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1 **Evaluation of the *Toxoplasma* ELITE MGB[®] real-time PCR assay for the diagnosis of**
 2 **toxoplasmosis**

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24 **Running title:** Commercial PCR assay for toxoplasmosis diagnosis

25

26 **ABSTRACT**

27

28 Molecular diagnosis of congenital toxoplasmosis or disseminated toxoplasmosis is mainly
29 based on PCR. The repeated DNA element rep529 has become the main DNA target used in
30 most, whether laboratory-developed or commercial, PCR methods. In this multicenter study,
31 we evaluated the *Toxoplasma* ELITE MGB® (Elitech®) commercial kit, by comparison with
32 three reference quantitative PCR assays (RA) used in routine in three proficient laboratories
33 of the French National Reference Center for Toxoplasmosis network, using *Toxoplasma*
34 calibrated suspensions diluted to obtain a range of concentrations from 0.1 to 10,000
35 parasites/mL. These suspensions were extracted either with the DNA extraction kit
36 (EXTRAblood®, Elitech®) recommended by the manufacturer or the QIAamp DNA-minikit
37 (Qiagen). The *Toxoplasma* ELITE MGB® assay was also evaluated on a panel of 128 clinical
38 samples, including 56 amniotic fluid, 55 placenta, and various other samples, of which 95
39 originated from patients with proven toxoplasmosis. The ELITE MGB assay amplified less
40 frequently low-concentration replicates (<10 parasites/mL) of calibrated suspensions, than
41 the RA of 2/3 laboratories. Additionally, the combination EXTRAblood® / *Toxoplasma* ELITE
42 MGB® yielded poorer sensitivity than the combination QIAamp DNA-minikit/ ELITE MGB® for
43 low parasite concentrations ($p < 0.001$ for 1 parasite/mL). On clinical samples, the sensitivity
44 and the specificity of the commercial assay were 89% and 100%, respectively. The sensitivity
45 ranged from 79% to 100% with placenta and amniotic fluid samples, respectively. Overall,
46 this study shows that the *Toxoplasma* ELITE MGB® assay is suitable for the diagnosis of
47 toxoplasmosis from non-cell-rich or non-hemoglobin-rich samples, and that the
48 EXTRAblood® kit is not optimal.

49 **INTRODUCTION**

50 Toxoplasmosis is a worldwide parasitic disease due to the intracellular coccidian parasite
51 *Toxoplasma gondii*. Molecular diagnosis is an essential tool for the diagnosis of congenital
52 toxoplasmosis, as well as of acute disease in immunocompromised patients with primary
53 infection or reactivation of past infection (1). Prenatal diagnosis of congenital toxoplasmosis
54 relies on *Toxoplasma* DNA detection in amniotic fluid (AF), and has been largely evaluated in
55 field studies, particularly in French series, as a national prevention program implemented in
56 1992 requires a monthly serologic follow-up of seronegative pregnant women and
57 recommends the realization of amniocentesis when a primary infection is documented. In
58 France, 21 University Hospitals have a ministerial agreement for the prenatal diagnosis of
59 congenital toxoplasmosis. Since several years now, all of them have moved for the use of
60 real-time PCR (rtPCR) methods targeting the repeated DNA element *rep529* (GenBank
61 accession number AF146527), as the sensitivity provided using this DNA target has proved
62 higher than that of the formerly used B1 gene in most studies (2-5). About three quarters of
63 these reference laboratories still use 'in-house' or laboratory-developed rtPCR techniques
64 which have been evaluated in clinical studies; but there is an increasing trend to use
65 commercial assays, which, in spite of being more expensive, are easier to use and allow a
66 better quality management than the former. The manufacturers of these kits announce a
67 sensitivity threshold, but the performance of these assays may be altered by the tested
68 sample type or the DNA extraction method used. Indeed, *Toxoplasma* may be searched not
69 only for the prenatal diagnosis of congenital toxoplasmosis, but also in
70 immunocompromised patients in other sample types, such as blood, broncho-alveolar
71 lavage fluid, cerebrospinal fluid (CSF), aqueous humor (AH) or various biopsies (6). In view of
72 the globally excellent performances of 'in-house' methods in proficient diagnostic centers

73 (7), the evaluation of the analytical and clinical performances of commercial kits is absolutely
74 needed to ensure the quality of results in routine use. Moreover, some commercial assays
75 are validated for *Toxoplasma* detection in AF, but not in other sample types. In this
76 multicenter study, we evaluated the performances of the *Toxoplasma* ELITE MGB® kit
77 (Elitech®, Puteaux, France), a *rep529*-targeting assay, using serial dilutions of calibrated
78 *Toxoplasma* suspensions in AF, and clinical samples including AF, placenta and various other
79 types of samples.

80

81 **MATERIALS & METHODS**

82 **Participating centers**

83 The three participating centers (University Hospitals of Paris-Cochin, Rennes and Grenoble,
84 France) are part of the Molecular Biology workgroup of the French National Reference
85 Center for Toxoplasmosis (NRCT: <http://cnrttoxoplasnose.chu-reims.fr/?lang=en>), and have a
86 ministerial agreement for the prenatal diagnosis of toxoplasmosis. They participate to
87 national external quality controls on a regular basis with satisfactory results.

88

89 **Samples**

90 ***Calibrated Toxoplasma suspensions***

91 The three participating centers used a calibrated *Toxoplasma* (type II) suspension produced
92 by the Molecular Biology workgroup of the NRCT (University Hospital of Montpellier)(8).
93 DNA was previously extracted from the suspension in each center using their routine
94 method for molecular diagnosis (here, QIAamp DNA mini-kit®, Qiagen, Les Ulis, France). The
95 three labs compared the sensitivity of detection of serial dilutions (from 10,000
96 tachyzoites/mL to 0.1/mL) after amplification using their own in-house PCR method (so-

97 called reference assay (RAs, Table 1) and the *Toxoplasma* ELITE MGB® kit, following the
98 manufacturer's instructions. All dilution points were amplified in triplicate or quadruplicate.
99 Another batch of calibrated suspension was extracted in parallel, using either the manual
100 extraction device recommended by the manufacturer, i.e. EXTRAblood® (Elitech), or with the
101 QIAamp DNA mini-kit®. In this experiment, the internal control was added at the time of
102 extraction, following the manufacturer's instructions (see below).
103 Additionally, one center also evaluated a batch of 10 samples of external quality controls
104 (EQC) from QCMD® (2014). This QC batch (TGDNA 14) consisted of 5 vials of lyophilized
105 amniotic fluid samples spiked with various concentrations of *T. gondii* or unspiked
106 (negative), and 5 vials of lyophilized plasma samples spiked with various concentrations of *T.*
107 *gondii* or unspiked (negative), and was extracted using EXTRAblood® (Elitech).

108

109 **Clinical samples**

110 This part of the work used stored DNA from clinical samples obtained during routine
111 molecular diagnosis (2005-2015). The three reference laboratories selected *Toxoplasma*-
112 positive and -negative DNA preserved at -20°C or -80°C following routine molecular diagnosis
113 (9). Thanks to the French prevention program for congenital toxoplasmosis, reference
114 centers have a collection of samples (mainly AF and placenta samples) from patients with
115 confirmed diagnosis. As already evaluated in previous studies, long-term storage of DNA at -
116 20°C or below does not alter the result of *Toxoplasma* real-time PCR (3, 9), thus allowing the
117 use of collections for diagnostic evaluations. Placenta and AF DNA samples from fetuses with
118 suspected congenital toxoplasmosis were classified as true-positive or true-negative on the
119 basis of the newborn serological follow-up (detection of specific IgM or IgA, and comparison
120 of mother and newborn antibody profiles by western-blotting during a one-year follow-up),

121 so that the diagnosis could be definitely confirmed or ruled out, as previously described (10-
122 13). The Elitech assay was certified CE-IVD in 2013 and validated for amniotic fluid samples
123 and whole blood, thus we considered that placenta samples were comparable to blood
124 samples. Other samples were collected from patients with retinochoroiditis or from
125 immunocompromised patients, for whom the clinical diagnosis was recorded (disseminated,
126 cerebral, or toxoplasmosis excluded). Overall, 128 DNA samples were included: 55 isolated
127 from placentas, 56 from AF, 4 from AH, 6 leukocyte pellets isolated from buffy-coats, 2 from
128 biopsies, and 5 from CSF. Of these, 33 samples were classified as PCR-negative and 95 as
129 PCR-positive, according to serological and clinical follow-up of patients.

130

131 **Molecular techniques**

132 ***DNA extraction of clinical specimens***

133 Clinical specimens were processed in the setting of routine diagnosis. After appropriate pre-
134 analytical steps (centrifugation of fluids, buffy-coat, pre-digestion of placenta or biopsies
135 with proteinase K), 200 μ L of clinical samples were extracted using the QIAamp DNA mini-
136 kit[®] according to the manufacturer's instructions, and eluted in 100 μ L (all three centers
137 used the same technique for routine diagnosis).

138 ***DNA extraction of Toxoplasma calibrated suspensions***

139 In each center, the same batch of *Toxoplasma* calibrated suspension was extracted using
140 QIAamp DNA mini-kit[®] and amplified in parallel with the RA and the ELITE MGB assay. In
141 one center, one batch of calibrated suspension was also extracted using EXTRAblood[®], with
142 or without adding 5 μ L of internal control (IC)(CPE-DNA Internal Control, Elitech[®]), following
143 the manufacturer's instructions. Briefly, 200 μ L of samples were mixed with 25 μ L of
144 proteinase K, 200 μ L of lysis buffer, 10 μ L of carrier RNA and 5 μ L of IC, incubated at 70°C for

145 10 min, then centrifuged for 5 sec at 11,000 rpm. After addition of 210 μ L of absolute
146 ethanol and a brief centrifugation, the lysate was loaded into a column. After several
147 washing steps, DNA was eluted in 60 μ L of buffer. Quality control samples (QCMD[®] 2014)
148 were also extracted using this technique.

149

150 ***DNA amplification***

151 Reference methods

152 The in-house methods used by the three participants targeted the *rep529* sequence and has
153 been previously evaluated and published (5, 8, 10, 14). Technical details can be found in
154 Table 1. All three RA satisfy annual external quality control program, managed by the
155 National Reference Center for Toxoplasmosis (Centre Hospitalier Universitaire de
156 Montpellier). All DNA from clinical samples were re-amplified in parallel with the RA method
157 and the ELITE MGB[®] assay. Newly-extracted *Toxoplasma* suspensions were amplified with
158 both techniques.

159 *Toxoplasma* ELITE MGB[®] method

160 The *Toxoplasma* ELITE MGB[®] assay was performed according to the manufacturer's
161 instructions, using 10 μ L of the DNA template and 20 μ L of mix. When the Elitech IC was not
162 added during extraction (calibrated suspensions or clinical samples previously extracted
163 using the QIAamp DNA mini-kit[®]), 2 μ L of Elitech IC was added in 10 μ L of template DNA, and
164 10 μ L of this solution were used for amplification, as suggested by the manufacturer.

165 The assay has been validated only on Applied Biosystems devices. In this study, ELITE MGB[®]
166 amplification was performed using a StepOnePlus[®] device or ABI[®] Prism 7000
167 (ThermoFisher), and the following program: 2 min at 50°C, 2 min at 94°C, and 45 cycles of 10
168 sec at 94°C, 30 sec at 60°C and 20 sec at 72°C. Each clinical sample was analyzed in a single

169 reaction, as well as the QCMD® EQC DNA. If the result was not concordant with that of the
170 previous routine diagnosis, a second PCR was performed with the Elitech® method and the
171 in-house PCR. The LOD (95% sensitivity) announced by the manufacturer in whole blood
172 samples is 34.29 or 88.72 *T. gondii* /mL, and 5.47 or 1.91 *T. gondii*/extraction in AF samples,
173 using manual extraction (EXTRAblood®, Elitech) or automated extraction (NucliSENS
174 EasyMAG®, BioMérieux), respectively.

175 Performance score

176 For each technique, a performance score was calculated from the results obtained with
177 calibrated suspensions, as follows: number of positive replicates/total number of
178 amplifications.

179

180 **Statistical analysis**

181 The Mann-Whitney test was used to compare the mean Ct obtained with qPCR assays for
182 the calibrated *Toxoplasma* suspensions. When the number of positive replicates was below
183 three, a two-way ANOVA analysis was used instead.

184 For clinical samples, the analysis of qualitative results obtained with both techniques was
185 analyzed using a Chi-square test or a Fisher's exact test.

186 Statistical analysis was made using GraphPad® Prism V5 (GraphPad software, USA). P < 0.05
187 was considered significant.

188

189 **RESULTS**

190 ***Comparative testing using Toxoplasma calibrated suspensions***

191 The first step of the study was to determine the PCR performance scores using serial
192 dilutions of calibrated *Toxoplasma* DNA suspension. PCR performance scores were

193 calculated as described elsewhere (7, 15), using the *T. gondii* (Tg) DNA serial dilution assay,
194 and are reported in Table 2. In all three centers, the scores obtained using the in-house
195 method and the Elitech® assay were close, but the RA method had a higher score than the
196 commercial assay in two out of three centers ($p < 0.01$, Table 2). Taken together, the lowest
197 parasite concentrations, i.e. “1” and “0.1” *Toxoplasma*/mL, were inconstantly amplified. For
198 “1 T.g./mL, the difference was statistically significant (5 positive out of 10 replicates and 8
199 out of 10 replicates with ELITE MGB and RAs, respectively)($p < 0.01$).

200 The mean cycle threshold (Ct) of amplification obtained with *Toxoplasma* ELITE MGB® was
201 calculated for concentrations of calibrated suspensions between 1 and 10,000 Tg/mL, and
202 the results were compared for the two DNA extraction methods. Surprisingly, the mean Cts
203 obtained with the combination EXTRAblood®/Elite MGB® (recommended by the
204 manufacturer) were significantly higher than those obtained with the combination QIAamp
205 ®/Elite MGB® (29.2±0.17 versus 27.8±0.18, $p < 0.05$ for 100 Tg/mL and 39.3±0.07 versus
206 34.6±0.4, $p < 0.001$ for 1 Tg/mL) (Figure 1).

207

208 ***Comparative testing using clinical samples***

209 When using clinical samples, the concordance between the in-house method and the Elite
210 MGB® assay was 92% (118/128). All negative DNAs were tested negative (33/33); but false
211 negative results were obtained using the Elite MGB® kit as compared with the RAs for 10 out
212 of 95 positive samples, yielding a specificity of 100% and a relative sensitivity of 89.5% for
213 the kit. This sensitivity was 100% for AF (38/38) and other fluid samples (AH, CSF), but only
214 79% (34/43) for placenta and 80% (4/5) for buffy coat samples (Table 3). In 9/10 cases, the
215 false negative results were obtained for placenta samples with high Ct values (>37). To rule
216 out PCR inhibition, samples were diluted to 1/10th and re-tested. Additionally, as the nature

217 of the Elitech® IC was not known and could be suspected to interfere with small amounts of
218 parasite DNA, these false negative samples were also tested without IC. Amplification was
219 restored from plain DNA in the absence of IC (2 cases, with Ct of 39.7 and 40.7) and after
220 dilution to 1/10th and absence of IC in one case (Ct = 36.7). No amplification was observed
221 after dilution to 1/10th when IC was not removed.

222 Overall, the sensitivity for fluid samples was higher than for cellular samples (100% versus
223 80%, $p < 0.01$)(Table 3).

224 Finally, the qualitative results obtained using the QCMD® 2014 quality control samples were
225 concordant for the RA1 and Elite MGB® assays (7/7 positive samples and 3/3 negative
226 samples; Table 3). Mean Cts obtained with both techniques were not statistically different
227 ($p = 0.8$, data not shown).

228

229 ***Influence of IC on amplification performances***

230 As the observation above suggested a competing effect of the IC, another series of DNA
231 extractions of a calibrated *Toxoplasma* suspension using different conditions was launched
232 to confirm this hypothesis. It appeared that DNA extraction with EXTRAblood® (with 5 µL of
233 IC added in the sample as recommended) followed by amplification with Elite MGB®
234 performed as well as extraction with EXTRAblood® followed by addition of IC at the time of
235 amplification with ELITE MGB® (Figure 2A). Surprisingly, at the “1 Tg/mL” concentration, the
236 mean Ct was significantly lower when the IC was withdrawn from the extraction and
237 amplification steps than that obtained after extraction with EXTRAblood® including IC (33.57
238 ± 0.93 versus 38.75 ± 1.18 , $p < 0.001$) (Figure 2A). Similarly, the addition of the IC to the mix of
239 amplification also led to an increase of the mean Ct at the “1 Tg/mL” concentration (33.57
240 ± 0.93 versus 37.97 ± 0.007 , $p < 0.001$). In another experiment, both extraction methods

241 (QIAamp DNA mini-kit® and EXTRAblood®) were compared using the same amplification
242 method (RA1). Whatever the parasite concentration, Ct values were much lower when DNA
243 was extracted with the QIAamp DNA mini-kit® than with EXTRAblood® (36.62 ± 1.38 versus
244 45, and 21.52 ± 0.08 versus 25.35 ± 0.2 , for the lowest and highest parasite concentrations,
245 respectively; $p < 0.001$) (Figure 2B).

246

247 **DISCUSSION**

248 In the diagnosis of congenital toxoplasmosis, a high sensitivity of the PCR assay is needed, as
249 parasite loads in amniotic fluids from congenitally infected infants are frequently as low as
250 10 tachyzoites/mL or less (16). Early treatment of pregnant women who benefit from
251 serological screening in France, could account for such low parasite loads (17). High
252 performance of molecular assays is also a key issue in immunocompromised patients, for
253 whom rapid and accurate diagnosis is essential. Furthermore, toxoplasmosis is of increasing
254 importance in HIV-negative immunocompromised patients, due to the growing number of
255 transplantations and to the use of immunosuppressive drugs for the treatment of chronic
256 inflammatory diseases (6). Here, when applied to calibrated DNA samples, the *Toxoplasma*
257 ELITE MGB® assay showed similar performance as the three laboratory-developed reference
258 methods down to 10 T.g/mL, but the lowest concentrations were inconstantly detected
259 (Table 2), despite a higher DNA input into the amplification reaction (10 µL) than in the
260 reference methods (Table 1).

261 When using clinical samples, the *Toxoplasma* ELITE MGB® assay showed a 100% sensitivity
262 for amniotic fluids, and yielded congruent results on a batch of external quality controls
263 (QCMD® 2014). Although the kit is commercialized, i.e. validated, only for amniotic fluids

264 and whole blood samples, we included other sample types which are frequently sent to
265 laboratories for diagnosis, such as aqueous humor or CSF. We also included placenta
266 samples, which offer the advantage to mimic blood samples and to be linked to a clinical
267 history and follow-up of neonates, thus can be definitely classified as true-positive or not.
268 The sensitivity for other fluid samples was as good as for AF, although the number of
269 samples included was small. However, the sensitivity was much lower for other sample
270 types, in particular placenta samples. These results show that this commercial assay
271 performs better on samples with low cellularity or reduced contamination with red blood
272 cells, which confirms the lower sensitivity using whole blood, reported by the manufacturer
273 himself. As routinely done in the three reference laboratories, when a PCR inhibition is
274 suspected from the observation of high Ct values with their IC, samples were re-tested after
275 sample dilution. This strategy was thus applied to the false-negative samples obtained with
276 the ELITE MGB assay, but it didn't fit for amplification restoration. Additionally, as the nature
277 of the Elitech® IC was not known and could be suspected to interfere with small amounts of
278 parasite DNA, these false-negative samples were also tested without IC. This allowed to
279 restore *Toxoplasma* amplification in 30% of these samples, thus demonstrating that
280 competition of the Elitech IC could partially explain the false negative results. The inhibitory
281 effect of the Elitech® IC was definitely confirmed using low concentrations of *Toxoplasma*
282 calibrated suspensions (Figure 2A). However, the technical instructions from the
283 manufacturer do not mention that the reactions should be performed in duplicate, plain and
284 diluted, or with and without IC, as this significantly augments the cost of the test. With
285 respect to the interpretation of IC amplification, it is stated in the manufacturer's
286 instructions that a Ct \leq 35 is suitable for a correct interpretation of results. During our

287 assays, the Ct obtained for IC was always < 30, thus it could be reasonably considered that
288 the test was valid; yet false negative results have been found.

289 As the DNA extraction method is also known to influence the performance of PCR
290 amplification (18), we decided to compare the Ct results of calibrated parasite suspensions,
291 extracted using either the extraction kit (EXTRAblood®) as recommended by the
292 manufacturer, or the QIAamp DNA blood mini-kit®, as routinely done in the three labs.
293 Subsequent amplification with the RA1 showed that the combination QIAamp®
294 extraction/RA1 performed much better than the combination EXTRAblood®/RA1 (Figure 2B),
295 stressing the need to evaluate the extraction and PCR methods together, as emphasized
296 earlier (19). Moreover, the combination EXTRAblood®/Elite MGB (recommended by the
297 manufacturer) performed significantly worse than the combination QiaAmp®/Elite MGB to
298 amplify low parasite concentrations (Figure 1), thus should be avoided. The evaluation of the
299 combination extraction / amplification methods is of peculiar importance for off-label use of
300 commercial PCR assays, particularly with cell-rich samples types, as its efficiency is
301 determining to detect very low parasite amounts. Furthermore, the validation of the entire
302 process is necessary to meet the requirements of quality assurance system.

303 Regarding the handiness and good laboratory practices, the mix provided in the kit contains
304 uracil-*N*-glycosylase (UNG) to limit carryover contaminations from previously amplified PCR
305 products. The kit does not include a standard to perform a curve to quantify parasite loads.
306 The PCR assay, the internal control and the positive control are purchased in three separate
307 vials, thus the management of batch traceability is not easy.

308 Several qPCR commercial assays are available to date to detect *Toxoplasma* DNA, but very
309 few have been evaluated using clinical samples or in routine use. A previous multicenter

310 study conducted by the Molecular Biology Group of the French NRCT evaluated another
311 commercial kit by Bio-Evolution® using 157 amniotic fluid samples, and found a 99%
312 concordance for *T. gondii*-infected samples (13). In the present study, we enrolled a smaller
313 amount of amniotic fluid samples (56), but the *Toxoplasma* ELITE MGB® assay showed also a
314 good concordance using these samples.

315 Taken together, this study showed that the *Toxoplasma* ELITE MGB® assay appears suitable
316 for prenatal diagnosis. However, a note of caution is in order when using cell-rich or
317 hemoglobin-rich samples, as this carries the risk of false negative results. In our hands, the
318 use of the EXTRAblood® DNA extraction lowered the performance of the *Toxoplasma* ELITE
319 MGB® assay for low parasite concentrations, and dramatically lowered the sensitivity of the
320 in-house PCR method tested. Like for all molecular diagnostic methods, clinical
321 microbiologists who would aim at implementing this technique in their laboratory should
322 evaluate the combination of extraction and amplification methods before changing one or
323 the other.

324

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329

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339

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410 **LEGENDS TO FIGURES**

411 **Figure 1:** Evaluation of the *Toxoplasma* Elite MGB® kit using serial dilutions of *Toxoplasma*
412 calibrated suspensions. Calibrated suspensions were extracted using either QIAamp DNA
413 mini-kit® or Extrablood®, before being amplified using *Toxoplasma* Elite MGB®.
414 Amplifications were performed in triplicate. Data are expressed as means ± SEM; *, p<0.05,
415 ***, p<0.001.

416

417 **Figure 2:** Evaluation of the impact of the internal control (IC) and of the extraction method
418 on amplification efficacy. (A) Comparison of the Ct of amplification obtained with Elite MGB®
419 PCR on DNA extracted with EXTRAblood®, with addition of internal control (+IC) in the
420 sample before extraction, or without internal control (-IC), or after addition of IC in DNA at
421 the time of amplification; (B) Comparison of the Ct obtained with the reference assay 1
422 (RA1) after DNA extraction with EXTRAblood® or with QIAamp DNA mini-kit®. Amplifications
423 were performed in triplicate; data are expressed as means ± SEM; **, p<0.01, ***, p<0.001.

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429 **Table 1:** Technical description of the four PCR methods used

430

Technique	DNA target	Type of probe	DNA input (μ L)	Internal control	No of cycles	PCR device
RA1	rep529	Taqman (FAM-TAMRA)	5	Universal extraction & inhibition DNA control (Diagenode®)	40	StepOnePlus® (ThermoFisher)
RA2	rep529	Taqman	5	non-competitive exo- genous DNA inserted in PCR 2.1 vector	50	AB7000® (ThermoFisher)
RA3	rep529	FRET	7	PhiX (DNA Bacteriophage)	40	LC2.0® (ThermoFisher)
Toxoplasma Elite MGB®	rep529	Taqman (FAM-MGB)	10	Artificial DNA sequence	45	StepOnePlus® (ThermoFisher)

431 RA: reference assay

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434 **Table 2:** Performance scores (No of positive reactions/No of reactions performed) for the
 435 four PCR assays using DNA serial dilutions of a calibrated *Toxoplasma* suspension

436

Concentration	Lab 1		Lab 2		Lab 3		
	RA1	Elite MGB	Elite MGB	RA2	Elite MGB	RA3	Elite MGB
	(Qiagen)	(Qiagen)	(Extrablood+IC)	(Qiagen)	(Qiagen)	(Qiagen)	(Qiagen)
10,000 Tg/mL	3/3	3/3	3/3	3/3	3/3	4/4	4/4
1,000 Tg/mL	3/3	3/3	3/3	3/3	3/3	4/4	4/4
100 Tg/mL	3/3	3/3	3/3	3/3	3/3	4/4	4/4
10 Tg/mL	3/3	3/3	3/3	3/3	3/3	4/4	4/4
1 Tg/mL	3/3	2/3	2/3	1/3	0/3	4/4	3/4
0.1 Tg/mL	0/3	0/3	0/3	0/3	1/3	1/4	1/4
PCR	15/18*	14/18	14/18	13/18	13/18	21/24*	20/24
performance	(83.3)	(77.8)	(77.8)	(72.2)	(72.2)	(87.5)	(83.3)
score (%)							

437 Tg, *Toxoplasma gondii*; RA, reference assay

438 *p<0.01, compared to Elite MGB/Qiagen

439

440 **Table 3:** Performances of the *Toxoplasma* ELITE MGB[®] assay using clinical samples,
441 according to the sample type

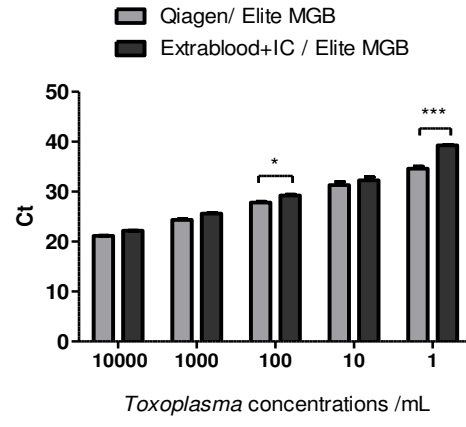
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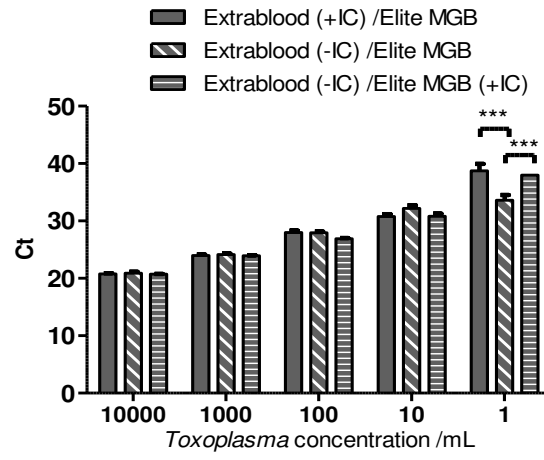
Sample type	Sensitivity % (n/N)	Specificity % (n/N)
Clinical samples (n = 128)	89.5 (85/95)	100 (33/33)
Amniotic fluid (n = 56)	100 (38/38)	100 (18/18)
Other fluids ^a (n = 9)	100 (7/7)	100 (2/2)
Placenta (n = 55)	79 (34/43)	100 (12/12)
Blood (buffy coat) (n = 6)	80 (4/5)	100 (1/1)
Biopsies (n = 2)	100 (2/2)	na
QCMD [®] samples (n = 10)	100 (7/7)	100% (3/3)

444 ^a consisting in 5 cerebrospinal fluid and 4 aqueous humor samples

445 na, not applicable



A



B

