Supplementary Material for

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

Running Title: COVID Antigen Tests Predict Infectivity

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Supplementary Methods

Study-specific performance of antigen testing and interpretation of antigen test results in relationship to quantitative metrics.

The antigen tests were performed according to the manufacturer's instructions with the exception that 250 uL of patient sample (nasopharyngeal swab sample eluted into 3 mL of saline or viral transport medium) was pipetted into the extraction vial provided with each kit rather than direct insertion of the nasal swab into the extraction vial. Therefore, only ~1/12 of the nasopharyngeal swab sample was added to sample extraction buffer. The LumiraDx test contained ~600 uL of extraction buffer; the other methods used ~500 uL of extraction reagent. The addition of the 250 µL volume to the total volume of the extraction buffer, further diluted the sample, as only a defined volume of the extraction buffer now containing the added volume of sample is added to the antigen test microfluid device or lateral flow assay card. This resulted in an overall ~17-fold dilution of the sample for the Lumira method and ~18-fold dilution of the sample for the other antigen test methods compared with direct elution of a specimen swab in extraction buffer that is normally performed with each testing kit.

For example, we estimate that if swab samples were tested directly without dilution in viral transport medium, LFA antigen tests would have reliably detected individuals with viral loads > ~5.5 x 10^5 genome copies/mL, and the LumiraDx test would have reliably detected individuals with viral loads > ~6 x 10^4 copies/mL (see Fig. 2), and presumably would have shifted detection of culture positive specimens down to a lower log10 bin (Tables 2 and 3, Tables S1 and S2). However, these estimates may be high or low based on pre-analytical (e.g., sample elution efficiency) and analytical variables not appreciated.

Table S1. Adjusted Antigen Test Results Versus Viral Culture Stratified by Viral Load Bins. Shifted viral load 17-fold downwards to adjust for dilution of specimens used for antigen testing based on our protocol (see methods section). The correction assumes that complete elution of a swab sample directly in antigen test extraction buffer following each antigen test method's Instructions for Use would result in analysis of a ~17-fold more concentrated specimen than tested in our study. The viral load in genome copies/mL used as a comparator was determined from a sample swab eluted in a standard 3 mL of transport media.

LumiraDx

Viral load bin (genome copies/mL)	n	Sensitivity	95% CI	Specificity	95% CI
10^8-10^{10}	1	1.00	0.05 to 1.0	N.D.	
10^7 to $<10^8$	16	1.00	0.80 to 1.00	0.00	0.00 to 0.95
10^6 to $<10^7$	39	1.00	0.91 to 1.00	0.00	0.00 to 0.82
10^5 to $<10^6$	33	1.00	0.88 to 1.00	0.00	0.00 to 0.49
10^4 to $<10^5$	36	0.72	0.52 to 0.86	0.27	0.10 to 0.57
10^3 to $< 10^4$	25	0.25	0.013 to 0.70	0.62	0.41 to 0.79
$<10^3$	39	0.00	0.00 to 0.95	0.97	0.87 to 1.00

BD Veritor

Viral load bin	n	Sensitivity	95% CI	Specificity	95% CI
10^8-10^{10}	1	1.00	0.05 to 1.0	N.D.	
10^7 to $<10^8$	16	1.0	0.80 to 1.00	0.00	0.00 to 0.95
10^6 to $<10^7$	39	0.95	0.82 to 0.99	0.50	0.03 to 0.97
10^5 to $<10^6$	32	0.86	0.69 to 0.94	0.50	0.09 to 0.91
10^4 to $<10^5$	36	0.86	0.69 to 0.94	0.50	0.09 to 0.91
10^3 to $< 10^4$	24	0.28	0.14 to 0.48	0.91	0.62 to 1.00
$<10^3$	39	0.00	0.00 to 0.49	0.95	0.76 to 1.00

CareStart

Viral load bin	n	Sensitivity	95% CI	Specificity	95% CI
10^8-10^{10}	1	1.0	0.05 to 1.00	N.D.	
10^7 to $<10^8$	15	1.00	0.78 to 1.00	0.00	0.00 to 0.95
10^6 to $<10^7$	39	1.00	0.90 to 1.00	0.00	0.00 to 0.82
10^5 to $<10^6$	32	0.82	0.64 to 0.92	0.50	0.09 to 0.91
10^4 to $<10^5$	34	0.22	0.10 to 0.42	0.81	0.52 to 0.97
10^3 to $< 10^4$	24	0.00	0.00 to 0.49	1.00	0.84 to 1.00
$<10^3$	39	0.00	0.00 to 0.95	1.00	0.91 to 1.00

Oscar Corona

Viral load bin	n	Sensitivity	95% CI	Specificity	95% CI
10^8-10^{10}	1	1.00	0.05 to 1.00	N.D.	
10^7 to $<10^8$	15	1.00	0.78 to 1.00	0.00	0.00 to 0.95
10^6 to $<10^7$	36	1.00	0.90 to 1.00	0.00	0.00 to 0.82

10^5 to $<10^6$	31	0.85	0.67 to 0.94	0.50	0.09 to 0.91
10^4 to $<10^5$	33	0.22	0.10 to 0.43	0.91	0.62 to 1.00
10^3 to $< 10^4$	24	0.00	0.000 to 0.49	1.00	0.84 to 1.00
$<10^3$	38	0.00	0.00 to 0.95	1.00	0.91 to 1.00

N.D. = not defined as there are no true negatives and false positives.

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Table S2. Adjusted Viral Culture Positivity Versus Viral Load Bin. Shifted viral load 12-fold downwards to predict the percent viral culture detection in any given viral load bin if the whole sample, rather than the one-twelfth (250 µL) of a 3 mL sample had been cultured, i.e., as performed in our protocol (see methods section). Therefore, both antigen detection in Table S1 and viral culture detection Table S2 have been adjusted to predict detection from a whole sample and should to an approximation be comparable. The viral load in genome copies/mL used as a comparator was determined from a sample swab eluted in a standard 3 mL of transport media.

Viral load bin	Percent positivity (95% C.I.)
10^8-10^{10}	100% (51-100%)
10^7 to $<10^8$	94% (74-100%)
10^6 to $<10^7$	93% (81-97%)
10^5 to $<10^6$	84% (68-93%)
10^4 to $<10^5$	68% (50-81%)
10^3 to $< 10^4$	16% (6-35%)
$<10^3$	3% (0-13%)

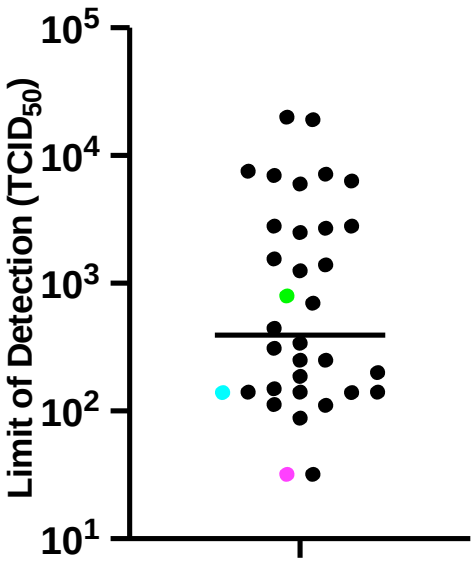

Figure S1. Limits of Detection for Emergency Use Authorization Antigen Tests. The limits of detection listed in the Instructions for Use for Emergency Use Authorized SARS-CoV-2 antigen tests available as of December 2021 are plotted. $TCID_{50}$ values represent the lowest dilution of viral stock material detected in at least 95% of 20 replicate measurements. Notably, the $TCID_{50}$ for the LumiraDx (purple), BD Veritor (cyan), and CareStart (green) antigen tests are among the more sensitive antigen tests on the market. Presumably, less sensitive tests would have a correspondingly lower ability to identify potentially infectious individuals.