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

Mariem Gdoura, Habib Halouani, Mehdi Mrad, Sahli Donia, Wafa Chamsa, Manel Mabrouk, Kamel Ben Salem, Nahed Hogga, Henda Triki

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

4   **Authors:** Mariem Gdoura[1,2], Habib Halouani[1], Mehdi Mrad[3], Sahli Donia[1], Wafa Chamsa[1],

5   Manel Mabrouk[3], Kamel Ben Salem[4], Nahed Hogga[1], Henda Triki[1].

6   **Authors affiliations:**

1. Laboratory of Clinical Virology, Institut Pasteur de Tunis/University Tunis El Manar/Tunis, Tunisia
2. Faculty of Pharmacy of Monastir/University of Monastir/ Monastir, Tunisia
3. Laboratory of Biochemistry and hormonology, Institut Pasteur de Tunis/University Tunis El Manar/Tunis, Tunisia
4.

17   **Abstract:**

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

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

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

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

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

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 *Herpessimplex 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 *Herpessimplex 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%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 *Herpessimplex 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 (paiwise comparison of

137   ROC curves for the 3 combinations *p*>0,05). However, Access® had an accuracy of

138   0,778CI95%[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 ou 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 consistenly 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.

7

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 socities

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).

8

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).

9

243   In contrast, cross reactivity with pre-pandemic pregnant women sera, and patients positive for

244   H*erpessimplex 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 coutry is

248   invited to evaluate regularily 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

10

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

11

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 Caeseele P, Bailey D, Forgie SE, Dingle TC, Krajden 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

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. Riester 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-
349   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-
352   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-
355   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-
365   canada/services/drugs-health-products/covid19-industry/medical-
366   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-
369   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-
373   19-work/target-product-profile-antibody-tests-to-help-determine-if-people-have-recent-infection-
374   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-
405   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-
434   eng.pdf?sequence=1&isAllowed=y

435
436
437

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®Biomérieux®	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®Mindray®	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®Roche®	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]

1

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]

2

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

	Original cut-off	Proposed cut-off	sensitivity% CI95%	sensitivity ≥ 14 days% CI95%	specificity% CI95%	AUC CI95%	*P*
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

3

**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
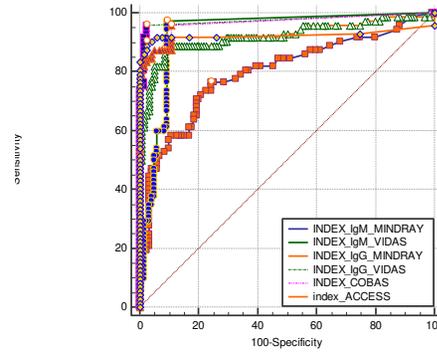
Figure 1: ROC curves for the 4 tests

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

1
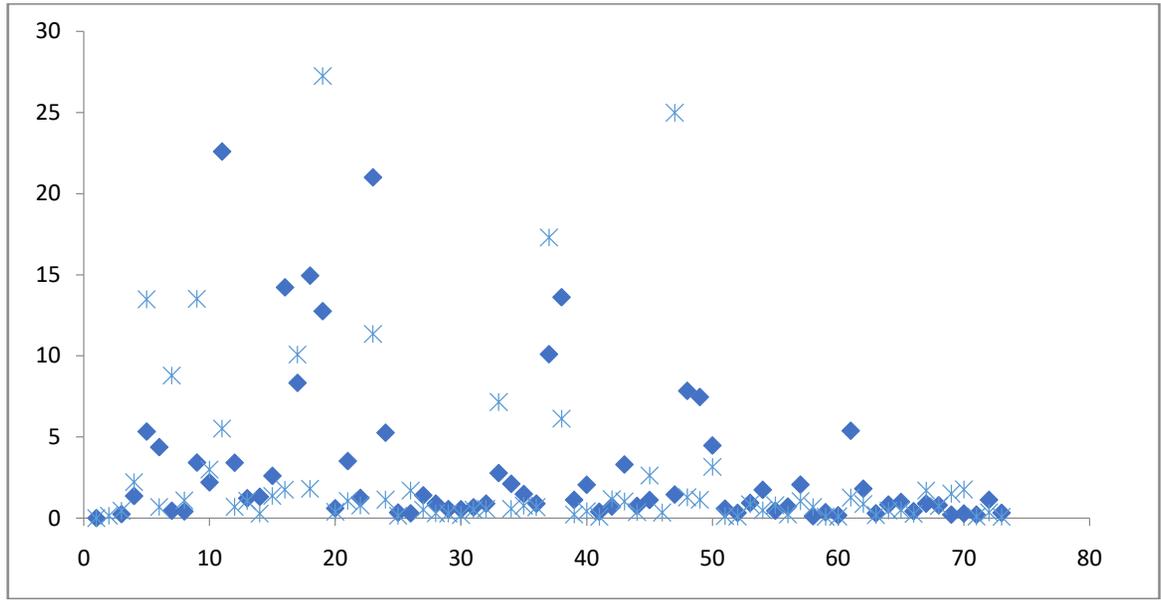
A
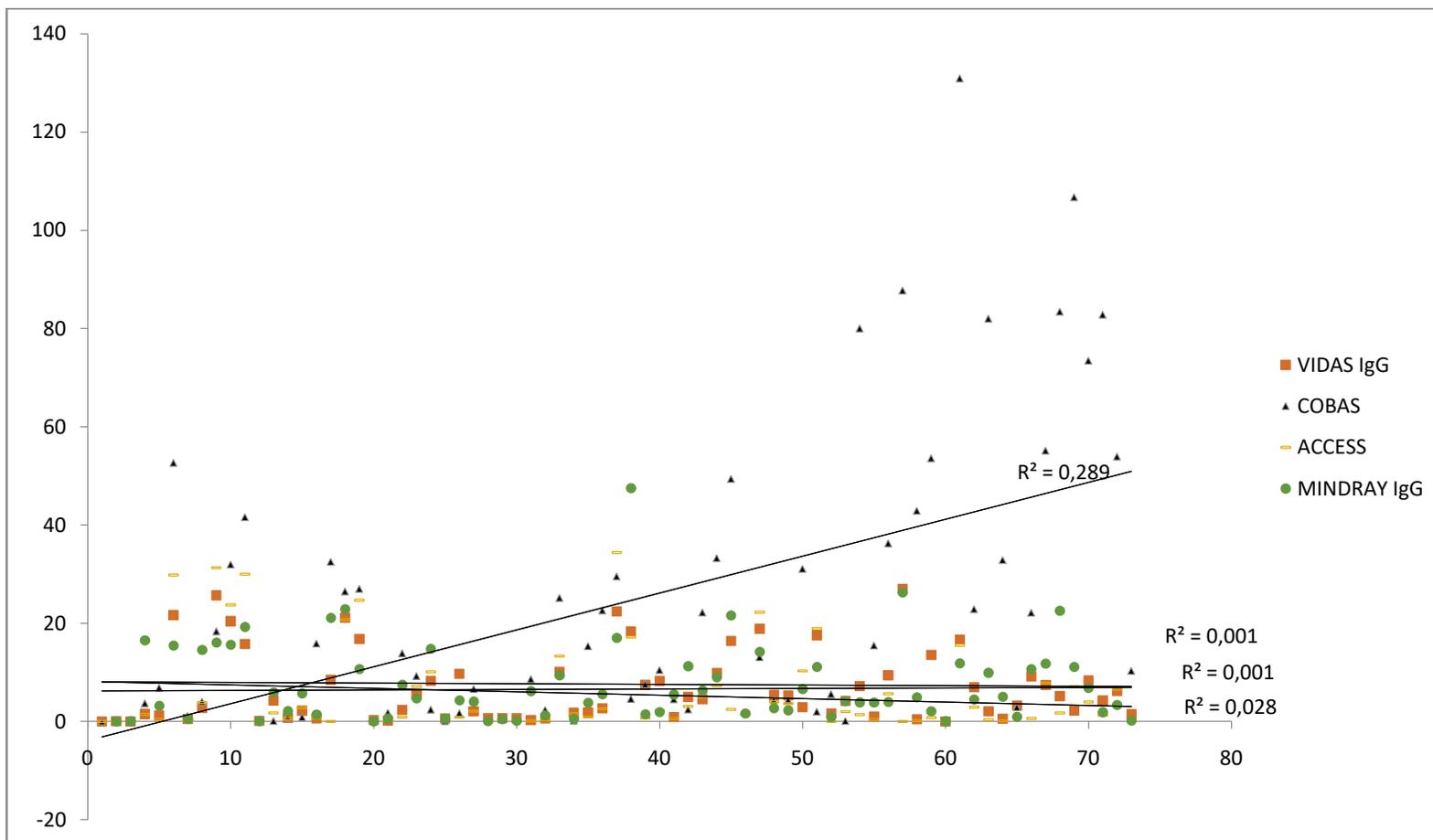
B

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