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Clinical evaluation of the BioFire® Respiratory Panel 2.1 and detection of SARS-CoV-2

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ABSTRACT

We evaluated the performance of the BioFire® Respiratory Panel 2.1 (RP2.1) in the detection of SARS CoV-2 in comparison against three other SARS CoV-2 EUA assays. In these studies, the RP2.1 panel had 98 % positive percent agreement (48/49) and 100 % negative percent agreement (49/49). Since 30 % of nasopharyngeal swab specimens have a SARS CoV-2 Ct > 30 and thus detection of virus in low titers is clinically relevant, a sample with a high titer was diluted and each 10 fold dilution was tested in triplicate and compared against 6 other EUA approved SARS CoV-2 assays. These data suggested that the BioFire® RP2.1 panel, along with four other SARS CoV-2 assays (Roche cobas, Cepheid Xpert Xpress, BioFire® Defense COVID19, and NECoV19), consistently detected viral RNA at the $10-7$ dilution. Overall, these studies suggest that the BioFire® RP2.1 assay can be used to detect acute cases of SARS CoV2 in addition to patients with low viral titer later in disease presentation.

1. Introduction

The gold standard for SARS-CoV-2 diagnosis is detection of viral RNA in nasopharyngeal (NP) swab specimens. Sample-to-answer nucleic acid amplification assays for the detection of SARS-CoV-2 RNA are available for a limited number of high-throughput diagnostic platforms including the Roche cobas 6800/8800 [1,2], the Hologic Panther and Panther Fusion [3–6], and the Abbott m2000 [7]. High-throughput platforms are mostly utilized in larger reference laboratories, state public health laboratories, and academic medical centers, but these assays are not well-suited to use in other settings that lack large testing volumes or the capacity to perform high complexity tests. This has led to the centralization of SARS-CoV-2 testing, meaning that turnaround time may be prolonged by the need to transport specimens over long distances. As SARS-CoV-2 prevalence increases, decentralized testing capability is needed to facilitate rapid identification of SARS-CoV-2 cases. To date, the BioFire COVID-19, Cepheid Xpert Xpress SARS-CoV-2 [8], DiaSorin Simplexa [9] and the GenMark ePlex SARS-CoV-2 tests [6] have emerged as rapid COVID19 testing platforms that fill this niche.

The BioFire® FilmArray® System (BioFire Diagnostics, LLC, Salt Lake City, UT, "BioFire") is another testing platform that is widely used in multiple laboratory environments. This multiplex, sample-to-answer, nucleic acid amplification platform utilizes syndromic panels for infectious disease diagnosis. BioFire recently received FDA Emergency Use Authorization (EUA) to distribute the syndromic BioFire® Respiratory Panel 2.1 (RP2.1) for use on BioFire® FilmArray® 2.0 and BioFire® Torch Systems. The BioFire RP2.1 was created by adding primers for the membrane (M) and spike (S) genes of SARS-CoV-2 to the existing FDA-cleared and CE-marked BioFire® Respiratory Panel 2 (RP2) test. It therefore detects 22 viral and bacterial respiratory pathogens including SARS-CoV-2. In this study, we clinically evaluated the BioFire RP2.1 SARS-CoV-2 assay in comparison to three other SARS-CoV-2 EUA assays.

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2. Materials and methods

2.1. SARS-CoV-2 archived clinical specimens

Residual natural nasopharyngeal swab in transport media (NPS) specimens leftover from SARS-CoV-2 testing performed as part of patient care were collected during March and April of 2020 at the University of Washington (Seattle, WA), University of Nebraska Medical Center (Omaha, NE), and ARUP laboratories (Salt Lake City, UT). Original specimen testing for SARS-CoV-2 was conducted according to manufacturer's instructions at ARUP laboratories using the Hologic Panther Fusion SARS-CoV-2 assay (FDA EUA), at the University of Nebraska Medical Center using the Roche cobas SARS-CoV-2 assay (FDA EUA), or at the University of Washington using a laboratory developed test based on the CDC N1 and N2 SARS-CoV-2 assays (Washington EUA) conducted as described in Perchetti et al. [10]. Specimens were frozen upon study enrollment to allow for storage and shipping. Additional NPS specimens collected before December 2019 and therefore presumed to be negative for SARS-CoV-2 were provided by BioFire Diagnostics.

2.2. SARS-CoV-2 dilution series

Ten-fold serial dilutions of a natural nasopharyngeal swab specimen with known high positivity for SARS-CoV-2 RNA (E gene detected at a cycle threshold (Ct) of 16.6 by the cobas SARS-CoV-2 assay) were prepared with a diluent of pooled NPS. Diluent was prepared from samples that tested negative for SARS-CoV-2 using an assay developed at Nebraska Medicine (NECoV19; FDA EUA) and was confirmed to be PCR negative prior to use. These samples were not tested for other respiratory viruses prior to pooling. On two separate subsequent occasions, an aliquot of the 10^{-4} or 10^{-5} diultions was thawed and added to newly generated pools of NPS to create additional intermediate dilutions between 10^{-6} and 10^{-8}. Single-use aliquots of each dilution were stored at −80 °C and thawed immediately prior to use.

2.3. Laboratory developed testing

Conventional RT-PCR testing was performed by a Nebraska Medicine LDT for SARS-CoV-2 E gene detection adapted from Corman [11]; 600 nM and 800 nM concentrations of the forward and reverse primers, respectively, were used. RNA was extracted from 400 µL of sample using a KingFisher Flex automated extractor and the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Applied Biosystems) and eluted in 50 µL. For all samples in which amplification of the E gene target was not detected, successful RNA extraction was confirmed by an identical PCR reaction using primers and probe specific for the cellular RNaseP gene that have been described by the Centers for Disease Control and Prevention [12]. The EDX SARS-CoV-2 RNA standard (Exact Diagnostics), which contains quantitated synthetic RNA transcripts comprised of five SARS-CoV-2 gene targets (E, N, ORF1ab, RdRp, and S) was diluted in Ambion RNA storage solution (ThermoFisher Scientific) to 5000, 2000, 1000, 500, 250, and 125 RNA copies/mL and extracted in triplicate. The QuantStudio Dx and QuantStudio Test Development Software version 1.0.3 (Applied Biosystems, Foster City, CA) were used for thermal cycling and data acquisition. A standard curve was generated to estimate the quantity of viral RNA in NP swab specimens based on LDT Ct values. Linear regression was performed in GraphPad Prism 8 (version 8.4.1).

2.4. Comparator SARS-CoV-2 testing

Testing by commercial assays (BioFire RP2.1 [BioFire Diagnostics], BioFire COVID-19 Test [BioFire Defense], cobas SARS-CoV-2 Test [Roche], GeneXpert Xpert Xpress SARS-CoV-2 [Cepheid], Aptima SARS-CoV-2 Assay [Hologic], and ID NOW COVID 19 [Abbot]) was performed according to manufacturer's instructions with the exception of the Abbot ID NOW. The instructions for use for this assay have been revised and now limit testing to swab specimens that can be used to directly inoculate the sample cup. Because comparison between platforms required use of a liquid specimen, we followed the instructions for use associated with the original EUA for the Abbot ID NOW assay and used transfer pipettes from the kit to add 200 µL of NP swab specimens in transport medium to the sample cup.

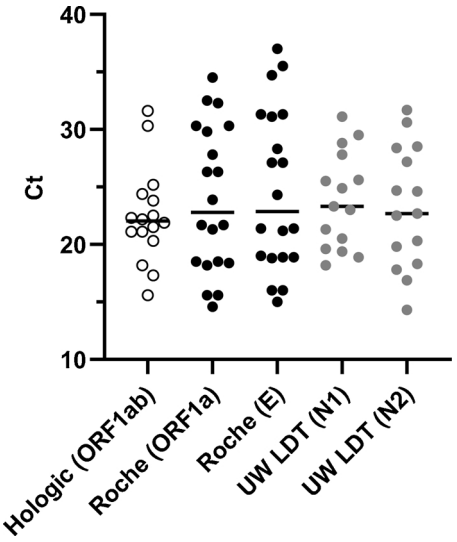

Fig. 1. Ct distribution of specimens tested in clinical study.
Ct values are shown for each assay used for characterizing clinical specimens, as indicated on the X axis. Horizontal bars represent Ct median values for each assay.

3. Results

3.1. Performance comparison between the SARS-CoV-2 component of the BioFire RP2.1 assay and reference SARS-CoV-2 assays

One hundred archived NPS specimens were tested as part of a clinical comparison study to evaluate the performance of the newly added SARS-CoV-2 assays on the BioFire RP2.1 Panel. Fifty of these archived specimens were identified as positive by another SARS-CoV-2 assay with FDA EUA and covered a range of Ct values, representative for COVID-19 viral titer in clinical samples within the reference FDA EUA assays Ct ranges (Fig. 1). These included 15 specimens tested by the Hologic Panther Fusion SARS-CoV-2 assay at ARUP Laboratories in Salt Lake City, Utah (median Ct of 22.1, IQR: 20.1–24.3); 15 specimens tested by an FDA EUA version of the CDC N1 (median Ct of 23.3, IQR: 19.6–27.8) and N2 (22.7, 18.3–28.4) assays at the University of Washington in Seattle, Washington, and 20 specimens tested using the Roche cobas SARS-CoV-2 assay (ORF1a Ct median of 22.8, IQR: 18.4–30.2 and E Ct median of 22.9, IQR: 18.0–31.2) at the University of Nebraska Medical Center in Omaha, Nebraska (Table 1). The remaining fifty specimens were expected to test negative for SARS-CoV-2 because

Table 1
BioFire RP2.1 SARS-CoV-2 target clinical performance.

Comparator Assay	PPA	NPA[a]
Roche cobas	19/19	–
Hologic Fusion	14/15	–
Univ Wash LDT	15/15	–
Total	48/49 (98.0 %)	49/49 (100 %)

[a] NPA compared against presumptive SARS-CoV-2 negative specimens collected prior to December 2019.

they were collected prior to December 2019. Testing of one positive and one negative specimen yielded invalid results due to instrument errors; these could not be retested according to the instructions for use due to insufficient specimen volume. These specimens were excluded from the analyses, resulting in reduction of sample size to 49 valid positive and 49 valid negative specimens.

The 49 negative specimens tested negative for SARS-CoV-2 on the BioFire RP2.1 assay (100 % negative percent agreement, Table 1). The BioFire RP2.1 assay detected SARS-CoV-2 in 48/49 positive specimens (98 % positive percent agreement, Table 1). Testing was repeated on the one positive specimen (Fusion assay Ct of 21.1) in which SARS-CoV-2 was not detected and the repeat test yielded a positive result. Five SARS-CoV-2 positive specimens (10 %) also tested positive for a second analyte on the panel. Adenovirus was detected in one (2 %) while human rhino/enterovirus was present in four specimens (8 %).

3.2. Performance comparison at low viral load levels

The clinical comparison study included only a limited number of samples with high Ct values. In order to better evaluate the performance of the BioFire RP2.1 SARS-CoV-2 assay in samples with low levels of viral RNA we tested serial dilutions of a high-titer positive patient specimen using both the BioFire RP2.1 and Nebraska Medicine NECoV19 assay as well as the SARS-CoV-2 assays for the following platforms: Roche cobas, Cepheid GeneXpert, Hologic Panther (Aptima assay), BioFire (BioFire COVID-19 Test made by BioFire Defense) and Abbott ID NOW. Testing with the BioFire RP2.1 demonstrated that the pooling of a large number of SARS-CoV-2 RNA negative specimens for use as diluent generated a complex background matrix for SARS-CoV-2 testing. Coronavirus NL63, coronavirus 229E, coronavirus HKU1, coronavirus OC43, human metapneumovirus, human rhino/enterovirus, influenza A H1 − 2009, influenza B, parainfluenza virus 3, respiratory syncytial virus, and *Chlamydia* pneumonia were detected in most or all aliquots, while parainfluenza virus 4 (10-fold dilution series), adenovirus, parainfluenza virus 2, and *Mycoplasma pneumoniae* (intermediate dilution series between 10 − 7 and 10 − 8) were detected in two or fewer aliquots suggesting presence at only low levels.

Performance of the SARS-CoV-2 assay on the BioFire RP2.1 was comparable to that of NECoV19, cobas, GeneXpert, and BioFire Defense assays (Table 2, Supplemental Table 1) with detection of SARS-CoV-2 in all replicates down to the 10^{-7} dilution. Applying Ct values from the LDT to a standard curve generated from extracted SARS-CoV-2 quantitated RNA standard showed that this dilution contains approximately 10^3 copies/mL. Virus detection was inconsistent at lower concentrations. SARS-CoV-2 detection dropped off below the 10^{-6} dilution and 10^{-5} dilutions for the Hologic Aptima assay and Abbott ID NOW assay, respectively (Table 2, Supplemental Table 1).

4. Discussion

Our studies show that the BioFire RP2.1 has similar performance to high throughput assays used for the detection of COVID19. A clinical comparison study demonstrated 98 % positive percent agreement and 100 % negative percent agreement in residual NP swab specimens between the BioFire RP2.1 and three comparison assays. The BioFire RP2.1 assay also demonstrated comparable sensitivity to the NECoV19, Roche cobas, Cepheid GeneXpert, and BioFire Defense FilmArray tests for detection of low levels of viral RNA in a clinical specimen dilution series. This suggests that the BioFire RP2.1 can reliably be used to detect SARS-CoV-2 not only in acute presentation of infection, but also later in the course of disease when viral titers in the nasopharynx wane. Detection tapered off at lower concentrations rather than falling off abruptly; it appears that stochasticity plays a role in viral detection when low levels of virus are present, precluding our ability to determine the significance of slight variations in rates of virus detection in more dilute specimens without a large number of replicates. Unlike the

Table 2
Results of dilution series testing.

Dilution	NECoV19[a] (E[b])	Roche cobas[a] (E[b])	Cepheid Xpert Xpress[a] (N2[b])	Hologic Aptima[a]	BioFire RP 2.1[a]	BioFire Defense COVID-19 Test[a]	Abott ID NOW[a]
1×10^{-5}	6/6 (27.3 ± 0.5)	2/2 (29.9, 30.8)	NA	NA	NA	NA	3/3
1×10^{-6}	7/7 (30.7 ± 0.6)	3/3 (33.2, 33.3, 33.5)	3/3 (35.6, 35.8, 36.5)	6/6	3/3	3/3	1/3
1×10^{-7}	7/7 (34.0 ± 0.4)	3/3 (34.8, 35.3, 36.0)	3/3 (38.2, 39.5, 39.6)	2/6	3/3	3/3	0/3
5×10^{-8}	7/9 (36.3 ± 2.8)	3/3 (35.3, 36.7, 37.6)	3/3 (39.5, 40.6, 44.2)	2/6	1/3	2/3	NA
2.5×10^{-8}	6/9 (35.1 ± 2.6)	2/3 (36.3, 37.6)	3/3 (39.3, 40.8, 41.6)	0/6	1/3	2/3	NA
1.25×10^{-8}	3/8 (32.5, 32.9, 36.4)	2/3 (37.5, 39.6)	2/3 (40.5, 40.7)	0/6	1/3	0/3	NA
1×10^{-8}	3/7 (38.1, 37.1, 36.5)	0/3	1/3 (42.1)	1/12	0/3	1/3	0/3

[a] Number of replicates which tested positive divided by total number of replicates per test at a given dilution.
[b] Ct values for this target (individual values or mean ± standard deviation of positive replicates are shown in parentheses). For multi-target assays, the target which was most commonly detected in specimens with high Ct values is shown).

BioFire RP2.1, the Hologic Aptima and ID NOW assays did not reliably detect low levels of virus that correspond to the NECoV19 and Roche cobas Ct values in the upper (Aptima) and lower (ID NOW) 30 s. An analysis of E Ct values for specimens which tested positive in Nebraska Medicine laboratories from March 11 to May 17 showed that 30 % were above 30, and 10 % exceeded 35, suggesting that this lower analytical sensitivity likely affects clinical sensitivity (Supplemental Fig. 1). The impact of such reduced clinical sensitivity remains unclear. Specimens with low viral loads are often those collected from patients whose infections have improved or resolved clinically, but who require a negative test result for removal of isolation measures, release to another facility or a return to work. If these patients remain capable of transmitting virus, a false negative result may facilitate spread of the virus. However, if, as suggested by Wolfel et al. and Bullard et al, the presence of viral RNA in their specimens does not reflect shedding of live virus, or if it reflects shedding at levels unlikely to result in transmission, a false negative result would be less consequential [13,14].

The addition of a SARS-CoV-2 test to a commonly used multiplex PCR panel will expand the number of laboratories able to test for SARS-CoV-2 and will allow detection of coinfection as well as of alternative diagnoses. Once the northern hemisphere respiratory season arrives, the ability to test for influenza, RSV, and SARS-CoV-2 simultaneously on the BioFire RP2.1 will greatly benefit hospitals as an important infection control management tool.

Credit author statement

H.M.C., S.H.H, M.J.B., D.D., U.S, M.R., K.M.B. and P.D.F. designed the study. H.M.C., K.M.B, M.J.B. and P.D.F. wrote the manuscript. All authors edited the manuscript. B.C., A.S., J.L.C., A.M.C-V, S.M.S., K.D.T., M-L.H., K.R.J., A.L.G., and P.D.F. performed testing and provided samples for the study. This study was designed and funded by BioFire Diagnostics.

Declaration of Competing Interest

The Corresponding author has acted as a consultant and received grant funding from BioFire Diagnostics.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2020.104538.

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