Journal of Infection 85 (2022) e149–e151

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Letters to the Editor

BioFire FilmArray respiratory panel RP2.1 for SARS-CoV-2 detection: The pitfalls

Dear Editor,

We read with great interest the Livingstone et al. article describing the results of testing 4,640 patients for SARS-CoV-2 through the acute medical admissions pathway with BioFire® FilmArray Respiratory PCR Panel 2.1 plus (BioFire RP2.1 plus, BioFire Diagnostics, bioMérieux, Marcy l'Etoile, France).[1] The authors concluded that the use of BioFire RP2.1 for COVID-19 significantly reduced the time to obtain results spent on assessment cohort wards and the proportion of healthcare-ssociated-COVID-19 infection.[1] BioFire RP2.1 plus is a multiplex nested PCR allowing the simultaneous detection of four bacteria and 19 viruses, including SARS-CoV-2 and Middle East Respiratory Syndrome Coronavirus (MERS-CoV). BioFire RP2.1 (not including MERS-CoV) was launched for emergency use authorization (EUA) in Taiwan in May 2020 and introduced in the China Medical University Hospital (CMUH), a 2,100-bed university-affiliated hospital located in Taichung, Taiwan, to replace the BioFire RP panel in February 2021.

From May 2021 to 5th July 5, 2022, a total of 3,710 nasopharyngeal swab specimens from 3,710 patients with respiratory tract infection or suspected COVID-19 were submitted for respiratory pathogen detection using the BioFire RP2.1 panel in the CMUH. Among these specimens, 561 (15.1%) were positive for one of the target pathogens in the panel, and 56 (10.0%) were positive for SARS-COV-2 (Table 1). Among the 56 SARS-CoV-2 positive specimens, 11 (19,6%) were also positive for other pathogens. The concomitant pathogens identified, along with SARS-CoV-2, were adenovirus plus human rhinovirus/enterovirus (n = 3), human rhinovirus/enterovirus plus parainfluenza virus (n = 2), human rhinovirus/enterovirus alone (n = 3), adenovirus alone (n = 2), and coronavirus HKU1 alone (n = 2). Among the 56 specimens positive for SARS-CoV-2 by BioFire RP2.1, 47 (83.9%) were rechecked by either cobas® Liat® or cobas® 6800 systems (Roche Diagnostics Basel, Switzerland) due to the request of cycle threshold (Ct) values by the attending physicians (Table 1), and 20 (42.6%, 20/47) of them became negative by either system.

A multicenter evaluation of BioFire RP2.1 for the detection of SARS-CoV-2 in 524 nasopharyngeal swab samples was conducted by Berry et al. In this study, one or more targets on the panel were detected in 19.3% (n = 101) of specimens tested, with SARS-CoV-2 detected in 12.6% (n = 66) of specimens.[3] Human rhinovirus/enterovirus was also detected in 32.7% (n = 33) and adenovirus in 3.0% (n = 3) of positive specimens, with one dual positive for both SARS-CoV-2 and adenovirus being detected. They revealed that SARS-CoV-2 results obtained from the BioFire RP2.1 were highly concordant with the composite reference results by three SARS-CoV-2 EUA tests, exhibiting 98.4% (61/62) positive percent agreement (PPA) and 98.9% (457/462) negative percent agreement (NPA).[3] They concluded that the BioFire RP2.1 exhibited excellent performance in the detection of SARS-CoV-2.[2] In this study, the five false positive results by BioFire RP2.1 were further analyzed and the authors demonstrated that the concentration of SARS-CoV-2 in the specimens was near the limit of detection (LOD) for both the BioFire RP2.1 and the comparator assays. [2]

Creager et al. evaluated the performance of the BioFire® Respiratory Panel 2.1 (RP2.1) in the detection of SARS CoV-2 in comparison to three other SARS CoV-2 EUA assays.[3] In the studies, the RP2.1 panel had 98 % PPA (48/49) and 100 % NPA (49/49), suggesting that the BioFire® RP2.1 assay can be used to detect acute cases of SARS CoV2, even among patients with a low viral titer later in disease presentation.[3]

Eckbo et al. compared BioFire RP2.1 and the laboratory-developed test for 57 nasopharyngeal swab samples, including 30 clinical specimens (E gene Ct values <25 [n = 5], Ct 21-δ35 [n = 10], Ct >35-δ40 [n = 10], and negative [n = 5] and 27 tests for limit of detection.[4] They demonstrated 100% concordance between the tests, and acceptable performance of BioFire RP2.1 at their stated limits of detection. [4]

However, Tazi et al compared two PCR assays, BioFire RP2.1 plus and their laboratory's reference test, MAScIR SARS-CoV-2 M kit 2.0, a triplex real-time RT-PCR, using TaqMan technology, targeting SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) and S genes.[5] The results were compared, and each discrepant sample with sufficient volume underwent a third test using ARGENE® SARS-CoV-2 R-GENE kit, a triplex real-time RT-PCR, which also used TaqMan technology, targeting SARS-CoV-2 N (Nucleocapsid) and RdRp genes. Of the 80 specimens positive for BioFire RP2.1 Plus, 21 (26.3%) had discordant results on MAScIR, and only 11 could be tested on ARGENE, revealing negative results in five cases.[4] These results led to them consequently retaining the SARS-CoV-2 positive results of these discordant samples on BioFire RP2.1 plus, regardless of the detection of one or both targets.[5]

Although RT-PCR is the gold standard for the diagnosis of COVID-19, its diagnostic performance can vary widely owing to the lack of standardization of assays. The target genes (LOD, copies/mL) of BioFire RP2.1, cobas® Liat® and cobas® 6800 were spike (S) and transmembrane glycoproteins (M) (160), orf 1ab and nucleocapsid protein (12), and orf 1ab and envelope protein (46), respectively. Additionally, for BioFire RP2.1, SARS-CoV-2 is reported qualitatively as detected if either the S or M gene assays are positive and Ct values are provided. As a result, it is difficult to conclude the false-negative or -positive results created by different assays because different gene targets and LODs are present in different assays. However, there is a clinical dilemma due to the change in the positive report by BioFire RP2.1 to negative results by another quantitative RT-PCR assay. In this study, 42.6% of BioFire RP2.1 SARS-CoV-2 positive results became negative by using

https://doi.org/10.1016/j.jinf.2022.07.030

Table 1
Detection of pathogens from nasopharyngeal swab specimens using the BioFire® FilmArray Respiratory PCR Panel 2.1 (BioFire RP2.1) and/or cobas® Liat® or cobas® 6800 Systems and from 14th May 2021 to 5th July 5 2022.

No.	Age/sex	Date of test	BioFire RP2.1detected	Additional tests	
				cobas® Liat System results for SARS-CoV-2 (cycle threshold value)	cobas® 6800System results for SARS-CoV-2 (cycle threshold value, orf1ab/E genes)
1	27/F	2021/5/20	Coronavirus HKU1 SARS-CoV-2	ND	Positive (33.20/33.73)
2	72/M	2021/5/20	Coronavirus HKU1 SARS-CoV-2	Positive (28)	Positive (-/36.71)
3	75/M	2021/5/22	SARS-CoV-2	ND	Positive (18.89/18.62)
4	44/M	2021/5/23	SARS-CoV-2	Positive (13.4)	ND
5	37/F	2021/5/29	SARS-CoV-2	ND	Positive (30.97/32.22)
6	**44/F**	2021/6/1	**Human rhinovirus/enterovirus SARS-CoV-2**	**ND**	**Negative**
7	**32/F**	2022/4/20	**Human rhinovirus/enterovirus SARS-CoV-2**	**ND**	**Negative**
8	**43/M**	2022/4/25	**SARS-CoV-2**	**Negative**	**ND**
9	**1/M**	2022/5/5	**SARS-CoV-2**	**Negative**	**ND**
10	2/F	2022/5/7	SARS-CoV-2 Adenovirus Human rhinovirus/enterovirus	ND	ND
11	44/F	2022/5/11	SARS-CoV-2	Positive (29.2)	ND
12	2/M	2022/5/11	SARS-CoV-2 Human rhinovirus/enterovirus	ND	ND
13	1/M	2022/5/14	SARS-CoV-2	Positive (11.7)	ND
14	**3/F**	2022/5/16	**SARS-CoV-2 Human rhinovirus/enterovirus Parainfluenza virus 4**	**Negative**	**ND**
15	72/M	2022/5/21	SARS-CoV-2	Positive (11.4)	ND
16	**2/M**	2022/5/21	**SARS-CoV-2**	**Negative**	**ND**
17	**2/M**	2022/5/23	**SARS-CoV-2**	**Negative**	**ND**
18	**17/M**	2022/5/24	**SARS-CoV-2**	**Negative**	**ND**
19	**72/F**	2022/5/24	**SARS-CoV-2**	**Negative**	**ND**
20	81/F	2022/5/24	SARS-CoV-2	Positive (34.2)	ND
21	**17/M**	2022/5/24	**SARS-CoV-2**	**Negative**	**ND**
22	8/M	2022/5/25	SARS-CoV-2	ND	ND
23	1/M	2022/5/25	SARS-CoV-2	ND	ND
24	6/F	2022/5/25	Adenovirus SARS-CoV-2 H Human rhinovirus/enterovirus	Positive (14.0)	ND
25	1/F	2022/5/29	SARS-CoV-2 Parainfluenza virus 3	ND	ND
26	9/F	2022/5/30	SARS-CoV-2	ND	ND
27	**2/M**	2022/6/5	**Adenovirus SARS-CoV-2 Human rhinovirus/enterovirus**	**Negative**	**ND**
28	**64/M**	2022/6/7	**SARS-CoV-2**	**ND**	**Negative**
29	9/F	2022/6/9	Adenovirus SARS-CoV-2	ND	ND
30	56/M	2022/6/10	SARS-CoV-2	ND	ND
31	**1/F**	2022/6/10	**SARS-CoV-2**	**Negative**	**ND**
32	2/M	2022/6/12	SARS-CoV-2	ND	ND
33	59/M	2022/6/13	SARS-CoV-2	Positive (31.3)	ND
34	78/F	2022/6/20	SARS-CoV-2	Positive (16.4)	ND
35	3/F	2022/6/21	SARS-CoV-2	Positive (15)	ND
36	5/M	2022/6/22	SARS-CoV-2	Positive (32.6)	ND
37	1/M	2022/6/22	SARS-CoV-2	Positive (29.8)	ND
38	71/M	2022/6/23	SARS-CoV-2	Positive (23.6)	ND
39	70/F	2022/6/23	SARS-CoV-2	Positive (29.5)	ND
40	3/F	2022/6/23	SARS-CoV-2	Positive (33.0)	ND
41	4/F	2022/6/24	SARS-CoV-2	Positive (36.2)	ND
42	3/F	2022/6/24	SARS-CoV-2	Positive (31.2)	ND
43	15/F	2022/6/28	SARS-CoV-2	Positive (27.3)	ND
44	69/M	2022/6/29	SARS-CoV-2	Positive (14.7)	ND
45	**33/M**	2022/6/30	**SARS-CoV-2**	**Negative**	**ND**
46	5/M	2022/6/30	SARS-CoV-2	Positive (20.3)	ND
47	59/M	2022/7/1	SARS-CoV-2	Positive (12.3)	ND
48	4/F	2022/7/1	SARS-CoV-2	Positive (16.6)	ND
49	**32/M**	2022/7/2	**SARS-CoV-2**	**Negative**	**ND**
50	8/M	2022/7/2	SARS-CoV-2	Positive (32.6)	ND
51	**2/M**	2022/7/4	**SARS-CoV-2**	**Negative**	**ND**

(*continued on next page*)

Table 1 (*continued*)

No.	Age/sex	Date of test	BioFire RP2.1detected	Additional tests	
				cobas® Liat System results for SARS-CoV-2 (cycle threshold value)	cobas® 6800System results for SARS-CoV-2 (cycle threshold value, orf1ab/E genes)
52	**1/M**	2022/7/5	**SARS-CoV-2**	**Negative**	**ND**
53	**4/M**	2022/7/5	**SARS-CoV-2 Human rhinovirus/enterovirus Parainfluenza virus 4**	**Negative**	**ND**
54	**13/F**	2022/7/5	**SARS-CoV-2**	**Negative**	**ND**
55	**3/F**	**2022/7/7**	**SARS-CoV-2**	**Negative**	**ND**
56	98/M	2022/7/7	SARS-CoV-2	Positive (15.4)	ND

The results in boldface indicate the presence of negative results by either the cobas® Liat or cobas® 6800 system
ND, not done.

other PER systems. Further studies are needed to investigate this discrepancy.

In conclusion, we agree with Tazi et al. 's recommendation that SARS-CoV-2 positive results by BioFire RP2.1, regardless of the detection of one or both targets, should be retained, and other quantitative RT-PCR assays should be performed to confirm the results.

Funding

The authors have not declared a specific grant for this research from any funding agency in the public, commercial, or not-for-profit sector.

Patient and public involvement

Patients and/or the public were not involved in the design, conduct, reporting, or dissemination of this research.

Patient consent for publication

Not required.

Ethical approval information

Not required.

Data sharing statement

Not required.

Declaration of Competing Interest

The authors declare no conflict of interest.

References

1. Livingstone R, Lin H, Brendish NJ, Poole S, Tanner AR, Borca F, et al. Routine molecular point-of-care testing for SARS-CoV-2 reduces hospital-acquired COVID-19. *J Infect* 2022;**84**(4):558–65.
2. Berry GJ, Zhen W, Smith E, Manji R, Silbert S, Lima A, et al. Multicenter evaluation of the BioFire Respiratory Panel 2.1 (RP2.1) for detection of SARS-CoV-2 in nasopharyngeal swab samples. *J Clin Microbiol* 2022;**60**(5):e0006622.
3. Creager HM, Cabrera B, Schnaubelt A, Cox JL, Cushman-Vokoun AM, et al. Clinical evaluation of the BioFire® Respiratory Panel 2.1 and detection of SARS-CoV-2. *J Clin Virol* 2020;**129**:104538.
4. Eckbo EJ, Locher K, Caza M, Li L, Lavergne V, Charles M. Evaluation of the BioFire® COVID-19 test and Respiratory Panel 2.1 for rapid identification of SARS-CoV-2 in nasopharyngeal swab samples. *Diagn Microbiol Infect Dis* 2021;**99**(3):115260.
5. Tazi S, Kabbaj H, Zirar J, Zouaki A, El Amin G, El Himeur O, et al. Comparative performance evaluation of FilmArray BioFire RP2.1 and MAScIR 2.0 assays for SARS-CoV-2 detection. *Adv Virol* 2022;**2022**:4510900.

Yu-Chang Chang, Chiung-Tzu Hsiao, Wan-Li Chen, Yang-Di Su
Department of Laboratory Medicine, China Medical University Hospital, Taichung, Taiwan

Po-Ren Hsueh*
Departments of Laboratory Medicine and Internal Medicine, China Medical University Hospital, Taichung, Taiwan
School of Medicine, China Medical University, Taichung, Taiwan

*Corresponding author.
E-mail address: hsporen@gmail.com (P.-R. Hsueh)