

1 **Comparison of seven commercial SARS-CoV-2 rapid Point-of-Care Antigen**
2 **tests**

3

4 Victor M. Corman^{1,2}, Verena Claudia Haage¹, Tobias Bleicker¹, Marie Luisa
5 Schmidt¹, Barbara Mühlemann¹, Marta Zuchowski³, Wendy Karen Jó Lei¹, Patricia
6 Tscheak¹, Elisabeth Möncke-Buchner¹, Marcel A. Müller^{1,2}, Andi Krumbholz⁴, Jan
7 Felix Drexler^{1,2}, Christian Drosten^{1,2}.

8 1: Institute of Virology, Charité-Universitätsmedizin Berlin, Corporate Member of
9 Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health,
10 10117 Berlin, Germany.

11 2: German Centre for Infection Research (DZIF), 10117 Berlin, Germany.

12 3: Labor Berlin GmbH, Berlin, Germany

13 4: Institute for Infection Medicine, Christian-Albrecht University and University
14 Medical Center Schleswig-Holstein, Kiel, Germany and Labor Dr. Krause und
15 Kollegen MVZ GmbH, Kiel, Germany

16

17

18 Address correspondence to:

19 Christian Drosten

20 Charite-Universitätsmedizin Berlin

21 Institute of Virology

22 Charitéplatz 1

23 10117 Berlin

24 Germany

25 christian.drosten@charite.de

26

27 **Abstract**

28 **Background**

29 Antigen point of care tests (AgPOCT) can accelerate SARS-CoV-2 testing. As first
30 AgPOCT are becoming available, there is a growing interest in their utility and
31 performance.

32 **Methods**

33 Here we compare AgPOCT products by seven suppliers: the Abbott Panbio™
34 COVID-19 Ag Rapid Test; the RapiGEN BIOCREDIT COVID-19 Ag; the Healgen®
35 Coronavirus Ag Rapid Test Cassette (Swab); the Coris Bioconcept Covid.19 Ag
36 Respi-Strip; the R-Biopharm RIDA®QUICK SARS-CoV-2 Antigen; the NAL von
37 minden NADAL COVID19-Ag Test; and the Roche/SD Biosensor SARS-CoV Rapid
38 Antigen Test. Tests were evaluated on recombinant nucleoprotein, cultured endemic
39 and emerging coronaviruses, stored clinical samples with known SARS-CoV-2 viral
40 loads (n=138), stored samples from patients with respiratory agents other than
41 SARS-CoV-2 (n=100), as well as self-sampled swabs from healthy volunteers
42 (n=35).

43 **Findings**

44 Limits of detection in six of seven tested products ranged between 2.08×10^6 and
45 2.88×10^7 copies per swab, the outlier at 1.58×10^{10} copies per swab. Specificities
46 ranged between 98.53% and 100% in five products, with two outliers at 94.85% and
47 88.24%. False positive results were not associated with any specific respiratory
48 agent. As some of the tested AgPOCT were early production lots, the observed
49 issues with specificity are unlikely to persist.

50 **Interpretation**

51 The sensitivity range of most AgPOCT overlaps with viral load figures typically
52 observed during the first week of symptoms, which marks the infectious period in the
53 majority patients. AgPOCTs with a limit of detection that approximates the virus
54 concentration above which patients are infectious may enable shortcuts in decision-
55 making in various areas of healthcare and public health.

56

57 **Background**

58 The ongoing SARS-CoV-2 pandemic challenges public health systems worldwide. In
59 absence of effective vaccines or drugs, virus detection by RT-PCR has been widely
60 adopted to enable nonpharmaceutical interventions based on case finding and
61 contact tracing. Because of its superior sensitivity and specificity, RT-PCR is the gold
62 standard for SARS-CoV-2 detection (1).

63 RT-PCR is a laboratory-based procedure that requires sophisticated equipment,
64 trained personnel, as well as logistics for sample shipment and results
65 communication. Timeliness of results is critical for the control of onward transmission
66 due to the concentration of viral shedding around the time of symptoms (2). The
67 widespread limitation of timely laboratory results is aggravated by the increasing
68 demand for RT-PCR tests certified for *in-vitro* diagnostic application, creating supply
69 bottlenecks and shortenings of overall testing capacity in many countries (3).

70 Antigen detection is usually inferior to RT-PCR in terms of sensitivity and specificity
71 (4, 5). Nevertheless, the possibility to perform point of care testing can provide
72 essential information when it is needed, even if in some situations the obtained
73 information has to be amended by an RT-PCR result obtained at a later point. As
74 first industry-manufactured antigen point of care test (AgPOCT) devices are
75 becoming available, there is a growing interest in their performance with particular
76 respect to sensitivity and overall specificity, two essential parameters that can guide
77 decisions over fields of application (6). Because of the intense but short-lived nature
78 of SARS-CoV-2 shedding from the upper respiratory tract, the clinical validation of
79 AgPOCT requires great attention to the timing of infection in studied subjects (7, 8).
80 If subjects are tested late in the course of infection, such as in the second week after
81 onset of symptoms, incongruences between RT-PCR and AgPOCT will cause an
82 apparently low clinical sensitivity for AgPOCT that is not necessarily relevant when
83 using these tests to diagnose early acute infections. From a practical perspective,
84 knowledge of the analytical- rather than clinical sensitivity of AgPOCT may be
85 sufficient to judge their utility in various fields of application, as compared to the well-
86 established RT-PCR as a reference method (9).

87 Here we aimed to compare seven available AgPOCT devices against an established
88 RT-PCR assay (10) by conducting a single-center evaluation in a laboratory setting.

89 Evaluation of analytical sensitivity relied on recombinant SARS-CoV-2 nucleoprotein,
90 SARS-CoV-2 cell culture supernatants, as well as stored clinical samples with
91 established SARS-CoV viral loads. Specificity was evaluated on cell culture
92 supernatants containing endemic and emerging human Coronaviruses, clinical
93 samples that earlier tested positive for respiratory pathogens, as well as fresh
94 nasopharyngeal self-swabs of healthy subjects.

95

96 **Material and methods**

97 Clinical samples

98 All stored specimens were taken for routine diagnostic testing with no extra
99 procedures required for the study. Specimens were stored in phosphate-buffered
100 saline (PBS) or universal transport medium (Copan UTM™) at -20°C. Respiratory
101 samples for specificity testing were obtained during 2019 from patients hospitalized
102 at Charité medical center and tested by the NxTAG® Respiratory Pathogen Panel
103 (Luminex). SARS-CoV-2 positive samples were collected between March and
104 October 2020 and tested and quantified by the SARS-CoV-2 E-gene assay as
105 published previously (10, 11). RNA was extracted from clinical samples by using the
106 MagNA Pure 96 system (Roche). The viral RNA extraction was performed using
107 100µl of sample, eluted in 100µl. Viral RNA of human coronaviruses (CoVs) other
108 than SARS-CoV-2 was quantified by real-time RT-PCR using specific *in vitro*
109 transcribed RNA standards (10, 12, 13). Virus RNA concentrations are given as
110 copies per mL.

111 SARS-CoV-2 negative healthy subjects

112 Healthy volunteers were employees of the institute of virology, between 22 and 61
113 years of age (median, 34.7 years). All subjects received instructions as well as
114 material to conduct self-testing with all AgPOCT at one point of time. All testing was
115 done under supervision of trained personal. Of note, most manufacturers do not list
116 self-test in their instructions for use. However, in recent study, self-sampling was
117 shown to be a reliable alternative to professional nasopharyngeal swabs for
118 AgPOCT (14). All manufacturers' instructions were exactly followed during self-
119 sampling.

120 AgPOCT testing

121 For the evaluation of the AgPOCTs, 50µl of stored respiratory samples (swab
122 resuspended in 1-3 mL of phosphate-buffered saline or universal transport medium)
123 were mixed with sample buffer volume as specified in the manufacturers'
124 instructions. Results in the form of a band on immunochromatography paper were
125 scored independently by two persons. In case of discrepant evaluations, a third
126 person was consulted to reach a final decision. In case of test failure indicated by
127 absence of a visible positive control band, the test procedure was repeated on the
128 same sample. All SARS-CoV-2 RNA negative samples that showed a false-positive
129 result in POCTs were retested.

130

131 Recombinant SARS-CoV-2 nucleoprotein (SARS-CoV-2-N)

132 The coding sequence of the SARS-CoV-2 nucleoprotein was amplified, purified and
133 cloned into the expression vector pET151/D-TOPO (ThermoFisher Scientific). E. coli
134 BL21 (DE3) cells were transformed with the pET151/D-TOPO-SARS-CoV-2 N
135 plasmid. Protein purification was performed by affinity chromatography under native
136 conditions as described previously with minor modifications (15). A second
137 purification step was included using heparine sepharose columns. N protein was
138 eluted with a NaCl gradient. For analytical sensitivity experiments SARS-CoV-2-N
139 protein was diluted in PBS and 50 µl of each dilution were applied to each test.
140 Three replicates per test were performed.

141

142 Cell culture samples

143 Cell culture supernatants containing all endemic human coronaviruses
144 (HCoV) 229E, NL63, OC43 and HKU1 as well as MERS-CoV, SARS-CoV, and
145 SARS-CoV-2 were tested in duplicates. Viral RNAs were extracted from cell culture
146 supernatants by the viral RNA mini kit (Qiagen) according to the manufacturer's
147 instructions. RNA concentration in all samples was determined by specific real-time
148 RT-PCR and *in vitro*-transcribed RNA standards designed for absolute quantification
149 of viral load. In the case of SARS-CoV-2 additional quantification was done by
150 plaque titration (11).

151 **Statistical analysis**

152 Logistic regression analyses were run using the PyMC3 package in Python (16). The
153 logistic regression model was implemented as follows:

154 $y \sim \text{Bernoulli}(\theta)$

155 $\theta = \text{logistic}(\alpha + \beta * X)$

156 $\alpha \sim \text{Normal}(0, 15)$

157 $\beta \sim \text{Normal}(0, 15)$

158

159 Where X is the observed \log_{10} SARS-CoV-2 RNA / mL, and y is the AgPOCT result.
160 Models were run for 25000 iterations with 5000 tuning steps using the automatically
161 assigned No-U-Turn sampler and an acceptance rate of 0.95. Models were
162 assessed for convergence using the Gelman Rubin statistic and visualization of
163 posterior traces. Posterior predictive distributions were used to assess model fit.

164

165 **Ethical statement**

166 The use of stored clinical samples for validation of diagnostic methods without
167 person-related data is covered by section 25 of the Berlin hospital law and does not
168 require ethical or legal clearance. The ethical committee has been notified of the
169 study and acknowledged receipt under file number EA1/369/20. The testing of
170 employees is part of an ongoing study on SARS-CoV-2 infection in employees under
171 Charité ethical review board file number EA1/068/20.

172

173 **Results**

174 **Analytical Sensitivity**

175 Initial comparisons of analytical sensitivity relied on purified bacterially-expressed
176 viral nucleocapsid protein, the target protein of all assays. Protein concentrations
177 between 5 and 25 ng/mL were detectable by most assays, corresponding to 250 to
178 1250 ng of protein per 50 μ l sample volume (**Table 1**). To confirm these figures on
179 viral protein, we tested cell culture supernatants from SARS-CoV-2-infected Vero
180 cells at defined concentrations of infectious (plaque-forming) units (PFU) of virus.
181 Almost all AgPOCT reliably detected ca. 44 PFU of virus per assay (**Table 1**). The
182 assays by manufacturers I, III, V, and VII detected as little as 4.4 PFU of virus per
183 test. The assay by manufacturer II was considerably less sensitive in detecting
184 recombinant protein as well as virus.

185

186 **Analytical sensitivity using clinical samples**

187 To determine the analytical sensitivity in clinical samples, we used stored swabs
188 obtained in universal transport medium (Copan UTM™) or without any medium. Dry
189 swabs were suspended in phosphate-buffered saline and all swab suspensions were
190 tested by RT-PCR as described (10). Of each suspension, 50 μ l were introduced into
191 the recommended volume of lysis reagent for each AgPOCT.

192

193 It should be noted that this procedure introduces a pre-dilution step (ca. 1:20) not
194 normally applied in AgPOCT protocols, resulting in a loss of sensitivity as opposed to
195 RT-PCR. On the contrary, the swabs used for this study are standard-gauge flocked
196 swabs that are not provided with AgPOCT. The swabs provided with AgPOCT
197 consist of the same material but are considerably thinner and thus carry less sample
198 volume. To estimate the relative sample input in the present procedure, we inserted
199 standard flocked swabs as well as the swabs included in AgPOCT kits in a solution
200 of 50% sucrose and determined the relative sample volume contained in each swab
201 by weighing. The resulting relative sample volume carried on AgPOCT swabs was
202 ca. 40% (range, ca. 10-90%) of that in standard-gauge swabs. Taking the above-
203 mentioned pre-dilution into account, this results in an approximately 8-fold lesser
204 sample input in AgPOCT in the present study, as opposed to direct application as

205 per manufacturer's instructions. This factor should be accounted for when directly
206 comparing against RT-PCR sensitivity in the following. It should be noted that the
207 piece-to-piece variability of swabs in some supplier's AgPOCT assays is
208 considerable.

209

210 A total of 138 SARS-CoV-2 RT-PCR positive samples were tested (**Figure 1A**).
211 Median virus load was 2.49×10^6 (range: 1.88×10^4 - 2.75×10^9) copies per mL of
212 swab suspension. Depending on initial testing and available volume per clinical
213 sample, up to 115 clinical samples per assay were used to evaluate AgPOCT assays
214 (**Figure 1B**). Only 45 samples were used for the assay by manufacturer II, which
215 detected only 4 of 45 samples correctly, each of these four samples containing more
216 than 2×10^8 RNA copies/mL, leading us to terminate further sensitivity testing for this
217 product. The distribution of test samples across all AgPOCT products is shown in
218 **Figure 1B**.

219 Based on this testing, a binary logistic regression analysis was performed to
220 determine 50% and 95% limits of detection per AgPOCT (**Supplementary Figure 1**).
221 Without correction for the lower sample input as opposed to standard AgPOCT
222 protocols in our study, the RT-PCR-quantified virus concentrations at which 95% hit
223 rates are achieved ranged between 3.4×10^6 and 7.41×10^7 copies per ml of swab
224 suspension for the five most sensitive assays. With correction for sample input, these
225 figures are lower by a factor of approximately 8 (**Table 2**).

226 **Exclusivity testing**

227 To determine any systematic cross-reactivity with relevant viral antigens, we tested
228 cell- or tissue culture supernatants containing known concentrations of the four
229 endemic human coronaviruses (HCoVs) as well as MERS- and SARS-CoV, applying
230 50 μ l of supernatant into the lysis buffer of each AgPOCT (**Table 3**). With one
231 exception that was not reproducible, none of the assays showed cross-reactivity
232 towards HCoVs and MERS-CoV. SARS-CoV was cross-detected by all assays.

233 We tested 100 stored clinical samples from patients with known acute infections
234 caused by respiratory viruses other than SARS-CoV-2, including some samples
235 containing mycoplasma and legionella. With one exception, all assays detected
236 either none, one, or two false positive results in 100 tests (**Table 4**). Of note, about
237 half of all false positive results were reproducible upon re-testing of the same

238 sample, while there was no association with any specific known pathogen in the
239 samples. This suggests a specific factor other than the tested pathogens to cause
240 positive signals. In 15 samples that tested false positive in total, one sample caused
241 a positive signal in two different assays.

242

243 **Testing of healthy volunteers**

244 In view of the rates of false positive results in clinical samples with two of the assays,
245 we conducted a self-testing exercise using all AgPOCT, employing healthy
246 laboratory members without signs of respiratory tract infection. As summarized in
247 **Table 5**, the same AgPOCT that generated false positive results with stored clinical
248 samples also showed increased rates of positives during testing of healthy subjects.
249 All positive results were resolved to false positive through immediate testing by RT-
250 PCR.

251

252 **Cumulative specificity**

253 The cumulative specificities from exclusivity testing as well as testing of healthy
254 volunteers were: Abbott Panbio™ COVID-19 Ag Rapid Test (99.26%), RapiGEN
255 BIOCREDIT COVID-19 Ag (100%); Healgen® Coronavirus Ag Rapid Test Cassette
256 (88.24%); Coris Bioconcept Covid.19 Ag Respi-Strip (100%); R-Biopharm
257 RIDA®QUICK SARS-CoV-2 Antigen (94.85%); NAL von minden NADAL COVID19-
258 Ag Test (99.26%); Roche/SD Biosensor SARS-CoV Rapid Antigen Test (98.53%).

259

260

261 **Discussion**

262 We provide a comparison of performance of seven AgPOCT assays that have
263 recently become available on the European market. These medical diagnostic
264 devices are cleared in many countries for use outside the laboratory as long as
265 testing results are supervised by medical personnel. The short turnaround time of
266 these tests is expected to enable major changes in clinical and public health
267 practice, given that sensitivity and specificity is sufficient. Because of the strong
268 demand during a constantly evolving situation, the latter question has not been
269 thoroughly clarified for most AgPOCT products.

270 The aim of the present study was to ease some of the challenges associated with
271 the clinical evaluation of AgPOCTs during the present pandemic situation. As the
272 arrival of prototype tests coincided with a time of low incidence over the summer
273 months in the Northern hemisphere, the recruitment of freshly infected subjects for
274 clinical evaluation has been difficult. Due to the rapid change of viral loads over the
275 acute phase of COVID-19 illness (11, 17), AgPOCT have a narrow timeframe for
276 their useful application that basically comprises the first week of symptoms. In view
277 of the growing experience with RT-PCR testing during this timeframe, we aimed to
278 mainly provide a reflection of test performance based on analytical properties, i.e.,
279 the approximate viral concentrations that can be detected by the assays as well as
280 their propensity to generate false positive results.

281 In terms of sensitivity, the detection range of most tests seemed to range between
282 one and ten million copies per swab (accounting for a systematic pre-dilution as
283 explained above) and thus corresponds to a concentration that predicts a virus
284 isolation success rate of ca. 20% in cell culture (11, 18, 19). In the cited studies, this
285 level of isolation success is typically reached by the end of the first week of
286 symptoms. He et al. have shown that this point in time also correlates with the end of
287 factual transmissibility (17). Although many caveats remain, the point in the course of
288 the first week of symptoms at which AgPOCT results turn negative may thus indicate
289 the time at which infectivity resolves. In a situation marked by transition to higher
290 incidence rates, the immediate availability of test results could enable novel public
291 health concepts in which decisions to isolate or maintain isolation are based on
292 infectivity testing rather than infection screening. Upon first patient contact, a positive
293 result in AgPOCT could also help physicians decide on immediate isolation
294 measures based on the identification of individuals who shed particularly large
295 amounts of virus. In hospitalized patients at the end of their clinical course, negative
296 AgPOCT results may provide an additional criterion to safely discharge patients.

297 Screening of asymptomatic subjects with the expectation of absence of virus is more
298 difficult. Given the limitations of sensitivity, the results of AgPOCT should be
299 understood as a momentary assessment of infectiousness rather than a diagnosis
300 with power to exclude infection. As there is a steep incline of virus concentration
301 around or before the onset of symptoms, guidelines for using AgPOCT should
302 mention that a negative test results may reflect a lack of sensitivity, particularly when

303 symptoms occur short after testing. Instructions that limit the validity of a negative
304 test result in healthy subjects to the day of application could be used to address this
305 challenge.

306 Also, the limited specificity of most AgPOCT should trigger RT-PCR confirmation of
307 positive tests whenever possible. We have seen acceptable rates of false positive
308 results with most AgPOCT but rates around 10% with two assays in particular. One
309 of these assays (R-Biopharm) was tested here as a preliminary version predating the
310 marketed product. The other assay may suffer from lot-to-lot variability as an
311 independent study of the same product does not show comparable issues with false
312 positives (information based on product insert by the distributor, HealGen).

313 There are clear limitations to our study. For instance, we can only provide an
314 approximate sensitivity assessment for individual AgPOCT as we used stored
315 samples on which we had to apply equal preanalytical treatments despite slight
316 differences between kits in terms of the size of the swab samples. An absolute
317 assessment of limits of detection for each test, as well as a strict comparison of
318 relative sensitivities is therefore not possible. Also, the encountered issues with
319 specificity of two products are likely to be transitory issues that can likely be
320 amended by adjustments of reagent concentrations and improvements of production
321 processes in the very near future, perhaps even before some products become
322 widely available. Our study finally does not compare practical differences between
323 assays, for instance, whether sample buffers come as bulk volume or are pre-filled in
324 reaction tubes. These issues are a main subject to the qualification of products as
325 consumer-grade tests (home tests), a process that is underway for some but not all
326 products. There are other limitations, including the absence of clinical information
327 due to anonymization of samples. Nevertheless, the present contribution provides an
328 early impression about the performance of AgPOCT of several major distributors.

329

330

331

332 **Acknowledgements**

333 Parts of this work was funded by European Union DG Research through projects
334 Prepare (GA602525) and Compare (GA643476) to CD, the German Ministry of
335 Research through projects RAPID (01KI1723A) and DZIF (301-4-7-01.703) to CD,
336 by the Federal Ministry for Economic Affairs and Energy (ZIM 16KN073824) to VMC,
337 and by the German Ministry of Health (Konsiliarlabor für Coronaviren) to CD and
338 VMC. This study is based on research funded in part by the Bill & Melinda Gates
339 Foundation (grant ID INV-005971) to JFD and CD. The findings and conclusions
340 contained within are those of the authors and do not necessarily reflect positions or
341 policies of the Bill & Melinda Gates Foundation.

342

343

344 **Table 1.** Outcome of testing by using serial dilutions of recombinant SARS-CoV-2
345 nucleoprotein and SARS-CoV-2 cell culture supernatant (triplicates). Protein and
346 virus were diluted in PBS. 50µl was used for testing; PFU, plaque-forming unit.

		AgPOCT assay ^a						
		I	II	III	IV	V	VI	VII
Recombinant	1,000	3	3	3	3	3	3	3
N-protein	250	3	3	3	3	3	3	3
Concentration	50	3	0	3	3	3	3	3
[ng/mL]	25	3	0	3	3	3	3	3
	10	3	0	3	0	3	3	3
	5	2	0	3	0	3	2	3
	2.5	0	0	3	0	3	0	0
SARS-CoV-2	8,800	3	3	3	3	3	3	3
[PFU/mL]	880	3	2	3	3	3	3	3
	88	3	0	3	0	3	1	3
	8,8	0	0	0	0	0	0	0
	0,88	0	0	0	0	0	0	0

347

348 ^aI: Abbott Panbio™ COVID-19 Ag Rapid Test; II. RapiGEN BIOCREDIT COVID-19 Ag; III: Healgen®
349 Coronavirus Ag Rapid Test Cassette (Swab); IV Coris Bioconcept Covid.19 Ag Respi-Strip; V: R-
350 Biopharm RIDA@QUICK SARS-CoV-2 Antigen; VI NAL von minden; NADAL COVID19-Ag Test; VII:
351 Roche/SD Biosensor SARS-CoV Rapid Antigen Test

352

353

354

355

356

357

358 **Table 2.** Limits of detection.

Assay ^a	N. of tested samples	Limit of detection ^b		Adjusted limit of detection ^d
		50% positive AgPOCT results	95% positive AgPOCT results	95% mean hit rate X 0.125
I	105	5.61 (5.27 - 5.95)	7.45 (6.79 - 8.20)	6.55 copies/swab
II ^c	45	9.51 (8.84 - 12.26)	11.10 (9.71 - 17.01)	10.20 copies/swab
III ^c	105	4.48 (3.41 - 5.32)	7.27 (6.27 - 8.40)	6.37 copies/swab
IV	105	7.60 (7.37 - 7.82)	8.36 (8.00 - 8.76)	7.46 copies/swab
V	105	5.40 (4.99 - 5.77)	7.22 (6.57 - 7.96)	6.32 copies/swab
VI	105	7.19 (6.97 - 7.43)	7.87 (7.52 - 8.23)	6.97 copies/swab
VII	115	5.64 (5.28 - 6.00)	7.68 (6.96 - 8.50)	6.78 copies/swab

359 ^aI: Abbott Panbio™ COVID-19 Ag Rapid Test; II. RapiGEN BIOCREDIT COVID-19 Ag; III: Healgen®
 360 Coronavirus Ag Rapid Test Cassette (Swab); IV Coris Bioconcept Covid.19 Ag Respi-Strip; V: R-
 361 Biopharm RIDA@QUICK SARS-CoV-2 Antigen; VI NAL von minden; NADAL COVID19-Ag Test; VII:
 362 Roche/SD Biosensor SARS-CoV Rapid Antigen Test

363 ^bMean concentration that yields 50% or 95% positive results according to a binary logistic regression
 364 analysis. Numbers in parenthesis denote the 95% highest posterior density interval determined by the
 365 Bayesian binary logistic regression model. Concentration per swab presumes that swabs are
 366 resuspended in 1 mL of fluid during preanalytical processes in RT-PCR used to determine viral loads.

367 ^cModel fit was suboptimal due to a large difference in the number of positive and negative test results.

368 ^dDue to a systematic preanalytical dilution factor in our AgPOCT evaluations, the projected mean
 369 concentrations at which 95% hit rate are achieved were corrected to be 8-fold (0.9 Log₁₀) lower. This
 370 correction factor is an average over all correction factors between the actual volume input in our
 371 validation studies and the volume input as per manufacturer's instruction. Input volumes in all cases
 372 are subject to great variability due to the undefined volumes of viscous respiratory tract specimens
 373 taken up by swab sampling devices. The here-provided statistical evaluation suggests a level of
 374 precision that does not reflect the clinical reality in AgPOCT use.

375

376 **Table 3. Specificity in testing using cell culture supernatants of other human**
377 **coronaviruses.**

Virus	Concentration [RNA copies/mL]	AgPOCT assay ^a						
		I	II	III	IV	V	VI	VII
HCoV-229E	2.87E+07	0/2	0/2	0/2	0/2	0/2	0/2	0/2
HCoV-OC43	1.0E+06	0/2	0/2	0/2	0/2	0/2	0/2	0/2
HCoV-NL63	1.70E+06	0/2	0/2	0/2	0/2	0/2	0/2	0/2
HCoV-HKU1	1.30E+07	0/2	0/2	1/3	0/2	0/2	0/2	0/2
MERS-CoV	1.87E+08	0/2	0/2	0/2	0/2	0/2	0/2	0/2
SARS-CoV	2.12E+09	2/2	2/2	2/2	2/2	2/2	2/2	2/2

378 ^aTests were performed by using non-inactivated cell culture supernatants in duplicates. Product
379 identities, I: Abbott Panbio™ COVID-19 Ag Rapid Test; II. RapiGEN BIOCREDIT COVID-19 Ag; III:
380 Healgen® Coronavirus Ag Rapid Test Cassette (Swab); IV Coris Bioconcept Covid.19 Ag Respi-Strip;
381 V: R-Biopharm RIDA®QUICK SARS-CoV-2 Antigen; VI NAL von minden; NADAL COVID19-Ag Test;
382 VII: Roche/SD Biosensor SARS-CoV Rapid Antigen Test

383

384

385 **Table 4. Specificity in the testing of clinical samples, without SARS-CoV-2**
 386 **detection:**

Pathogen	AgPOCT assay ^a							
	N	I	II	III	IV	V	VI	VII
Adenovirus	9	-	-	1 ^b	-	-	-	-
Bocavirus	9	-	-	-	-	-	-	-
HCoV-NL63	1	-	-	-	-	-	-	-
HCoV-OC43	1	-	-	-	-	-	-	-
Entero/Rhinovirus	9	-	-	1 ^b	-	-	-	-
Influenzavirus A H1	10	-	-	2 ^b	-	1 ^c	-	-
Influenzavirus A H3	9	-	-	2 ^{b,c}	-	1 ^c	-	-
Influenzavirus B	1	-	-	-	-	-	-	-
Metapneumovirus	1	-	-	-	-	-	-	-
Parainfluenzavirus 1	8	-	-	3 ^b	-	-	-	-
Parainfluenzavirus 2	3	-	-	2 ^{b,c}	-	-	-	-
Parainfluenzavirus 3	10 ^d	-	-	1 ^c	-	-	-	1 ^b
RSV-A	7	1 ^b	-	-	-	-	-	-
RSV-B	7	-	-	-	-	-	-	-
Mycopla. pneumon.	8	-	-	-	-	-	-	-
Legion. Pneumophila	7	-	-	-	-	-	-	-
Total	100	1	0	12	0	2	0	1

387 ^aI: Abbott Panbio™ COVID-19 Ag Rapid Test; II. RapiGEN BIOCREDIT COVID-19 Ag; III: Healgen®
 388 Coronavirus Ag Rapid Test Cassette (Swab); IV Coris Bioconcept Covid.19 Ag Respi-Strip; V: R-
 389 Biopharm RIDA@QUICK SARS-CoV-2 Antigen; VI NAL von minden; NADAL COVID19-Ag Test; VII:
 390 Roche/SD Biosensor SARS-CoV Rapid Antigen Test

391 ^bThe non-specific positive reaction was reproduced in a repeat test.

392 ^cThe non-specific positive reaction was reproduced in a repeat test in one of the two replicates.

393 ^dOne of these samples also was positive in assays III and VII

394

395

396 **Table 5. False positive results in 35 SARS-CoV-2 negative employees**

397

AgPOCT ^a	I	II	III	IV	V	VI	VII
False positives	-	-	3 ^c	-	5 ^c	1	1
Specificity (%) ^b	100	100	91.42	100	82.86	97.12	97.12

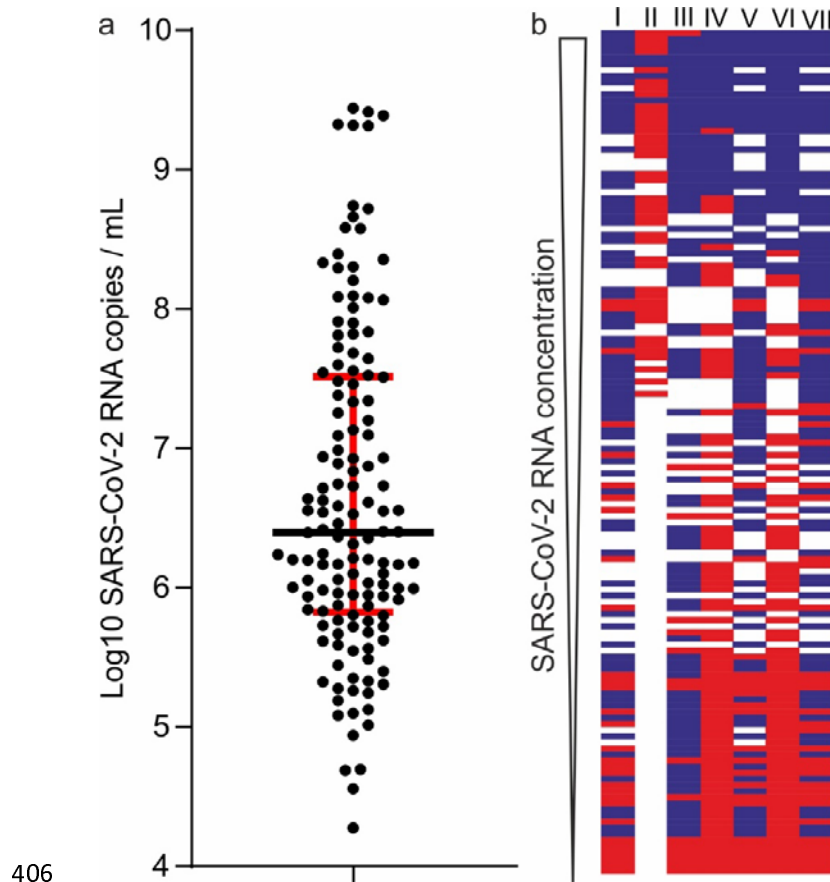
398

399 ^aI: Abbott Panbio™ COVID-19 Ag Rapid Test; II. RapiGEN BIOCREDIT COVID-19 Ag; III: Healgen®
400 Coronavirus Ag Rapid Test Cassette (Swab); IV Coris Bioconcept Covid.19 Ag Respi-Strip; V: R-
401 Biopharm RIDA@QUICK SARS-CoV-2 Antigen; VI NAL von minden; NADAL COVID19-Ag Test; VII:
402 Roche/SD Biosensor SARS-CoV Rapid Antigen Test;

403 ^bIn 35 subjects, 30 conducting nasopharyngeal swabs and 5 conducting pharyngeal swabs

404 ^cOne person tested false positive in assays III and V

405



406

407 **Figure 1**

408 a) Distribution of SARS-CoV-2 viral RNA concentrations across clinical samples
409 used for AgPOCT testing. b) Overview of tested samples and corresponding
410 outcomes in the seven AgPOCT (per column). Blue fields correspond to a positive
411 AgPOCT result, red fields to a negative result. Empty fields represent samples that
412 were not tested in the corresponding test.

413

414 References

- 415 1. Organization. WH. Coronavirus disease (COVID-19) technical guidance: Laboratory testing for
416 2019-nCoV in humans. 2020 [Available from: [https://www.who.int/emergencies/diseases/novel-](https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance)
417 [coronavirus-2019/technical-guidance/laboratory-guidance](https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance). .
- 418 2. Kretzschmar ME, Rozhnova G, Bootsma MCJ, van Boven M, van de Wijgert J, Bonten MJM.
419 Impact of delays on effectiveness of contact tracing strategies for COVID-19: a modelling study.
420 *Lancet Public Health*. 2020;5(8):e452-e9.
- 421 3. Guglielmi G. The explosion of new coronavirus tests that could help to end the pandemic.
422 *Nature*. 2020;583(7817):506-9.
- 423 4. Mak GC, Cheng PK, Lau SS, Wong KK, Lau CS, Lam ET, et al. Evaluation of rapid antigen test
424 for detection of SARS-CoV-2 virus. *J Clin Virol*. 2020;129:104500.
- 425 5. Bruning AHL, Leeflang MMG, Vos J, Spijker R, de Jong MD, Wolthers KC, et al. Rapid Tests for
426 Influenza, Respiratory Syncytial Virus, and Other Respiratory Viruses: A Systematic Review and Meta-
427 analysis. *Clin Infect Dis*. 2017;65(6):1026-32.
- 428 6. Mertens P, De Vos N, Martiny D, Jassoy C, Mirazimi A, Cuypers L, et al. Development and
429 Potential Usefulness of the COVID-19 Ag Respi-Strip Diagnostic Assay in a Pandemic Context. *Front*
430 *Med (Lausanne)*. 2020;7:225.
- 431 7. Dinnes J, Deeks JJ, Adriano A, Berhane S, Davenport C, Dittrich S, et al. Rapid, point-of-care
432 antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. *Cochrane Database Syst*
433 *Rev*. 2020;8:CD013705.
- 434 8. Krüger LJ, Gaeddert M, Köppel L, Brümmer LE, Gottschalk C, Miranda IB, et al. Evaluation of
435 the accuracy, ease of use and limit of detection of novel, rapid, antigen-detecting point-of-care
436 diagnostics for SARS-CoV-2. *medRxiv*. 2020:2020.10.01.20203836.
- 437 9. Ghebremedhin B, Engelmann I, König W, König B. Comparison of the performance of the
438 rapid antigen detection actim Influenza A&B test and RT-PCR in different respiratory specimens. *J*
439 *Med Microbiol*. 2009;58(Pt 3):365-70.
- 440 10. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019
441 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill*. 2020;25(3).
- 442 11. Wolfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological
443 assessment of hospitalized patients with COVID-2019. *Nature*. 2020;581(7809):465-9.
- 444 12. Corman VM, Eckerle I, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, et al. Detection of a
445 novel human coronavirus by real-time reverse-transcription polymerase chain reaction. *Euro*
446 *Surveill*. 2012;17(39).
- 447 13. Owusu M, Annan A, Corman VM, Larbi R, Anti P, Drexler JF, et al. Human coronaviruses
448 associated with upper respiratory tract infections in three rural areas of Ghana. *PLoS One*.
449 2014;9(7):e99782.
- 450 14. Lindner AK, Nikolai O, Kausch F, Wintel M, Hommes F, Gertler M, et al. Head-to-head
451 comparison of SARS-CoV-2 antigen-detecting rapid test with self-collected anterior nasal swab
452 versus professional-collected nasopharyngeal swab. *medRxiv*. 2020:2020.10.26.20219600.
- 453 15. Dijkman R, Jebbink MF, El Idrissi NB, Pyrc K, Müller MA, Kuijpers TW, et al. Human
454 coronavirus NL63 and 229E seroconversion in children. *J Clin Microbiol*. 2008;46(7):2368-73.
- 455 16. Salvatier J WT, Fonnesbeck C. Probabilistic programming in Python using PyMC3. *PeerJ*
456 *Computer Science* 2016;2:e55
- 457 17. He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, et al. Temporal dynamics in viral shedding and
458 transmissibility of COVID-19. *Nat Med*. 2020;26(5):672-5.
- 459 18. van Kampen JJA, van de Vijver DAMC, Fraaij PLA, Haagmans BL, Lamers MM, Okba N, et al.
460 Shedding of infectious virus in hospitalized patients with coronavirus disease-2019 (COVID-19):
461 duration and key determinants. *medRxiv*. 2020:2020.06.08.20125310.

462 19. Perera R, Tso E, Tsang OTY, Tsang DNC, Fung K, Leung YWY, et al. SARS-CoV-2 Virus Culture
463 and Subgenomic RNA for Respiratory Specimens from Patients with Mild Coronavirus Disease. *Emerg*
464 *Infect Dis.* 2020;26(11):2701-4.

465

466

467

468

469

470

