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## **Clinical performance of four immunoassays for antibodies to SARS-CoV-2, including a prospective analysis for the diagnosis of COVID-19 in a real-life routine care setting**

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1        **Clinical performance of four immunoassays for antibodies to SARS-CoV-2,**  
2        **including a prospective analysis for the diagnosis of COVID-19 in a real-life**  
3        **routine care setting**

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18       Keywords: SARS-CoV-2; COVID-19; serology; immunoassay; antibody; ELISA; CLIA;  
19       RT-PCR

20

21 **ABSTRACT**

22 **Objectives:** The aim of the present study was to evaluate the clinical performance of  
23 four SARS-CoV-2 immunoassays and their contribution in routine care for the  
24 diagnosis of COVID-19, in order to benefit of robust data before their extensive use.

25 **Methods:** The clinical performance of Euroimmun ELISA SARS-CoV-2 IgG, Abbott  
26 SARS-CoV-2 IgG, Wantai SARS-CoV-2 Ab ELISA, and DiaPro COVID-19 IgG  
27 confirmation were evaluated in the context of both a retrospective and a prospective  
28 analysis of COVID-19 patients. The retrospective analysis included plasma samples  
29 from 63 COVID-19 patients and 89 control (pre-pandemic) patients. The prospective  
30 study included 203 patients who tested either negative (n=181) or positive (n=22) by  
31 RT-PCR before serology sampling.

32 **Results:** The specificity was 92.1%, 98.9%, 100% and 98.9% and the sensitivity 14  
33 days after onset of symptoms was 95.6%, 95.6%, 97.8% and 95.6% for Euroimmun  
34 IgG, Abbott IgG, Wantai Ab, and DiaPro IgG confirmation SARS-CoV-2  
35 immunoassays, respectively. The low specificity of Euroimmun IgG (for ratio <5) was  
36 not confirmed in routine care setting (98.5% negative agreement). Serology was  
37 complementary to RT-PCR in routine care and lead to identification of false positive  
38 (Ct>38, <2 targets detected) and false negative RT-PCR results (>1 month post onset  
39 of symptoms).

40 **Conclusions:** Serology was complementary to RT-PCR for the diagnosis of COVID-  
41 19 at least 14 days after onset of symptoms. First line serology testing can be  
42 performed with Wantai Ab or Abbott IgG assays, while DiaPro IgG confirmation assay  
43 can be used as an efficient confirmation assay.

44

45 **INTRODUCTION**

46 The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) firstly  
47 reported in late 2019 in Wuhan [1] and causing coronavirus disease (COVID-19) has  
48 spread across the world and lead to a worldwide sanitary crisis. Detection of viral RNA  
49 using reverse-transcriptase polymerase chain reaction (RT-PCR) in respiratory  
50 samples is the gold standard for early diagnosis of COVID-19. However, sensitivity of  
51 this molecular diagnosis starts to decrease at week 3 after onset of symptoms [2].  
52 Complementary to RT-PCR in respiratory samples, SARS-CoV-2 serology allows  
53 identification of COVID-19 cases with a higher sensitivity than RT-PCR several days  
54 after onset of symptoms [3]. In addition, it can be used to determine the fraction of the  
55 population that has been exposed to the virus [3]. However, results of such  
56 serosurveys depend on the performance of immunoassays and on the seroprevalence  
57 of SARS-CoV-2 which remains quite low, even in COVID-19 hotspots [4]. Given this  
58 low prevalence, it is crucial to have robust data evaluating those assays before clinical  
59 or epidemiological use. Four immunoassays were evaluated in our study: Euroimmun  
60 ELISA SARS-CoV-2 IgG (Euroimmun, Lübeck, Germany), Abbott SARS-CoV-2 IgG  
61 (Abbott Diagnostics, Illinois, USA), Wantai SARS-CoV-2 Ab ELISA (Beijing Wantai  
62 Biological Pharmacy Enterprise, Beijing, China), and DiaPro COVID-19 IgG  
63 Confirmation (Diagnostic Bioprobes, Milano, Italy). The latter assay was used to  
64 determine the specificity of SARS-CoV-2 Ab against S1, S2 and N Ag. The first aim  
65 was to evaluate the performance of these SARS-CoV-2 immunoassays on a series of  
66 63 COVID-19 patients and 89 pre-pandemic control patients. The second aim was to  
67 evaluate their contribution in routine care to confirm or infirm the diagnosis of COVID-  
68 19.

69

## 70 MATERIAL AND METHODS

### 71 Patients and samples

72 Two complementary studies were performed. First, clinical performance of  
73 immunoassays were evaluated on 63 COVID-19 patients tested positive for SARS-  
74 CoV-2 RNA by RT-PCR at Tours University Hospital (Table 1). SARS-CoV-2 RT-PCR  
75 were performed in respiratory samples using Allplex™ 2019-nCoV assay (Seegene,  
76 Seoul, Republic of Korea), Abbott RealTime SARS-CoV-2 assay (Abbott Molecular,  
77 Illinois, USA) or Bosphore 2019-nCoV detection kit (Anatolia GeneWorks, Istanbul,  
78 Turkey) depending on reagents and systems availability. Among the positive RT-PCR  
79 results, inconclusive RT-PCR results were defined as results positive only for one gene  
80 (E, ORF1ab or N). All 63 patients required an in-patient hospital stay for COVID-19  
81 and had plasma samples collected between April 8<sup>th</sup> and May 11<sup>th</sup> 2020. Retrospective  
82 testing for SARS-CoV-2 Ab was performed on these samples, collected between 2 to  
83 36 days after the onset of symptoms. Mild and critical COVID-19 cases were defined  
84 according to WHO [5]. Specificity was evaluated on plasma collected before the end  
85 of 2019 in 89 patients from occupational medicine (n=30), emergency or pneumology  
86 departments (n=26) or from patients tested positive by RT-PCR (Allplex™ RP3,  
87 Seegene) for seasonal coronaviruses (n=33, OC43, 229E or NL63) between 3 to 82  
88 weeks before serology sampling. Positive and negative predictive values of  
89 immunoassays were estimated in a context of low (2.4%) and high seroprevalence  
90 (9.8%) of SARS-CoV-2 Ab. These estimates were based on prevalence of SARS-CoV-  
91 2 Ab in healthcare professionals (2.4%, 108/4 444 tested from June to August 2020)  
92 or from patients (9.8%, 6/61 tested from May to June 2020).

93 Second, the contribution of SARS-CoV-2 serology in routine care for the diagnosis of  
94 COVID-19 was evaluated on 203 patients tested for SARS-CoV-2 infection by RT-PCR  
95 between April 8<sup>th</sup> and June 11<sup>th</sup> 2020 (Table 1). Most of these patients were healthcare  
96 professionals (167/203, 82.3%) who did not require an in-patient hospital stay  
97 (125/167, 74.9%). Other patients required an in-patient hospital stay (31/203) or had  
98 ambulatory testing (6/203). These patients were tested for SARS-CoV-2 antibodies at  
99 least 14 days after RT-PCR testing (unless otherwise specified) between June 1<sup>st</sup> and  
100 June 25<sup>th</sup> 2020. The entire study was performed according to French Reference  
101 Methodology MR-004, after patient information and anonymization of data. Samples  
102 were obtained from the registered biological collection DC-2020-3961.

103

104 *Table 1: Clinical presentation of patients*

	First study (clinical performance)		Second study (routine care)	
	RT-PCR+	Pre-pandemic control group	RT-PCR-	RT-PCR+
Nb	63	89	181	22
Age (median/IQR)	79/67-90	30/11-54	39/30-50	49/31-58
Sex (F:M)	1.52	1.17	1.51	1.75
Severe outcome	19/63 (30.2%)	N/A	N/A	0
ICU	18/63 (28.6%)	NA	N/A	0
Death	3/63 (4.7%)	NA	N/A	0

105 SARS-CoV-2 RT-PCR assays: Allplex™ 2019-nCOV (Seegene), Abbott RealTime  
 106 SARS-CoV-2 or Bosphore 2019-nCoV (Anatolia GeneWorks); NA: not available; ICU:  
 107 intensive care unit

108

109

## 110 **SARS-CoV-2 immunoassays**

111 Euroimmun ELISA SARS-CoV-2 IgG, Abbott SARS-CoV-2 IgG (Alinity-I analyzer),  
112 Wantai SARS-CoV-2 Ab ELISA, and DiaPro COVID-19 IgG Confirmation assays were  
113 performed according to the manufacturer's recommendations. Euroimmun IgG assay  
114 was used as first line immunoassay in routine care setting. Positive or undetermined  
115 results and results discordant with RT-PCR were confirmed with other assays. For  
116 statistical analysis, Euroimmun IgG and Wantai Ab uninterpretable results were  
117 considered negative. DiaPro IgG confirmation assay was considered positive when Ab  
118 against at least two targets (S1, S2 or nucleoprotein) were detected.

## 119 **Statistical analysis**

120 Statistical analysis was carried out using Graphpad Prism v5. Comparison of sensitivity  
121 and specificity were performed using McNemar's test. All tests were two-sided at the  
122 0.05 significance level.

## 123 **RESULTS**

### 124 **Clinical performance of SARS-CoV-2 immunoassays in the retrospective study.**

125 The sensitivity of Euroimmun IgG, Abbott IgG, Wantai Ab, and DiaPro IgG confirmation  
126 SARS-CoV-2 assays seven to thirteen days after the onset of symptoms were 30.8,  
127 46.2, 84.6 and 61.5% (Figure 1). In this timeframe, the DiaPro IgG confirmation  
128 demonstrated an excellent sensitivity for anti-N Ab (100%), higher than that for anti-S2  
129 Ab (15.4%,  $p=0.003$ ) and higher, although not significantly, than that for anti-S1 Ab  
130 (53.8%;  $p=0.13$ ).

131 The sensitivity of Euroimmun IgG, Abbott IgG, Wantai Ab and DiaPro IgG confirmation  
132 SARS-CoV-2 assays 14 days after the onset of symptoms were 95.6%, 95.6%, 97.8%

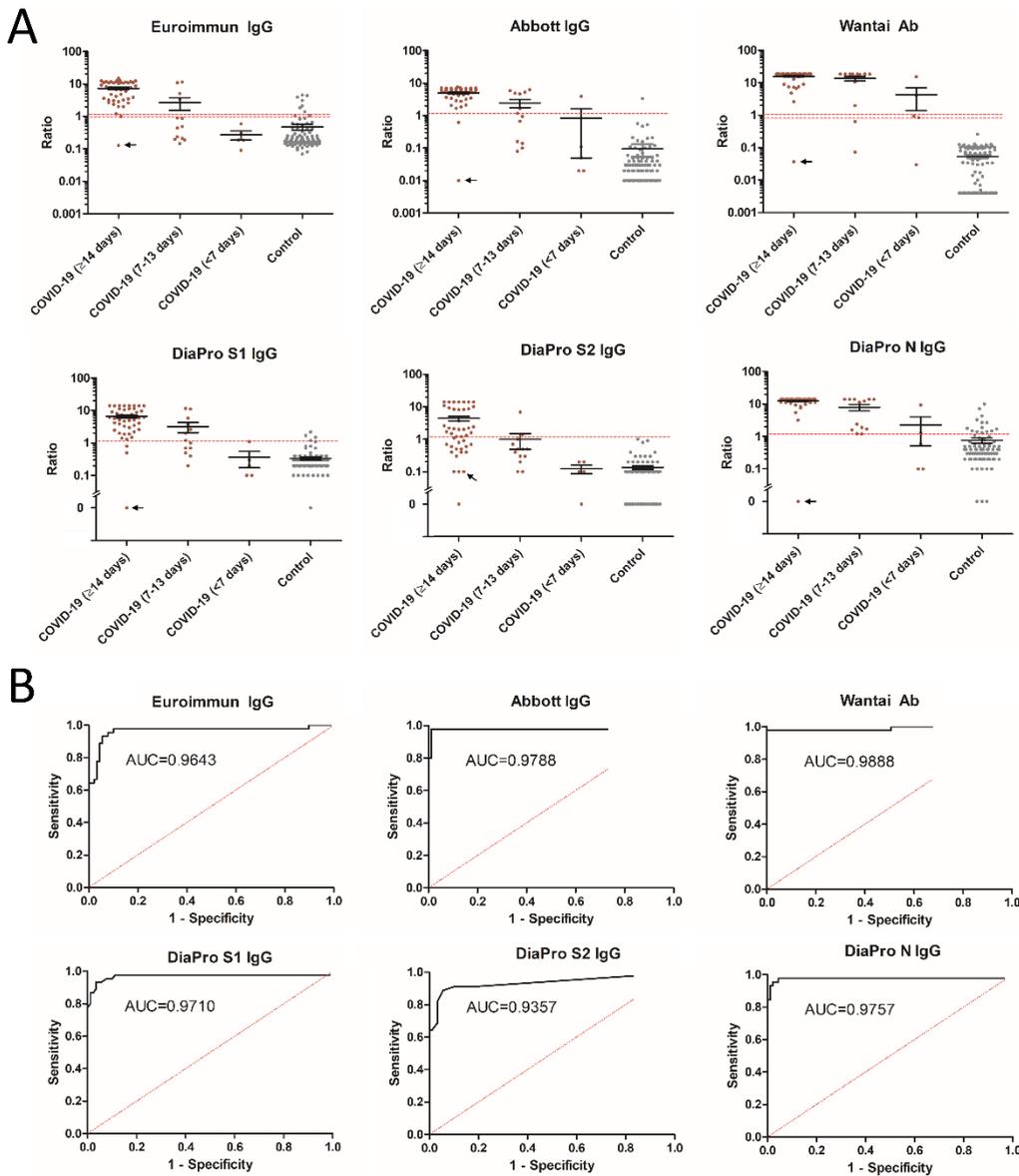
133 and 95.6%, respectively (Figure 1). The DiaPro IgG confirmation assay demonstrated  
134 good and similar sensitivities for anti-S1 and anti-N Ab (93.3% and 97.8%), both higher  
135 than that for anti-S2 Ab (62.2%,  $p \leq 0.002$ ). The single patient who tested negative with  
136 all immunoassays was a 61 years old heart transplant patient who experienced fever  
137 and myocarditis and had inconclusive RT-PCR result (positive only for the N gene) 26  
138 days post-onset of symptoms (Figure 1). This patient could have had a false positive  
139 RT-PCR result or, less likely false negative serology results because of its  
140 immunosuppressive treatments.

141 The specificity of Euroimmun IgG, Abbott IgG, Wantai Ab and DiaPro IgG confirmation  
142 SARS-CoV-2 immunoassay were 92.1%, 98.9%, 100% and 98.9%, respectively  
143 (Figure 1). Specificity of the DiaPro IgG confirmation assay was lower for anti-N Ab  
144 (84.3%) than for anti-S1 Ab (95.5%,  $p=0.02$ ) and anti-S2 Ab (100.0%,  $p=0.0005$ ).  
145 Specificity of Euroimmun IgG assay was lower than other immunoassays, with a  
146 significant difference versus Wantai Ab assay ( $p=0.02$ ) but not versus Abbott IgG  
147 ( $p=0.08$ ). False positive Euroimmun IgG results were observed in in an equivalent  
148 manner in the different groups of control patients: those with seasonal coronaviruses  
149 infections (2/33, both OC43), those from emergency or pneumology departments  
150 (3/26) and those from occupational medicine (2/30). False positive Euroimmun IgG  
151 results in control patients were associated with a lower ratio (median of 3.13; IQR 1.90-  
152 4.40, maximum 5) than those from the COVID-19 patients (median of 7.60, IQR 3.20-  
153 11.14,  $p=0.02$ ) (Figure 1). The two different patients with false positive Abbott IgG  
154 (1/89) or DiaPro IgG confirmation (1/89) results had a history of infection with  
155 Coronavirus 229E.

156 Positive predictive values based on a 2.4% and a 9.8% prevalence rate were  
157 22.9%/56.8%; 68.1%/90.4%; 100%/100%, and 68.1%/90.4% for Euroimmun IgG,

158 Abbott IgG, Wantai Ab and DiaPro IgG confirmation SARS-CoV-2 immunoassays.  
159 Negative predictive values based on a 2.4% and a 9.8% prevalence rate were  
160 99.9%/99.5% for Euroimmun IgG, Abbott IgG and DiaPro IgG confirmation, and  
161 99.9%/99.8% for Wantai Ab SARS-CoV-2 immunoassays.

162



163

164

165 *Figure 1: Clinical performance of SARS-CoV-2 immunoassays.*

166 *A) Comparison of SARS-CoV-2 immunoassays results between COVID-19 patients*  
 167 *and control patients. Black arrow indicates the heart transplant patient who tested*  
 168 *negative with all immunoassays more than 14 days after onset of symptoms. B) ROC*  
 169 *curves for evaluation of performances of SARS-CoV-2 immunoassays for samples ≥14*  
 170 *days post onset of symptoms.*

171 Table 2: Clinical performance of SARS-CoV-2 immunoassays relative to delay with onset of symptoms

Assay		Euroimmun IgG	Abbott IgG	Wantai Ab	DiaPro IgG confirmation			
					ELISA	CLIA (Alinity-i)	ELISA	ELISA
Platform		ELISA	CLIA (Alinity-i)	ELISA				
Antigen		S1	N	S (RBD)	S1	S2	N	≥2 Ag
<b>Sensitivity</b>								
≥7-13 dps	n/N	4/13	6/13	11/13	7/13	2/13	13/13	8/13
	%	30.8	46.2	84.6	53.8	15.4	100	61.5
	95% CI	9.1-61.4	19.2-74.9	54.6-98.1	25.1-80.8	1.9-4.5	75.3-100.0	31.6-86.1
≥14 dps	n/N	43/45	43/45	44/45	42/45	28/45	44/45	43/45
	%	95.6	95.6	97.8	93.3	62.2	97.8	95.6
	95% CI	84.9-99.5	84.9-99.5	88.2-99.9	81.7-98.6	46.5-76.2	88.2-99.9	84.9-99.5
<b>Specificity</b>								
	n/N	82/89	88/89	89/89	85/89	89/89	75/89	88/89
	%	92.1	98.9	100.0	95.5	100.0	84.3	98.9
	95% CI	84.5-96.8	93.9-100.0	95.9-100.0	88.9-98.8	95.9-100.0	75.0-91.1	93.9-100.0

172 dps: days post-onset of symptoms

173 **Contribution of SARS-CoV-2 serology in routine care to confirm or infirm the**  
174 **diagnosis of COVID-19**

175 Agreement between RT-PCR and Euroimmun IgG was 68% (15/22) and 96%  
176 (173/181) for patients who tested positive and negative by RT-PCR, respectively.  
177 Results of other immunoassays (Figure 2), the delay between RT-PCR and serology  
178 as well as Ct and targets of RT-PCR results were analyzed for these patients (Figure  
179 3). Patients were considered as suffering from COVID-19 if they tested positive with  
180 RT-PCR (all targets positive with Ct<38) and/or with at least two out of four SARS-  
181 COV-2 immunoassays (Figure 2). This allowed an accurate definition of 23 COVID-19  
182 cases and 180 non-COVID-19 patients. It lead to the identification of factors associated  
183 with false negative and false positive RT-PCR or serology results.

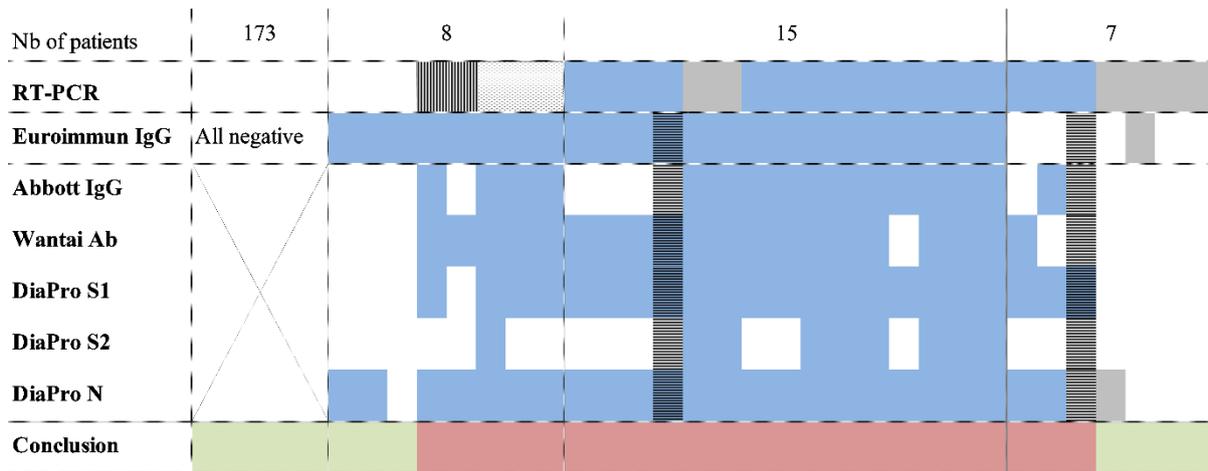
184 Among the 23 COVID-19 patients, 18/23 were from occupational medicine (8 in-patient  
185 hospital stay, non-critical disease). Overall, positive and negative agreement between  
186 RT-PCR and COVID-19 diagnosis was 94.6% (194/203), 78.3% (18/23), and 97.8%  
187 (176/180), respectively. Overall, positive and negative agreement between Euroimmun  
188 IgG and COVID-19 diagnosis was 97.0% (197/203), 87.0% (20/23), and 98.3%  
189 (177/180), respectively.

190 We considered that false positive RT-PCR results occurred in four patients. They were  
191 characterized by inconclusive RT-PCR results. Only the N gene was detected, with  
192 Ct>38. These patients tested negative with all immunoassays, suggesting the absence  
193 of exposure to SARS-CoV-2. Importantly, such inconclusive RT-PCR results were also  
194 observed in two COVID-19 patients confirmed positive with all serological assays  
195 (Figure 2), precluding any systematic interpretation of inconclusive RT-PCR results as  
196 false positive.

197 False negative RT-PCR results were observed in two asymptomatic patients, and in  
198 three symptomatic patients with late RT-PCR sampling (>1 month after onset of  
199 symptoms). These three symptomatic individuals were positive with all immunoassays,  
200 confirming the benefit of serology testing for patients with late presentation after onset  
201 of symptoms.

202 False negative serology results were observed in 2/23 COVID-19 patients who were  
203 sampled too early for serology testing (<14 days after onset of symptoms, Figure 2 and  
204 Figure 3). Among the 21 remaining COVID-19 patients tested for antibody to SARS-  
205 CoV-2 at least 14 days post onset of symptoms, positive agreement with COVID-19  
206 diagnosis was 90.5% (19/21), 76.2% (16/21), 90.5% (19/21) and 95.2% (20/21) for  
207 Euroimmun IgG, Abbott IgG, Wantai Ab and DiaPro IgG confirmation assay,  
208 respectively. The DiaPro IgG confirmation assay had the highest positive agreement  
209 with COVID-19 diagnosis. It detected anti-S1, -S2 and -N Ab in 20, 12 and 21 of these  
210 21 patients, respectively. In contrast, 5 out of 21 COVID-19 patients were not detected  
211 with the Abbott IgG assay. Interestingly, these five COVID-19 patients were also  
212 negative for anti-S2 Ab with the DiaPro IgG confirmation assay (Figure 2). The low  
213 positive agreement of Abbott IgG (16/21) with COVID-19 confirmed cases might  
214 suggest a lower sensitivity. However, the data could be due to the small sample size  
215 and would deserve to be confirmed in larger studies.

216



**Legend:**

- Negative
- Positive
- Undetermined/inconclusive
- Late RT-PCR sampling (>30 dps)
- RT-PCR in asymptomatic patient
- Early serology sampling (<14 dps)
- COVID-19 confirmed
- COVID-19 excluded

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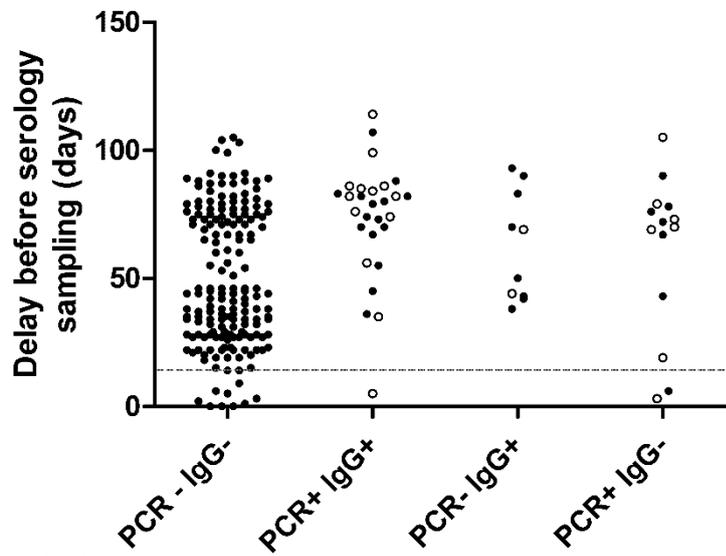
219 *Figure 2 : Agreement between RT-PCR and serology for 203 patients.*

220 *COVID-19 confirmed if positive RT-PCR (>1 gene) and/or Ab detected with two or*

221 *more immunoassays.*

222

223



224

225

226 *Figure 3: Delay between serology and first RT-PCR or onset of symptoms.*

227 *Delay between serology and first RT-PCR (plain dots) or onset of symptoms (empty*

228 *dots). The dashed line represents early serology testing before 14 days after onset of*

229 *symptoms.*

230

231

## 232 **DISCUSSION**

233 In the first part of our analysis corresponding to the retrospective evaluation, the  
234 specificity was 92.1%, 98.9%, 100%, and 98.9% for Euroimmun IgG, Abbott IgG,  
235 Wantai Ab, and DiaPro IgG confirmation SARS-CoV-2 assays, respectively. The  
236 sensitivity 14 days after onset of symptoms was 95.6%, 95.6%, 97.8%, and 95.6% for  
237 Euroimmun IgG, Abbott IgG, Wantai Ab, and DiaPro IgG confirmation SARS-CoV-2  
238 assays, respectively. The sensitivity between 7 and 13 days was suboptimal for Wantai  
239 Ab (84.6%) and inadequate (<75%) for other assays. Our results are in accordance  
240 with the available data for which the sensitivity ranges 14 days after onset of symptoms  
241 have been described as 61.7-96.0%, 77.8-100.0%, 98-100% and specificity ranges as  
242 86.6-100.0%, 95.1-100.0%, 98.0-99.1% for Euroimmun IgG [6–19], Abbott IgG  
243 [9,10,13,19–21] and Wantai Ab immunoassays [15,22,23], respectively. Although we  
244 included a collection of serum samples from patients for whom a recent infection by  
245 seasonal coronaviruses was documented, we did not notice any specific clustering of  
246 false positive results that could be attributed to a particular cross-reactivity.

247 Clinical performance of the DiaPro IgG confirmation assay (combining S1, S2 and N  
248 Ag) were similar to other immunoassays and to the manufacturer's statement  
249 (sensitivity 98%, specificity 90%). Interestingly, a higher sensitivity was observed for  
250 anti-N Ab and anti-S1 Ab than for anti-S2 Ab (97.8 and 93.3 vs 62.2%,  $p \leq 0.002$ ). This  
251 confirmed previous studies based on in-house ELISAs [24]. In contrast, a higher  
252 specificity was observed for anti-S2 Ab (100%) than for anti-N Ab (84.3%,  $p = 0.0005$ ).  
253 As suggested by previous studies [25,26], combination of S and N Ag probably  
254 contributed to the overall good performances of this assay, which had the highest  
255 positive agreement of all immunoassays (20/21) with COVID-19 diagnosis in our sub-

256 study performed in real-life routine conditions. Furthermore, this assay has the  
257 advantage to allow comparison of the ratio between anti-N IgG and anti-S IgG, which  
258 has been associated with the prognosis [26].

259 We observed a lack of specificity for the Euroimmun IgG assay (92.1%, CI 95%: 84.5-  
260 96.8%) in comparison to other assays in our retrospective study. This low specificity  
261 was not so problematic in the routine care setting since 98.5% of the non-COVID-19  
262 patients tested negative with Euroimmun IgG. There are conflicting data in the  
263 literature regarding the specificity of this assay. It demonstrated a good specificity ( $\geq$   
264 95%) in most studies [7,10–12,14,18,19], while a minority of studies suggested  
265 otherwise [6,9,17]. In this context and given the low prevalence of SARS-CoV-2 Ab,  
266 confirmation of positive Euroimmun IgG results seems reasonable. RT-PCR also  
267 demonstrated a relative lack of specificity in routine care setting (negative agreement  
268 of 97.8%, 176/180) especially for inconclusive RT-PCR results (only one gene  
269 detected, with Ct>38).

270 Some false negative serology results were associated with early serology sampling  
271 (<14 dps), while all false negative RT-PCR results were associated with late RT-PCR  
272 sampling (>30dps). This confirms that timing of testing is critical for good sensitivity of  
273 SARS-CoV-2 RT-PCR and serology [3]. However, early serology sampling does not  
274 explain the relative lack of agreement with COVID-19 diagnosis for Euroimmun IgG  
275 (19/21), Abbott IgG (16/21), Wantai Ab (19/21), and DiaPro IgG confirmation assay  
276 (20/21) in routine care setting. Similar observations have been made in populations of  
277 healthcare professionals, with false negative rates ranging from 1% [27] to 20% [28–  
278 30] several weeks after disease. This could be due to the high proportion of patients  
279 with mild COVID-19, resulting in low rates of seroconversion [28].

280 Our study confirms that Abbott IgG, Wantai Ab and DiaPro IgG confirmation assays  
281 are suitable assays for the diagnosis of COVID-19 at least 14 days after onset of  
282 symptoms. Main advantages of these assays are their automation (Abbott IgG), their  
283 optimal clinical performance (Wantai Ab) and their ability to differentiate between anti-  
284 N, -S1 and -S2 Ab (DiaPro IgG confirmation). DiaPro IgG confirmation assay has a  
285 low throughput (four wells per patient) which is adequate for confirmation testing.  
286 Euroimmun IgG assay can also be used for the diagnosis of COVID-19 if positive  
287 results are confirmed with another assay to compensate for its low specificity.

288 The huge impact of the SARS-CoV-2 emergence in public health justifies extensive  
289 seroepidemiological studies to survey its spread in various populations and numerous  
290 settings. There is a burst of serologic assays rolling out in different formats, including  
291 simple rapid tests. Our study shows that specificity may be highly variable among  
292 available immunoassays for antibody to SARS-CoV-2. Poor specificity of an assay in  
293 a population where prevalence and incidence of COVID-19 are low will lead to  
294 irrelevant data. Our study, as others, stresses on the absolute necessity to use only  
295 carefully validated assays to provide epidemiological data useful to public health  
296 decision makers.

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## 306 **CONTRIBUTION**

307 ER carried out the experiment. JM, CP, ER and YA analyzed the data. JM, CP, FB, KS  
308 and CGG designed the study and wrote the manuscript with support from AL.

309 AG, SM, AL, LB, GD and HB contributed to the design, patient management and data  
310 collection.

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