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SARS-CoV2 serology assays: utility and limits of different antigen based tests through the evaluation and the comparison of four commercial tests

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1 **Title:** SARS-CoV2 serology assays: utility and limits of different antigen based tests through
2 the evaluation and the comparison of four commercial tests

3 **Running title:** Four commercial SARS-CoV2 serology tests

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13 **Acknowledgment:** We would like to thank Dr Malika Ben Ahmed from the laboratory of
14 immunology of the Institut Pasteur of Tunis, Tunisia, for providing pre-pandemic sera
15 positive for rheumatoid factors.

16
17 **Abstract:**

18 Introduction: SARS-CoV2 serology testing is multipurpose provided to choose an efficient
19 test. We evaluated and compared 4 different commercial serology tests, three of them had the
20 Food and Drug Administration (FDA) approval. Our goal was to provide new data to help to
21 guide the interpretation and the choice of the serological tests.

22 Methods: Four commercial tests were evaluated: Cobas®Roche®(total anti-N antibodies),
23 VIDAS®Biomerieux®(IgM and IgG anti-RBD antibodies), Mindray®(IgM and IgG anti-N
24 and anti-RBD antibodies) and Access®Beckman Coulter®(IgG anti-RBD antibodies). Were
25 tested: a positive panel (n=72 sera) obtained from COVID-19 confirmed patients and a
26 negative panel (n=119) of pre-pandemic sera. Were determined the analytical performances
27 and was drawn the ROC curve to assess the manufacturer's threshold.

28 Results: A large range of variability between the tests was found. Mindray®IgG and Cobas®
29 tests showed the best overall sensitivity 79,2%CI95% [67,9-87,8]. Cobas® showed the best
30 sensitivity after D14; 85,4%CI95% [72,2-93,9]. The best specificity was noted for Cobas®,
31 VIDAS®IgG and Access® IgG(100%CI95% [96,9-100]). Access® had the lower sensitivity
32 even after D14 (55,5% CI95% [43,4-67,3]). VIDAS®IgM and Mindray®IgM tests showed
33 the lowest specificity and sensitivity rates. Overall, only 43 out of 72 sera gave concordant
34 results (59,7%). Retained cut-offs for a significantly better sensitivity and accuracy, without
35 altering significantly the specificity, were: 0,87 for Vidas®IgM($p=0,01$), 0,55 for
36 Vidas®IgG($p=0,05$) and 0,14 for Access®($p<10^{-4}$).

37 Conclusion: Although FDA approved, each laboratory should realize its own evaluation for
38 commercial tests. Tests variability may raise some concerns that seroprevalence studies may
39 vary significantly based on the used serology test.

40 **Key words:** SARS-CoV2, serology, commercial tests, false positive, false negative

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NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.

44
45
46 **Main text:**
47 **Introduction:**

48 The severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) is an emerging virus that
49 was first reported in December 2019 in Wuhan, China (1,2). Rapidly, the virus has spread
50 across the globe and has become a major public health concern. In 11 March 2020, the World
51 Health Organisation (WHO) announced the COVID-19 disease as a pandemic (3). To date,
52 millions of infections by SARS-CoV-2 and hundreds of thousands of deaths have been
53 attributed to the COVID-19. According to WHO COVID-19 dashboard as of 15 September
54 2021, the total of infections and deaths numbers are 225 680 357 and 4 644 740, respectively
55 (4). Molecular testing by real time PCR is the angular stone in the diagnosis of the COVID-
56 19 as it is playing a crucial role in testing, monitoring and contact tracing (5). However, as
57 screening indications are mainly limited to symptomatic patients, documented cases represent
58 probably only the visible part of the iceberg. For these reasons, other diagnostic methods are
59 needed to better estimate COVID-19 spread (6,7). Serology testing suits ideally for this
60 purpose as the detection of specific anti SARS-CoV2 antibodies offers valuable information
61 about previous contact with the virus, helps to assess the herd immunity at a large or specific
62 population, and recently, have decisive role to monitor vaccinated patients (8–10). A
63 worldwide laboratories and companies competition was launched soon after the virus
64 emergence, to develop efficient serology tests with good sensitivity and specificity, ease to
65 use, rapid result and reasonable cost-effectiveness balance (11). Today many different tests
66 are commercially available: enzyme-linked immune-sorbent assay (ELISA), enzyme-linked
67 fluorescent assay (ELFA), eletrochimiluminescent assay (ECLIA). These tests detect
68 different isotypes; IgM, IgG, IgA, total antibodies and use different antigens; the full spike
69 glycoprotein or sub-units S1 and S2, the receptor-binding protein (RBD) or nucleoprotein N
70 (11–17). International health authorities such as the Food and Drug Administration and the

71 World Health Organization agreed to grant what they called an emergency use authorization
72 (FDA-EUA) and an emergency use list (WHO-EUL), respectively (18–20). Nowadays,
73 serology has become widely used with many indications(21,22). However, some tests may
74 lack sufficient clinical evaluation which made specialists establishing their own evaluation
75 and sharing concerns toward their performances(12,14–17).

76 In the present study we conducted a head-to-head comparison of 4 different commercial
77 serology tests targeting either the N protein or the RBD protein or both of them; three of them
78 had an FDA-EUA. Our goal was to provide experimental data which help to guide the choice
79 of the serological tests according to their indication, as well as the interpretation of the
80 serology profiles.

81 **Methods:**

82 The study was done in the Laboratory of Virology of the Institut Pasteur of Tunis, Tunisia
83 and was approved by institutional review boards at the Institut Pasteur of Tunis. The selection
84 of samples followed the guidelines of the French “*Centre National de Reference des Virus*
85 *des Infections Respiratoires*” published on December 4th 2020 (23), i.e. the evaluation needs
86 at least 50 true positive sera and at least 50 true negative sera. A positive panel was
87 composed of a total of 72 unique, non duplicated serum samples obtained from COVID-19
88 confirmed patients on the basis of a positive RT-PCR on nasopharyngeal swab. Samples were
89 collected from the first day (D0) until 162 day (D162) after molecular confirmation. Serum
90 samples included 29 sera collected from D1 to D14, 16 sera from D16 to D30, 14 sera from
91 D31 to D60 and 13 sera after D60 (until D162). Along with the positive panel, a negative
92 panel was included in the study; it is composed of 119 pre-pandemic sera collected before
93 December 2019 and served as negative controls. Four commercial tests were evaluated:
94 Cobas®Roche® (ECLIA) detecting total anti-N antibodies, VIDAS®Biomerieux® (ELFA)
95 detecting specific IgM and IgG anti-RBD antibodies, Mindray® (CLIA) detecting specific

96 IgM and IgG anti-N and anti-RBD antibodies and Access®Beckman Coulter® (CLIA)
97 detecting specific IgG anti-RBD antibodies. All sera, the 72 from confirmed cases and the
98 119 pre-pandemic sera were tested by the 4 tests and manipulations were carried out
99 according to the manufacturers' instructions. The intrinsic characteristics and the
100 manufacturer's performances of each test are summarized in Table 1.

101 Were determined: the overall sensitivity, the sensitivity after D14, the specificity, the positive
102 predictive value PPV, the negative predictive value NPV, the area under the curve (AUC)
103 and the correlation between the results (in ratio index/cut-off index (ratios)) with the days
104 after COVID-19 confirmation. Based on the obtained ratios, Receiving operating
105 characteristic (ROC) curve for each test has been drawn using the obtained ratios, in order to
106 assess the manufacturer's threshold to achieve the best performances. Finally, the 4 tests were
107 compared between each other and pooled results of different tests were statistically studied.

108 *Statistical Analysis:* PPV and NPV were calculated using the FDA calculator available on its
109 website, accessed 22 July 2021 and arbitrary fixed the prevalence of the disease at 5%. Using
110 RT-PCR as the reference standard, sensitivity, specificity, AUC were calculated to assess the
111 performance of each assay and T-test was used to compare AUC for each test. The optimal
112 cutoff point was selected based on the point with the highest Youden index J. Agreement was
113 determined between all the tests, two by two, using the Cohen's Kappa statistic test.
114 Correlation between ratios was calculated by determining the Pearson Coefficient r. The
115 significance level was set at 5%, and a 95% confidence interval (CI95%) was reported for
116 each measure. All calculations were performed using MEDCALC®V18.2.1.

117 **Results:**

118 The performances of each test were evaluated by calculating the sensitivity, specificity, PPV,
119 NPV and AUC. All experimental results are shown in Table 2. For IgM tests, VIDAS®IgM
120 and Mindray®IgM tests showed the lowest specificity and sensitivity rates for all the sera

121 and those collected after D14. The PPV and NPV were also the lowest. False positive tests
122 were obtained for 7 pre-pandemic patients for Vidas®IgM: 3 patients having rheumatoid
123 factors and 4 patients positive for *Herpes simplex virus*; the ratios ranged from 1,08 to 13,94.
124 False positive tests were obtained for 3 pre-pandemic patients for Mindray®IgM: 2 patients
125 having auto-immune disease and 1 patient positive for *Herpes simplex virus*; the ratios ranged
126 from 1,94 to 4,97. These two tests had good and similar accuracies ($p=0,587$). For IgG and
127 total antibody tests, Mindray®IgG and Cobas® tests showed the best overall sensitivity
128 79,2% CI95% [67,9-87,8] (57 positive out of 72 true positive). Cobas® showed the best
129 sensitivity after D14 85,4% CI95% [72,2-93,9]. For the other tests, the sensitivity increased
130 considerably after D14 except for Access® (Table 2). The best specificity and PPV were
131 noted for Cobas®, VIDAS®IgG and Access® IgG: 100% CI95% [96,9-100] (119 negative out
132 of 119 true negative). Mindray®IgG was slightly less specific (95,8% CI95% [90,5-
133 98,6]), and PPV was low 49,8% CI95% [29,4-70,2]. Positive results were obtained for 5 pre-
134 pandemic patients: 1 having rheumatoid factors, 2 patients positive for *Herpes simplex virus*
135 and 2 pregnant women, ratios ranged from 1,3 to 3,8. Considering accuracy, Cobas®,
136 VIDAS®IgG and Mindray®IgG had very good and similar accuracy (pairwise comparison of
137 ROC curves for the 3 combinations $p>0,05$). However, Access® had an accuracy of
138 0,778 CI95% [0,712-0,835] which is good but statistically lower than the other tests ($p=0,587$).
139 ROC curves for each test were drawn based on the obtained ratios (Figure 1) as shown in
140 Tables 3. Retained cut-offs for a significantly better sensitivity and accuracy, without altering
141 significantly the specificity, were: 0,87 instead of 1 for Vidas®IgM ($p=0,01$), 0,55 instead of
142 1 for Vidas®IgG ($p=0,05$) and 0,14 instead of 1 for Access® ($p<10^{-4}$). For Cobas® and
143 Mindray®IgM and IgG, the new proposed cut offs did not give better analytical
144 performances than the original cut-offs ($p>0,05$, Table 3).

145 All experimental results obtained for the positive panel were summarized in Table 4
146 including concordance and discordance between the tests and in connexion also with the days
147 after confirmation. Overall, 43 out of 72 sera gave concordant results (59,7% concordance).
148 Of them, 35 were positive, sampled between D4 and D140 and 8 were negative, sampled
149 between D0 and D60. Discordant results represented 40,3% of the panel (29 out of 72). They
150 were divided into 3 groups: the first group (n=17) contains samples that were positive by 3
151 tests over 4, Access® failed to detect 13 samples collected between D6 and D90 and Cobas®
152 failed to detect 4 samples collected between D8 and D39 d. The second group (n=11)
153 included samples that were positive by only 2 tests over 4. We obtained positive results by
154 Mindray® in concordance with another test (Vidas® or Cobas®) in 7 cases out of 11. The
155 third group contains one sample collected at D7 detected positive by only Vidas® and
156 negative by the other tests.

157 To further understand the differences between the tests results, we determined the agreement
158 between the results and the correlation between the ratios. In this section, the comparison was
159 focused on the isotype and on the antigen. For the IgM tests, Vidas® and Mindray® present
160 moderate agreement ($k=0,570$) and weak correlation ($r=0,484$). For the other tests, the results
161 of the agreement and correlation are grouped in Figure 2. For tests detecting exclusively
162 antibodies against RBD, Vidas®IgG and Access®, concordance was important and
163 correlation is positive and strong. For Cobas®, the test that detects antibodies against N
164 exclusively, agreement was perfect with Vidas® and important with Access® and correlation
165 is positive and moderately strong with Vidas® but negative with Access®. Considering the
166 Mindray®, the test that detects both N and RBD specific antibodies, it presents a perfect
167 agreement with all tests except Access® and a positive correlation with all tests.

168 Figure 3 shows the scatterplots of the 4 tests' ratios against d. Figure 3.a shows the
169 distribution of the IgM tests indexes (Vidas® and Mindray®), which is heterogenous et does

170 not fit a specific pattern however it is obvious that high indexes were obtained during the first
171 21 days after infection. Figure 3.b illustrates the distribution of total antibodies and IgG
172 antibodies tests indexes (Cobas®, Vidas®, Mindray® and Access®) and shows that there is
173 no correlation with days after infection. However, for Cobas®, ratios seem to be increasing
174 over days. Different test results combinations' were studied by pooling the obtained results.
175 No improvement of performances was noted for [Cobas®, *versus* Cobas® and Vidas® IgG
176 and IgM] , for [Cobas® *versus* Mindray®IgM+IgG], for [Vidas®IgG+IgM *versus*
177 Mindray®IgM+IgG] (pairwise comparison of ROC curves $p>0,05$). Only the combination
178 Vidas®IgG+IgM and Cobas® was consistently more accurate than Mindray®IgM and IgG
179 (pairwise comparison of ROC curves $p=0,0399$).

180 **Discussion:**

181 SARS-CoV2 serology tests were developed and optimized in a record time after the virus
182 emergence. Thanks to the the softened authorization procedure, they were rapidly
183 commercialized and used worldwide(18–20). In this study, we evaluated and compared 4
184 serology commercial automated tests: Cobas®Roche® (ECLIA) detecting total anti-N
185 antibodies, IgG+++ (anti-N), VIDAS®Biomerieux® (ELFA) detecting specific IgM and IgG
186 anti-RBD antibodies, Mindray® (CLIA) detecting specific IgM and IgG anti-N and anti-
187 RBD antibodies and Access®Beckman Coulter® (CLIA) detecting specific IgG anti-RBD
188 antibodies. Our evaluation revealed a gap between claimed and experimental analytical
189 performances in terms of sensitivity and specificity and, accordingly, we propose new
190 analytical criteria. In addition, the comparison between the evaluated tests showed a
191 significant divergence between the obtained qualitative results in 40,3% of the positive tested
192 sera (29 out of 72). Our findings suggest that the most sensitive test, after D14 is Cobas®
193 (85,4%IC95%[72,2-93,9]) which detects high ratios until 4 months after primo-infection.

194 Besides, we found that combining RBD and N tests from different tests gives the best
195 accuracy.

196 We tested 72 RT-PCR confirmed patients and 119 pre-pandemic sera. Our work stands out
197 from the rest of the literature by studying tests of different antigen and having international
198 approved certificate, by proposing new significant cut-offs to improve the analytical
199 performances and by a deep assessment of the origins behind discordances of the obtained
200 results as well as the discussion of the utility and the limits of each test. Thus, our work
201 provides original and helpful data serving the health care professionals in their routine
202 practice. Even our panel is not too large, the number of tests is not big and the impact of the
203 disease severity was not studied, our results are extrapolable given that the panel is
204 representative (from D0 until D162), the tests are diversified (different antigens and isotypes)
205 and the conclusions are applicable for a diagnosis laboratory receiving all type of indications.
206 The evaluation of commercial tests was widely reported for SARS-CoV2 virus as well as for
207 other pathogens. International recommendations were published by several scientific societies
208 and instances such as *Haute Autorité Sanitaire France* HAS, FINDXX and PHE and Health
209 Canada in order to harmonize the criteria of validation of the tests (23–26). In our study, the
210 evaluation of all tests gave lower performances than the claimed ones and did not respond to
211 the HAS validation criteria, the most flexible one, in terms of sensitivity (Table 1,2).
212 According to the HAS, the sensitivity of detecting IgG and total antibodies must exceed 90%
213 after D14 from disease onset while for IgM antibodies, the sensitivity must exceed 90% after
214 D7. All tests were studied specifically after D14, based on a large review published by
215 Cochrane on 15976 samples which found that all the results for IgG, IgM, IgA, total
216 antibodies showed low sensitivity during the first week after the symptoms onset (all less
217 than 30.1%), it rises in the second week and reaches its highest values in the third week (36).

218 In our series, the best sensitivity after D14 were the one of Cobas® (85,4%CI95%[72,2-
219 93,9]) followed by Vidas®IgG and Mindray®IgG (83,3%CI95%[69,8-92,5]) and Access®
220 came in the last position(55,5% CI95% [43,4-67,3]) (Table 2). For IgM detection Mindray®
221 and Vidas® had very low sensitivity even after D14. High sensitivity for Cobas® confirms
222 the findings of other authors (12,15,16,27). This could be explained, first, by the used antigen
223 which is exclusively the N protein, known to be the most abundantly expressed immune-
224 dominant protein (21). Second is the ability of Cobas® to detect all immunoglobulin classes
225 and such data was reported for Siemens Atellica® that detects total antibodies anti-
226 glycoprotein S1(28). Third is the ECLIA Elecsys® technology developed by Roche® which
227 is highly efficient regardless of the measured analyte(29,30). For the other tests, such
228 unsatisfactory sensitivities were reported by other studies for the same tests. Similar low
229 sensitivities for the Vidas® test were reported by Younes et al. (88,3 for Vidas®IgG after
230 D21) and by Wolf et al. (over all sensitivity of 64,9%CI95% [55,2-73,7] for Vidas®IgM and
231 73%CI95% [63,7-81] for Vidas®IgG) (31,32). Padoan et al. also reported a sensitivity of 86.4
232 (77.0-93.0) for both Mindray®IgM and IgG tests but a new Mindray® generation would give
233 much better performances: 99% and 96% from D1 to D41 for IgG and IgM, respectively
234 (33,34). This new version of Mindray® was not available at the study writing time and merits
235 to be evaluated. Access® showed very low sensitivity for IgG detection (55,5%CI95% [43,4-
236 67,3]) and this sensitivity did not increase after D14. Other authors reported similar results
237 for Access® 39.6%CI 95% [32.5–47.3%] and 69% CI95% [59.0-77.9] (15,35). Beckman® has
238 developed a new Access® test allows semi quantified detection of antibodies against RBD
239 and that has obtained the FDA-EUA and thus merits to be evaluated.

240 Regarding the specificity, Vidas®IgM and Mindray®IgM and IgG tests gave positive signals
241 for few pre-pandemic sera and this was also reported by other authors (31,33,34). Cross
242 reactivity with pre-pandemic auto-immune disease patients sera was previously reported(37).

243 In contrast, cross reactivity with pre-pandemic pregnant women sera, and patients positive for
244 *Herpes simplex virus* is reported for the first time. Results of Vidas®IgM and Mindray®IgM
245 and IgG should be interpreted with caution; indeed, PPV of these tests are less than 50%,
246 which mean that half of tested patients are susceptible to be false positive. Here, PPV and
247 NPV were calculated by the FDA calculator fixing the prevalence at 5%. Each country is
248 invited to evaluate regularly the PPV according to the prevalence evolution. The tests
249 Cobas®, Access® and Vidas® IgG are the extremely specific (100%IC95%[96,9-100]) and
250 the PPV is 100%, as reported by other studies (16,27,31).

251 The pre-defined tests' thresholds were experimentally optimized and adjusted for an
252 improved sensitivity with very little loss in specificity. This approach is being widely used
253 and reported by many authors for better interpretation of commercial tests, for COVID-19
254 tests as well as other pathogens (31,38,39). We found out that decreasing the cut-off signals
255 for Vidas®IgM, Vidas®IgG and Access® improves significantly the sensitivity and the
256 accuracy (Table3). As none of the tests propose a grey zone for borderline results, which is
257 unusual, we propose that any results between the proposed cut-off and the original cut-off
258 (i.e: [0,87 to 1] for Vidas®IgM, [0,55–1] for Vidas®IgG and [0,14–1] for Access®) should
259 be retested or, better, the patient should be re-sampled after 10 to 15 days to follow the
260 antibody kinetic. More generally, we suggest that any weak signal less than 2 times the cut-
261 off, should be interpreted with caution.

262 Comparison between the four tests showed concordant results in 59,7% of the samples
263 collected in confirmed cases (43 out of 72) among which, 8 were negative by all the 4 tests;
264 they were sampled between D0 and D60, median=14. As the 4 different tests using different
265 antigens, gave negative results, it is suggested that this negativity is inherent to the
266 individuals. Indeed, this may be explained by either a late sero-conversion, or a rapid sero-
267 reversion (40). Some authors has suggested that 5 to 10% of infected persons do not develop

268 antibodies at all (41). A non negligible proportion of discordant results was found in 40,3%
269 sera (n=29 out of 72). Access® was the test that fails the most to detect positive results (13
270 cases out of 29 discordant results, Table 4). For the rest of the tests, although general
271 agreement between qualitative results is important (figure 2), they gave various discordant
272 patterns.

273 A dominated discordant pattern is interesting, it is about positive results for Mindray® (24
274 cases out of 29 cases), indicating that a combination of N and RBD antigens would increase
275 the number of true positives sera. Indeed, Mindray® is not an FDA-EUA test, but was
276 introduced in this study for its originality as it is multiplex (N+RBD). We demonstrated that
277 Mindray®IgM and IgG offers similar good sensitivity to Cobas®, but the combinaison
278 [Cobas®+Vidas®IgM+IgG] exceeds Mindray® IgM+IgG in accuracy. So, a two steps
279 strategy starting by testing Cobas® then Vidas®IgM+IgG could improve significantly the
280 sensitivity, and offers separate comprehension of antibodies specificity.

281 Questions regarding the magnitude and the longevity of the antibody response remain
282 unanswered. Many literature reviews tried to propose a general kinetic of antibodies and
283 recognize a big variability between individuals and proportionality with COVID-19 severity
284 (42,43). In our study, Figures 3.a showed that scatter plots of the two IgM tests are high and
285 condensed among the first 3 weeks, suggesting that their detection is in line with an ongoing
286 or acute infection. However, we found that IgM may still detectable even until D162,
287 Regarding IgG (Figure 2 and 3.b) anti RBD antibodies follow the same decay contrasting
288 with the anti-N that persists positive with high ratios for longer time. This is explained by
289 half life time for IgG anti RBD which is 49 days versus the half time of the IgG anti-N is 75
290 days (43).

291 In conclusion, although serological assays do not replace molecular tests in diagnosing active
292 infection, they are multipurpose provided to choose the most efficient test and to properly

293 interpret the results. We characterized the performance of four commercial antibody
294 platforms and found out and explained the variability between them. Although FDA
295 approved, each laboratory should realize its own evaluation for commercial tests, and health
296 professionals should be aware about false negative rate before 14 to 21 days after primo-
297 infection. Finally, this variability may raise some concerns that seroprevalence studies may
298 vary significantly based on the used serology test.

299 **References:**

- 300
301 1. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak
302 associated with a new coronavirus of probable bat origin. *Nature*. mars 2020;579(7798):270-3.
- 303 2. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and
304 naming it SARS-CoV-2. *Nat Microbiol*. 2020;5(4):536-44.
- 305 3. Chronologie de l'action de l'OMS face à la COVID-19 [Internet]. [cited 25 sept 2021]. available
306 on : <https://www.who.int/fr/news/item/29-06-2020-covidtimeline>
- 307 4. WHO Coronavirus (COVID-19) Dashboard [Internet]. [cited 25 sept 2021]. Available on:
308 <https://covid19.who.int>
- 309 5. Esbin MN, Whitney ON, Chong S, Maurer A, Darzacq X, Tjian R. Overcoming the bottleneck to
310 widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection.
311 *RNA N Y N*. juill 2020;26(7):771-83.
- 312 6. Nishiura H, Kobayashi T, Miyama T, Suzuki A, Jung S, Hayashi K, et al. Estimation of the
313 asymptomatic ratio of novel coronavirus infections (COVID-19). *Int J Infect Dis*. mai
314 2020;94:154-5.
- 315 7. Van Caeselele P, Bailey D, Forgie SE, Dingle TC, Kraiden M. SARS-CoV-2 (COVID-19)
316 serology: implications for clinical practice, laboratory medicine and public health. *CMAJ Can*
317 *Med Assoc J*. 24 août 2020;192(34):E973-9.
- 318 8. Sidiq Z, Hanif M, Dwivedi KK, Chopra KK. Benefits and limitations of serological assays in
319 COVID-19 infection. *Indian J Tuberc*. déc 2020;67(4):S163-6.
- 320 9. Dimeglio C, Herin F, Martin-Blondel G, Miedougé M, Izopet J. Antibody titers and protection
321 against a SARS-CoV-2 infection. *J Infect* [Internet]. 20 sept 2021 [cited 25 sept 2021];0(0).
322 Available on: [https://www.journalofinfection.com/article/S0163-4453\(21\)00483-7/fulltext](https://www.journalofinfection.com/article/S0163-4453(21)00483-7/fulltext)
- 323 10. Crotty S. Hybrid immunity. *Science*. 25 juin 2021;372(6549):1392-3.
- 324 11. Petherick A. Developing antibody tests for SARS-CoV-2. *Lancet Lond Engl*.
325 2020;395(10230):1101-2.
- 326 12. Schnurra C, Reiners N, Biemann R, Kaiser T, Trawinski H, Jassoy C. Comparison of the
327 diagnostic sensitivity of SARS-CoV-2 nucleoprotein and glycoprotein-based antibody tests. *J*
328 *Clin Virol*. août 2020;129:104544.
- 329 13. Younes N, Al-Sadeq DW, AL-Jighefee H, Younes S, Al-Jamal O, Daas HI, et al. Challenges in
330 Laboratory Diagnosis of the Novel Coronavirus SARS-CoV-2. *Viruses*. juin 2020;12(6):582.

- 331 14. Nandakumar V, Profaizer T, Lozier BK, Elgort MG, Larragoite ET, Williams ESCP, et al.
332 Evaluation of a Surrogate ELISA- Based Severe Acute Respiratory Syndrome Coronavirus 2
333 (SARS-CoV-2) cPass Neutralization Antibody Detection Assay and Correlation with IgG
334 Commercial Serology Assays. *Arch Pathol Lab Med.* 28 juin 2021;
- 335 15. Tan SS, Saw S, Chew KL, Huak CY, Khoo C, Pajarillaga A, et al. Head-to-head evaluation on
336 diagnostic accuracies of six SARS-CoV-2 serological assays. *Pathology (Phila).* déc
337 2020;52(7):770-7.
- 338 16. Riestler E, Majchrzak M, Mühlbacher A, Tinguely C, Findeisen P, Hegel JK, et al. Multicentre
339 Performance Evaluation of the Elecsys Anti-SARS-CoV-2 Immunoassay as an Aid in
340 Determining Previous Exposure to SARS-CoV-2. *Infect Dis Ther.* 9 août 2021;1-17.
- 341 17. Murray MJ, McIntosh M, Atkinson C, Mahungu T, Wright E, Chatterton W, et al. Validation of a
342 commercially available indirect assay for SARS-CoV-2 neutralising antibodies using a
343 pseudotyped virus assay. *J Infect.* mai 2021;82(5):170-7.
- 344 18. Ravi N, Cortade DL, Ng E, Wang SX. Diagnostics for SARS-CoV-2 detection: A comprehensive
345 review of the FDA-EUA COVID-19 testing landscape. *Biosens Bioelectron.* 1 oct
346 2020;165:112454.
- 347 19. Diagnostics laboratory emergency use listing [Internet]. [cited 25 sept 2021]. Available on:
348 [https://www.who.int/teams/health-product-and-policy-standards/about/regulation-and-](https://www.who.int/teams/health-product-and-policy-standards/about/regulation-and-prequalification)
349 [prequalification](https://www.who.int/teams/health-product-and-policy-standards/about/regulation-and-prequalification)
- 350 20. Health C for D and R. Emergency Use Authorizations for Medical Devices [Internet]. FDA. FDA;
351 2021 [cited 25 sept 2021]. Available on: [https://www.fda.gov/medical-devices/emergency-](https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations-medical-devices)
352 [situations-medical-devices/emergency-use-authorizations-medical-devices](https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations-medical-devices)
- 353 21. CDC. Labs [Internet]. Centers for Disease Control and Prevention. 2020 [cited 25 sept 2021].
354 Available on: [https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-](https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html)
355 [guidelines.html](https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html)
- 356 22. on behalf of the Canadian Public Health Laboratory Network (CPHLN) Serology Working Group,
357 Charlton C, Kanji J, Tran V, Kus J, Gubbay J, et al. Practical guidance for clinical laboratories
358 for SARS-CoV-2 serology testing. *Can Commun Dis Rep.* 7 mai 2021;47(04):171-83.
- 359 23. Arrêté du 3 décembre 2020 modifiant l'arrêté du 10 juillet 2020 prescrivant les mesures
360 d'organisation et de fonctionnement du système de santé nécessaires pour faire face à
361 l'épidémie de covid-19 dans le cadre de l'état d'urgence sanitaire - Légifrance [Internet]. [cited
362 25 sept 2021]. Available on: <https://www.legifrance.gouv.fr/jorf/id/JORFTEXT000042607794>
- 363 24. Canada H. COVID-19 serological testing devices: Notice on sensitivity and specificity values
364 [Internet]. 2021 [cited 25 sept 2021]. Available on: [https://www.canada.ca/en/health-](https://www.canada.ca/en/health-canada/services/drugs-health-products/covid19-industry/medical-devices/testing/serological/notice-sensitivity-specificity-values.html)
365 [canada/services/drugs-health-products/covid19-industry/medical-](https://www.canada.ca/en/health-canada/services/drugs-health-products/covid19-industry/medical-devices/testing/serological/notice-sensitivity-specificity-values.html)
366 [devices/testing/serological/notice-sensitivity-specificity-values.html](https://www.canada.ca/en/health-canada/services/drugs-health-products/covid19-industry/medical-devices/testing/serological/notice-sensitivity-specificity-values.html)
- 367 25. 20200427-COVID-19-IA-Evaluation-Synopsis.pdf [Internet]. [cited 25 sept 2021]. Available on:
368 [https://www.finddx.org/wp-content/uploads/2020/04/20200427-COVID-19-IA-Evaluation-](https://www.finddx.org/wp-content/uploads/2020/04/20200427-COVID-19-IA-Evaluation-Synopsis.pdf)
369 [Synopsis.pdf](https://www.finddx.org/wp-content/uploads/2020/04/20200427-COVID-19-IA-Evaluation-Synopsis.pdf)
- 370 26. Target Product Profile: antibody tests to help determine if people have recent infection to SARS-
371 CoV-2: Version 2 [Internet]. GOV.UK. [cited 25 sept 2021]. Available on:
372 [https://www.gov.uk/government/publications/how-tests-and-testing-kits-for-coronavirus-covid-](https://www.gov.uk/government/publications/how-tests-and-testing-kits-for-coronavirus-covid-19-work/target-product-profile-antibody-tests-to-help-determine-if-people-have-recent-infection-to-sars-cov-2-version-2)
373 [19-work/target-product-profile-antibody-tests-to-help-determine-if-people-have-recent-infection-](https://www.gov.uk/government/publications/how-tests-and-testing-kits-for-coronavirus-covid-19-work/target-product-profile-antibody-tests-to-help-determine-if-people-have-recent-infection-to-sars-cov-2-version-2)
374 [to-sars-cov-2-version-2](https://www.gov.uk/government/publications/how-tests-and-testing-kits-for-coronavirus-covid-19-work/target-product-profile-antibody-tests-to-help-determine-if-people-have-recent-infection-to-sars-cov-2-version-2)
- 375 27. Tan SS, Saw S, Chew KL, Wang C, Pajarillaga A, Khoo C, et al. Comparative Clinical
376 Evaluation of the Roche Elecsys and Abbott Severe Acute Respiratory Syndrome Coronavirus 2

- 377 (SARS-CoV-2) Serology Assays for Coronavirus Disease 2019 (COVID-19). *Arch Pathol Lab*
378 *Med.* 1 janv 2021;145(1):32-8.
- 379 28. Pflüger LS, Bannasch JH, Brehm TT, Pfefferle S, Hoffmann A, Nörz D, et al. Clinical evaluation
380 of five different automated SARS-CoV-2 serology assays in a cohort of hospitalized COVID-19
381 patients. *J Clin Virol.* sept 2020;130:104549.
- 382 29. Douet T, Armengol C, Charpentier E, Chauvin P, Cassaing S, Iriart X, et al. Performance of
383 seven commercial automated assays for the detection of low levels of anti-Toxoplasma IgG in
384 French immunocompromised patients. *Parasite Paris Fr.* 2019;26:51.
- 385 30. Schmidt M, Jimenez A, Mühlbacher A, Oota S, Blanco L, Sakuldamrongpanich T, et al. Head-to-
386 head comparison between two screening systems for HBsAG, anti-HBc, anti-HCV and HIV
387 combination immunoassays in an international, multicentre evaluation study. *Vox Sang.* août
388 2015;109(2):114-21.
- 389 31. Younes S, Al-Jighefee H, Shurrab F, Al-Sadeq DW, Younes N, Dargham SR, et al. Diagnostic
390 Efficiency of Three Fully Automated Serology Assays and Their Correlation with a Novel
391 Surrogate Virus Neutralization Test in Symptomatic and Asymptomatic SARS-COV-2
392 Individuals. *Microorganisms.* 25 janv 2021;9(2):245.
- 393 32. Wolff F, Dahma H, Duterme C, Van den Wijngaert S, Vandenberg O, Cotton F, et al. Monitoring
394 antibody response following SARS-CoV-2 infection: diagnostic efficiency of 4 automated
395 immunoassays. *Diagn Microbiol Infect Dis.* 1 nov 2020;98(3):115140.
- 396 33. Padoan A, Cosma C, Zaupa P, Plebani M. Analytical and diagnostic performances of a high-
397 throughput immunoassay for SARS-CoV-2 IgM and IgG [Internet]. 2020 nov [cited 25 sept 2021]
398 p. 2020.11.20.20235267. Available on:
399 <https://www.medrxiv.org/content/10.1101/2020.11.20.20235267v1>
- 400 34. Pieri M, Nuccetelli M, Nicolai E, Sarubbi S, Grelli S, Bernardini S. Clinical validation of a second
401 generation anti-SARS-CoV-2 IgG and IgM automated chemiluminescent immunoassay. *J Med*
402 *Virol.* avr 2021;93(4):2523-8.
- 403 35. COVID-19: laboratory evaluations of serological assays [Internet]. GOV.UK. [cited 25 sept
404 2021]. Available on: [https://www.gov.uk/government/publications/covid-19-laboratory-](https://www.gov.uk/government/publications/covid-19-laboratory-evaluations-of-serological-assays)
405 [evaluations-of-serological-assays](https://www.gov.uk/government/publications/covid-19-laboratory-evaluations-of-serological-assays)
- 406 36. Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests
407 for identification of current and past infection with SARS-CoV-2. *Cochrane Database Syst Rev*
408 [Internet]. 2020 [cited 25 sept 2021];(6). Available on:
409 <https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD013652/full>
- 410 37. Jääskeläinen AJ, Kuivanen S, Kekäläinen E, Ahava MJ, Loginov R, Kallio-Kokko H, et al.
411 Performance of six SARS-CoV-2 immunoassays in comparison with microneutralisation. *J Clin*
412 *Virol Off Publ Pan Am Soc Clin Virol.* août 2020;129:104512.
- 413 38. Meyer B, Torriani G, Yerly S, Mazza L, Calame A, Arm-Vernez I, et al. Validation of a
414 commercially available SARS-CoV-2 serological immunoassay. *Clin Microbiol Infect.* oct
415 2020;26(10):1386-94.
- 416 39. Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark
417 comparison. *Lancet Infect Dis.* déc 2020;20(12):1390-400.
- 418 40. Milani GP, Dioni L, Favero C, Cantone L, Macchi C, Delbue S, et al. Serological follow-up of
419 SARS-CoV-2 asymptomatic subjects. *Sci Rep.* 18 nov 2020;10(1):20048.
- 420 41. Petersen LR, Sami S, Vuong N, Pathela P, Weiss D, Morgenthau BM, et al. Lack of antibodies
421 to SARS-CoV-2 in a large cohort of previously infected persons. *Clin Infect Dis Off Publ Infect*
422 *Dis Soc Am.* 4 nov 2020;ciaa1685.

- 423 42. Huang AT, Garcia-Carreras B, Hitchings MDT, Yang B, Katzelnick LC, Rattigan SM, et al. A
424 systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of
425 protection, and association with severity. *Nat Commun.* 17 sept 2020;11(1):4704.
- 426 43. Piccoli L, Park Y-J, Tortorici MA, Czudnochowski N, Walls AC, Beltramello M, et al. Mapping
427 Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain
428 by Structure-Guided High-Resolution Serology. *Cell.* 12 nov 2020;183(4):1024-1042.e21.
- 429 44. Zhang W, Du R-H, Li B, Zheng X-S, Yang X-L, Hu B, et al. Molecular and serological
430 investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg*
431 *Microbes Infect.* 1 janv 2020;9(1):386-9.
- 432 45. WHO-2019-nCoV-lab-testing-2021.1-eng.pdf [Internet]. [cited 25 sept 2021]. Available on:
433 [https://apps.who.int/iris/bitstream/handle/10665/342002/WHO-2019-nCoV-lab-testing-2021.1-](https://apps.who.int/iris/bitstream/handle/10665/342002/WHO-2019-nCoV-lab-testing-2021.1-eng.pdf?sequence=1&isAllowed=y)
434 [eng.pdf?sequence=1&isAllowed=y](https://apps.who.int/iris/bitstream/handle/10665/342002/WHO-2019-nCoV-lab-testing-2021.1-eng.pdf?sequence=1&isAllowed=y)

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Table 1: Characteristics of the automated analyzers used for SARS-CoV-2 antibodies detection

Automated Analyzer	Certifications and EUA	detection Method	antibody detected	targeted antigen	sample volume	cut-off	result interpretation	reported sensitivity	reported specificity
Vidas®Bio mérieux®	CE-IVD, FDA-EUA	ELFA	IgM IgG	S1 RBD	100 uL including the dead volume	1 for both	<1 Negative > or = 1 Positive	IgG: n=71 out of 120, PPA 59,2% CI95%[49,8-68] 100% [66.4; 100.0] IgM: n=69 out of 111, PPA 62,2% CI95%[52,5-71,2], > 8 days 100% [63.1; 100.0]	IgG: 99.9% [99.4-100.0] , IgM: 99.4% [97.7-99.9].
CL- 900i®Mind ray®	CE-IVD	CLIA	IgM IgG	S and N protein	10 uL with a minimum of 100 uL of dead volume	1 for IgM 1 for IgG	<1 Negative > or = 1 Positive for IgM and <10 Negative > or = 10 Positive	82.22%	87.60%
Elecsys®R oche®	CE-IVD, FDA-EUA, WHO-EUL	ECLIA	Total antibodies: IgG+++, IgM and IgA	N protein	10 uL with a minimum of 100 uL of dead volume	1	<1 Negative > or = 1 Positive	100% [88,1-100] after 14 days	99.8 %.[99,7-99,9]
Access® Beckman®	CE-IVD, FDA-EUA	CLIA	IgG	S1 RBD	10 uL with a minimum of 100 uL of dead volume	1	<1 Negative > or = 1 Positive	100% [93,8-100] after 18 days	99.8 %.[99,4-99,9]

Table2: Experimental analytical performances for the 4 automated analyzers according to the manufacture criteria and to the new proposed criteria

	false -	false – ≥ 14 days	true +	true + ≥ 14 days	Sensitivity % CI95%	sensitivity ≥ 14 days % CI95%	false +	true -	Specificity % CI95%	AUC CI95%	VPP % CI95%	VPN % CI95%
Vidas® IgM	35	29	37	19	51,4 [39,3-63,3]	39,6 [25,7-54,7]	7	112	94,1 [88,2-97,6]	0,728 [0,659-0,789]	31,5 [17,8-49,4]	97,4 [96,6-97,9]
Vidas® IgG	17	8	55	40	76,4 [64,9-85,6]	83,3 [69,8-92,5]	0	119	100 [96,9-100]	0,882 [0,828-0,924]	100	98,7 [98,1-99,2]
Cobas®	15	7	57	41	79,2 [68-87,8]	85,4 [72,2-93,9]	0	119	100 [96,9-100]	0,896 [0,844-0,935]	100	98,9 [98,3-99,3]
Access®	32	20	40	28	55,5 [43,4-67,3]	41,7 [27,6-56,8]	0	119	100 [96,9-100]	0,778 [0,712-0,835]	100	97,7 [97-98,2]
Mindray® IgM	40	32	32	16	44,4 [32,7-56,6]	66,7 [51,6-79,6]	3	116	97,5 [92,8-99,5]	0,710 [0,640-0,773]	48,1 [22,7-74,5]	97 [96,4-97,6]
Mindray® IgG	15	8	57	40	79,2 [68-87,8]	83,3 [69,7-92,5]	5	114	95,8 [90,5-98,6]	0,875 [0,819-0,918]	49,8 [29,4-70,2]	98,8 [98,2-99,3]

Table 3: Results of the ROC analysis for new cut-offs

	Original cut-off	Proposed cut-off	sensitivity% CI95%	sensitivity \geq 14 days% CI95%	specificity% CI95%	AUC CI95%	<i>P</i>
Vidas® IgM	1	>0,87	59,7 [47,5-71,1]	52 [37,2 66,7]	94,1 [88,3-97,6]	0,767 [0,703-0,827]	0,01
Vidas® IgG	1	>0,55	84,7 [74,3-92,1]	91,2 [80 97,7]	98,3 [94,1-99,8]	0,922 [0,875-0,956]	0,05
Cobas®	1	>0,725	81,94 [71,1-90]	85,4 [72,2 93,9]	99,2 [95,4-100]	0,906 [0,855-0,943]	0,36
Access®	1	>0,14	83,3 [72,7-91,1]	83,3 [69,8 92,5]	100 [96,9-100]	0,917 [0,868-0,952]	$<10^{-4}$
Mindray® IgM	1	>0,83	47,2 [35,3-59,3]	37,5 [23,9 52,6]	95,8 [90,5-98,6]	0,715 [0,645-0,778]	0,63
Mindray® IgG	10	>7,94	81,9 [72,7-91,9]	87,5 [74,7 95,2]	94,1 [88,3-97,6]	0,887 [0,834-0,928]	0,34

Table 4: Concordance and discordance between the 4 evaluated tests results for the positive panel (n=72)

Group	N	VIDAS® IgG and/or IgM	Cobas®	Access®	Mindray® IgG and/or IgM	DAYS*
C1	35	+	+	+	+	25 [4-140]
C2	8	-	-	-	-	14 [0-60]
3 positive tests over 4 n=17	0	+	+	+	-	NA
	13	+	+	-	+	21 [6-90]
	4	+	-	+	+	20 [8-39]
	0	-	+	+	+	NA
2 positive tests over 4 n=11	3	+	+	-	-	39, 90, 162
	1	+	-	+	-	17
	1	+	-	-	+	9
	0	-	+	+	-	NA
	6	-	+	-	+	23 [15-86]
	0	-	-	+	+	NA
1 positive test over 4 n=01	1	+	-	-	-	7
	0	-	+	-	-	NA
	0	-	-	+	-	NA
	0	-	-	-	+	NA

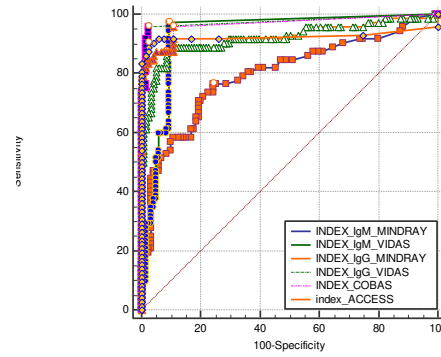
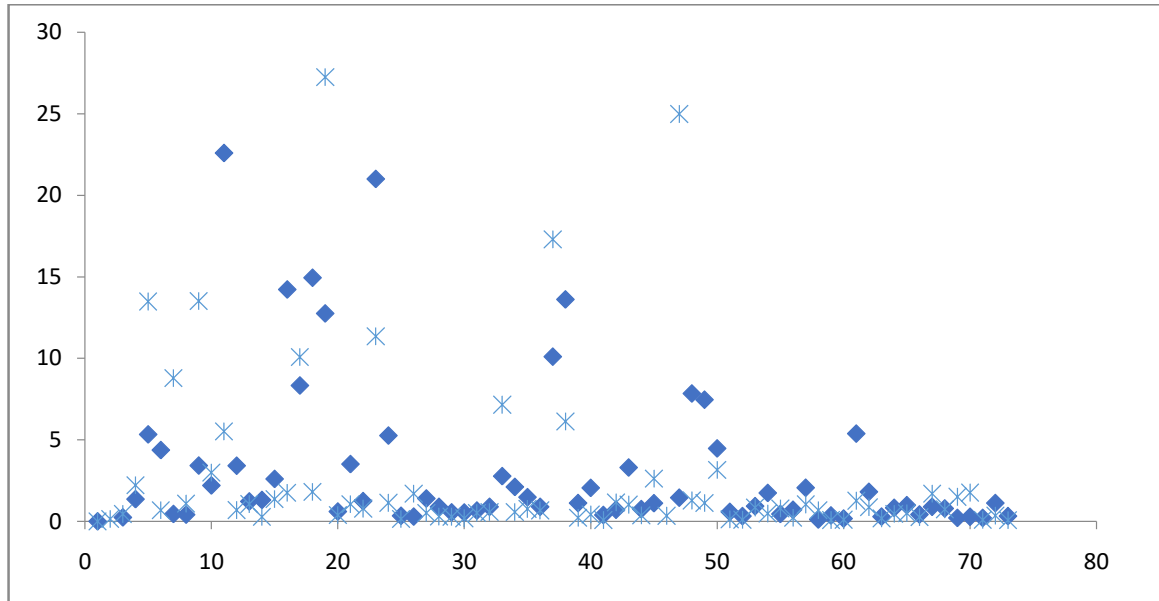


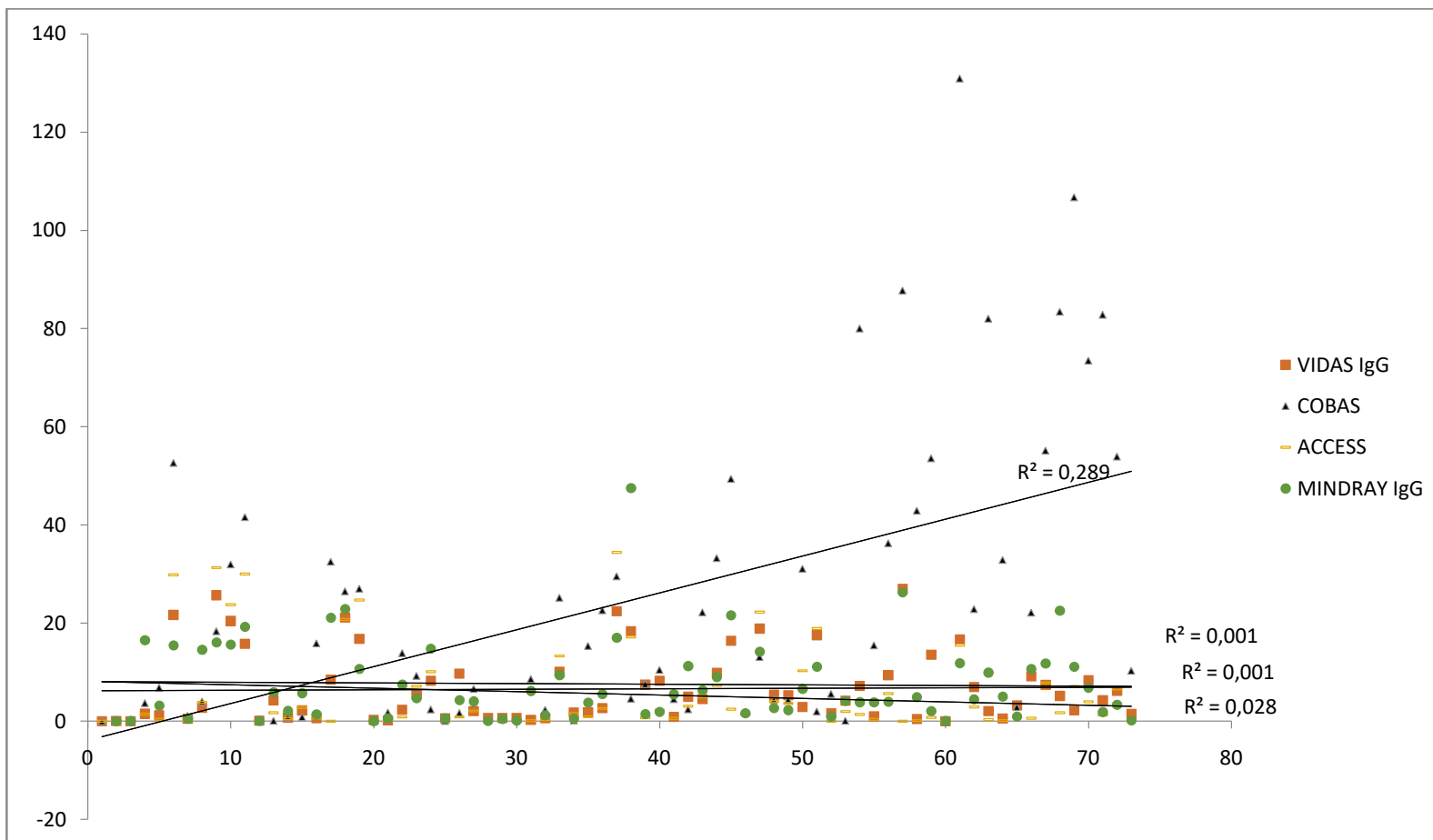
Figure 1: ROC curves for the 4 tests

Cohen's Kappa statistic	VIDAS® IgG	COBAS®	MINDRAY® IgG	
ACCESS®	0,792	0,630	0,684	
MINDRAY® IgG	0,815	0,817	0,564	COBAS®
COBAS®	0,848	0,526	0,790	MINDRAY® IgG
	0,647	0,380	0,832	ACCESS®
	MINDRAY® IgG	COBAS®	VIDAS® IgG	Pearson correlation coefficient

Figure 2: Agreement between qualitative results and correlation between COI/COI cut-offs ratios



A



B
Figure 3 Scatterplot tests indexes plotted against day of RT-PCR positive results. A: IgM tests, B: other tests.