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1 **Could PLATELIA™ Toxo IgM be the new gold standard for the serological diagnosis of**
2 **congenital toxoplasmosis: a French multicenter study**

3
4 **Running title: PLATELIA Toxo IgM and congenital toxoplasmosis**

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25 **ABSTRACT**

26 **Objective:** To assess the performance of PLATELIA™ Toxo IgM (Biorad) and Toxo ISAGA®
27 (BioMérieux) to detect anti-Toxoplasma IgM in infants at risk of congenital toxoplasmosis.

28 **Study design:** A retrospective multicenter study comparing serologic results obtained in the
29 framework of routine diagnosis work-up for congenital toxoplasmosis. All infants born to
30 mothers infected with *T. gondii* during pregnancy from 2010 to 2020, with at least 6 months
31 of serologic follow-up were included (n=1010).

32 **Results:** One thousand and ten cases were included, of which 250 infants (24.75%) had
33 congenital toxoplasmosis. A total of 1039 sera were included. The concordance between the
34 two techniques was 96%, with kappa coefficient of 0.87, showing an almost perfect agreement
35 between ISAGA and PLATELIA. Cumulative sensitivity and specificity were 73.2% and 99.5%,
36 and 74.8% and 100%, for ISAGA and PLATELIA, respectively. The mean time to detect IgM using
37 ISAGA and PLATELIA tests was 6.9+/-20.1 days and 5.6+/-14.7 days, respectively (ns). Finally,
38 the sensitivity of ISAGA and PLATELIA to detect IgM antibodies in infected neonates at 5 days
39 of life was 62% and 64%, respectively.

40 **Conclusion:** Performances of PLATELIA™ Toxo IgM assay were comparable to the gold
41 standard ISAGA. This ELISA is suitable for routine serology for the diagnosis of congenital
42 toxoplasmosis in newborns.

43

44 **KEYWORDS:** congenital toxoplasmosis, *Toxoplasma gondii*, serology, IgM, neonatal diagnosis

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47

48 **Introduction**

49 Toxoplasmosis is a widespread zoonotic infection caused by *Toxoplasma gondii*, an obligate
50 intracellular protozoan parasite. Its prevalence varies widely according to geographical area
51 (1). *T. gondii* infection is acquired primarily through ingestion of cysts in infected undercooked
52 meat or oocysts that may contaminate soil, water, and food. Infection by *T. gondii* is typically
53 asymptomatic or subclinical in healthy humans. However, in case of primary infection during
54 pregnancy, the parasite may pass through the placenta and lead to congenital infection, with
55 a variable burden of disease in the world (2,3). The clinical severity for the fetus decreases and
56 the transmission rate increases as gestational age at the time of maternal infection progresses
57 (1,4). Thus fetal damage can range from asymptomatic to severe neurological alterations, such
58 as cerebral calcifications, ventricular dilatation, hydrocephalus and/or mental retardation,
59 and to retinal lesions with possible relapses lifelong (4).

60 In France, pregnant women benefit since 1992, from a monthly systematic screening if they
61 are seronegative until delivery, and from information on how to avoid infection. The objective
62 is to promptly identify and treat maternal infection in order to prevent fetal infection and
63 decrease sequelae after birth (3,5). Thanks to this prevention program, about 90% of
64 congenitally infected infants are asymptomatic at birth (6). As the risk of developing
65 retinochoroiditis during the first year of life or in early adulthood is high, infected infants must
66 be treated as soon as they are diagnosed infected (1). Indeed, in the absence of treatment,
67 some cohort studies showed that 15% to 80% of children with prenatal toxoplasmosis
68 developed lifelong ocular disease (7,8).

69 Prenatal diagnosis of congenital toxoplasmosis (CT) relies on the detection of parasite DNA by
70 PCR in amniotic fluid samples. Several studies (9–13) showed that about 90% of infected

71 fetuses were diagnosed by PCR. Thus, a negative result from PCR performed on amniotic fluid
72 cannot definitely rule out congenital infection.

73 For that reason, clinical and biological checkup of neonates must be performed, and
74 serological follow-up must be conducted during the first year of life, in the aim of starting
75 pyrimethamine - sulfadiazine therapy as soon as possible, to reduce the incidence of ocular
76 lesions and improve neurological outcome (14,15). Neonatal biological checkup usually relies
77 on the detection of parasite DNA in the placenta and/or amniotic fluid collected at delivery,
78 and serological tests (16,17). Serological examination relies on the detection of specific anti-
79 *Toxoplasma* IgM, IgA, and IgG antibodies, in the cord blood and serum of the newborn (9,18–
80 20). IgM detection is a simple pivotal test to make a diagnosis of congenital infection, provided
81 that it is confirmed by comparative western-blot showing a pattern different from the
82 mother's, when positive in cord blood (20). Western-blotting can also have added value by
83 detecting neosynthesized IgG in the infant' serum (21–23).

84 It is imperative that IgM assays used for the diagnosis of congenital toxoplasmosis be sensitive,
85 specific and exhibit high positive and negative predictive values (PPV, NPV) in order to
86 promptly identify infected newborns and start treatment. Until now, the Toxo ISAGA® assay
87 (BioMérieux, Marcy-l'Etoile, France) is considered the gold standard for the diagnosis of CT in
88 newborns, but it will be no longer marketed in 2024. Thus alternative methods are needed,
89 but few assays are validated on cord blood by manufacturers. The aim of this study was to
90 assess the compare the performance of PLATELIA™ Toxo IgM (PLATELIA) (Biorad, Marnes-la-
91 Coquette, France) and Toxo ISAGA® (ISAGA) for the diagnosis of CT .

92

93

94 **Materials and Methods**

95 **Ethics.**

96 All routine analyses were performed during routine work-up. Data were recorded
97 anonymously. The study was approved by the ethics committee of the CHU de Rennes
98 (approval number #23.39).

99

100 **Study population**

101 This is a retrospective multicentric study aiming at comparing serologic results obtained in the
102 framework of routine diagnosis of congenital diagnosis in Cochin-Port Royal Hospital (Paris,
103 France) and Rennes University Hospital (Rennes, France). All infants born to mothers infected
104 with *T. gondii* during pregnancy from 2010 to 2020 were included, if they benefited from
105 serologic follow-up for at least 6 months, allowing to exclude or confirm the diagnosis of CT.

106 Prenatal diagnosis of CT was performed by the detection of *T. gondii* DNA in amniotic fluid, 4
107 to 5 weeks after the estimated date of infection and after 18 weeks of amenorrhea, after
108 informed consent was granted. Amniotic fluid was tested for *Toxoplasma* by means of real-
109 time PCR assays, as previously described (17,24). At birth, all newborns were assessed for
110 possible congenital toxoplasmosis by clinical, neurologic, imaging and ophthalmologic
111 examinations, according to usual recommendations (1) and by the following serological tests:
112 detection of anti-*Toxoplasma* IgM, IgA and IgG antibodies, in cord blood and/or neonatal
113 blood by PLATELIA and ISAGA, comparison of the maternal and neonatal IgG and IgM antibody
114 patterns by WB (WB Toxoplasma IgG/IgM®, LDBio, Lyon, France), and detection of the parasite
115 in the placenta tissue or amniotic fluid at delivery. All serologic techniques were combined
116 during the first three months of life, then only IgG and IgM were monitored for at least six
117 months or until clearance of maternal IgG on two consecutive samples.

118 Congenital toxoplasmosis was defined by the presence of specific anti-*Toxoplasma* IgM and/or
119 IgA antibodies in the neonate serum > 3 days (D3) after birth, or persistent or increase of IgG
120 titers in infants at or beyond 1 year of age, or positive *T. gondii* qPCR in amniotic fluid.

121 The following information was collected for each case: date of birth, date of serum sampling,
122 results obtained with ISAGA and PLATELIA, trimester of maternal infection, result of prenatal
123 diagnosis when performed, outcome (infected or non-infected), period of CT diagnosis
124 (antenatal, at birth, during postnatal follow-up).

125

126 **Samples**

127 The first sample collected at birth (D0 and/or D3-5) was included for each neonate. When cord
128 blood sample was contaminated by maternal blood, as demonstrated by similar IgM profiles
129 obtained by paired WB analysis of mother's and cord blood, this one was excluded from
130 analysis, to the profit of the next newborn's sample collected at D3 or later. In some cases,
131 another sample could be included, if a positive IgM result was obtained during follow-up with
132 one or the other technique.

133

134 **Detection of anti-*Toxoplasma*-IgM**

135 Anti-*Toxoplasma* IgM was detected by the two methods, according to the supplier's
136 recommendations. The Toxo-ISAGA® assay is a manual semi-quantitative assay based on
137 immunoagglutination of whole *Toxoplasma* tachyzoites, needing an overnight incubation, and
138 is considered a reference method for IgM detection. According to the manufacturer's
139 instruction, newborn serum was diluted to 1/20 and incubated in three anti-IgM-coated wells,
140 for 2 hours at 37°C. After several washing steps, *Toxoplasma* antigen was added, then the
141 wells were covered and incubated overnight at 37°C. The agglutination of tachyzoites was

142 observed and rated from 0 (total sedimentation) to 4 (total agglutination) by comparison to
143 negative and positive controls, and the scores obtained for the three wells was summed up.
144 The usual threshold of positivity for adults is ≥ 9 , while rates of 6-8 are considered doubtful.
145 However, the manufacturer underlines that an index = 3 can be considered in favor of CT
146 (children ≤ 6 months). Therefore, a threshold index ≥ 3 was used in the present study.
147 The PLATELIA™ Toxo IgM is a quantitative immunocapture ELISA assay, using 96-well
148 microplates coated with anti-human μ -chains antibodies and the reaction steps were
149 automated using an Evolis® device (Bio-Rad), using the manufacturer protocol. Results are
150 expressed as an index (ratio relative to calibrators). An index < 0.8 is considered negative,
151 while an index ≥ 1.00 is considered positive for anti-*Toxoplasma* IgM. Values from 0.8 to 0.99
152 are considered equivocal (grey zone).

153

154 **Statistical methods**

155 Concordance test and kappa coefficient were used to assess agreement between the two
156 techniques for IgM antibody detection in all sera. The crude specificity was calculated on all
157 sera from uninfected infants (including the control sera in case of a previous positive result).
158 The sensitivity for the diagnosis of CT was calculated using the first positive sample obtained
159 with each technique. In case of negative IgM at birth, the mean time until positive result, if
160 any, was calculated for each technique. For easier comparison, all results in the grey zone, i.e.
161 Toxo-ISAGA® ≥ 3 and PLATELIA Toxo IgM® ≥ 0.8 , were considered positive.
162 Mean time to positive IgM detection was compared among techniques using t-test.
163 Proportions of IgM detection with each technique, by various patient categories were
164 compared using Chi-square test. Data were analyzed using GraphPad Prism software v6.

165

166 **Results**

167 One thousand and ten infants were included in the study, of whom 250 (25%) were diagnosed
168 with congenital toxoplasmosis. In total, 1039 sera were included for analysis, consisting of 275
169 sera and 764 sera from infected and non-infected infants, respectively.

170 The crude concordance between the two techniques was 96% (1003/1039) (Table 1), yielding
171 a kappa coefficient of 0.87, i.e. an almost perfect agreement between ISAGA and PLATELIA.
172 Most discrepancies (32/36) were observed in infected infants, for whom one or the other
173 technique was negative. A false positive IgM result was observed with ISAGA in four infants
174 on day 0 (D0), D3, D4 and D37, respectively (index 6, 6, 3 and 3, respectively), who were later
175 proven non-infected. These were true false positive results, as IgM were not detected using
176 western-blot assay. A control sample drawn 12 to 28 days later was negative. No false positive
177 results were observed using PLATELIA IgM. The specificity of ISAGA and PLATELIA was 99.5%
178 and 100%, respectively (Table 2). Among the 250 infected babies (275 sera), 200 (80%) had
179 anti-*Toxoplasma* IgM detected with any technique at any time; 15 out of 200 (7.5%) infants
180 were ISAGA-positive/PLATELIA-negative, while 17 (8%) were ISAGA-negative/PLATELIA-
181 positive during follow-up. In two cases with discordant results at first sampling, the technique
182 yielding initial false-negative result (both with PLATELIA) turned out positive on a next sample
183 obtained at day 13 and 42, respectively. In 13 neonates with no IgM detection at first sampling,
184 both techniques turned positive simultaneously on the next sample. The overall sensitivity of
185 ISAGA and PLATELIA during follow-up was 73.2% and 74.8%, respectively (Table 2). The
186 negative and positive predictive values are also presented in Table 2.

187 In 5/15 samples yielding a false negative result with PLATELIA only, the IgM index was close to
188 the grey zone threshold (>0.6), while only 2/765 sera from non-infected neonates had a
189 negative result with an index >0.6 , suggesting that such results close to the PLATELIA threshold

190 should trigger tightened follow-up of newborns. Of the 17 samples with an isolated false
191 negative ISAGA result, only two sera had an index value different from 0 (1 and 2,
192 respectively).

193 The diagnostic performance at birth, i.e. at ≤ 5 days of life, was evaluated on the earliest
194 samples available. The sensitivity of ISAGA and PLATELIA for the early diagnosis of congenital
195 toxoplasmosis was 62% (155/250) and 64% (159/250), respectively (Table 3).

196 The mean time to detect IgM (when positive) in infected neonates during follow-up, with
197 ISAGA and PLATELIA was 6.9 +/- 20.1 days and 5.6 +/- 14.7 days, respectively ($p=0.56$). In all
198 but one case, IgM were detected within the first three months of life; in the remaining case,
199 only ISAGA yielded a positive result (index=6) at six month.

200 Among the 200 infected babies with positive IgM detected during serological follow-up, 166
201 (83%) and 169 (84.5%) were diagnosed within one month after birth with ISAGA and PLATELIA,
202 respectively (Table 4). Overall, the time to IgM detection did not differ for PLATELIA and ISAGA
203 ($p=0.972$, Table 4).

204 Besides, there was no differences in the frequency of IgM detection with ISAGA and PLATELIA
205 according to the trimester of maternal infection ($p= 0.696$), nor according to the result of
206 prenatal diagnosis ($p=0.724$) (Table 4).

207 IgM were undetected in 32 (46%) cases and 39 (56%) cases with PLATELIA and ISAGA, for 70
208 maternal infections with positive prenatal diagnosis, which occurred during the 1st or 2nd
209 trimester. By contrast, in 27 cases of maternal infection during the third trimester and positive
210 prenatal diagnosis, PLATELIA and ISAGA were negative in 8 and 9 cases, respectively.

211

212

213 **Discussion**

214 Biological and clinical investigations to diagnose congenital infection must be performed at
215 birth, when maternal primary toxoplasmosis occurred during pregnancy, or when newborns
216 present with clinical features suggestive of congenital toxoplasmosis, such as retinal lesions,
217 intracranial calcifications, hepatosplenomegaly, or cerebral ventricular dilation, when the
218 mother didn't benefit from serologic screening.

219 The added value of postnatal diagnosis is primarily to detect infected neonates who were not
220 diagnosed by prenatal diagnosis (25). This can occur in about 10% of cases, as a result of low
221 parasite concentrations or delayed transfer of the parasite across the placenta. Here, 10 cases
222 (4%) had a false negative antenatal result, half of them had IgM detected during follow-up,
223 with no significant difference according to the assay. On the other side, analysis of amniotic
224 fluid may not be performed when serological seroconversion occurred at a late stage of
225 pregnancy, representing 22 to 52% of cases, according to case series reported in the literature
226 (1), and 139/250 (56%) cases in the present study. For that reason, post-natal serological
227 follow-up of infants is essential to monitor the clearance of maternal anti-*Toxoplasma* IgG and
228 the absence of anti-*Toxoplasma* IgM, to rule out congenital toxoplasmosis (26,27).

229 IgM detection must be sensitive, but above all, very specific, in order to safely prompt early
230 treatment in infected newborns and improve prognosis (28,29). Here, we observed four false
231 positive results with ISAGA, which could be related to the difficulty of reading and scoring the
232 agglutination in the wells.

233 Sensitivity of IgM detection can be an issue, as many series published in the literature account
234 for a sensitivity ranging from 44% to 87% and from 44% to 81%, using ISAGA or ELISA assays,
235 respectively (20). The lack of IgM detection could be at least partially attributed to maternal
236 treatment (25,30). In the present study, we could detect IgM in 73.2% and 74.8% of infected

237 infants with ISAGA and PLATELIA, respectively, which is in agreement with other studies
238 evaluating postnatal diagnosis (18,30,31). As already shown in other studies, the rate of IgM
239 detection was higher when maternal infection occurred during the third trimester of
240 pregnancy, but was not linked to the IgM assay (25,32). We previously showed that IgM were
241 more often undetected using ISAGA than PLATELIA, when the mother received anti-
242 *Toxoplasma* antenatal therapy (25). Indeed, out of 70 infants born to 54 treated and 16
243 untreated mothers, the sensitivity of ISAGA was 51.9% and 81.2%, respectively ($p < 0.05$), but
244 that of PLATELIA was not significantly altered. Here, ISAGA also appeared to be more likely
245 negative in the neonate when the mother received prolonged pyrimethamine-sulfadiazine
246 therapy, i.e. when infection occurred during the first or second trimester of pregnancy, but
247 the current study was not designed to evaluate the impact of treatment.

248 In the literature, ISAGA is commonly recommended for IgM testing in young infants, because
249 i) it is considered a gold standard for the diagnosis of CT, ii) it is blood-sparing, as it needs only
250 10 μ L serum, and iii) it is very well-suited for small series. In the perspective of end of
251 commercialization of Toxo-ISAGA[®] by bioMérieux, it is important to validate which technique
252 could replace it for the diagnosis of congenital toxoplasmosis. Some old studies reported that
253 ISAGA was more sensitive than immunoenzymatic methods for the detection of IgM
254 antibodies (21,32). However, the present study showed that the PLATELIA[™] Toxo IgM assay
255 had a very similar sensitivity and a 100% specificity, thus could be a valuable alternative.
256 Additionally, it also requires only 10 μ L serum, if the sample is diluted manually. Overall, the
257 concordance of both techniques was very good, and the precocity of IgM detection with
258 PLATELIA was at least as good as with ISAGA (64% versus 62%, respectively, at <5 days of life).
259 Additionally, our results highlighted that IgM index close to the grey zone threshold (>0.6), is
260 highly suggestive of congenital infection and should trigger tightened follow-up of newborns.

261 A recent study by Avignon et al. (31) retrospectively evaluated PLATELIA™ Toxo IgM among
262 five assays, in 432 infants, and showed that it had the best sensitivity, and a specificity
263 comparable to ISAGA. Our study included 1010 infants and allowed to draw strong conclusions
264 about the high performances of PLATELIA™ Toxo IgM.

265 In conclusion, sensitivity and specificity of PLATELIA Toxo-IgM observed in this study on an
266 important cohort of duly followed-up infants, mainly born to treated mothers, were
267 comparable to the gold standard ISAGA. Complementary studies could be undertaken to
268 confirm the high performances of PLATELIA in neonates from untreated mothers, although
269 IgM are usually more easily detected in such situation.

270 While Toxo-ISAGA® is an expensive and manual technique, with an overnight time-to-result,
271 which requires advanced expertise for scoring and is usually reserved to reference centers for
272 toxoplasmosis, PLATELIA-Toxo IgM® can be used for routine diagnosis on a 96-well automated
273 device, and is well-adapted for clinical laboratories to provide a result in about 5 hours. Thus,
274 PLATELIA-Toxo IgM® is highly suitable for routine diagnosis of congenital toxoplasmosis in
275 newborns.

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392 **Table 1.** Results of anti-Toxoplasma IgM detection by ISAGA and PLATELIA in 1010 newborns
393 (N=1039 sera).

		PLATELIA		
		Negative test	Positive test	Total
ISAGA	Negative test	833	17	850
	Positive test	19 ^a	170	189
Total		852	187	1039

394 ^a of which 4 were false positive results

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397 **Table 2.** Performance of IgM detection using ISAGA and PLATELIA assays for the diagnosis of
398 congenitally-infected (N = 250) and uninfected (N = 760) infants

	ISAGA	PLATELIA
Sensitivity % (n/N)	73.2 (183/250)	74.8 (187/250)
Specificity % (n/N)	99.5 (756/760)	100 (760/760)
PPV % (n/N)	97.8 (183/187)	100 (187/187)
NPV % (n/N)	97.8 (756/773)	98 (760/775)

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402 **Table 3.** Sensitivity and specificity of ISAGA and PLATELIA tests for diagnosing CT within 5 days
403 after birth

Technique	Sensitivity \leq 5 days of life % (n/N)	Specificity \leq 5 days of life % (n/N)
ISAGA	62 (155/250)	99.7 (758/760)
PLATELIA	64 (159/250)	100 (760/760)

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407 **Table 4.** IgM detection with ISAGA and PLATELIA according to the trimester of maternal

408 infection (N= 250 cases)

	IgM positive ^a (ISAGA)	IgM positive ^a (PLATELIA)	p-value ^c
Trimester of infection, n/N			
First trimester (n=10)	5	3	0.696 (ns)
Second trimester (n=92)	47	56	
Third trimester (n=129)	114	111	
Unknown ^b (n=19)	17	15	
Time to positive result			0.972 (ns)
≤5 days	155	159	
6 to 30 days	11	10	
31 to 60 days	13	13	
>60 days	4	3	
Prenatal diagnosis			0.724 (ns)
Positive (n=101)	53	60	
Negative (n=10)	5	4	
Not done or unknown (n=139)	124	119	

409 ^a Only the 1st positive sample was considered410 ^b Of which 2 cases were consecutive to reinfection or reactivation411 ^c Chi-square test

412 ns; not significant

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