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2 **SARS-CoV-2 Antigen Tests Predict Infectivity Based on Viral Culture: Comparison of**  
3 **Antigen, PCR Viral Load, and Viral Culture Testing on a Large Sample Cohort**

4

5 Short Title: SARS-CoV-2 Antigen Predicts Viral Culture Infectivity

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31 infectivity, sensitivity

32

### 33 **Abstract**

34 The relationship of SARS-CoV-2 antigen testing results, viral load, and viral culture detection  
35 remains to be fully defined. Presumptively, viral culture can provide a surrogate measure for  
36 infectivity of sampled individuals, and thereby inform how and where to most appropriately  
37 deploy available diagnostic testing modalities. We therefore determined the relationship of  
38 antigen testing results from three lateral flow and one microfluidics assay to viral culture  
39 performed in parallel in 181 nasopharyngeal swab samples positive for SARS-CoV-2. Sample  
40 viral loads, determined by RT-qPCR, were distributed across the range of viral load values  
41 observed in our testing population. We found that antigen tests were predictive of viral culture  
42 positivity, with the LumiraDx method showing enhanced sensitivity (90%; 95% confidence  
43 interval (95% CI) 83-94%) compared with the BD Veritor (74%, 95% CI 65-81%), CareStart  
44 (74%, 95% CI 65-81%) and Oscar Corona (74%, 95% CI 65-82%) lateral flow antigen tests.  
45 Antigen and viral culture positivity were also highly correlated with sample viral load, with areas  
46 under the receiver-operator characteristic curves (ROCs) of 0.94-0.97 and 0.92, respectively. In  
47 particular, a viral load threshold of 100,000 copies/mL was 95% sensitive (95% CI, 90-98%) and  
48 72% specific (95% CI, 60-81%) for predicting viral culture positivity. Taken together, the  
49 detection of SARS-CoV-2 antigen identified highly infectious individuals, some of whom may  
50 harbor 10,000-fold more virus in their samples than those with any detectable infectious virus.  
51 As such, our data support use of antigen testing in defining infectivity status at the time of  
52 sampling.

## 53 **Introduction**

54 Ongoing management of the SARS-CoV-2 pandemic will require judicious use of  
55 available diagnostic testing modalities. Testing may be used in two general contexts, either for  
56 diagnosis of symptomatic illness or to identify asymptomatic carriers so that appropriate  
57 isolation precautions can be instituted and thereby mitigate the infectious risk to others.

58 There are two major SARS-CoV-2 diagnostic technologies: (1) nucleic acid amplification  
59 tests (NAATs), typically real-time polymerase chain reaction tests (PCR), and (2) antigen tests,  
60 which generally detect the presence of viral nucleocapsid (N) antigen. For the purposes of  
61 discussion, we will use PCR to represent all types of NAAT tests, recognizing that there are  
62 several widely used alternative detection technologies such as transcription mediated  
63 amplification. PCR tests are highly sensitive, and generally target conserved regions in more  
64 than one viral gene to ensure robustness even in the context of ongoing evolution of genetic  
65 variants. However, they are expensive compared to antigen tests and have slower turn-around  
66 time (TAT) when performed on large automated diagnostic platforms, which are usually located  
67 in central laboratories. Most antigen tests currently on the market are based on lateral flow  
68 immunoassay methods and detect the conserved N protein (1). In contrast to NAAT tests, antigen  
69 tests are fast, highly amenable to point-of-use, and inexpensive. Although antigen tests have  
70 lower sensitivity compared with PCR tests, it should be noted that antigen and PCR tests detect  
71 different components of the SARS-CoV-2 virus, neither of which may necessarily reflect live  
72 replicating virus.

73 With regard to appropriate selection and use of these alternative testing modalities, the  
74 ultimate question is how sensitive does a test need to be to serve specific goals of the pandemic  
75 response, given cost constraints and speed of testing requirements. A test with lower sensitivity

76 may offer compelling benefit if it were able to reliably identify individuals who present an  
77 immediate infectious risk to others. Arguably, more frequent and repetitive testing of individuals  
78 using a test with lower sensitivity, may provide equivalent utility to more sensitive NAAT  
79 technology at lower cost and greater convenience (2).

80 An individual's infectivity presumptively may be approximated by the infectivity of a  
81 diagnostic sample. Diagnostic samples may contain viable virus, non-viable/non-replicating  
82 virus components, and/or free viral nucleic acid. Antigen and PCR tests target different  
83 components of the SARS-CoV-2 virus, and detection by either technology does not necessarily  
84 reflect the presence of replication-competent, infectious virus and therefore infectiousness.  
85 However, viable virus can be cultured, i.e., it will infect and replicate in tissue culture cells.  
86 Therefore, tissue culture assays can provide an assessment of the presence and amount of viable  
87 virus, and, by inference, the infectivity of individuals from whom samples were obtained.

88 Therefore, we sought to compare the results of antigen and quantitative PCR viral load  
89 determination with viral culture detection of SARS-CoV-2 to further inform decisions on best  
90 utilization of antigen and PCR testing.

91

## 92 **Results**

93 In our study design, the same patient specimens were tested by reverse-transcription  
94 (RT)-qPCR; antigen detection using four different commercial methods, three with FDA  
95 Emergency Use Authorization; and viral culture for SARS-CoV-2. This avoided the need for six  
96 separate sample collections from each patient and the inherent variation introduced during  
97 collection of multiple specimens from the same individual (3). Of note, typically, the instructions  
98 for use (IFU) for antigen tests indicate that sample from a single nasal collection swab should be

99 eluted directly in the test specific extraction buffer. Therefore, we estimate that samples used for  
100 antigen testing in our protocol were ~17 to 18-fold more dilute (see materials and methods  
101 section) than they would have been had a separate, dedicated swab been used directly for these  
102 tests.

103 A total of 206 samples positive for SARS-CoV-2 by RT-qPCR using the Abbott M2000  
104 or Abbott Alinity m platforms (limit of detection ~100 genome copies/mL) were analyzed by  
105 LumiraDx (n= 206), BD Veritor (n=204), CareStart (n=201), and Oscar Corona (n=193) antigen  
106 tests and by viral culture (n=181). For all but thirteen samples, sufficient sample volume was  
107 available to test using all four antigen testing methods. Samples were preselected to span the  
108 distribution of viral loads observed during PCR testing from March to June, 2021.

109 We first compared categorical agreement between antigen test and viral culture results.  
110 Viral cultures were performed by adding sample to VeroE6 cells and cultured for 13-14 days.  
111 Viral culture positivity was scored both qualitatively and quantitatively using RT-qPCR to detect  
112 SARS-CoV-2 in viral culture supernatants as described in the materials and methods section.  
113 Two-by-two contingency tables, sensitivity, specificity, positive and negative predictive values  
114 for qualitative comparisons of each antigen testing method to viral culture are shown in Tables 1-  
115 4. Sensitivity was 90% for LumiraDx and 74% for the other methods, using viral culture  
116 positivity as the gold standard. Specificity was 70% for LumiraDx and 91-92% for the other  
117 antigen test methods. Negative predictive values for the LumiraDx were approximately 10%  
118 higher than for other methods.

119 We then examined the quantitative relationship between sample viral load and the  
120 presence and quantity of culturable virus. We observed that levels of virus in day 3 viral culture  
121 supernatant were reasonably correlated ( $R^2 = 0.55$ ) with original sample viral load, with a sharp

122 loss of detection of viable virus in samples with viral loads of less than  $\sim 10^5$  genome copies/mL  
123 (Fig. 1).

124 Distributions of viral load results determined by RT-qPCR in samples, testing positive or  
125 negative, respectively, by respective antigen tests are shown in Fig. 2. Lateral flow assay (LFA)-  
126 based antigen tests detected samples with a viral load  $> \sim 10^7$  genome copies/mL, with the  
127 exception of a small number of outliers. The microfluidic LumiraDx test detected samples with  
128 lower viral loads compared to the other antigen testing methods with a cutoff for consistent  
129 detection closer to  $10^6$  genome copies/mL. Differences between  $\log_{10}$  transformed mean viral  
130 loads of samples positive by LumiraDx and positive by each of the LFA methods, respectively,  
131 were statistically significant for all pairwise comparisons (adjusted  $P < 0.05$ ), as were differences  
132 in (geometric) mean viral loads of samples testing negative by LumiraDx and negative by each  
133 of the LFA methods, respectively, using the Holm-Sidak's multiple comparison test. However,  
134 BD Veritor, CareStart and Oscar Corona tests were indistinguishable in all pairwise comparisons  
135 with one another (adjusted  $P = 0.99$ ).

136 Receiver-operator characteristic curves (ROC) were plotted to determine the viral load  
137 cutoffs which would reasonably predict detection by viral culture and antigen testing,  
138 respectively (Fig. 3). Notably, a viral load cutoff of  $\sim 10^5$  was highly sensitive for predicting a  
139 positive viral culture without undue loss of specificity. A viral load cutoff of  $\sim 10^4$ - $10^5$  was  
140 reasonably sensitive for predicting a positive LumiraDx result without undue loss of specificity.  
141 Viral load cutoffs of  $10^5$ - $10^6$  likewise were reasonably predictive of positive BD, Oscar and  
142 CareStart test results. The ROC area under the curve (AUC) was  $> 0.94$  for all comparisons (Fig.  
143 3B-E), indicating that viral loads could serve as a reasonable surrogate for predicting the  
144 presence of culturable infectious virus and detectable antigen (and vice versa). Notably, viral

145 load cutoffs for detecting positive viral cultures and positive antigen tests were similar, further  
146 supporting similar qualitative detection by viral culture and antigen tests.

147 Three hundred SARS-CoV-2 RT-qPCR negative samples were also tested by all four  
148 antigen tests. All antigen tests were negative, supporting high specificity of the antigen assays.

149

## 150 **Discussion**

151 The evolving COVID-19 pandemic has brought a growing need for more and diverse  
152 diagnostic test methods. The detection of SARS-CoV-2 RNA by RT-qPCR is the gold standard  
153 for laboratory diagnosis of COVID-19, yet it is well recognized that the assay may detect RNA  
154 fragments or viral debris that do not correlate with viable infectious virus. For example, in the  
155 Syrian golden hamster model, transmissibility of SARS-CoV-2 correlated well with detection of  
156 infectious virus by culture, but not with positive RNA results by qPCR (4). Therefore, qPCR  
157 may identify a large number of patients who are infected, but who may not necessarily be  
158 infectious to others. SARS-CoV-2 viral culture may be a better surrogate of infectivity, but is  
159 currently impractical and rarely used as a primary testing modality due to its stringent biosafety  
160 requirements (Biosafety Level 3; BL3) requirements, assay complexity, and low throughput (5,  
161 6).

162 Antigen test methods are an inexpensive and more rapid test method compared to most  
163 NAAT tests and are amenable to point-of-care settings, for example, use at schools and for self-  
164 testing. We therefore compared performance of antigen tests to PCR and viral culture to assess  
165 the ability of antigen tests to identify infected and infectious individuals. Our general findings  
166 were that antigen tests largely predicted ability to culture live virus. Furthermore, culture and

167 antigen tests were both consistently positive at higher viral loads as determined by RT-qPCR and  
168 negative at lower viral loads.

169 Previous studies have also correlated viral culture with qPCR testing, describing positive  
170 cultures from upper respiratory tract samples between 6-9 days after infection (6-9). In our study,  
171 a viral load of cutoff of 100,000 copies/mL was 95% sensitive (95% CI, 90-98%) and 72%  
172 specific (95% CI, 60-81%) for predicting viral culture positivity. Other studies of SARS-CoV-2  
173 culture have reported similar findings, where viable virus culture was described from samples  
174 with 250,000-1,000,000 copies/mL (9, 10) or expressed alternatively as cycle threshold (Ct)  
175 value cutoffs of 24-35 (11, 12). Note, we converted from Ct values to viral loads (in units of  
176 genome copies/mL), since Ct values cannot be compared from assay to assay (3).

177 Presumptively, individuals with the highest amount of culturable virus pose the greatest  
178 degree of infectivity and risk to others. It should be stressed that viral loads for SARS-CoV-2  
179 vary over nine orders of magnitude. The difference between the lowest viral loads, where virus is  
180 consistently detected by culture ( $10^5$  copies/mL, Fig. 1, 3), and the highest observed viral loads  
181 (~1 billion copies/mL) is at least four orders of magnitude. Furthermore, above the viral culture  
182 detection threshold, we found that the amount of viable virus in day 3 culture supernatants was  
183 roughly proportional to sample viral load determined by qPCR (Fig. 1), suggesting that the large  
184 range of viral loads determined by RT-qPCR corresponds to the range and degree of sample  
185 infectivity. Importantly, antigen test sensitivity is noted to be near 100% in the upper three orders  
186 of magnitude of viral loads observed (Fig. 2, 3, 4), suggesting that antigen tests are quite good in  
187 detecting individuals who shed larger amounts of virions and therefore would pose significant  
188 risk to others during casual contact.

189           It has been argued by some that Ct values should not be made available to providers  
190 based on a number of reasons, including differences in the correlation between Ct and viral load  
191 generated from different assays (reviewed in (13)). Our data support the meaningful association  
192 of viral load with infectivity, and therefore further argue for conversion of ambiguous Ct values  
193 to unambiguous viral loads, calibrated to a universal standard, as we have proposed previously  
194 (3).

195           Interestingly, the viral load threshold associated with consistent LFA detection in our  
196 clinical samples was similar to the previously described analytical limit of detection (LoD) of the  
197 Quidel Sofia antigen test determined using SARS-CoV-2 infected Vero cell quality control  
198 material (14). The LoD for the Sofia test in viral genome copies/mL was inferred from the  
199 TCID<sub>50</sub> provided by the manufacturer in its Instructions for Use (IFU) (14), and the conversion  
200 factor between TCID<sub>50</sub> and viral genome copies/mL provided in documentation for the lots  
201 (70033548, 70034991) of BEI Resources, NR-52286 quality control material available during  
202 the time the IFU studies were performed (3, 14). This correspondence suggests that quantitative  
203 relationships between detectable antigen and viral genomic material are similar for human  
204 nasopharyngeal specimens and virus grown in cell culture.

205           Our study design had several strengths as well as some limitations. It is known that there  
206 may be variability in results obtained from repeat sampling of the same patient, for example, due  
207 to collection technique (3, 15). We sought to eliminate this source of confounding variability by  
208 performing antigen, RT-qPCR, and viral culture testing on the same samples. Therefore, the per  
209 sample performance characteristics of each methodology could be directly compared.

210           However, this led to the need to perform antigen testing outside of direct swab sampling  
211 testing recommendations, potentially leading to underestimation of antigen test sensitivity. Based

212 on the dilution factor in viral transport medium, and the amount of sample used in antigen testing  
213 in our study, we estimate that antigen tests, had they been performed by directly eluting swab  
214 samples into antigen test extraction buffer, would have detected samples with approximately 17  
215 to 18-fold lower viral loads. As such, the antigen tests would have identified samples from  
216 individuals with the lowest viral loads associated with a positive viral culture. For example, we  
217 estimate that if swab samples were tested directly without dilution in viral transport medium,  
218 LFA antigen tests would have reliably detected individuals with viral loads  $> \sim 550,000$  genome  
219 copies/mL, and the LumiraDx test would have reliably detected individuals with viral loads  $>$   
220  $\sim 60,000$  copies/mL (see Fig 2). It is possible though that this estimate is too high or too low  
221 based on pre-analytical (e.g., sample elution efficiency) and analytical variables not appreciated.

222 Both a strength and weakness of the approach was the selection of samples based on the  
223 distribution of viral loads observed during clinical testing (3). Samples were not selected based  
224 on patients' clinical symptoms, timing of symptoms, and potential exposures. Our testing centers  
225 obtain samples from community and hospital settings, both for diagnosis of illness and screening  
226 purposes. Thus, this is likely to reflect a real-world situation in which testing is performed for a  
227 variety of purposes. Therefore, without knowledge of the purpose and timing of specimen  
228 collection, it is possible that results may not be fully representative of performance  
229 characteristics in all diagnostic settings. Also, samples were obtained in March through June  
230 2021 prior to emergence of the Delta or Omicron variants in our region. Relationships between  
231 viral load, antigen, and culture results are likely to be maintained. However, experimental  
232 verification during emergence of current and future variants would be desirable. Lastly, we used  
233 nasopharyngeal swab samples rather than nasal swab samples that are more often used for  
234 antigen testing assays. Of note, we previously found in a clinical trial, in which nasal swabs and

235 nasopharyngeal swabs were collected from the same patients in parallel, that viral loads between  
236 these sample types were congruent for samples with viral loads > 1000 genome copies/mL (15),  
237 the range of interest in our study. Therefore, we believe that the use of nasopharyngeal swabs  
238 necessitated by our study design had negligible impact on our comparisons.

239 One goal was to determine whether gaps in specificity for each antigen testing method  
240 (i.e., false positive results) would or would not overlap. If the latter were found, then antigen  
241 tests could potentially be used sequentially for screening and confirmatory analysis. However, no  
242 false positives were noted for any method. Therefore, the number of samples analyzed was  
243 insufficient to address whether further improvements in aggregate specificity could be achieved  
244 by use of tandem antigen testing strategies. It is possible that our sampling strategy, resulting in  
245 specimen dilution compared with direct nasal sampling, may have been biased towards increased  
246 specificity.

247 Three of the antigen assays analyzed in our study (BD Veritor, CareStart, and Oscar  
248 Corona) were chromatographic LFAs. The BD Veritor system interprets results using an  
249 automated reader, while the CareStart and Oscar Corona assays was read visually. The Oscar  
250 Corona assay is not approved for use in the United States, but is widely used in India. The  
251 performance of these three assays in our study was essentially identical. In contrast, the  
252 LumiraDx assay used a microfluidic immunofluorescent detection technology and appeared  
253 significantly more sensitive than the other antigen test methods (see Fig. 1-3, Table 1), consistent  
254 with high level detection of infected patients during the first twelve days of symptom onset, as  
255 previously described, using PCR as the gold standard for infection (16). The observed lower  
256 sensitivity of antigen tests relative to PCR has also been described previously (17-20).

257 Informed use of SARS-CoV-2 testing is crucial to current and future control of the  
258 SARS-CoV-2 pandemic. The available testing options have different attributes in terms of TAT,  
259 potential for point-of-care deployment, cost, and performance. Our study finds that inexpensive,  
260 point-of-care deployable LFA assays have sufficient sensitivity to detect individuals whose  
261 diagnostic samples contain culturable virus and who therefore pose a potential transmission risk  
262 to others (Fig. 4). The performance characteristics of these LFA tests are outstanding in  
263 identifying the most highly infectious specimens. This attribute may be especially important  
264 during the SARS-CoV-2 Delta variant surge, characterized by individuals with viral load skewed  
265 to even higher levels, whether symptomatic or asymptomatic (21, 22).

266 Therefore, our data support use of antigen testing to identify infectious individuals at the  
267 time of sampling. These tests would presumably be highly efficacious at identifying and  
268 allowing isolation of significantly infectious individuals from communal events, same-day  
269 healthcare procedures, communal travel arrangements, and other settings with significant person-  
270 to-person contact where universal masking is not feasible or desired, as has been demonstrated in  
271 recent epidemiological studies (23). As a point-of-use testing modality, results can be  
272 immediately available and inform timely mitigation of infectious risk to others and/or clinical  
273 management. However, the sensitivity of antigen tests is low relative to PCR. They, therefore,  
274 will not identify recently infected individuals whose viral load and infectious burden has yet to  
275 climb into a detectable range, nor identify patients who have been infected in the recent past and  
276 whose results may inform contact tracing efforts. Accordingly, antigen tests lack the power of  
277 PCR for screening programs intended to secure populations through regular testing at longer  
278 spaced time intervals and testing of patients being admitted to hospitals where the best available  
279 analytical sensitivity is desirable to prevent outbreaks in at risk populations. The improved

280 detection by the LumiraDx test shows that new testing modalities should be evaluated on a  
281 sliding scale relative to a quantitative standard such as viral load, for as in this case, they may  
282 provide an enhanced safety zone for screening individuals who will have contact with vulnerable  
283 populations or healthcare settings.

284         The identification of individuals at high risk for transmitting SARS-CoV-2 is a major  
285 public health goal to limit community spread. While viral culture may be the standard diagnostic  
286 method to determine infectivity; cost, complexity, and BL3 requirements prohibit its use as a  
287 routine clinical diagnostic method. Overall, our study supports use of antigen detection tests for  
288 specific purposes, where immediate detection of potential infectivity and especially highly  
289 infectious individuals is desired. Furthermore, although significantly less sensitive than PCR for  
290 detecting infected individuals, they provide a point-of-use alternative which may, through  
291 repeated testing at closely spaced time intervals and more rapid results, provide equivalent power  
292 to address goals of the pandemic at much lower cost.

293

## 294 **Materials and Methods.**

295         **Samples.** The 206 SARS-CoV-2 positive and 300 SARS-CoV-2 negative samples  
296 analyzed in this study were nasopharyngeal swabs obtained in 3 mL of saline or viral transport  
297 medium at COVID-19 testing sites at Beth Israel Deaconess Medical Center (Boston, MA) for  
298 purposes of diagnosis unrelated this study. Samples were collected from March 2021 through  
299 June 2021, and selected for analysis solely based on viral load distribution. Specimens generally  
300 were collected at drive-through testing sites in Boston and several surrounding communities  
301 affiliated with our medical center (15). After PCR testing for clinical purposes, samples were  
302 stored at 4°C until testing by viral culture and antigen testing. Human subjects research in this

303 study was approved by the Institutional Review Boards at Beth Israel Deaconess Medical Center  
304 and the Harvard T.H. Chan School of Public Health.

305 **RT-qPCR testing.** SARS-CoV-2 RT-qPCR testing of samples and Vero cell culture  
306 supernatants was performed using the Abbott Molecular M2000 Real-Time or Alinity m SARS-  
307 CoV-2 assays according to the manufacturer's instructions. Both assays had received Emergency  
308 Use Authorization (EUA) for qualitative diagnosis of SARS-CoV-2 infection and detect identical  
309 SARS-CoV-2 N and RdRp gene targets. In addition, they both output a quantitative fractional  
310 cycle number (FCN), a type of cycle threshold described in detail elsewhere (24). An extended  
311 panel of standards ranging from 300 to  $10^6$  viral genome copies/mL (provided by LGC Seracare,  
312 Milford, MA) was used to establish a calibration curve and convert FCN values to viral genome  
313 copies/mL. The standards consist of replication-incompetent, enveloped, positive singled-  
314 stranded RNA Sindbis virus into which the whole genome of SARS-CoV-2 was cloned and titers  
315 determined using digital droplet PCR analysis by LGC SeraCare (Russell Garlick, LGC  
316 SeraCare, personal communication). The standards therefore model SARS-CoV-2 virus and were  
317 run through all stages of sample preparation and extraction to allow appropriate comparison with  
318 identically processed patient samples. Coefficients of determination ( $R^2$ ) for comparison of FCN  
319 values with  $\log_{10}$  transformed viral load values obtained from analysis of standards were 0.997  
320 for both assays. Slope and intercepts defined linear regression equations that were used to  
321 convert FCN to viral load values including extension above and below the level of calibrators  
322 tested. Evaluation of the accuracy and modeling of viral load conversions were described  
323 previously (3, 25).

324 **Antigen testing.** The BD Veritor (Franklin Lakes, NJ), LumiraDx (Waltham, MA),  
325 CareStart (Access Bio, Inc., Somerset, NJ), and Oscar Corona (Oscar Medicare Pvt. Ltd, New

326 Delhi, India) SARS-CoV-2 antigen tests were performed according to the manufacturer's  
327 instructions with the exception that 250 uL of patient sample (nasopharyngeal swab sample  
328 eluted into 3 mL of saline or viral transport medium) was pipetted into the extraction vial  
329 provided with each kit rather than direct insertion of the nasal swab into the extraction vial. The  
330 LumiraDx test contained ~600 uL of extraction buffer; the other methods used ~500 uL of  
331 extraction reagent. Therefore, approximate dilution of sample compared with direct sample  
332 assuming complete elution from direct swab sampling was ~17 fold for the Lumira method and  
333 ~18-fold for the other antigen test methods.

334 **SARS-CoV-2 viral culture.** Vero E6 (ATCC CRL-1586) cells were seeded on a 6-well  
335 flat bottom plate at  $0.3 \times 10^6$  cells per well in Eagle's minimum essential media (EMEM)  
336 containing 1% antibiotic-antimycotic, 1% HEPES and 5% fetal calf serum (FCS, Gibco), and  
337 grown to confluence at approximately  $1 \times 10^6$  cells per well (9-11).

338 Vero E6 cells were inoculated with 250ul of patient sample and incubated at 37°C for 24  
339 hours for viral adsorption. Simultaneously, a negative control was also inoculated with 250uL of  
340 viral growth media. Carryover of non-viable viral RNA present in samples was limited by  
341 washing cell cultures after the 24-hour viral adsorption and adding fresh EMEM composite  
342 media with reduced FCS to 2% for viral growth. Therefore, detectable virus should represent  
343 viable replicating virus. On days, 3, 6, and 13-14 days of culture, 800 uL of cell culture  
344 supernatant was removed and added to 800 ul of VXL buffer (QIAGEN, German, MD) (1:1  
345 ratio) for subsequent nucleic acid extraction and SARS-CoV-2 real-time RT-qPCR. Cultures  
346 were re-fed with addition of 1 mL of EMEM with reduced FCS after sampling at each time  
347 point.

348 Supernatant viral loads below the LoD of the PCR assay were scored negative at that  
349 timepoint. Samples with two of three sequential supernatant viral loads exceeding the LoD were  
350 considered positive, and, conversely, samples with either one or no viral loads exceeding the  
351 LoD were considered negative.

352 **Statistics.** Statistical comparisons were performed with Stata version 13.1 (Stata  
353 Corporation, College Station, TX) and/or Prism 9 for MacOS (GraphPad, San Diego, CA).  
354 Sensitivity and specificity for ROC curve analysis was determined through standard formulas in  
355 Microsoft Excel and imported into Prism for graphical representation.

356

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368

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## 477 **Figure Legends**

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### 479 **Figure 1. Quantitative Relationship Between Culturable Virus and Sample Viral Load.** Day

480 3 viral culture supernatant from each cultured sample and its corresponding respiratory sample

481 were each analyzed by RT-qPCR to determine respective viral loads (n=181). The viral load in

482 log<sub>10</sub> genome copies/mL of culture supernatant is plotted against the log<sub>10</sub> viral load in genome

483 copies/mL of the original patient sample. Linear regression (solid line) with 95% confidence

484 intervals (dashed lines) is shown.  $R^2 = 0.55$ . Samples with negative viral cultures (see materials

485 and methods) for representation are assigned a y-axis, log<sub>10</sub> value of 0 and are demarcated as

486 colored brown dots.

487

### 488 **Figure 2. Antigen Testing Results Compared with Log<sub>10</sub> Viral Load.** Viral load in log<sub>10</sub>

489 genome copies/mL. POS = positive antigen test result. NEG = negative antigen test result.

490 Lumira = LumiraDx antigen test; BD = BD Veritor antigen test; Oscar = Oscar Corona antigen

491 test.

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### 493 **Figure 3. Receiver operator characteristic curves (ROC) comparing SARS-CoV-2 sample**

494 **viral load levels as a predictor of viral culture and antigen detection.** For each plot,

495 sensitivity versus 1-specificity was plotted for each viral load value (genome/copies/mL)

496 determined by RT-qPCR for each sample in our study when used as a lower limit threshold for

497 scoring positive and negative detection for all other viral load results with qualitative viral

498 culture or antigen test determinations, respectively, as the comparators. (A) Log<sub>10</sub> viral load

499 (v.l.) in genome copies/mL versus detection by viral culture. (B) Log<sub>10</sub> viral load versus

500 LumiraDx antigen detection. (C) Log<sub>10</sub> viral load versus BD Veritor antigen detection. (D)  
501 Log<sub>10</sub> viral load versus Oscar Corona antigen detection. (E) Log<sub>10</sub> viral load versus CareStart  
502 antigen detection. Viral load values along the ROC curves are labeled in log<sub>10</sub> intervals and  
503 demarcated in color as indicated in accompany heatmap legend bar. AUC (area under the curve)  
504 for each ROC curve is denoted on respective plots.

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506 **Figure 4. Model of Infectious Risk versus SARS-CoV-2 Detection by RT-qPCR and**  
507 **Antigen Tests.** Both LumiraDx and lateral flow-based antigen tests (e.g., BD Veritor, CareStart,  
508 and Oscar Corona) were able to detect individuals with viable, culturable virus and who  
509 therefore pose an immediate infectious risk to others. Dotted lines indicate reliable detection  
510 threshold predicted for each method. Presumptively, infectious risk is proportional to the amount  
511 of culturable virus which is roughly proportional to the viral load in samples. Antigen tests were  
512 excellent in detecting patients with the highest viral loads which may be four to five log<sub>10</sub>-fold  
513 greater than viral loads detected at the lowest levels where virus can be consistently cultured.  
514 PCR and, to a lesser extent, the LumiraDx test can detect individuals before and after the  
515 expected infectious period and therefore may be more appropriate for screening programs where  
516 regular testing is performed at longer time intervals. The viral load curve shown is for  
517 representational purposes and may not reflect viral load kinetics in any specific individual.

518 **Table 1. LumiraDx Antigen Results Versus Viral Culture**  
519

	Culture POS	Culture NEG	Effect size	Value	95% CI
Lumira POS	101	23	Sensitivity	0.90	0.83 to 0.94
Lumira NEG	11	54	Specificity	0.70	0.59 to 0.79
			Positive Predictive Value	0.81	0.74 to 0.87
			Negative Predictive Value	0.83	0.72 to 0.90

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522 **Table 2. BD Veritor Antigen Results Versus Viral Culture**  
523

	Culture POS	Culture NEG	Effect size	Value	95% CI
BD POS	82	6	Sensitivity	0.74	0.65 to 0.81
BD NEG	29	70	Specificity	0.92	0.84 to 0.96
			Positive Predictive Value	0.93	0.86 to 0.97
			Negative Predictive Value	0.71	0.61 to 0.79

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526 **Table 3. CareStart Antigen Results Versus Viral Culture**  
527

	Culture POS	Culture NEG	Effect size	Value	95% CI
CareStart POS	80	7	Sensitivity	0.74	0.65 to 0.81
CareStart NEG	28	69	Specificity	0.91	0.82 to 0.95
			Positive Predictive Value	0.92	0.84 to 0.96
			Negative Predictive Value	0.71	0.61 to 0.79

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531 **Table 4. Oscar Corona Antigen Results Versus Viral Culture**  
532

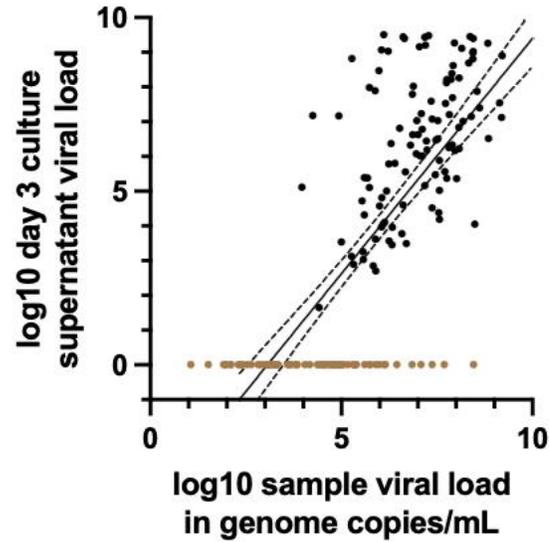
	Culture POS	Culture NEG	Effect size	Value	95% CI
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Oscar POS	75	6	Sensitivity	0.74	0.65 to 0.82
Oscar NEG	26	69	Specificity	0.92	0.84 to 0.96
			Positive Predictive Value	0.93	0.85 to 0.97
			Negative Predictive Value	0.73	0.63 to 0.81

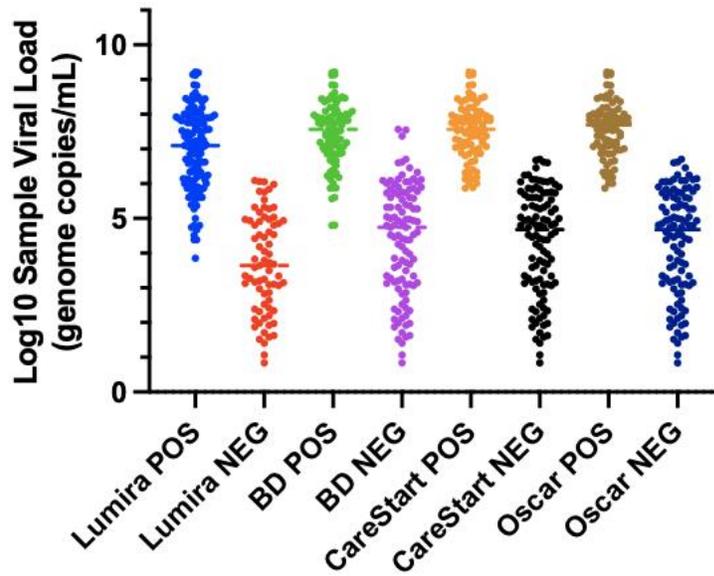
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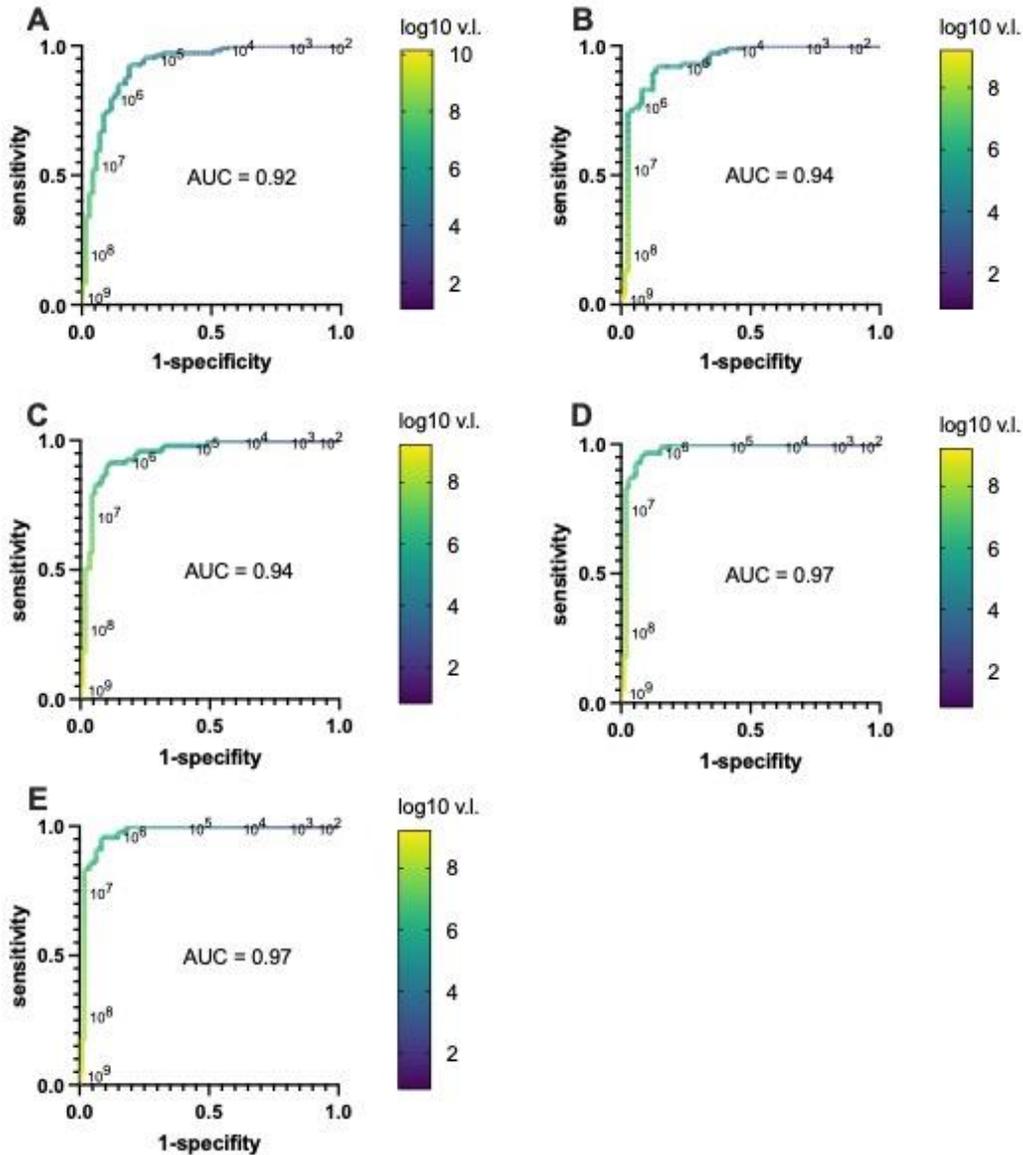


**Figure 1. Quantitative Relationship Between Culturable Virus and Sample Viral Load.** Day 3 viral culture supernatant from each cultured sample and its corresponding respiratory sample were each analyzed by RT-qPCR to determine respective viral loads (n=181). The viral load in log<sub>10</sub> genome copies/mL of culture supernatant is plotted against the log<sub>10</sub> viral load in genome copies/mL of the original patient sample. Linear regression (solid line) with 95% confidence intervals (dashed lines) shown.  $R^2 = 0.55$ . Samples with negative viral cultures (see materials and methods) for representation are assigned a y-axis, log<sub>10</sub> value of 0 and are demarcated as colored brown dots.

537



**Figure 2. Antigen Testing Results Compared with Log10 Viral Load.** Viral load in log10 genome copies/mL. POS = positive antigen test result. NEG = negative antigen test result. Lumira = LumiraDx antigen test; BD = BD Veritor antigen test; Oscar = Oscar Corona antigen test.



538

539 **Figure 3. Receiver operator characteristic curves (ROC) comparing SARS-CoV-2 sample**

540 **viral load levels as a predictor of viral culture and antigen detection.** For each plot,

541 sensitivity versus 1-specificity was plotted for each viral load value (genome/copies/mL)

542 determined by RT-qPCR for each sample in our study when used as a lower limit threshold for

543 scoring positive and negative detection for all other viral load results with qualitative viral

544 culture or antigen test determinations, respectively, as the comparators. (A) Log10 viral load

545 (v.l.) in genome copies/mL versus detection by viral culture. (B) Log10 viral load versus

546 LumiraDx antigen detection. (C) Log10 viral load versus BD Veritor antigen detection. (D)

547 Log10 viral load versus Oscar Corona antigen detection. (E) Log10 viral load versus CareStart

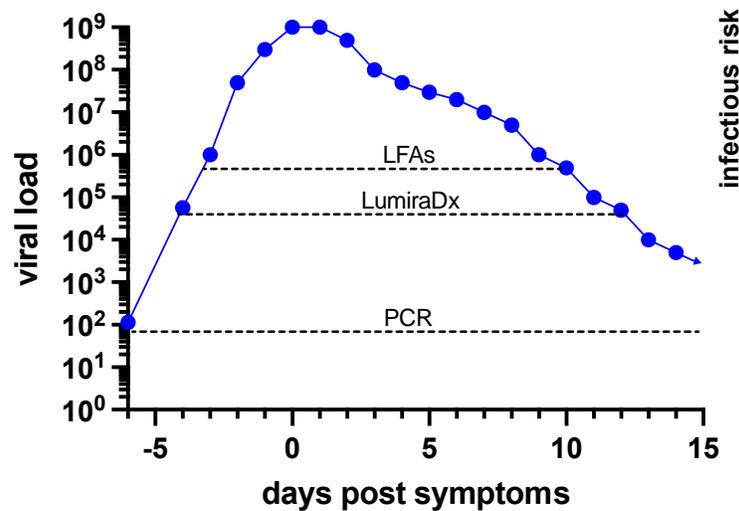
548 antigen detection. Viral load values along the ROC curves are labeled in log10 intervals and

549 demarcated in color as indicated in accompany heatmap legend bar. AUC (area under the curve)

550 for each ROC curve is denoted on respective plots.

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