Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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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

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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.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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