Evaluating the Effect of a Modified Sample Preparation on SARS-CoV-2 Detection in a Cartridge-Based Platform

Wei Zhen,[a,#] Natalie N. Whitfield,[b,#] Elizabeth Smith,[a,#] Christine Shaw,[b] Cristina Ivy,[b] Caylee Douglas,[b] Stephanie Bickford,[b] and Gregory J. Berry[a,c,*]

Introduction: The ePlex® SARS-CoV-2 emergency use authorization (EUA) test is a cartridge-based assay for the detection of SARS-CoV-2 in nasopharyngeal specimens. Since performance data has been previously published on this platform, the manufacturer has modified the workflow design in order to improve assay performance. Evaluation of the new workflow, which eliminated the sample delivery device (SDD), led to a dramatic improvement of assay performance while saving time and making cartridge loading more convenient.

Methods: 145 confirmed positive nasopharyngeal swab specimens were used to evaluate the assay analytical sensitivity, accuracy, and overall time-saving for the 2 workflows that is with and without the use of SDD on the ePlex SARS-CoV-2 test.

Results: Elimination of the SDD step led to a dramatic increase in accuracy and the overall limit of detection when using 145 previously defined and valid SARS-CoV-2 positive specimens with relatively low, medium, and high cycle thresholds (C_T). This simple workflow change led to an overall detection from 94/145 (64.8%) to 131/145 (90.3%), with an additional 37 specimens being detected. C_T value ranges revealed that 90% of the specimens in the $33 \leq C_T < 35.3$ C_T range were detected, whereas with the SDD workflow, only 30% of positive specimens were detected in this same range. Hands-on time for each specimen also improved and showed overall time savings.

Conclusion: The simple workflow modification eliminating the SDD led to an overall improvement in the detection of positive specimens and also simplified workflow and reduced hands-on time.

IMPACT STATEMENT

This study evaluates the GenMark ePlex SARS-CoV-2 EUA assay and the impact of a simple workflow modification on SARS-CoV-2 detection. The removal of the SDD from the assay setup dramatically increased assay performance, leading to an overall detection increase from 94/145 (64.8%) to 131/145 (90.3%). In addition, this modification improved assay workflow and turnaround time. Laboratories using this assay can

[a] Infectious Disease Diagnostics, Northwell Health Laboratories, Lake Success, NY [b] GenMark Diagnostics, LLC [c] Department of Pathology and Laboratory Medicine, The Donald and Barbara Zucker School of Medicine at Hofstra/Northwell.
* Address correspondence to this author at: Infectious Disease Diagnostics, Northwell Health Laboratories, 450 Lakeville Rd., Lake Success, NY 11042. Fax 718-224-6585; e-mail gberry1@northwell.edu .
[#] These authors contributed equally to this work.
Received November 19, 2020; accepted February 1, 2021.
DOI: 10.1093/jalm/jfab007
© American Association for Clinical Chemistry 2021. All rights reserved.
For permissions, please email: journals.permissions@oup.com.

implement this workflow immediately for better assay performance. These data also support the use of this same workflow modification in the new multiplex respiratory panel, which includes SARS-CoV-2 as a new target.

INTRODUCTION

At the end of 2019, the outbreak of viral pneumonia that swept across Wuhan, China was found to be caused by the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with the resulting disease later defined as coronavirus disease 2019 (COVID-19). Since December 2019 when the SARS-CoV-2 outbreak began, it has developed into a global pandemic. According to the Center for System Science and Engineering (CSSE) at Johns Hopkins University, more than 50 million confirmed cases have been documented and more than 1.25 million people have died, the United States alone now accounts for approximately one-fifth of cases and deaths (1).

The diagnosis and effective management of COVID-19 relies on a combination of epidemiological criteria, clinical symptoms, clinical imaging tests, and especially diagnostic laboratory tests. With the rapid spread of SARS-CoV-2, robust diagnostic testing of SARS-CoV-2 infection is crucial for appropriate patient management and to contain the viral spread. Real-time PCR (RT–PCR) has become the most widely used diagnostic test for COVID-19 worldwide (2). The first available RT–PCR test authorized for use in the United States by the FDA for emergency use authorization (EUA) was from the Centers for Disease Control and Prevention (CDC) (3). Shortly thereafter, several RT–PCR tests became commercially available. One of the first sample-to-answer SARS-CoV-2 tests available, the GenMark ePlex® SARS-CoV-2 test (GenMark Diagnostics), was validated for clinical use at Northwell Health Laboratories. The ePlex test is authorized for the qualitative detection of SARS-CoV-2 in clinical nasopharyngeal swab specimens from symptomatic patients.

When granted FDA EUA status, the ePlex SARS-CoV-2 test followed the same established workflow as the ePlex Respiratory Pathogen Panel, in which an aliquot of the viral transport media from the primary nasopharyngeal swab specimen is transferred to a sample delivery device (SDD). The SDD contains a lysis buffer designed to standardize the sample and minimize the viscosity of extremely thick, or very mucoid specimens (4). After the sample is loaded on the ePlex cartridge for processing, an additional internal lysis buffer is added to the sample prior to nucleic acid extraction, followed by amplification and detection.

The performance of the ePlex SARS-CoV-2 test was evaluated and compared to multiple other SARS-CoV-2 EUA assays (5–7), with findings showing a slightly lower sensitivity in these comparison studies. In response, the manufacturer has updated the instructions for use to include (*a*) a second optional workflow for nasopharyngeal swab specimens that excludes the use of the sample delivery device (non-SDD), and (*b*) an improved limit of detection (LoD) for the assay using the updated workflow (8). In this study, we evaluated the analytical performance of the 2 workflows, the original SDD workflow and new non-SDD workflow, for the ePlex SARS-CoV-2 test in 145 previously known positive nasopharyngeal swab specimens from symptomatic patients. The time savings associated with this new workflow were also assessed.

MATERIALS AND METHODS

Specimen collection and storage: nasopharyngeal swab specimens (NPS) from symptomatic patients were collected with sterile swabs made of Dacron, nylon, or rayon. After collection, each swab was placed into 3 mL of sterile Universal Transport Medium (various manufacturers). NPS were transported to the laboratory and tested as soon as possible after collection. After transport and before initial testing, the samples were stored for up to 72 h at 2–8 °C. Following routine testing, samples were aliquoted and stored at −80 °C. This study was submitted to the Northwell Institutional Review Board (HSRD HSRD21-0025) and was determined to be exempt from review and approval.

Reference and Discordant Analysis Assays

The Hologic Panther Fusion® SARS-CoV-2 assay (Hologic Inc.) was considered the reference standard in this study and the Simplexa COVID-19 Direct EUA assay (Diasorin Molecular LLC) was used to evaluate the discordant results [nasopharyngeal swab specimens were tested according to the manufacturer's package insert as previously described (4, 5) for both assays].

Study Design

A total of 150 residual NP swab specimens, that were initially SARS-CoV-2 positive by the Panther Fusion EUA assay at Northwell Health Laboratories between April and May 2020, were archived for this study. The randomly preselected 150 SARS-CoV-2 positive specimens included patients without bias to age or gender and contained relatively low viral load with higher cycle threshold (C_T) SARS-CoV-2 specimens ($30 \leq C_T \leq 37.1$, $n = 100$) and medium-to-high viral load SARS-CoV-2 specimens ($12.9 \leq C_T < 30$, $n = 50$) for ORF1ab using the reference standard assay. Five specimens that were initially deemed positive when archived, but were subsequently negative when tested by both of the ePlex sample processing workflows and the Simplexa assay were excluded from the analysis. The study was performed only on positive specimens tested previously with the ePlex SARS-CoV-2 EUA test to compare the clinical performance of the test using the original SDD vs the new non-SDD testing workflow.

GenMark ePlex SARS-CoV-2 Test EUA

Two workflows for testing of NPS with the ePlex SARS-CoV-2 test were performed according to the manufacturer's package insert. SDD began with briefly vortexing the specimen for 3–5 seconds and transferring 200 μL of the primary NPS sample into the SDD provided with the ePlex SARS-CoV-2 test kit. The SDD was vortexed for 10 seconds and the entire volume of the SDD was dispensed into the sample loading port of the SARS-CoV-2 test cartridge. For the non-SDD workflow, the SDD was excluded from the procedure, and 200 μL of the primary NPS sample was briefly vortexed and directly dispensed into the sample loading port of the SARS-CoV-2 test cartridge. After the sample was dispensed into the cartridge, using either workflow, the cartridge cap was firmly pushed down to securely seal the sample delivery port. Each cartridge was bar-coded, scanned at the ePlex instrument, and inserted into an available ePlex bay. The ePlex instrument generated a report for the detection of SARS-CoV-2 after the completion of each test. The GenMark ePlex SARS-CoV-2 test amplifies and detects 2 conserved regions of the 2019-nCoV virus nucleocapsid (N) gene, with either one or both regions leading to a positive detection.

Analytical Sensitivity

The LoD studies for the ePlex SARS-CoV-2 test were completed using a heat-inactivated virus procured from Zeptometrix (USA-WA1/2020)

using the 2 ePlex sample preparation workflows. A range-finding study was performed to characterize the preliminary LoD concentrations to be tested with the ePlex SARS-CoV-2 test SDD workflow. A dilution panel was created from a starting concentration of 1 median tissue culture infectious dose ($TCID_{50}$)/mL of the heat-inactivated virus. The dilution panel was prepared in pooled known-negative NPS samples and aliquoted for testing with replicates at 1, 0.33, 0.1, 0.03, and 0.01 $TCID_{50}$/mL. The $TCID_{50}$/mL was converted to copies/mL by quantifying the lowest concentration using digital droplet PCR (ddPCR). ddPCR was performed by the UCSD Genomics and Sequencing Core laboratory using a Bio-Rad QX200 droplet digital PCR instrument with the primers and probes from the CDC SARS-CoV-2 assay (N2 gene). The LoD was established as the lowest concentration at which 95% positivity was achieved.

Discordant Analysis

The following 2 scenarios were considered discordant results. First, if the results were negative from the SDD or non-SDD workflow, the specimen was tested with the DiaSorin Simplexa COVID-19 EUA assay. If positive by Simplexa, the sample result was reported as discordant on ePlex for whichever workflow (SDD or non-SDD) that resulted as negative. If negative by Simplexa, the specimen was considered negative and was therefore excluded from the study. In addition, if the ePlex test result for the SDD and non-SDD was discordant, the assay was repeated using a new cartridge to confirm the result.

Workflow Evaluation

A comparison of the setup between the SDD and non-SDD workflows was conducted using a stopwatch to measure the amount of time needed for hands-on time (HoT) per sample.

Statistical Methods

The reference standard for each sample was the result obtained from the Panther Fusion SARS-CoV-2 assay. The percentage agreement, median C_T values, and 95% confidence intervals were calculated using Microsoft Office Excel 365 software (Microsoft).

RESULTS

A total of 150 NPS specimens, previously identified as positive for SARS-CoV-2, were tested to evaluate the performance of the non-SDD workflow for the ePlex SARS-CoV-2 test as compared to the standard with SDD workflow. The positive NPS specimens included a range of C_T values of 12.9–37.1 for ORF1ab generated by the Hologic Panther Fusion SARS-CoV-2 assay [median C_T of 32, interquartile range (IQR): 27.13–34.20]. Five specimens deemed to be initially positive were negative by both of the ePlex sample processing workflows and by the Simplexa assay, and were therefore excluded from the study (Fig. 1). After exclusion, the median C_T value of the remaining 145 included samples was 31.9 (IQR 26.8–34.0).

When NPS were tested after processing with the ePlex SDD workflow, the ePlex detected 94/145 (64.8%), while the non-SDD workflow resulted in 131/145 (90.3%) samples detected as positive for SARS-CoV-2 (Table 1). Of the 51 samples not detected when tested on the ePlex with SDD, 37 of these samples were detected when tested on the ePlex using the non-SDD workflow. Two of the remaining 14 samples that were not detected when tested on the ePlex with the non-SDD workflow, were detected on repeat testing (Fig. 1). Overall, 12 samples were not detected by the assay using the non-SDD, 10 of which were positive for both Simplexa targets (S-gene and ORF1ab), while 2 were positive only for the S-gene. The median C_T values for the samples tested by the ePlex without the SDD shifted to higher C_T values

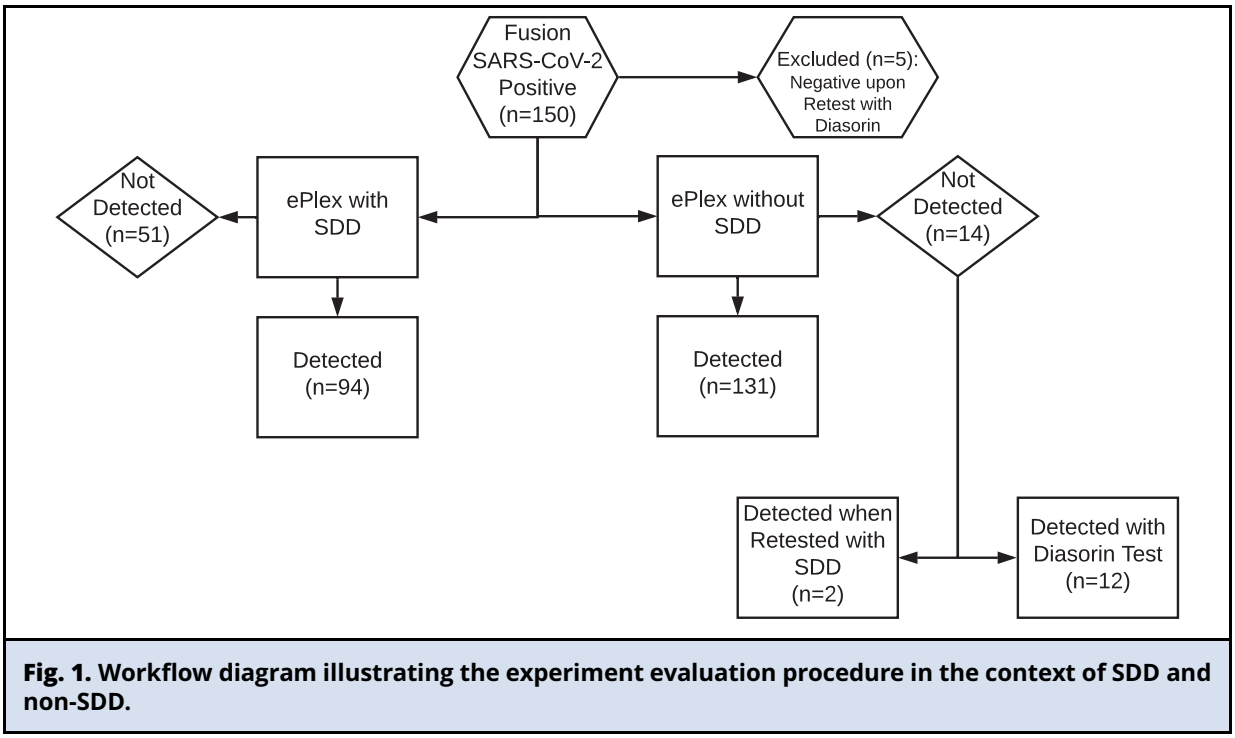

Fig. 1. Workflow diagram illustrating the experiment evaluation procedure in the context of SDD and non-SDD.

Table 1. Results from ePlex assessment of 2 workflows for 145 preselected Fusion SARS-CoV-2 positive nasopharyngeal swab specimens.

	Processing	
	SDD	**Non-SDD**
Detected by ePlex	94/145	131/145
Overall percentage agreement	64.8%	90.3%
Median C_T value "detected"	29.75 (95 CI 27.2–30.65)	31.4 (95 CI 30.6–32.3)
Median C_T value "not detected"	34.4 (95 CI 33.7–35.2)	35.3 (95 CI 34.45–36.25)

representing lower viral titer specimens when compared to the SDD workflow (Table 1).

The detection of SARS-CoV-2 by the ePlex SARS-CoV-2 test was improved when samples with higher Fusion C_T values were tested using the non-SDD sample processing workflow (Fig. 2). To evaluate the ePlex detection rate in the context of the known C_T values when comparing the 2 workflows, the overall agreement stratified by the Fusion C_T value was calculated (Table 2). At low C_T values <30, the percentage agreement for each workflow was 97% with the SDD and 100% without the SDD. However, as the C_T values increased for samples tested with the SDD, $30 < C_T < 33$, $33 \leq C_T < 35$, and $C_T \geq 35$ the agreement decreased to 78, 30, and 20% respectively. In contrast, when the same samples were tested using non-SDD processing, only samples with C_T values

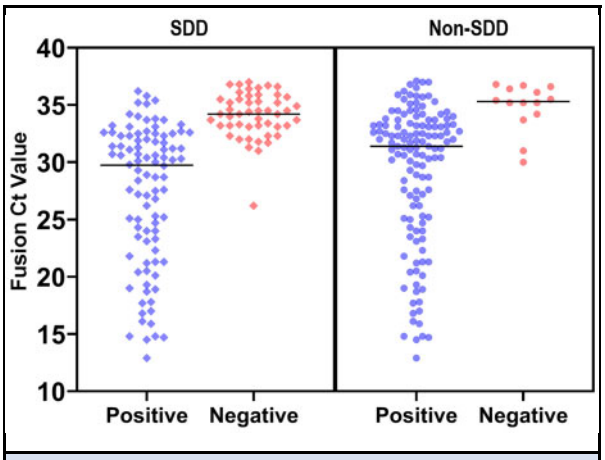

Fig. 2. Results for the detection of SARS-CoV-2 for each workflow by individual sample and cycle threshold value. C_T values are shown for the Panther Fusion and each ePlex workflow indicated as ◆ for samples tested with SDD and ● for samples tested using non-SDD. Blue represents Fusion positive samples also positive by ePlex, while red represents Fusion positive samples negative by ePlex. Horizontal bars represent median C_T values for each condition.

Table 2. Percentage agreement of the ePlex SARS-CoV-2 test compared to Fusion of positive nasopharyngeal swab specimens processed with SDD and non-SDD stratified by Fusion C_T value.

C_T value	SDD	Non-SDD
<20	100% (15/15)	100% (15/15)
20–<30	97% (33/34)	100% (34/34)
30–<33	78% (32/41)	95% (39/41)
33–<35	30% (9/30)	93% (28/30)
≥35	20% (5/25)	60% (15/25)
Total	64.8% (94/145)	90.3% (131/145)

of $33 \leq C_T < 35$ and $C_T \geq 35$ showed a decrease in the percentage agreement of 93 and 60%, respectively.

The analytical sensitivity or LoD of the ePlex SARS-CoV-2 test using each workflow was determined using heat-inactivated SARS-CoV-2 virus diluted in a negative NP specimen matrix. The positivity rate at which ≥95% detection was observed for SARS-CoV-2 using each workflow was a concentration of 1 $TCID_{50}$/mL for replicates tested with the SDD workflow and a concentration of 0.03 $TCID_{50}$/mL for replicates tested without the SDD (Table 3). The verified LoD concentration for detection of SARS-CoV-2 using the non-SDD workflow was determined to be 0.03 $TCID_{50}$/mL, which corresponds to 750 genomic copies per mL, as determined by digital droplet PCR.

HoT was also evaluated between the SDD and non-SDD workflow. The setup for the SDD workflow takes approximately 120 seconds from the time the sample is picked up until the time the cartridge is ready for loading onto the instrument. In contrast, when analyzing the approximate time to load one specimen using the non-SDD workflow, it takes approximately 40 seconds.

DISCUSSION

In this study, we analyzed the original SDD and modified Non-SDD workflows for the GenMark ePlex SARS-CoV-2 test using 150 previously tested SARS-CoV-2 positive specimens to determine whether the non-SDD modification would lead to increased sensitivity. We also compared operational efficiency between these 2 workflows. This simple change led to a clear improvement in LoD, an overall median C_T value shift of 1.65 (from 29.75 to 31.4) for those specimens detected, indicating that largely, more positive specimens were detected in the higher C_T category. Overall detection also increased from 94/145 (64.8%) to 131/145 (90.3%), with an additional 37 specimens being detected. The mean C_T values of those specimens "not detected" also shifted later, from 34.4 with SDD to 35.3 non-SDD, indicating that not detected specimens were in the later C_T value range. Ct value ranges also show that 90% of the specimens that fell in the 33 to <35.3 C_T range

Table 3. Analytical sensitivity of ePlex SARS-CoV-2 for 2 workflows established with Zeptometrix USA-WA1/2020 Attenuated Virus.

	Percent replicates detected (no. positive/total no.)	
$TCID_{50}$/mL	**SDD**	**Non-SDD**
1	100% (20/20)	NT
0.33	80% (16/20)	100% (20/20)
0.1	70% (14/20)	NT
0.03	NT	95% (19/20)
0.01	NT	80% (16/20)

NT = not tested.

were detected, whereas, with the original SDD workflow, only 30% of positive specimens were detected in this range. Based on our findings, it is clear that the removal of the SDD from the assay led to a substantially increased overall sensitivity and accuracy. These findings further reinforce that many factors can influence the performance characteristics of a molecular assay, including specimen preprocessing and assay buffers, as well as the established LoD of the assay. The workflow for this change also added efficiency, shaving ~80 seconds off the HoT for each specimen. In an 8-hour shift where 96 specimens could be tested (assuming one 24-bay instrument), this represents >2 hours (128 minutes) of saved time in overall assay prep.

In addition, the new ePlex Respiratory Pathogen Panel 2 (RP2) also uses the non-SDD workflow, partially based on the knowledge garnered from this study, making these findings relevant to the new panel design as well. RP2 expands the ePlex's FDA-cleared Respiratory Pathogen Panel to include the SARS-CoV-2 target on a broad multiplex panel allowing for rapid differentiation of SARS-CoV-2 from other circulating respiratory pathogens with similar symptomology. With the same processing workflow as the SARs-CoV-2 test, the RP2 workflow modification will provide similar overall efficiency improvements for laboratories that use a multiplex panel during the respiratory illness season when test volumes can be high.

Previously, we analyzed the ePlex SARS-CoV-2 test and showed that the assay missed 5 out of 58 total positive specimens. Further analysis of discordant specimens revealed that the missed positive specimens range from C_T values of 32 to 38.5, with 3 of those specimens having a C_T value of ≤34 (5). Based on this current analysis of the non-SDD workflow, it is probable the ePlex SARS-CoV-2 test may have detected these specimens, suggesting overall improved performance of the assay.

Limitations of this study include that this was a single-site study and also that positive specimen C_T values to analyze were selected, instead of prospectively chosen. While this is the case, we selected 150 positive specimens that were in the range expected to have the most impact from a change in the workflow, which allowed us to focus on this range while still saving testing resources.

In conclusion, the non-SDD workflow showed superior performance when compared to the original SDD workflow. This simple change has increased the sensitivity of the SARS-CoV-2 assay with a minor change that also simplified the HoT and workflow. These time savings are also generalizable to the RP2 Panel, which is replacing the standalone SARS-CoV-2 test.

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.*

N.N. Whitfield, statistical analysis; E. Smith, statistical analysis, administrative support, provision of study material or patients; C. Shaw, administrative support, provision of study material or patients; C. Ivy, statistical analysis.

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:** N.N. Whitfield, GenMark Dx; C. Shaw, Genmark Diagnostics; C. Ivy, GenMarkDx; C. Douglas, GNMK. **Consultant or Advisory Role:** None declared. **Stock Ownership:** N.N. Whitfield, GenMark Dx; C. Shaw, Genmark Diagnostics; C. Ivy, GenMarkDx; C. Douglas, GNMK; S. Bickford, Genmark Dx. **Honoraria:** None declared. **Research Funding:** This work was performed with the support of the Genomics and Sequencing Core at the San Diego Center for AIDS Research (P30 AI036214), the VA San Diego Healthcare System, and the Veterans Medical Research Foundation. This work was performed with the support of the James B. Pendleton Charitable Trust. **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.

REFERENCES

1. Johns Hopkins Coronavirus Resource Center-COVID-19 Dashboard. https://coronavirus.jhu.edu/map.html. (Accessed November 2020).
2. World Health Organization. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance. https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2. (Accessed November 2020).
3. Centers for Disease Control and Prevention. CDC 2019-nCoV real-time RT-PCR diagnostic panel instructions for use. https://www.cdc.gov/coronavirus/2019-ncov/lab/virus-requests.html. (Accessed November 2020).
4. Babady NE, England MR, Jurcic Smith KL, He T, Wijetunge DS, Tang YW, et al. Multicenter evaluation of the ePlex respiratory pathogen panel for the detection of viral and bacterial respiratory tract pathogens in nasopharyngeal swabs. J Clin Microbiol 2017;56:e01658–17.
5. Zhen W, Smith E, Manji R, Schron D, Berry GJ. Clinical evaluation of three sample-to-answer platforms for the detection of SARS-CoV-2. J Clin Microbiol 2020;58:e00783–20.
6. Zhen W, Manji R, Smith E, Berry GJ. Comparison of four molecular in vitro diagnostic assays for the detection of SARS-CoV-2 in nasopharyngeal specimens. J Clin Microbiol 2020;58:e00743–20.
7. Mostafa HH, Hardick J, Morehead E, Miller JA, Gaydos CA, Manabe YC. Comparison of the analytical sensitivity of seven commonly used commercial SARS-CoV-2 automated molecular assays. J Clin Virol 2020;130:104578.
8. ePlex SARS-CoV test EUA, Package insert. https://www.fda.gov/media/136282/download. (Accessed November 2020).