Predicting Direct-Specimen SARS-CoV-2 Assay Performance Using Residual Patient Samples

Running title: Predicting SARS-CoV-2 assay performance

Lee F Schroeder
University of Michigan, Michigan Medicine, Department of Pathology, Ann Arbor, Michigan, USA

Michael A Bachman
University of Michigan, Michigan Medicine, Department of Pathology, Ann Arbor, Michigan, USA

Allison Idoni
University of Michigan, Michigan Medicine, Department of Pathology, Ann Arbor, Michigan, USA

Jennifer Gegenheimer-Holmes
University of Michigan, Michigan Medicine, Department of Emergency Services, Ann Arbor, Michigan, USA

Steven L Kronick
University of Michigan, Michigan Medicine, Department of Emergency Services, Ann Arbor, Michigan, USA

Riccardo Valdez
University of Michigan, Michigan Medicine, Department of Pathology, Ann Arbor, Michigan, USA

Paul R Lephart
University of Michigan, Michigan Medicine, Department of Pathology, Ann Arbor, Michigan, USA

Corresponding author:
Lee F Schroeder
619-459-2804
leeschro@med.umich.edu
Department of Pathology & Clinical Labs
NCRC, Bldg. 35
2800 Plymouth Road
Ann Arbor, MI 48109-2800

Keywords: Point of Care Testing Systems, Infectious Disease, Laboratory Methods and Tools

Nonstandard Abbreviations:
VTM: viral transport media
pPPA: predicted positive percent agreement
LOD: limit-of-detection
CT: cycle threshold
Sn: sensitivity

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Abstract

Background

Diagnostic sensitivities of point-of-care SARS-CoV-2 assays depend on specimen type and population-specific viral loads. Evaluation of these assays require 'direct' specimens from paired-swab studies rather than more accessible residual specimens in viral transport media (VTM).

Methods

Residual VTM and limit-of-detection studies were conducted on Abbott ID NOW™ COVID-19, Quidel Sofia 2™ SARS Antigen FIA, and DiaSorin Simplexa™ COVID-19 Direct assays, with cycle threshold (CT) adjustments to approximate direct-specimen testing based on gene-target doubling each PCR cycle. Logistic regression was used to model assay performance by specimen CT. These models were applied to CT distributions of symptomatic and asymptomatic populations presenting to emergency services to predict the percent of specimens that would be detected by each assay. A 96-sample paired-swab study was conducted to confirm model results.

Results

When using direct nasopharyngeal samples and fit with either VTM or limit-of-detection data, percent positivities for ID NOW (symptomatic 94.9%/97.4%; asymptomatic 88.4.0%/89.6%) and Simplexa (symptomatic 97.8%/97.2%; asymptomatic 91.1%/90.8%) were predicted to be similar. Likewise, fit with VTM data, percent positivities for ID NOW with direct nasal specimens (symptomatic 77.8%; asymptomatic 64.5%) and Sofia 2 with direct nasopharyngeal specimens (symptomatic 76.6%, asymptomatic 60.3%) were similar. The paired-swab study comparing direct nasopharyngeal specimens on ID NOW and nasopharyngeal VTM specimens on Simplexa showed 99% concordance.

Conclusions

Assay performance can be modeled as dependent on viral load, fit using laboratory bench study results, and adjusted to account for direct-specimen testing. When using nasopharyngeal specimens, direct testing on Abbott ID NOW and VTM testing on DiaSorin Simplexa have similar performance.

Impact statement

Throughout the COVID-19 pandemic, there has been a proliferation of SARS-CoV-2 assays with Emergency Use Approval. There is significant variation in the analytic sensitivities of these diagnostics as well as a strong dependence of diagnostic sensitivity on patient population and specimen type, making it difficult for institutions to evaluate tests for implementation. Furthermore, many point-of-care tests require direct-specimens, rather than residual viral transport media, presenting additional challenges for verification. This study demonstrates a model that can use data from limit-of-detection and residual viral transport media studies to predict the performance of direct-specimen assays in different patient populations.

Introduction

SARS-CoV-2 diagnostic testing throughout much of the pandemic has been defined by reagent scarcity and testing delays.(1–4) Nonetheless, there has been a proliferation of assay options on the market, from central laboratory instruments to near-patient and point-of-care formats, including multiple specimen types, and both nucleic acid as well as antigen targets.(5,6) Importantly, the accuracy of these assays varies substantially according to viral RNA concentration in samples, with a strong dependence on specimen type and patient population.(7–10) While the availability of numerous assays and specimen types is desirable, verification of assay accuracy with different specimen types in different patient populations is challenging. When assays are approved for use with viral transport media (VTM), verification is straight-forward, as residual samples can be used. However, many point-of-care assays require 'direct' or 'dry' swabs, where swabs are placed directly into assay reagents without first diluting in VTM. For these assays, verification requires paired-swab studies, where two swabs are collected from each patient, one for the index and one for the reference test. Paired-swab designs, however, are time-consuming and difficult to conduct when positivity is low. Considering the large set of assay-, specimen type-, and patient population-combinations that an institution must consider, paired-swab designs as the initial evaluation are often untenable.

The goal of this study was to identify a rapid point-of-care assay with similar performance to the rapid real-time reverse transcription polymerase chain reaction (rRT-PCR) testing used throughout the pandemic at our institution, the DiaSorin Simplexa™ COVID-19 Direct (DiaSorin, Cypress, CA). Recently, another study demonstrated the relationship of assay performance with viral load, calculated positive percent agreements (PPAs) for two assays for different categories of viral load, and finally estimated overall performance in their entire patient population.(11) We expand on this by developing a logistic regression model of assay performance to quickly estimate performance of several assay/specimen type/population combinations before dedicating resources for a clinical paired-swab study. We use residual VTM samples and LOD studies to evaluate two direct-swab assays, making adjustments to account for the benefit of direct-swab testing based on gene target doubling in each PCR cycle. As viral load varies by population being studied and by specimen type, the model can predict performance of assays under a variety of test settings. Data are presented for the Abbott ID NOW™ COVID-19 (Abbott Molecular, Des Plaines, IL), Quidel Sofia 2™ SARS Antigen FIA (Quidel, Inc., San Diego, CA) and the deployed Simplexa assay. A targeted paired-swab study was prospectively conducted between the ID NOW and Simplexa assays to verify model predictions before implementation.

Methods

The underlying assumption of this model is that specimen viral load is the primary determinant of SARS-CoV-2 assay diagnostic sensitivities, whether it be nucleic acid testing or antigen testing. In turn, PCR cycle threshold (CT) is assumed to be an estimator of viral load. The higher the viral load, the lower the CT will be in PCR assays that detect SARS-CoV-2, and the more likely any assay will detect virus in that sample. By using CT distributions of positive cases in different patient populations, one can estimate the performance expected for various assays in use, and potentially to be used, in a health system. This performance measure is calculated by mapping CT-specific analytic sensitivities to the CT distribution of the population-of-interest. Development of the model followed five steps:

Step 1: The model assumes negligible CT-bias between assays used to generate analytic sensitivity curves and the population-specific CT distributions. Accordingly, assay CTs were compared for bias with residual VTM samples (Table 1, #1 and #2).

Step 2: Data from multiple sources were used to fit models of CT-specific analytic sensitivity. Positive cases from a study conducted in April, 2020(7) were used for ID NOW and Simplexa characterization. Briefly, twenty-four foam nasal swabs were tested directly with ID NOW (Table 1, #3), and the paired nasopharyngeal VTM samples were tested on central laboratory assay (see below) as well as ID NOW (Table 1, #4) and Simplexa (Table 1, #7).(7) An LOD study was also conducted on the ID NOW (Table 1, #5) and Simplexa (Table 1, #8).(7) To simulate direct testing on the ID NOW for the LOD study, reagent buffer viral particle concentrations were increased to equal that in dilution aliquots used for other assays.(7) In addition, 32 residual VTM samples that were positive by our central laboratory assays were run on the Sofia 2 (Table 1, #6). As the Sofia 2 SARS Antigen FIA assay has recently lost approval for nasopharyngeal samples, such testing would be considered off-label. However, the Sofia 2 Flu+SARS Antigen FIA EUA does include nasopharyngeal samples. For each assay and study, the performance (detection/no detection) was compared to CTs of these same specimens derived from either the Abbott RealTime m2000™ SARS-CoV-2 assay (April, 2020 study) or Abbot Alinity™ m SARS-COV-2 assay (Abbott Molecular, Des Plaines, IL; Table 1). All m2000 CTs were increased by 10 cycles to account for the unique reporting on that instrument that does not count the first 10 cycles.

Analytic sensitivity curves were generated through logistic regression, using CT as the independent variable, and ID NOW, Sofia 2, or Simplexa result (positive/negative) as the dependent variable, along with 95% point-wise confidence intervals and p-values calculated by the likelihood ratio test.(12) Because ID NOW and Sofia 2 require direct-swabs in clinical practice, an adjustment was made to account for the VTM dilution that will not typically occur. In the ID NOW study, 200 ul of VTM were transferred, representing 6.6% of the total VTM volume (3 ml tube), and therefore 6.6% of total viral particles. For Sofia 2, 50 ul of VTM were transferred, representing 1.6% of viral particles. Assuming direct-swabbing would transfer 100% of viral particles into the assay, there would be 1/.066 = 15 times (ID NOW) and 1/.016 = 60 times (Sofia 2) more viral particles than in VTM studies. As PCR approximately doubles gene targets each cycle, dilutions were estimated to cause a 3.9 CT shift of CTs on ID NOW, and a 5.9 CT shift for Sofia 2. For example, if ID NOW detected a VTM specimen of CT 25.0, we added 3.9 CT for a final value of 28.9 CT for that specimen to be used in the ID NOW logistic regression, as direct-swab testing should detect lower viral loads.

Step 3: For patient populations-of-interest, distributions of CTs from October 10th, 2020 through January 31st, 2021 were queried from our institution's electronic medical record (supplemental Figure S1). These CTs were from different instruments as, e.g., the emergency services CTs derived from Simplexa S-gene CT, while outpatient CTs derived from central laboratory PCR assays. The analyses in this study pertain to emergency services patients only.

Step 4: Logistic regressions from Step 2 for the different assays and specimen types were then applied to the historic CT distributions for emergency services symptomatic and asymptomatic patients. For example, if logistic regression predicted 50% analytic sensitivity for samples positive at CT 35.0, then it was predicted the assay would detect 50% of cases of CT 35.0 in the population-of-interest. This was repeated for each CT value in the distribution and total detected cases were summed and divided by the total distribution count to calculate a predicted positive percent agreement (pPPA). Since the emergency services CT distribution was generated by Simplexa, it is a pPPA against the Simplexa. We also calculate the pPPA of the Simplexa against the historic Simplexa CT distribution, which is expected to be below 100% when some samples are near the LOD. This allows use of the pPPA as an index to compare the performance of proposed assays with the current assay (i.e., the Simplexa). While the Simplexa

pPPA with itself would be expected to be less than 100%, if all positive and negative samples from emergency services testing were to be rerun on the Simplexa, the absolute number of positives would be expected to be the same between runs.

Step 5: Finally, estimates of missed cases per 1,000 tested were made for varying prevalences as a measure that can be benchmarked against Infectious Disease Society of America (IDSA) guidelines. As pPPA is not the same as diagnostic sensitivity, and since the pPPA was calculated against the routine assay in use for emergency services patients (i.e., the Simplexa rapid assay) that has a lower analytic sensitivity than typical reference standards, an adjustment was made to account for likely additional missed cases. This was achieved by binning emergency services CT-distributions into 10 bins based on the Simplexa analytic sensitivity curve fit with LOD data such that the CT-bins represented equally spaced sensitivity windows (i.e., windows with midpoints of 95%, 85%, etc). Then, a multiplier for each CT-value was calculated as the inverse of the Simplexa analytic sensitivity for the midpoint of the relevant CT-window, rounded to two decimal places (i.e., 1.05, 1.18), and multiplied by 100 (i.e. 105, 118). Finally, new CT distributions were created such that each original CT value was instead represented by replicates according to this multiplier. This process accounts for cases likely missed in routine testing and will enrich the CT-distribution with higher values. Total missed cases per 1000 tested were calculated using analytic sensitivity curves against these enriched CT-distributions. This was estimated for all missed cases and for cases < 33.0 CT,(13,14) although the exact cutoff for infectiousness is contentious.(15)

A paired-swab study was conducted after these modeling exercises were complete. IRB approval was not required as per institutional policy of clinical quality improvement projects. In 96 patients presenting to adult emergency services, a direct nasopharyngeal swab was collected for the ID NOW and testing was performed within 1 hour, and another nasopharyngeal swab was collected simultaneously in VTM and performed on the Simplexa. ID NOW positive results were communicated to those performing the Simplexa assay to facilitate patient care. As this was a study meant to compare ID NOW to Simplexa (the institutional standard for emergency services) only discrepant specimens were sent to the central laboratory for confirmation on either the Alinity or m2000.

For all clinical and laboratory testing, each assay was performed according to manufacturer's EUA instructions, with the exception of the use of residual VTM samples for ID NOW(7) and Sofia 2 (as described above). In all analyses, patient status (symptomatic vs asymptomatic) was determined from an institutional checklist based on federal reporting guidance.(16) All statistical analyses were conducted in R statistical environment (R Foundation for Statistical Computing, Vienna, Austria), and dot plots were generated in GraphPad Prism 9 (GraphPad Software, San Diego, USA). A bootstrap version of the univariate Kolmogorov-Smirnov test was used to compare CT distributions from patients enrolled in the paired-swab study and those not enrolled (10,000 bootstraps).

Results

Bias between PCR assays in this study was less than 1 CT. Simplexa showed a -0.15 bias with m2000, and Alinity showed a 0.82 bias with m2000 (supplemental Figure S2, see difference plot). Analytic sensitivity curves of point-of-care assays, across CTs generated by nasopharyngeal VTM specimens on our central laboratory assays, are shown in Figure 1. Logistic regression estimated direct-swab nasal ID NOW analytic sensitivity, fit with direct-swab nasal specimen data as 95% at CT 20.1, 50% at 28.8, and 5% at 37.4. It estimated direct-swab nasopharyngeal ID NOW analytic sensitivity, fit with patient VTM data, as 95% at CT 27.5, 50%

at CT 36.2, and 5% at CT 44.9 and fit with LOD data as 95% at CT 33.6, 50% at CT 34.5, and 5% at CT 35.5. Direct-swab nasopharyngeal Sofia 2 analytic sensitivity estimates, fit with patient VTM data, were 95% at CT 23.7, 50% at CT 27.8, and 5% at CT 32.0. The model estimated VTM nasopharyngeal Simplexa analytic sensitivity, fit with patient VTM data, as 95% at CT 32.9, 50% at CT 35.2, and 5% at CT 37.6 and fit with LOD data as 95% at CT 31.4, 50% at CT 35.6, and 5% at CT 39.9. All p-values for CT as predictor were significant.

Applying these logistic regression models to the emergency services symptomatic population (Figure 2, Table 2), overall pPPA for ID NOW direct nasal specimens (compared to Simplexa nasopharyngeal VTM testing as per routine clinical care) was estimated to be 77.8% (95% confidence interval of the model was 50.2%−89.1%). ID NOW pPPA for direct nasopharyngeal specimens was estimated to be 94.9% (65.8%−98.5%) (fit with patient VTM data) and 97.4% (66%−98.8%) (fit with LOD data). Sofia 2 pPPA for direct nasopharyngeal specimens was estimated as 76.6% (56.4%−84.7%), fit with patient VTM data. Simplexa pPPA for nasopharyngeal VTM testing was estimated as 97.8% (2.7%−99.7%) (fit with patient VTM data) and 97.2% (83.3%−98.9%) (fit with LOD data).

Similarly, for the asymptomatic population presenting for emergency services (Figure 3, Table 2), overall pPPA for ID NOW using direct nasal specimens was estimated to be 64.5% (40.8%−79.8%). ID NOW pPPA for direct nasopharyngeal specimens was estimated to be 88.4% (59.8%−95.5%) (fit with patient VTM data) and 89.6% (57.4%−94.7%) (fit with LOD data). Sofia 2 pPPA for direct nasopharyngeal specimens was estimated as 60.3% (45%−71.4%), fit with patient VTM data. Simplexa pPPA for nasopharyngeal VTM testing was estimated as 91.1% (6.2%−99%) (fit with patient VTM data) and 90.8% (75.2%−96.2%) (fit with LOD data).

When adjusting for cases likely missed in the routine testing that generated the historic CT-distributions, the number of predicted missed cases, and potentially infectious missed cases with CT<33.0, per 1,000 tested were plotted by prevalence in Figure 4. In symptomatic emergency services patients at 15% SARS-CoV-2 prevalence, ID NOW direct nasal testing was predicted to miss 33.8 (16.7-75.0) infected cases per 1,000. Using direct nasopharyngeal specimens, ID NOW was predicted to miss 8.3 (2.6-51.8) (fit with patient VTM data) and 4.5 (2.3-51.6) (fit with LOD data) infected cases per 1,000 tested and Sofia 2 was predicted to miss 35.7 (23.6-65.9) cases. Using nasopharyngeal VTM samples, Simplexa was predicted to miss 4.1 (0.5-146.0) (fit with patient VTM data) and 4.8 (1.8-25.7) (fit with LOD data) cases.

In asymptomatic emergency services patients and at 3% prevalence, ID NOW direct nasal testing was predicted to miss 15.0 (9.3-20.7) infected cases per 1,000. Using direct nasopharyngeal specimens, ID NOW was predicted to miss 7.1 (3.3-15.2) (fit with patient VTM data) and 9.2 (5.4-16.8) (fit with LOD data) infected cases per 1,000 tested and Sofia 2 was predicted to miss 16.4 (12.8-19.8) cases. Using nasopharyngeal VTM samples, Simplexa was predicted to miss 8.3 (0.5-28.3) (fit with VTM data) and 7.6 (3.3-12.3) (fit with LOD data) cases.

In the paired-swab study of 96 emergency services patients (average age 53 years), 25 positives (18 symptomatic and 7 asymptomatic) were identified, with nearly 100% concordance between the ID NOW and Simplexa results (Table S1). The only discrepancy was an ID NOW positive/Simplexa negative sample that was confirmed in the clinical laboratories as positive at CT 34.6. One sample was excluded from analysis due to a specimen aliquoting error when tested on Simplexa. The distribution of CT values detected by Simplexa ranged from 11.0 CT to 32.1 CT (supplemental Figure S3, a dotplot of positives on the Simplexa, whether enrolled in the

study or not, is shown for comparison to evaluate representativeness of the enrolled patients; K-S test p = 0.86 consistent with no selection bias).

Discussion

In this study we develop and demonstrate a model to translate analytic sensitivity of SARS-CoV-2 assays into predictions of PPA as well as missed cases in different patient populations using different specimen types. We also show this can be performed for direct-swab assays using data derived from VTM samples, and allows predictions for multiple settings when data for only one patient population or one specimen type are available. This approach should not replace a validation study. Instead, it increases efficiency and reduces cost by quickly evaluating direct-specimen assays under a number of different conditions, before devoting resources for clinical paired-swab studies. Two different methods (via residual VTM studies, and LOD studies) predicted that if using direct nasopharyngeal samples, ID NOW would have a similar performance to VTM nasopharyngeal testing on Simplexa, the routine standard for rapid emergency services testing at our institution. In contrast, ID NOW direct nasal testing and Sofia 2 direct nasopharyngeal testing were predicted to perform with a clinically meaningful lower performance. These data are consistent with other studies, where ID NOW direct nasal testing performed with a range of 48%-88% positive percent agreement (PPA).(7,17–19) No studies of direct nasopharyngeal samples on ID NOW or Sofia 2 were identified. The prediction of equivalence for ID NOW and Simplexa was confirmed with 99% concordance in a 96-patient paired-swab study that included 25 positive-cases with a wide range of CT values. In published reports, Simplexa has demonstrated a range of LODs (cps/ml): 37(20), 167(21), 501(22), and 521(7). In clinical specimens, PPAs have been reported as 100% in symptomatic populations,(20,23) 88% and 88.1% in mixed populations,(7,9) and 96% and 100% in undescribed popluations,(22,24) with most false negative occurring at CT>33.0.(9)

Using this approach, other data sources could be incorporated. For instance, the CT difference between testing nasal versus nasopharyngeal specimens could potentially be estimated.(10) Using that CT benefit, one could map the nasal specimen analytic sensitivity curve (from a clinical nasal specimen study) to a predicted nasopharyngeal specimen curve. Similarly, one could use this method to predict performance with specimen-pooling in different populations, either based on a clinical study, LOD study, or simple CT adjustment of 2.3 cycles knowing that there will be a dilution of five times the concentration of viral particles (if pooling 5 specimens). Another data source is literature, when CTs of positive cases are reported.

A quality target for diagnostic sensitivity of SARS-COV-2 assays is not universally accepted. However, IDSA determined an acceptable benchmark for symptomatic patients at 10-20 missed cases per 1,000 tested, while >60 per 1,000 was deemed unacceptable (recommendations 5, 6).(25) In our modeling, ID NOW and Simplexa using nasopharyngeal specimens were not expected to exceed 20 missed cases per 1,000 in symptomatic patients up to (and beyond) a prevalence of 20% and in asymptomatic patients up to a prevalence of 6%). In contrast, ID NOW nasal testing and Sofia 2 nasopharyngeal testing were expected to exceed 20 missed cases per 1,000 tested in symptomatic patients at 8% prevalence and in asymptomatic patients at 3-4% prevalence. Importantly, missed cases of CT<33.0, with presumably higher infectious risk, were much lower for all assays.

It should be noted that the values in Figure 4 represent estimated total missed cases for each diagnostic, and not additional missed cases in comparison to the institutional standard. Also, while missed cases by the institutional standard will be lower, even the highest sensitivity

central laboratory assays will by definition miss samples with viral loads at and beyond their 95% LOD. It should also be mentioned that Simplexa was not estimated to detect 100% of emergency services cases, even though the Simplexa was the assay that resulted the CT distributions used for the modeling. This is once again because the assay is detecting cases at and below the 95% LOD in routine practice and therefore is expected to be missing some cases.

Finally, this modeling approach could be employed for other types of qualitative diagnostic testing where there is an associated quantitative output (e.g. CT value) that acts as a surrogate for the biomarker concentration and when the primary driver of sensitivity is thought to be the biomarker's concentration in the specimen. This would likely apply to other infectious disease testing such as influenza and RSV.

There are limitations to this study. First, to estimate the benefit of direct-swab testing compared to VTM, we assumed nucleic acid doubling per PCR cycle. This is expected during the exponential PCR phase, when reagents exceed template. At high CT values, this relationship can degrade. Furthermore, there will be dispersion around this doubling, as can be seen in the LOD study, where CT values ranged across ~7 CT when theoretically 5 doubling dilutions would cover 5 CT. Second, PPAs are typically calculated against the highest sensitivity assay at an institution. In our study, the emergency services CT distributions were generated by the highest sensitivity *rapid* assay at our institution, but still with a sensitivity lower than the central laboratory. The pPPAs are still valid, but should not be equated to diagnostic sensitivity. Had CT-distributions of the same patients been generated by central laboratory instruments, pPPAs of Figures 2 and 3 would be lower. Nevertheless, the pPPA is valuable as an index to compare the relative performance of different assays in various specimen types and populations. Third, estimation of total missed cases required another data manipulation step to quantify likely missed cases not present in the electronic health record, thus creating additional uncertainty in those results. Fourth, CTs are not specifically harmonized between instruments and therefore CT distributions between different patient populations may not be directly comparable, to the extent that CT values between two assays show bias. While one could consider mapping assay CTs to viral load, this was not done as bias was deemed minimal and using CTs directly reduced the burden of model deployment.

The impetus for this study came from our institutional need to develop a model to initially assess the viability of several COVID assays, particularly those that cannot be evaluated with residual specimens. Early in the pandemic we implemented the Simplexa COVID-19 assay where rapid testing was critical (e.g., in emergency services), as it had a 90-minute turn-around time. As the pandemic continued and the need for more rapid testing became critical, we sought to identify a new assay with a shorter turn-around time but similar performance characteristics to the Simplexa. The model presented here permitted an efficient evaluation of several assay/specimen-type/population combinations and predicted the ID NOW using a direct nasopharyngeal specimen would perform similarly to Simplexa. The predictive modeling data provided us the evidence needed for the commitment of significant resources in a clinical paired-swab study that ultimately demonstrated equivalence of the two assays in our patient population.

Acknowledgments: We are grateful for contributions from Amy Rosendaul and the laboratory staff of the Emergency Department Laboratory, the nursing staff of the Michigan Medicine Adult Emergency Services, Scott McClellen and Michele Garrasi in the Microbiology Laboratory, and Karen Barron and Nick Wesener from Point-of-Care Testing Services. We also thank the

leadership team of the C3PO trial, a convalescent plasma study funded by the National Heart, Lung, and Blood Institute (OTA number: 1OT2HL156812-01), and Biomedical Advanced Research and Development Authority, for supporting our clinical study through lending of instruments and reagents.

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: S.L. Kronick received consulting fees from Seraph Biosciences.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: Biomedical Advanced Research and Development Authority lent instruments and reagents.
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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Figure 1. Logistic regression for assay analytic sensitivity (dependent variable) by PCR cycle threshold (independent variable) for ID NOW, Sofia 2, and Simplexa. X-axis PCR cycle threshold determined by central laboratory instruments as described in methods (Abbott Alinity or m2000). CT values were adjusted for ID NOW and Sofia 2 where regressions are fit with VTM or LOD data, as described in methods. Source of data for fitting the logistic regression is listed in parentheses; predictions are for use with direct specimens in the case of ID NOW and Sofia 2 but VTM in the case of Simplexa, as per package insert. Orange circles represent assay result (100=positive, 0=negative), thin grey lines represent 95% confidence intervals of logistic regression.

Figure 2. Overlaying the analytic sensitivity curves and the distribution of CT values from routine clinical testing in emergency department (ED) symptomatic patients. Curves are reproduced from Figure 1. The CT-specific analytic sensitivity for any given bar in the histogram of CT values is estimated by the logistic regression curve at the particular CT value. Source of data for each assay model is listed in parentheses. Overall pPPA (predicted positive percent agreement) with confidence interval is listed for each assay model.

Figure 3. Overlaying the analytic sensitivity curves and the distribution of CT values from routine clinical testing in emergency department (ED) asymptomatic patients. Curves are reproduced from Figure 1. The CT-specific analytic sensitivity for any given bar in the histogram of CT values is estimated by the logistic regression curve at the particular CT value. Source of data for each assay model is listed in parentheses. Overall pPPA (predicted positive percent agreement) with confidence interval is listed for each assay model.

Figure 4. Predicting missed cases per 1,000 patients tested in emergency department symptomatic and asymptomatic patients (A, B) and limiting missed cases only to those patients with cycle thresholds < 33.0 cycles (C, D).

Table 1. Description of laboratory and clinical evaluations conducted

#	Study name	Study design	Index assay	Index assay specimen	Reference assay	Reference assay specimen	N*	Source
1	**CT bias**	CT bias assessment	Simplexa	Nasopharyngeal, VTM	Abbott m2000	Nasopharyngeal, VTM	14	This study
2	**CT bias**	CT bias assessment	Abbot Alinity	Nasopharyngeal, VTM	Abbott m2000	Nasopharyngeal, VTM	59	This study
3	**ID NOW, direct-swab nasal**	Paired-swab	ID NOW	Nasal swab, direct	Abbott m2000	Nasopharyngeal, VTM	24 **	(7)
4	**ID NOW, VTM nasopharyngeal**	VTM	ID NOW	Nasopharyngeal, VTM	Abbott m2000	Nasopharyngeal, VTM	24 **	(7)
5	**ID NOW, LOD**	LOD, viral concentrations adjusted to account for direct testing	ID NOW	Dilutions in VTM, with concentration in reaction buffer equal to concentration of VTM used for the reference swab.	Abbott m2000	Dilutions in VTM	30	(7)
6	**Sofia, VTM nasopharyngeal**	VTM	Sofia II	Nasopharyngeal, VTM	Abbott Alinity	Nasopharyngeal, VTM	32	This study
7	**Simplexa, VTM nasopharyneal**	VTM	Simplexa	Nasopharyngeal, VTM	Abbott m2000	Nasopharyngeal, VTM	24 **	(7)
8	**Simplexa, LOD**	LOD	Simplexa	Dilutions in VTM	Abbott m2000	Dilutions in VTM	30	(7)
9	**ID NOW/Simplexa paired-swab study, nasopharngeal**	Paired-swab	ID NOW	Nasopharyngeal swab, direct	Simplexa	Nasopharyngeal, VTM	96	This study

LOD: limit of detection

*For all studies other than #9, only positive samples of the reference assay are included as only sensitivity is being evaluated

**There were 25 positive samples in Lephart et al, but only 24 were positive by m2000

Table 2. Summary of pPPAs with confidence intervals

	ED symptomatic pPPA (95% CI)	ED asymptomatic pPPA (95% CI)
ID NOW, nasal (direct-swab data)	77.8% (50.2%−89.1%)	64.5% (40.8%−79.8%)
ID NOW, np (VTM data)	94.9% (65.8%−98.5%)	88.4% (59.8%−95.5%)
ID NOW, np (LOD data)	97.4 (66%−98.8%)	89.6% (57.4%−94.7%)
Sofia 2, np (VTM data)	76.6% (56.4%−84.7%)	60.3% (45%−71.4%)
Simplexa, np (VTM data)	97.8% (2.7%−99.7%)	91.1% (6.2%−99%)
Simplexa, np (LOD data)	97.2% (83.3%−98.9%)	90.8% (75.2%−96.2%)

Abbr: pPPA (predicted positive percent agreement)

Figure 1

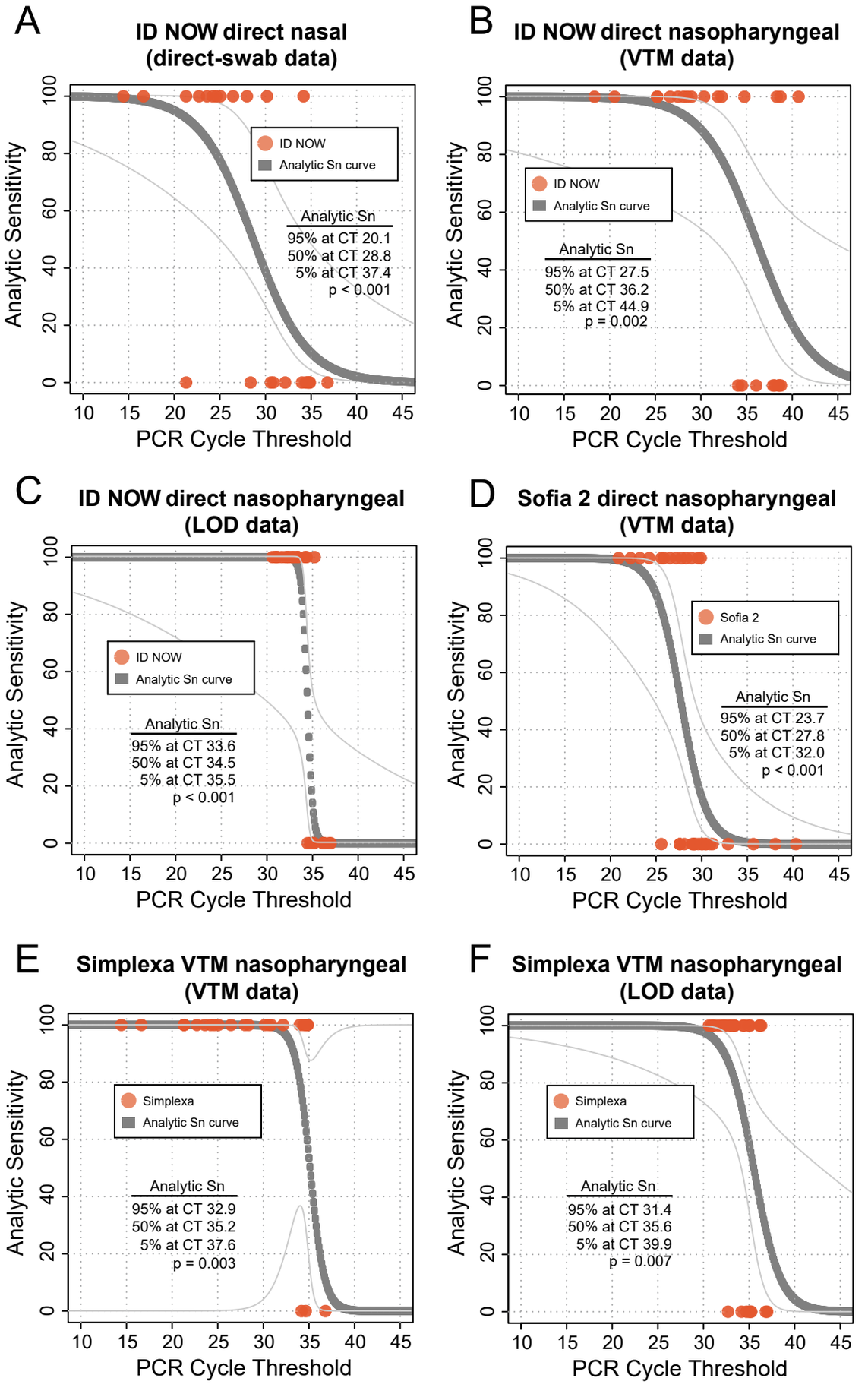

A
ID NOW direct nasal (direct-swab data)
Analytic Sensitivity
PCR Cycle Threshold
ID NOW
Analytic Sn curve
Analytic Sn
95% at CT 20.1
50% at CT 28.8
5% at CT 37.4
p < 0.001
B
ID NOW direct nasopharyngeal (VTM data)
Analytic Sensitivity
PCR Cycle Threshold
ID NOW
Analytic Sn curve
Analytic Sn
95% at CT 27.5
50% at CT 36.2
5% at CT 44.9
p = 0.002
C
ID NOW direct nasopharyngeal (LOD data)
Analytic Sensitivity
PCR Cycle Threshold
ID NOW
Analytic Sn curve
Analytic Sn
95% at CT 33.6
50% at CT 34.5
5% at CT 35.5
p < 0.001
D
Sofia 2 direct nasopharyngeal (VTM data)
Analytic Sensitivity
PCR Cycle Threshold
Sofia 2
Analytic Sn curve
Analytic Sn
95% at CT 23.7
50% at CT 27.8
5% at CT 32.0
p < 0.001
E
Simplexa VTM nasopharyngeal (VTM data)
Analytic Sensitivity
PCR Cycle Threshold
Simplexa
Analytic Sn curve
Analytic Sn
95% at CT 32.9
50% at CT 35.2
5% at CT 37.6
p = 0.003
F
Simplexa VTM nasopharyngeal (LOD data)
Analytic Sensitivity
PCR Cycle Threshold
Simplexa
Analytic Sn curve
Analytic Sn
95% at CT 31.4
50% at CT 35.6
5% at CT 39.9
p = 0.007

Figure 2

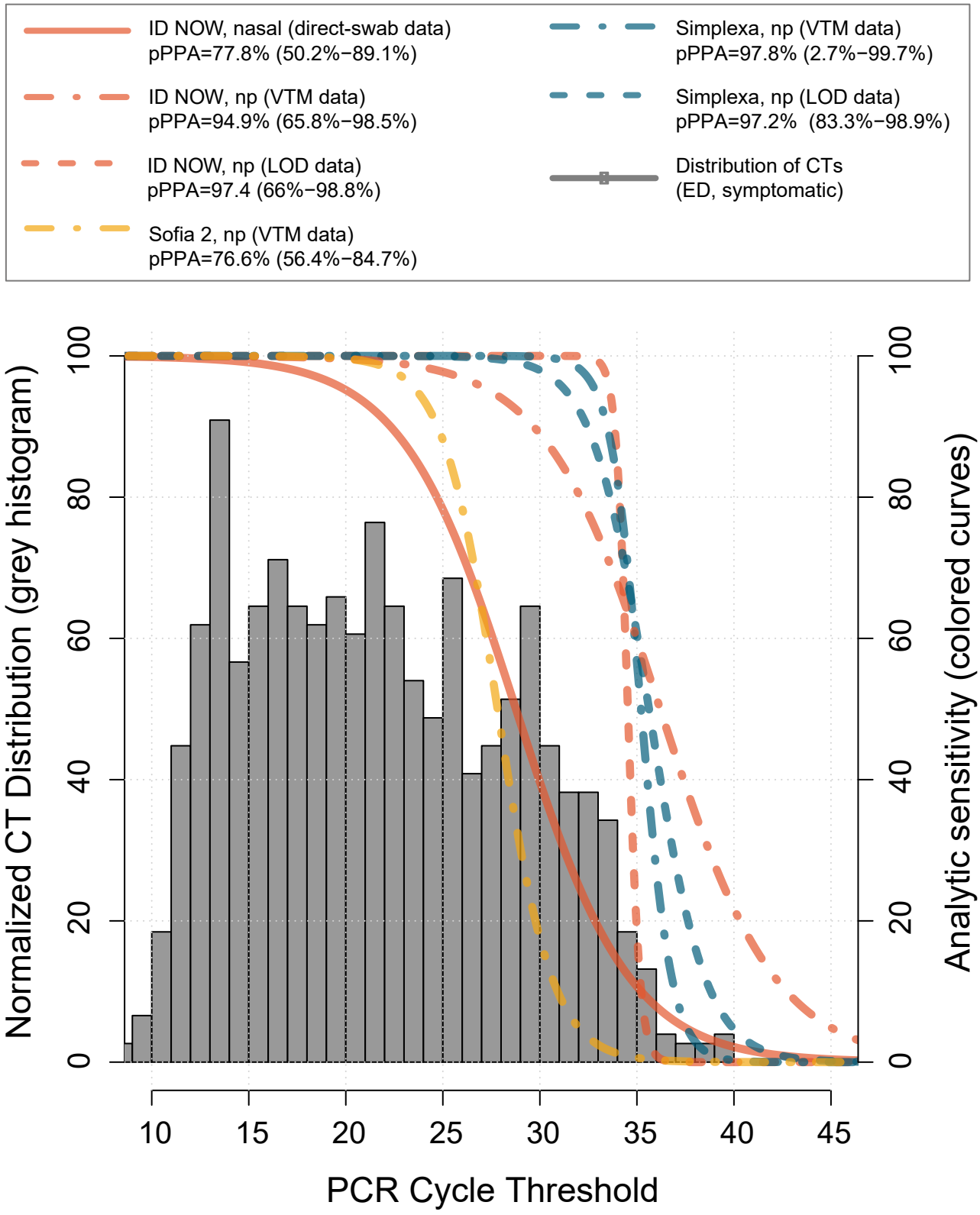
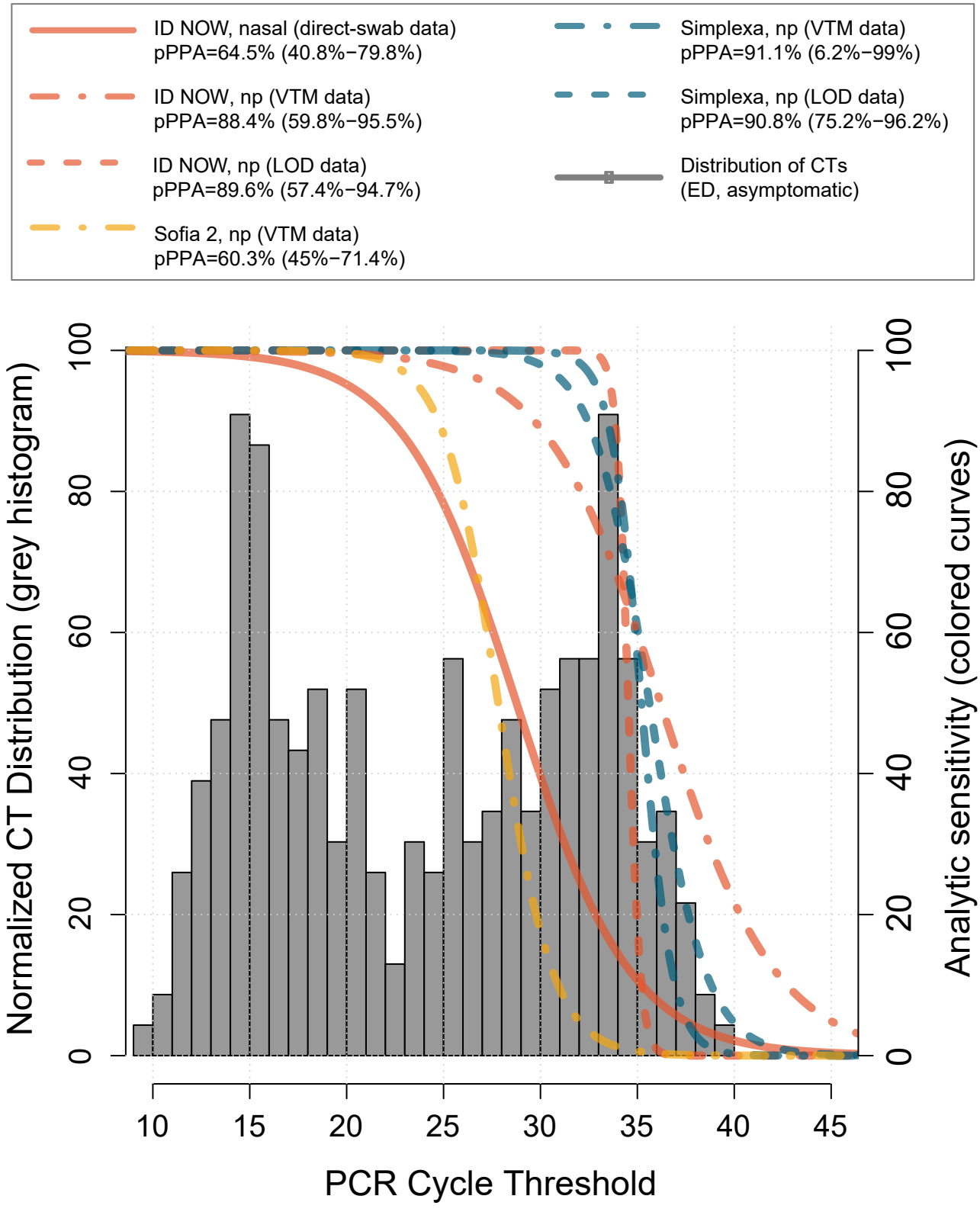

ID NOW, nasal (direct-swab data)
pPPA=77.8% (50.2%−89.1%)
ID NOW, np (VTM data)
pPPA=94.9% (65.8%−98.5%)
ID NOW, np (LOD data)
pPPA=97.4 (66%−98.8%)
Sofia 2, np (VTM data)
pPPA=76.6% (56.4%−84.7%)
Simplexa, np (VTM data)
pPPA=97.8% (2.7%−99.7%)
Simplexa, np (LOD data)
pPPA=97.2% (83.3%−98.9%)
Distribution of CTs
(ED, symptomatic)
Normalized CT Distribution (grey histogram)
Analytic sensitivity (colored curves)
0
20
40
60
80
100
10
15
20
25
30
35
40
45
PCR Cycle Threshold

Figure 3


ID NOW, nasal (direct-swab data)
pPPA=64.5% (40.8%−79.8%)
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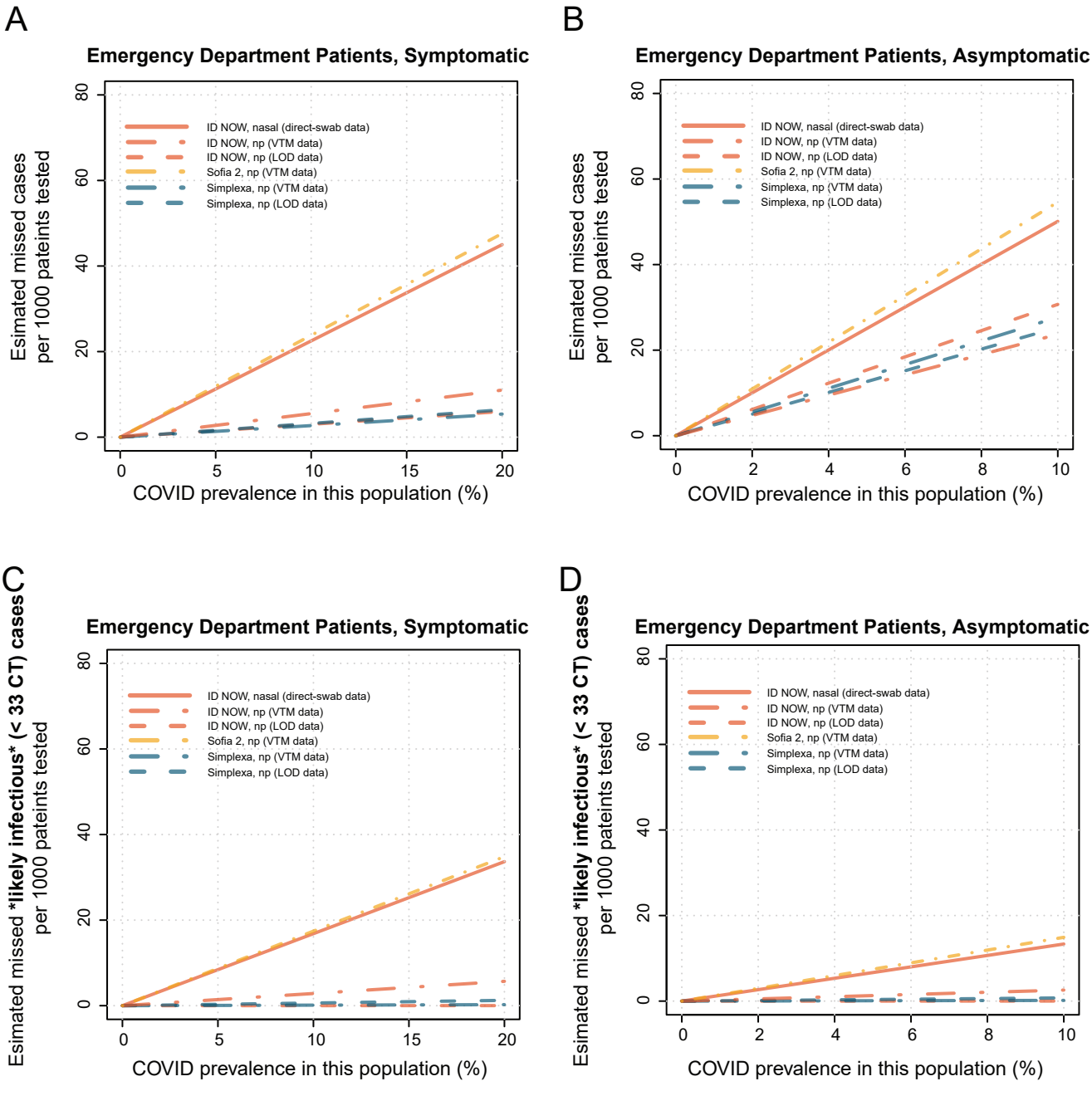
Simplexa, np (LOD data)
pPPA=90.8% (75.2%−96.2%)
Distribution of CTs
(ED, asymptomatic)
Normalized CT Distribution (grey histogram)
Analytic sensitivity (colored curves)
0
20
40
60
80
100
10
15
20
25
30
35
40
45
PCR Cycle Threshold

Figure 4


A
Emergency Department Patients, Symptomatic
Esimated missed cases per 1000 pateints tested
COVID prevalence in this population (%)
B
Emergency Department Patients, Asymptomatic
Esimated missed cases per 1000 pateints tested
COVID prevalence in this population (%)
C
Emergency Department Patients, Symptomatic
Esimated missed *likely infectious* (< 33 CT) cases per 1000 pateints tested
COVID prevalence in this population (%)
D
Emergency Department Patients, Asymptomatic
Esimated missed *likely infectious* (< 33 CT) cases per 1000 pateints tested
COVID prevalence in this population (%)
ID NOW, nasal (direct-swab data)
ID NOW, np (VTM data)
ID NOW, np (LOD data)
Sofia 2, np (VTM data)
Simplexa, np (VTM data)
Simplexa, np (LOD data)