EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR BIOFIRE RESPIRATORY PANEL 2.1 DECISION SUMMARY

A. De Novo Number:

DEN200031

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the BioFire Respiratory Panel 2.1 (RP2.1).

C. Measurands:

The assay detects and identifies nucleic acids of the following respiratory pathogens: Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2), Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, including subtypes H1, H1-2009, and H3, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus, *Bordetella parapertussis* (*IS*1001), *Bordetella pertussis* (*ptxP*), *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*.

D. Type of Test:

A multiplexed nucleic acid test intended for use with the BioFire FilmArray 2.0 or FilmArray Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections, including COVID-19.

E. Applicant:

BioFire Diagnostics, LLC

F. Proprietary and Established Names:

BioFire Respiratory Panel 2.1 (RP2.1)

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3981

2. Classification:

   Class II (special controls)

3. Product code(s):

   QOF

4. Panel:

   Microbiology (83)

H. Indications for Use:

1. Indication(s) for use:

   The BioFire Respiratory Panel 2.1 (RP2.1) is a PCR-based multiplexed nucleic acid test intended for use with the BioFire FilmArray 2.0 or BioFire FilmArray Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections, including COVID-19.

   The following organism types and subtypes are identified using the BioFire RP2.1:

   - Adenovirus,
   - Coronavirus 229E,
   - Coronavirus HKU1,
   - Coronavirus NL63,
   - Coronavirus OC43,
   - Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2),
   - Human Metapneumovirus,
   - Human Rhinovirus/Enterovirus,
   - Influenza A, including subtypes H1, H1-2009, and H3,
   - Influenza B,
   - Parainfluenza Virus 1,
   - Parainfluenza Virus 2,
   - Parainfluenza Virus 3,
   - Parainfluenza Virus 4,
   - Respiratory Syncytial Virus,
   - *Bordetella parapertussis* (IS1001),
   - *Bordetella pertussis* (ptxP),
   - *Chlamydia pneumoniae*, and
   - *Mycoplasma pneumoniae*

   Nucleic acids from the respiratory viral and bacterial organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection is indicative of the presence of the identified microorganism and aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test

should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by an NPS specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the BioFire RP2.1 may not be the definite cause of disease. Additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.

2. Special conditions for use statement(s):

   For prescription use only.

   For *in vitro* diagnostic use only.

3. Special instrument requirements:

   FilmArray Respiratory Panel 2.1 (RP2.1) is performed on the FilmArray 2.0 or the FilmArray Torch systems.

I. Device Description:

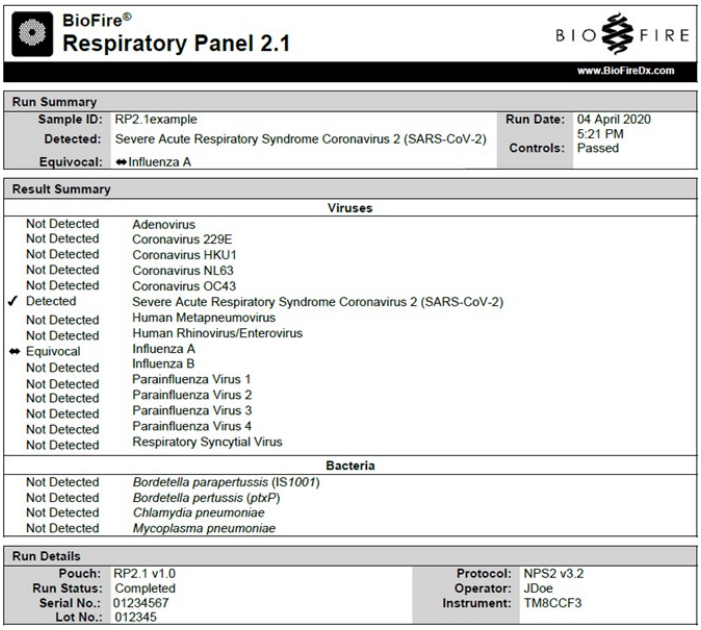
The BioFire Respiratory Panel 2.1 is designed to simultaneously identify 22 different potential pathogens of the respiratory tract infection, including the novel coronavirus Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), from a single NPS specimen in transport medium or saline. BioFire RP2.1 is compatible with BioFire's PCR-based in vitro diagnostic BioFire FilmArray 2.0 and BioFire FilmArray Torch systems for infectious disease testing. A specific software module (i.e., BioFire RP2.1 Pouch Module Software) is used to perform BioFire RP2.1 testing on these systems.

The RP2.1 reagent kit contains all the materials required to complete tests and includes the RP2.1 pouch, hydration solution, sample buffer, and sample handling components such as transfer pipettes. The RP2.1 pouches are used to test patient samples and is a closed-system disposable that stores all the necessary reagents for sample preparation reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from multiple pathogens within a single NPS specimen. The rigid plastic component ("fitment") of the pouch contains reagents in freeze-dried form. The flexible plastic portion of the pouch is divided into discrete segments ("blisters") where the required chemical processes are carried out. After sample collection, the user injections hydration solution and sample combined with BioFire Sample Buffer into the pouch, places the pouch into a FilmArray instrument, and starts the run. All other operations are automated.

The FilmArray instruments (FilmArray 2.0 and FilmArray Torch systems) interact with the pouch mechanically, thermally, and optically to drive the multi-step chemical process

required for purification and detection of specific nucleic acid targets from the patient sample. FilmArray instruments follow a protocol defined in the BioFire RP2.1 Pouch Module Software that is downloaded from the host computer prior to runtime. The instrument protocol defines the specific sequence of the testing process, including the times and temperatures, as the instrument performs bead-based extraction/isolation/purification of nucleic acids, performs reverse transcription and a 2-stage nested PCR reaction, executes DNA melt and fluorescent signal detection, and monitors system performance in real time, and communicates results and errors to the user via software. The primary difference between the FilmArray 2.0 and FilmArray Torch systems is the external configuration of multiple modules in a system. Up to eight FilmArray 2.0 modules can be connected to one computer and pouch loading station, while up to 12 FilmArray Torch modules can be connected to one system base in a vertical stack to a computer and pouch loading station. In addition, the pouches are front-loaded via an automated mechanism for the Torch system whereas the pouches are manually inserted, removed, and there is pouch and lid sensing in the FilmArray 2.0.

Once a test run is completed, the software automatically interprets the results and displays a test report. The report can be printed and/or saved as a file. The test report is a single page containing three sections: Run Summary, Result Summary, and Run Details. An additional section, Change Summary, is present in specific situations. The overall layout of the report was previously described in the BioFire RP2 510(k) [K170604] and remains unchanged for the BioFire RP2.1—



BioFire®
Respiratory Panel 2.1

BIOFIRE
www.BioFireDx.com

Run Summary

Sample ID:	RP2.1example	Run Date:	04 April 2020 5:21 PM
Detected:	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	Controls:	Passed
Equivocal:	⬌Influenza A		

Result Summary

	Viruses
Not Detected	Adenovirus
Not Detected	Coronavirus 229E
Not Detected	Coronavirus HKU1
Not Detected	Coronavirus NL63
Not Detected	Coronavirus OC43
✓ Detected	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)
Not Detected	Human Metapneumovirus
Not Detected	Human Rhinovirus/Enterovirus
⬌ Equivocal	Influenza A
Not Detected	Influenza B
Not Detected	Parainfluenza Virus 1
Not Detected	Parainfluenza Virus 2
Not Detected	Parainfluenza Virus 3
Not Detected	Parainfluenza Virus 4
Not Detected	Respiratory Syncytial Virus
	Bacteria
Not Detected	*Bordetella parapertussis* (IS*1001*)
Not Detected	*Bordetella pertussis* (*ptxP*)
Not Detected	*Chlamydia pneumoniae*
Not Detected	*Mycoplasma pneumoniae*

Run Details

Pouch:	RP2.1 v1.0	Protocol:	NPS2 v3.2
Run Status:	Completed	Operator:	JDoe
Serial No.:	01234567	Instrument:	TM8CCF3
Lot No.:	012345		

Test results for the organisms included in the BioFire RP2.1 are provided in two locations on the report. The Result Summary section provides a complete list of the test results. Possible results include “Detected,” “Not Detected,” “Equivocal,” and “Invalid.” Positive (Detected) and Equivocal results are also displayed in the Run Summary section. The following table

provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

Table 1. Explanation of Reported Results and Required Actions

Result	Explanation	Action
Detected[a]	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism were POSITIVE (i.e., met the requirements for a positive result)	Report results.
Not Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism were NEGATIVE (i.e., did not meet the requirements for a positive result)	Report results.
Equivocal	The run was successfully completed AND The pouch controls were successful (Passed) AND The combination of positive and negative assay results for Influenza A were inconclusive	Retest the original sample ONCE and report the result of the retest.
Invalid	The pouch controls were not successful (Failed) OR The run was not successful (Run Status displayed as: Aborted, Incomplete, Instrument Error, or Software Error)	See *Interpretation of control fields on the BioFire RP2.1 test report* for instruction.

[a] If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.

For most organisms detected by the BioFire RP2.1, the organism is reported as Detected if a single corresponding assay is positive. For example, Human Metapneumovirus will have a test report result of "Human Metapneumovirus Detected" if at least two of the three replicates of the one Human Metapneumovirus assay (hMPV) have similar positive melt peaks with Tm values that are within the assay-specific Tm range.

In contrast, the test results for SARS-CoV-2, Adenovirus, and Influenza A depend on the interpretation of results from more than one assay. Interpretation results for all organisms detected by the BioFire RP2.1, except for SARS-CoV-2, are previously described in the BioFire RP2 510(k) submission [K170604] and remain unchanged for the BioFire RP2.1.

The BioFire RP2.1 pouch contains two different assays for the detection of the SARS-CoV-2 microorganism. The assays each target a spike protein (S) gene and membrane protein (M) gene respectively. The BioFire FilmArray software interprets each of these assays independently and the results are combined as a final test result for the virus. An assay is called positive if at least two of the three replicates within the pouch have similar positive melt peaks with Tm values that are within the assay-specific Tm range. If either one or both of the assays is called

positive, the test report will show Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2) as Detected. If all assays are called negative, the test report will be Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2) Not Detected.

J. Standard/Guidance Document Referenced (if applicable):

General

- Guidance for Clinical Laboratories, Commercial Manufactures, and FDA Staff – Policy for Coronavirus Disease-
- 2019 Tests During the Public Health Emergency (2020)
- GHTF, Clinical Evidence for IVD Medical Devices - Clinical Performance Studies for In Vitro Diagnostic Medical
- Devices (November 2012)
- WMA Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects
- 2017/746 Regulation EU 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU
- 2016/679 GDPR, Regulation EU 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation)
- Guidance for Industry – Part 11, Electronic Records; Electronic Signatures – Scope and Application (August 2003)
- Guidance for Industry – Computerized Systems Used in Clinical Investigations (May 2007)
- Guidance for Industry – Oversight of Clinical Investigations – A Risk-Based Approach to Monitoring (August 2013)
- Guidance for Industry – Electronic Source Data in Clinical Investigations (September 2013)
- Guidance for IRBs, Clinical Investigators, and Sponsors – Informed Consent Information Sheet (July 2014)
- FDA Draft Guidance – Use of Electronic Records and Electronic Signatures in Clinical Investigations Under 21 CFR Part 11 – Questions and Answers (June 2017)
- Guidance for Industry and FDA Staff – Acceptance of Clinical Data to Support Medical Device Applications and Submissions – Frequently Asked Questions (February 2018)
- Guidance for Sponsors, Investigators, and IRBs – Impact of Certain Provisions of the Revised Common Rule on FDA-Regulated Clinical Investigations (October 2018)
- ICH E6(R1) Guideline for Good Clinical Practice E6(R1) – June 1996
- ICH E6(R2) Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2) – November 2016
- Guidance for Industry and Food and Drug Administration Staff – Highly Multiplexed Microbiological/Medical Countermeasure In Vitro Nucleic Acid Based Diagnostic Devices, (August 27, 2014)
- Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests, FDA Guidance Document (March 13, 2007)
- User Protocol for Evaluation of Qualitative Test Performance, Clinical and Laboratory Standards Institute (CLSI) Approved Guideline – Second Edition, EP12-A2 (January 2008)
- Molecular Diagnostic Methods for Infectious Diseases, Clinical and Laboratory Standards Institute (CLSI) Proposed Guideline, MM3-P2 (February 2006)

- Interference Testing in Clinical Chemistry, 3rd Edition, Clinical and Laboratory Standards Institute (CLSI) Approved Guideline, EP07 (April 2018).
- CLSI EP25-A, 'Evaluation of stability of in vitro diagnostic reagents; Approved Guidelines'.
- Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff – Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable, (April 2006)

Software

- Guidance for Industry and FDA Staff, Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices (May 11, 2005)
- Off-The-Shelf Software Use in Medical Devices, Guidance for Industry and Food and Drug Administration Staff (September 27, 2019)
- General Principle of Software Validation; Final Guidance for Industry and FDA Staff (January 11, 2002)
- Content of Premarket Submissions for Management of Cybersecurity in Medical Devices, Guidance for Industry and Food and Drug Administration Staff (October 2, 2014)

Labeling

- Use of Symbols on Labels and in Labeling of In Vitro Diagnostic Devices Intended for Professional Use, FDA Guidance Document (November 30, 2004)
- Guidance for Industry and FDA on Alternative to Certain Prescription Device Labeling Requirements (January 1, 2000)

FDA-recognized Standards

- ISO 14971:2007 'Medical devices – Application of risk management to medical devices'
- EN 62366:2008/IEC 62366-1:2015, 'Medical device – Application of usability engineering to medical devices'
- ISO 62304:2006, 'Medical device software – Software life-cycle processes' – IEC 62304:2006, November 27, 2008
- ISO 15223-1:2012, 'Medical Devices – Symbols to be used with medical device labels, labeling and information to be supplied – Part 1: General requirements'

Non-recognized Standards

- ISO 13485:2016/EN ISO 13485:2016, 'Medical devices – Quality Management System – Requirements for regulatory purposes'
- ISO 20916:2019, 'In vitro diagnostic medical devices – Clinical performance studies using specimens from human subjects – Good study practice'
- EN 13612:2002, Performance evaluation of in vitro diagnostic medical devices (European Commission)
- EN ISO 18113-1:2011, '*In vitro* diagnostic medical devices – Information supplied by the manufacturer (labeling) – Part 1: Terms, definition and general requirements'
- EN ISO 18113-2:2011, '*In vitro* diagnostic medical devices – Information supplied by the manufacturer (labeling) – Part 2: In vitro diagnostic reagents for professional use'
- EN ISO 23640:2015, 'In vitro diagnostic medical devices – Evaluation of stability of in vitro diagnostic reagents'

K. Test Principle:

The BioFire RP2.1 test takes approximately 2 minutes of hands-on-time from the point of collection to the initiation of the automated test. Once the test is initiated, a test result is produced in approximately 45 minutes.

During a test, the FilmArray instrument, software, and pouch work together to generate assay results. The test works through automated sample processing and nested multiplex nucleic acid amplification (including reverse transcription as appropriate) followed by high-resolution melt analysis to confirm the identity of the amplified product. The basic sequence of actions and their associated instrument functions are outlined in Figure 1—

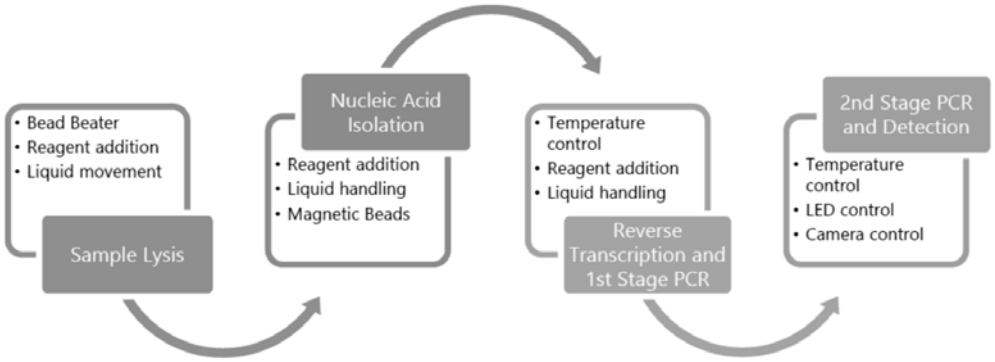


Figure 1. Basic steps performed during BioFire RP2.1 testing

The pouch contains all the necessary PCR reagents and is where samples are automatically processed to generate test results. The instrument communicates with the host computer and the FilmArray software. The software provides instructions to the instrument to control the various test steps. The instrument drives the testing process by applying mechanical force on the pouch exterior to actuate liquid movement to various compartments and to seal or block off flow in particular channels. The instrument also thermally interacts with the pouch to perform the subsequent 2-stage nested PCR reactions.

Optical systems on-board the instrument that include a LED and digital camera allow illumination and recording of fluorescence generated in the second stage PCR. The fluorescence signal generated during DNA melting is automatically analyzed by the FilmArray software from replicate wells of each assay for the detection of amplicons with a specific Tm. The detection denotes the presence of specific bacterial or viral targets.

The BioFire RP2.1 pouch contains the same sample preparation and PCR reaction chemistry as the previously cleared BioFire FilmArray Respiratory Panel 2 (RP2) (K170604; cleared for use on both FilmArray 2.0 and FilmArray Torch Systems). The PCR1 primer multiplex is also the same, with the addition of SARS-CoV-2 primers. The PCR2 array is similar except with the additions and minor reconfiguration of wells to accommodate the two SARS-CoV-2 assays. In addition, the instrument protocol and the analysis parameters in the panel-specific pouch module are the same as for FilmArray RP2, with the additional analysis of the SARS-CoV-2 assays.

The BioFire RP2.1 procedure occurs in six steps below. This simple procedure minimizes specimen manipulation and reduces operator error.

- **Step 1** - Place pouch into the FilmArray Pouch Loading Station.
- **Step 2** - Hydrate pouch using a blue Hydration Injection Vial.
- **Step 3** - Prepare sample in the red Sample Injection Vial:
    - Dispense the Sample Buffer tube into the Sample Injection Vial.
    - With a transfer pipette, draw the NPS in transport media or saline sample to the third line, then add it to the Sample Injection Vial.
    - Mix by inversion.
- **Step 4** - Load sample mix in pouch.
- **Step 5** - Insert pouch into the instrument.
- **Step 6** - Enter sample information and start the run. The BioFire RP2.1 protocol will be automatically selected upon scanning the pouch barcode.

The FilmArray software uses the following steps to interpret the melt curve data generated from each FilmArray RP2.1 assay—

- Analysis of Melt Curves
    - The FilmArray RP2.1 Melt Detector first performs basic calculations on the melt data to determine if a PCR reaction occurred in each well. If the melt profile indicates that a PCR product is present, then the analysis software calculates one or two Tm values, depending on the number of melt curves present in the data, and the Tm values are compared against an expected melt range for the associate assay. If the software determines that the melt is positive and the melt curve falls inside the assay's specific melt range, thent he curve is called positive. If the software determines that the melt is negative or that it is not in the appropriate range, then the curve is called negative.
- Analysis of Replicates
    - The analysis software evaluates the replicates for each assay (target and control) to determine if the assay is positive or negative. For a positive, at least two of the three wells associated with an assay must have a positive melt curve and the Tm for the positive curves must be similar (i.e., within 1°C). Assays that do not meet these criteria are called negative.
- Analysis of Controls
    - Results for control assays are compared to their expected values and are reported as "Passed", "Failed" or "Invalid". Passed control result is for successful run completion AND both pouch controls were successful. Failed result is when the run was successfully completed BUT at least one of the pouch controls (RNA Process Control and/or PCR2 Control) failed. If the instrument detects an out-of-specification condition or a significant error, it will automatically abort the run. If this happens or if user aborts the run, the control result will display "Invalid" and all results in the Result Summary of the report will also be displayed as "Invalid." A Run Status indicating "Incomplete", "Aborted", "Software Error", or "Instrument Error" will be reported to the user and the operator is asked to consult with the manual for

specific instructions on resolving the error. The test should be repeated once error is corrected.

- Interpretation of Assay Results
    - Once the results for the individual assays are determined, the software applies interpretation rules to determine the final test results. For most organisms detected by the BioFire RP2.1, the organism is reported as Detected if a single corresponding assay is positive. The BioFire RP2.1 also includes test results for organisms (i.e., SARS-CoV-2, Adenovirus, and Influenza A) that depend on interpretation of results from more than one assay. See the Interpretation of Results section for more information on interpreting these test results.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A multi-variable study was performed to evaluate the reproducibility of BioFire RP2.1 analyte detection on FilmArray 2.0 and FilmArray Torch systems. This study was additive to the reproducibility evaluation performed for the BioFire RP2 device, with overlapping data for certain analytes to bridge results from the two panels and collect data for select analytes including the newly added SARS-CoV-2.

Contrived samples were used in this study to evaluate variability in between run, system, site, day, or lot. Three samples were prepared in a matrix of viral transport medium (Table 2) and data were collected representing a negative (no analyte) and those containing analytes at low positive (1x LoD) or moderate positive (3x LoD) concentrations. The positive samples included inactivated SARS-CoV-2, Coronavirus NL63, Influenza A H1-2009, and three analytes that had been previously evaluated for the BioFire RP2 reproducibility study (i.e., Adenovirus, *Bordetella parapertussis* (IS*1001*) and Respiratory Syncytial Virus).

Each sample was tested repeatedly in three (3) different testing sites over five days by different operators (at least two per site), on different systems (60 per system) and modules, using three different reagent kit lots. Twenty replicates per sample were tested at each site on both FilmArray systems for a total of 120 valid runs per sample and 360 valid runs in total for the entire study. Reproducibility of analyte detection was assessed as percent agreement with the expected Detected and Not Detected results for the positive and negative samples.

The performance of the FilmArray systems and BioFire RP2.1 Controls are summarized as follow. Valid results were obtained in 361 of the 363 runs that were initiated (361/363, 99.4%). There were 181 and 182 runs initiated on the FilmArray 2.0 and FilmArray Torch systems, respectively. There was one instrument error (FilmArray 2.0) and one aborted run (FilmArray Torch). This showed that performance of the controls was reproducible (no control failures) and valid results were obtained for all completed runs.

Reproducibility data for each BioFire RP2.1 analyte are summarized in Table 2. Results are organized by system type (i.e., FilmArray 2.0 or Torch), test site (Site A, B, C), and all sites/systems with the corresponding 95% confidence interval. The summary data are presented as a combination of results collected for reproducibility studies with the BioFire RP2.1 (gray highlight) and the previous RP2 devices.

Table 2. Reproducibility of Detection Results for BioFire RP2.1 Analytes

Highlighted data were collected with the BioFire RP2.1. Non-highlighted data was collected with the BioFire FilmArray RP2. The same number of replicates (120) were tested per sample on both panels, but testing was distributed differently between sites and systems.

Analyte (Isolate Source ID)		Concentration Tested	Agreement with Expected Result							
			FilmArray 2.0				FilmArray Torch			
			Site A	Site B	Site C	System Total	Site A	Site B	Site C	System Total
Adenovirus[a] (NIBSC 16/324) WHO International Standard		Negative (no analyte)	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**
		Moderate Positive (3× LoD) 9.0E+03 IU/mL	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**
		Low Positive (1× LoD) 3.0E+03 IU/mL	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**
Coronavirus 229E		Negative (no analyte)	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**
Coronavirus HKU1		Negative (no analyte)	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**
Coronavirus NL63 (BEI NR-470)		Negative (no analyte)	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**
		Moderate Positive (3× LoD) 7.5E-01 $TCID_{50}$/mL (1.6E+02 copies/mL)	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**
		Low Positive (1× LoD) 2.5E-01 $TCID_{50}$/mL (5.4E+01 copies/mL)	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**
Coronavirus OC43	Coronavirus OC43	Negative (no analyte)	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**
	Coronavirus OC43 (ATCC VR-759)	Moderate Positive (3× LoD) 9.0E+01 $TCID_{50}$/mL	-	29/30 (96.7%)	30/30 (100%)	**59/60 (98.3%)**	29/30 (96.7%)	-	29/30 (96.7%)	**58/60 (96.7%)**
		Low Positive	-	30/30 (100%)	27/30 (90.0%)	**57/60 (95.0%)**	30/30 (100%)	-	30/30 (100%)	**60/60 (100%)**

Analyte (Isolate Source ID)		Concentration Tested	Agreement with Expected Result							
			FilmArray 2.0				FilmArray Torch			
			Site A	Site B	Site C	System Total	Site A	Site B	Site C	System Total
		(1× LoD) 3.0E+01 $TCID_{50}$/mL								
Human Metapneumovirus	Human Metapneumovirus	Negative (no analyte)	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**
	Human Metapneumovirus (Zeptometrix 0810161CF)	Moderate Positive (3× LoD) 3.0E+01 $TCID_{50}$/mL	-	30/30 (100%)	30/30 (100%)	**60/60 (100%)**	30/30 (100%)	-	30/30 (100%)	**60/60 (100%)**
		Low Positive (1× LoD) 1.0E+01 $TCID_{50}$/mL	-	28/30 (93.3%)	30/30 (100%)	**58/60 (96.7%)**	30/30 (100%)	-	30/30 (100%)	**60/60 (100%)**
Human Rhinovirus/Enterovirus	Human Rhinovirus/ Enterovirus	Negative (no analyte)	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**
	Human Rhinovirus (Zeptometrix 0810012CFN)	Moderate Positive (3× LoD) 3.0E-01 $TCID_{50}$/mL (1.1E+02 copies/mL)	-	28/30 (93.3%)	30/30 (100%)	**58/60 (96.7%)**	30/30 (100%)	-	30/30 (100%)	**60/60 (100%)**
		Low Positive (1× LoD) 1.0E-01 $TCID_{50}$/mL (3.8E+01 copies/mL)	-	30/30 (100%)	30/30 (100%)	**60/60 (100%)**	30/30 (100%)	-	30/30 (100%)	**60/60 (100%)**
Influenza A H1		Negative (no analyte)	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**
Influenza A H1-2009 (Zeptometrix 0810109CFN)		Negative (no analyte)	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**
		Moderate Positive (3× LoD) 1.5E+00 $TCID_{50}$/mL (9.9E+02 copies/mL)	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**
		Low Positive (1× LoD) 5.0E-01 $TCID_{50}$/mL (3.3E+02 copies/mL)	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**
Infl	Influenza A H3	Negative (no analyte)	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**

Analyte (Isolate Source ID)		Concentration Tested	Agreement with Expected Result							
			FilmArray 2.0				FilmArray Torch			
			Site A	Site B	Site C	System Total	Site A	Site B	Site C	System Total
	Influenza A H3 (ATCC VR-810)	Moderate Positive (3× LoD) 3.0E-01 $TCID_{50}$/mL	-	29/30 (96.7%)	30/30 (100%)	**59/60 (98.3%)**	30/30 (100%)	-	30/30 (100%)	**60/60 (100%)**
		Low Positive (1× LoD) 1.0E-01 $TCID_{50}$/mL	-	30/30 (100%)	30/30 (100%)	**60/60 (100%)**	30/30 (100%)	-	30/30 (100%)	**60/60 (100%)**
Influenza B	Influenza B	Negative (no analyte)	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**
	Influenza B (Zeptometrix 0810037CF)	Moderate Positive (3× LoD) 1.5E+01 $TCID_{50}$/mL	-	30/30 (100%)	30/30 (100%)	**60/60 (100%)**	30/30 (100%)	-	30/30 (100%)	**60/60 (100%)**
		Low Positive (1× LoD) 5.0E+00 $TCID_{50}$/mL	-	30/30 (100%)	30/30 (100%)	**60/60 (100%)**	30/30 (100%)	-	30/30 (100%)	**60/60 (100%)**
Parainfluenza Virus 1		Negative (no analyte)	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**
Parainfluenza Virus 2	Parainfluenza Virus 2	Negative (no analyte)	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**
	Parainfluenza Virus 2 (Zeptometrix 0810015CF)	Moderate Positive (3× LoD) 1.5E+00 $TCID_{50}$/mL	-	29/30 (96.7%)	30/30 (100%)	**59/60 (98.3%)**	30/30 (100%)	-	29/30 (96.7%)	**59/60 (98.3%)**
		Low Positive (1× LoD) 5.0E-01 $TCID_{50}$/mL	-	30/30 (100%)	27/30 (90.0%)	**57/60 (95.0%)**	30/30 (100%)	-	29/30 (96.7%)	**59/60 (98.3%)**
Parainfluenza Virus 3		Negative (no analyte)	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**
Parainfluenza Virus 4	Parainfluenza Virus 4	Negative (no analyte)	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**
	Parainfluenza Virus 4 (Zeptometrix 0810060CF)	Moderate Positive (3× LoD) 1.5E+02 $TCID_{50}$/mL	-	30/30 (100%)	30/30 (100%)	**60/60 (100%)**	30/30 (100%)	-	30/30 (100%)	**60/60 (100%)**
		Low Positive (1× LoD) 5.0E+01 $TCID_{50}$/mL	-	29/30 (96.7%)	30/30 (100%)	**59/60 (98.3%)**	30/30 (100%)	-	29/30 (96.7%)	**59/60 (98.3%)**
		Negative (no analyte)	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**

Analyte (Isolate Source ID)	Concentration Tested	Agreement with Expected Result							
		FilmArray 2.0				FilmArray Torch			
		Site A	Site B	Site C	System Total	Site A	Site B	Site C	System Total
Respiratory Syncytial Virus[b] (Zeptometrix 0810040ACF)	**Moderate Positive (3× LoD) 6.0E-02 $TCID_{50}$/mL (2.7E+01 copies/mL)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**
	Low Positive (1× LoD) 2.0E-02 $TCID_{50}$/mL (9.0E+00 copies/mL)	19/20 (95%)	20/20 (100%)	18/20 (90%)	**57/60 (95%)**	20/20 (100%)	20/20 (100%)	19/20 (95%)	**59/60 (98.3%)**
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (ATCC VR-1986HK)	**Negative (no analyte)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**
	Moderate Positive (3× LoD) 1.5E+03 copies/mL	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**
	Low Positive (1× LoD) 5.0E+02 copies/mL	20/20 (100%)	19/20 (95%)	19/20 (95%)	**58/60 (96.7%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**
***Bordetella parapertussis*[c] (IS*1001*) (Zeptometrix 0801461)**	**Negative (no analyte)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**
	Moderate Positive (3× LoD) 1.8E+02 IS*1001* copies/mL	19/20 (95%)	20/20 (100%)	20/20 (100%)	**59/60 (98.3%)**	19/20 (95%)	19/20 (95%)	20/20 (100%)	**58/60 (96.7%)**
	Low Positive (1× LoD) 6.0E+01 IS*1001* copies/mL	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	19/20 (95%)	20/20 (100%)	**59/60 (98.3%)**
***Bordetella pertussis* (*ptxP*)**	***Bordetella pertussis* (*ptxP*)**	**Negative (no analyte)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**
	Bordetella pertussis (*ptxP*) (Zeptometrix 0801459)	**Moderate Positive (3× LoD) 3.0E+03 CFU/mL**	-	30/30 (100%)	30/30 (100%)	**60/60 (100%)**	30/30 (100%)	-	30/30 (100%)	**60/60 (100%)**
		Low Positive (1× LoD) 1.0E+03 CFU/mL	-	30/30 (100%)	30/30 (100%)	**60/60 (100%)**	28/30 (93.3%)	-	30/30 (100%)	**58/60 (96.7%)**

Analyte (Isolate Source ID)	Concentration Tested	Agreement with Expected Result							
		FilmArray 2.0				FilmArray Torch			
		Site A	Site B	Site C	System Total	Site A	Site B	Site C	System Total
Chlamydia pneumoniae	**Negative (no analyte)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**
Mycoplasma pneumoniae	**Negative (no analyte)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**

[a] Reproducibility of Adenovirus detection with the BioFire RP2 was 98.3% and 99.2% for the low and moderate positive samples, respectively and 100% for the negative sample(s).
[b] Respiratory Syncytial Virus results in the BioFire RP2 reproducibility study agreed with the expected result in 98.3 – 100% of the positive sample replicates in 100% of the negative sample replicates.
[c] *Bordetella parapertussis* (IS1001) was detected in 93.3% of the low positive sample replicates tested and in 99.2% of the moderate positive sample replicates tested in the BioFire RP2 reproducibility study. Agreement with the expected Not Detected result was 100% for the negative sample(s).

For the three analytes that had been evaluated in both studies, the reproducibility of detection observed for the BioFire RP2.1 was overall similar to what was observed for BioFire RP2. Overall, there were ten Not Detected results when the analyte was known to be present in the test sample in the reproducibility evaluations for the BioFire RP2.1. The observed Not Detected frequency is consistent with the test levels (<5% Not Detected results when testing at or above LoD). No pattern in Not Detected results was observed in the study variables (site, system, day instrument/modules, operator, or reagent lot).

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Controls

Two process controls are included in each pouch:

RNA Process Control
The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and becomes rehydrated when sample is loaded. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, reverse transcription, PCR1, dilution, PCR2, and DNA melting. A positive control result indicates that all steps carried out in the FilmArray RP2.1 pouch were successful.

PCR2 Control
The PCR2 Control assay detects a DNA target that is dried in the array along with the corresponding primers. A positive result indicates that PCR2 was successful.

Both control assays must be positive for the test run to pass. If the controls fail, the sample should be retested using a new pouch.

The FilmArray Software automatically fails the run if the melting temperature for either the RNA Process Control or the PCR2 Control is outside of an acceptable range.

The following is also described in the product package insert regarding to external controls:

External controls should be used in accordance with laboratory protocols and the appropriate accrediting organization requirements, as applicable. Transport media can be used as an external negative control. Previously characterized positive samples or negative samples spiked with well-characterized organisms can be used as external positive controls. Commercial external control materials may be available from other manufacturers; these should be used in accordance with the manufacturers' instructions and appropriate accrediting organization requirements, as applicable.

Calibrators

This device does not contain calibrators.

Specimen Stability

The BioFire RP2.1 test requires approximately 0.3mL of NPS specimen, collected according to standard technique and placed in transport media or saline. Samples in medium should be tested as soon as possible, but they may be stored at room temperature (approximately 23°C) for up to four hours, under refrigeration (approximately 4°C) for up to three days, or frozen (≤-15 °C or ≤-70°C) for up to 30 days.

Detailed documentation concerning NPS sample storage and transport was provided in the original FilmArray RP submissions (K103175, K110764, K120267) for NPS specimens stored in viral transport media. The BioFire RP2 and the BioFire RP2.1 utilize this same sample type and test principles and the additional organisms detected (*B. parapertussis* and SARS-CoV-2) are biologically similar to others detected by the FilmArray RP (i.e., a representative bacteria and virus). Therefore, the original FilmArray RP specimen stability study data are applicable to the BioFire RP2 and BioFire RP2.1 panels for samples stored in viral transport media. However, for establishing sample stability and storage conditions for NPS specimens in saline, an additional study was performed to validate claims.

For the study, natural NPS in saline matrix was prepared by eluting two NPS specimens collected from a single anonymous, asymptomatic volunteer in 6mL of 0.9% saline. Contrived organism mixes (Table 3) were prepared using analyte-

negative individual donor natural NPS in saline matrices. For each contrived mix, a total of ten unique donor NPS in saline matrices were individually spiked with organisms corresponding to the RP2.1 panel to a final concentration based on the LoD (up to 5x). LoD of the analytes were determined in a separate limit of detection for saline samples study as described in the Limit of Detection section. Immediately following sample preparation (T0), ten replicates (one from each donor) were tested to serve as a no storage control and to establish the expected Detected and Not Detected results.

Table 3. Organism composition of each contrived sample mix for stability studies with saline samples

Sample Mix	Organism[a]	Strain/Isolate/ Serotype	Concentration Tested[b]	Units	Concentration Relative to LoD[c]
M1	Adenovirus Species C	Serotype 2 WHO Int Std	1.5E+04 (1.5E+04)	IU/mL[d] (copies/mL)[e]	5×
	Coronavirus NL63	NL63	1.3E+00 (2.7E+02)	$TCID_{50}$/mL (copies/mL)	5×
	Influenza A H1N1pdm09 (H1-2009)	A/SwineNY/ 3/2009	2.6E+00 (1.7E+03)	$TCID_{50}$/mL (copies/mL)	5×
	Bordetella parapertussis	A747	2.1E+02 (3.0E+02)	CFU/mL (IS1001 copies/mL)	5×
	Parainfluenza Virus 3	Type 3	8.1E+01 (1.9E+02[g])	$TCID_{50}$/mL (copies/mL)	5×
	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	USA-WA1/2020 (heat inactivated)	2.5E+03[h]	copies/mL	5×
M4	Coronavirus 229E	229E	2.0E+00 (3.3E+02)	$TCID_{50}$/mL (copies/mL)	5×
	Human Metapneumovirus	Type 16, IA10-2003, A1	5.0E+01	$TCID_{50}$/mL[i]	5×
	Human Rhinovirus	1A	3.8E+00 (1.9+01[g])	$TCID_{50}$/mL (copies/mL)	5×
	Influenza A H3N2 (H3)[j]	Hong Kong/4801/14	1.3E+00 (1.1E+00[g])	$TCID_{50}$/mL (copies/mL)	5×
	Parainfluenza Virus 4	Type 4a	2.5E+02 (8.0E+03)	$TCID_{50}$/mL (copies/mL)	5×
	Mycoplasma pneumoniae	M129	6.3E+00[k] (4.7E+02[k])	CCU/mL copies/mL	60×[k]
	Middle East Respiratory Syndrome Coronavirus	EMC/2012 (heat inactivated)	6.7E+02	copies/mL	5×
M5	Coronavirus OC43	OC43	7.1E-02 (2.8E+02[g])	$TCID_{50}$/mL (copies/mL)	5×
	Enterovirus	D68	6.0E+02 (5.2E+01)	$TCID_{50}$/mL (copies/mL)	2×[l]
	Influenza A H3N2 (H3)[j]	Hong Kong/4801/14	1.4E+00 (1.1E+00[g])	$TCID_{50}$/mL (copies/mL)	5×
	Parainfluenza Virus 2	Type 2	2.5E+00 (1.5E+02)	$TCID_{50}$/mL (copies/mL)	5×
	Chlamydia pneumoniae	AR-39	9.0E+01 (6.7E+02[g])	IFU/mL (copies/mL)	5×

Sample Mix	Organism[a]	Strain/Isolate/ Serotype	Concentration Tested[b]	Units	Concentration Relative to LoD[c]
M6	Coronavirus HKU1	Clinical NPS Specimen 53727	1.0E+04[h]	RNA copies/mL	5×
	Influenza A H1N1 (H1)	A/New Caledonia/20/99	5.0E+03 (7.0E+02)	$TCID_{50}$/mL (copies/mL)	5×
	Influenza B	B/FL/04/06	2.5E+01 (1.7E+02)	$TCID_{50}$/mL (copies/mL)	5×
	Parainfluenza Virus 1	Type 1	1.6E+00 (5.0E+02[g])	$TCID_{50}$/mL (copies/mL)	5×
	Bordetella pertussis	A639	2.0E+03	CFU/mL	2×[l]
	Respiratory Syncytial Virus[n]	Type A	2.1E-01 (9.0E+01)	$TCID_{50}$/mL (copies/mL)	10×

Following testing at T0, individual sample aliquots were prepared from each contrived donor mix and these were stored at ambient (25°C), refrigerated (8°C), or frozen (≤-15°C) temperatures for the durations indicated in Table 4. Note, the T0 time point was collected on different days for different analytes but the frozen time point for all analytes was collected on the same day. This resulted in analytes in the different sample mixes to be tested for at least 30 days after the no storage time point.

At each time point, ten replicates (one from each donor) evenly distributed between the FilmArray 2.0 and FilmArray Torch instruments were tested. The reported results of Detected (D), Equivocal (E), and Not Detected (ND) were evaluated for each analyte across the storage conditions and compared to the results observed at T0.[(b) (4)] (b) (4)

Table 4. Storage conditions and sample size to be tested at each condition.

Storage Condition		Sample Size
Temperature	**Time**	
N/A	No Storage Control (T0)	10
Ambient (25°C)	4 hours	10
Refrigerated (8°C)	2 days	10
	3 days	10
Frozen (≤-15°C)	≥30 days	10
	Total	**50**

A valid result (i.e., all internal pouch controls passing) was required for each pouch tested. Pouches with invalid results due to a control failure, instrument error, or software error were retested until a valid result was obtained. Only results from the valid pouches were considered in subsequent analyses. Samples with Influenza A/subtype Equivocal or Influenza A (no subtype detected) results were retested according to the intended result interpretation algorithm (see Table 1).

Any observed trending across conditions (i.e., time- and/or temperature-dependent shift in assay parameters) would be indicative of possible impact on sample stability.

The below table provides a summary of the saline sample stability study. As indicated in the summary table, some observations across different analytes and storage conditions did not meet the expected Detected results. Some of the missed detections did not correspond to the same donor samples and they were distributed such that it appears no trend was observed (e.g., an unexpected Not Detected result for T0 but Detected result for later time points and other storage conditions, etc.). However, for three analytes detected by the RP2.1 device (RSV, Parainfluenza Virus 2, and *Mycoplasma Pneumoniae*) an additional study was conducted to clarify any possible negative trends.

Table 5. Summary of analyte detection results observed for samples tested at T0 (no storage control), ambient, refrigerated, and frozen conditions. Results are reported as expected Detected results (D) in samples that contained the relevant analyte and as expected Not Detected (ND) results in samples that did not contain the relevant analyte.

Organism	Source ID	Concentration Tested[a]	No Storage Control		Ambient (25°C)		Refrigerated (8°C)				Frozen (≤-15°C)	
			T0		4 hours		2 days		3 days		≤30 days[b]	
			D	ND	D	ND	D	ND	D	ND	D	ND
VIRUSES												
Adenovirus C (WHO IS)[c]	NIBSC 16/324	1.5E+04 IU/mL[d]	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30
Coronavirus 229E	ATCC VR-740	3.3E+02 copies/mL	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30
Coronavirus HKU1	Clinical NPS Specimen 53727	1.0E+04 copies/mL	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30
Coronavirus NL63	BEI NR-470	2.7E+02 copies/mL	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30
Coronavirus OC43	Zeptometrix 0810024CF	2.8E+02 copies/mL	10/10	30/30	10/10	30/30	9/10	30/30	10/10	30/30	9/10	30/30
Human Metapneumovirus	Zeptometrix 0810161CF	5.0E+01 $TCID_{50}$/mL	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30
Human Enterovirus	ATCC VR-1823	2.6E+02 copies/mL[e]	10/10	20/20[g]	10/10	20/20[g]	10/10	20/20[g]	10/10	20/20[g]	10/10	20/20[g]
Human Rhinovirus	Zeptometrix 0810012CFN	1.9E+01 copies/mL	10/10		10/10		9/10		9/10		8/10	
Influenza A H1N1	Zeptometrix 0810036CF	7.0E+02 copies/mL	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30
Influenza A H1N1-2009	Zeptometrix 0810249CF[f]	1.7E+03 copies/mL	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30
Influenza A H3N2[h]	Zeptometrix 0810526CF	1.1E+00 copies/mL	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Influenza B	Zeptometrix 0810255CF	1.7E+02 copies/mL	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30
Parainfluenza Virus 1	Zeptometrix 0810014CF	5.0E+02 copies/mL	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30

Organism	Source ID	Concentration Tested[a]	No Storage Control		Ambient (25°C)		Refrigerated (8°C)				Frozen (≤-15°C)	
			T0		4 hours		2 days		3 days		≤30 days[b]	
			D	ND	D	ND	D	ND	D	ND	D	ND
Parainfluenza Virus 2	Zeptometrix 0810015CF	1.5E+02 copies/mL	7/10	30/30	7/10	30/30	9/10	30/30	9/10	30/30	5/10	30/30
Parainfluenza Virus 3	Zeptometrix 0810016CF	1.9E+02 copies/mL	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30
Parainfluenza Virus 4	Zeptometrix 0810060CF	8.0E+03 copies/mL	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30
Severe Acute Respiratory Syndrome Coronavirus 2	ATCC VR-1986HK	2.5E+03 copies/mL[e]	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30
BACTERIA												
Bordetella parapertussis	Zeptometrix 0801461	3.0E+02 copies/mL	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30
Bordetella pertussis	Zeptometrix 0801459	1.0E+04 CFU/mL[e]	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30
Chlamydia pneumoniae	ATCC 53592	6.7E+02 copies/mL	9/10	30/30	8/10	30/30	9/10	30/30	10/10	30/30	8/10	30/30
Mycoplasma pneumoniae	Zeptometrix 0801579	4.7E+03 copies/mL[e]	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30	10/10	30/30

[a] Unless otherwise noted, the concentration tested is 5× LoD where LoD was determined in the LoD study for saline sample type with the BioFire Respiratory Panel 2.1 (RP2.1).

[b] The storage claim is that samples can be frozen for up to 30 days. Although the T0 time point was collected on different days for different analytes, the frozen time point for all analytes was collected on the same day; this resulted in analytes being stored for variable durations and tested at different intervals after T0. All analytes were tested at least 30 days after T0. Samples containing Adenovirus, *Bordetella parapertussis*, Coronavirus NL63, Influenza A H1N1-2009, Parainfluenza Virus 3, and Severe Acute Respiratory Syndrome Coronavirus 2 were tested 45 days after T0. Samples containing *Chlamydia pneumoniae*, Coronavirus OC43, Human Enterovirus, Parainfluenza Virus 2 were tested 31 days after T0. Samples containing *Bordetella pertussis*, Coronavirus 229E, Coronavirus HKU1, Human Metapneumovirus, Human Rhinovirus, Influenza A H1N1, Influenza A H3N2, Influenza B, *Mycoplasma pneumoniae*, Parainfluenza Virus 1, Parainfluenza Virus 4, Middle East Respiratory Syndrome Coronavirus, and Respiratory Syncytial Virus were tested 30 days after T0.

[c] WHO IS = World Health Organization International Standard

[d] IU = International Units. BioFire Diagnostics quantified the WHO IS by quantitative real-time PCR and demonstrated that 1.5E+04 IU/mL is equivalent to 1.5E+04 copies/mL.

[e] Indicated concentration is 10× LoD.

[f] Catalog or Source ID from Zeptometrix was previously 0810109CFN, as indicated on the Certificate of Analysis when the stock was received.

[g] Human Enterovirus and Human Rhinovirus cannot be distinguished by the BioFire RP2.1 Panel. A detection of either virus is reported as Human Rhinovirus/Enterovirus. Two organism mixes did not contain either virus, resulting in 20 expected Not Detected results at each time point.

[h] Influenza A H3 was incorrectly spiked into one sample mix, resulting in a lower than expected organism concentration. It was then included in an additional mix at the appropriate concentration. Influenza A H3 results for the incorrectly formulated sample mix were excluded, resulting in 10 expected Detected results and only 20 expected Not Detected results at each time point.

Additional saline specimen stability study (*Mycoplasma pneumoniae*, Parainfluenza Virus 2, and Respiratory Syncytial Virus (RSV))

In the original saline specimen stability study, three analytes were evaluated in concentrations that were not in accordance with the original study design and therefore yielded results that did not clarify possible stability trends. Therefore, an additional study was performed at the indicated 5x LoD as originally intended for those three analytes. A summary of the additional data collected for the three analytes are summarized in the below table (Table 6). Note that the conditions that were evaluated corresponded to sample storage claims, and included no storage control (T0), ambient (4 hours at 25°C), refrigerated (2-3 days at 8°C), and frozen (30 days at ≤-15°C) conditions. Replicates at each test condition were evaluated and the performance was compared to the results obtained from the no storage control (T0) to indicate if there were any adverse sample stability observations in the saline media.

For this evaluation, unique natural NPS in saline specimen matrices were individually spiked with the three organisms to result in a final concentration of 5x LoD. The organism stock of *Mycoplasma pneumoniae* for this mix was the same used in previous evaluations for sample stability. However, due to insufficient volume remaining of the Parainfluenza Virus 2 and RSV stocks used in the initial testing, alternate stocks with the same source ID but different lot numbers were used in this mix. Replacement organism stock lots were used and quantified in copies/mL. Due to the slight variations in quantification methods used for the original and reestablished stock concentrations, different absolute values in copies/mL were observed compared to the original values. Therefore, the updated stock concentration was used to evaluate equivalency in panel performance with saline media for these analytes.

Table 6. Summary of analyte detection results observed for the additional sample mix at T0 (no storage control), ambient, refrigerated, and frozen conditions. Results are reported are number of detections vs. total replicates tested.

Organism	Source ID	Concentration Tested[a]	No Storage Control	Ambient (25°C)	Refrigerated (8°C)		Frozen (≤-15°C)
			T0	4 hours	2 days	3 days	30 days
Mycoplasma pneumoniae	Zeptometrix 0801579	2.3E+03 copies/mL	9/9	9/9	9/9	9/9	9/9
Parainfluenza Virus 2	Zeptometrix 0810015CF	5.0E+03 copies/mL	9/9	9/9	9/9	9/9	9/9
Respiratory Syncytial Virus	Zeptometrix 0810040ACF	4.5E+02 copies/mL	9/9	9/9	9/9	9/9	9/9

[a] All concentrations are 5× the limit of detection (LoD) with LoD updated from new stock evaluations for Parainfluenza Virus 2 and Respiratory Syncytial Virus (see Table 30).

For the *Mycoplasma pneumoniae* analyte, there were no observed trends that would indicate a sample stability issue with the saline specimens over the range of storage conditions. Compared to the Cp observed for the no storage control (mean Cp (b) (4)) the change in Cp ranged from (b) (4) to (b) (4) with no notable trends. Therefore, it appeared that the storage conditions evaluated did not impact test performance for the analyte.

For the Parainfluenza Virus 2 analyte, compared to the Cp observed for the no storage control (mean Cp (b) (4)) the change in mean Cp ranged from (b) (4) to (b) (4) without any notable trends across the different storage conditions evaluated. All replicates were detected at the various test conditions.

For the RSV analyte, compared to the T0 baseline (mean Cp (b) (4)) the change in mean Cp ranged from (b) (4) to (b) (4) . The changes across the different storage conditions evaluated do not show significant trends and all replicates were detected as expected. Further, based on the assumption that a 2-fold difference in concentration results in one cycle shift in Cp value, the variation is within the expected range for stochastic detection results.

Cumulatively, the reevaluations for these three analytes indicate no significant trends in sample stability in the saline media for the indicated sample storage conditions.

Fresh vs. Frozen Study
Detailed documentation concerning fresh vs. frozen NPS in VTM specimens was provided in the original FilmArray RP submissions (K103175, K110764, and K120267). The results of the analytical study conducted for the original FilmArray RP submissions demonstrated that preservation of samples (by freezing at ≤-70°C) does not affect the accuracy of test results compared to freshly collected or freshly prepared samples. Therefore, it is appropriate to utilize frozen archived prospective and retrospective clinical samples in the evaluation of BioFire RP2.1 to supplement the prospective (fresh) clinical study data, and to use frozen simulated samples in analytical studies for this submission.

Single-Spiked vs. Multi-Spiked Specimen Study
Detailed documentation concerning analytical performance with samples composed of multiple organisms in a specimen was provided in the original FilmArray RP submissions (K103175, K110764, and K120267). The results of the analytical study demonstrated that the LoDs from testing single-spiked and multi-spiked specimens were comparable. Therefore, it wasdeemed appropriate to utilize the multi-spiked approach in conducting the analytical studies and the clinical study testing contrived clinical specimens in support of this submission.

d. Detection limit:

The Limit of Detection (LoD) for all analytes on the BioFire RP2.1 were evaluated based on whether the analytes were new (i.e., SARS-CoV-2) or previously included in the BioFire RP2 panel, and in terms of the specimen media type (i.e., nasopharyngeal swab matrix in saline or viral transport media). LoD confirmation testing consisted of twenty replicates at the estimated LoD concentration (1×) and twenty replicates of a ten-fold dilution of the LoD sample (0.1×). Samples were contrived in either viral transport media (VTM) or in saline. In addition, saline specimens contained an artificial nasopharyngeal swab (aNPSs) matrix, and equivalency in detection between the previously established LoD of samples in VTM was evaluated for validation of NPS in saline as an appropriate sample type. When possible, testing was performed with the same stock/lot used to verify and confirm the LoD in VTM for the saline samples.

The LoD concentration was confirmed by detection in at least 95% of 20 replicates and detection in less than 95% of 20 replicates at a concentration below the LoD (0.1x) on FilmArray 2.0 and FilmArray Torch systems. The saline sample matrix was considered equivalent when the LoD was verified by detection in ≥95% (≥19/20) of replicates at the LoD (1×) concentration and detection in <95% (≤18/20) of replicates below the LoD (0.1×) on FilmArray 2.0 and FilmArray Torch systems. When these criteria were not met, additional side-by-side testing was performed to compare

samples prepared in both saline/artificial NPS and VTM at the same concentrations in order to demonstrate that no significant difference existed between the sample matrices.

Limit of Detection for SARS-CoV-2

LoD estimate and confirmation testing for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was performed with both inactivated and infectious cultures of the same virus isolate (USA-WA1/2020); see Table 8). Samples containing the inactivated virus (ATCC VR-1986HK, USA-WA1/2020) were prepared in Remel M4 viral transport medium as the sample matrix. Samples with cultured infectious virus (USA-WA1/2020) were prepared in a matrix of pooled clinical nasopharyngeal swab specimens (archived NPS specimens collected prior to the SARS-CoV-2 outbreak in Dec 2019). For aNPSs samples, heat inactivated virus was used to verify the LoD in contrived specimens.

The stock concentration of inactivated virus (VR-1986HK) in copies/mL and $TCID_{50}$/mL was provided by ATCC. The concentration was assessed to be 1.60E+05 (i.e., 1.60 x 10^5)$TCID_{50}$/mL and 1.16E+9 copies/mL, respectively by digital droplet PCR (ddPCR). The concentration of infectious virus (in genomic copies/mL and $TCID_{50}$/mL) was provided by the contracted laboratory that cultured and tested the virus. For the infectious virus, the copies/mL concentration was determined by two different methods. Quantitative real-time PCR (qPCR) was performed on intact virus (not extracted) using a WHO qualified assay with primers and probe targeting the *E* gene (WHO E, Charité, Germany; stock culture concentration of 2.4E+09 copies/mL) and qPCR on extracted genomic RNA was performed using the CDC 2019-Novel Coronavirus (2019-nCoV) Diagnostic Panel N1 assay primers and probe (CDC N1; stock culture concentration of 7.4E+08 copies/mL). Both copies/mL concentrations are indicated in the above table on isolate/culture information (Table 8), but the higher of the two stock concentrations (2.4E+09 copies/mL determined with the WHO/Charité E assay) was applied for determining the LoD concentration.

LoD estimate testing for inactivated and infectious SARS-CoV-2 consisted of multiple replicates of a ten-fold serial dilution. The SARS-CoV-2 Detected (D) or Not Detected (ND) results are shown for each replicate, (b) (4)

(b) (4)

Table 7. LoD Estimate Test Results for Inactivated SARS-CoV-2 (ATCC VR-1986HK[a])
The boxed data indicates the estimated LoD concentration.

Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) Assay and Detection Results
(b) (4)

[a] The concentration of inactivated virus (VR-1986HK) in copies/mL and $TCID_{50}$/mL was provided by ATCC. The copies/mL value was determined by digital droplet PCR (ddPCR).

Based on the results of the LoD estimate testing, an estimated LoD concentration of 5.0E+02 copies/mL was selected for LoD confirmation testing.

LoD confirmation testing of contrived samples containing inactivated virus (prepared in Remel M4 viral transport medium) consisted of twenty replicates at the estimated LoD concentration (1×) and twenty replicates of a ten-fold dilution of the LoD sample (0.1×). The testing confirmed a LoD of **5.0E+02 copies/mL** with detection of Severe Acute Respiratory Syndrome Coronavirus (SARS-COV-2) in 20/20 (100%) replicates at the 1× concentration and detection in <95% of the replicates (5/20, 25%) at the 0.1× concentration (Table 8).

Table 8. LoD BioFire RP2.1 LoD Confirmation Results for Inactivated SARS-CoV-2 (ATCC VR-1986HK)

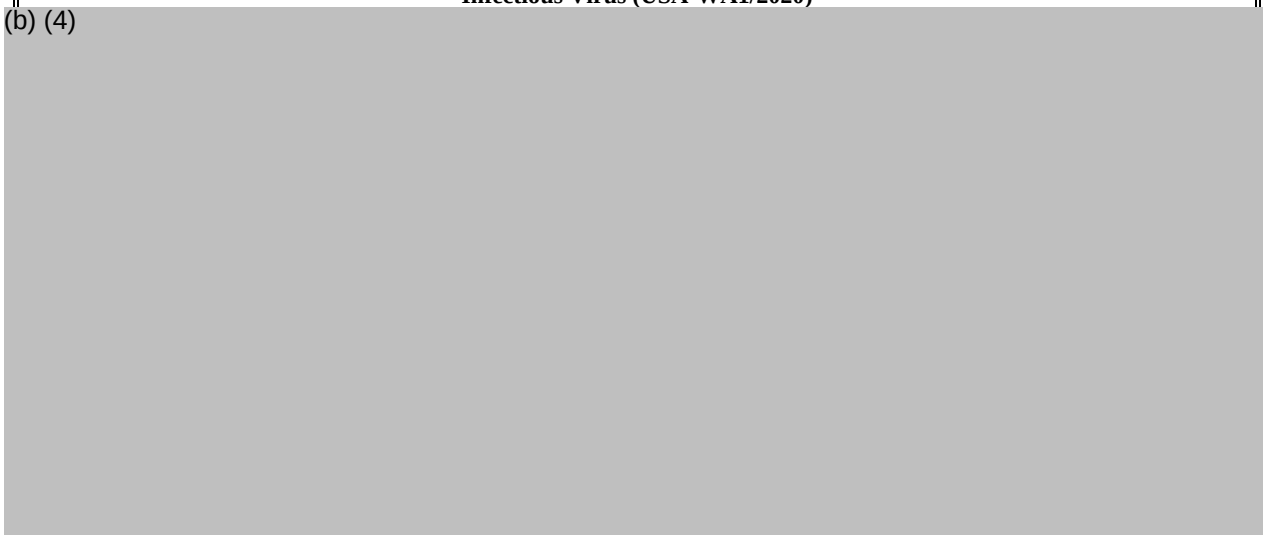
(b) (4)

(b) (4)

The same method of LoD estimate and confirmation testing was carried out with contrived samples containing infectious virus in pooled clinical NPS (unknown transport media/medium). Six replicates were tested per dilution. Results from the LoD estimate titration are shown in Table 9 and an estimated LoD of 1.6E+02 copies/mL was selected for confirmation testing.

Table 9. LoD Estimate Results for Infectious SARS-CoV-2 (USA-WA1/2020; WRCEVA)
Samples were prepared in pooled clinical NPS matrix.[a] The boxed data indicates the estimated LoD concentration

Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) Detection and Assay Results Infectious Virus (USA-WA1/2020)
(b) (4)



[a] Additional analyte detection (Influenza B, Parainfluenza Virus 3, and Human Rhinovirus/Enterovirus) from the sample matrix was observed in some replicates.

Additional testing at the estimated LoD concentration and below confirmed a LoD of **1.6E+02 copies/mL** for the infectious virus, with detection of Severe Acute Respiratory Syndrome Coronavirus (SARS-COV-2) in 20/20 (100%) replicates at the 1× concentration and detection in <95% of the replicates (11/20, 55%) at the 0.1× concentration (Table 10).

Table 10. LoD Confirmation Results for Infectious SARS-CoV-2 (USA-WA1/2020; WRCEVA)
Samples were prepared in pooled clinical NPS matrix.[a]

(b) (4)

[a] Additional analyte detection (Parainfluenza Virus 4, and Human Rhinovirus/Enterovirus) from the sample matrix was observed in some replicates.

The confirmed LoD concentrations for inactivated and infectious virus (**5.0E+02 copies/mL** and **1.6E+02 copies/mL**, respectively) in transport media specimens are within a ~3-fold difference and therefore are considered similar.

Initial testing of the heat-inactivated virus in saline sample evaluations generated only 90% detection (18/20) at the LoD concentration of 5.0E+02 copies/mL. A second round of testing was performed where replicates at 1x LoD concentration in samples prepared in NPS and VTM were compared. (b) (4)

(b) (4)

Table 11. SARS-CoV-2 LoD Verification Results – Round 2

(b) (4)

Limit of Detection for other (non-SARS-CoV-2) BioFire RP2.1 Analytes (VTM specimens)

For all other analytes, the assays are shared with the previous version of the panel (i.e., BioFire RP2) and the new BioFire RP2.1. Therefore, LoD concentrations of VTM samples were evaluated and confirmed in the context of the BioFire RP2 LoD concentrations. To minimize uncertainty due to variability in isolate/culture quantification, LoD confirmation samples were prepared in transport medium at 1x and 0.1x LoD concentrations, using the same isolate culture stocks used in the BioFire RP2 LoD study, whenever possible. A clinical NPS specimen was used as the source material for the un-culturable Coronavirus HKU1 (viral RNA quantified in copies/mL by qPCR).

Substitution was required for another four of the original BioFire FilmArray RP2 study isolate cultures (Human Metapneumovirus, Human Rhinovirus, *Bordetella*

pertussis, and *Mycoplasma pneumoniae*) due to the original culture having been consumed in other testing. When a replacement culture was required, the analyte concentration tested on BioFire RP2.1 matched only one unit of measure for LoD concentration established with the BioFire FilmArray RP2 (i.e., either quantification by molecular methods in copies/mL or quantification by culture methods in $TCID_{50}$/mL or CFU/mL) because the ratio of viable/infectivity units per mL to copies per mL varies from culture event to culture event. The units used to prepare the BioFire RP2.1 LoD confirmation samples for analytes other than SARS-CoV-2 are indicated in Table 13 below.

All samples (including those containing substituted isolate cultures) were first tested on BioFire FilmArray RP2 and then tested with the BioFire RP2.1. Data from both panels are shown in Table 12 on a combination of FilmArray 2.0 and FilmArray Torch systems. The analytes with additional information to note in the LoD evaluations between the FilmArray RP2 and BioFire RP2.1 panels are discussed below.

Table 12. Limit of Detection (LoD) for BioFire Respiratory Panel 2.1 (RP2.1) Analytes
When a substitute culture was tested (grey shading), the LoD concentration is listed in only one unit.

Analyte	Isolate	LoD Concentration	FilmArray RP2 Detection Results		BioFire RP2.1 Detection Results	
			1×LoD	0.1×LoD	1×LoD	0.1×LoD
Viruses						
Adenovirus	Species C Serotype 2 WHO Int'l Standard NIBSC 16/324	**3.0E+03 IU/mL**[a] (3.0E+03 copies/mL)[b]	**20/20 100%**	12/20 60.0%	**20/20 100%**	14/20 70.0%
Coronavirus 229E	ATCC VR-740	**4.0E-01 $TCID_{50}$/mL** 6.5E+01 copies/mL	**19/20 95.0%**	12/20 60.0%	**20/20 100%**	10/20 50.0%
Coronavirus HKU1	Clinical NPS specimen (53727)	**2.0E+03 copies/mL**	**20/20 100%**	3/20 15.0%	**19/20**[c] **95.0%**	6/20 30.0%
Coronavirus NL63	BEI NR-470	**2.5 E-01 $TCID_{50}$/mL** 5.4E+01 copies/mL	**20/20 100%**	4/20 20.0%	**20/20 100%**	5/20 25%
Coronavirus OC43	ATCC VR-759	**3.0E+01 $TCID_{50}$/mL** 5.6E+02 copies/mL	**20/20 100%**	6/20 30.0%	**20/20 100%**	8/20 40.0%
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	USA-WA1/2020 ATCC VR-1986HK (heat inactivated)	**5.0E+02 copies/mL**[d] 6.9E-02 $TCID_{50}$/mL	**N/A**		**20/20 100%**	5/20 25.0%
	USA-WA1/2020 WRCEVA[e] (infectious)	**1.6E+02 copies/mL**[f] 1.1E-02 $TCID_{50}$/mL			**20/20 100%**	11/20 55.0%
Human Metapneumovirus	16, Type A1 IA10-2003 Zeptometrix 0810161CF	**1.0E+01 $TCID_{50}$/mL** (1.2E+03 copies/mL)[g]	**19/20 100%**	20/20 100%	**20/20 100%**	20/20 100%
Human Rhinovirus/ Enterovirus	Human Rhinovirus Type 1A Zeptometrix 0810012CFN	**3.8E+01 copies/mL**	**19/20 95.0%**	10/20 50.0%	**19/20 95.0%**	9/20 45.0%
	Enterovirus D68 ATCC VR-1823	**3.0E+02 $TCID_{50}$/mL** 2.6E+01 copies/mL	**20/20 100%**	11/20 55.0%	**20/20 100%**	14/20 70.0%
Influenza A H1	Influenza A H1N1 A/New Caledonia/20/99 Zeptometrix 0810036CF	**1.0E+03 $TCID_{50}$/mL** 1.4E+02 copies/mL	**20/20 100%**	12/20[h] 60.0%	**20/20 100%**	10/20[h] 50.0%
Influenza A H1-2009	Influenza A H1N1pdm09 A/Swine/NY/03/2009 Zeptometrix 0810249CF	**5.0E-01 $TCID_{50}$/mL** 3.3E+02 copies/mL	**20/20 100%**	5/20[h] 25.0%	**20/20 100%**	3/20[h] 15.0%

Analyte	Isolate	LoD Concentration	FilmArray RP2 Detection Results		BioFire RP2.1 Detection Results	
			1×LoD	0.1×LoD	1×LoD	0.1×LoD
Influenza A H3	Influenza H3N2 A/Port Chalmers/1/73 ATCC VR-810	**1.0E-01 $TCID_{50}$/mL** 2.1E+01 copies/mL	**18/20[i] 90.0%**	0/20[h] 0.0%	**20/20 100%**	4/20[h] 20.0%
Influenza B	B/FL/04/06 Zeptometrix 0810255CF	**5.0E+00 $TCID_{50}$/mL** 3.4E+01 copies/mL	**20/20 100%**	15/20 75.0%	**20/20 100%**	17/20 85.0%
Parainfluenza Virus 1	Type 1 Zeptometrix 0810014CF	**5.0E+00 $TCID_{50}$/mL** 1.0E+03 copies/mL	**20/20 100%**	14/20 70.0%	**20/20 100%**	14/20 70.0%
Parainfluenza Virus 2	Type 2 Zeptometrix 0810015CF	**5.0E-01 $TCID_{50}$/mL** 3.0E+01 copies/mL	**20/20 100%**	5/20 25.0%	**19/20 95.0%**	6/20 30.0%
Parainfluenza Virus 3	Type 3 Zeptometrix 0810016CF	**2.5E+00 $TCID_{50}$/mL** 3.8E+01 copies/mL	**19/20 95.0%**	12/20 60.0%	**20/20 100%**	6/20 30.0%
Parainfluenza Virus 4	Type 4a Zeptometrix 0810060CF	**5.0E+01 $TCID_{50}$/mL** 1.6E+03 copies/mL	**20/20 100%**	8/20 40.0%	**19/20 95.0%**	5/20 25.0%
Respiratory Syncytial Virus	Type A Zeptometrix 0810040ACF	**2.0E-02 $TCID_{50}$/mL** 9.0E+00 copies/mL	**20/20 100%**	6/20 30.0%	**20/20 100%**	3/20 15.0%
Bacteria						
***Bordetella parapertussis* (IS*1001*)**	A747 Zeptometrix 0801461	**6.0E+01 IS*1001* copies/mL** 4.1E+01 CFU/mL	**20/20 100%**	6/20 30%	**20/20 100%**	3/20 15.0%
***Bordetella pertussis* (*ptxP*)**	A639 Zeptometrix 0801459	**1.0E+03 CFU/mL**	**20/20 100%**	13/20 65.0%	**19/20 95.0%**	10/20 50%
***Chlamydia pneumoniae*[k]**	TW183 ATCC VR-2282	**1.0E-01 $TCID_{50}$/mL** 6.6E+01 copies/mL	**40/40 100%**	9/40 22.5%	**32/40[j] 80.0%**	10/40 25.0%
		2.0E-01 $TCID_{50}$/mL 1.3E+02 copies/mL	-	-	**20/20 100%**	9/20 45.0%
Mycoplasma pneumoniae	M129 Zeptometrix 0801579	4.6E+02 copies/mL	**20/20** 100%	**8/20** 40.0%	**20/20** 100%	7/20 35.0%

[a] IU = International Units.

[b] BioFire Diagnostics quantified the WHO International Standard by quantitative real-time PCR to demonstrate that 3.0E+03 IU/mL=3.0E+03 copies/mL. Two other adenovirus serotypes (B7 and F41) were also tested for LoD verification and confirmation. Each was detected in ≥95% of replicates on both panels at a 1×LoD concentration less than 3.0E+03 copies/mL (8.7E+02 and 1.1E+03 copies/mL, respectively).

[c] Results are from a second sample containing Coronavirus HKU1 at the 1× LoD concentration. In the first 1× sample tested on the BioFire RP2.1, Coronavirus HKU1 was detected in 18/20 (90%) replicates (5/20, 25.0% for the 0.1× sample).

[d] Concentration of viral RNA in the culture was determined by digital droplet PCR, as indicated on the Certificate of Analysis from ATCC.

[e] World Reference Center for Emerging Viruses and Arboviruses; contributed by the United States Centers for Disease Control (CDC).

[f] Concentration of viral RNA in the culture was determined by quantitative real-time PCR using E gene primers and probe as described on the World Health Organization (WHO) website: https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf.

[g] A copies/mL concentration for the substitute culture of Human Metapneumovirus that was tested has not been determined. The corresponding BioFire FilmArray RP2 LoD concentration for Human Metapneumovirus at 1.0E+01 $TCID_{50}$/mL is 1.2E+03 copies/mL (based on testing of a different culture of the same isolate).

[h] Detection for Influenza A subtypes is calculated based only on the correct Influenza A (subtype) Detected result. An Equivocal or Influenza A (no subtype detected) result is tallied as a Not Detected result.

[i] 2/20 replicates were Influenza A H3 Equivocal.

[j] Two different samples containing *C. pneumoniae* at the FilmArray RP2 LoD concentration of 6.6E+01 copies/mL were prepared and both samples were tested with BioFire FilmArray RP2 and BioFire RP2.1. Detection at the 1× level was 20/20 with BioFire FilmArrayRP2 and 16/20 with BioFire RP2.1 for both samples.

[k] The LoD concentration for *Chlamydia pneumoniae* on the BioFire RP2.1 is two-fold higher than the LoD concentration on the BioFire FilmArray RP2.

LoD testing for Adenovirus was performed with a World Health Organization (WHO) International Standard developed by NIBSC (National Institute for Biological

Standards and Control) that was released in late 2018 (NIBSC 16/324, 1st WHO International Standard for human adenovirus DNA for nucleic acid amplification techniques, version 1.0, dated 12/13/2018). This is a whole virus preparation of HAdV type 2 with a standardized concentration expressed in International Units (IU)[1]. All adenovirus cultures previously quantified by different species-specific qRT-PCR kits and assays were re-evaluated using a single quantitative assay (Adenovirus R-GENE) that was verified against the international standard.

LoD confirmation for the Adenovirus international standard was first performed in 2019 with the FilmArray RP2 to establish an Adenovirus LoD concentration of 3.0E+03 IU/mL (data provided in Table 13 and Table 14; equivalent to 3.0E+03 copies/mL when quantified with the Adenovirus R-GENE (bioMerieux) quantitative real-time PCR kit). The same samples used in establishing the Adenovirus LoD on the FilmArray RP2 with the international standard was tested with the BioFire RP2.1. Similar results were observed (Table 15), confirming identical LoD for Adenovirus (3.0E+03 IU/mL or 3.0E+03 copies/mL) on each panel.

Table 13. LoD Estimate Test Results for WHO Adenovirus International Standard (NIBSC 16/324) on BioFire FilmArray RP2

The boxed data indicates the estimated LoD concentration

(b) (4)

Table 14. LoD Confirmation Results for WHO Adenovirus International Standard (NIBSC 16/324) on BioFire FilmArray

(b) (4)

[1] Fryer JF, Hockley JG, Govind S, Morris CL and the Collaborative Study Group. Collaborative Study to Evaluate the Proposed 1st WHO International Standard for Human Adenovirus (HAdV) DNA for Nucleic Acid Amplification Techniques (NAT). WHO ECBS Report 2018; WHO/BS/2018.2346

(b) (4)

Table 15. Adenovirus LoD Verification and Confirmation Results (Species C Serotype 2; WHO International Standard, NIBSC 16/32)

(b) (4)

Additional Adenovirus LoD Data

The BioFire RP2.1 contains five assays for Adenovirus detection and the WHO International Standard (type C2) is amplified by the Adeno2 and Adeno6 assays. Therefore, for completeness two additional Adenovirus isolates (serotypes B7A and F41) were also tested at the RP2 1x LoD concentrations (8.7E+02 copies/mL and 1.1E+03 copies/mL, respectively by R-GENE quantification) and at 0.1x LoD, in order to collect data for each of the five adenovirus assays. In these Adenovirus isolates testing, twenty replicates of each sample were evaluated with both panels and demonstrated 100% detection of replicates (20/20) at the 1x LoD concentration for the FilmArray RP2 and BioFire RP2.1 devices. The inclusion of two additional isolates in the testing allowed for data to be collected from each assay (Adeno2 and Adeno7.1 for B7 and Adeno 3 and Adeno8 for F41). Both of the additional isolates were tested and detected as expected (Table 16) at a copies/mL concentration lower than that of the WHO International Standard. The LoD for the Adenovirus WHO International Standard is the Adenovirus LoD claim for the panel.

Table 16. Additional Adenovirus LoD Testing Data on FilmArray RP2 and BioFire RP2.1

Analyte	Isolate	LoD Concentration [a,b]	FilmArray RP2 Detection Results		BioFire RP2.1 Detection Results	
			1×LoD	0.1×LoD	1×LoD	0.1×LoD
Adenovirus	Species B Serotype 7A Zeptometrix 0810021CF	**5.0E-02 $TCID_{50}$/mL** 3.9E+01 copies/mL[a] [8.7E+02 copies/mL][b]	**20/20** **100%**	12/20 60.0%	**20/20** **100%**	14/20 70.0%
	Species F Serotype 41 ATCC VR-930	**1.0E+00 $TCID_{50}$/mL** 1.2E+02 copies/mL[a] [1.1E+03 copies/mL] [b]	**20/20** **100%**	14/20 70.0%	**20/20** **100%**	13/20 65.0%

[a] Concentration based on quantification with the Genesig Adenovirus B kit (PrimerDesign, Ltd.) as described in K170604
[b] Concentration based on quantification with Adenovirus R-GENE (BioMerieux, ref. 69-010B)

The sample containing Human Metapneumovirus required a substitute culture that is only quantified in $TCID_{50}$/mL units (same isolate, different culture event/lot#). Attempts to accurately quantify the substitute culture in copies/mL (similar to the original stock used in the RP2 LoD study) were not successful and therefore the sample could only be prepared based on the RP2 $TCID_{50}$/mL LoD (1.0E+01 $TCID_{50}$/mL). Testing at this concentration resulted in detection of the analyte in 19/20 and 20/20 replicates on FilmArray RP2 and BioFire RP2.1, respectively. However, the analyte was also detected in all replicates (20/20) on both panels at the 0.1× LoD concentration.

(b) (4)

(b) (4) more robust detection at the 0.1x level (Table 19) suggests that the substitute culture has a slightly higher nucleic acid concentration compared to the original culture at the same $TCID_{50}$/mL. Based on the

data provided, it appears that the BioFire RP2.1 hMPV amplification and detection are equivalent to FilmArray RP2 and the existing claimed LoD concentration (1.0E+01 $TCID_{50}$/mL and 1.2E+03 copies/mL) appears to be applicable to FilmArray RP2 and BioFire RP2.1.

Table 17. Comparison of Results at 1.0E+01 $TCID_{50}$/mL for Different Cultures of Human Metapneumovirus (Zeptometrix 0810161CF)

(b) (4)

Table 18. Human Metapneumovirus LoD Verification and Confirmation Results (Zeptometrix 0810161CF) (BioFire RP2 and BioFire RP2.1)

(b) (4)

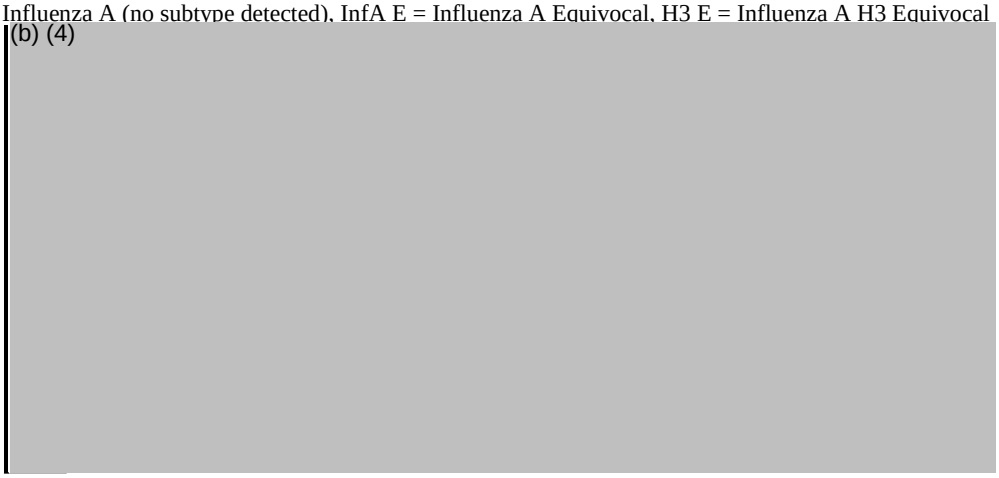
(b) (4)

For Influenza A detection, the BioFire RP2.1 contains five assays, and including three assays used to differentiate the Influenza A hemagglutinin type. Testing for Influenza A H3 with BioFire FilmArray RP2 at the BioFire FilmArray RP2 LoD concentration generated 18/20 Influenza H3 Detected results, with Influenza A H3 Equivocal results for the other two replicates. Retesting is typically recommended for an Equivocal result, however, the retesting was not performed for these two sample replicates (Table 19). The BioFire RP2.1 data (20/20 at the 1× concentration and 4/20 at the 0.1× concentration) confirm that the concentration tested is an appropriate LoD for Influenza A H3 detection by BioFire RP2.1. The 2.1E+01 copies/mL confirmed LoD concentration is the same for each panel.

Table 19. Influenza A H3 LoD Verification and Confirmation Results (A/Port Chalmers/1/73, ATCC VR-810)

NSD = Influenza A (no subtype detected), InfA E = Influenza A Equivocal, H3 E = Influenza A H3 Equivocal

(b) (4)



(b) (4)

Chlamydia pneumoniae is the only analyte tested where results indicate a potential difference in LoD between the panels. The first sample prepared was detected in 20/20 replicates (100%) with the BioFire FilmArray RP2 test and in only 16/20 replicates in the BioFire RP2.1 testing. (b) (4) , a second sample was prepared at the same concentration and tested on both panels again, with the same detection results (20/20 BioFire FilmArray RP2 and 16/20 BioFire RP2.1).

Table 20. *Chlamydia pneumoniae* LoD Testing Data on BioFire FilmArray RP2 and BioFire RP2.1 Pouches for Two Samples Prepared at the Same Concentration (1x LoD; 6.6E+01 copies/mL, 1.0E-01 $TCID_{50}$/mL)

(b) (4)

(b) (4)

Testing was then performed with the BioFire RP2.1 pouches with a sample containing *C. pneumoniae* at a 2-fold higher concentration (1.3E+02 copies/mL) and the analyte was detected in all replicates (20/20, 100%) at the 1× concentration and in <95% of the replicates tested at the 0.1× concentration (9/20, 45% at 1.3E+01 copies/mL; Table 21). The data confirm a **revised LoD for *C. pneumoniae* of 1.3E+02 copies/mL**. Analytical reactivity (inclusivity) testing was performed for *C. pneumoniae* based on the revised LoD concentration.

Table 21. *Chlamydia pneumoniae* LoD Testing Data on BioFire RP2.1 at a Revised LoD Concentration (1.3E+02 copies/mL)

(b) (4)

Limit of Detection for other (non-SARS-CoV-2) BioFire RP2.1 Analytes (Saline specimens)

When possible, testing was completed with the same stock/lot used to verify and confirm the LoD of the BioFire RP2.1 in VTM (results for 18/25 analytes of the RP2.1 panel are shown in Table 22). Among these, five analytes did not initially meet the acceptance criteria. These are indicated in the table by the addition of results from comparing to samples prepared in VTM. Additional evaluations were performed for these analytes including Adenovirus C, Coronavirus HKU1, and Respiratory Syncytial Virus. The SARS-CoV-2 detection rate in aNPSs is detailed above.

Table 22. Limit of Detection (LoD) for BioFire Respiratory Panel 2.1 (RP2.1) Analytes in Saline.

Analyte	Isolate	LoD Concentration	Detection Results			
			Saline[a]		VTM[b]	
			1× LoD	0.1× LoD	1× LoD	0.1× LoD
Viruses						
Adenovirus	Species C Serotype 2 WHO Int'l Standard NIBSC 16/324	3.0E+03 IU/mL[c] (3.0E+03 copies/mL)[e]	**20/20[d] 100%**	**20/20[d] 100%**	**20/20 100%**	**18/20 90%**
	Species F Serotype 41 ATCC VR-930	1.0E+00 $TCID_{50}$/mL 1.1E+03 copies/mL	**20/20 100%**	**7/20 35%**		
Coronavirus 229E	ATCC VR-740	4.0E-01 $TCID_{50}$/mL 6.5E+01 copies/mL	**20/20 100%**	**8/20 40%**		
Coronavirus HKU1	Clinical NPS specimen (53727)	2.0E+03 copies/mL	**18/20 90%**	**6/20 30%**	**17/20 85%**	**5/20 25%**
Coronavirus NL63	BEI NR-470	2.5 E-01 $TCID_{50}$/mL 5.4E+01 copies/mL	**20/20 100%**	**10/20 50%**		
			100%	**10%**		
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	USA-WA1/2020 ATCC VR-1986HK (heat inactivated)	5.0E+02 copies/mL[f] 2.1E-01 $TCID_{50}$/mL	**17/20[g] 85%**	**10/20[g] 50%**	**20/20 100%**	**5/20 25%**
Human Metapneumovirus	16, Type A1 IA10-2003 Zeptometrix 0810161CF	1.0E+01 $TCID_{50}$/mL[h]	**20/20 100%**	**20/20 100%**		
Human Rhinovirus/ Enterovirus	Enterovirus D68 ATCC VR-1823	3.0E+02 $TCID_{50}$/mL 2.6E+01 copies/mL	**19/20 95%**	**6/20 30%**		
Influenza A H1[i]	Influenza A H1N1 A/New Caledonia/20/99 Zeptometrix 0810036CF	1.0E+03 $TCID_{50}$/mL 1.4E+02 copies/mL	**20/20 100%**	**14/20 70%**		
Influenza A H1-2009[i]	Influenza A H1N1pdm09 A/Swine/NY/03/2009 Zeptometrix 0810249CF	5.0E-01 $TCID_{50}$/mL 3.3E+02 copies/mL	**19/20 95%**	**0/20 0%**		
Influenza B	B/FL/04/06 Zeptometrix 0810255CF	5.0E+00 $TCID_{50}$/mL 3.4E+01 copies/mL	**19/20 95%**	**10/20 50%**		
Parainfluenza Virus 2	Type 2 Zeptometrix 0810015CF	5.0E-01 $TCID_{50}$/mL 3.0E+01 copies/mL	**20/20 100%**	**6/20 30%**		
Parainfluenza Virus 4	Type 4a Zeptometrix 0810060CF	5.0E+01 $TCID_{50}$/mL 1.6E+03 copies/mL	**20/20 100%**	**4/20 20%**		
Respiratory Syncytial Virus	Type A Zeptometrix 0810040ACF	2.0E-02 $TCID_{50}$/mL 9.0E+00 copies/mL	**16/20[j] 80%**	**2/20[j] 10%**	**16/20 80%**	**7/20 35%**
Bacteria						
			20/20	**11/20**		

***Bordetella parapertussis* (IS*1001*)**	A747 Zeptometrix 0801461	6.0E+01 IS*1001* copies/mL 4.1E+01 CFU/mL	**100%**	**55%**	
***Bordetella pertussis* (*ptxP*)**	A639 Zeptometrix 0801459	1.0E+03 CFU/mL	**20/20 100%**	**12/20 60%**	
Mycoplasma pneumoniae	M129 Zeptometrix 0801579	4.6E+02 copies/mL	**19/20 95%**	**9/20 45%**	

[a] Testing was performed in aNPSs.
[b] Comparison to samples prepared in VTM are shown when initial testing results did not meet the acceptance criteria
[c] IU = International Units.
[d] Results shown are from a second sample containing Adenovirus C tested alongside sample prepared in VTM. In the first 0.1× sample tested on the BioFire RP2.1/RP2.1*plus*, Adenovirus was Detected in 19/20 (95%) replicates (20/20,100% for the 1× sample).
[e] BioFire Diagnostics quantified the WHO International Standard by quantitative real-time PCR to demonstrate that 3.0E+03 IU/mL=3.0E+03 copies/mL.
[f] Concentration of viral RNA in the culture was determined by digital droplet PCR, as indicated on the Certificate of Analysis from ATCC.
[g] Results shown are from a second sample containing SARS-CoV-2 tested alongside sample prepared in VTM. In the first 1× sample tested on the BioFire RP2.1, SARS-CoV-2 was Detected in 18/20 (90%) replicates (3/20, 15% for the 0.1× sample).
[h] A copies/mL concentration for the culture of Human Metapneumovirus that was tested has not been determined.
[i] Detection for Influenza A subtypes is calculated based only on the correct Influenza A (subtype) Detected result. An Equivocal or Influenza A (no subtype detected) result is tallied as a Not Detected result.
[j] Results shown are from a second sample containing Respiratory Syncytial Virus tested alongside sample prepared in VTM. In the first 1× sample tested on the BioFire RP2.1, Respiratory Syncytial Virus was Detected in 15/20 (75%) replicates (4/20, 20% for the 0.1× sample).

For seven analytes (Adenovirus, Coronavirus OC43, Human Rhinovirus/Enterovirus, Influenza A H3, Parainfluenza Virus 1, Parainfluenza Virus 3, and *Chlamydia pneumoniae*), it was necessary to perform testing using different organism stock cultures or lots than were previously evaluated on the RP2.1 device. For these analytes, LoD verification was completed by testing organism at a range of concentrations intended to bracket the LoD in samples prepared in both VTM and aNPSs. This procedure was used to preclude potential quantification differences in stocks used in the original study and subsequent saline media evaluations, that might confound a determination of the potential impact of media type on device performance. Detection results for these analytes indicated overall concordance at similar organism concentrations in both saline and VTM (Table 23).

Table 23. Limit of Detection (LoD) for BioFire Respiratory Panel 2.1 (RP2.1) Analytes that were Evaluated with New Isolates in Saline and VTM.

Analyte	Isolate	1× LoD Concentration	Detection Results			
			Saline[a]		VTM	
			1× LoD	0.1× LoD	1× LoD	0.1× LoD
Adenovirus	Species B Serotype 3 Zeptometrix 0810062CF	1.2E+07 TCID50/mL 3.9E+02 copies/mL[b]	**20/20 100%**	**9/20 45%**	**19/20 95%**	**12/20 60%**
Coronavirus OC43	Zeptometrix 0810024CF	3.6E+05 $TCID_{50}$/mL 5.6E+01 copies/mL[c]	**20/20 100%**	**11/20 55%**	**19/20 95%**	**12/20 60%**
Human Rhinovirus/ Enterovirus	Human Rhinovirus Type 1A Zeptometrix 0810012CFN	1.3E+06 $TCID_{50}$/mL 3.8E+01 copies/mL[d]	**20/20 100%**	**20/20 100%**	**20/20 100%**	**15/20 75%**
Influenza A H3	Hong Kong/4801/14 ZeptoMetrix 0810526CF	7.2E+05 $TCID_{50}$/mL 2.1E-01 copies/mL[e]	**19/20 95%**	**1/20 5%**	**20/20 100%**	**3/20 15%**
Parainfluenza Virus 1	Type 1 Zeptometrix	4.2E+05 $TCID_{50}$/mL 1.0E+02 copies/mL[c]	**20/20 100%**	**3/20 15%**	**19/20 95%**	**8/20 40%**

Analyte	Isolate	1× LoD Concentration	Detection Results			
			Saline[a]		VTM	
			1× LoD	0.1× LoD	1× LoD	0.1× LoD
	0810014CF					
Parainfluenza Virus 3	Type 3 Zeptometrix 0810016CF	3.4E+07 $TCID_{50}$/mL 3.8E+01 copies/mL[d]	**20/20 100%**	**9/20 45%**	**20/20 100%**	**12/20 60%**
Chlamydia pneumoniae	AR-39 ATCC 53592	2.9E+07 IFU/mL 1.3E+02 copies/mL[d]	**19/20 95%**	**7/20 35%**	**18/20 90%**	**0/20 0%**

[a] Testing was performed in aNPSs.
[b] 1× LoD value is approximately 2-fold lower than the value established when testing original stock in VTM.
[c] 1× LoD value is 10-fold lower than value established when testing original stocks in VTM.
[d] 1× LoD value is equivalent to value established when testing original stocks in VTM.
[e] 1× LoD value is 100-fold lower than value established when testing an original stock in VTM.

e. Analytical Reactivity (Inclusivity):

The BioFire RP2.1 contains assays for the detection of SARS-CoV-2 and multiple other viral and bacterial respiratory pathogens. Assays are designed to detect sequences of clinically relevant strains, serotypes, and/or genotypes of species that cause respiratory illness.

The reactivity of each assay in the BioFire RP2.1 device has been previously evaluated by *in silico* analysis and laboratory testing in studies for the performance evaluation of the BioFire RP2, except for SARS-CoV-2. The reactivity assessment and limitations defined in the previous studies also apply to the same assays in the BioFire RP2.1 because the sample type, assay primers, pouch chemistry and reaction conditions are unchanged. In brief, similar to the previous BioFire RP2 analytical reactivity study, when testing the BioFire RP2.1, each isolate that was evaluated with RP2.1 was prepared as a contrived sample in transport medium at a concentration near (3x) LoD and then tested in triplicate. Reactivity was established when the isolate was detected at a near-LoD concentration in 3/3 or 4/5 replicates, and any isolate or sequence with an observed or predicted issue with detection (i.e., requiring more than 10-fold LoD concentration) is defined as an assay limitation.

The Detection Limit section describe the limits of detection for the analytes in the RP2.1 panel. The focus of this reactivity study for the RP2.1 device was on evaluating the reactivity of the SARS-CoV-2 assays and revising or expanding the reactivity assessment for a small number of analytes with updated limits of detection (i.e., Adenovirus and *C. pneumoniae*).

***In Silico* Reactivity Analysis of SARS-CoV-2 Sequences**

Evaluation of inclusivity (analytical reactivity) for the BioFire RP2.1 SARS-CoV-2 assays (SARSCoV2-1 and SARSCoV2-2) was based on *in silico* analysis of sequences from the NCBI and GISAID databases as of February 21, 2021.

In total, 467,066 sequences from around the globe were aligned to the assay primers. The sequences evaluated included the following lineages and variants of

concern (VOC) or variants under investigation (VUI) that may have important epidemiological, immunological, or pathogenic properties from a public health perspective:

- A.23 lineage (Uganda)
  - VUI-202102/01 (A.23.1 with E484K in Spike)
- B.1.1, B1.1.7, B.1.258 lineages (United Kingdom; Δ69-70 and N501Y in Spike)
  - VOC-202012/01 (B.1.1.7)
  - VOC-202102/02 (B.1.1.7 with E484K in Spike)
- B.1.1.28 lineage (Brazil)
  - VOC-202101/02 - P1 variant (Brazil/Japan)
  - VUI-202101/01 - P2 variant (Brazil)
- B.1.1.318 (United Kingdom)
  - VUI-202102/04
- B.1.351 lineage (South Africa)
  - VOC-202012/02 (501Y.V2 in Spike)
- B.1.429 lineage (United States)
  - CAL.20C variant
- B.1.525 lineage (United Kingdom)
  - VUI-202102/03 or UK1188
- B.1.526 (United States)

All lineages and variants of public health interest identified as of February 2021 are predicted to be detected. Approximately 1.2% of the sequences (5,405/467,066) have a mismatched base within the 3' half of a primer that may affect one assay, but will be detected by the second assay. Both SARS-CoV-2 assays of the BioFire RP2.1 device are predicted to be impaired for nine sequences (9/467,066) evaluated. In summary, this analysis determined that 99.998% (467,057/467,066) of the database sequences evaluated will be amplified by at least one of the SARS-CoV-2 assays in the BioFire RP2.1 device.

The analysis is summarized in the following table—

Table 24. *In silico* Prediction of SARS-CoV-2 Detection by the BioFire RP2.1 Assays[a]

Predicted Assay Result / # sequences		SARSCoV2-1 +	SARSCoV2-1 -	# (%) sequences predicted to be detected with no limitations (one or both assays positive)
SARSCoV2-2	+	461,652	4581[b]	467,057/467,066 (99.998%)**
	-	824	9[c]	

[a] +/+ indicates detected by both assays with no impairment, +/- indicates detection by one assay with no impairment and potential for impaired detection by the other assay, -/- indicates potential for impaired detection by both assays.
[b] Includes sequences of lineage B.1.525 (VUI-202102/03), which has a mutation in the Spike gene that is predicted to impair detection by the SARS-CoV2-1 assay, but detection by the SARSCoV2-2 (Membrane gene) assay is predicted to be unaffected.
[c] Nine sequences have mismatches in the 3' half of primer(s) for both the SARSCoV2-1 and SARSCoV2-2 assays. The mismatches are predicted to impair detection for these sequences.

Empirical Evaluations for the Reactivity Analysis of SARS-CoV-2 Sequences

The *in silico* reactivity predictions were supplemented with empirical experiments to estimate the actual detection impact of the mismatches observed in two sequences (as indicated in the above *In silico* Prediction of SARS-CoV-2 Detection by the BioFire RP2.1 Assays table summary). Briefly, synthetic double-stranded gene fragments, (i.e., gBlocks) were synthesized for the regions of the Spike (S) gene amplified by the SARSCoV2-1 assay and the Membrane (M) gene amplified by the SARSCoV2-2 assay. One construct for each gene carried the consensus sequence with a perfect match to the assay primers (control) while the other was generated with the mismatches to primers, as indicated by the Oct 24, 2020 database alignment. Dilutions of the control and mismatch constructs were tested with the BioFire RP2.1 device.

Testing verified that the 3' terminal mismatch to one of the inner primers of the SARSCoV2-1 assay had a significant (10,000-fold) impact on amplification and detection relative to the control sequence. Further, a mismatch that is 7 bases in from the 3' end of a SARS-CoV2-2 inner primer had a much less impact (10-fold) on amplification and detection relative to the control sequence.

The analysis indicated that as of Oct 24, 2020, the BioFire RP2.1 device can detect the analyzed sequences though an impairment or limit of detection is predicted at low concentrations (≤10x LoD) for 2/130,788 sequences.

Reactivity for Other BioFire RP2.1 Analytes

The BioFire RP2.1 differs from the BioFire FilmArray RP2 by the addition of primers for the detection of SARS-CoV-2. The assay primers and reaction conditions for the viral and bacterial analytes shared between panels are unchanged, and testing has demonstrated that, with the exception of the updated Adenovirus evaluations based on the WHO International Standard and *C. pneumoniae*, the analytical LoD is unchanged for the assays shared between the two panels. The analytical inclusivity testing performed for the assessment of FilmArray RP2 assays (see K170604) were applied to the corresponding BioFire RP2.1 analytes with the following exceptions—

- Testing of adenovirus isolates (same as in the RP2 study) but based on the BioFire RP2.1 Adenovirus LoD newly established with the WHO International Standard (3.0E+03 IU/mL or copies/mL)
- Testing of *C. penumoniae* isolates (same isolates as in RP2) but based on the LoD established for the BioFire RP2.1

Analytical reactivity testing for adenovirus with the BioFire RP2.1 included evaluating over 25 different isolates representing all but two for the known serotypes within the species associated with respiratory infection (i.e., B, C, and E) and representative serotypes for the other species. Adenovirus B serotype 55 and Adenovirus C serotype 57 were not tested but predicted to be detected efficiently with sequence analysis. At least one representative of each serotype within the Species A and F was tested, while only three different serotypes were tested as representative of species D supplemented with sequence analysis that predicted all other D serotypes

would be detected by the Adenovirus assays. An isolate of species G was not available for testing but *in silico* evaluation predicts that the Adenovirus assays react with species G, serotype 52. Overall, testing in triplicate demonstrated that all isolates were detected as expected on the BioFire RP2.1 at a concentration at or below 3x LoD (Table 26). Note that isolates tested in the Detection Limit study were not re-tested in this inclusivity testing but are listed for reference.

Table 25. Results for Adenovirus Inclusivity Testing on BioFire RP2.1
Isolates highlighted grey were tested in the BioFire RP2.1 LoD study and results are compiled from 20 replicates.

Species	Serotype	Isolate ID	Strain	Adenovirus				(b) (4)
				xLoD	Test Concentration (copies/mL)[a]	#Detected /Total	Result	
A	Serotype 12	**ATCC VR-863**	Huie	3x	9.0E+03 copies/mL	**3/3**	D D D	
	Serotype 18	**ATCC VR-19**	Washington D.C./1954	3x	9.0E+03 copies/mL	**3/3**	D D D	
	Serotype 31	**Zeptometrix 0810073CF**	-	3x	9.0E+03 copies/mL	**3/3**	D D D	
B	Serotype 3	**Zeptometrix 0810062CF**	-	3x	9.0E+03 copies/mL	**3/3**	D D D	
	Serotype 7A	**Zeptometrix 0810021CF**	-	0.3x	8.7E+02 copies/mL	**20/20**	D	
	Serotype 7d/d2	**UIRF**	Iowa/2001	3x	9.0E+03 copies/mL	**3/3**	D D D	
	Serotype 7h	**UIRF**	Iowa/1999	3x	9.0E+03 copies/mL	**3/3**	D D D	
	Serotype 11	**ATCC VR-12**	-	3x	9.0E+03 copies/mL	**3/3**	D D D	
	Serotype 14	**ATCC VR-15**	-	3x	9.0E+03 copies/mL	**3/3**	D D D	
	Serotype 16	**ATCC VR-17**	CH.79/Saudi Arabia/1955	3x	9.0E+03 copies/mL	**3/3**	D D D	
	Serotype 21	**ATCC VR-1833**	-	3x	9.0E+03 copies/mL	**3/3**	D D D	
	Serotype 34	**ATCC VR-716**	Compton/1972	3x	9.0E+03 copies/mL	**3/3**	D D D	
	Serotype 35	**ATCC VR-718**	Holden	3x	9.0E+03 copies/mL	**3/3**	D D D	

(b) (4)

	Serotype 50	**ATCC VR-1602**	Wan/Amsterdam/1988	3x	9.0E+03 copies/mL	**3/3**	D D D
C	Serotype 1	**Zeptometrix 0810050CF**	-	3x	9.0E+03 copies/mL	**3/3**	D D D
	Serotype 2	**ATCC VR-846**	Adenoid 6	3x	9.0E+03 copies/mL	**3/3**	D D D
		NIBSC 16/324	WHO International Standard	1x	3.0E+03 copies/mL	**20/20**	D
	Serotype 5	**Zeptometrix 0810020CF**		3x	9.0E+03 copies/mL	**3/3**	D D D
	Serotype 6	**ATCC VR-6**	Tonsil 99/Wash DC	3x	9.0E+03 copies/mL	**3/3**	D D D
D	Serotype 8	**Zeptometrix 0810069CF**	-	3x	9.0E+03 copies/mL	**3/3**	D D D
	Serotype 20	**Zeptometrix 0810115CF**	-	3x	9.0E+03 copies/mL	**3/3**	D D D
	Serotype 37	**Zeptometrix 0810119CF**	-	3x	9.0E+03 copies/mL	**3/3**	D D D
E	Serotype 4a	**UIRF 4a**	S. Carolina/2004	3x	9.0E+03 copies/mL	**3/3**	D D D
	Serotype 4	**Zeptometrix 0810070CF**	-	3x	9.0E+03 copies/mL	**3/3**	D D D
F	Serotype 40	**Zeptometrix 0810084CF**	-	3x	9.0E+03 copies/mL	**4/5**	D ND D D D
	Serotype 40	**NCPV 0101141v**	-	3x	9.0E+03 copies/mL	**3/3**	D D D
	Serotype 41	**ATCC VR-930**	Tak/73-3544/Netherlands/1973	0.4x	1.1E+03	**20/20**	D
		Zeptometrix 0810085CF	-	3x	9.0E+03 copies/mL	**3/3**	D D D

[a] All isolates were quantified by a quantitative real-time PCR assay (R-GENE, BioMerieux).

Analytical reactivity testing of *C. pneumoniae* isolates was performed according to the testing procedure followed for the FilmArray RP2 inclusivity study. Each isolate was prepared in VTM matrix at 3× the 1.3E+02 copies/mL BioFire RP2.1 LoD for this analyte and each was detected in the requisite 3/3 or 4/5 replicates tested (Table 27). The LoD isolate data (in grey) are shown for reference.

Table 26. BioFire RP2.1 Inclusivity Testing Results for *C. pneumoniae* Based on the Revised LoD

Source/ Isolate ID	Strain/Location/ Year Isolated	Concentration Tested	xLoD	# Detected /Total	Result
ATCC[a] VR-2282	TW-183	1.3E+02 copies/mL	1x	20/20	D
ATCC VR-1310	CWL-029	3.9E+02 copies/mL	**3x**	**4/5**	ND
					D
					D
					D
					D
ATCC VR-1360	CM-1/Georgia		**3x**	**3/3**	D
					D
					D
ATCC 53592	AR-39/Seattle/1983		**3x**	**3/3**	D
					D
					D

(b) (4)

Summary of BioFire RP2.1 Analytical Reactivity

Cumulatively, through the *in silico* and empirical analytical reactivity assessments, including those from shared assays in the BioFire RP2, 179 different viral and bacterial isolates were detected within 10x LoD.

It is notable that the Influenza A assays will react variably with non-human influenza A viruses and some rarely encountered human influenza A viruses that are not H1, H1-2009, or H3; generally producing Influenza A Equivocal or Influenza A (no subtype detected) results. Also, not evaluated in this study but in previous BioFire RP2 evaluations, the BioFire RP2.1 *Bordetella pertussis*, Influenza A (subtype), Influenza B and Human Rhinovirus/Enterovirus assays are predicted to react with the viral nucleic acids in *B. pertussis*, influenza, and poliovirus vaccines respectively. Thus, results should be interpreted with caution for specimens obtained from patients with such vaccinations.

The BioFire RP2.1 analytical reactivity test data is summarized as follows—

Table 27. Summary of BioFire RP2.1 Analytical Reactivity Testing and *in silico* Analysis[a]

RP2.1 Analyte/Result		# of Isolates Detected	xLoD Detected	Description of Isolates Tested and Detected	Limitations
Viruses					
Adenovirus	Species A	3	3x	A12, A18, A31	None[b]
	Species B	11	3x	B3, B7, B11, B14, B16, B21, B34, B35, B50	
	Species C	5	3x	C1, C2, C5, C6	
	Species D	3	3x	D8, D20, D37	

RP2.1 Analyte/Result		# of Isolates Detected	xLoD Detected	Description of Isolates Tested and Detected	Limitations
	Species E	2	3x	E4 and E4a	
	Species F	4	3x	F40 and F41	
Coronavirus 229E		2	3x	Group 1 Coronavirus CoV-229E	None
Coronavirus HKU1		5	3x	Group 2 Coronavirus HKU1 Clinical specimens from 2010 and 2015	
Coronavirus NL63		2	3x	Group 1 Coronavirus CoV-NL63	
Coronavirus OC43		2	3x	Group 2 Coronavirus CoV-OC43	
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)		1[c]	1x	USA-WA1/2020	
Human Metapneumovirus		11	3x	Genotypes: A1, A2, B1, B2 Subtypes: 3, 4, 5, 8, 9, 13, 16, 18, 20, 22, 27	None
Human Rhinovirus/ Enterovirus	Human Rhinovirus	14	3x	Species A: A1, A2, A7, A16, A34, A57, A77, A85 Species B: B3, B14, B17, B27, B42, B83	None[d]
	Enterovirus	11	3x	Species: A, B, C, D Coxsackievirus A9, A10, A21, A24 Coxsackievirus B3, B4 Echovirus 6, 9, 11 Enterovirus 71, D68 (2014)	
Influenza A H1		13	Up to 10x[e]	Human and swine Influenza A H1N1 isolates from the 1930s to 2007 and H1N2 recombinant virus	None for Human isolates. Variable detection of swine and avian isolates, depending on strain and concentration
Influenza A H1-2009		9	Up to 10x[f]	Influenza A H1N1pdm09 isolates from 2009 and 2012	None
Influenza A H3		10	3x	Influenza A H3N2 isolates from the 1960s to 2012, attenuated vaccine/recombinant H3N2 viruses, and swine variant H3N2 (H3N2v)	None
Influenza A (no subtype detected) or Influenza A (Equivocal)		10	3x[g]	Human H2N2 recombinant Avian H2N3, H5N1, H5N2, H5N3, H5N8, H7N7, H7N9, H10N7 (including isolates from human infection)	Variable results reported, depending on strain and concentration
Influenza B		13	3x	Influenza B isolates from the 1940s to 2012 and attenuated vaccine/recombinant viruses	None
Parainfluenza Virus 1		4	3x	Multiple strains of PIV1	None
Parainfluenza Virus 2		2	3x	Multiple strains of PIV2	
Parainfluenza Virus 3		3	3x	Multiple strains of PIV3	
Parainfluenza Virus 4		4	3x	Subtypes: A and B	
Respiratory Syncytial Virus		7	Up to 10x[h]	Subtypes: A and B	None
Bacteria					
***Bordetella parapertussis* (IS*1001*)**		6	3x	Strains of *B. parapertussis* and *B. bronchiseptica* containing IS*1001*[i]	None[j]
Bordetella pertussis (ptxP)		9	3x	Strains of *B. pertussis*	None
Chlamydia pneumoniae		4	3x	Strains of *C. pneumoniae*	None
Mycoplasma pneumoniae		9	3x	Strains of *M. pneumoniae*	None

[a] Includes limitations observed in testing and/or predicted by *in silico* analyses. Data for Adenovirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), and *C. pneumoniae* are from testing with BioFire RP2.1. The remaining reactivity summary data are from the BioFire FilmArray RP2 inclusivity study.

[b] *In silico* analysis of available sequences predicts that the BioFire RP2.1 will react with all currently characterized serotypes of Adenovirus, including B55, C57, several species D serotypes and G52, which were not tested.
[c] The reactivity assessment for SARS-CoV-2 also included fifty clinical specimens from the 2020 pandemic collected in three geographic regions of the United States.
[d] *In silico* analysis of available sequences predicts that the HRV/EV assay will react with all currently characterized species and serotypes of Human Rhinovirus and Enterovirus, including Polioviruses.
[e] Influenza A H1N1 isolates ATCC VR-95 (H1N1 PR/8/1934) and swine variant Hsw1N1, ATCC VR-897 (HswN1 A/New Jersey/8/76) were detected as Influenza A H1 at 10×LoD. All other H1N1 isolates were detected as Influenza A H1 at 1× or 3× LoD.
[f] Influenza A H1N1pdm09 isolate BEI NR-44345 (Hong Kong/H090-761-V1(0)/2009) was detected as Influenza A H1-2009 at 10× LoD. All other H1N1pdm09 isolates tested were detected as Influenza A H1-2009 at 1× or 3× LoD.
[g] All human and avian isolates (or genomic RNA) tested were reported as either Influenza A Equivocal or Influenza A (no subtyped detected) at 3× LoD. Three strains reported as Influenza A Equivocal at the 3× LoD concentration were reported as Influenza A (no subtype detected) when tested at higher concentrations of 10× LoD (H2N2 strains, BEI NR-9679 and BEI NR-2775) or 100× LoD (Avian H2N3 Mallard/Alberta/79/2003). Avian H10N7 (Chicken/Germany/N/49; BEI NR-2765) was only reported as Influenza A Equivocal, even at a concentration equal to 100× LoD.
[h] Respiratory Syncytial Virus, subtype B, ATCC VR-1580 (Washington DC/1962) was detected at 10× LoD. All other RSV isolates tested (subtypes A and B) were detected at 1× or 3× LoD.
[i] Reactivity with IS*1001* sequences in *B. bronchiseptica* represents the intended reactivity of the assay, but the analyte will be inaccurately reported as *B. parapertussis*. The assay is not expected to react with IS*1001*-like sequences in *B. holmesii*.

f. Analytical Specificity/Cross-reactivity:

The potential for cross-reactivity between all BioFire RP2.1 assays (including SARS-CoV-2) and various on-panel or off-panel organisms that may be present in clinical respiratory specimens was evaluated with a combination of empirical testing and *in silico* analysis. The isolates tested represent all organisms evaluated on the previous-generation respiratory panel (i.e., BioFire FilmArray Respiratory Panel 2 (RP2)) as well as additional isolates selected to assess the specificity of the novel SARS-CoV-2 assays.

Over 25 on-panel organisms (Table 29) were tested at high concentrations to assess the potential for intra-panel cross-reactivity. For off-panel testing, organisms were selected that represent normal respiratory flora and pathogens that may be present in the respiratory tract, as well as near-neighbors or species genetically related to the organisms detected by the panel. The off-panel isolates included more than 65 bacterial, viral, and fungal species, including common causes of pharyngitis and upper or lower respiratory infection as well as other SARS or SARS-like coronaviruses (Table 30). On-panel and off-panel isolates were tested at the highest concentration possible (generally ≥1.0E+07 units/mL for bacteria and fungi and ≥1.0E+05 units/mL for viruses). These concentrations are equal to or greater than those tested on the FilmArray RP2. Each isolate was tested in triplicate (once on each of the three different pouch/reagent lots) according to standard testing procedure.

Most bacterial and fungal isolates were grown in-house using traditional culture methods and quantified in CFU/mL (plate counting or turbidity standards) or cells/mL (optical density (OD600) reading). Some difficult-to-culture organisms, such as obligate intracellular bacteria, were cultured by an outside source laboratory and documented, with a Certificate of Analysis, in standard quantification units (CFU/mL or cells/mL), infectivity units (TCID50 or IFU), or other measures such as color changing units (CCU) or cell counts (i.e. nuclei/mL).

Viruses were cultured by outside source laboratories and provided as culture fluids quantified in TCID50/mL or other relevant units (provided on a Certificate of Analysis from the culture collection). Five coronaviruses (SARS-CoV, SARS-CoV-2, MERS-CoV, and two bat SARS-like CoVs) were cultured and tested in a contracted biosafety level 3 laboratory. Two viruses (bocavirus and CoV HKU1) that are not amenable to *in vitro* culture were acquired for testing as clinical specimens and quantified in RNA copies/mL.

Organisms that could not be acquired as intact organism cultures were tested as purified preparations of genomic DNA (gDNA) and reported in units of genomic equivalents per mL (GE/ mL) or were evaluated via directed *in silico* analysis of publicly available whole genome sequences.

Any samples generating unexpected results were retested to determine if the results were reproducible and/or investigated to rule in or rule out contamination, isolate misidentification, or cross-reactivity. Confirmed cross-reactivities or limitations identified in this and previous studies are included in the descriptions for the BioFire RP2.1.

Over 90 organisms were tested with the BioFire RP2.1, and only those few cross-reactivities previously described for the BioFire RP2 assays were identified in the testing (e.g. *Bordetella pertussis* ptxP assay cross-reactivity with the *ptxP* pseudogene in other *Bordetella* species; see Table 32). *In silico* evaluation of the SARS-CoV-2 assays did indicate a risk of cross-reactivity with closely related SARS-like coronaviruses of bat and pangolin origin, although these viruses are not predicted to be present in human clinical specimens.

All known or predicted risks of cross-reactivity for the BioFire RP2.1 are summarized in Table 31. No additional cross-reactivities were identified in this study for previously existing panel assays or for the novel SARS-CoV-2 assays.

Table 28. On-Panel Organisms Tested for Evaluation of BioFire RP2.1 Analytical Specificity

Organism		Isolate ID	Concentration Tested	Cross-Reactivity Detected
Bacteria				
Bordetella parapertussis		Zeptometrix 0801462	6.43E+09 CFU/mL	***Bordetella pertussis* (*ptxp*)** [a]
Bordetella pertussis		ATCC 9797	5.50E+09 CFU/mL	**Human Rhinovirus/Enterovirus** [b]
Chlamydia pneumoniae		ATCC 53592	1.93E+07 IFU/mL	None
Mycoplasma pneumoniae		Zeptometrix 0801579	2.65E+07 CCU/mL	None
Viruses				
Adenovirus	7A (species B)	Zeptometrix 0810021CF	1.02E+07 $TCID_{50}$/mL	None
	1 (species C)	Zeptometrix 0810050CF	2.26E+07 $TCID_{50}$/mL	None
	4 (species E)	ATCC VR-1572	1.58E+06 $TCID_{50}$/mL	None

Organism	Isolate ID	Concentration Tested	Cross-Reactivity Detected
Coronavirus 229E	Zeptometrix 0810229CF	1.13E+05 $TCID_{50}$/mL	None
Coronavirus HKU1	Clinical specimen	8.94E+06 RNA copies/mL	None
Coronavirus NL63	Zeptometrix 0810228CF	2.34E+05 $TCID_{50}$/mL	None
Coronavirus OC43	Zeptometrix 0810024CF	6.37E+06 $TCID_{50}$/mL	None
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	USA-WA1/2020	2.4E+09 copies/mL	None
Human Metapneumovirus	Zeptometrix 0810159CF	1.05E+06 $TCID_{50}$/mL	None
Human Rhinovirus (Type 1A)	Zeptometrix 0810012CFN	8.40E+05 $TCID_{50}$/mL	None
Enterovirus (D68)	ATCC VR-1823	1.58E+07 $TCID_{50}$/mL	None
Influenza A H1N1 (A1/FM/1/47)	ATCC VR-97	1.58E+08 $CEID_{50}$/mL	None
Influenza A Hsw N1 (A/NewJersey/8/76)	ATCC VR-897	8.89E+06 $CEID_{50}$/mL	**Influenza A H1-2009** [c]
Influenza A (H1N1) pdm09 (Michigan/45/15)	Zeptometrix 0810538CF	9.40E+04 $TCID_{50}$/mL	None
Influenza A H3N2 (A/Alice)	ATCC VR-776	3.33E+08 $CEID_{50}$/mL	None
Influenza B (Massachusetts/2/12)	Zeptometrix 0810239CF	9.55E+05 $TCID_{50}$/mL	None
Parainfluenza Virus 1	Zeptometrix 0810014CF	6.80E+07 $TCID_{50}$/mL	None [d]
Parainfluenza Virus 2	Zeptometrix 0810357CF	4.57E+06 $TCID_{50}$/mL	None [d]
Parainfluenza Virus 3	ATCC VR-93	6.80E+07 $TCID_{50}$/mL	None
Parainfluenza Virus 4	ATCC VR-1377	4.17E+04 $TCID_{50}$/mL	None
Respiratory Syncytial Virus	Zeptometrix 0810040ACF	7.00E+05 $TCID_{50}$/mL	None

[a] *Bordetella pertussis* (*ptxP*) assay may amplify pertussis toxin pseudogene sequences from some strains of *B. parapertussis* at high concentration (>1.2E+09 CFU/mL).

[b] Human Rhinovirus/Enterovirus assay may amplify non-target sequences from *Bordetella* species (*B. pertussis*, *B. parapertussis*, and *B. bronchiseptica*) at a concentration ≥4.5E+07 CFU/mL.

[c] The H1 hemagglutinin (HA) gene of Influenza A H1N1 strains of swine origin (prior to 2009) will be amplified by the H1 assay (Influenza A H1 Detected). However, some strains/sequences of swine origin may also be amplified by the H1-2009 assay (Influenza A H1-2009 Detected) at higher concentrations. Testing of this strain at 8.89E+06 $CEID_{50}$/mL generated an Influenza A H1 Detected result in 1/3 replicates and an Influenza A H1-2009 Detected in 2/3 replicates.

[d] Parainfluenza Virus 3 (PIV3) was detected in 2/5 replicates of the Parainfluenza Virus 1 (PIV1) isolate tested and in 2/4 replicates of the Parainfluenza 2 isolate tested. Sequencing of the amplicons generated match PIV3 sequences, indicating contamination of both the PIV1 and PIV2 isolate stocks with PIV3 nucleic acid.

Table 29. Off-Panel Organisms Tested for Evaluation of BioFire RP2.1 Analytical Specificity

Organism	Isolate ID	Concentration Tested	Cross-Reactivity Detected/Predicted
Bacteria			
Acinetobacter calcoaceticus	ATCC 23055	5.15E+09 CFU/mL	None
Arcanobacterium haemolyticum	ATCC 9345	5.70E+09 CFU/mL	None
Bacillus anthracis	Evaluated *in silico*		None
Bordetella avium	ATCC 35086	1.88E+09 cells/mL	None
Bordetella bronchiseptica	ATCC 10580	2.09E+09 cells/mL	***Bordetella pertussis* (*ptxp*)** [a]

Organism	Isolate ID	Concentration Tested	Cross-Reactivity Detected/Predicted
Bordetella hinzii	ATCC 51783	4.30E+06 CFU/mL	None
Bordetella holmesii	ATCC 700052	3.15E+07 CFU/mL	None
Burkholderia cepacia	ATCC 17762	5.04E+09 CFU/mL	None
Chlamydia trachomatis	Zeptometrix 0801775	1.67E+08 IFU/mL	None
Chlaymdia psittaci	Evaluated *in silico*		None
Corynebacterium diphtheriae	Zeptometrix 0801882	7.47E+08 CFU/mL	None
Corynebacterium striatum	ATCC BAA-1293	5.20E+09 CFU/mL	None
Coxiella burnetii	Evaluated *in silico*		None
Escherichia coli	CDC AR Bank #0538	5.53E+09 CFU/mL	None
Fusobacterium necrophorum	ATCC 27852	1.33E+08 cells/mL	None
Haemophilus influenzae	ATCC 33391	5.85E+09 CFU/mL	None
Klebsiella (Enterobacter) aerogenes	CDC AR Bank #0074	6.83E+09 CFU/mL	None
Klebsiella oxytoca	JMI 7818	5.60E+09 CFU/mL	None
Klebsiella pneumoniae	NCTC 13465	1.75E+08 CFU/mL	None
Lactobacillus acidophilus	Zeptometrix 0801540	1.60E+08 CFU/mL	None
Lactobacillus plantarum	Zeptometrix 0801507	1.20E+09 CFU/mL	None
Legionella (Fluoribacter) bozemanae	ATCC 33217	3.24E+09 cells/mL	None
Legionella (Fluoribacter) dumoffii	ATCC 33279	2.65E+09 cells/mL	None
Legionella feeleii	ATCC 35849	1.49E+09 cells/mL	None
Legionella longbeachae	Zeptometrix 0801577	1.93E+08 CFU/mL	None
Legionella (*Tatlockia*) *micdadei*	Zeptometrix 0801576	1.80E+09 CFU/mL	None
Legionella pneumophila	Zeptometrix 0801530	1.75E+09 CFU/mL	None [b]
Leptospira interrogans	ATCC BAA-1198D-5 (genomic DNA)	7.89E+08 GE/mL	None
Moraxella catarrhalis	ATCC 8176	5.73E+09 CFU/mL	None
Mycobacterium tuberculosis	Zeptometrix 0801660 (avirulent strain)	9.07E+06 CFU/mL	None
Mycoplasma genitalium	ATCC 33530D (genomic DNA)	8.40E+07 GE/mL	None
Mycoplasma hominis	Zeptometrix 0804011	2.11E+09 CCU/mL	None
Mycoplasma orale	ATCC 19524	1.00E+07 CCU/mL	None
Neisseria elongata	Zeptometrix 0801510	1.99E+08 CFU/mL	None [c]
Neisseria gonorrhoeae	ATCC 19424	2.31E+09 CFU/mL	None
Neisseria meningitidis	ATCC 13090	1.99E+09 CFU/mL	None
Proteus mirabilis	ATCC 12453	5.60E+09 CFU/mL	None
Pseudomonas aeruginosa	ATCC 27853	4.33E+09 CFU/mL	None
Serratia marcescens	JMI 697	4.75E+09 CFU/mL	None
Staphylococcus aureus (MRSA)	ATCC 10832	1.88E+08 CFU/mL	None
Staphylococcus epidermidis	ATCC 29887	4.95E+09 CFU/mL	None
Stenotrophomonas maltophilia	ATCC 700475	4.93E+09 CFU/mL	None
Streptococcus agalactiae	ATCC 13813	5.45E+09 CFU/mL	None
Streptococcus dysgalactiae	ATCC 43078	5.70E+09 CFU/mL	None

Organism	Isolate ID	Concentration Tested	Cross-Reactivity Detected/Predicted
Streptococcus pneumoniae	ATCC BAA-341	5.20E+09 CFU/mL	None
Streptococcus pyogenes	ATCC 19615	5.46E+07 CFU/mL	None
Streptococcus salivarius	ATCC 13419	4.92E+09 CFU/mL	None
Ureaplasma urealyticum	ATCC 27618	1.00E+08 CCU/mL	None
Viruses (SARS-CoV-2 Related Coronaviruses)			
Bat SARS-like Coronavirus	BEI NR-44009 (Recombinant with SARS Urbani RBD)	3.15E+06 $TCID_{50}$/mL	None
Bat SARS-like Coronavirus HKU5	BEI NR-48814 (Recombinant with SARS Urbani SE)	1.95E+06 $TCID_{50}$/mL	None
Severe Acute Respiratory Syndrome Coronavirus (SARS)	BEI NR-18925 Urbani strain	5.3E+09 copies/mL	None
Viruses			
Bocavirus	Clinical specimen	1.40E+08 copies/mL	None
Cytomegalovirus (CMV)	Zeptometrix 0810003CF	7.67E+06 $TCID_{50}$/mL	None
Epstein-Barr Virus (EBV)	Zeptometrix 0810008CF	3.65E+07 copies/mL	None
Herpes Simplex Virus 1 (HSV1)	ATCC VR-1778	3.30E+08 copies/mL	None
Herpes Simplex Virus 2 (HSV2)	Zeptometrix 0810217CF	1.30E+07 $TCID_{50}$/mL	None
Human Herpes Virus 6 (HHV6)	Zeptometrix 0810072CF	4.11E+08 copies/mL	None
Human Parechovirus (HPeV)	Zeptometrix 0810147CF	2.26E+07 $TCID_{50}$/mL	None
Influenza C	Evaluated *in silico*		None
Measles Virus	Zeptometrix 0810025CF	1.63E+05 $TCID_{50}$/mL	None
Mumps	Zeptometrix 0810079CF	4.83E+05 units/mL	None
Fungi			
Aspergillus flavus	Zeptometrix 0801598	1.15E+08 CFU/mL	None
Aspergillus fumigatus	Zeptometrix 0801716	5.47E+07 CFU/mL	None
Blastomyces dermatitidis	ATCC 26199D-2 (genomic DNA)	7.05E+07 GE/mL	None
Candida albicans	ATCC 10231	1.19E+06 CFU/mL	None
Cryptococcus neoformans	ATCC MYA-4564	6.00E+07 CFU/mL	None
Histoplasma capsulatum	Evaluated *in silico*		None
Pneumocystis jirovecii (carinii)	ATCC PRA-159	6.67E+07 nuclei/mL	None

[a] *Bordetella pertussis* (*ptxP*) assay may amplify pertussis toxin pseudogene sequences from some strains of *B. bronchiseptica* at high concentration (≥1.2E+09 CFU/mL).

[b] *Bordetella parapertussis* (IS*1001*) was detected in 3/3 replicates. Amplification with an alternate *B. parapertussis*/IS*1001* PCR assay confirmed the presence of IS*1001* nucleic acid in the *L. pneumophila* stock (contamination).

[c] *Mycoplasma pneumoniae* was detected in 3/3 replicates. Amplification with an alternate *M. pneumoniae* PCR assay (*gyrB* gene) confirmed the presence *M. pneumoniae* nucleic acids in the *N. elongata* stock (contamination).

Table 30. Predicted and Observed Cross-Reactivity of the BioFire RP2.1

Cross-reactive Organism(s)/Sequence(s)	BioFire RP2.1 Result	Description
Bat coronavirus RaTG13[2] (accession# MN996532) **Pangolin coronavirus**[3] (accession# MT08407) **Bat SARS-like coronavirus** (accession# MG772933 and MG772934)	**Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)**	The SARS-CoV-2 assays can amplify a small selection of sequences from closely related Sarbecoviruses isolated from bats and pangolin. The SARSCoV2-2 assay is predicted to cross-react with all four sequences, while the SARSCoV2-1 assay will likely only cross-react with the bat coronavirus_RaTG13.
Non-pertussis *Bordetella* species *(e.g. Bordetella parapertussis, Bordetella bronchiseptica*[a])	***Bordetella pertussis* (*ptxP*)**[b]	The *Bordetella pertussis* (*ptxP*) assay can amplify pertussis toxin pseudogene sequences in *B. bronchiseptica* and *B. parapertussis*, primarily when present at high concentrations (≥1.2E+09 CFU/mL).
***Bordetella bronchiseptica*[a]** (with IS*1001* sequences)	***Bordetella parapertussis* (IS*1001*)**	Some strains of *B. bronchiseptica* carry IS*1001* insertion sequences identical to those carried by *B. parapertussis*. These sequences will be efficiently amplified by the IS1001 assay and reported by BioFire RP2.1 as *Bordetella parapertussis* (IS*1001*).
Bordetella pertussis ***Bordetella parapertussis*** ***Bordetella bronchiseptica***	**Human Rhinovirus/Enterovirus**[c] [d]	The Human Rhinovirus/Enterovirus assay may amplify off-target sequences found in strains of *B. pertussis*, *B. bronchiseptica*, and *B. parapertussis* when present at high concentration. Cross-reactivity with *B. pertussis* was observed at a concentration of 4.5E+07 CFU/mL or higher.
Influenza A H1N1 (swine origin)	**Influenza A H1-2009**[e]	The Influenza A H1-2009 assay may react with H1 hemagglutinin gene sequences from viruses of swine origin. BioFire RP2.1 will report either Influenza A H1 or Influenza A H1-2009, depending on the strain and concentration in the sample.

[a] *B. bronchiseptica* infection is rare in humans and more common in domesticated animals ('kennel cough').

[b] Cross-reactivity between the *Bordetella pertussis* (ptxP) assay and *B. parapertussis* will be reported as a co-detection (*Bordetella parapertussis* (IS*1001*) Detected and *Bordetella pertussis* (*ptxP*) Detected); while cross-reactivity with most strains of *B. bronchiseptica* (that do not carry IS*1001*) will be reported only as *Bordetella pertussis* (*ptxP*) Detected.

[c] Cross-reactivity with *B. parapertussis* and *B. bronchiseptica* is predicted based on *in silico* analysis but was not observed when tested at a concentration of 1.2E+09 CFU/mL.

[d] Cross-reactivity between the Human Rhinovirus/Enterovirus assays and *B. pertussis* or *B. parapertussis* will be reported as a co-detection (*Bordetella pertussis* (*ptxP*) Detected and Human Rhinovirus/Enterovirus Detected or *Bordetella parapertussis* (IS*1001*) Detected and Human Rhinovirus/Enterovirus Detected); while cross-reactivity with most strains of *B. bronchiseptica* (that do not carry IS*1001*) will be reported (falsely) only as Human Rhinovirus/Enterovirus Detected.

[e] Swine origin Hsw1N1 (A/New Jersey/8/1976 ; ATCC VR-897) was detected as either Influenza A H1 or Influenza A H1-2009 at a concentration of 8.9E+06 CEID50/mL.

Select organisms that could not be acquired for empirical testing were evaluated via *in silico* analysis to assess the potential for cross-reactivity between the novel SARS-CoV-2 assay primers and the organism whole genome sequences. The isolates evaluated include medically relevant select agents (*Bacillus anthracis* and

[2] Peng Zhou et al., "A Pneumonia Outbreak Associated with a New Coronavirus of Probable Bat Origin," *Nature* 579, no. 7798 (March 2020): 270–73, https://doi.org/10.1038/s41586-020-2012-7.

[3] Rachele Cagliani et al., "Computational Inference of Selection Underlying the Evolution of the Novel Coronavirus, SARS-CoV-2," *Journal of Virology*, April 1, 2020, JVI.00411-20, jvi;JVI.00411-20v1, https://doi.org/10.1128/JVI.00411-20.

Coxiella burnetii), a BSL3 fungal respiratory pathogen (*Histoplasma capsulatum*), a rare zoonotic fungal pathogen (*Chlamydia psittaci*), and a relatively underdiagnosed cause of influenza infection (Influenza C virus).

Analysis involved retrieval of at least three representative whole genome sequences for each organism from the publicly available GenBank database and subsequent BLAST analysis to identify regions with greater than 80% homology to the SARS-CoV-2 assay primers. Homology was evaluated for both outer and inner assay primers, although only reactivity with inner primers is essential to generate a cross-reactive result using the nested FilmArray system. When regions with >80% homology to at least one inner assay primer were identified, the sequences were further assessed to determine the potential for bidirectional amplification and generation of a detectable amplicon sequence (<2000 bp).

Although some regions with >80% homology to individual outer or inner primer sequences were identified (see Table 32), no primer binding sites were located in an orientation to allow for bi-directional amplification of complementary strands nor within sufficient proximity to generate an appropriately sized, detectable amplicon. As a result, no risk of cross-reactivity was identified for the organisms evaluated.

Table 31. *In silico* Evaluation of Potential Cross-Reactivity Between SARS-CoV-2 Assay Primers and Select Off-Panel Organisms

Percent homology under SARS-CoV-2 assay outer forward (OF), inner forward (IF), inner reverse (IR), and outer forward (OF) primers. Results for outer reaction primers are shaded grey. Sequences with >80% homology to an inner assay primer are shaded green. Sequences with no homology to assay primers are denoted NH (no homology).

Organism	GenBank Accession No.	SARSCoV2-1 Assay Primers				SARSCoV2-2 Assay Primers				Predicted Cross-reactivity
		OF	IF	IR	OR	OF	IF	IR	OR	
Bacillus anthracis	NC_005945.1	NH	NH	NH	NH	85%	83%	NH	81%	None
	CP012728.1	NH	NH	NH	NH	85%	83%	NH	81%	
	NZ_KN050648.1	NH	NH	NH	NH	85%	83%	NH	81%	
Chlamydia psittaci	NC_020248.1	NH	NH	NH	NH	85%	NH	NH	81%	None
	NC_015470.1	NH	NH	NH	NH	85%	NH	NH	81%	
	NZ_KE355746.1	NH	NH	NH	NH	85%	NH	NH	81%	
Coxiella burnetii	NC_002971	81%	NH	NH	NH	80%	NH	NH	NH	None
	CP000733	81%	NH	NH	NH	80%	NH	NH	NH	
	CP007555	81%	NH	NH	NH	80%	NH	NH	NH	
	CP018150	81%	NH	NH	NH	80%	NH	NH	NH	
	HG825990	81%	NH	NH	NH	80%	NH	NH	NH	
	NC_004704	NH	NH	NH	NH	80%	NH	NH	NH	
	CP000735	NH	NH	NH	NH	80%	NH	NH	NH	
	CP000914	NH	NH	NH	NH	80%	NH	NH	NH	
Histoplasma capsulatum	ABBT00000000	81%	NH	NH	NH	80%	83.3%	81.8%	81%	None
	AAJI000000000	81%	NH	NH	NH	80%	83.3%	81.8%	81%	

Organism	GenBank Accession No.	SARSCoV2-1 Assay Primers				SARSCoV2-2 Assay Primers				Predicted Cross-reactivity
		OF	IF	IR	OR	OF	IF	IR	OR	
	ABBS00000000	81%	NH	NH	NH	80%	83.3%	81.8%	81%	
	ABRJ00000000	81%	NH	NH	NH	80%	83.3%	81.8%	81%	
	ABRK00000000	81%	NH	NH	NH	80%	83.3%	81.8%	81%	
Influenza C Virus	1419 Sequences Evaluated[a]	NH	NH	NH	NH	NH	NH	NH	NH	None

[a] GenBank accession numbers evaluated for Influenza C cross-reactivity analysis were provided separately.

g. *Assay cut-off:*

Not applicable

h. *Interfering Substances:*

Testing for possible interference from select substances that may be present in nasopharyngeal swabs (NPS) was performed with the BioFire RP2.1. The majority of interference data was collected in the interference study performed with the predecessor panel, BioFire FilmArray Respiratory Panel 2 (RP2). Both the BioFire RP2.1 and BioFire RP2 test the same sample type using the same pouch chemistry and cycling conditions. The only difference between the panels is the addition of the SARS-CoV-2 assays; therefore, the previous BioFire RP2 interference study results remain relevant and are applied to the BioFire RP2.1 as appropriate.

Substances evaluated for interference generally were categorized as—endogenous substances (i.e., biological substances naturally in NPS samples), competing microorganisms (i.e., pathogens or natural flora tested at high concentrations to evaluate polymicrobial NPS specimens), exogenous substances (i.e., non-native substances in NPS samples), or technique-specific substances (i.e., substances introduced during sample processing, collection, or testing).

The endogenous and technique-specific substances, as well as competing microorganisms, evaluated on the BioFire RP2.1 in this study were selected to assess the risk of possible interference with the SARS-CoV-2 assays as well as the other panel assays. Substances were also selected to reproduce a subset of the data collected in the BioFire RP2 study in order to verify that the data collected with one panel could be applied to the other.

Each substance was added to contrived samples containing representative organisms at concentrations near (2-3x) LoD (Table 33). The concentration of substance added to the samples was equal to or greater than the highest level expected to be in NPS specimens. The organisms in the sample included the new analyte (SARS-CoV-2), two analytes included in the RP2 interference study (Adenovirus and *Bordetella parapertussis*), and three additional analytes not previously evaluated in the RP2 interference study (Coronavirus NL63, Influenza A H1N1pdm09, and Respiratory

Syncytial Virus). The test sample was composed to represent the types of analytes detected by the panel, including bacteria and enveloped or non-enveloped viruses with DNA and RNA genomes.

Table 32. Contrived Sample Composition for BioFire RP2.1 Interference Testing

Organism	Description	Source	3x LoD (Sample) Concentration
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	New analyte for RP2.1 RNA genome (+ strand) enveloped virus	ATCC VR-1986HK (heat inactivated)	**1.5E+03 copies/mL**
Adenovirus (Type 2)	DNA genome (double-stranded) non-enveloped virus	WHO International Standard NIBSC 16/324	**9.0E+03 IU/mL**
Coronavirus NL63	RNA genome (+ strand) enveloped virus	BEI NR-470	**7.5E-01 $TCID_{50}$/mL**
Influenza A H1N1pdm09 (H1-2009)	RNA genome (- strand) enveloped virus	Zeptometrix 0810109CFN	**1.5E+00 $TCID_{50}$/mL**
Respiratory Syncytial Virus	RNA genome (+ strand) enveloped virus	Zeptometrix 810040ACF	**6.0E-02 $TCID_{50}$/mL**
Bordetella parapertussis	Gram-negative bacterium DNA genome	Zeptometrix 0801461	**1.8E+02 IS*1001* copies/mL**

Testing near LoD was to identify the effects of even minor interference on analyte detection. A control sample with no substance (positive control) was tested on each day of evaluation to demonstrate the expected detection without any potential interference. Each potential interferent was also added to a negative sample (substance only negative control) and tested in tandem with the corresponding positive sample to serve as a control for the substance alone (e.g. detection of an on-panel competing microorganism). Each type of sample (positive control, negative control, and sample with interfering substance) was tested in triplicate, with one replicate on each of three different pouch lots. The pouch control and analyte results from the spiked sample with potential interferents and negative control samples were compared to the results from the positive control sample replicates to evaluate whether there was interference in detection.

In addition, *in silico* analysis of the SARS-CoV-2 assays during assay development identified some closely related coronaviruses with >80% homology under one or more assay primers. Of those, three (Bat_CoV KY770858, Bat_CoV GU190215 and SARS-like coronavirus KR559017) show the indicated homology to only the outer forward primer and are, therefore, not expected to react with the SARS-CoV-2 assays nor present a risk of interference. However, four sequences from the proposed precursors to SARS-CoV-2 (Bat_CoV_RTG13 MN996532, Pangolin CoV MT084071, and Bat SARS-like coronavirus MG772933 and MG772934) are predicted to cross-react with one or more of the BioFire RP2.1 SARS-CoV-2 assays. These viruses could not be acquired for interference testing. However, none of these

viruses have been isolated from human infections. Therefore, the risk of interference with the presence of these coronaviruses in polymicrobial infections with SARS-CoV-2 appears to be negligible.

The combination of BioFire RP2.1 and BioFire RP2 interference testing evaluated a total of forty-three substances (Table 34). The notable results from testing are summarized as follows—a total of 41 pouch runs were performed on both FilmArray Torch and FilmArray 2.0 systems and all completed with valid results (i.e., no errors). Overall, testing with potentially interfering substances at high, "worst-case scenario" concentrations (Table 35) demonstrated no interference with pouch controls or detection of panel analytes. The only exception was with bleach, resulting in missed detection of various panel analytes likely due to damaged nucleic acids (this was also observed in the previous BioFire RP2 panel). A general warning to avoid contact between samples and bleach is noted.

Endogenous substance interference testing with the BioFire RP2.1 confirmed that presence of blood and human genomic DNA in samples had no effect on detection of low-level SARS-CoV-2 or other analytes, consistent with results obtained with these substances in the BioFire RP2 evaluation. In addition, no interference with SARS-CoV-2 detection was observed when testing a clinically relevant concentration of human peripheral blood mononuclear cells (PBMCs).

Competing microorganism testing with the BioFire RP2.1 showed that there was no effect with a high concentration of another human beta-coronavirus that can cause respiratory illness (Coronavirus OC43), nor a high concentration of a bacterium representing normal flora of the respiratory tract (*Streptococcus salivarius*). During evaluation of the potential *Streptococcus salivarius* interferent, one spiked substance replicate yielded a no detection with the Respiratory Syncytial Virus analyte. However, the lack of detection was not reproducible in the two repeated replicates on the same pouch lot.

For technique-specific substance testing, no interference was observed when samples were prepared and tested in PrimeStore Molecular Transport Medium (MTM).

Table 33. Results from the Evaluation of Potentially Interfering Substance Effects on Analyte Detection - FilmArray RP2 or BioFire RP2.1

Substances tested with the BioFire RP2.1 are in **bold** font. Results for substances tested only with the BioFire FilmArray RP2 previously are applied to BioFire RP2.1

Substance Tested	Concentration Tested	Result
Endogenous Substances		
Human Whole Blood	**10% v/v**	**No Interference**
Human Mucus (Sputum)	1 swab/mL sample	No Interference
Human Genomic DNA	**20 ng/µL**	**No Interference**
Human Peripheral Blood Mononuclear Cells (PBMCs)	**1.0E+03 cells/µL**	**No Interference**
Competitive Microorganisms		
Coronavirus 229E	1.7E+04 $TCID_{50}$/mL	No Interference

Substance Tested	Concentration Tested	Result
Coronavirus OC43 (betacoronavirus)	**9.6E+05 $TCID_{50}$/mL**	**No Interference**
Adenovirus A12	8.9E+05 $TCID_{50}$/mL	No Interference
Parainfluenza Virus 3	6.6E+05 $TCID_{50}$/mL	No Interference
Bordetella pertussis	5.8E+08 CFU/mL	No Interference
Enterovirus D68	1.6E+07 $TCID_{50}$/mL	No Interference
Echovirus 6	1.0E+07 $TCID_{50}$/mL	No Interference
Respiratory Syncytial Virus	4.2E+04 $TCID_{50}$/mL	No Interference
Staphylococcus aureus	2.5E+07 CFU/mL	No Interference
Streptococcus pneumoniae	1.7E+07 CFU/mL	No Interference
Streptococcus salivarius	**2.5E+09 CFU/mL**	**No Interference**
Haemophilus influenzae	6.2E+07 CFU/mL	No Interference
Candida albicans	1.0E+06 CFU/mL	No Interference
Herpes Simplex Virus 1	1.6E+06 $TCID_{50}$/mL	No Interference
Cytomegalovirus	1.2E+06 $TCID_{50}$/mL	No Interference
Exogenous Substances [a]		
Tobramycin (systemic antibiotic)	0.6 mg/mL	No Interference
Mupirocin (active ingredient in anti-bacterial ointment)	2% w/v	No Interference
Saline Nasal Spray with Preservatives (0.65% NaCl, Phenylcarbinol, Benzalkonium chloride)	1% v/v	No Interference
Nasal Decongestant Spray (Oxymetazoline HCl 0.05%, Benzalkonium chloride, phosphate)	1% v/v	No Interference
Analgesic ointment (Vicks®VapoRub®)	1% w/v	No Interference
Petroleum Jelly (Vaseline®)	1% w/v	No Interference
Snuff (Tobacco)	1% w/v	No Interference
Disinfecting/Cleaning Substances		
Bleach	1% and 2% v/v [up to 1024 ppm chlorine]	Interference [b]
Disinfecting wipes (ammonium chloride)	½ in^2	No Interference
Ethanol	7% v/v	No Interference
DNA*Zap* (Ambion AM9891G & AM9892G)	1% v/v	No Interference
RNase*Zap* (Ambion AM9782)	1% v/v	No Interference
Specimen Collection Materials		
Rayon Swabs (Copan 168C)	N/A	No Interference
Nylon Flocked Swabs (Copan 553C)	N/A	No Interference
Polyester Swabs (Copan 175KS01)	N/A	No Interference
Calcium Alginate Swabs (Puritan 25-801 A 50)	N/A	No Interference
M4 Transport Medium (Remel)	100%	No Interference
M4-RT Transport Medium (Remel)	100%	No Interference
M5 Transport Medium (Remel)	100%	No Interference
M6 Transport Medium (Remel)	100%	No Interference
Universal Viral Transport vial (BD)	100%	No Interference
PrimeStore Molecular Transport Medium	**70% v/v**	**No Interference**
Sigma-Virocult Viral Collection and Transport System	100%	No Interference

Substance Tested	Concentration Tested	Result
(Swab and Transport Medium)		
Copan ESwab Sample Collection and Delivery System (Swab and Liquid Amies Medium)	100%	No Interference

[a] Nasal vaccines (e.g. FluMist) were not evaluated, but are predicted to be reactive with the BioFire FilmArray RP2 and BioFire RP2.1 Influenza A (subtype) and Influenza B assays.

[b] Not Detected results were reported for several analytes after incubation of the sample with 2% bleach for 10 minutes or overnight. It was concluded that interference resulted primarily from damage to the organisms/nucleic acids in the sample, rather than inhibition or interference with pouch function(s).

i. Carry-Over Contamination:

A formal carry-over study in support of this regulatory submission for the BioFire RP2.1 was not performed since carry-over studies with high positive samples followed by negative samples have been performed for other FDA-cleared FilmArray Panels that are similar to the RP2.1 (i.e., BioFire FilmArray RP, BCID, and GI) for both the FilmArray 2.0 and the FilmArray Torch systems, and no significant carry-over has been observed.

2. Comparison studies:

a. FDA SARS-CoV-2 Reference Panel Testing:

Quality assessments such as evaluating the BioFire RP2.1 device with the FDA SARS-CoV-2 reference panel, provide additional information on the relative sensitivity and specificity of the included SARS-CoV-2 assays. An evaluation of SARS-CoV-2 sensitivity and MERS-CoV cross-reactivity was performed using reference material, blinded samples, and a standard protocol provided by FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD.

The results are summarized in the following table—

Table 34. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	LoD Concentration	Cross-Reactivity
SARS-CoV-2	NPS in transport medium	**6.0E+03 NDU/mL[a]**	**N/A**
MERS-CoV		**N/A**	**Not Detected**

[a] NDU/mL = RNA NAAT detectable units/mL

b. Matrix comparison:

Not applicable

3. Clinical studies:

Prospective Clinical Study

A clinical evaluation of the BioFire RP2.1 panel was performed with prospectively collected NPS specimens. Specimens that were residual NPS in transport media left over from standard of care testing for SARS-CoV-2, and those that were either held at room temperature for ≤4 hours or 4°C for ≤3 days before enrollment were accepted for this study. Specimens that were other than NPS in transport media or specimens that could not be tested within the defined storage parameters were excluded. Three collection sites were used in this prospective clinical study.

The BioFire RP2.1 was evaluated by comparing the test results for SARS-CoV-2 with a composite comparator of three U.S. FDA EUA tests. All results were interpreted according to the test's IFU.

Concordance for two out of three of the EUA tests were considered the final result for the comparator, and the interpretation rules for the reference method were outlined as follows—

Table 35. BioFire RP2.1 Prospective Clinical Evaluation Composite Comparator Interpretations Rules[a]

Rule #	EUA Results	Composite Result
1	Pos/Pos/Any	Positive
2	Neg/Neg/Any	Negative
3	Pos/Neg/Inv	*specimen excluded*
4	Inv/Inv/Any	*specimen excluded*

[a] 'Any' may be positive, negative, or invalid. 'Inv' (invalid) results include any non-definitive result such as equivocal, indeterminate, unresolved, or inconclusive.

A total of 534 NPS specimens were acquired for the clinical study. In terms of the inclusion criteria for the study, a total of 527 specimens initially were included in the analysis. Of these 311 (59.0%) were run on BioFire 2.0 systems and 216 (41.0%) were run on BioFire Torch systems. Two tests did not complete the initial run resulting in a total instrument success rate of 99.6% (525/527). One specimen was able to be rerun. Ten (10) NPS specimens were excluded for reasons that included—not meeting inclusion criteria after enrollment (insufficient volume, N=1; stored at incorrect temperature, N=6), a run failure with insufficient volume for retesting (N=1), and inability to determine composite comparator interpretation for a specimen due to invalid comparator results (N=2).

The final data was comprised of 524 valid specimens with the following demographic information—

Table 36. Demographic Data of Prospectively Collected Specimens

		Overall
Sex	Male	270 (52%)
	Female	251 (48%)
	Unknown	3 (<1%)
Age	0-18 years	55 (10%)
	19-40 years	170 (32%)
	41-60 years	146 (28%)
	61+ years	153 (29%)
Total		**524**

Note that two ECMs were provided to study sites for daily testing (one each as positive and negative controls). Instrument operators were required to complete a valid ECM run on each day of specimen testing. All ECM sample runs were completed and yielded the expected results.

A summary of the BioFire RP2.1 SARS-CoV-2 prospective clinical study performance is provided in Table 38. Positive Percent Agreement (PPA) was calculated as 100% × (TP / (TP + FN)). True positive (TP) indicates that both the BioFire RP2.1 and the comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the BioFire RP2.1 was negative while the comparator result was positive. Negative Percent Agreement (NPA) was calculated as 100% × (TN / (TN + FP)). True negative (TN) indicates that both the BioFire RP2.1 and the comparator method had negative results, and false positive (FP) indicates that the BioFire RP2.1 was positive while the comparator result was negative.

Table 37. BioFire RP2.1 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Overall Prospective Study Performance

Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/ (TP + FN)	%	95%CI	TN/ (TN + FP)	%	95%CI
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	61/62[a]	98.4	91.4-99.7%	457/462[b]	98.9	97.5-99.5%

[a] SARS-CoV-2 was detected in the single FN specimen with all three composite comparator methods.

[b] SARS-CoV-2 was detected in 4/5 FP specimens with only one of the three composite comparator methods. SARS-CoV-2 was detected in the remaining FP specimen (1/5) using an additional independent molecular method.

Overall, a single (1) False Negative and five (5) False Positive discrepant results were observed between the RP2.1 and the reference comparator methods in the prospective clinical study. Regarding the observed False Negative result, further investigation

indicated that all comparator assays had positive results, but the respective Ct and Cp values suggested that the analyte concentration in the sample may have been near the LoD according to the manufacturers' IFUs. Due to insufficient sample volume, this specimen could not be further investigated.

Table 38. False Negative SARS-CoV-2 RP2.1 Comparator Prospective Study Result

SCN	Discrepancy	Subject Information		Comparator EUA PCR Tests								
					Comparator 1			Comparator 2		Comparator 3		
		Age (years)	Sex	Composite EUA Result	Result	T1 (*Nsp2*) Ct	T2 (*N*) Ct	Result	(b) (4) (b) (4)	Result	N1 Ct	N2 Ct
01-0301	FN	19-40	M	Positive	Pos	31.98	31.25	D		Pos	34.1	36.0

Investigation of the five False Positive specimens indicated that analyte concentrations may have been near LoD based on observed Cp values for all five RP2.1 runs. The SOC assay was negative for all five specimens. Four specimens had a Detected or Equivocal result on the COVID-19 reference assay with a varying number of positive assays for that device. For the fifth specimen, the study site initiated additional SOC testing based on their internal review of the amplification data. That specimen was further tested with another FDA authorized test, with a subsequent positive result. It is also noted that the observed mean Ct values for both reference tests suggested an analyte concentration near LoD, according to the manufacturer IFUs. Due to insufficient sample volume, these specimens could not be further investigated.

Table 39. False Positive SARS-CoV-2 RP2.1 Comparator Prospective Study Results

SCN	Discrepancy	Subject Information		BioFire RP2.1		Comparator EUA PCR Tests					Additional Investigation		
				Median Cp				Comparator 2			FDA authorized test		
		Age (years)	Sex	SARS CoV2-1	SARS CoV2-2	Composite EUA Result	Comparator 1	Result	(b) (4) (b) (4)	Comparator 3	Result	T1 (*N2*) Ct	T2 (*E*) Ct
01-0042	FP	41-60	M	(b)	(b)	Negative	Neg	E		Neg	NT[a]		
01-0107	FP	0-18	F	(b)	(b)	Negative	Neg	D		Neg	NT[a]		
01-0113	FP	19-40	F	(b)	(b)	Negative	Neg	D		Neg	NT[a]		
01-0143	FP	19-40	F	(b)	(b)	Negative	Neg	E		Neg	NT[a]		
01-0236	FP	61+	F	(b)	-	Negative	Neg	ND		Neg	Pos	38.2	36.0

[a] NT = Not Tested

Retrospective Clinical Study

A clinical evaluation of the BioFire RP2.1 panel was performed using 50 natural retrospective leftover (archived) clinical specimens. All SARS-CoV-2 positive specimens were collected during March and April of 2020. Known demographic information for these collected samples is summarized in (Table 41). These specimens had been

previously characterized as positive for SARS-CoV-2 using the respective Emergency Use Authorization (EUA) assay employed at the collection site (i.e., the comparator method listed in the archived specimen testing). Specimens were obtained from three geographically distinct laboratories in the United States. Positive specimens were randomized and tested alongside 50 NPS specimens that were collected before December 2019 (i.e., expected to be negative for SARS-CoV-2). Positive Percent Agreement (PPA) was determined by comparing the observed test result to the expected test result based on previous laboratory testing, and Negative Percent Agreement (NPA) was determined by comparing the observed test result for SARS-CoV-2 negative specimens to the expected result of Not Detected. In addition, the LoD of RP2.1 SARS-CoV-2 assays were comparable to the reported LoDs of the EUA comparator tests (according to the manufacturer IFU) used during this retrospective clinical study.

Table 40. Demographic Summary for Positive SARS-CoV-2 Archived Specimens

Specimen Demographics (N=50)		
Sex	Male (%)	15 (30%)
	Female (%)	20 (40%)
	Unknown	15 (30%)
Age Range	0-18 years	1 (2%)
	19-40 years	13 (26%)
	41-60 years	13 (26%)
	61+ years	8 (16%)
	Unknown	15 (30%)

In the course of testing, two specimens (one positive and one negative) were excluded due to instrument errors. Results from the remaining 98 evaluable specimens are shown in Table 46 below. The PPA was 98% (48/49) and NPA was 100% for the SARS-CoV-2 assay. One false negative (FN) result was observed (the specimen was positive upon retest). Further, 10.4% (5/48) of the 48 specimens with SARS-CoV-2 Detected results had additional analytes identified by BioFire RP2.1 in this retrospective study (Table 43).

Table 41. BioFire RP2.1 SARS-CoV-2 Archived Specimen Performance Data Summary

Agreement with known analyte composition						
Comparator Method	**PPA:** TP/ (TP+FN)	**%**	**95% CI**	**NPA:** TN/ (TN+FP)	**%**	**95% CI**
EUA 1	14/15[a]	93.3	[70.2-98.8%]	N/A	N/A	N/A
EUA 2	15/15	100	[79.6-100%]	N/A	N/A	N/A
EUA 3	19/19	100	[83.3-100%]	N/A	N/A	N/A
Negative Specimens	N/A	N/A	N/A	49/49	100	[92.7 – 100%]
Overall Agreement	**48/49[a]**	**98.0**	**[89.3 – 99.6%]**	**49/49**	**100**	**[92.7 – 100%]**

[a] One FN specimen was positive upon BioFire RP2.1 retest

Table 42. Additional Analytes identified by BioFire RP2.1 in 48 specimens with SARS-CoV-2 Detected Results

Additional Analytes	Number Observed (%)
Adenovirus	1 (2.1%)
HRV/EV	4 (8.3%)

Contrived Clinical Specimen Study

The BioFire RP2.1 clinical specimen study included testing of 50 contrived clinical specimens spiked with inactivated SARS-CoV-2 isolate USA-WA1/2020 at various levels of LoD (25 at 2× LoD, 15 at 3× LoD, and 10 at 5× LoD) and randomized with ten non-spiked specimens. Each specimen was a unique NPS specimen which had been collected before December 2019, and was therefore expected to be negative for SARS-CoV-2. PPA was determined by comparing the observed test results for samples contrived in unique clinical specimens to the expected Detected result. PPA and NPA are shown in Table 48. For SARS-CoV-2 contrived testing, both the PPA and NPA were 100%.

Table 43. Contrived SARS-CoV-2 Testing

	Agreement with known analyte composition			
	PPA: TP/(TP+FN)	**%**	**NPA:** TN/(TN+FP)	**%**
Overall Agreement	50/50	100%	10/10	100%
95% CI	**[92.9 – 100%]**		**[72.2-100%]**	

(b) (4)

(b) (4)

Clinical Comparison Study

A clinical comparison study was performed to demonstrate equivalency between the existing BioFire RP2 and the new BioFire RP2.1 panel that is a modification of the RP2 panel with the addition of assays for the detection of SARS-CoV-2. The clinical comparison study used 210 clinical archived nasopharyngeal swab (NPS) specimens eluted in transport media. Note, the firm also evaluated 10 MERS-CoV contrived specimens as part of their internal characterizations for the comparison study. However, the BioFire RP2.1 does not report results for MERS-CoV. The clinical NPS specimens were previously obtained during prospective evaluations of other BioFire respiratory panels or acquired from external sources for the BioFire specimen repository. Specimens were chosen solely based on the analyte content, and analyte levels (if known) were not used as part of the specimen selection process. Specimens for use in this study were collected prior to December 2019, and were therefore presumed to be negative for SARS-CoV-2.

Specimens were stored frozen and maintained at <-70°C until testing and were split into two aliquots for parallel testing with each panel. An attempt was made to test at least ten archived clinical specimens for every analyte shared between RP2 and RP2.1 panels. The archived and contrived specimens were evaluated at BioFire laboratories using FilmArray 2.0 and Torch systems. Prior to testing, specimens were randomized and the analyte contents remained blinded to the personnel performing the test procedures. Specimen

aliquots were subject to the same number of freeze/thaw cycles to prevent bias in testing.

The comparison performance is reported as Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of the BioFire RP2 to BioFire RP2.1 panels. The results from the comparison evaluation is summarized in the below table and it was observed that there was overall a 97.7% positive percent agreement (PPA) and 99.8% negative percent agreement (NPA) (Table 45). The majority of discrepant results were due to unexpected positives by either panel (i.e., detection of analytes not previously identified by the source lab in addition to the expected analyte). These discrepant results are summarized below along with the corresponding expected analyte detection and Cp values (Table 46).

Table 44. Performance Comparison of the Modified BioFire RP2.1 to the Original BioFire FilmArray RP2 using Archived and Contrived Specimens

Analyte	Positive Agreement				Negative Agreement			
	RP2 (+) RP2.1 (+)	RP2 (+) RP2.1 (-)	PPA	95% CI	RP2 (-) RP2.1 (-)	RP2 (-) RP2.1 (+)	NPA	95% CI
Viruses								
Adenovirus	14	1	14/15 (93.3%)	70.2-98.8%	203	2	203/205 (99%)	96.5-99.7%
Coronavirus 229E	10	1	10/11 (90.9%)	62.3-98.4%	209	0	209/209 (100%)	98.2-100%
Coronavirus HKU1	10	0	10/10 (100%)	72.2-100%	208	2	208/210 (99%)	96.6-99.7%
Coronavirus NL63	10	0	10/10 (100%)	72.2-100%	210	0	210/210 (100%)	98.2-100%
Coronavirus OC43	10	0	10/10 (100%)	72.2-100%	210	0	210/210 (100%)	98.2-100%
Human Metapneumovirus	12	0	12/12 (100%)	75.8-100%	208	0	208/208 (100%)	98.2-100%
Human Rhinovirus/Enterovirus	19	3	19/22 (86.4%)	66.7-95.3%	195	3	195/198 (98.5%)	95.6-99.5%
Influenza A	30	0	30/30 (100%)	88.6-100%	190	0	190/190 (100%)	98.0-100%
Influenza A H1	5	0	5/5 (100%)	56.6-100%	215	0	215/215 (100%)	98.2-100%
Influenza A H1-2009	12	0	12/12 (100%)	75.8-100%	208	0	208/208 (100%)	98.2-100%
Influenza A H3	13	0	13/13 (100%)	77.2-100%	207	0	207/207 (100%)	98.2-100%
Influenza B	10	0	10/10 (100%)	72.2-100%	210	0	210/210 (100%)	98.2-100%

Analyte	Positive Agreement				Negative Agreement			
	RP2 (+) RP2.1 (+)	RP2 (+) RP2.1 (-)	PPA	95% CI	RP2 (-) RP2.1 (-)	RP2 (-) RP2.1 (+)	NPA	95% CI
Parainfluenza Virus 1	9	0	9/9 (100%)	70.1-100%	211	0	211/211 (100%)	98.2-100%
Parainfluenza Virus 2	11	0	11/11 (100%)	74.1-100%	209	0	209/209 (100%)	98.2-100%
Parainfluenza Virus 3	10	1	10/11 (90.9%)	62.3-98.4%	208	1	208/209 (99.5%)	97.3-99.9%
Parainfluenza Virus 4	11	0	11/11 (100%)	74.1-100%	209	0	209/209 (100%)	98.2-100%
Respiratory Syncytial Virus	10	0	10/10 (100%)	72.2-100%	210	0	210/210 (100%)	98.2-100%
Middle East Respiratory Syndrome Coronavirus (MERS-CoV)[a]	10	0	10/10 (100%)	72.2-100%	210	0	210/210 (100%)	98.2-100%
Bacteria								
***Bordetella parapertussis* (IS*1001*)**	10	0	10/10 (100%)	72.2-100%	210	0	210/210 (100%)	98.2-100%
***Bordetella pertussis* (*ptxP*)**	10	0	10/10 (100%)	72.2-100%	210	0	210/210 (100%)	98.2-100%
Chlamydia pneumoniae	10	0	10/10 (100%)	72.2-100%	210	0	210/210 (100%)	98.2-100%
Mycoplasma pneumoniae	10	0	10/10 (100%)	72.2-100%	210	0	210/210 (100%)	98.2-100%
Overall								
Overall	256	6	256/262 (97.7%)	95.1-98.9%	4570	8	4570/4578 (99.8%)	99.7-99.9%

[a] MERS-CoV is not reported by the RP2.1 device. The analyte was evaluated in contrived specimens with inactivated virus.

Table 45. Summary of Discrepant Results in Comparison Testing between BioFire RP2.1 and RP2 devices.
Discrepant analytes are shown in **bold** text and highlighted specimens were positive for the expected analyte.

Sample Number	All Reported Results: Expected Analyte Based on Source Lab Result	(b) (4)
Expected Analyte Detected by Both Methods		
053953-026	Coronavirus OC43	(b) (4)
053953-033	Parainfluenza Virus 1	
053953-078	*Mycoplasma pneumoniae*	
053953-083	Human Metapneumovirus	

Sample Number	All Reported Results	
	Expected Analyte Based on Source Lab Result	(b) (4)
053953-085	Human Metapneumovirus	(b) (4)
053953-099	Parainfluenza Virus 4	
053953-123	RSV	
053953-134	Parainfluenza Virus 1	
053953-178	*Bordetella parapertussis*	
053953-199	Negative	
053953-204	*Bordetella parapertussis*	
053953-219	Coronavirus 229E	
Expected Analyte Not Detected by At Least One Method		
053953-002	Parainfluenza Virus 3	(b) (4)
053953-112	Coronavirus 229E	

This study also served to demonstrate SARS-CoV-2 assay specificity with testing of 220 NPS specimens collected before December 2019 (i.e., presumptively negative for SARS-CoV-2). The NPA for SARS-CoV-2 was 100% (220/220) as observed for the BioFire RP2.1 panel (Table 47).

Table 46. Overall BioFire RP2.1 NPA (Specificity) for SARS-CoV-2 in Clinical Comparison Study

NPA: TN/(TN+FP)	%	95% CI
220/220	100%	98.3 - 100%

4. Clinical cut-off:

   Not applicable

5. Expected values:

   Prevalence of RP2.1 results in the prospective study stratified by study site are presented in the following table. Only three analytes were observed—Adenovirus, SARS-CoV-2, and Human Rhinovirus/Enterovirus.

Table 47. Prevalence of Detected Analytes Stratified by Site; # = Number; EV = Expected Value

BioFire RP2.1 Result	Overall (N=524)		Site 1 (N=309)		Site 2 (N=110)		Site 3 (N=105)	
	#	EV	#	EV	#	EV	#	EV
Viruses								
Adenovirus	3	0.6%	3	1.0%	0	0%	0	0%
Coronavirus 229E	0	0%	0	0%	0	0%	0	0%
Coronavirus HKU1	0	0%	0	0%	0	0%	0	0%
Coronavirus NL63	0	0%	0	0%	0	0%	0	0%
Coronavirus OC43	0	0%	0	0%	0	0%	0	0%
Middle East Respiratory Syndrome Coronavirus (MERS-CoV)	0	0%	0	0%	0	0%	0	0%
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	66	12.6%	46	14.9%	12	10.9%	8	7.6%
Human Metapneumovirus	0	0%	0	0%	0	0%	0	0%
Human Rhinovirus/Enterovirus	33	6.3%	12	3.9%	11	10.0%	10	9.5%
Influenza A	0	0%	0	0%	0	0%	0	0%
Influenza A H1	0	0%	0	0%	0	0%	0	0%
Influenza A H1-2009	0	0%	0	0%	0	0%	0	0%
Influenza A H3	0	0%	0	0%	0	0%	0	0%
Influenza B	0	0%	0	0%	0	0%	0	0%
Parainfluenza Virus 1	0	0%	0	0%	0	0%	0	0%
Parainfluenza Virus 2	0	0%	0	0%	0	0%	0	0%
Parainfluenza Virus 3	0	0%	0	0%	0	0%	0	0%
Parainfluenza Virus 4	0	0%	0	0%	0	0%	0	0%
Respiratory Syncytial Virus	0	0%	0	0%	0	0%	0	0%
Bacteria								
Bordetella parapertussis (IS*1001*)	0	0%	0	0%	0	0%	0	0%
Bordetella pertussis (*ptxP*)	0	0%	0	0%	0	0%	0	0%
Chlamydia pneumoniae	0	0%	0	0%	0	0%	0	0%
Mycoplasma pneumoniae	0	0%	0	0%	0	0%	0	0%

The prevalence of RP2.1 detected analytes stratified by age are as follows—

Table 48. Prevalence of Detected Analytes Stratified by Age; # = Number; EV = Expected

BioFire RP2.1 Result	Overall (N=524)		0-18 years (N=55)		19-40 years (N=170)		41-60 years (N=146)		61+ years (N=153)	
	#	EV	#	EV	#	EV	#	EV	#	EV
Viruses										
Adenovirus	3	0.6%	1	1.8%	2	1.2%	0	0%	0	0%
Coronavirus 229E	0	0%	0	0%	0	0%	0	0%	0	0%
Coronavirus HKU1	0	0%	0	0%	0	0%	0	0%	0	0%
Coronavirus NL63	0	0%	0	0%	0	0%	0	0%	0	0%
Coronavirus OC43	0	0%	0	0%	0	0%	0	0%	0	0%
Middle East Respiratory Syndrome Coronavirus (MERS-CoV)	0	0%	0	0%	0	0%	0	0%	0	0%
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	66	12.6%	5	9.1%	24	14.1%	22	15.1%	15	9.8%
Human Metapneumovirus	0	0%	0	0%	0	0%	0	0%	0	0%
Human Rhinovirus/Enterovirus	33	6.3%	19	34.5%	5	2.9%	7	4.8%	2	1.3%
Influenza A	0	0%	0	0%	0	0%	0	0%	0	0%
Influenza A H1	0	0%	0	0%	0	0%	0	0%	0	0%
Influenza A H1-2009	0	0%	0	0%	0	0%	0	0%	0	0%
Influenza A H3	0	0%	0	0%	0	0%	0	0%	0	0%
Influenza B	0	0%	0	0%	0	0%	0	0%	0	0%
Parainfluenza Virus 1	0	0%	0	0%	0	0%	0	0%	0	0%
Parainfluenza Virus 2	0	0%	0	0%	0	0%	0	0%	0	0%
Parainfluenza Virus 3	0	0%	0	0%	0	0%	0	0%	0	0%
Parainfluenza Virus 4	0	0%	0	0%	0	0%	0	0%	0	0%
Respiratory Syncytial Virus	0	0%	0	0%	0	0%	0	0%	0	0%
Bacteria										
Bordetella parapertussis (IS*1001*)	0	0%	0	0%	0	0%	0	0%	0	0%
Bordetella pertussis (*ptxP*)	0	0%	0	0%	0	0%	0	0%	0	0%
Chlamydia pneumoniae	0	0%	0	0%	0	0%	0	0%	0	0%
Mycoplasma pneumoniae	0	0%	0	0%	0	0%	0	0%	0	0%

During the prospective study, the RP2.1 reported a single specimen with multiple organism detections (1/524 total specimens). The distinct co-detection consisted of the SARS-CoV-2 analyte (True Positive result by comparator testing) and Adenovirus.

M. Instrument Name:

FilmArray 2.0 or FilmArray Torch System

N. System Descriptions:

1. Modes of Operation:
   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes _____ or No ____X____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ___X_____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X____ or No _____

3. Specimen Identification:

   Specimen identification can be entered manually or via barcode

4. Specimen Sampling and Handling:

   The BioFire RP2.1 is intended for use with nasopharyngeal swab (NPS) collected in transport media or saline. The operator places a Hydration Injection Vial and a Sample Injection Vial into the BioFire Pouch Loading Station. The operator first hydrates the test pouch with the Hydration Injection Vial and then adds Sample Buffer into the Sample Injection Vial using the provided Sample Buffer ampoule. Using a transfer pipette provided in the kit, the operator adds ~300µl of specimen into the Sample Injection Vial, closes the Sample Injection Vial, removes the Sample Injection Vial containing the sample mixture from the Loading station, inverts the vial at least three times to mix, and then inserts it into the Loading Station port where the proper amount of specimen is pulled into the BioFire RP2.1 pouch by vacuum. The BioFire RP2.1 pouch is then placed in the FilmArray 2.0 instrument or the available module of a FilmArray Torch system for testing.

5. Calibration:

   Not applicable.

6. Quality Control:

   See Quality Control Section above ["Traceability, Stability, Expected Values (controls, calibrators, or methods)"]

O. Other Supportive Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not Applicable

P. Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device. It consists of the instructions for use, a quick reference guide and Fact Sheets for Healthcare Providers and Patients.

Q. Identified Risks to Health and Mitigation Measures

Identified Risks to Health	Mitigation Measures
Risk of an inaccurate test result (false positive or false negative result) leading to improper patient management	Certain labeling information, including limitations, warnings, device descriptions, explanation of procedures, and performance information identified in special controls (1), (3), (5), and (6). Use of certain specimen collection devices identified in special control (2). Certain design verification and validation, documentation of certain analytical studies and clinical studies, risk analysis strategies, and device descriptions identified in special control (4). Testing of characterized viral samples and labeling information identified in special control (7).
Misinterpretation of test results leading to misdiagnosis and associated risk of false test results	Certain labeling information, including limitations, warnings, device descriptions, explanation of procedures, results interpretation information, and performance information identified in special controls (1), (3), and (5). Certain design verification and validation, documentation of certain analytical studies and clinical studies, risk analysis strategies, and device descriptions identified in special control (4).
Failure to correctly operate the device leading to inaccurate test results	Certain labeling information, including limitations, warnings, device descriptions, explanation of procedures, and performance information identified in special controls (1), (3), (5), and (6). Use of certain specimen collection devices identified in special control (2). Certain design verification and validation, documentation of certain analytical studies and clinical studies, risk analysis strategies, and device descriptions identified in special control (4).

R. Benefit/Risk Analysis:

Summary of the Assessment of Benefit

The benefit of the assay is the ability to use a well-validated device for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections, including COVID-19. The device can detect and concurrently distinguish common and the novel respiratory pathogen, SARS-CoV-2, in human clinical specimens to aid in the differential diagnosis of the respiratory infections in conjunction with other clinical, epidemiological, and laboratory data. An assay to detect SARS-CoV-2 and distinguish it from multiple common viral and bacterial respiratory pathogens in

nasopharyngeal swabs that is properly validated and consistently provides accurate test results is a critical diagnostic element in the evaluation of patients suspected of having SARS-CoV-2. The analyte added to the existing FilmArray RP2 device and tested by the FilmArray RP2.1, SARS-CoV-2 PCR, is a pathogen known to cause novel infection that can lead to outbreaks, where rapid detection would have compelling public health and safety considerations. Aid in the diagnosis of this pathogen could help guide outbreak investigations. The device may also benefit laboratory personnel by helping to rapidly identify samples that require more stringent protective measures for staff to help avoid accidental exposure to contagious pathogens. The purpose of a correct diagnostic result is to guide the appropriate patient management and therapy when used in conjunction with other clinical and laboratory information. The benefit of a correct SARS-CoV-2 result and of results for multiple common viral and bacterial respiratory pathogens have the potential to be long lasting for those tested, particularly if the illness was life threatening. Positive SARS-CoV-2 results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status and guide patient management. The test results may improve infection control measures and may aid in tracking and reducing transmission of infection. There is currently no acute SARS-CoV-2 diagnostic device that has undergone full FDA premarket review (i.e., not EUA authorized) to most definitively determine clinical truth for the method comparison study, however this uncertainty is acceptable, particularly because the sponsor used a composite comparator method comprised of multiple EUA-authorized devices, which represents the most reasonable alternative to establish clinical truth in the clinical study.

Summary of the Assessment of Risk

The risks associated with the device, when used as intended, are those related to the risk of false test results, failure to correctly interpret the test results, and failure to correctly operate the device. In general, the risks associated with inaccurate results for SARS-CoV-2 and the common viral and bacterial respiratory pathogens detected by the FilmArray RP2.1 in nasopharyngeal swabs are error in diagnosis and error or delay in treatment, and delay in diagnosing the patient's true disease. False positive SARS-CoV-2 results may lead to include improper patient management, including treatment for SARSCoV-2 with antiviral medication, monoclonal antibody treatment, or convalescent plasma. These treatments have risks including toxicity and more rarely allergic reactions. False positive SARS-CoV-2 results may also lead to unnecessary isolation or quarantine and additional health monitoring, mis-allocation of resources used for surveillance and prevention, and delayed diagnosis and treatment of other infections or health conditions. False negative SARS-CoV-2 results may lead to missing and not appropriately treating or monitoring a patient who has SARS-CoV-2 infection. Missing and not treating or appropriately monitoring a patient with SARS-CoV-2 infection whose clinical picture warrants treatment could result in the known sequelae of SARS-CoV-2 infection and may result in high morbidity and mortality in these patients. False negative SARS-CoV-2 results may also lead to unnecessary additional diagnostic evaluation or treatment and delay in correct diagnosis or further spread of disease, with the potential for novel cases of infection and concomitant increase in patient morbidity and mortality.

Summary of the Assessment of Benefit-Risk

The clinical benefits outweigh the risks for the proposed assay, considering the mitigations of the risks provided in the De Novo application, product labeling, risk analysis and documentation, special controls, as well as general controls. The required De Novo application helps to ensure that errors will be uncommon and will facilitate accurate assay implementation and interpretation of results. Further, a post-market monitoring plan was provided by the sponsor for continuous monitoring of the quality, safety, and performance during the entire lifecycle of the device. The plan includes assessment of publicly available information such as sequence analysis and other post-market activities including further benchtop evaluations where appropriate. The plan summary appeared acceptable and addressed special controls for the device. In addition, the device's performance observed in the clinical study suggests that errors will be uncommon and that the assay will provide substantial benefits to patients as an aid in the diagnosis of SARS-CoV-2 or other respiratory infections when used in conjunction with other laboratory results and clinical information.

S. Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

T. Conclusion

The De Novo request is granted and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code(s): QOF

Device Type: Device to detect and identify nucleic acid targets in respiratory specimens from microbial agents that cause the SARS-CoV-2 respiratory infection and other microbial agents when in a multi-target test

Class: II (special controls)

Regulation: 21 CFR 866.3981