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1 # Evaluation of a SARS-CoV-2 rapid antigen test: potential to help reduce community
2 spread?

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9 ## ABSTRACT

10

11 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can spread from symptomatic
12 patients with COVID-19, but also from asymptomatic individuals. Therefore, robust surveillance
13 and timely interventions are essential for the control of virus spread within the community. In this
14 regard the frequency of testing and speed of reporting, but not the test sensitivity alone, play a
15 crucial role. In order to reduce the costs and meet the expanding demands in real-time RT-PCR
16 (rRT-PCR) testing for SARS-CoV-2, complementary assays, such as rapid antigen tests, have
17 been developed. Rigorous analysis under varying conditions is required to assess the clinical
18 performance of these tests and to ensure reproducible results. We evaluated the sensitivity and
19 specificity of a recently licensed rapid antigen test using 137 clinical samples in two institutions.
20 Test sensitivity was between 88.2-89.6% when applied to samples with viral loads typically seen
21 in infectious patients. Of 32 rRT-PCR positive samples, 19 demonstrated infectivity in cell culture,
22 and 84% of these samples were reactive with the antigen test. Seven full-genome sequenced
23 SARS-CoV-2 isolates and SARS-CoV-1 were detected with this antigen test, with no cross-
24 reactivity against other common respiratory viruses. Numerous antigen tests are available for
25 SARS-CoV-2 testing and their performance to detect infectious individuals may vary. Head-to-

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26 head comparison along with cell culture testing for infectivity may prove useful to identify better
27 performing antigen tests. The antigen test analyzed in this study is easy-to-use, inexpensive, and
28 scalable. It can be helpful in monitoring infection trends and thus has potential to reduce
29 transmission.

30

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31 ## 1. INTRODUCTION

32 Since the beginning of COVID-19 outbreak in December 2020, the global demand for the
33 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing has been steadily
34 increasing. Already back in March 2020, hospitals and laboratories around the world announced
35 their concerns about reagent, consumable material shortages, and limited personal protective
36 equipment. Yet, timely detection and isolation of SARS-CoV-2 infected cases and identification of
37 their contacts are pivotal to slowing down the pandemic.

38 The main public health strategy during a pandemic relies on robust and easy to perform
39 diagnostic tools that can be used to test large number of samples in a short time. To date the gold
40 standard diagnostic method for SARS-CoV-2 detection [1] is based on real time reverse
41 transcription-PCR (rRT-PCR) technology which has been promptly implemented by the World
42 Health Organization (WHO) [2], Center for Disease Control and Prevention (CDC) [3] protocols,
43 and a number of commercial assays [4]. The SARS-CoV-2 rRT-PCR has high specificity and
44 sensitivity [5, 6]. However, the type and quality of the patient specimen [7, 8], stage of the disease,
45 and the degree of viral replication and/or clearance have an impact on the test outcome [9]. These
46 factors are critical not only for PCR-based but also for other diagnostic test systems aiming to
47 detect the presence of the virus. Hence interpreting a test result for SARS-CoV-2 depends on the
48 accuracy of the test, but the prevalence and the estimated risk of disease before testing should
49 also be taken into consideration.

50 In many countries SARS-CoV-2 testing is extended to asymptomatic population, e.g. in
51 schools, airports, nursing-homes, and workplaces. This leads to a growing gap between the large
52 number of demand and the laboratory capacities to preform rRT-PCR tests, especially in
53 developing countries. Despite high specificity and sensitivity, rRT-PCR has a disadvantage in point
54 of care testing, because it usually requires professional expertise, expensive reagents and
55 specialized equipment. Therefore, alternative assays, such as rapid antigen detection tests, which

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56 can also detect the presence of the virus directly in respiratory samples, have been developed [4]
57 and tested by different groups [10-14]. However, it is vital to determine the sensitivity, specificity
58 of such tests relative to standard rRT-PCR in order to identify the ideal circumstances that their
59 application would be beneficial.

60 This study was performed to evaluate a novel antigen test produced by R-Biopharm for
61 the detection of SARS-CoV-2 in different specimens and to identify its limitations and potential
62 usage. Different types of materials and verification analysis were used by two institutions
63 independently to assure the reproducibility of the testing and to analyze the potential caveats.

64

65 **2. MATERIALS AND METHODS**

66 *2.1 Specimen collection*

67 At the Institute of Virology, Charité Berlin stored specimens taken after routine diagnostic were
68 used with no extra procedures required for the study. Cell culture supernantants of respiratory
69 viruses other than SARS-CoV-2 were available at the institute of virology, Charite through a EVD-
70 LabNet EQA (https://www.evd-labnet.eu/; Fischer/Mögling, unpublished data).

71 At the Institute of Virology, Frankfurt, the clinical samples were collected from subjects as part of
72 registered protocols. Combined oropharyngeal/nasal swabs were collected, stored in 2 ml PBS at
73 4°C and processed for further analysis within 24 hours.

74

75 *2.2 Cell culture and virus stocks*

76 Caco-2 (human colon carcinoma) were cultured in Minimum Essential Medium (MEM)
77 supplemented with 10% fetal calf serum (FCS). 100 IU/mL of penicillin and 100 g/mL of
78 streptomycin. All culture reagents were purchased from Sigma (St. Louis. MO. USA). The Caco-
79 2 cells were originally obtained from DSMZ (Braunschweig, Germany, no.: ACC 169) differentiated
80 by serial passaging and selected for high permissiveness to virus infection. Caco-2 cells were

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81 infected with different viral isolates (FFM1-FFM7) [15] at an MOI 0.1. Cell culture supernatant was
82 harvested 48 h after infection, precleared at 2000 x g for 10 min at room temperature. Aliquots of
83 virus particle containing supernatant were kept at -80°C.
84
85 *2.3 Detection of infectious virus in cell culture*
86 Of the swab-dilution, 500 µL were mixed with 1.5 ml of MEM containing 1% FCS (Sigma-Aldrich;
87 St. Louis, Missouri, USA), 7.5 µg/ml Amphotericin B, and 0.1 mg/ml Primocin, (InvivoGen; San
88 Diego, California, USA). Swab-inoculums were transferred to Caco-2 cells seeded in 5.5 cm2
89 culture tubes. Cytopathogenic effect (CPE) was assessed daily for up to seven days or until cell
90 lysis occurred.
91
92 *2.4 Rapid Antigen Test*
93 Rapid antigen test was provided by R-Biopharm. Test was performed according to the
94 manufacturer's recommendations and evaluated visually by four or six-eye principle. Briefly,
95 samples were vortexed for 20 sec. 50 µl from Solution A (blue) and B (yellow) were dispensed in
96 clean 1.5 ml reaction tubes which leads to green coloring. Immediately 50 µl of the test samples
97 were added to the reaction mixture. Samples were then mixed briefly and incubated for 10 min at
98 room temperature. Test strips were placed in to mixture vertically to allow absorption. Test results
99 were evaluated after 10 min. Intensities of the test bands were compared to control band
100 categorized as follows: +++ (test band intensity stronger than the control), ++ (test and control
101 bans intensity are similar), + (test band intensity is weaker than the control). Antigen testing for
102 viable SARS-CoV-2 and SARS-CoV-1 cell culture supernatants was performed in a BSL-3
103 laboratory.
104
105 *2.5 RNA extraction and rRT-PCR analysis*

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106 At the Institute of Virology, Charité Berlin, stored samples (swab resuspended in 1.5 mL of
107 phosphate-buffered saline) were anonymized before testing. After thawing at RT all samples were
108 analyzed by antigen test and rRT-PCR in parallel. RNA extraction for rRT-PCR was done by using
109 the MagNA Pure 96 system, using 100 µl of sample, eluted in 100 µl. rRT-PCR was done as
110 published previously [1].
111 At the Institute of Virology in Frankfurt the SARS-CoV-2 test (Cobas, Roche, Basel, Switzerland)
112 was performed on the rRT-PCR automated Cobas 6800 system. Of the swab-dilution, 1000 µl
113 aliquots were mixed with lysis buffer (1:1 ratio) and 500 µL aliquots were transferred to barcoded
114 secondary tubes, loaded on the Cobas 6800 system, and tested with Cobas SARS-CoV-2 master
115 mix containing an internal RNA control and primer-probe sets towards ORF1 and E-gene
116 according to the manufacturer's instructions.
117 Within seven days of virus inoculation using clinical sample material, culture supernatant was
118 collected to perform rRT-PCR in order to confirm productive virus replication. RNA was isolated
119 from 100 µL cell culture supernatant using the QIAcube HT instrument and QIAamp 96 Virus
120 QIAcube HT Kit (Qiagen; Hilden, Germany) according to the manufacturer's instructions. SARS-
121 CoV-2 RNA was analyzed by rRT- PCR using the Luna Universal One-Step RT-qPCR Kit (New
122 England Biolabs; Ipswich, Massachusetts, USA) and primers targeting RNA-dependent RNA
123 polymerase (RdRp) [15]. RdRP_SARSr-F2 (GTGARATGGTCATGTGTGGCGG)
124 RdRP_SARSr-R1 (CARATGTTAAASACACTATTAGCATA).
125

126 *2.6 Statistical Analysis*

127 The number of positive samples were compared two by two contingency table. The agreement
128 between the antigen test and rRT-PCR techniques was evaluated using the Cohen's weighted
129 kappa index (K value) [16]. K value interpretations were categorized as follows: <0.20 is poor,
130 0.21- 0.40 is fair, 0.41-0.60 is moderate agreement, 0.61- 0.80 is substantial agreement and 0.81-
131 1.00 is almost perfect agreement [17].

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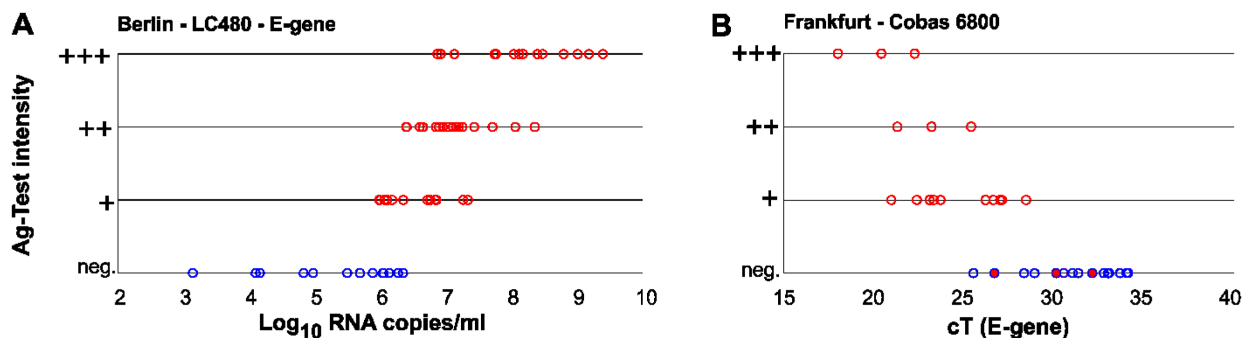
132 *2.7 Ethical Statement*

133 The use of stored clinical samples for validation of diagnostic methods without person related data
134 is covered by section 25 of the Berlin hospital law and does not require ethical or legal clearance.
135 The use of anonymized clinical samples for validation of diagnostic methods does not require
136 ethical clearance by the Goethe University, Frankfurt.

137

138 3. RESULTS

139 Rapid antigen test sensitivity and specificity were evaluated by two independent institutions
140 using various number of clinical samples. rRT-PCR was used as a reference test system. We
141 deemed individuals to be uninfected with SARS-CoV-2 when a negative result was obtained by
142 rRT-PCR.



143

144 **Figure 1. Antigen test analysis performed in Berlin (A) and Frankfurt (B). A.** Log_{10} RNA
145 copies/ml and corresponding antigen (Ag) detection test results (red circles positive n: 45, blue
146 circles negative n: 13) intensity for each rRT-PCR positive sample (n: 58). **B**. Cycle threshold (cT)
147 value and corresponding antigen (Ag) detection test results (red circles positive n: 16, blue circles
148 negative n: 16) intensity for each rRT-PCR positive sample (n: 32). 32 rRT-PCR positive samples
149 were tested in cell culture for infectivity. All Ag-test positive (n:16, red circles) and three Ag-test
150 negative (red-filled blue circles) samples displayed CPEs after inoculating in Caco-2 cells (**Table**
151 **S2**). Intensities of the test bands were compared to control band and designated as follows: +++

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152 (test band intensity stronger than the control), ++ (test and control bans intensity are similar), +
153 (test band intensity is weaker than the control).

154 In the Institute of Virology, Charité, Berlin, a total of 67 stored patient samples were
155 available for the study. Of these, 58 were rRT-PCR positive with cycle threshold (cT) range
156 between 18.77-40 corresponding to 2.5×10^9 -1380 RNA genome copies/ml (**Table S1**),
157 representing 86.6% (58/67) of the clinical samples analyzed (**Figure 1A**). When the rRT-PCR
158 results were used as a reference, the antigen test diagnosed SARS-CoV-2 infection status with a
159 sensitivity of 77.6% (45/58) and a specificity of 100% (9/9) (**Table 1**). After re-evaluating the data
160 based on the acceptable analytic sensitivity and limit of detection suggested by WHO [18], we
161 identified 48 samples with $\geq 10^6$ RNA genome copies/ml. Rapid antigen test performed with 89.6%
162 sensitivity for this sample set (**Table S1**). Of these, 40 samples had $\sim 2.23 \times 10^6$ or more RNA
163 genome copies/ml and reacted positive with the antigen test (**Table 1**). In contrast samples with
164 less than 7.63×10^5 RNA copies/ml were negative (**Figure 1A, Table S1**). Cohen's weighted kappa
165 value of 0.482 indicated moderate agreement between the rRT-PCR and the rapid antigen test
166 (**Table 2**). The overall concordance between the rRT-PCR and the antigen test was 80.6% (54/67).

167

		Antigen test				
		Negative	Positive	Marginal row	Sensitivity (%)	Specificity (%)
rRT-PCR	Negative	9	0	9		100
	Positive	13	45	58	77.6	
genome copies/ml	$\geq 2.23 \times 10^6$	0	40	40	100	
	1.38×10^3-2.21×10^6	13	5	18	27.7%	

168

169 **Table 1**. Sensitivity and specificity of the antigen detection test in comparison to rRT-PCR

170

171

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	Antigen test		
rRT-PCR	Negative	Positive	Row marginal
Negative	9	0	9 (13.4%)
Positive	13	45	58 (86.5%)
Column marginal	22 (32.8%)	45 (67.2%)	67

Weighted Kappa	0.482
Standard error	0.110
95% CI	0.266 to 0.698

172

173 **Table 2**. Cohen's weighted kappa coefficient between rapid antigen test and rRT-PCR.

174 Certain rapid tests may be used at the point-of-care and thus offer benefits for the detection
175 and management of infectious diseases. In order to assess the potential of the rapid antigen test
176 in this context, 70 nasopharyngeal samples freshly collected from individuals living in a shared
177 housing were analyzed head to head by rRT-PCR using Cobas 6800 system, rapid antigen test,
178 and cell culture using Caco-2 cells to determine the infectivity (Institute of Medical Virology,
179 Goethe University, Frankfurt). 45.7% (32/70) of the clinical samples were diagnosed positive for
180 SARS-CoV-2 by rRT-PCR with cT values ranging between 18.01-35.98 (**Figure 1B, Table S2**).
181 The antigen test diagnosed the infection status with a sensitivity of 50% (16/32) and a specificity
182 of 100% (**Table 3**). Re-evaluating the data based on the limit of detection, sensitivity was
183 determined to be 88.2% for samples with cT values <28, and it was reduced in the group of
184 samples with cT values ≥ 28 (6.7%) (**Table 3**). Cohen's weighted kappa value of 0.521 indicated
185 moderate agreement between rRT-PCR and the rapid antigen test (**Table 4**). The overall
186 concordance between the rRT-PCR and the antigen test was 77.1% (54/70) (**Table 4**).

187 rRT-PCR is a highly sensitive method to detect viral RNA molecules from clinical samples.
188 However, viral RNA can persist in different body parts and can be detected in specimens for much
189 longer than the presence of viable virus [19]. Thus demonstration of infectivity on permissive cell
190 lines *in vitro* is a more reliable surrogate for infectivity and virus transmission. Therefore, we

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191 attempted virus isolation by inoculating rRT-PCR positive samples in Caco-2 cells. Cytopathic
192 changes were monitored daily by microscopy for a week and subsequently aliquots of culture
193 supernatant were tested to verify viral RNA copies (**Table S2**). For samples that are positive for
194 both antigen test and rRT-PCR (16/32, cT 18.01-28.45), we observed cytopathic effects (CPE) in
195 cell culture 1-3 days after inoculation (**Figure 1B, Table S2**). Three samples that had a negative
196 result in the antigen test, but were positive by rRT-PCR (cT values 26.69, 30.12, and 32.13)
197 displayed CPE as well. Other 13 antigen-test negative samples with higher cT values (indicating
198 lower viral load) between 28.34-34.12 were not infectious in cell culture. Interestingly, one sample
199 with a relatively low cT value 25.53, did not show any CPE in cell culture and was also negative
200 for the antigen test (**Table S2**).

201

		Antigen test				
		Negative	Positive	Marginal row	Sensitivity (%)	Specificity (%)
rRT-PCR	Negative	38	0	38		100
	Positive	16	16	32	50	
cT<28	Positive	2	15	17	88.2	
cT≥28	Positive	14	1	15	6.7	

202

203 **Table 3**. Comparison of the clinical diagnostic performance of rapid antigen test with rRT-PCR.

204

	Antigen test		
rRT-PCR	Negative	Positive	Row marginal
Negative	38	0	38 (54.3%)
Positive	16	16	32 (45.7%)
Column marginal	54 (77.1%)	16 (22.9%)	70

Weighed Kappa	0.521
Standard error	0.092
95% CI	0.339 to 0.702

205

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206 **Table 4**. Cohen's weighted kappa coefficient between rapid antigen test and rRT-PCR.

207 In order to investigate potential cross reactivity among common coronaviruses and other
208 respiratory viruses, infectious and heat inactivated (4 h at 60°C) cell culture supernatants were
209 tested (**Table 5**). SARS-CoV-1 and SARS-CoV-2 tested positive with the antigen test, as
210 expected. The antigen test did not display any cross-reactivity with the other respiratory and
211 endemic corona viruses listed in **Table 5**.

212

Cell culture supernatant with virus	Ag-Test
SARS-CoV-1	+
SARS-CoV-2	+
HCoV-229E	-
HCoV-NL63	-
MERS	-
Enterovirus	-
Rhinovirus	-
Parainfluenzavirus 1	-
Parainfluenzavirus 2	-
Parainfluenzavirus 3	-
Parainfluenzavirus 4	-
hMPV A	-
hMPV B	-
RSV	-
Influenzavirus A H1N1	-
Influenzavirus A H3N2	-
Influenza B	-

213

214 **Table 5**. Rapid Antigen Test results using different respiratory virus cell culture supernatant
215 stocks.

216 We further evaluated the detection sensitivity among different SARS-CoV-2 isolates. Here
217 we used cell culture supernatant collected from Caco-2 cells infected with seven different isolates
218 [15] and SARS-CoV-1 (**Figure 3**). The virus stocks were thawed at room temperature and a total

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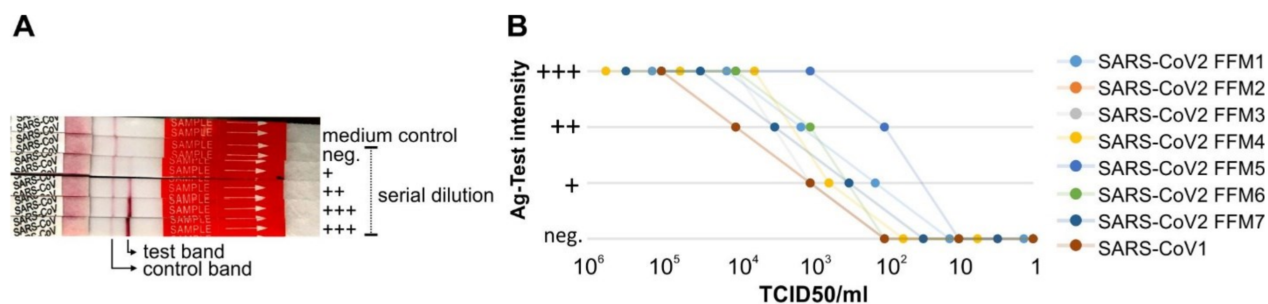
219 of six 10-fold dilutions were prepared in PBS. The antigen test was performed and evaluated
220 immediately (**Figure 3A**). In parallel, aliquots of the dilutions were mixed with lysis buffer used for
221 RNA extraction to inactivate the virus. rRT-PCR was performed for two different gene targets
222 ORF1 and E-gene that resulted in similar cT values (**Figure 3B, Table S3**). 10-fold serial dilutions
223 led to ~3 cT difference in rRT-PCR for each set as anticipated. According to our results the limit
224 of detection was between 100-560 RNA copies/ml which is in line with the manufacturer's findings.
225 We previously identified RG203KR mutations in FFM3, FFM4 and FFM6 and S→L mutation in
226 FFM1 within the nucleocapsid protein coding region [15]. According to GISAID classification the
227 GR clade, carrying the combination of Spike D614G and nucleocapsid RG203KR mutations, is
228 currently the most common representative of the SARS-CoV-2 population worldwide [20]. Our
229 results suggest that the presence of the RG203KR mutation did not interfere with the antigen test
230 performance.



231

232 **Figure 3.** Rapid Antigen Test Results for SARS-CoV-1 and SARS-CoV-2 isolates. **A.**
233 Representative lateral flow assay using serially diluted virus stock. Intensities of the test bands
234 were compared to control band and designated as follows: +++ (test band intensity stronger than
235 the control), ++ (test and control bans intensity are similar), + (test band intensity is weaker than
236 the control). **B.** TCID50/ml values and corresponding antigen (Ag) detection test intensity for
237 serially diluted SARS-CoV-2 isolates FFM1-7 and SARS-CoV-1 are shown. Representative result
238 of two experiments.

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240 **4. DISCUSSION**

241 In this study we validated the assay performance of a recently approved rapid antigen test
242 in two independent institutions using a total of 137 clinical samples. Although the test specificity
243 was 100% for this particular sample set, overall sensitivity was low (50-77.6%), yet re-analyzing
244 samples with higher viral loads showed good correlation (88.2-89.6%). Previous studies reported
245 that lower cT values are associated with higher viral culture positivity [21, 22]. There is currently
246 no direct evidence whether cell culture positivity or higher viral load correlates with contagiousness
247 of an individual, however, it is commonly recognized as the surrogate of infectivity [23]. Since an
248 important aspect of using point-of-care testing is to able to identify infected individuals who are
249 infectious and can potentially transmit the virus, we performed correlation analysis within a group
250 of clinical samples tested. 19 out of 32 SARS-CoV-2 infected individuals were positive in cell
251 culture. The antigen test detected 16 out of 19 these (84%). In contrast 43.7% (14/32) of the
252 samples were not infectious in cell culture, yet positive by rRT-PCR, probably due to persisting
253 genomic and subgenomic viral RNA within the collected sample. We detected an excess amount
254 of viral RNA in cell culture supernatants due to high replication capacity of the virus in permissive
255 cells, despite a negative antigen test result. This might explain the cT discrepancy between the
256 cell culture supernatant and clinical samples. Limited clinical sample size is the major limitation of
257 this study. Future efforts should aim to monitor frequent sampling of larger groups and to compare
258 different rapid antigen tests, different sampling sites along with infectivity correlation in cell culture.

259 Our results suggest that the rapid antigen test can detect SARS-CoV-2 infected individuals
260 with high viral loads and has potential in determining highly contagious individuals. Despite low
261 analytic sensitivity, rapid antigen tests are inexpensive and therefore can be used frequently for
262 detecting infected individuals who are asymptomatic, pre-symptomatic and without known or
263 suspected exposure to SARS-CoV-2 [24]. They can be beneficial in congregate settings, such as
264 a long-term care facility or a correctional facility, workplace, or a school testing its students, faculty,

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265 and staff. Rapid antigen tests probably perform best during the early stages of SARS-CoV-2
266 infection when the viral load is higher.
267

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278

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280 **SUPPLEMENTARY TABLES**

281 **Table S1.** Summary of rRT-PCR and antigen testing using clinical samples (Charite, Berlin). neg:
282 negative antigen test. N/D: not detectable.

283

Sample ID	rRT-PCR		Antigen test
	E-gene	Log10 RNA copies/mL	Intensity
#65	18,77	9,40	+++
#19	19,48	9,18	+++
#60	20,02	9,01	+++
#56	20,72	8,80	+++
#22	21,78	8,47	+++
#66	22,01	8,40	+++
#25	22,02	8,40	+++
#67	22,16	8,35	++
#21	22,74	8,17	+++
#20	22,94	8,11	+++
#57	23,11	8,06	++
#15	23,19	8,04	+++
#53	24,07	7,76	+++
#26	24,15	7,74	+++
#8	24,24	7,71	++
#12	25,15	7,43	++
#27	25,47	7,33	+
#58	25,7	7,26	+
#14	25,74	7,25	++
#1	25,92	7,19	++
#18	26,04	7,16	++
#64	26,12	7,13	+++
#4	26,14	7,13	+++
#10	26,22	7,10	++
#11	26,44	7,03	++
#23	26,71	6,95	++
#3	26,81	6,92	+++
#28	26,89	6,90	++
#29	26,89	6,90	++
#61	26,98	6,87	+++
#5	27,03	6,85	+
#13	27,05	6,85	++

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#59	27,09	6,83	+
#55	27,32	6,76	+
#49	27,47	6,72	+
#2	27,69	6,65	++
#17	27,85	6,60	++
#6	28,49	6,40	++
#7	28,52	6,39	++
#9	28,67	6,35	+
#46	28,68	6,34	neg
#50	28,92	6,27	neg
#51	29,21	6,18	+
#47	29,36	6,14	neg
#24	29,46	6,10	+
#32	29,59	6,06	+
#33	29,64	6,05	neg
#31	29,68	6,04	neg
#16	29,83	5,99	+
#54	29,87	5,98	+
#52	30,18	5,88	neg
#48	30,81	5,69	neg
#30	31,45	5,49	neg
#39	33,13	4,97	neg
#43	33,61	4,83	neg
#62	35,76	4,16	neg
#63	35,98	4,10	neg
#41	40	3,14	neg
#34	N/D	N/D	neg
#35	N/D	N/D	neg
#36	N/D	N/D	neg
#37	N/D	N/D	neg
#38	N/D	N/D	neg
#40	N/D	N/D	neg
#42	N/D	N/D	neg
#44	N/D	N/D	neg
#45	N/D	N/D	neg

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287 **Table S2**. Summary of rRT-PCR (Cobas), antigen testing, virus isolation in cell culture, rRT-PCR
288 with cell culture supernatant of 70 clinical samples (University Hospital Frankfurt). neg: negative
289 antigen test. N/D: not detectable.

Sample ID	Roche (COBAS) rRT-PCR		Antigen test	CPE in cell culture	Luna One-step RdRP rRT-PCR with culture supernatant
	ORF1	E-gene			
1	18,01	18,01	+++	+	20,67
2	20,22	20,42	+++	+	21,27
3	21,45	21,31	++	+	20,69
4	21,9	20,97	+	+	19,89
5	21,96	22,27	+++	+	19,66
6	22,2	22,39	+	+	20,72
7	23,31	23,33	+	+	20,68
8	23,33	23,21	++	+	20,41
9	23,51	23,1	+	+	20,64
10	23,76	23,7	+	+	20,22
11	24,91	25,4	++	+	20,99
12	25,3	25,53	neg	-	36,72
13	26,4	26,63	+	+	20,93
14	26,56	26,69	neg	+	20,32
15	26,91	26,2	+	+	20,74
16	27,04	27,01	+	+	20,31
17	27,46	27,11	+	+	20,23
18	27,78	28,45	+	+	21,16
19	28,39	28,92	neg	-	36,11
20	28,8	28,34	neg	-	40,18
21	29,32	30,12	neg	+	20,64
22	29,84	30,54	neg	-	38,55
23	30,15	31,03	neg	-	41,26
24	30,45	31,35	neg	-	37,28
25	31,49	32,77	neg	-	36,69
26	31,89	32,99	neg	-	37,30
27	32,19	33,06	neg	-	37,39
28	32,34	34,03	neg	-	36,73
29	33,69	32,13	neg	+	20,50
30	35,01	33,04	neg	-	38,44
31	35,39	33,67	neg	-	39,52
32	35,78	34,12	neg	-	36,94
33	36,3	N/D	neg	-	36,68
34	N/D	N/D	neg		
35	N/D	N/D	neg		
36	N/D	N/D	neg		
37	N/D	N/D	neg		
38	N/D	N/D	neg		
39	N/D	N/D	neg		
40	N/D	N/D	neg		
41	N/D	N/D	neg		
42	N/D	N/D	neg		
43	N/D	N/D	neg		
44	N/D	N/D	neg		
45	N/D	N/D	neg		
46	N/D	N/D	neg		
47	N/D	N/D	neg		
48	N/D	N/D	neg		

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49	N/D	N/D	neg	
50	N/D	N/D	neg	
51	N/D	N/D	neg	
52	N/D	N/D	neg	
53	N/D	N/D	neg	
54	N/D	N/D	neg	
55	N/D	N/D	neg	
56	N/D	N/D	neg	
57	N/D	N/D	neg	
58	N/D	N/D	neg	
59	N/D	N/D	neg	
60	N/D	N/D	neg	
61	N/D	N/D	neg	
62	N/D	N/D	neg	
63	N/D	N/D	neg	
64	N/D	N/D	neg	
65	N/D	N/D	neg	
66	N/D	N/D	neg	
67	N/D	N/D	neg	
68	N/D	N/D	neg	
69	N/D	N/D	neg	
70	N/D	N/D	neg	

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293 **Table S3**. Cycle threshold (cT) value for ORF1 and E-gene rRT-PCR and rapid antigen test results

294 for serially diluted SARS-CoV-2 isolates and SARS-CoV-1. neg: negative antigen test.

295

SAMPLE	**TCID50/ml**	**rRT-PCR (Cobas)**		**Ag-Test**
		ORF1	E-gene	Intensity
SARS-CoV-2 FFM1	1,3E+05	13,12	13,86	+++
	1,3E+04	16,56	16,79	+++
	1,3E+03	20,22	20,52	++
	1,3E+02	23,86	24,10	+
	1,3E+01	27,24	27,63	neg
	1,3E+00	30,50	31,02	neg
SARS-CoV-2 FFM2	1,0E+05	20,12	20,37	+++
	1,0E+04	23,23	23,62	++
	1,0E+03	27,07	27,29	+
	1,0E+02	30,25	30,53	neg
	1,0E+01	33,24	33,83	neg
	1,0E+00	34,78	36,23	neg
SARS-CoV-2 FFM3	1,0E+05	16,36	16,63	+++
	1,0E+04	19,78	19,95	+++
	1,0E+03	23,45	23,54	+
	1,0E+02	26,72	27,14	neg
	1,0E+01	30,15	30,68	neg
	1,0E+00	33,17	33,87	neg
SARS-CoV-2 FFM4	5,6E+05	12,7	13,4	+++
	5,6E+04	16,3	16,78	+++
	5,6E+03	19,46	19,73	+++
	5,6E+02	23,3	23,5	+
	5,6E+01	26,61	26,93	neg
	5,6E+00	29,7	30	neg
SARS-CoV-2 FFM5	1,0E+05	13,96	14,08	+++
	1,0E+04	16,66	16,81	+++
	1,0E+03	20,13	20,33	+++
	1,0E+02	23,26	23,64	++
	1,0E+01	27,01	27,08	+
	1,0E+00	29,74	30,11	neg
SARS-CoV-2 FFM6	1,0E+05	16,1	16,35	+++
	1,0E+04	19,57	19,83	+++
	1,0E+03	23,43	23,47	++
	1,0E+02	26,36	26,63	+
	1,0E+01	30,04	30,64	neg
	1,0E+00	33,36	34,05	neg
SARS-CoV-2 FFM7	3,0E+05	14,87	15,12	+++
	3,0E+04	17,71	18,02	+++
	3,0E+03	21,95	22,4	++
	3,0E+02	25,4	26,12	+
	3,0E+01	28,81	29,26	neg
	3,0E+00	32,11	32,72	neg
SARS-CoV-1	1,0E+05	n/d	17	+++
	1,0E+04	n/d	20,2	++
	1,0E+03	n/d	24,54	+
	1,0E+02	n/d	27,71	neg
	1,0E+01	n/d	31,1	neg
	1,0E+00	n/d	34,21	neg

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