Category: Infectious Disease

Analytical sensitivity and specificity of four point of care rapid antigen diagnostic tests for SARS-CoV-2 using real-time quantitative PCR, quantitative droplet digital PCR, and a mass spectrometric antigen assay as comparator methods

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Running Title: Analytical sensitivity and specificity of SARS-CoV-2 antigen tests

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Key words: SARS-CoV-2, antigen, point of care, rapid diagnostic test

Abbreviations: Ag RDT**:** rapid antigen diagnostic tests; RT-qPCR: reverse transcription quantitative polymerase chain reaction; ddPCR: droplet digital PCR; PBS: phosphate buffered saline; LF: lateral flow; FIA: fluorescence immunoassay; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; NAAT: nucleic acid amplification tests; LC-480: Roche Light Cycler 480; EUA: emergency use authorization; FDA: U.S Food and Drug Administration; Ct: cycle threshold; POC: point of care; PRM: parallel reaction monitoring; MS: mass spectrometric

Abstract

Background: We evaluated the analytical sensitivity and specificity of four rapid antigen diagnostic tests (Ag RDTs) for SARS-CoV-2, using reverse transcription quantitative PCR (RT-qPCR) as the reference method; and further characterizing samples using droplet digital quantitative PCR (ddPCR) and a mass spectrometric antigen test.

Methods: 350 (150 negative and 200 RT-qPCR positive) residual phosphate buffered saline (PBS) samples were tested for antigen using the BD Veritor lateral flow (LF), ACON LF, ACON fluorescence immunoassay (FIA), and LumiraDx FIA. ddPCR was performed on RT-qPCR positive samples to quantitate the viral load in copies/mL applied to each Ag RDT. Mass spectrometric antigen testing was performed on PBS samples to obtain a set of RT-qPCR positive, antigen positive samples for further analysis.

Results: All Ag RDTs had nearly 100% specificity compared to RT-qPCR. Overall analytical sensitivity varied from 66.5% to 88.3%. All methods detected antigen in samples with viral load >1,500,000 copies/mL RNA, and detected ≥75% of samples with viral load of 500,000 to 1,500,000 copies/mL. The BD Veritor LF detected only 25% of samples with viral load between 50,000-500,000 copies/mL, compared to 75% for the ACON LF device and >80% for LumiraDx and ACON FIA. The ACON FIA detected significantly more samples with viral load <50,000 copies/mL compared to the BD Veritor. Among samples with detectable antigen and viral load <50,000 copies/mL, sensitivity of the Ag RDT varied between 13.0% (BD Veritor) and 78.3% (ACON FIA).

Conclusions: Ag RDTs differ significantly in analytical sensitivity, particularly at viral load <500,000 copies/mL.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes a mild to severe respiratory illness(1). While most infections are asymptomatic or cause mild symptoms, individuals with underlying health conditions and the elderly are at highest risk of developing severe complications(2). Nucleic acid amplification tests (NAAT), primarily reverse transcription quantitative polymerase chain reaction (RT-qPCR), are considered the gold standard for diagnosis, but most take several hours to complete. Cost and instrumentation are also barriers to providing NAAT testing; especially for screening in communities, schools or long-term care facilities.

Rapid diagnostic tests that detect SARS-CoV-2 antigen (Ag RDTs) are one solution to expand screening for SARS-CoV-2 infection, but large prospective studies have reported variable clinical sensitivity of Ag RDT. Depending upon the population studied and reference NAAT method, sensitivities of Ag RDTs have ranged from 35.8-97.6% in independent studies (3-11). Most studies have used lateral flow (LF) immunoassay devices, though fluorescence immunoassay (FIA) devices also have been found to have variable clinical sensitivity ranging from 41.2-97.6% (8, 12).

Differing results for clinical sensitivity of Ag RDTs may be due to factors such as patient population studied, reference test used, or differences in the analytical sensitivity of Ag RDTs. For influenza rapid antigen testing, FIA methods have shown improved clinical and analytical sensitivity compared to LF methods (13, 14). In contrast few studies have evaluated the analytical sensitivity of Ag RDTs (15-18). One study compared an FIA Ag RDT to three LF devices and found that the FIA had higher sensitivity in specimens with viral loads >1,000,000 copies/mL(15). Comparing the analytical sensitivity of Ag RDTs is challenging because archived samples must

be used that are not the intended sample type, and there is currently no reference method for antigen detection. The clinical significance of SARS-CoV-2 NAAT positive, antigen negative samples remains uncertain.

We compared the analytical sensitivity and specificity of four SARS-CoV-2 Ag RDTs, two LF and two FIA assays, using 150 negative and 200 positive phosphate buffered saline (PBS) samples tested by an RT-qPCR reference method. Ag RDTs were performed after 10-fold dilution of PBS sample into method-specific extraction buffer, after validation of the dilution protocol. To help address the lack of a reference method for antigen measurement, we also used a laboratory-developed, mass spectrometric measurement for SARS-CoV-2 antigen to define a subset of RT-qPCR positive, antigen positive samples. We further characterized RT-qPCR positive samples by performing quantitative droplet digital PCR (ddPCR), allowing us to quantitate the viral load applied to each Ag RDT for direct comparison of analytical sensitivity. The results allow direct comparison of the analytical sensitivity of Ag RDT devices stratified by viral load applied to each test; and provide information about the extent to which differences in analytical sensitivity of Ag RDT may impact interpretation of clinical studies or limit the clinical utility of these devices.

2. Materials and Methods

Sample selection and reference RT-qPCR testing: This was a retrospective study using residual clinical samples tested for SARS-CoV-2 by RT-qPCR. Sterile, nylon fiber, non-flocked nasopharyngeal swabs were collected from symptomatic and asymptomatic patients with a clinical order for SARS-CoV-2 NAAT at Mayo Clinic, Rochester MN. Swabs were placed in 3 mL phosphate-buffered saline (PBS) for transportation to the testing lab. PBS samples were kept at 4º C for up to 3 days after RT-qPCR testing, then frozen at -80º C until point of care (POC) Ag RDT testing. Although we could find no published data on nucleocapsid antigen stability in PBS samples, mass spectrometry experiments demonstrated no loss of nucleocapsid peptides (as measured by peak height) after 5 days at room temperature (data not shown).

Reference RT-qPCR testing was performed with a laboratory-developed test using the TaqMan assay on a Roche Light Cycler 480 (LC480) performed in the Mayo Clinic Rochester laboratories. For LC480 testing, nucleic acid is first extracted on the bioMerieux easyMAG/eMAG, Hamilton STAR, or Roche MP96. This TaqMan assay employs a reverse transcriptase reaction to convert RNA to complementary DNA followed by amplification of the nucleocapsid gene. A Taqman probe specific for SARS-CoV-2 RNA is labeled with the fluorophore FAM. The dye labeled probe allows for detection of SARS-CoV-2 virus in the corresponding detector channel of the LC480 instrument. The test has received Emergency Use Authorization (EUA) by the Food and Drug Administration (FDA). RT-qPCR samples were selected non-consecutively to obtain 150 negative and 200 positive samples. Positive samples were chosen to span cycle threshold (Ct) values of 20-35, with most samples selected to be in Ct ranges of 25.01-30.00 or 30.01-35.00 (**Table 1**). The study protocol was approved by the Mayo Clinic Institutional Review Board.

POC Ag RDTs: POC tests used included the BD Veritor (Becton, Dickinson and Co), an immunochromatographic (LF) assay with an opto-electronic interpretation instrument (reader). The test detects nucleocapsid proteins from SARS-CoV-2 by capturing antigen-conjugate complexes as they migrate across a reaction area coated by antibodies to nucleocapsid proteins. After placing sample in the extraction tube, 3 drops of extracted sample are applied to the test cassette, and the cassette is placed in the reader. Results take 15 minutes, and the test has received FDA EUA. The ACON SARS-CoV-2 Antigen Rapid Test (ACON Laboratories) is a LF immunoassay for the qualitative detection of nucleocapsid protein antigen to SARS-CoV-2. The test consists of an extraction tube and test cassette, takes 15 minutes, and has not received FDA EUA. ACON SARS-CoV-2 Antigen Test Kit FIA is an immunofluorescent sandwich assay (FIA) that uses lateral flow technology with fluorescent detection of nucleocapsid antigens to SARS-CoV-2. The test uses the same extraction tube as the LF device, but extracted sample (4 drops) is applied to an FIA test cassette and the cassette placed in an FIA reader. The test has not received FDA EUA. LumiraDx (LumiraDx) is a microfluidic immunofluorescence (FIA) assay for qualitative detection of nucleocapsid antigens to SARS-CoV-2. The test requires a disposable test strip and reader. Sample is introduced into the extraction tube, then a single drop of extracted sample is applied to the strip. Results take approximately 12 minutes and the test has received FDA EUA.

For all Ag RDTs, residual PBS samples were added to vendor-specific extraction buffer in a 1:9 ratio (one part PBS residual sample, nine parts vendor-specific extraction buffer), such that samples applied to Ag RDT consisted primarily of extraction buffer to optimize performance of each assay. To ensure that results of the visually read test (ACON LF) would be unbiased, ACON LF results were read and recorded by a separate technologist prior to recording results of the other

Ag RDTs. ACON LF results were also photographed to resolve any faint or uncertain control or test lines (there were none in the study).

Quantitative, digital droplet PCR: After extracted RNA samples were tested for RT-qPCR, they were stored at -80C until ddPCR testing. The assay utilizes the QX200 AutoDG Droplet Digital PCR System (Bio-Rad) following the Bio-Rad SARS-CoV-2 ddPCR Kit EUA Instructions for Use. Further information on the ddPCR method can be found in the online **Supplemental Data Appendix 1**.

Mass spectrometric (MS) antigen detection: After POC Ag RDT testing, PBS samples were frozen at -80C until MS testing. PBS samples underwent viral inactivation followed by antibody-based purification using an anti-nucleocapsid protein monoclonal antibody coupled to MSIA D.A.R.T.'S™. The bound material was eluted with 100 µL of 0.2% TFA and 0.002% Z3-16 in water and immediately subjected to in-solution trypsin digestion. Parallel reaction monitoring (PRM) analysis was performed on an Exploris 480™ mass spectrometer (ThermoFisher Scientific)(19). A detailed description of the MS method is provided in the online **Supplemental Data Appendix 2**.

Statistical analysis: The primary outcome was sensitivity and specificity of Ag RDT for detection of SARS-CoV-2 antigen in PBS samples, using RT-qPCR as the reference method. Sensitivity was estimated (with 95$^{th\%}$ confidence intervals) using McNemar tests (for all specimens combined), and with exact McNemar tests when stratifying by copies/mL (<50,000; 50,000 to <500,000; 500,000 to <1,500,000; and ≥1,500,000). We used 200 positive samples to achieve >90% power to detect differences in overall sensitivity ≥10% between methods (McNemar test, 5% type-I error, assuming >10% discordant samples). Viral load brackets were developed post hoc

after measuring viral load and keeping roughly equal numbers of samples in the lowest two viral load brackets. *P*-values less than 0.005 were considered statistically significant to account for multiple comparisons between methods. Analyses were performed using SAS version 9.4 (SAS Institute Inc).

3. Results:

Familiarization and dilution experiments: Familiarization experiments consisted of running the vendor-specific positive and negative controls, PBS blank (PBS diluted 1:9 into vendor-specific extraction buffer), and extraction buffer blank, following manufacturer's directions for use of each test. All tests recovered intended positive (positive QC) and negative (negative QC, PBS blank and extraction buffer blank) results. Next, we used 13 PBS pools (pools made from RT-qPCR positive PBS samples) with Ct values of 22-34, with all samples (except positive and negative QC) diluted 1:9 into method-specific extraction buffer. The BD Veritor was positive for PBS pools with Ct 22-27, and negative for PBS pools 28-34. The ACON FIA and ACON LF were positive for PBS pools Ct 22-28; while the LumiraDx was positive up to a Ct of 32. Two technologists reviewed and photographed LF results for BD Veritor and ACON LF. The background of the LF devices were not different between extraction buffer blanks, negative QC, and negative or positive PBS pool samples diluted 1:9 into extraction buffer. For the LumiraDx FIA, raw fluorescence values were available in development mode of the software; and raw fluorescence values for PBS blanks or negative PBS pools did not differ from background fluorescence observed for negative QC or extraction buffer blanks (data not shown).

Specificity of Ag RDTs: 150 PBS samples that previously tested negative by RT-qPCR were used as the negative sample set. The specificity (95% CI) of the Ag RDT was 100 (97.5-100)% for BD Veritor, LumiraDx and ACON FIA tests, and 97.3 (93.3-99.0) % for the ACON LF. The MS antigen test was positive in two RT-qPCR negative samples. One of these samples tested positive by ddPCR with a viral load (in the PBS sample) of 6509 copies/mL (651 copies/mL applied to Ag RDT); while the other tested negative by ddPCR initially but upon repeat was just above the

detection threshold at 332 copies/mL in the PBS sample. These two samples were negative by all four Ag RDT and were considered true negative samples for calculation of Ag RDT specificity.

Sensitivity of Ag RDTs: 200 PBS samples previously testing positive by RT-qPCR were used as the positive sample set. There were three samples that tested positive by RT-qPCR, but negative by ddPCR and all five (including the MS antigen) antigen tests. These samples were excluded from further analysis because the presence of SARS-CoV-2 RNA or antigen could not be confirmed. Among the remaining 197 positive RT-qPCR samples, the sensitivity (95% CI) of the MS antigen test was 90.9 (86.0-94.1)%. The sensitivities of the Ag RDT were 66.5 (59.6-72.7)% for BD Veritor, 77.7 (71.4-82.9)% for ACON LF, 83.2 (77.4-87.8)% for LumiraDx, and 88.3 (83.1-92.1)% for ACON FIA (**Figure 1**). The BD Veritor detected significantly fewer RT-qPCR positive samples than did the ACON LF, Lumira Dx FIA, or ACON FIA (P <0.0001). Differences between the ACON LF and LumiraDx FIA (P=0.0045) and ACON LF and ACON FIA (P <0.0001) were also statistically significant, while differences between the LumiraDx and ACON FIA were not statistically significant (P=0.008). The MS antigen test was significantly more sensitive ($P \leq$ 0.0001) than all Ag RDT except the ACON FIA (P=0.10).

When Ag RDT results are broken down into brackets of applied viral load, all Ag RDT are very sensitive when > 1,500,000 copies/mL of viral RNA are applied (**Figure 1**). All the Ag RDT demonstrated ≥75% sensitivity for detection of antigen in samples with 500,000 to 1,500,000 million copies/mL RNA, though the BD Veritor showed a trend towards lower sensitivity in this group that did not reach statistical significance (P >0.005 all comparisons). In contrast in the range of 50,000 to 500,000 copies/mL, the BD Veritor detected only 25 (13.1-42.1)% of samples compared to the ACON LF which detected 75 (57.9-86.7)%. The BD Veritor was significantly

less sensitive ($P < 0.0001$) than the other Ag RDT in the range of 50,000-500,000 copies/mL. The two FIA tests demonstrated >80% sensitivity in this viral load range. At the lowest viral load range (< 50,000 copies/mL), both the BD Veritor ($P < 0.0001$) and ACON LF ($P = 0.0001$) were significantly less sensitive than the ACON FIA (**Figure 1**), while other comparisons did not yield statistically significant differences. Three samples failed repeated runs by ddPCR for unknown reasons and are included in overall sensitivity but not in any viral load bracket.

A scatterplot showing the viral load of detected and undetected samples by each method for all RT-qPCR positive samples with <1,500,000 copies/mL is shown in **Figure 2**. On the BD Veritor platform, only four PBS samples with viral load <200,000 copies/mL were detected. Compared to the BD Veritor, the ACON LF method detected more samples with viral load between 30,000-100,000, and missed only 5 samples with viral load >100,000 (**Figure 2**). The LumiraDx FIA was able to detect approximately half (12 of 26) of samples in the 10,000-100,000 copies/mL range; while only the ACON FIA detected a majority (18 of 26) of specimens with viral load between 10,000-100,000 copies/mL (**Figure 2**).

Viral load of antigen negative samples and comparison of Ag RDT at low viral load: There were 16 samples that were RT-qPCR positive, ddPCR positive, but tested negative by all antigen tests. The Ct and dd-PCR copy number are shown in **Table 2**. Excluding the 16 samples that were negative by all antigen tests, the sensitivity of Ag RDTs for detecting samples with <50,000 copies/mL varied from 13.0% (3/23, BD Veritor), 17.4% (4/23, ACON LF), 52.1% (12/23 LumiraDx), to 78.3% (18/23 ACON FIA). Differences between the BD Veritor and ACON FIA ($P < 0.0001$) and ACON LF and ACON FIA ($P = 0.0001$) were statistically significant.

Relationship between Ct value and viral load by ddPCR: Previous studies have used Ct value as a surrogate for viral load, both to estimate the limit of detection of antigen tests(15, 16) or to characterize RT-qPCR positive, Ag RDT negative samples(5-7). We investigated the relationship between Ct and viral load by quantitative ddPCR in PBS samples. Viral load decreases as a function of Ct between values of 29 to 33, although with considerable variability. Above Ct 33, the viral load is highly variable with values between <10,000 and >1,000,000 copies/mL (Figure 3). The ddPCR viral load of samples with Ct value of 35.00 varied from 378 to 1,119,259 copies/mL (**Figure 3**). We have observed a similar relationship between Ct and viral load by ddPCR for the Roche 6800 RT-qPCR method (data not shown).

4. Discussion

One reason for differing clinical sensitivities of Ag RDT is that the tests may vary in analytical sensitivity. A few comparisons of Ag RDT analytical sensitivity have been performed using RNA standards or other methods to quantitate viral load as a function of Ct value of RT-qPCR results(15-17). In RT-qPCR, differences in amplification efficiencies between individual samples significantly affects the accuracy and reproducibility of measured target concentration derived from calibration curves(20). In contrast, ddPCR partitions each sample into independent sub-reactions, allowing the accurate and precise quantitation of targets without use of a calibration curve(20). In one study ddPCR exhibited 4-20 fold increased precision for quantitation of HIV DNA compared to RT-qPCR(21). We found that the relationship between Ct and viral load by ddPCR was poor for samples with Ct values >33. These samples could have between several hundred to > 1,000,000 copies/mL. This limits the use of Ct to interpret the potential significance of RT-qPCR positive, Ag RDT negative samples.

Other studies have used serial dilution of samples with known RNA content to compare analytical sensitivity of AgRDTs (18, 22). Estimates for the limit of detection of Ag RDT range from 40,000 copies/mL(16) to >50,000,000 copies/mL(18), with the latter study finding a 50-fold difference in analytical sensitivity between Ag RDTs evaluated (18). ddPCR is a quantitative method capable of precise measurement of low viral loads, and has been found to be more sensitive analytically than RT-qPCR for detection of SARS-CoV-2 RNA(23). We used RT-qPCR as the reference method for SARS-CoV-2, but also performed ddPCR to allow direct comparison of the analytical sensitivity of Ag RDT across a range of measured viral loads.

Another obstacle to direct analytical comparisons of Ag RDTs is that no reference method exists for presence of SARS-CoV-2 antigen in residual PBS samples. In this study we used a laboratory-developed, MS antigen test that uses antibody enrichment followed by tryptic digestion to allow detection of SARS-CoV-2 antigen. While we do not propose the MS antigen method as a reference method, detection of antigen by four POC Ag RDT methods as well as the MS antigen method allowed us to compare Ag RDT sensitivity among a set of NAAT positive patient samples with antigen detectable by one or more methods. Most previous studies have reported results as positive and negative percent agreement, rather than sensitivity and specificity, due to the lack of reference methods for SARS-CoV-2 antigen or RNA. We chose to report analytical sensitivity and specificity because our study design, while not perfect, has gone far beyond previous studies in addressing the lack of reference methods.

One of the major findings from our study is that the two LF methods varied substantially in analytical sensitivity. All samples with >50,000 copies/mL had antigen detected by at least one antigen-based detection method. Among samples with viral load between 50,000-500,000 copies/mL, the BD Veritor LF method detected only 25% of samples compared to 75% for the ACON LF test. The two FIA tests had >80% sensitivity in this range, consistent with studies performed on Ag RDT for influenza demonstrating better analytical sensitivity using FIA technology(13, 14).

The differences in analytical sensitivity between Ag RDTs were even greater at viral load <50,000 copies/mL. Analytical sensitivity ranged from 13.0% for BD Veritor to 78.3% for ACON FIA in this range. Because RT-qPCR positive, Ag RDT negative samples tend to have higher Ct value, some investigators have suggested that these represent non-infectious samples. Viral culture has been used to help define the sensitivity of Ag RDTs (9, 15) among presumably infectious

samples; but both biological and environmental limitations to viral culture for SARS-CoV-2 make interpretation of NAAT positive, culture negative samples unclear(9). We used ddPCR to quantitatively measure viral load, and found that Ag RDTs vary significantly in analytical sensitivity when viral loads are <500,000 copies/mL. We found that RT-qPCR positive samples without detectable antigen by any method had viral load <50,000 copies/mL and high Ct value. However, the majority of samples with high (33-35) Ct values had detectable antigen by one or more methods.

We speculate that samples with no detectable antigen by more sensitive methods and low viral load are least likely to be infectious. To design studies to identify infectious patients, investigators will need to be aware of both the differential sensitivity of POC antigen tests and the imperfect relationship between Ct and viral load (if Ct value will be used as a surrogate for viral load). Our study shows that use of more sensitive FIA methods allows detection of antigen in the majority of RT-qPCR positive samples, even with very low viral loads. By using more sensitive Ag RDT methods, studies can eventually determine whether the absence of antigen really does imply reduced or no risk of infectivity. Use of more sensitive Ag RDTs clinically will diminish discrepancies in patient classification between Ag RDTs and RT-qPCR.

Limitations: The primary limitations of our study are the lack of a reference methods for SARS-CoV-2 antigen detection or RNA detection, and inability to compare multiple Ag RDTs using the intended sample type (nasal swab direct to vendor-specific extraction buffer). We addressed these limitations by validating a dilution protocol using PBS samples and further characterizing PBS samples by ddPCR and a MS antigen method. We also compared the overall sensitivity of the MS antigen test using undiluted PBS samples to Ag RDT using diluted samples, which may have underestimated the overall sensitivity of the POC tests. Lastly, we studied only four (2 LF and 2

FIA) Ag RDT methods. Though we demonstrated significant variability in analytical sensitivity, we do not know if these results represent lower and upper bounds for assay performance or whether some Ag RDTs demonstrate better or worse performance.

Conclusions: Ag RDTs differ significantly in analytical sensitivity, especially among specimens with <500,000 copies/mL RNA content. Interpreting the significance of NAAT positive, Ag RDT negative samples requires knowledge of the analytical sensitivity of the Ag RDT used. When antigen is present more sensitive methods can detect it in the majority of specimens with viral load <50,000 copies/mL; while less sensitive methods will fail to detect antigen in most samples with <500,000 copies/mL RNA.

Acknowledgments

We thank LumiraDx and ACON for providing readers, disposables, extraction buffer, and controls for these devices. We also thank Sarah Jenkins for help with statistical analysis. None of the authors have disclosures to report for this study. We thank Eric and Wendy Schmidt for supporting the Advanced Diagnostic Laboratory at Mayo Clinic, where the mass spectrometric antigen test was developed.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: LumiraDx and ACON provided readers, disposables, extraction buffer, and controls for the devices.
Expert Testimony: None declared.
Patents: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

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Table 1: Cycle threshold (Ct) values of positive RT-qPCR specimens by Ct bracket used for sample selection for Ag RDT testing, viral load by ddPCR, and MS antigen analysis.

Ct bracket	Number of samples selected
20.00 – 25.00	24
25.01 – 30.00	88
30.01 – 35.00*	88

* Positive RT-qPCR specimens exceeded the fluorescence threshold within 40 cycles, but all positive samples with cycle threshold greater than or equal to 35.00 cycles are reported as Ct 35.00.

Table 2: Viral load (copies/mL by ddPCR) and cycle threshold (Ct) value for 16 samples that were positive by RT-qPCR and ddPCR, but negative by all five antigen tests (including the MS antigen test).

Sample	ddPCR viral load (copies/mL)	RT-qPCR cycle threshold (Ct)
1	49,443	34.36
2	38	35.00
3	1941	35.00
4	880	33.68
5	4366	35.00
6	10,817	35.00
7	831	35.00
8	4655	35.00
9	5876	35.00
10	1299	32.18
11	35,025	32.13
12	427	35.00
13	12,792	35.00
14	2041	35.00
15	10,425	35.00
16	90	35.00

Figure Legends

Figure 1: Sensitivity (% samples detected by antigen tests) for the BD Veritor LF, ACON LF, LumiraDx FIA, and ACON FIA rapid antigen tests and mass spectrometric antigen test for SARS-CoV-2 among 197 RT-qPCR positive samples. Sensitivity among samples with viral load (measured by ddPCR) of >1,500,000 copies/mL, 500,000 to 1,500,000 copies/mL, 50,000 to 500,000 copies/mL, and <50,000 copies/mL is also shown for the four rapid antigen tests. The percent sensitivity is shown above each bar except for tests showing 100% sensitivity. * indicates statistically significant ($P < 0.005$) difference between paired comparisons for comparisons within viral load brackets (overall sensitivity not shown, see results).

Figure 2: Scatterplot showing the viral load (copies/mL measured by ddPCR) among RT-qPCR positive PBS samples with antigen detected (D) or undetected (U) by the BD Veritor LF, ACON LF, LumiraDx FIA, and ACON FIA methods. Note the Y axis is displayed in logarithmic scale.

Figure 3: Viral load (copies/mL by ddPCR) vs cycle threshold (Ct) by RT-qPCR for all samples with Ct greater than 29.00 that did not saturate the ddPCR method (ddPCR saturated at 15,000,000 copies/mL in PBS samples). Most samples with Ct less than 29.00 saturated the ddPCR method.

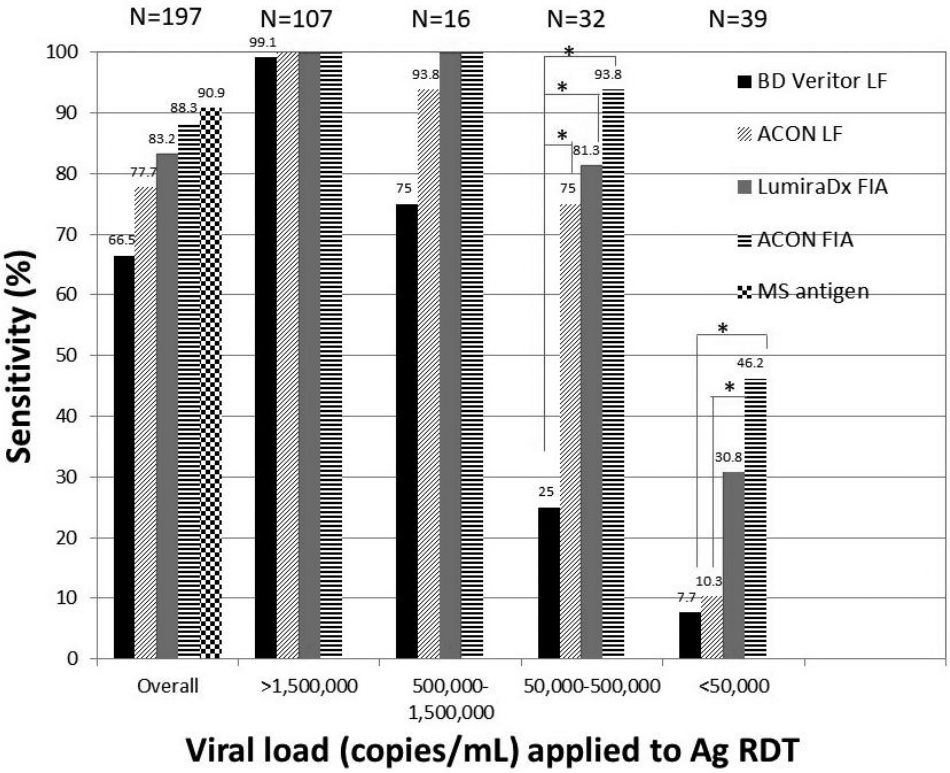

Figure 1

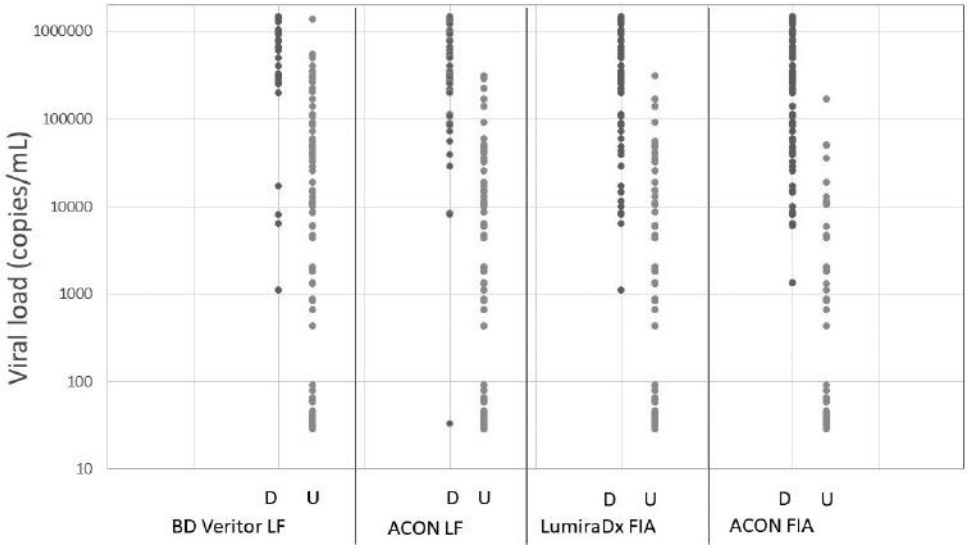

Figure 2

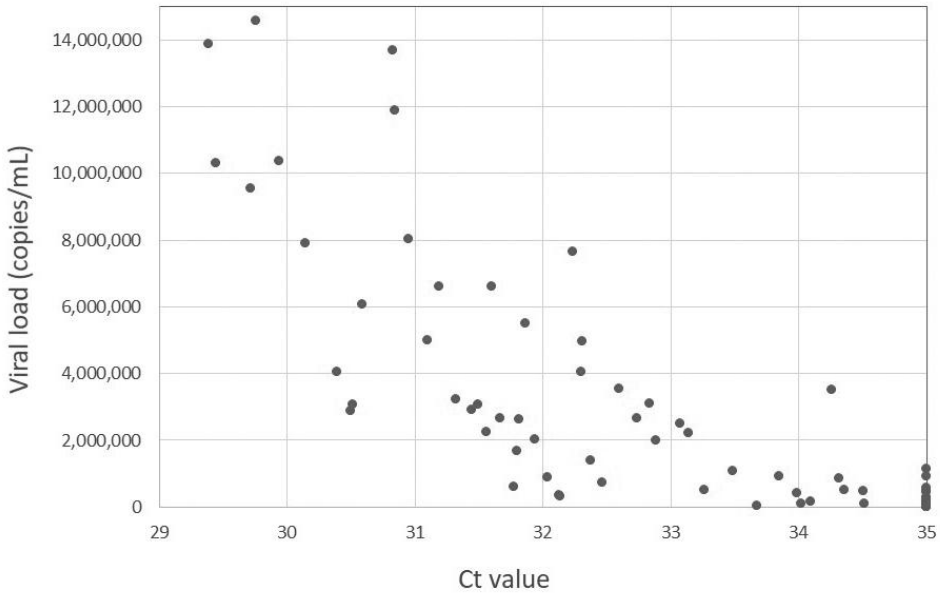

Figure 3