**5 (P)**	Absence	**Presence**
Patient 18	0	**28.1 (P)**	**33.61 (P)**	0.26 (N)	0.11 (N)				
	0.5	**22.7 (P)**	**24.12 (P)**	0.19 (N)	0.12 (N)	0.17 (N)	0 (N)	Absence	Absence
	2.5	**6.3 (P)**	**9.95 (P)**	0.19 (N)	0.20 (N)	0.15 (N)			
	4	1.5 (N)	5.71 (GZ)	0.19 (N)	0.11 (N)	0.06 (N)			
Patient 19	0	**27.7 (P)**	**19.94 (P)**	0.09 (N)	0.09 (N)	0.2 (N)			
	0.5	**26.2 (P)**	**19.83 (P)**	0.07 (N)	0.39 (N)	0.17 (N)	0 (N)	Absence	Absence
	1.5	**7.2 (P)**	**7.08 (P)**	0.07 (N)	0.20 (N)	0.2 (N)			
	3	**3.4 (P)**	2.98 (N)	0.1 (N)	0.09 (N)	0.24 (N)			
	6	0.6 (N)	0.95 (N)	0.09 (N)	0.29 (N)	0.1 (N)			
Patient 20	0	**25.6 (P)**	**38.51 (P)**	0.22 (N)	0.12 (N)				
	0.5	**12.5 (P)**	**19.57 (P)**	0.27 (N)	0.11 (N)	0.16 (N)	**3 (P)**	Absence	Absence
	2	**4.6 (P)**	**12.05 (P)**	0.2 (N)	0.46 (N)	0.21 (N)			
	4	1.4 (N)	3.26 (N)	0.13 (N)	0.23 (N)	0.08 (N)			

The following titers were considered positive, negative, or equivocal, respectively, in the various tests: IgG Toxo-pGOLD, ≥6.7, <4.5, 4.5–6.6; IgM Toxo-pGOLD Newborn Infant cutoff, ≥0.50; IgG Architect Abbott (IU/ml), ≥3, <1.6, 1.6–2.9; IgM Architect Abbott (index), ≥0.60, <0.50, 0.50–0.59, IgA Platelia BIO-RAD, ≥1, <0.8, 0.8–0.9, Toxo-ISAGA IgM bioMérieux cutoff when suspicion of congenital toxoplasmosis, ≥3.

(Table 4), the results on the pGOLD platform were not conclusive of a congenital infection. Based on only IgG and IgM Architect results, it would also be difficult to establish the diagnosis of congenital toxoplasmosis on the samples tested. Indeed, at birth (day 0), the positive IgM Architect test result could have been due to a contamination by maternal blood (Pomares and Montoya, 2016). The positive IgG and gray zone IgM Architect test results on follow-up sample collected at 15 days of life (0.5 months) also did not lead to a diagnosis of congenital toxoplasmosis. For this patient, the IgG and IgM tested on Toxo-pGOLD platform were positive and negative, respectively on both samples. In contrast, the positive ISAGA IgM (bioMérieux) and the neosynthesized IgM detected by Western blot were the only assays allowing the diagnosis of congenital toxoplasmosis for this patient. In the follow-up samples of newborns and infants tested in our study, the Toxo-pGOLD platform displayed similar performance as Architect. Taken together, this was the first time the Toxo-pGOLD platform was used for newborn and infant population. Similar to the IgM ISAGA (bioMérieux), the cutoff value for IgM test on Toxo-pGOLD platform needed to be adapted to the studied population. More samples should be tested in order to validate the pGOLD assay for newborns. The choice of other antigens for IgG and IgM detection on the Toxo-pGOLD platform could also improve the performance of the assay.

In addition to the very good agreement of the Toxo-pGOLD platform compared to clinical interpretation of the serological profile, the platform allowed detection of both IgG and IgM simultaneously in ~1 microliter of serum. The capacity of detection of these 2 antibodies subtypes in such a low volume of serum represents a major advantage in newborns and infants population where sampling can be significantly limited by volume. Several multiplexed platforms exist but can only detect IgG and IgM in 2 separate assays and the tests are of high cost, which makes their applications for the mass screening difficult (Guigue et al., 2014; Wang et al., 2016), as the cost of an assay is an important factor in mass screening (Stillwaggon et al., 2011). Based on the Disability Adjusted Life Years (DALYs), the screening strategy for *T. gondii* should be re-considered as the cost of illness of infection is high (Mangen et al., 2015; Scallan et al., 2015).

Currently under development, the Toxo-pGOLD platform is being investigated to expand its capacity to perform *T. gondii* avidity and detect simultaneously in addition to *T. gondii* other pathogens such as cytomegalovirus, rubella virus, herpes virus and human immunodeficiency virus in around ~1 microliter of serum. In addition to serum, the Toxo-pGOLD is being explored for its use in whole blood and saliva.

The Toxo-pGOLD platform is novel platform with a very good sensitivity and specificity in clinical conditions in different groups of French patients (acutely infected, chronically infected, non-infected patients and newborns and infants whom mothers seroconverted during pregnancy) for *T. gondii* IgG and IgM serology. In addition, the very low volume of sample required (~ 1 microliter of serum) represents a real

Table 5
Comparison of Toxo-pGOLD platform test results to the clinical interpretation in adult population.

		Corrected value according to the serological profile		Sensitivity % [95% CI]	Specificity % [95% CI]	Agreement % [95% CI]	K coefficient [95% CI]	PPV % [95% CI]	NPV % [95% CI]
		Positive	Negative						
IgG tested by Toxo-pGOLD	Positive	108	0	99.1% [94.3–99.9]	100% [95.8–100]	99.5% [97.1–100]	0.99 [0.98–1]	100% [95.7–100]	99.1% [94.4–99.9]
	Negative	1	111						
IgM tested by Toxo-pGOLD	Positive	48	3	96% [85.1–99.3]	98.2% [91.6–99]	97.7% [94.5–99.2]	0.94 [0.89–0.98]	94.1% [82.8–98.5]	98.8% [95.3–99.8]
	Negative	2	167						

CI = confidence interval.
PPV = Positive predictive value.
NPV = Negative predictive value.

advantage in clinical practice for patient sampling. With such good performances in clinical conditions, the Toxo-pGOLD platform is a strong candidate to be used as a screening test for *T. gondii* infection.

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Conflict of Interest

Pr. H. Dai is a scientific adviser of Nirmidas Biotech and served as a consultant for this work.

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