Submission ID: 911137

Rapid Development of a Multiplexed PCR Prototype Method that Offers a Syndromic Diagnostic Option by Integrating Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Detection with Twenty-One Other Common Respiratory Pathogens

Usha Spaulding[1], Jeremiah Antosch[1], Jessica Stone[1], Tanner Robinson[1], Kerrin Halo[1], Iryna Kavetska[1], Tyler Healy[1], Alex Taylor[1], Matthew Jones[1], Toma Todorov[1], Zhenmei Lu[1], Joann Cloud[1], Maggie Buccambuso[1], Brad Graham[1], Jeremy Boone[2], Hillary Wood[2], Margarita Rogatcheva[1]

[1]BioFire Diagnostics, LLC, Salt Lake City, Utah; [2]MRIGlobal, 425 Volker Blvd., Kansas City, MO 64110

Contact Information:
Usha K Spaulding
BioFire Diagnostics, LLC
515 Colorow Drive, SLC, UT 84108
Usha.spaulding@BioFireDX.com

BIOFIRE® BY BIOMÉRIEUX

FLM1-MKT-XXXX

Background: The US Food and Drug Administration (FDA) has granted Emergency Use Authorization (EUA) for multiple PCR-based tests to aid in the diagnosis and containment of COVID-19. A vast majority of these tests detect only SARS-CoV-2 which causes symptoms similar to those caused by other respiratory pathogens. Hence, other etiologies or co-infections requiring a different therapy may be missed. The BioFire® Respiratory Panel 2.1 (RP2.1) continues the syndromic approach of the FDA-cleared BioFire® Respiratory Panel 2 (RP2), to provide the ability to simultaneously detect 22 respiratory pathogens, including SARS-CoV-2, from nasopharyngeal swab (NPS) specimens. The goal of this study was to rapidly develop a RP2.1 prototype that contains high-performing SARS-CoV-2 assays and maintains the performance of assays retained from RP2.

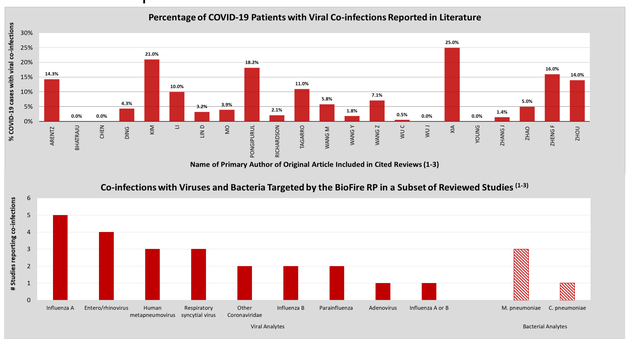
The BioFire Respiratory Panel 2.1 (RP2.1)

Viruses	
Adenovirus	Influenza A
Coronavirus HKU1	Influenza A H1
Coronavirus NL63	Influenza A H3
Coronavirus 229E	Influenza A H1-2009
Coronavirus OC43	Influenza B
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	Parainfluenza Virus 1
Human Metapneumovirus	Parainfluenza Virus 2
Human Rhinovirus/Enterovirus	Parainfluenza Virus 3
Respiratory Syncytial Virus	Parainfluenza Virus 4
Bacteria	
Chlamydia pneumoniae	*Bordetella pertussis* (*ptxP*)
Mycoplasma pneumoniae	*Bordetella parapertussis* (IS*1001*)

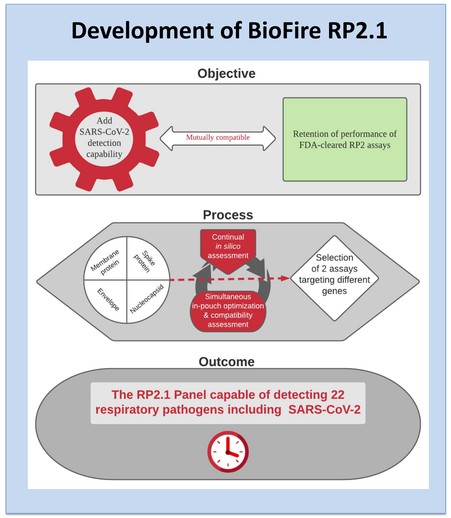
Methods: Twelve assays targeting regions of four SARS-CoV-2 genes were tested for compatibility with the RP2 assays and chemistry conditions. All retained RP2 assays were evaluated to verify retention of the established performance of the IVD/commercial RP2 test. The sensitivity of the novel SARS-CoV-2 assays was estimated with nucleic acids and inactivated virus at BioFire Diagnostics, LLC, and with live virus in contrived pooled NPS samples at MRIGlobal. Periodic *in silico* inclusivity evaluation by assessment of primer homology of SARS-CoV-2 assays to SARS-CoV-2 genomes from accessible databases is being performed. The specificity of the SARS-CoV-2 assays was evaluated against high titers of 47 non-target analytes including 6 other Coronaviruses and 25 off-Panel organisms, both pathogens and commensals, that may be occur in patient samples.

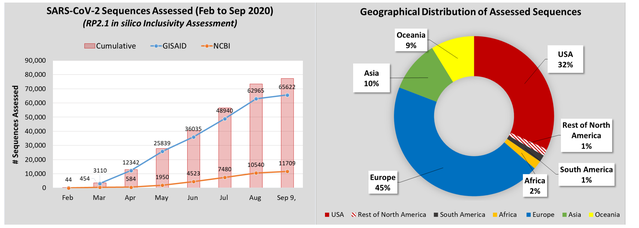
Strong case for a comprehensive Panel to rule in/rule out COVID-19

- COVID-19 symptoms can be indistinguishable from symptoms caused by other respiratory pathogens[(1-3)]
- In three systematic reviews[(1-3)], multiple cited studies reported viral and bacterial co-infections/secondary infections in confirmed COVID-19 cases
- Co-infections in severe COVID-19 cases were associated with adverse outcomes for patients[2]



- Several viruses and bacteria on the RP2 were detected from COVID-19 patient samples in 6 of the studies reviewed that performed tests to identify co-infection/secondary infection pathogens





Predicted *in silico* Inclusivity				
		SARSCoV2-1		# (%) sequences predicted to be detected (one or both assays positive)
# sequences		+	-	
SARSCoV2-2	+	76,973	297	77,331/77,331
	-	61	0	(100%)

- ~2000-fold increase in sequence data since Feb 2020 (SARS-CoV-2 assays were designed with ~ 40 NCBI sequences)
  - Assays remain robust with 100% inclusivity predicted *in silico*
  - Global representation of sequence diversity from NCBI and GISAID[4]
- The RP2.1 prototype has a limit of detection of 10^2 -10^3 genome copies/mL
  - Live virus testing in pooled NPS matrix performed in BL3 (MRIGlobal)
- Other assays performed comparably in RP2 and RP2.1 Panels.
  - Predict retention of the IVD/Commercial RP2 sensitivity in RP2.1

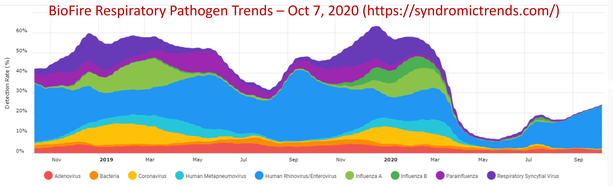
Limit of detection of SARS-CoV-2 (USA-WA1/2020)			
Test Material	**Matrix**	**Concentration**	**Units**
Live virus	Pooled NPS in VTM	1.00E+02	GC/mL[a]
Inactivated virus	VTM	1.50E+02	$TCID_{50}$/mL[b]
Extracted viral RNA	BioFire RNA diluent	1.00E+03	GC/mL

[a] *Genome copies/mL* [b] *Tissue culture infective dose 50/mL*

Organism	Concentration	Unit	RP2.1 Detected/Tested	RP2 Detected/Tested
Adenovirus	0.427	TCID50/mL	5/5	5/5
	12.53	TCID50/mL	5/5	5/5
	0.0158	TCID50/mL	5/5	5/5
Coronavirus 229E	0.275	TCID50/mL	5/5	5/5
Coronavirus HKU1	1X	Clinical Sample	5/5	5/5
Coronavirus NL63	0.016	TCID50/mL	5/5	5/5
Coronavirus OC43	1.917	TCID50/mL	5/5	5/5
Influenza A H1	0.232	TCID50/mL	5/5	5/5
Influenza A H1-2009	0.2317	TCID50/mL	5/5	5/5
Influenza A H3	0.833	TCID50/mL	5/5	5/5
Influenza B	0.188	TCID50/mL	5/5	5/5
Human Metapneumovirus	8.933	TCID50/mL	5/5	4/5
Human Rhinovirus/Enterovirus	0.167	TCID50/mL	5/5	5/5
Parainfluenza Virus 1	0.192	TCID50/mL	5/5	5/5
Parainfluenza Virus 2	1.917	TCID50/mL	5/5	5/5
Parainfluenza Virus 3	19.17	TCID50/mL	5/5	5/5
Parainfluenza Virus 4	0.958	TCID50/mL	5/5	5/5
Respiratory Syncytial Virus	0.417	TCID50/mL	4/5	5/5
Bordetella pertussis	3000	CFU/mL[a]	5/5	5/5
Bordetella parapertussis	25	CFU/mL	5/5	5/5
Chlamydia pneumoniae	1.667	TCID50/mL	5/5	5/5
Mycoplasma pneumoniae	1.867	CCU/mL[b]	5/5	5/5

[a] *Colony forming units/mL* [b] *Color changing units/mL*

- Specificity of RP2.1 assays, including the two SARS-CoV-2 assays, was assessed with high titers of 22 on-Panel analytes and 25 off-Panel analytes that encompassed a variety of near-neighbor as well as commensal and pathogenic organisms likely to be present in the patient sample
  - Near-neighbors, MERS-CoV and SARS-CoV, were tested using live viruses at titers >10^9 GC/mL at MRIGlobal
  - No unexpected results were encountered for any RP2.1 assays
- No non-specific amplifications in pre-screened negative matrices, with high loads of human DNA, or with negative transport media



Conclusion : With the onset of the respiratory ailments season, and relaxation of shelter-in-place directives, an elevation in the detection rates of respiratory viruses by the RP2 has been observed in the BioFire Syndromic Trends (https://syndromictrends.com/). The results of this study indicate a strong potential for the RP2.1 to serve as a sensitive comprehensive syndromic option to aid in the diagnosis of COVID-19 as well as respiratory diseases caused by other pathogens, including co-infections. This feature is expected to aid in effective triage and timely treatment of patients presenting with symptoms consistent with SARS-CoV-2 infection.

The BioFire RP2.1 was granted an Emergency Use Authorization (EUA) from US FDA on May 1, 2020

Citations:

1. Chen X, Liao B, Cheng L, et al. The microbial coinfection in COVID-19. *Appl Microbiol Biotechnol*. Published online August 11, 2020:1-9. doi:10.1007/s00253-020-10814-6
2. Lansbury L, Lim B, Baskaran V, Lim WS. Co-infections in people with COVID-19: a systematic review and meta-analysis. *J Infect*. 2020;81(2):266-275. doi:10.1016/j.jinf.2020.05.046
3. Lai C-C, Wang C-Y, Hsueh P-R. Co-infections among patients with COVID-19: The need for combination therapy with non-anti-SARS-CoV-2 agents? | Elsevier Enhanced Reader. doi:10.1016/j.jmii.2020.05.013
4. https://www.gisaid.org/