BioFire® Respiratory Panel 2.1 (RP2.1)

IVD

For Emergency Use Authorization (EUA) only

Rx Only

Instructions for Use	https://www.biofiredx.com/e-labeling/ITI0101
Quick Guide	https://www.biofiredx.com/e-labeling/ITI0072
Safety Data Sheet (SDS)	https://www.biofiredx.com/e-labeling/ITI0060

Customer and Technical Support Information *For more information on how to contact Customer and Technical Support, refer to Appendix B.	**U.S. Customers**	Phone: 1-800-735-6544 (toll free) E-mail: support@BioFireDX.com Website: www.biofiredx.com
	Outside of the U.S.	Contact the local bioMérieux sales representative or an authorized distributor.

INTENDED USE

The BioFire Respiratory Panel 2.1 (RP2.1) is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and differentiation of nucleic acids from multiple viral and bacterial respiratory organisms, including nucleic acid from Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), in nasopharyngeal swabs (NPS) obtained from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity or moderate complexity tests.

The BioFire Respiratory Panel 2.1 (RP2.1) is intended for the detection and differentiation of nucleic acid from SARS-CoV-2 and the following organism types and subtypes identified using the BioFire RP2.1.

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

SARS-CoV-2 RNA and nucleic acids from the other respiratory viral and bacterial organisms identified by this test are generally detectable in nasopharyngeal swabs (NPS) 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. Positive results are indicative of the presence of the identified organism, but do not rule out co-infection with other pathogens. The agent(s) detected by the BioFire RP2.1 may not be the definite cause of disease.

Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.

Negative results in the setting of a respiratory illness may be due to infection with pathogens not detected by this test, or lower respiratory tract infection that may not be detected by an NPS specimen. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative SARSCoV-2 results must be combined with clinical observations, patient history, and epidemiological information. Negative results for other organisms identified by the test may require additional laboratory testing (eg, bacterial and viral culture, immunofluorescence and radiography) when evaluating a patient with possible respiratory tract infection.

The BioFire RP2.1 is intended for use by laboratory personnel who have received specific training on the use of the FilmArray 2.0 and/or the FilmArray Torch Systems. The BioFire RP2.1 is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY AND EXPLANATION OF THE TEST

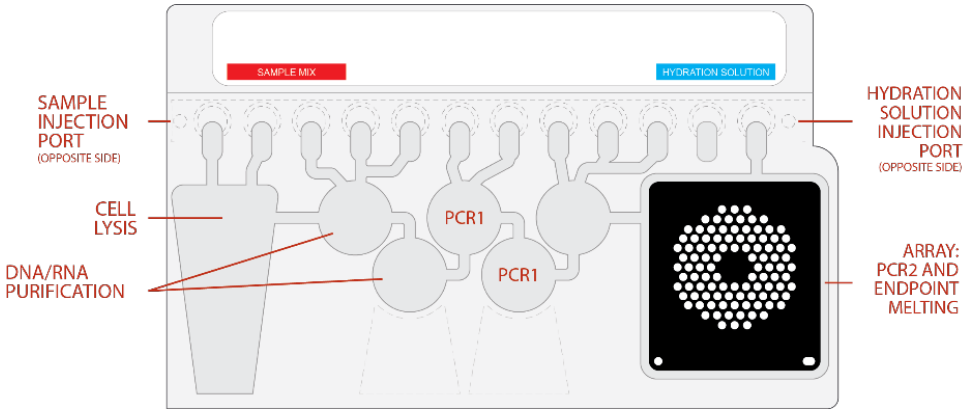
The BioFire RP2.1 is a real-time, nested multiplexed polymerase chain reaction test designed to simultaneously identify nucleic acids from 22 different viruses and bacteria associated with respiratory tract infection, including SARS-CoV-2, from a single nasopharyngeal swab (NPS) specimen. Specifically, the SARS-CoV-2 primers contained in the BioFire RP2.1 are designed to detect RNA from the SARS-CoV-2 in nasopharyngeal swabs in transport media from patients who are suspected of COVID-19. Internal controls are used to monitor all stages of the test process.

PRINCIPLE OF THE PROCEDURE

The BioFire® RP2.1 pouch 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 respiratory pathogens within a single NPS specimen. After sample collection, the user injects hydration solution and sample combined with Sample Buffer into the pouch, places the pouch into a BioFire® FilmArray® System instrument module, and starts a run. The entire run process takes about 45 minutes. Additional detail can be found in the appropriate BioFire FilmArray System Operator's Manual.

During a run, the FilmArray® system:

- Lyses the sample by agitation (bead beating) in addition to chemical lysis mediated by the Sample Buffer.
- Extracts and purifies all nucleic acids from the sample using magnetic bead technology.
- Performs nested multiplex PCR by:
    - First performing reverse transcription, followed by a multiplexed first stage PCR reaction (PCR1).
    - Then performing multiple simultaneous second-stage PCR reactions (PCR2) in the array to amplify sequences within the PCR1 products.
- Uses endpoint melting curve data to detect target-specific amplicons and analyses the data to generate a result for each analyte on the BioFire RP2.1.



MATERIALS PROVIDED

Each kit contains sufficient reagents to test 30 samples (30-test kit; 423738):

- Individually packaged BioFire RP2.1 pouches
- Single-use (1.0 mL) Sample Buffer ampoules
- Single-use pre-filled (1.5 mL) Hydration Injection Vials (blue)
- Single-use Sample Injection Vials (red)
- Individually packaged Transfer Pipettes

MATERIALS REQUIRED BUT NOT PROVIDED

- BioFire® FilmArray® System including:
  - BioFire® FilmArray® 2.0 or BioFire® FilmArray® Torch Systems, including panel-specific software module, and accompanying system-specific software
  - BioFire® FilmArray® Pouch Loading Station
- 10% bleach solution or a similar disinfectant

WARNINGS AND PRECAUTIONS

General Precautions

1. For *in vitro* diagnostic use under Emergency Use Authorization only.
2. This test has not been FDA cleared or approved; but has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high or moderate complexity tests
3. This test has been authorized only for the detection and differentiation of nucleic acid of SARS-CoV-2 from multiple respiratory viral and bacterial organisms.
4. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
5. A trained healthcare professional should carefully interpret the results from the BioFire RP2.1 in conjunction with a patient's signs and symptoms, results from other diagnostic tests, and relevant epidemiological information.
6. BioFire RP2.1 pouches are only for use with BioFire FilmArray 2.0 and BioFire FilmArray Torch systems.
7. Always check the expiration date on the pouch. Do not use a pouch after its expiration date.
8. BioFire RP2.1 pouches are stored under vacuum in individually wrapped canisters. To preserve the integrity of the pouch vacuum for proper operation, be sure that a FilmArray instrument/module will be available and operational before unwrapping any pouches for loading.
9. If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.

Safety Precautions

1. Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable clean powder-free gloves and lab coats. Protect skin, eyes, and mucus membranes. Change gloves often when handling reagents or samples.
2. Handle all samples and waste materials as if they were capable of transmitting infectious agents. Observe safety guidelines such as those outlined in:
   - CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*[1]
   - CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*[2]

- Refer to Interim Laboratory Safety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.

3. Follow your institution's safety procedures for handling biological samples.
4. If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to a state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
5. Dispose of materials used in this assay, including reagents, samples, and used buffer vials, according to federal, state, and local regulations.
6. Sample Buffer is assigned the following classifications:
   - Acute toxicity (Category 4)
   - Serious Eye damage (Category 1)
   - Skin irritation (Category 2).

   Please refer to the BioFire RP2.1 Safety Data Sheet (SDS) for more information.
7. Sample Buffer will form hazardous compounds and fumes when mixed with bleach or other disinfectants.

   WARNING: Never add bleach to Sample Buffer or sample waste.
8. Bleach, a recommended disinfectant, is corrosive and may cause severe irritation or damage to eyes and skin. Vapor or mist may irritate the respiratory tract. Bleach is harmful if swallowed or inhaled.
   - Eye contact: Hold eye open and rinse with water for 15-20 minutes. Remove contact lenses after the first 5 minutes and continue rinsing eye. Seek medical attention.
   - Skin contact: Immediately flush skin with plenty of water for at least 15 minutes. If irritation develops, seek medical attention.
   - Ingestion: Do not induce vomiting. Drink a glassful of water. If irritation develops, seek medical attention.
   - Please refer to the appropriate Safety Data Sheet (SDS) for more information.

Laboratory Precautions

1. **Preventing organism contamination**

   Due to the sensitive nature of the BioFire RP2.1, it is important to guard against contamination of the sample and work area by carefully following the testing process outlined in this instruction document, including these guidelines:

   - Laboratory personnel may carry or shed common respiratory pathogens asymptomatically and can inadvertently contaminate the specimen while it is being processed. Careful adherence to the sample processing steps described in this document is recommended to avoid possible contamination. Samples should be processed in a clean biosafety cabinet if available, or according to local laboratory guidelines. If a biosafety cabinet is not used, a dead air box (e.g., AirClean PCR workstation), a splash shield (e.g., Bel-Art Scienceware Splash Shields), or a face shield can be used when preparing samples instead.
   - Laboratory personnel with active respiratory symptoms (runny nose, cough) should wear a standard surgical mask (or equivalent) and should avoid touching the mask while handling specimens.

- It is recommended to avoid handling specimens or pouches in an area used to routinely process respiratory pathogen culture, and/or immunofluorescence testing.
- Prior to processing specimens, thoroughly clean both the work area and the BioFire® Pouch Loading Station using a suitable cleaner such as freshly prepared 10% bleach or a similar disinfectant. To avoid residue build-up and potential damage to the specimen or interference from disinfectants, wipe disinfected surfaces with water.
- Specimens and pouches should be handled and/or tested one-at-a-time. Always change gloves and clean the work area between each pouch and specimen.
- Use clean gloves when removing Sample Buffer ampoules and Sample/Hydration Injection Vials from bulk packaging bags, and reseal bulk packaging bags when not in use.
- Avoid collecting or handling specimens in areas that are exposed to vaccine material for pathogens detected by the BioFire RP2.1 (*e.g.* influenza and *Bordetella pertussis*). Particular care should be taken during these processes to avoid contamination. Some *B. pertussis* acellular vaccines (i.e. Pentacel®, Daptacel®, and Adacel®) contain PCR-detectable DNA. Contamination of specimens or testing materials with vaccine can cause false-positive *B. pertussis* results (http://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-pcr-bestpractices.html).

2. Preventing amplicon contamination

A common concern with PCR-based assays is false positive results caused by contamination of the work area with PCR amplicon. Because the BioFire RP2.1 pouch is a closed system, the risk of amplicon contamination is low provided that pouches remain intact after the test is completed. Adhere to the following guidelines, in addition to those above, to prevent amplicon contamination:

- Discard used pouches in a biohazard container immediately after the run has completed.
- Avoid excessive handling of pouches after test runs.
- Change gloves after handling a used pouch.
- Avoid exposing pouches to sharp edges or anything that might cause a puncture.

WARNING: If liquid is observed on the exterior of a pouch, the liquid and pouch should be immediately contained and discarded in a biohazard container. The instrument and workspace must be decontaminated as described in the appropriate BioFire FilmArray Operator's Manual.

DO NOT PERFORM ADDITIONAL TESTING UNTIL THE AREA HAS BEEN DECONTAMINATED.

Precautions Related to Public Health Reporting

Local, state, and federal regulations for notification of reportable disease are continually updated and include a number of organisms for surveillance and outbreak investigations.[3,4] Additionally, the Centers for Disease Control and Prevention (CDC) recommends that when pathogens from reportable diseases are detected by a culture independent diagnostic test (CIDT), the laboratory should facilitate obtaining the isolate or clinical materials for submission to the appropriate public health laboratory to aid in outbreak detection and epidemiological investigations. Laboratories are responsible for following their state and/or local regulations and should consult their local and/or state public health laboratories for isolate and/or clinical sample submission guidelines.

Pertussis is a nationally notifiable infectious condition in the U.S. If *Bordetella pertussis* is detected, notify the state and/or local health departments.

Laboratories in the U.S. are required to report all positive SARS-CoV-2 results to the appropriate public health authorities.

REAGENT STORAGE, HANDLING, AND STABILITY

1. Store the test kit, including reagent pouches and buffers, at room temperature (15–25 ºC). **DO NOT REFRIGERATE.**
2. Avoid storage of any materials near heating or cooling vents or in direct sunlight.
3. All kit components should be stored and used together. Do not use components from one kit with those of another kit. Discard any extra components from the kit after all pouches have been consumed.
4. Do not remove pouches from their packaging until a sample is ready to be tested. Once the pouch packaging has been opened, the pouch should be loaded as soon as possible (within approximately 30 minutes).
5. Once a pouch has been loaded, the test run should be started as soon as possible (within approximately 60 minutes). Do not expose a loaded pouch to temperatures above 40°C (104°F) prior to testing.

SAMPLE REQUIREMENTS

The following table describes the requirements for specimen collection, preparation, and handling that will help ensure accurate test results.

Specimen Type	**Nasopharyngeal Swab** (NPS) collected according to standard technique and immediately placed in up to 3 mL of transport media
Minimum Sample Volume	0.3 mL (300 µL)
Transport and Storage	Specimens should be processed and tested with the BioFire RP2.1 as soon as possible. If storage is required, specimens can be held: • At room temperature for up to 4 hours (15-25 °C) • Refrigerated for up to 3 days (2-8 °C) • Frozen (≤-15 °C or ≤-70°C) (for up to 30 days)[a]

[a] Frozen storage for up to 30 days was evaluated for this sample type. However, longer frozen storage may be acceptable. Please follow your institutions rules and protocols regarding sample storage validation.

NOTE: NPS specimens should not be centrifuged before testing.

NOTE: Bleach can damage organisms/nucleic acids within the specimen, potentially causing false negative results. Contact between bleach and specimens during collection, disinfection, and testing procedures should be avoided.

PROCEDURE

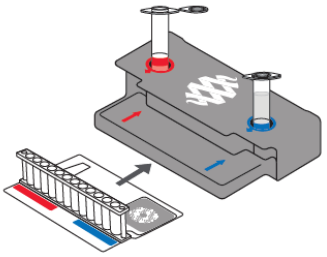
Use clean gloves and other Personal Protective Equipment (PPE) when handling pouches and samples. Only prepare one BioFire RP2.1 pouch at a time and change gloves between samples and pouches. Once sample is added to the pouch, promptly transfer to the instrument to start the run. After the run is complete, discard the pouch in a biohazard container.

Step 1: Prepare Pouch

1. Thoroughly clean the work area and the BioFire Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse.
2. Remove the pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective canister.

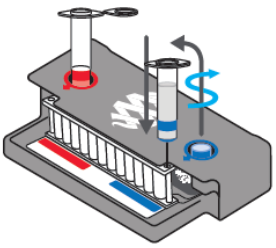
NOTE: The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.

3. Check the expiration date on the pouch. Do not use expired pouches.
4. Insert the pouch into the Pouch Loading Station, aligning the red and blue labels on the pouch with the red and blue arrows on the Pouch Loading Station.
5. Place a red-capped Sample Injection Vial into the red well of the Pouch Loading Station.
6. Place a blue-capped Hydration Injection Vial into the blue well of the Pouch Loading Station.



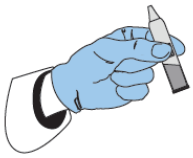
Step 2: Hydrate Pouch

1. Unscrew the Hydration Injection Vial from the blue cap.
2. Remove the Hydration Injection Vial, leaving the blue cap in the BioFire Pouch Loading Station.
3. Insert the Hydration Injection Vial's cannula tip into the pouch hydration port located directly below the blue arrow of the Pouch Loading Station.
4. Forcefully push down in a firm and quick motion to puncture seal until a faint "pop" is heard and there is an ease in resistance. Wait as the correct volume of Hydration Solution is pulled into the pouch by vacuum.
    - If the hydration solution is not automatically drawn into the pouch, repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is again not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.
5. Verify that the pouch has been hydrated.
    - Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen.
    - If the pouch fails to hydrate (dry reagents appear as white pellets), repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is still not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.



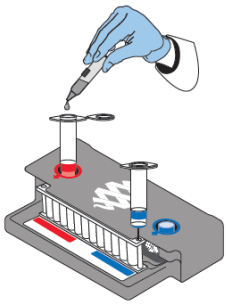
Step 3: Prepare Sample Mix

1. Add Sample Buffer to the Sample Injection Vial.
    - Hold the Sample Buffer ampoule with the tip facing up.



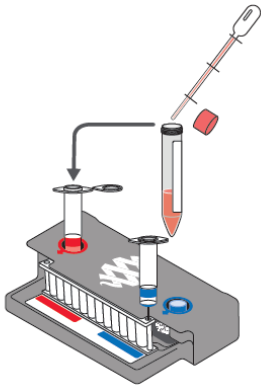
NOTE: Avoid touching the ampoule tip during handling, as this may introduce contamination.

- Firmly pinch at textured plastic tab on the side of the ampoule until the seal snaps.
- Invert the ampoule over the red-capped Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

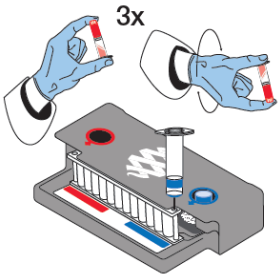


NOTE: Avoid squeezing the ampoule additional times. This will generate foaming, which should be avoided.

WARNING: The Sample Buffer is harmful if swallowed and can cause serious eye damage and skin irritation.



2. Thoroughly mix the NPS specimen by vortex or inversion.
3. Use the transfer pipette provided in the test kit to draw specimen to the third line (approximately 0.3 mL) of the transfer pipette.
4. Add the specimen to the Sample Buffer in the Sample Injection Vial.
5. Tightly close the lid of the Sample Injection Vial and discard the transfer pipette in a biohazard waste container.

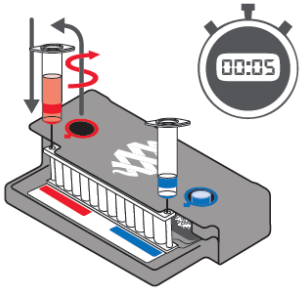


NOTE: DO NOT use the Transfer Pipette to mix the sample once it is loaded into the Sample Injection Vial.

6. Remove the Sample Injection Vial from the Pouch Loading Station and invert the vial at least 3 times to mix.
7. Return the Sample Injection Vial to the red well of the Pouch Loading Station.

Step 4: Load Sample Mix

1. Slowly twist to unscrew the Sample Injection Vial from the red cap and wait for 5 seconds with the vial resting in the cap.



NOTE: Waiting 5 seconds decreases the risk of dripping and contamination from the sample.

2. Lift the Sample Injection Vial, leaving red cap in the well of the Pouch Loading Station, and insert the Sample Injection Vial cannula tip into the pouch sample port located directly below the red arrow of the Pouch Loading Station.
3. Forcefully push down in a firm and quick motion to puncture seal (a faint “pop” is heard) and sample is pulled into the pouch by vacuum.

4. Verify that the sample has been loaded.
    - Flip the barcode label down and check to see that fluid has entered the reagent well next to the sample loading port.
    - If the pouch fails to pull sample from the Sample Injection Vial, the pouch should be discarded. Retrieve a new pouch and repeat from *Step 1: Prepare Pouch*.
5. Discard the Sample Injection Vial and the Hydration Injection Vial in appropriate biohazard sharps container.
6. Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the FilmArray Pouch Loading Station.

Step 5: Run Pouch

The FilmArray® Software includes step-by-step, on-screen instructions that guide the operator through performing a run. Brief instructions for FilmArray 2.0 and FilmArray Torch Systems are given below. Refer to the appropriate BioFire FilmArray System Operator's Manual for more detailed instructions.

FilmArray 2.0

1. Ensure that the FilmArray 2.0 system (instrument and computer) is powered on and the software is launched.
2. Follow on-screen instructions and procedures described in the Operator's Manual to place the pouch in a module, enter pouch, sample, and operator information.
3. Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type, and Protocol can be manually entered from the information provided on the pouch label into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

NOTE: When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the BioFire RP2.1 pouch.

4. Enter the Sample ID. The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.
5. If necessary, select and/or confirm the appropriate protocol for your sample type from the Protocol drop down list. The BioFire RP2.1 has a single protocol available in the drop down list.
6. Enter a user name and password in the Name and Password fields.

NOTE: The font color of the username is red until the user name is recognized by the software.

7. Review the entered run information on the screen. If correct, select Start Run.

    Once the run has started, the screen displays a list of the steps being performed by the instrument and the number of minutes remaining in the run.

NOTE: The bead-beater apparatus makes an audible, high-pitched noise during the first minute of operation.

8. When the run is finished, follow the on-screen instructions to remove the pouch, then immediately discard it in a biohazard waste container.

9. The run file is automatically saved in the FilmArray database, and the test report can be viewed, printed, and/or saved as a PDF file.

BioFire FilmArray Torch

1. Ensure that the BioFire FilmArray Torch System is powered on.
2. Select an available module on the touch screen or scan the barcode on the pouch using the barcode scanner.
3. Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type, and Protocol can be manually entered from the information provided on the pouch label into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

NOTE: When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the BioFire RP2.1 pouch.

4. Enter the Sample ID. The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.
5. Insert the pouch into the available module.
    - Ensure that the pouch fitment label is lying flat on top of pouch and not folded over. As the pouch is inserted, the module will grab onto the pouch and pull it into the chamber.
6. If necessary, select and/or confirm the appropriate protocol for your sample type from the Protocol drop down list. The BioFire RP2.1 has a single protocol available in the drop down list.
7. Enter operator user name and password, then select Next.

NOTE: The font color of the username is red until the user name is recognized by the software.

8. Review the entered run information on the screen. If correct, select Start Run.

    Once the run has started, the screen displays a list of the steps being performed by the module and the number of minutes remaining in the run.

NOTE: The bead-beater apparatus can be heard as a high-pitched noise during the first minute of operation.

9. At the end of the run, remove the partially ejected pouch, then immediately discard it in a biohazard waste container.
10. The run file is automatically saved in the FilmArray database, and the test report can be viewed, printed, and/or saved as a PDF file.

QUALITY CONTROL

Process Controls

Two process controls are included in each pouch:

1. **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 BioFire RP2.1 pouch were successful.

2. **PCR2 Control**

   The PCR2 Control assay detects a DNA target that is dried into wells of 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.

Monitoring Test System Performance

The FilmArray software will automatically fail the run if the melting temperature (Tm) for either the RNA Process Control or the PCR2 Control is outside of an acceptable range (80.3-84.3°C for the RNA Process Control and 73.8-77.8°C for the PCR2 Control). If required by local, state, or accrediting organization quality control requirements, users can monitor the system by trending Tm values for the control assays and maintaining records according to standard laboratory quality control practices.[5,6] Refer to the appropriate BioFire FilmArray Operator's System Manual for instructions on obtaining control assay Tm values. The PCR2 Control is used in several FilmArray pouch types (e.g., RP, BCID2, BCID, GI, ME, and RP2) and can therefore be used to monitor the system when multiple pouch types are used on the same FilmArray System.

External Controls

Good laboratory practice recommends running external positive and negative controls regularly. 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.

Due to the COVID-19 pandemic and the resulting shortage of external control material, BioFire recommends that all laboratories perform external QC with each new lot and shipment of reagents, at a minimum, while running the BioFire RP2.1 under Emergency Use Authorization (EUA).

INTERPRETATION OF RESULTS

Assay Interpretation

When PCR2 is complete, the instrument performs a high resolution DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well (for more information see appropriate BioFire FilmArray Operator's System manual). The BioFire FilmArray Software then performs several analyses and assigns a final assay result. The steps in the analyses are described below.

Analysis of melt curves. The BioFire FilmArray Software evaluates the DNA melt curve for each well of the PCR2 array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (Tm) of the curve and compares it against the expected Tm range for the assay. If the software determines that the Tm falls inside the assay-specific Tm range, the melt curve is called positive. If the software determines that the melt curve is not in the appropriate Tm range, the melt curve is called negative.

Analysis of replicates. Once melt curves have been identified, the software evaluates the three replicates for each assay to determine the assay result. For an assay to be called positive, at least two of the three associated melt curves must be called positive, and the Tm for at least two of the three positive melt curves must be similar (within 1°C). Assays that do not meet these criteria are called negative.

Organism Interpretation

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 the hMPV assay is positive (at least two of the three hMPV assay wells on the array have similar positive melt peaks with Tm values that are within the assay-specific Tm range). The test results for SARS-CoV-2, Adenovirus, and Influenza A depend on the interpretation of results from more than one assay. Interpretation and actions for these three multi-assay results are provided below.

SARS-CoV-2

The BioFire RP2.1 pouch contains two different assays for the detection of the SARS-CoV-2. The target of each assay is shown in Table 1 below. The BioFire FilmArray software interprets each assay independently and if either one or both of the assays is positive, the test report will show Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) as Detected. If both assays are negative, the test report result will be Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Not Detected.

Table 1. Gene Targets for SARS-CoV-2 Assays on the BioFire RP2.1

Assay Name	Gene Target
SARSCoV2-1	Spike protein (S) gene
SARSCoV2-2	Membrane protein (M) gene

Adenovirus

The BioFire RP2.1 pouch contains five assays (Adeno2, Adeno3, Adeno6, Adeno7.1, and Adeno8) for the detection of Adenovirus. The FilmArray Software interprets each of these assays independently (as described above) and the results are combined as a final test result for the virus. If one assay or any combination of assays is positive, the test report result will be Adenovirus Detected. If all assays are negative, the test report result will be Adenovirus Not Detected.

Influenza A

The assays in the BioFire RP2.1 are designed to both detect Influenza A and to differentiate commonly occurring hemagglutinin subtypes. To accomplish this, the BioFire RP2.1 uses two Influenza A assays, (FluA-pan-1 and FluA-pan-2) and three subtyping assays directed at the hemagglutinin gene (FluA-H1-2, FluA-H1-2009, and FluA-H3). Each of the individual assays is interpreted independently (as described above) and the test result reported for Influenza A is based on the combined results of the five assays as outlined in Table 2. Specimens with an Equivocal result or multiple Influenza A subtypes detected should be retested once.

Table 2. Possible Assay Results for Influenza A and the Corresponding Interpretation

Result \ Assay	FluA pan Assays (n 2)	FluA H1 2	FluA H1 2009	FluA H3	Action
Influenza A Not Detected	Negative	Negative	Negative	Negative	None
Influenza A H1	**≥1 positive**	**Positive**	Negative	Negative	
Influenza A H3	**≥1 positive**	Negative	Negative	**Positive**	
Influenza A H1-2009	**≥1 positive**	Any result	**Positive**	Negative	
Influenza A H1 **Influenza A H3**	**≥1 positive**	**Positive**	Negative	**Positive**	Multiple infections are possible but rare [a], retest to confirm result [b]
Influenza A H1-2009 **Influenza A H3**	**≥1 positive**	Any result	**Positive**	**Positive**	
Influenza A (no subtype detected)	**2 positive**	Negative	Negative	Negative	Retest (see below)
Influenza A Equivocal	**1 positive**	Negative	Negative	Negative	Retest once (see Result Summary section below for further instruction).
Influenza A H1 Equivocal	Negative	**Positive**	Negative	Negative	
Influenza A H3 Equivocal	Negative	Negative	Negative	**Positive**	
Influenza A H1-2009 Equivocal	Negative	Any result	**Positive**	Negative	

[a] The BioFire RP2.1 can simultaneously detect multiple influenza viruses contained in multivalent vaccines (see Limitations).

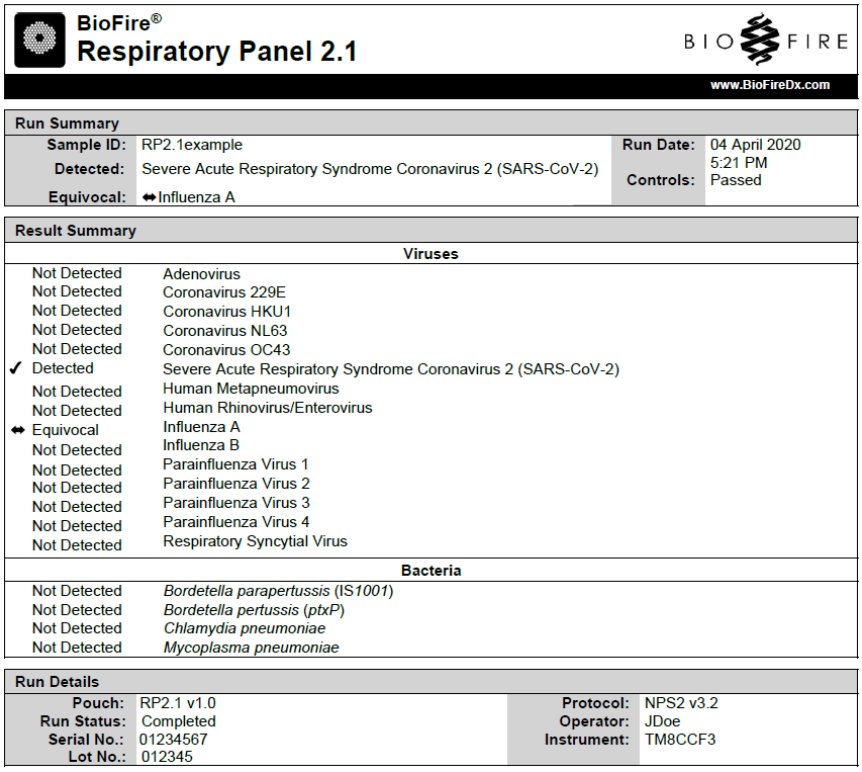
[b] Repeated multiple subtype positives should be further confirmed by other FDA cleared Influenza subtyping tests.

Influenza A (no subtype detected)

If both FluA-pan assays are positive, but none of the hemagglutinin subtyping assays are positive, then the interpretation is Influenza A (no subtype detected). This result could occur when the titer of the virus in the specimen is low and not detected by the subtyping assays. This result could also indicate the presence of a novel Influenza A strain. In both cases, the sample in question should be retested. If the retest provides a different result, test the sample a third time to ensure the accuracy of the result. If the retest provides the same result, then the function of the BioFire RP2.1 pouches should be verified by testing with appropriate external control materials (known positive samples for Influenza A H1, Influenza A H3 and Influenza A H1-2009), and a negative control should also be run to test for PCR-product contamination. If the BioFire RP2.1 accurately identifies the external and negative controls, contact the appropriate public health authorities for confirmatory testing.

BioFire RP2.1 Test Report

The BioFire RP2.1 test report is automatically displayed upon completion of a run and can be printed or saved as a PDF file. Each report contains a Run Summary, a Result Summary, and a Run Details section.



BioFire® Respiratory Panel 2.1

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		

Run Summary

The Run Summary section of the test report provides the Sample ID, time and date of the run, control results and an overall summary of the test results. Any organism with a Detected result will be listed in the corresponding field of the summary. If all of the organism assays were negative then 'None' will be displayed in the Detected field. Controls are listed as Passed, Failed, or Invalid. Table 3 provides additional information for each of the possible control field results.

Table 3. Interpretation of Controls Field on the BioFire RP2.1 Test Report

Control Result	Explanation	Action
Passed	The run was successfully completed AND Both pouch controls were successful.	None Report the results provided on the test report
Failed	The run was successfully completed BUT At least one of the pouch controls (RNA Process Control and/or PCR2 Control) failed.	Repeat the test using a new pouch. If the error persists, contact Technical Support for further instruction.
Invalid	The controls are invalid because the run did not complete. (Typically this indicates a software or hardware error).	Note any error codes displayed during the run and the Run Status field in the Run Details section of the report. Refer to the appropriate FilmArray operator's manual or contact Technical Support for further instruction. Once the error is resolved, repeat the test or repeat the test using another instrument.

Result Summary

The Result Summary section of the test report lists the result for each target tested by the panel. Possible results for each organism are Detected, Not Detected, or Invalid (Equivocal is also a possible result for Influenza A and its subtypes). Table 4 provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

Table 4. Reporting of 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 described in the Assay Interpretation section above)	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 described in the Assay Interpretation section above)	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 (see Table 2)	Retest the original specimen and report the result. If the result of the retest is again 'Equivocal', the final result should be considered 'Detected'.
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 Table 3 , Interpretation of Control Field on the FilmArray Test Report for instruction.

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

Run Details

The **Run Details** section provides additional information about the run including: pouch information (type, lot number, and serial number), Run Status (Completed, Incomplete, Aborted, Instrument Error, or Software Error), the protocol that was used to perform the test, the identity of the operator that performed the test, and the instrument used to perform the test.

Change Summary

It is possible to edit the Sample ID once a run has completed. If this information has been changed, an additional section called **Change Summary** will be added to the test report. This Change Summary section lists the field that was changed, the original entry, the revised entry, the operator that made the change, and the date that the change was made. Sample ID is the only field of the report that can be changed.

Change Summary				
Field	**Changed To**	**Changed From**	**Operator**	**Date**
[1]Sample ID	New Example Id	Old Example Id	Anonymous	06 Apr 2020

LIMITATIONS

1. For prescription use only.
2. The use of this assay as an *in vitro* diagnostic under US FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high and moderate complexity tests.
3. BioFire RP2.1 performance has only been established on the BioFire FilmArray 2.0 and BioFire FilmArray Torch systems.
4. The BioFire RP2.1 is a qualitative test and does not provide a quantitative value for the organism(s) in the specimen.
5. Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
6. The performance of the BioFire RP2.1 has been evaluated for use with human specimen material only.
7. The BioFire RP2.1 has not been validated for testing of specimens other than nasopharyngeal swab (NPS) specimens in transport medium.
8. The performance of BioFire RP2.1 has not been established for specimens collected from individuals without signs or symptoms of respiratory infection.
9. The performance of the BioFire RP2.1 has not been specifically evaluated for NPS specimens from immunocompromised individuals.
10. The effect of antibiotic treatment on test performance has not been evaluated.
11. The performance of the BioFire RP2.1 has not been established with potentially interfering medications for the treatment of influenza or cold viruses. The effect of interfering substances has only been evaluated for those listed in the *Interference* section. Interference from substances that were not evaluated could lead to erroneous results.
12. The performance of the BioFire RP2.1 has not been established for monitoring treatment of infection with any of the panel organisms.
13. The performance of BioFire RP2.1 has not been established for screening of blood or blood products.
14. The detection of viral and bacterial nucleic acid is dependent upon proper specimen collection, handling, transportation, storage and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive or false negative values resulting from improperly collected, transported or handled specimens.
15. A negative BioFire RP2.1 result does not exclude the possibility of viral or bacterial infection. Negative test results may occur from the presence of sequence variants (or mutation) in the region targeted by the assay, the presence of inhibitors, technical error, sample mix-up, an infection caused by an organism not detected by the panel, or lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen. Test results may also be affected by concurrent antiviral/antibacterial therapy or levels of organism in the specimen that are below the limit of detection for the test. Negative results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.
16. If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.

17. Viral and bacterial nucleic acids may persist *in vivo* independent of organism viability. Detection of organism target(s) does not imply that the corresponding organisms are infectious or are the causative agents for clinical symptoms.
18. Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods when prevalence is moderate to low.
19. Performance characteristics for Influenza A were established when Influenza A H1-2009, A H1, and A H3 were the predominant Influenza A viruses in circulation. Performance of detecting Influenza A may vary if other Influenza A strains are circulating or a novel Influenza A virus emerges.
20. Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Bordetella parapertussis, Bordetella pertussis, Chlamydia pneumoniae,* Coronavirus 229E, Influenza A H1, Influenza A H3, Influenza B, Parainfluenza Virus 1, and Parainfluenza Virus 4 were established primarily with retrospective clinical specimens. Performance characteristics for Influenza A H1 was established primarily using contrived clinical specimens.
21. The BioFire RP2.1 influenza A subtyping assays target the influenza A hemagglutinin (H) gene only. The BioFire RP2.1 does not detect or differentiate the influenza A neuraminidase (N) subtypes.
22. The BioFire RP2.1 may not be able to distinguish between existing viral strains and new variants as they emerge. For example, the BioFire RP2.1 can detect Influenza A H3N2v (first recognized in August, 2011), but will not be able to distinguish this variant from Influenza A H3N2 seasonal. If variant virus infection is suspected, clinicians should contact their state or local health department to arrange specimen transport and request a timely diagnosis at a state public health laboratory.
23. Recent administration of nasal influenza vaccines (e.g. FluMist) prior to NPS specimen collection could lead to accurate virus detection by the BioFire RP2.1 of the viruses contained in the vaccine, but would not represent infection by those agents
24. Due to the genetic similarity between Human Rhinovirus and Enterovirus, the BioFire RP2.1 cannot reliably differentiate them. A BioFire RP2.1 Rhinovirus/Enterovirus Detected result should be followed-up using an alternate method (e.g. cell culture or sequence analysis) if differentiation between the viruses is required.
25. BioFire RP2.1 detects a single-copy Pertussis Toxin promoter target (*ptxP*, present at one copy per cell) in *B. pertussis*. Other PCR tests for *B. pertussis* target the multi-copy IS*481* insertion sequence (present in both *B. pertussis* and *B. holmesii*) and are therefore capable of detecting lower levels of *B. pertussis* (i.e. more sensitive).
    - BioFire RP2.1 should not be used if *B. pertussis* infection is specifically suspected; a *B. pertussis* molecular test that is FDA-cleared for use on patients suspected of having a respiratory tract infection attributable to *B. pertussis* only should be used instead.
    - Due to lower sensitivity, the BioFire RP2.1 *B. pertussis* assay is less susceptible than IS*481* assays to the detection of very low levels of contaminating *B. pertussis* vaccine material. However, care must always be taken to avoid contamination of specimens with vaccine material as higher levels may still lead to false positive results with the BioFire RP2.1 test (see contamination prevention guidelines).
    - The IS*481* sequence is also present in *B. holmesii* and to a lesser extent in *B. bronchiseptica*, whereas the BioFire RP2.1 assay (*ptxP*) was designed to be specific for *B. pertussis*. However, the BioFire RP2.1 *Bordetella pertussis* (*ptxP*) assay can also amplify pertussis toxin pseudogene sequences when present in *B. bronchiseptica* and *B. parapertussis*. Cross-reactivity was observed only at high concentration (e.g. ≥1.2E+09 CFU/mL).

26. There is a risk of false positive results due to cross-contamination with organisms, nucleic acids or amplified products. Particular attention should be given to the Laboratory Precautions noted under the *Warnings and Precautions* section.
27. Primers for both BioFire RP2.1 SARS-CoV-2 assays share substantial sequence homology with the Bat coronavirus RaTG13 (accession: MN996532) and cross-reactivity with this closely-related viral sequence is predicted. In addition, the SARSCoV2-2 assay may cross-react with Pangolin coronavirus (accession: MT084071) and two other bat SARS-like coronavirus sequences (accession MG772933 and MG772934). It is unlikely that these viruses would be found in a human clinical nasopharyngeal swab; but if present, the cross-reactive product(s) produced by the BioFire RP2.1 will be detected as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).
28. There is a risk of false positive results for *Bordetella* species and Human Rhinovirus/Enterovirus due to non-specific amplification and cross-reactivity with organisms that can be found in the respiratory tract. Observed and predicted cross-reactivity for BioFire RP2.1 is described in the *Analytical Specificity* section. Erroneous results due to cross-reactivity with organisms that were not evaluated or new variant sequences that emerge is also possible.
29. Some strains of *B. bronchiseptica* (rarely isolated from humans) do carry IS*1001* insertion sequences identical to those carried by most strains of *B. parapertussis*. These sequences will be amplified by the IS1001 assay and reported by BioFire RP2.1 as *Bordetella parapertussis* (IS*1001*).
30. The BioFire RP2.1 Human Rhinovirus/Enterovirus assay may amplify off-target sequences found in strains of *B. pertussis, B. bronchiseptica* and *B. parapertussis*. Cross-reactivity with *B. pertussis* was observed at a concentration of ≥4.5E+07 CFU/mL.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The BioFire RP2.1 Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

However, to assist clinical laboratories using the BioFire RP2.1 (“your product” in the conditions below), the relevant Conditions of Authorization are listed below:

- Authorized laboratories* using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents, and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and You (support@BioFireDX.com) any suspected occurrence of false positive or false negative results and

significant deviations from the established performance characteristics of your product of which they become aware.

- All laboratory personnel using your product must be appropriately trained in performing and interpreting the results of your product, use appropriate personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
- BioFire Diagnostics, LLC, authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

* The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high or moderate complexity tests" as "authorized laboratories."

BIOFIRE RP2 AND RP2.1

The BioFire RP2.1 (Ref #: 423738) was developed by adding the reagents required to detect the SARS-CoV-2 targets into the existing BioFire RP2 (Ref #: RFIT-ASY-0129, RFIT-ASY-0130). Assays for all analytes shared between the two panels and reaction conditions of the test were unchanged from BioFire RP2. Studies were performed to demonstrate the performance of the new SARS-CoV-2 assays and to demonstrate that the performance characteristics of the assays from BioFire RP2 are unaffected by the panel modification. The original studies of the BioFire RP2 remain relevant for the performance of the BioFire RP2.1.

EXPECTED VALUES

In the prospective clinical evaluation of the BioFire RP2, 1612 eligible specimens (NPS), including 918 prospective fresh (Category I) specimens and 694 prospective archived/frozen (Category II) specimens, were collected and tested at three study sites across the United States over approximately six months (January – March and September – November 2016). Expected value (as determined by BioFire RP2) summaries for Category I and II specimens respectively, stratified by specimen collection site are presented in Table 5 and Table 6.

NOTE: Expected values for SARS-CoV-2 have not been determined.

Table 5. Expected Value (As Determined by BioFire RP2) Summary by Collection Site for the BioFire RP2 Prospective Clinical Evaluation (Category I Fresh Prospective Specimens) (September 2016 – November 2016)

	Overall (n 918)		Site 1 (n 331) Salt Lake City, UT		Site 2 (n 284) Chicago, IL		Site 3 (n 303) Columbus, OH	
	No.	Expected Value (%)	No.	Expected Value (%)	No.	Expected Value (%)	No.	Expected Value (%)
Viruses								
Adenovirus	66	7.2%	25	7.6%	7	2.5%	34	11.2%
CoV- 229E	9	1.0%	4	1.2%	5	1.8%	0	0%
CoV-HKU1	1	0.1%	0	0%	1	0.4%	0	0%
CoV-NL63	1	0.1%	0	0%	0	0%	1	0.3%
CoV-OC43	12	1.3%	4	1.2%	1	0.4%	7	2.3%
hMPV	5	0.5%	2	0.6%	2	0.7%	1	0.3%
HRV/EV	378	41.2%	146	44.1%	69	24.3%	163	53.8%
Influenza A	3	0.3%	2	0.6%	0	0%	1	0.3%
Influenza A H1	0	0%	0	0%	0	0%	0	0%
Influenza A 2009-H1	0	0%	0	0%	0	0%	0	0%
Influenza A H3	3	0.3%	2	0.6%	0	0%	1	0.3%
Influenza B	0	0%	0	0%	0	0%	0	0%

Parainfluenza Virus 1	5	0.5%	3	0.9%	2	0.7%	0	0%
Parainfluenza Virus 2	54	5.9%	8	2.4%	13	4.6%	33	10.9%
Parainfluenza Virus 3	49	5.3%	20	6.0%	13	4.6%	16	5.3%
Parainfluenza Virus 4	8	0.9%	3	0.9%	1	0.4%	4	1.3%
RSV	50	5.4%	9	2.7%	5	1.8%	36	11.9%
Bacteria								
Bordetella parapertussis (IS1001)	4	0.4%	0	0%	0	0%	4	1.3%
Bordetella pertussis (ptxP)	3	0.3%	1	0.3%	0	0%	2	0.7%
Chlamydia pneumoniae	3	0.3%	1	0.3%	0	0%	2	0.7%
Mycoplasma pneumoniae	21	2.3%	2	0.6%	7	2.5%	12	4.0%

Table 6. Expected Value (As Determined by BioFire RP2) Summary by Collection Site for the BioFire RP2 Prospective Clinical Evaluation (Category II Archived Prospective Specimens) (January 2016 – March 2016)

	Overall (n 694)		Site 1 (n 250) Salt Lake City, UT		Site 2 (n 243) Chicago, IL		Site 3 (n 201) Columbus, OH	
	No.	Expected Value (%)	No.	Expected Value (%)	No.	Expected Value (%)	No.	Expected Value (%)
Viruses								
Adenovirus	52	7.5%	18	7.2%	20	8.2%	14	7.0%
CoV- 229E	7	1.0%	2	0.8%	3	1.2%	2	1.0%
CoV-HKU1	54	7.8%	28	11.2%	16	6.6%	10	5.0%
CoV-NL63	49	7.1%	24	9.6%	17	7.0%	8	4.0%
CoV-OC43	26	3.7%	8	3.2%	10	4.1%	8	4.0%
hMPV	76	11.0%	26	10.4%	25	10.3%	25	12.4%
HRV/EV	124	17.9%	43	17.2%	44	18.1%	37	18.4%
Influenza A	75	10.8%	9	3.6%	27	11.1%	38	18.9%
Influenza A H1	0	0%	0	0%	0	0%	0	0%
Influenza A 2009-H1	74	10.7%	9	3.6%	27	11.1%	38	18.9%
Influenza A H3	1	0.1%	0	0%	0	0%	1	0.5%
Influenza B	16	2.3%	3	1.2%	7	2.9%	6	3.0%
Parainfluenza Virus 1	5	0.7%	2	0.8%	2	0.8%	1	0.5%
Parainfluenza Virus 2	0	0%	0	0	0	0%	0	0%
Parainfluenza Virus 3	4	0.6%	2	0.8%	0	0%	2	1.0%
Parainfluenza Virus 4	8	1.2%	4	1.6%	2	0.8%	2	1.0%
RSV	149	21.5%	59	23.6%	51	21.0%	39	19.4%
Bacteria								
Bordetella parapertussis (IS*1001*)	2	0.3%	1	0.4%	1	0.4%	0	0%
Bordetella pertussis (*ptxP*)	0	0%	0	0%	0	0%	0	0%
Chlamydia pneumoniae	3	0.4%	0	0%	2	0.8%	1	0.5%
Mycoplasma pneumoniae	7	1.0%	3	1.2%	4	1.6%	0	0%

Expected value (as determined by BioFire RP2) summary by age group for the BioFire RP2 prospective clinical evaluation (Category I and II prospective specimens combined) (January – March and September – November 2016 and) is presented in Table 7.

Table 7. Expected Value (As Determined by BioFire RP2) Summary by Age Group for the BioFire RP2 Prospective Clinical Evaluation (Category I and II Prospective Specimens) (January – March and September – November 2016)

	Overall (N 1612)		≤5 years (N 885)		6 21 years (N 331)		22 49 years (N 128)		50+ years (N 268)	
Viruses										
Adenovirus	118	7.3%	96	10.8%	18	5.4%	2	1.6%	2	0.7%
CoV- 229E	16	1.0%	3	0.3%	7	2.1%	1	0.8%	5	1.9%
CoV-HKU1	55	3.4%	37	4.2%	9	2.7%	2	1.6%	7	2.6%
CoV-NL63	50	3.1%	41	4.6%	6	1.8%	2	1.6%	1	0.4%

	Overall (N 1612)		≤5 years (N 885)		6 21 years (N 331)		22 49 years (N 128)		50+ years (N 268)	
CoV-OC43	38	2.4%	28	3.2%	7	2.1%	0	0%	3	1.1%
hMPV	81	5.0%	60	6.8%	12	3.6%	3	2.3%	6	2.2%
HRV/EV	502	31.1%	379	42.8%	88	26.6%	16	12.5%	19	7.1%
Influenza A	78	4.8%	29	3.3%	20	6.0%	13	10.2%	16	6.0%
Influenza A H1	0	0%	0	0%	0	0%	0	0%	0	0%
Influenza A 2009-H1	74	4.6%	26	2.9%	19	5.7%	13	10.2%	16	6.0%
Influenza A H3	4	0.2%	3	0.3%	1	0.3%	0	0%	0	0%
Influenza B	16	1.0%	7	0.8%	7	2.1%	1	0.8%	1	0.4%
Parainfluenza Virus 1	10	0.6%	9	1.0%	0	0%	1	0.8%	0	0%
Parainfluenza Virus 2	54	3.3%	39	4.4%	10	3.0%	1	0.8%	4	1.5%
Parainfluenza Virus 3	53	3.3%	44	5.0%	6	1.8%	2	1.6%	1	0.4%
Parainfluenza Virus 4	16	1.0%	13	1.5%	1	0.3%	0	0%	2	0.7%
RSV	199	12.3%	168	19.0%	10	3.0%	8	6.3%	13	4.9%
Bacteria										
Bordetella parapertussis (IS*1001*)	6	0.4%	4	0.5%	2	0.6%	0	0%	0	0%
Bordetella pertussis (*ptxP*)	3	0.2%	0	0%	3	0.9%	0	0%	0	0%
Chlamydia pneumoniae	6	0.4%	1	0.1%	4	1.2%	1	0.8%	0	0%
Mycoplasma pneumoniae	28	1.7%	10	1.1%	14	4.2%	3	2.3%	1	0.4%

In addition, the most common multiple detections (as determined by BioFire RP2) during the BioFire RP2 prospective clinical evaluation (Category I and II prospective specimens combined) (January – March and September – November 2016 and), stratified by age group, is presented in Table 8. Overall, the BioFire RP2 detected at least one organism in a total of 1020 specimens (63.3% positivity rate; 1020/1612). Two or more organisms were detected by the BioFire RP2 in 24.0% of positive specimens (245/1020; 15.2% of all tested specimens, 245/1612).

Table 8. Expected Value (Multiple Detections with ≥ 5 occurrences as Determined by the BioFire RP2) Summary by Age Group for the Prospective Clinical Evaluation (January – March and September – November 2016)

Multiple Detection Combination	Overall (N 1612)	≤5 years (N 885)	6 21 years (N 331)	22 49 years (N 128)	50+ years (N 268)
Adenovirus + HRV/EV	30 (1.9%)	27 (3.1%)	3 (0.9%)	0 (0%)	0 (0%)
HRV/EV + RSV	22 (1.4%)	22 (2.5%)	0 (0%)	0 (0%)	0 (0%)
CoV-HKU1 + RSV	13 (0.8%)	12 (1.4%)	0 (0%)	0 (0%)	1 (0.4%)
CoV-NL63 + RSV	13 (0.8%)	12 (1.4%)	0 (0%)	0 (0%)	1 (0.4%)
HRV/EV + PIV2	11 (0.7%)	9 (1.0%)	1 (0.3%)	0 (0%)	1 (0.4%)
HRV/EV + PIV3	11 (0.7%)	10 (1.1%)	1 (0.3%)	0 (0%)	0 (0%)
Adenovirus + RSV	10 (0.6%)	8 (0.9%)	2 (0.6%)	0 (0%)	0 (0%)
Adenovirus + HRV/EV + RSV	9 (0.6%)	9 (1.0%)	0 (0%)	0 (0%)	0 (0%)
CoV-NL63 + HRV/EV	8 (0.5%)	7 (0.8%)	1 (0.3%)	0 (0%)	0 (0%)
CoV-HKU1 + HRV/EV	5 (0.3%)	3 (0.3%)	2 (0.6%)	0 (0%)	0 (0%)
CoV-OC43 + HRV/EV	5 (0.3%)	5 (0.6%)	0 (0%)	0 (0%)	0 (0%)
hMPV + HRV/EV	5 (0.3%)	5 (0.6%)	0 (0%)	0 (0%)	0 (0%)

PERFORMANCE CHARACTERISTICS

Clinical Performance of the BioFire RP2.1 SARS-CoV-2 and BioFire RP2 Assays

A three arm clinical evaluation was conducted to evaluate the performance of the new BioFire RP2.1 SARS-CoV-2 assays and to demonstrate equivalent performance of all other assays relative to the BioFire RP2 when testing clinical specimens.

The first arm involved testing of 50 archived NPS specimens that had previously been characterized as positive for SARS-CoV-2. Specimens were obtained from three geographically distinct laboratories in the United States (Table 9).

Table 9. Archived Source and Identification Methods

Site	Location	Positive Samples Tested	Sample ID Method
Site 1	Salt Lake City, Utah	15	Panther Fusion® SARS-CoV-2 (Hologic, Inc.; EUA)
Site 2	Seattle, Washington	15	Laboratory Developed Test (LDT) based on CDC N1 and N2 EUA assays (Washington State EUA)
Site 3	Omaha, Nebraska	20	cobas® SARS-CoV-2 (Roche Molecular Systems; EUA)

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 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 10). For SARS-CoV-2 archived specimens the PPA was 98% (48/49) and NPA was 100%.

Table 10. BioFire RP2.1 SARS-CoV-2 Archived NPS Specimen Performance Data Summary

Agreement with known analyte composition						
Comparator Method	**PPA:** TP/(TP+FN)	**%**	**95% CI**	**NPA:** TN/(TN+FP)	**%**	**95% CI**
Panther Fusion® SARS-CoV-2 (Hologic, Inc.)	14/15[1]	93.3	[70.2-98.8%]	N/A	N/A	N/A
LDT based on CDC N1 and N2 EUA assays	15/15	100	[79.6-100%]	N/A	N/A	N/A
cobas® SARS-CoV-2 (Roche Molecular Systems)	20/20	100	[83.9-100%]	N/A	N/A	N/A
Negative Specimens	N/A	NA	N/A	49/49	100	[92.7 – 100%]
Overall Agreement	**48/49[1]**	**98**	**[89.3 – 99.6%]**	**49/49**	**100**	**[92.7 – 100%]**

[1] One FN specimen was positive upon retest

Notably, of the 48 specimens with SARS-CoV-2 Detected results, 10.4% (5/48) had other analytes identified by the BioFire RP2.1 (Table 11).

Table 11. 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%)

Archived clinical specimens testing was complemented with 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 12. For SARS-CoV-2 contrived testing, both the PPA and NPA were 100%.

Table 12. Contrived SARS-CoV-2 Testing with the BioFireRP2.1

	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%]**	

The final arm was a clinical comparison study between the BioFire RP2 and modified BioFire RP2.1 using 220 archived clinical specimens. Archived specimens were chosen solely based on the analyte content. Analyte level, if known, was not used for specimen selection. Specimens were split for testing side-by-side with each test. This comparison of archived specimens demonstrates equivalent performance between the BioFire RP2 and BioFire RP2.1 for shared analytes with 97.6% PPA and 99.8% NPA overall (Table 13).

Table 13. Performance Comparison of the Modified BioFire RP2.1 to the Original BioFire RP2 using Archived Specimens

Analyte	RP2.1+ RP2+	RP2.1 RP2+	PPA	RP2.1 RP2	RP2.1+ RP2	NPA
Viruses						
Adenovirus	14	1	93.3%	203	2	99%
Coronavirus 229E	10	1	90.9%	209	0	100%
Coronavirus HKU1	10	0	100%	208	2	99%
Coronavirus NL63	10	0	100%	210	0	100%
Coronavirus OC43	10	0	100%	210	0	100%
Human Metapneumovirus	12	0	100%	208	0	100%
Human Rhinovirus/Enterovirus	19	3	86.4%	195	3	98.5%
Influenza A	30	0	100%	180	0	100%
Influenza A H1	5	0	100%	215	0	100%
Influenza A H1-2009	12	0	100%	208	0	100%
Influenza A H3	13	0	100%	207	0	100%
Influenza B	10	0	100%	210	0	100%
Parainfluenza Virus 1	9	0	100%	211	0	100%
Parainfluenza Virus 2	11	0	100%	209	0	100%
Parainfluenza Virus 3	10	1	90.9%	208	1	99.5%
Parainfluenza Virus 4	11	0	100%	209	0	100%
Respiratory Syncytial Virus	10	0	100%	210	0	100%
Bacteria						
***Bordetella parapertussis* (IS*1001*)**	10	0	100%	210	0	100%
***Bordetella pertussis* (*ptxP*)**	10	0	100%	210	0	100%

Analyte	RP2.1+ RP2+	RP2.1 RP2+	PPA	RP2.1 RP2	RP2.1+ RP2	NPA
Chlamydia pneumoniae	10	0	100%	210	0	100%
Mycoplasma pneumoniae	10	0	100%	210	0	100%
Overall	***246***	***6***	***97.6%***	***4350***	***8***	***99.8%***

All 220 specimens tested in the clinical comparison study were collected before December 2019 and were evaluated for SARS-CoV-2 specificity. This data is summarized in Table 14 along with the specificity values from the other studies. Overall NPA (specificity) for all three studies was 279/279 (100%; Table 14).

Table 14. Overall BioFire RP2.1 NPA (Specificity) for SARS-CoV-2

	NPA: TN/(TN+FP)	%	95% CI
Archived Specimens	49/49	100%	[92.7 - 100%]
Contrived Specimens	10/10	100%	[72.2 - 100%]
Comparison Specimens	220/220	100%	[98.3 - 100%]
Overall	**279/279**	**100%**	**[98.6 - 100%]**

Clinical Performance of BioFire RP2

Prospective Clinical Evaluation of BioFire RP2

The clinical performance of the BioFire RP2 was established during a multi-center study conducted at three geographically distinct U.S. study sites during portions of the 2015-2016 and 2016-2017 respiratory illness seasons. A total of 1635 residual NPS specimens in viral transport media (VTM) were acquired for the prospective clinical study. Between January and March 2016, specimens were prospectively collected from all comers meeting the study eligibility criteria and immediately frozen (N=695 specimens) for later testing as prospective archived/frozen (Category II) specimens. Between September and November 2016, specimens were prospectively collected from all comers meeting the study eligibility criteria and tested fresh (N=940 specimens) as prospective fresh (Category I) specimens. Category II specimens were distributed to study sites beginning in September 2016. Study sites also began testing Category I specimens at this time. At each site, Category II specimens were thawed and tested according to the study procedures as time permitted over the remaining duration of the clinical study. A total of 23 prospective specimens (Category I and II specimens) were excluded from the final performance data analysis due to incompliance with the study protocol. The most common reasons for specimen exclusion were that a valid external control was not completed on the day of testing, that specimens were tested outside the 3-day refrigerated storage window, or that the specimen was found to not meet the inclusion criteria after the specimen had been enrolled. The final data set consisted of 1612 prospective specimens. Table 15 provides a summary of demographic information for the 1612 specimens included in the prospective study.

Table 15. Demographic Summary for Prospective BioFire RP2 Clinical Evaluation

		Overall	Site 1	Site 2	Site 3
Sex	Male	867 (54%)	331 (57%)	271 (51%)	265 (53%)
	Female	745 (46%)	250 (43%)	256 (49%)	239 (47%)
Age	≤ 5 years	885 (55%)	379 (65%)	170 (32%)	336 (67%)
	6 - 21 years	331 (21%)	132 (23%)	89 (17%)	110 (22%)
	22 - 49 years	128 (8%)	27 (5%)	79 (15%)	22 (4%)
	50+ years	268 (17%)	43 (7%)	189 (36%)	36 (7%)
Status	Outpatient	329 (20%)	77 (13%)	66 (13%)	186 (37%)
	Hospitalized	640 (40%)	229 (39%)	197 (37%)	214 (42%)
	Emergency	643 (40%)	275 (47%)	264 (50%)	104 (21%)
Total		**1612**	**581**	**527**	**504**

The performance of the BioFire RP2 was evaluated by comparing the BioFire RP2 test results with those from an FDA-cleared multiplexed respiratory pathogen panel (the main comparator method) as well as with results from two analytically-validated PCR assays followed by bi-directional sequencing for *B. parapertussis* (this analyte is not detected by the FDA-cleared multiplexed respiratory pathogen panel). The *B. parapertussis* comparator assays were designed to amplify a different sequence than that amplified by the BioFire RP2. Any specimen that had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched organism-specific sequences deposited in the NCBI GenBank database (www.ncbi.nlm.nih.gov) with acceptable E-values was considered Positive. Any specimen that tested negative by both of the comparator assays was considered Negative.

Positive Percent Agreement (PPA) for each analyte was calculated as 100% x (TP / (TP + FN)). True positive (TP) indicates that both the BioFire RP2 and the comparator method had a positive result for this specific analyte, and false negative (FN) indicates that the BioFire RP2 result was negative while the comparator result was positive. Negative Percent Agreement (NPA) was calculated as 100% x (TN / (TN + FP)). True negative (TN) indicates that both the BioFire RP2 and the comparator method had negative results, and a false positive (FP) indicates that the BioFire RP2 result was positive but the comparator result was negative. The exact binomial two-sided 95% confidence interval was calculated. Samples for which false positive and/or false negative results (i.e., discrepant results) were obtained when comparing the BioFire RP2 results to the comparator method results were further investigated. The discrepancy investigation was mainly conducted by performing independent molecular methods with primers that are different from that of the BioFire RP2 and/or comparator method retesting. The prospective clinical study results are summarized in Table 16.

Table 16. BioFire RP2 Prospective Clinical Performance Summary

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Viruses							
Adenovirus[a]	Fresh	36/38	94.7	82.7-98.5	850/880	96.6	95.2-97.6
	Frozen	34/36	94.4	81.9-98.5	640/658	97.3	95.7-98.3
	Overall	***70/74***	***94.6***	***86.9-97.9***	***1490/1538***	***96.9***	***95.9-97.6***
CoV-229E[b]	Fresh	5/5	100	56.6-100	909/913	99.6	98.9-99.8
	Frozen	6/7	85.7	48.7-97.4	686/687	99.9	99.2-100
	Overall	***11/12***	***91.7***	***64.6-98.5***	***1595/1600***	***99.7***	***99.3-99.9***
CoV-HKU1[c]	Fresh	1/1	100	-	917/917	100	99.6-100
	Frozen	42/42	100	91.6-100	640/652	98.2	96.8-98.9
	Overall	***43/43***	***100***	***91.8-100***	***1557/1569***	***99.2***	***98.7-99.6***

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
CoV-NL63[d]	Fresh	0/0	-	-	917/918	99.9	99.4-100
	Frozen	40/40	100	91.2-100	645/654	98.6	97.4-99.3
	Overall	***40/40***	***100***	***91.2-100***	***1562/1572***	***99.4***	***98.8-99.7***
CoV-OC43[e]	Fresh	11/13	84.6	57.8-95.7	904/905	99.9	99.4-100
	Frozen	22/28	78.6	60.5-89.8	662/666	99.4	98.5-99.8
	Overall	***33/41***	***80.5***	***66.0-89.8***	***1566/1571***	***99.7***	***99.3-99.9***
hMPV[f]	Fresh	5/5	100	56.6-100	913/913	100	99.6-100
	Frozen	68/70	97.1	90.2-99.2	616/624	98.7	97.5-99.3
	Overall	***73/75***	***97.3***	***90.8-99.3***	***1529/1537***	***99.5***	***99.0-99.7***
HRV/EV[g]	Fresh	320/328	97.6	95.3-98.8	532/590	90.2	87.5-92.3
	Frozen	105/108	97.2	92.1-99.1	567/586	96.8	95.0-97.9
	Overall	***425/436***	***97.5***	***95.5-98.6***	***1099/1176***	***93.5***	***91.9-94.7***
FluA[h]	Fresh	3/3	100	43.9-100	915/915	100	99.6-100
	Frozen	75/75	100	95.1-100	616/616	100	99.4-100
	Overall	***78/78***	***100***	***95.3-100***	***1531/1531***	***100***	***99.7-100***
FluA H1	Fresh	0/0	-	-	918/918	100	99.6-100
	Frozen	0/0	-	-	691/691	100	99.4-100
	Overall	***0/0***	***-***	***-***	***1609/1609***	***100***	***99.8-100***
FluA H1-2009	Fresh	0/0	-	-	918/918	100	99.6-100
	Frozen	74/74	100	95.1-100	617/617	100	99.4-100
	Overall	***74/74***	***100***	***95.1-100***	***1535/1535***	***100***	***99.8-100***
FluA H3	Fresh	3/3	100	43.9-100	915/915	100	99.6-100
	Frozen	1/1	100	-	690/690	100	99.4-100
	Overall	***4/4***	***100***	***51.0-100***	***1605/1605***	***100***	***99.8-100***
FluB[i]	Fresh	0/0	-	-	918/918	100	99.6-100
	Frozen	14/14	100	78.5-100	678/680	99.7	98.9-99.9
	Overall	***14/14***	***100***	***78.5-100***	***1596/1598***	***99.9***	***99.5-100***
PIV1[j]	Fresh	5/5	100	56.6-100	913/913	100	99.6-100
	Frozen	4/4	100	51.0-100	689/690	99.9	99.2-100
	Overall	***9/9***	***100***	***70.1-100***	***1602/1603***	***99.9***	***99.6-100***
PIV2[k]	Fresh	46/47	97.9	88.9-99.6	863/871	99.1	98.2-99.5
	Frozen	0/0	-	-	694/694	100	99.4-100
	Overall	***46/47***	***97.9***	***88.9-99.6***	***1557/1565***	***99.5***	***99.0-99.7***
PIV3[l]	Fresh	40/42	95.2	84.2-98.7	867/876	99.0	98.1-99.5
	Frozen	3/3	100	43.9-100	690/691	99.9	99.2-100
	Overall	***43/45***	***95.6***	***85.2-98.8***	***1557/1567***	***99.4***	***98.8-99.7***
PIV4[m]	Fresh	6/6	100	61.0-100	910/912	99.8	99.2-99.9
	Frozen	3/3	100	43.9-100	686/691	99.3	98.3-99.7
	Overall	***9/9***	***100***	***70.1-100***	***1596/1603***	***99.6***	***99.1-99.8***
RSV[n]	Fresh	44/45	97.8	88.4-99.6	867/873	99.3	98.5-99.7
	Frozen	131/131	100	97.2-100	545/563	96.8	95.0-98.0
	Overall	***175/176***	***99.4***	***96.9-99.9***	***1412/1436***	***98.3***	***97.5-98.9***
Bacteria							
B. parapertussis (IS***1001***)[o]	Fresh	4/5	80.0	37.6-96.4	913/913	100	99.6-100
	Frozen	2/2	100	34.2-100	692/692	100	99.4-100

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
	Overall	***6/7***	***85.7***	***48.7-97.4***	***1605/1605***	***100***	***99.8-100***
***B. pertussis* (*ptxP*)[p]**	Fresh	2/2	100	34.2-100	915/916	99.9	99.4-100
	Frozen	0/1	0.0	-	693/693	100	99.4-100
	Overall	***2/3***	***66.7***	***20.8-93.9***	***1608/1609***	***99.9***	***99.6-100***
***C. pneumoniae*[q]**	Fresh	2/2	100	34.2-100	915/916	99.9	99.4-100
	Frozen	3/3	100	43.9-100	691/691	100	99.4-100
	Overall	***5/5***	***100***	***56.6-100***	***1606/1607***	***99.9***	***99.6-100***
***M. pneumoniae*[r]**	Fresh	17/17	100	81.6-100	897/901	99.6	98.9-99.8
	Frozen	6/7	85.7	48.7-97.4	686/687	99.9	99.2-100
	Overall	***23/24***	***95.8***	***79.8-99.3***	***1583/1588***	***99.7***	***99.3-99.9***

[a] Adenovirus was detected in 3/4 FN specimens using an independent molecular method. Adenovirus was detected in 38/48 FP specimens using an independent molecular method; an additional two FP specimens were indicated to have been collected from subjects with an acute history of adenovirus infection.

[b] The single FN specimen was negative for CoV-229E when tested using an independent molecular method. All five FP specimens were negative for CoV-229E when tested using an independent molecular method.

[c] CoV-HKU1 was detected in 3/12 FP specimens upon comparator method retest.

[d] CoV-NL63 was detected in 3/10 FP specimens during discrepancy investigation; two were detected using an independent molecular method and one was detected upon comparator method retest.

[e] Of the eight FN specimens, six were TP for CoV-HKU1. They were confirmed to be due to a known cross-reactivity with CoV-HKU1 by the comparator method; All six specimens were negative for CoV-OC43 when tested with two independent PCR assays; the remaining two FN specimens were negative for CoV-OC43 when tested using an independent molecular method. CoV-OC43 was detected in 2/5 FP specimens upon comparator method retest.

[f] Both FN specimens were negative for hMPV when tested using an independent molecular method. hMPV was detected in 6/8 FP specimens during discrepancy investigation; one was detected using an independent molecular method and five were detected upon comparator method retest.

[g] HRV/EV was detected in 5/11 FN specimens during discrepancy investigation; one was detected using an independent molecular method and four were detected upon BioFire RP2 retest. HRV/EV was detected in 33/77 FP specimens during discrepancy investigation; four were detected using an independent molecular method and 29 were detected upon comparator method retest.

[h] Three specimens were excluded from influenza A analysis: one with a comparator method result of Influenza A (No Subtype Detected) and two BioFire RP2 Influenza A (Equivocal) detections.

[i] FluB was detected in both FP specimens during discrepancy investigation; one was detected using an independent molecular method and one was detected upon comparator method retest.

[j] The single FP specimen was negative for PIV1 when tested using an independent molecular method.

[k] The single FN specimen was negative for PIV2 when tested using an independent molecular method. PIV2 was detected in 5/8 FP specimens during discrepancy investigation; one was detected using an independent molecular method and four were detected upon comparator method retest.

[l] PIV3 was detected in both FN specimens during discrepancy investigation; one was detected using an independent molecular method and one was detected upon BioFire RP2 retest. PIV3 was detected in 4/10 FP specimens during discrepancy investigation; two were detected using an independent molecular method and two were detected upon comparator method retest.

[m] PIV4 was detected in 1/7 FP specimens using an independent molecular method.

[n] The single FN specimen was negative for RSV when tested using an independent molecular method. RSV was detected in 8/24 FP specimens during discrepancy investigation; three were detected using an independent molecular method and five were detected upon comparator method retest.

[o] *B. parapertussis* was detected in the single FN specimen upon BioFire RP2 retest.

[p] *B. pertussis* was detected in the both the FN and FP specimens using an independent molecular method.

[q] *C. pneumoniae* was detected in the single FP specimen using an independent molecular method.

[r] *M. pneumoniae* was detected in the single FN specimen upon BioFire RP2 retest. *M. pneumoniae* was detected in all five FP specimens during discrepancy investigation; three were detected using an independent molecular method and two were detected upon comparator method retest.

BioFire RP2 reported a total of 245 specimens with discernible multiple organism detections (15.2% of all specimens, 245/1612; and 24.0% of positive specimens, 245/1020; Table 17). The majority of multiple detections (190/245; 77.6%) contained two organisms, while 20.0% (49/245) contained three organisms, 1.6% (4/245) contained four organisms, 0.4% (1/245) contained five organisms, and 0.4% (1/245) contained six organisms. Out of the 245 specimens with multiple detections, 124 specimens (50.6%; 124/245) were concordant with the comparator methods. One hundred twenty-one (121) specimens (49.4%; 121/245) contained one or more organisms that had not been detected by the comparator methods (i.e. false positive results).

The three organisms that were most prevalent in multiple detections were also the three most prevalent organisms in the study as a whole (i.e. HRV/EV, RSV, and adenovirus). The most prevalent multiple detections (≥5 instances) are shown in Table 18.

Table 17. Prevalence of Analytes in Multiple Detections as determined by the BioFire RP2

Analyte	Prevalence in Multiple Detections (N 245)	
Viruses		
Adenovirus	85	34.7%
CoV-229E	6	2.4%
CoV-HKU1	41	16.7%
CoV-NL63	31	12.7%
CoV-OC43	19	7.8%
hMPV	33	13.5%
HRV/EV	150	61.2%
FluA H1	0	0%
FluA H1-2009	9	3.7%
FluA H3	2	0.8%
FluB	6	2.4%
PIV1	5	2.0%
PIV2	15	6.1%
PIV3	21	8.6%
PIV4	12	4.9%
RSV	105	42.9%
Bacteria		
B. parapertussis (IS*1001*)	6	2.4%
B. pertussis (*ptxP*)	0	0%
C. pneumoniae	1	0.4%
M. pneumoniae	7	2.9%

The most prevalent multiple detection was adenovirus with HRV/EV (1.9% of all specimens; 30/1612) followed by HRV/EV with RSV (1.4% of all specimens; 22/1612); as previously stated these were also the most prevalent organisms detected in the study.

Table 18. Multiple Detection Combinations (≥5 instances) as Determined by the BioFire RP2

Distinct Multiple Detection Combinations			Total Multiple Detections	Number of Specimens with False Positive Detections	False Positive Analyte(s)[a]
Analyte 1	Analyte 2	Analyte 3			
Adenovirus	HRV/EV		30	15	Adenovirus (15), HRV/EV (1)
HRV/EV	RSV		22	7	HRV/EV (3), RSV (4)
CoV-HKU1	RSV		13	7	CoV-HKU1 (4), RSV (3)
CoV-NL63	RSV		13	3	CoV-NL63 (2), RSV (1)
HRV/EV	PIV2		11	7	HRV/EV (6), PIV2 (2)
HRV/EV	PIV3		11	6	HRV/EV (3), PIV3 (4)
Adenovirus	RSV		10	5	Adenovirus (4), RSV (1)
Adenovirus	HRV/EV	RSV	9	5	Adenovirus (2), HRV/EV (3), RSV (1)
CoV-NL63	HRV/EV		8	2	CoV-NL63 (2)

Distinct Multiple Detection Combinations			Total Multiple Detections	Number of Specimens with False Positive Detections	False Positive Analyte(s)[a]
Analyte 1	Analyte 2	Analyte 3			
CoV-HKU1	HRV/EV		5	2	CoV-HKU1 (1), HRV/EV (1)
CoV-OC43	HRV/EV		5	3	HRV/EV (3)
hMPV	HRV/EV		5	1	HRV/EV

[a] Of the 67 discrepant analytes (out of 293 total analytes), 32 (47.8%) were observed as being present in the specimen during discrepancy investigation; 22/67 (32.8%) were observed using an independent molecular method and 13/67 (19.4%) were observed upon comparator method retest.

The overall success rate for initial specimen tests in the prospective study was 99.3% (1611/1623) (95% CI: 98.7% - 99.6%); 12 tests were unsuccessful (one due to an incomplete test, one due to an instrument error, and ten due to control failures). Two tests (2/1623; 0.1%) did not complete on the initial run, resulting in an instrument success rate of 99.9% (1621/1623) (95% CI: 99.6% - 100%) for initial specimen tests. Both specimens were able to be retested and valid results were produced after a single retest. Ten tests (10/1621; 0.6%) did not produce valid pouch controls, resulting in a pouch control success rate of 99.4% (1611/1621) (95% CI: 98.9% - 99.7%) for completed runs in the initial specimen tests. Nine of the 10 invalid specimens were able to be retested and produced valid control results after a single retest; one was not able to be retested due to insufficient specimen volume.

Testing of Preselected Archived Specimens with BioFire RP2

Some of the analytes on the BioFire RP2 were of low prevalence and were not encountered in large enough numbers during the prospective study to adequately demonstrate system performance. To supplement the results of the prospective clinical study, an evaluation of preselected archived retrospective specimens was performed at BioFire. These specimens were archived NPS in VTM specimens that were selected because they had previously tested positive for one of the following analytes: coronavirus 229E, influenza A H1, influenza A H3, influenza B, parainfluenza virus 1, parainfluenza virus 4, *Bordetella parapertussis*, *B. pertussis*, and *Chlamydia pneumoniae.* Parainfluenza virus 2, parainfluenza virus 3, and *Mycoplasma pneumoniae* were also expected to be low prevalence based on BioFire data collected during the 2015-2016 respiratory season, therefore archived testing was performed for these analytes as well and included in the study data (although ultimately they were observed in larger numbers during the prospective clinical study).

A total of 217 preselected archived retrospective clinical specimens were initially received for testing in this retrospective study. Prior to testing with the BioFire RP2, the composition/integrity of the specimens was first confirmed with confirmatory molecular methods (PCR followed by bi-directional sequencing for *B. parapertussis* or an FDA-cleared multiplexed respiratory pathogens panel.

The specimens were divided into two different groups for testing based on the method of confirmation testing performed: all specimens containing analytes on the FDA-cleared multiplexed respiratory pathogens panel comparator method were tested in Group 1 and specimens containing *B. parapertussis* were tested in Group 2. Negative NPS specimens were also included in each group for testing.

The FDA-cleared multiplexed respiratory pathogen panel comparator method was performed on 197 of the 217 preselected archived retrospective clinical specimens only (Group 1). One of the 197 specimens was excluded from performance analysis because of an invalid BioFire RP2 run with insufficient volume to retest. Additionally, two of the 197 specimens were also excluded from performance analysis because a valid FDA-cleared multiplexed respiratory pathogens panel comparator method confirmation result was not obtained and there was insufficient specimen volume for retesting: one comparator run was incomplete and the other comparator run had a control failure. Valid comparator method and BioFire RP2 results were obtained for 194 of these 197 archived specimens (Group 1).

The *B. parapertussis* PCR followed by bi-directional sequencing comparator assays were performed on 20 of the 217 preselected archived retrospective clinical specimens only (Group 2). The FDA-cleared multiplexed respiratory

pathogens panel comparator method was not performed on Group 2 specimens. Valid comparator method and BioFire RP2 results were obtained for 20 of these 20 archived specimens.

A summary of the available demographic information of these 214 valid archived specimens is provided in Table 19.

Table 19. Available Demographic Summary for All Valid Archived Specimens

Total Specimens		214
Sex	Female (%)	75 (35%)
	Male (%)	81 (38%)
	Unknown	58 (27%)
Age Range	≤ 5 years	78 (36%)
	6 - 21 years	46 (21%)
	22 - 49 years	13 (6%)
	50+ years	19 (9%)
	Unknown	58 (27%)

All Group 1 and Group 2 positive archived specimens (as determined at the source laboratory) that were not confirmed by the respective comparator method were further excluded from the performance calculation for each of the respective analytes.

The BioFire RP2 retrospective specimens testing performance data against the comparator methods are provided in Table 20 by analyte.

Table 20. BioFire RP2 Archived Specimen Performance Data Summary

Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses						
Adenovirus	0/0	0	N/A	189/194	97.4	94.1-98.9
CoV- 229E [a]	15/15	100	79.6-100	175/175	100	97.9-100
CoV-HKU1	0/0	0	N/A	194/194	100	98.1-100
CoV-NL63	2/2	100	34.2-100	192/192	100	98.0-100
CoV-OC43	0/0	0	N/A	194/194	100	98.1-100
hMPV	1/1	100	20.7-100	192/193	99.5	97.1-99.9
HRV/EV	18/19	94.7	75.4-99.1	168/175	96.0	92.0-98.0
Influenza A	22/22	100	85.1-100	172/172	100	97.8-100
Influenza A H1	3/3	100	43.9-100	191/191	100	98.0-100
Influenza A 2009-H1	1/1	100	20.7-100	193/193	100	98.0-100
Influenza A H3	18/18	100	82.4-100	176/176	100	97.9-100
Influenza B [b]	16/16	100	80.6-100	177/177	100	97.9-100
Parainfluenza Virus 1	16/16	100	80.6-100	178/178	100	97.9-100
Parainfluenza Virus 2 [c]	16/16	100	80.6-100	177/177	100	97.9-100
Parainfluenza Virus 3	17/17	100	81.6-100	175/177	98.9	96.0-99.7
Parainfluenza Virus 4	17/17	100	81.6-100	174/177	98.3	95.1-99.4

Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
RSV	2/2	100	34.2-100	191/192	99.5	97.1-99.9
Bacteria						
Bordetella parapertussis (IS*1001*) [d]	16/16	100	80.6-100	4/4	100	51.0-100
Bordetella pertussis (*ptx*P) [e]	25/26	96.2	81.1-99.3	160/162	98.8	95.6-99.7
Chlamydia pneumoniae [f]	17/17	100	81.6-100	176/176	100	97.9-100
Mycoplasma pneumoniae [g]	16/16	100	80.6-100	171/173	98.8	95.9-99.7

[a] Four of 19 CoV-229E positive archived specimens by the source laboratory were not confirmed by the comparator method and therefore were excluded from the performance calculation for CoV-229E .

[b] One of the 17 Influenza B positive archived specimens by the source laboratory was not confirmed by the comparator method and therefore was excluded from the performance calculation for Influenza B.

[c] One of the 17 Parainfluenza Virus 2 positive archived specimens the source laboratory was not confirmed by the comparator method and therefore was excluded from the performance calculation for Parainfluenza Virus 2 .

[d] The comparator *B. parapertussis* PCR followed by sequencing assays were performed on 20 archived specimens only (Group 2). The comparator method for the other analytes was not performed on these 20 specimens.

[e] Six of the 31 *B. pertussis* positive archived specimens by the source laboratory were not confirmed by the comparator method and therefore were excluded from the performance calculation for *B. pertussis*.

[f] One of the 17 *C. pneumoniae* positive archived specimens by the source laboratory was not confirmed by the comparator method and therefore was excluded from the performance calculation for *C. pneumoniae*.

[g] Five of the 21 *M. pneumoniae* positive archived specimens by the source laboratory were not confirmed by the comparator method and therefore were excluded from the performance calculation for *M. pneumoniae*.

Testing of Contrived Specimens with BioFire RP2

Influenza A H1 is of such rarity that that both prospective and retrospective archived testing efforts were insufficient to demonstrate system performance. To supplement the prospective and retrospective data, an evaluation of contrived specimens was performed at one of the three clinical testing sites participating in the prospective evaluation. Contrived clinical specimens were prepared using individual unique residual NPS specimens that had previously tested negative by the FDA-cleared multiplexed respiratory pathogens panel (i.e., the same test as the comparator method employed in the prospective and retrospective clinical evaluations) at the source laboratory. Spiking was performed using multiple quantified isolates of Influenza A H1. The spiking scheme was such that at least 25 of the contrived positive specimens had analyte concentrations at 2 × the limit of detection (LoD), while the remaining 25 contrived positive specimens were at additional concentrations that spanned the clinically relevant range which was based on BioFire RP2 Cp observations of influenza A (A H1, A H-2009, and H3) from the prospective and archived specimen studies. Contrived positive specimens were prepared and randomized along with 50 un-spiked influenza A H1 negative specimens such that the analyte status of each contrived specimen was unknown to the users performing the testing. The results of the BioFire RP2 testing contrived specimens are presented in Table 21.

Table 21. BioFire RP2 Performance Using Contrived Specimens

Analyte	Positive Percent Agreement				Negative Percent Agreement		
	× LoD	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Influenza A H1	2	22/23 [a]	95.7%	79.0-99.2	50/50	100	92.9-100
	10	10/10	100%	72.3-100			
	50	5/5	100%	56.6-100			
	200	5/5	100%	56.6-100			
	1000	5/5	100%	56.6-100			
	Combined	47/48 [a]	97.9%	89.1-99.6			

[a] The FN specimen was spiked with influenza A/Weiss/43; this strain was detected at all other concentrations. Two specimens (also spiked with strain A/Weiss/43) had a result of Influenza A Equivocal or Influenza A H1 Equivocal and were excluded from Influenza A H1 performance calculation.

Limit of Detection

LoD for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

The BioFire RP2.1 limit of detection (LoD) for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was estimated by testing serial dilutions of contrived samples containing known concentrations of inactivated or infectious virus culture (concentration of viral RNA in the cultures determined by digital droplet (dd) PCR or quantitative real-time PCR, respectively). The estimated LoD was the lowest concentration tested with detection in all replicates. Confirmation of the LoD concentration for the inactivated virus (in transport medium) and infectious virus (in pooled clinical NPS) was achieved when the virus was detected in at least 19 of 20 replicates (≥95%) tested on FilmArray 2.0 and FilmArray Torch systems (Table 22).

Table 22. Limit of Detection (LoD) for SARS-CoV-2

BioFire RP2.1 Analyte	Isolate		LoD Concentration	#Detected/Total
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	USA-WA1/2020	Heat-inactivated virus ATCC VR-1986HK	**5.0E+02 copies/mL**[a] 6.9E-02 $TCID_{50}$/mL	**20/20** 100%
		Infectious virus[b]	**1.6E+02 copies/mL**[c] 1.1E-02 $TCID_{50}$/mL	**20/20** 100%

[a] Concentration of viral RNA determined by ddPCR, as indicated on the Certificate of Analysis from ATCC.

[b] Obtained for culturing in a biosafety level 3 laboratory from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA), contributed by the U.S. Centers for Disease Control (CDC).

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

FDA SARS-CoV-2 Reference Panel Testing

An evaluation of SARS-CoV-2 sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples, and a standard protocol provided by the 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 Table 23.

Table 23. 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

SARS-CoV-2 Comparison Testing Near LoD

Detection of SARS-CoV-2 by the BioFire RP2.1 was compared to detection of SARS-CoV-2 by two other tests that have received Emergency Use Authorization from the U.S. Food and Drug Administration (BioFire® COVID-19 Test from BioFire Defense, LLC and the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel from the U.S. Centers for Disease Control).

For comparison to the BioFire COVID-19 Test, contrived samples were prepared by spiking inactivated SARS-CoV-2 (USA-WA1/2020; ATCC VR-1986HK) into transport medium and serially diluting, with an intermediate dilution near the LoD of the BioFire COVID-19 Test. Five replicates of each sample were tested with the BioFire RP2.1 and BioFire COVID-19 Test according to the manufacturer's instructions for use. SARS-CoV-2 was detected equivalently at concentrations ≥3.0E+02 copies/mL (Table 24).

Table 24. Inactivated SARS-CoV-2 Detection Comparison Between BioFire RP2.1 and the BioFire COVID-19 Test

D = Detected, ND = Not Detected

	5.0E+04 copies/mL		5.0E+03 copies/mL		5.0E+02 copies/mL		3.0E+02 copies/mL		5.0E+01 copies/mL	
Replicate	**RP2.1**	**COVID-19**	**RP2.1**	**COVID-19**	**RP2.1**	**COVID-19**	**RP2.1**	**COVID-19**	**RP2.1**	**COVID-19**
1	D	D	D	D	D	D	D	D	D	ND
2	D	D	D	D	D	D	D	D	ND	Equivocal
3	D	D	D	D	D	D	ND	D	D	ND
4	D	D	D	D	D	D	D	ND	D	ND
5	D	D	D	D	D	D	D	D	ND	ND
#Detected /Total	**5/5**	**5/5**	**5/5**	**5/5**	**5/5**	**5/5**	**4/5**	**4/5**	**3/5**	**0/5**

For comparison to the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel, contrived samples were prepared by spiking infectious SARS-CoV-2 (USA-WA1/2020; WRCEVA) into pooled clinical NPS and serially diluting the sample. Six replicates of each sample were tested with the BioFire RP2.1 and CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel according to the manufacturer's instructions for use. The CDC 2019-nCOV Diagnostic Panel testing included all extraction and PCR controls, with three extraction events (QIAGEN QIAmp Viral RNA Mini Kit) and duplicate PCR reactions for each extract. Results from the N1 and N2 (and RP) assays were interpreted as Positive, Inconclusive or Negative. SARS-CoV-2 was detected by both panels equivalently at each concentration.

Table 25. Infectious SARS-CoV-2 Detection Comparison Between BioFire RP2.1 and CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel

D = Detected, ND = Not Detected

	1.6E+05 copies/mL		1.6E+04 copies/mL		1.6E+03 copies/mL		1.6E+02 copies/mL		1.6E+01 copies/mL	
Replicate	**BioFire RP2.1**	**CDC 2019-nCoV**	**BioFire RP2.1**	**CDC 2019-nCoV**	**BioFire RP2.1**	**CDC 2019-nCoV**	**BioFire RP2.1**	**CDC 2019-nCoV**	**BioFire RP2.1**	**CDC 2019-nCoV**
1	D	Positive	D	Positive	D	Positive	D	Positive	D	Positive
2	D	Positive	D	Positive	D	Positive	D	Positive	ND	Inconclusive
3	D	Positive	D	Positive	D	Positive	D	Positive	ND	Positive
4	D	Positive	D	Positive	D	Positive	D	Positive	D	Positive
5	D	Positive	D	Positive	D	Positive	D	Positive	D	Inconclusive
6	D	Positive	D	Positive	D	Positive	D	Positive	ND	Inconclusive
#Detected /Total	**6/6**	**6/6**	**6/6**	**6/6**	**6/6**	**6/6**	**6/6**	**6/6**	**3/6**	**3/6**

LoD for Other BioFire RP2.1 Analytes (non-SARS-CoV-2)

BioFire RP2.1 LoD confirmation testing for all other analytes was performed with contrived samples containing analytes spiked at the BioFire RP2 LoD concentration in transport medium. Confirmation of the LoD was achieved when detection was observed in at least 19 of 20 replicates (≥95%) tested on FilmArray 2.0 and FilmArray Torch systems. BioFire RP2.1 LoD concentration is provided in viable or infectious units (e.g. CFU/mL, CCU/mL or $TCID_{50}$/mL) and/or nucleic acid copies/mL based on a quantitative real-time PCR (Table 26). The confirmed LoD for all analytes is equivalent (within 2-fold) between BioFire RP2.1 and the BioFire RP2.

Table 26. Summary of Limit of Detection (LoD) for BioFire RP2.1 Analytes (non-SARS-CoV-2)

BioFire RP2.1 Analyte	Isolate	LoD Concentration	#Detected/Total
Viruses			
Adenovirus	Species C Serotype 2 WHO International Standard NIBSC 16/324	3.0E+03 IU/mL[a] (3.0E+03 copies/mL)	**20/20** 100%
Coronavirus 229E	ATCC VR-740	4.0E-01 $TCID_{50}$/mL 6.5E+01 copies/mL	**20/20** 100%
Coronavirus HKU1	Clinical specimen	2.0E+03 copies/mL	**19/20**[b] 95.0%
Coronavirus NL63	BEI NR-470		**20/20**

BioFire RP2.1 Analyte	Isolate	LoD Concentration	#Detected/Total
		2.5 E-01 $TCID_{50}$/mL 5.4E+01 copies/mL	100%
Coronavirus OC43	ATCC VR-759	3.0E+01 $TCID_{50}$/mL 5.6E+02 copies/mL	**20/20** 100%
Human Metapneumovirus	16, Type A1 IA10-2003 Zeptometrix 0810161CF	1.0E+01 $TCID_{50}$/mL[b] 1.2E+03 copies/mL[b]	**20/20** 100%
Human Rhinovirus/ Enterovirus[d]	Human Rhinovirus Type 1A Zeptometrix 0810012CFN	8.6E-02 $TCID_{50}$/mL[c] 3.8E+01 copies/mL[c]	**19/20** 95.0%
	Enterovirus D68 ATCC VR-1823	3.0E+02 $TCID_{50}$/mL 2.6E+01 copies/mL	**20/20** 100%
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%
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%
Influenza A H3	Influenza H3N2 A/Port Chalmers/1/73 ATCC VR-810	1.0E-01 $TCID_{50}$/mL 2.1E+01 copies/mL	**20/20** 100%
Influenza B	B/FL/04/06 Zeptometrix 0810255CF	5.0E+00 $TCID_{50}$/mL 3.4E+01 copies/mL	**20/20** 100%
Parainfluenza Virus 1	Type 1 Zeptometrix 0810014CF	5.0E+00 $TCID_{50}$/mL 1.0E+03 copies/mL	**20/20** 100%
Parainfluenza Virus 2	Type 2 Zeptometrix 0810015CF	5.0E-01 $TCID_{50}$/mL 3.0E+01 copies/mL	**19/20** 95.0%
Parainfluenza Virus 3	Type 3 Zeptometrix 0810016CF	2.5E+00 $TCID_{50}$/mL 3.8E+01 copies/mL	**20/20** 100%
Parainfluenza Virus 4	Type 4a Zeptometrix 0810060CF	5.0E+01 $TCID_{50}$/mL 1.6E+03 copies/mL	**19/20** 95.0%
Respiratory Syncytial Virus	Type A Zeptometrix 0810040ACF	2.0E-02 $TCID_{50}$/mL 9.0E+00 copies/mL	**20/20** 100%
Bacteria			
***Bordetella parapertussis* (IS*1001*)**	A747 Zeptometrix 0801461	6.0E+01 IS*1001* copies/mL[d] 4.1E+01 CFU/mL	**20/20** 100%
***Bordetella pertussis* (*ptxP*)**	A639 Zeptometrix 0801459	1.0E+03 CFU/mL	**19/20** 95.0%
Chlamydia pneumoniae	TW183 ATCC VR-2282	2.0E-01 $TCID_{50}$/mL 1.3E+02 copies/mL[e]	**20/20** 100%
Mycoplasma pneumoniae	M129 Zeptometrix 0801579	6.3E+00 CCU/mL[f] 4.6E+02 copies/mL[f]	**20/20** 100%

[a] IU = International Units. Adenovirus LoD in IU/mL was first established through estimate and confirmation testing on the BioFire RP2 and the BioFire RP2.1 LoD was confirmed at the same concentration. 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.

[b] The LoD sample for Human Metapneumovirus was prepared based on $TCID_{50}$/mL. The copies/mL LoD for Human Metapneumovirus is extrapolated from the LoD established when testing a different culture of the same isolate at the same $TCID_{50}$/mL concentration on the BioFire RP2. A copies/mL value for the culture tested on BioFire RP2.1 has not been determined.

[c] The BioFire RP2.1 copies/mL LoD concentration for Human Rhinovirus is the same as the LoD concentration in copies/mL on the BioFire RP2. The $TCID_{50}$/mL LoD concentration varies between the two panels because a different culture of the same isolate was tested on BioFire RP2.1 and the ratios of copies:$TCID_{50}$ differs between the culture events.

[d] IS*1001* sequences can be present in more than one copy per cell, so the relationship between CFU/mL and copies/mL may vary from strain to strain and culture to culture. LoD was determined based on the copy number of IS*1001* measured by an independent quantitative real-time PCR assay.

[e] The copies/mL LoD concentration for *Chlamydia pneumoniae* on BioFire RP2.1 is 2-fold higher than the LoD concentration on the BioFire RP2.

[f] The BioFire RP2.1 copies/mL LoD concentration for *Mycoplasma pneumoniae* is the same as the LoD concentration in copies/mL on the BioFire RP2. The LoD concentration in viable/infectious units varies between the two panels because a different culture of the same isolate was tested on BioFire RP2.1 and a different unit of measurement (CCU/mL vs. $TCID_{50}$/mL) was used between the culture events.

NOTE: LoD concentrations of the cultured viruses and the obligate intracellular bacterium M. pneumoniae are provided in units of TCID50 (50% Tissue Culture Infectious Dose) or CCU/mL (color changing units). $TCID_{50}$ and CCU are not a direct virus or cell count, but an indirect measure of viral or bacterial concentration based on infectivity and cytotoxicity and will therefore vary considerably depending on technique and

methodology (including cell type, culture media and conditions, cytotoxicity of the virus, etc.). It is not appropriate to make determinations on relative sensitivity of different molecular assays for detection of viruses and bacteria based on LoD values measured in $TCID_{50}$/mL or CCU/mL. Concentrations are also presented in estimated copies/mL based upon independent quantitative PCR assays (qPCR). Note that the accuracy of qPCR assays may also be affected by assay conditions and sequence variance between strains.

Analytical Reactivity (Inclusivity)

Reactivity of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Assays

Evaluation of analytical reactivity for the BioFire RP2.1 SARS-CoV-2 assays (SARSCoV2-1 and SARSCoV2-2) was based on *in silico* sequence analysis of all available sequences in the NCBI and GISAID databases as of April 6, 2020. In total, 4281 sequences from around the globe were aligned to the assay primers and less than 0.07% (3/4281) were found to have a mismatched base within the 3' half of one or more primer(s) that could have an impact on amplification by one of the assays. All three sequences with a 3' mismatched base in one assay are 100% identical to all primers of the other assay.

This analysis determined that all database sequences evaluated will be amplified by one or both SARS-CoV-2 assays and all sequences (4281/4281; 100%) are predicted to be detected by the BioFire RP2.1 (Table 27).

Table 27. *In silico* Prediction of SARS-CoV-2 Detection by BioFire RP2.1 Assays

+/+ 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

Predicted Assay Result / # sequences		SARSCoV2 1		# (%) sequences predicted to be detected (one or both assays positive)
		+	-	
SARSCoV2 2	+	4278	2	4281/4281 (100%)
	-	1	0	

NOTE: At this time, only one isolate of known sequence (USA-WA1/2020) has been tested analytically with the BioFire RP2.1 and it was detected at a concentration as low as 1.6E+02 copies/mL (see Limit of Detection section above).

Reactivity of the BioFire RP2 Assays (non-SARS-CoV-2)

Analytical reactivity (inclusivity) of the non-SARS-CoV-2 assays was evaluated by *in silico* analysis and testing on the BioFire RP2 with a collection of 177 isolates that represent temporal and geographic diversity of the analytes, including relevant species, strains, serotypes, or genotypes. All isolates were tested at a concentration near LoD and all isolates were detected by the BioFire RP2 at concentrations within 10× LoD. In addition, *in silico* analysis of sequence data was used to make predictions of assay reactivity for less common strains or serotypes that were not tested but that may be detected by the BioFire RP2 (and BioFire RP2.1).

Because the BioFire RP2 assays and reaction conditions are the same in the modified BioFire RP2.1 and testing has demonstrated the LoD for these analytes is equivalent between panels (see *Limit of Detection* section); the analytical reactivity data from the BioFire RP2 has been applied to BioFire RP2.1 (Table 28 –Table 39) in all cases except *C. pneumoniae*, which was tested on BioFire RP2.1 based on the BioFire RP2.1 LoD for this analyte (Table 38).

NOTE: BioFire RP2.1 influenza A assays will react variably with non-human influenza A viruses and 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.

NOTE: BioFire RP2.1 Influenza A (subtype) and Influenza B assays are predicted to react with attenuated viruses used in vaccines.

Table 28. Adenovirus Isolates Tested and Detected by BioFire RP2

Species	Serotype[a]	Isolate ID/Source	[Strain/Location/Year]	xLoD[b] Detected	Result
A	12	ATCC VR-863	[Huie/Massachusetts]	3x	Adenovirus Detected
	18	ATCC VR-19	[Washington DC/1954]	1x	
	31	Zeptometrix 0810073CF	-	3x	
B	3	Zeptometrix 0810062CF	-	3x	
	7A	Zeptometrix 0810021CF	-	1x	
	7d/d2	Univ of Iowa Research Foundation	[Iowa/2001]	3x	
	7h	Univ of Iowa Research Foundation	[Iowa/1999]	3x	
	11	Univ of Iowa Research Foundation	[Wisconsin/2005]	3x	
	14	Univ of Iowa Research Foundation	[Missouri/2005]	3x	
	16	ATCC VR-17	[CH.79/Saudia Arabia/1955]	3x	
	21	Univ of Iowa Research Foundation	[Missouri/2005]	3x	
	34	ATCC VR-716	[Compton/1972]	3x	
	35	ATCC VR-718	[Holden]	3x	
	50	ATCC VR-1602	[Wan/Amsterdam/1988]	3x	
C	1	Zeptometrix 0810050CF	-	3x	
	2	ATCC VR-846	[Adenoid 6]	1x	
	5	Zeptometrix 0810020CF	-	3x	
	6	ATCC VR-6	[Tonsil 99/Washington DC]	3x	
D	8	Zeptometrix 0810069CF	-	3x	
	20	Zeptometrix 0810115CF	-	3x	
	37	Zeptometrix 0810119CF	-	1x	
E	4a	Univ of Iowa Research Foundation	[S Carolina/2004]	1x	
	4	Zeptometrix 0810070CF	-	3x	
F	40	Zeptometrix 0810084CF	-	3x	
		NCPV 0101141v	-	3x	
	41	ATCC VR-930	[Tak/73-3544/Netherlands/1973]	1x	
		Zeptometrix 0810085CF	-	3x	

[a] *In silico* analysis of available sequences predicts that the BioFire RP2/RP2.1 will also react with Adenovirus B55, C57, species D serotypes, and G52.

[b] xLoD refers to the multiple of the BioFire RP2 LoD concentration for each adenovirus species that was tested and detected on BioFire RP2 pouches. These concentrations are equal to or less than the equivalent BioFire RP2.1 xLoD based on the LoD established in IU/mL and copies/mL with the Adenovirus WHO International Standard.

Table 29. Coronavirus (non-SARS-CoV-2) Isolates/Specimens Tested and Detected by BioFire RP2

Coronavirus Type	Isolate ID/Source	[Location/Year]	xLoD Detected	Result
229E	ATCC VR-740	-	1x	Coronavirus 229E
	Zeptometrix 0810229CF	-	3x	
HKU1	Clinical Specimen	[Utah/2015]	1x	Coronavirus HKU1
	Clinical Specimen	[Utah/2015]	3x	
	Clinical Specimen	[Utah/2015]	3x	
	Clinical Specimen	[S. Carolina/2010]	3x	
	Clinical Specimen	[Detroit/2010]	3x	
NL63	BEI NR-470[a]	[Amsterdam/2003]	1x	Coronavirus NL63
	Zeptometrix 0810228CF	-	3x	
OC43	ATCC VR-759[b]	-	1x	Coronavirus OC43
	Zeptometrix 0810024CF	-	3x	

[a] Organism obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Human Coronavirus NL63, NR-470.

[b] Discontinued part number; see ATCC VR-1558.

Table 30. Human Metapneumovirus Isolates Tested and Detected by BioFire RP2

Genotype	Serotype	Isolate ID/Source	[Location/Year]	xLoD Detected	Result
A1	16	Zeptometrix 0810161CF	[Iowa10/2003]	1x	Human Metapneumovirus
	9	Zeptometrix 0810160CF	[Iowa3/2002]	3x	
A2	20	Zeptometrix 0810163CF	[Iowa14/2003]	3x	
	27	Zeptometrix 0810164CF	[Iowa27/2004]	3x	
B1	3	Zeptometrix 0810156CF	[Peru2/2002]	3x	

Genotype	Serotype	Isolate ID/Source	[Location/Year]	xLoD Detected	Result
	5	Zeptometrix 0810158CF	[Peru3/2003]	3x	
	13	Univ of Iowa Research Foundation	[Iowa7/2003]	3x	
B2	4	Zeptometrix 0810157CF	[Peru1/2002]	3x	
	8	Zeptometrix 0810159CF	[Peru6/2003]	3x	
	18	Zeptometrix 0810162CF	[Iowa18/2003]	3x	
	22	Univ of Iowa Research Foundation	[Iowa16/2003]	3x	

Table 31. Human Rhinovirus and Enterovirus Isolates Tested and Detected by BioFire RP2

Species	Serotype	Isolate ID/Source	[Strain/Location/Year]	xLoD Detected	Result
Human Rhinovirus					
A	1	Zeptometrix 0810012CFN	[1A]	1x	Human Rhinovirus/ Enterovirus
	2	ATCC VR-482	[HGP]	3x	
	7	ATCC VR-1601	[68-CV11]	3x	
	16	ATCC VR-283	[11757/Washington DC/1960]	3x	
	34	ATCC VR-507[a]	[137-3]	3x	
	57	ATCC VR-1600	[Ch47]	3x	
	77	ATCC VR-1187	[130-63]	3x	
	85	ATCC VR-1195	[50-525-CV54]	3x	
B	3	ATCC VR-483	[FEB]	3x	
	14	ATCC VR-284	[1059/S Carolina/1959]	3x	
	17	ATCC VR-1663	[33342/N Carolina/1959]	3x	
	27	ATCC VR-1137	[5870]	3x	
	42	ATCC VR-338	[56822]	3x	
	83	ATCC VR-1193	[Baylor 7]	3x	
Enterovirus					
A	Coxsackievirus 10	ATCC VR-168	[NY/1950]	3x	Human Rhinovirus/ Enterovirus
	Enterovirus 71	ATCC VR-1432	[H]	3x	
B	Coxsackievirus A9	Zeptometrix 0810017CF	-	3x	
	Coxsackievirus B3	Zeptometrix 0810074CF	-	3x	
	Coxsackievirus B4	Zeptometrix 0810075CF	-	3x	
	Echovirus 6	Zeptometrix 0810076CF	-	3x	
	Echovirus 9	Zeptometrix 0810077CF	-	3x	
	Echovirus 11	Zeptometrix 0810023CF	-	3x	
C	Coxsackievirus A21	ATCC VR-850	[Kuykendall/California/1952]	3x	
	Coxsackievirus A24	ATCC VR-583	[DN-19/Texas/1963]	3x	
D	68	ATCC VR-1823	[US/MO/2014-18947]	1x	

[a] Discontinued part number; see ATCC VR-1365.

Table 32. Influenza A Isolates Tested and Detected by BioFire RP2

Type	Isolate ID/Source		[Strain/Location/Year]	xLoD Detected	Result
H1N1	Human	Zeptometrix 0810036CF	[New Caledonia/20/1999]	1x	Influenza A H1
		ATCC VR-219	[NWS/1933]	3x	
		ATCC VR-95	[PR/8/1934]	10x[a]	
		ATCC VR-96	[Weiss/1943]	3x	
		ATCC VR-97	[FM/1/1947]	3x	
		ATCC VR-98	[Mal/302/1954]	3x	
		ATCC VR-546	[Denver/1/1957]	3x	
		Zeptometrix 0810036CFN	[Solomon Isl/03/2006]	3x	
		Zeptometrix 0810244CF	[Brisbane/59/2007]	3x	
	Swine	ATCC VR-333	[A/Swine/Iowa/15/1930]	3x	
		ATCC VR-99	[A/Swine/1976/1931]	3x	
		ATCC VR-897	[A/New Jersey/8/76 (Hsw1N1)]	10x[a]	
H1N2	Recombinant	BEI NR-9677[b]	[Kilbourne F63, A/NWS/1934 (HA) x A/Rockefeller Institute/5/1957 (NA)]	3x	
H1N1 pdm09	Human	Zeptometrix 0810249CFN	[SwineNY/03/2009]	1x	Influenza A H1-2009
		Zeptometrix 0810248CFN	[SwineNY/01/2009]	3x	
		Zeptometrix 0810109CFN	[SwineNY/02/2009]	3x	
		Zeptometrix 0810109CFJ	[Canada/6294/2009]	3x	
		Zeptometrix 0810165CF	[California/07/2009]	3x	
		Zeptometrix 0810166CF	[Mexico/4108/2009]	3x	
		BEI NR-19823[c]	[Netherlands/2629/2009]	3x	
		BEI NR-44345[d]	[Hong Kong/H090-761-V1(0)/2009]	10x[e]	

Type	Isolate ID/Source		[Strain/Location/Year]	xLoD Detected	Result
		BEI NR-42938[f]	[Georgia/F32551/2012]	3x	
H3N2	Human	ATCC VR-810	[Port Chalmers/1/1973]	1x	Influenza A H3
		ATCC VR-776	[Alice (live attenuated vaccine)]	3x	
		Zeptometrix 0810238CF	[Texas/50/2012]	3x	
		ATCC VR-547	[Aichi/2/1968]	3x	
		ATCC VR-544	[Hong Kong/8/1968]	3x	
		ATCC VR-822	[Victoria/3/1975]	3x	
		Zeptometrix 0810252CF	[Wisconsin/67/2005]	3x	
		Zeptometrix 0810138CF	[Brisbane/10/2007]	3x	
	Recombinant	ATCC VR-777	[MCR2(A/England/42/72xA/PR8/34)]	3x	
H3N2v[g]	Human	Clinical Specimen	[Ohio/2012]	3x	
H2N2	Human	BEI NR-2775[h]	[Japan/305/1957]	10x[e]	Influenza A (no subtype detected)
	Recombinant	BEI NR-9679[i]	[Korea/426/1968xPuerto Rico/8/1934]	10x[e]	
H2N3	Avian	MRI Global[j]	Mallard/Alberta/79/2003	3x	Influenza A Equivocal
H5N1		MRI Global[j]	A/Chicken/Yunnan/1251/2003	3x	Influenza A (no subtype detected)
H5N2		MRI Global[j]	[A/Northern pintail/Washington/40964/2014]	3x	
H5N3		BEI NR-9682[k]	A/Duck/Singapore/645/1997	3x	
H5N8		MRI Global[j]	[AGyrfalcon/Washington/41088-6/2014]	3x	
H7N7		MRI Global[j]	A/Netherlands/219/2003	3x	
H7N9		MRI Global[j]	A/Anhui/01/2013	3x	
H10N7		BEI NR-2765[l]	Chicken/Germany/N/49	3x	Influenza A Equivocal

[a] Reported as Influenza A (no subtype detected) at 3× LoD.

[b] Genomic RNA obtained through BEI Resources NAID, NIH: Kilbourne F63: A/NWS/1934 (HA) x A/Rockefeller Institute/5/1957 (NA) (H1N2), Reassortant NWS-F, NR-9677.

[c] Virus obtained through BEI Resources, NIAID, NIH: Influenza A Virus, A/Netherlands/2629/2009 (H1N1)pdm09, NR-19823.

[d] Virus obtained through BEI Resources, NIAID, NIH: Influenza A Virus, A/Hong Kong/H090-761-V1(0)/2009 (H1N1)pdm09, NR-44345.

[e] Reported as Influenza A Equivocal or Influenza A (no subtype detected) at 3× LoD.

[f] Virus obtained through BEI Resources, NIAID, NIH: Influenza A Virus, A/Georgia/F32551/2012 (H1N1)pdm09, NR-42938.

[g] Human isolate of recent swine variant H3N2 virus.

[h] Genomic RNA obtained through BEI Resources, NIAID, NIH: Genomic RNA from Influenza A Virus, A/Japan/305/1957 (H2N2), NR-2775.

[i] Genomic RNA obtained through BEI Resources, NIAID, NIH: Genomic RNA from Kilbourne F38: A/Korea/426/1968 (HA, NA) x A/Puerto Rico/8/1934 (H2N2), NR-9679.

[j] Isolate provided and tested by MRI Global, Kansas City, MO.

[k] Genomic RNA obtained through BEI Resources, NIAID, NIH: Genomic RNA from Kilbourne F181: A/duck/Singapore/645/1997 (H5N3), Wild Type, NR-9682.

[l] Genomic RNA obtained through BEI Resources, NIAID, NIH: Genomic RNA from Influenza A Virus, A/chicken/Germany/N/1949 (H10N7), NR-2765.

Table 33. Influenza B Isolates Tested and Detected by BioFire RP2

Lineage	Isolate ID/Source	[Strain/Location/Year]	xLoD Detected	Result
N/A	ATCC VR-101	[Lee/1940]	3x	Influenza B
	ATCC VR-102	[Allen/1945]	3x	
	ATCC VR-103	[GL/1739/1954]	3x	
	ATCC VR-296	[1/Maryland/1959]	3x	
	ATCC VR-295	[2/Taiwan/1962]	3x	
	ATCC VR-786	[Brigit/Russia/1969]	3x	
Victoria	ATCC VR-823	[5/Hong Kong/1972]	3x	
	Zeptometrix 0810258CF	[2506/Malaysia/2004]	3x	
	CDC 2005743348	[1/Ohio/2005]	3x	
Yamagata	Zeptometrix 0810256CF	[07/Florida/2004]	3x	
	Zeptometrix 0810255CF	[04/Florida/2006]	1x	
	Zeptometrix 0810241CF	[1/Wisconsin/2010]	3x	
	Zeptometrix 0810239CF	[2/Massachusetts/2012]	3x	

Table 34. Parainfluenza Virus Isolates Tested and Detected by BioFire RP2

Type	Subtype	Isolate ID/Source	[Strain/Location/Year]	xLoD Detected	Result
1		Zeptometrix 0810014CF	-	1x	Parainfluenza Virus 1
		ATCC VR-94	[C-35/Washington DC/1957]	3x	
		BEI NR-3226[a]	[C39]	3x	
		BEI NR-48680[b]	[FRA/29221106/2009]	3x	

Type	Subtype	Isolate ID/Source	[Strain/Location/Year]	xLoD Detected	Result
2		Zeptometrix 0810015CF	-	1x	Parainfluenza Virus 2
		ATCC VR-92	[Greer/Ohio/1955]	3x	
3		Zeptometrix 0810016CF	-	1x	Parainfluenza Virus 3
		ATCC VR-93	[C-243/Washington DC/1957]	3x	
		BEI NR-3233[c]	[NIH 47885, Wash/47885/57]	3x	
4	A	Zeptometrix 0810060CF	-	1x	Parainfluenza Virus 4
		ATCC VR-1378	[M-25/1958]	3x	
	B	Zeptometrix 0810060BCF	-	3x	
		ATCC VR-1377	[CH-19503/Washington DC/1962]	3x	

[a] Discontinued part number.

[b] Virus obtained through BEI Resources, NIAID, NIH: Human Parainfluenza Virus 1, HPIV1/FRA/29221106/2009, NR-48680.

[c] Virus obtained through BEI Resources, NIAID, NIH: Human Parainfluenza Virus 3, NIH 47885, NR-3233.

Table 35. Respiratory Syncytial Virus Isolates Tested and Detected by BioFire RP2

Type	Source	[Strain/Location/Year]	xLoD Detected	Result
A	Zeptometrix 0810040ACF	[2006]	1x	Respiratory Syncytial Virus
	ATCC VR-26	[Long/Maryland/1956]	3x	
	ATCC VR-1540	[A2/Melbourne/1961]	3x	
B	Zeptometrix 0810040CF	[Ch-93 (18)-18]	3x	
	ATCC VR-1400	[WV/14617/1985]	3x	
	ATCC VR-955	[9320/Massachusetts/1977]	3x	
	ATCC VR-1580	[18537/Washington DC/1962]	10x	

Table 36. *Bordetella parapertussis* (and *Bordetella bronchiseptica*) Isolates Tested and Detected by BioFire RP2

Species	Source	[Strain/Location/Year]	xLoD Detected	Result
Bordetella parapertussis	Zeptometrix 0801461	[A747]	1x	*Bordetella parapertussis* (IS*1001*)
	Zeptometrix 0801462	[E595]	3x	
	ATCC 15237	[NCTC 10853]	3x	
	ATCC 15311	[NCTC 5952]	3x	
	ATCC BAA-587	[12822/Germany/1993]	3x	
Bordetella bronchiseptica (containing IS*1001*)	NRRL B-59909	[MBORD849/ Pig/Netherlands]	3x	

[a] Reactivity with IS*1001* sequences in *B. bronchiseptica* represents the intended reactivity of the assay, however the analyte will be inaccurately reported as *B. parapertussis*. The assay does not react with IS*1001*-like sequences in *B. holmesii* (see *Analytical Reactivity*).

Table 37. *Bordetella pertussis* Isolates Tested and Detected by BioFire RP2

Isolate ID/Source	[Strain]	xLoD Detected	Result
Zeptometrix 0801459	[A639]	1x	*Bordetella pertussis* (*ptxP*)
Zeptometrix 0801460	[E431]	3x	
ATCC 8467	[F]	3x	
ATCC 9340	[5,17921]	3x	
ATCC 9797	[18323/NCTC 10739]	3x	
ATCC 10380	[10-536]	3x	
ATCC 51445	[CNCTC Hp 12/63,623]	3x	
ATCC BAA-589	[Tohama]	3x	
ATCC BAA-1335	[MN2531]	3x	

Table 38. *Chlamydia pneumoniae* Isolates Tested and Detected by BioFire RP2.1[a]

Isolate ID/Source	[Strain/Location/Year]	xLoD Detected[a]	Result
ATCC VR-2282	[TW-183/Taiwan/1965]	1x	*Chlamydia pneumoniae*
ATCC VR-1310	[CWL-029]	3x	
ATCC VR-1360	[CM-1/Georgia]	3x	
ATCC 53592	[AR-39/Seattle/1983]	3x	

[a] xLoD refers to the multiple of the BioFire RP2.1 LoD concentration (1.3E+02 copies/mL) tested and detected on BioFire RP2.1 pouches.

Table 39. M*ycoplasma pneumoniae* Isolates Tested and Detected by BioFire RP2

Type	Isolate ID/Source	[Strain]	xLoD Detected	Result
1	Zeptometrix 0801579	[M129]	1x	*Mycoplasma pneumoniae*
	ATCC 29342	[M129-B7]	3x	

Type	Isolate ID/Source	[Strain]	xLoD Detected	Result
	ATCC 29085	[PI 1428]	3x	
2	ATCC 15531	[FH strain of Eaton Agent [NCTC 10119]	3x	
	ATCC 15492	[Mac]	3x	
unknown	ATCC 15293	[M52]	3x	
	ATCC 15377	[Bru]	3x	
	ATCC 39505	[Mutant 22]	3x	
	ATCC 49894	[UTMB-10P]	3x	

Analytical Specificity (Cross-Reactivity)

All known or predicted risks of cross-reactivity for the BioFire RP2.1 assays (including SARS-CoV-2) are summarized in Table 40 with the *in silico* and laboratory evaluations of analytical specificity described below.

Table 40. Predicted and Observed Cross-Reactivity of the BioFire RP2.1

Cross reactive Organism(s)/Sequence(s)	BioFire RP2.1 Result	Description
Bat coronavirus_RTG13[7] (accession# MN996532) **Pangolin coronavirus**[8] (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_RTG13.
Non-pertussis *Bordetella* species *(e.g., Bordetella parapertussis, Bordetella bronchiseptica*[a])	***Bordetella pertussis* (*ptxP*)** [b,c]	The *Bordetella pertussis* (*ptxP*) assay can amplify pertussis toxin pseudogene sequences in *B. bronchiseptica* and *B. parapertussis* primarily when present at high concentration (≥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**[d,e]	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**[f]	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* (IS1001) Detected and *Bordetella pertussis* (*ptx*P) Detected); while cross-reactivity with most strains of *B. bronchiseptica* (that do not carry IS1001) will be reported only as *Bordetella pertussis* (*ptx*P) Detected.

[d] 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.

[e] Cross-reactivity between the Human Rhinovirus/Enterovirus assays and *B. pertussis* or *B. parapertussis* will be reported as a co-detection (*Bordetella pertussis* (*ptx*P) Detected and Human Rhinovirus/Enterovirus Detected or *Bordetella parapertussis* (IS1001) Detected and Human Rhinovirus/Enterovirus Detected); while cross-reactivity with most strains of *B. bronchiseptica* (that do not carry IS1001) will be reported (falsely) only as Human Rhinovirus/Enterovirus Detected.

[f] 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 $CEID_{50}$/mL.

In Silico Analysis of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Assays

Cross-reactivity of the BioFire RP2.1 SARS-CoV-2 assays was evaluated using both *in silico* analysis and by testing whole organisms or purified nucleic acid from other organisms. *In silico* analysis included primer BLAST search of database sequences, and only sequences from SARS coronavirus and others in the Sarbecovirus subgenus of betacoronaviruses were returned. Based on this analysis, both assays are predicted to cross-react with a single Bat coronavirus sequence (accession number MN996532; isolated from bat species *Rhinolophus affinisin* in Yunan province, China[7]). A risk of possible cross-reactivity was also identified for two other bat SARS-like coronaviruses (accession numbers MG772933 and MG772934) and a pangolin coronavirus (accession number MT08407[8]). It is unlikely that these viruses would be found in a human clinical nasopharyngeal swab; but, if present, the cross-reactive product(s) produced by the BioFire RP2.1 assay(s) will be detected as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).

No other cross-reactivities with the BioFire RP2.1 SARS-CoV-2 assays were predicted by sequence analysis nor observed in high level on-panel and off-panel organism testing (including SARS-CoV, MERS-CoV, CoV-299E, CoV-HKU1, CoV-NL63, CoV-OC43 and two recombinant bat SARS-like coronaviruses; see Table 41 and Table 42).

Cross-Reactivity Testing for the BioFire RP2.1 Assays

The potential for non-specific amplification by assays for detection of SARS-CoV-2 and other analytes was evaluated by testing high concentrations of organisms or nucleic acids with the BioFire RP2.1. On-panel organisms were tested to assess the potential for intra-panel cross-reactivity, and off-panel organisms were tested to evaluate panel specificity. Off-panel organisms included normal respiratory flora and pathogens that may be present in NPS specimens as well as near-neighbors or species genetically related to the organisms detected by the BioFire RP2.1. The concentration of organism tested (in triplicate) was at least 1.0E+06 units/mL for bacteria and fungi and at least 1.0E+05 unit/mL for viruses. For the few organisms of interest that were not available for laboratory testing, results of *in silico* analysis of the organism whole genome sequences are indicated.

The on-panel and off-panel organisms and concentrations tested are shown in Table 41 and Table 42.

Table 41. 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
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		Zeptometrix 0810538CF	9.40E+04 $TCID_{50}$/mL	None

Organism	Isolate ID	Concentration Tested	Cross Reactivity Detected
(Michigan/45/15)			
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
Parainfluenza Virus 2	Zeptometrix 0810357CF	4.57E+06 $TCID_{50}$/mL	None
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.

Table 42. 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]**
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	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	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
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
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

Organism	Isolate ID	Concentration Tested	Cross Reactivity Detected/Predicted
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
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
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
Viruses (SARS-CoV-2 Related Coronaviruses)			
Bat SARS-like Coronavirus (recombinantt)	BEI NR-44009	Unknown[b] (undiluted culture)	None
Bat SARS-like Coronavirus HKU5 (recombinant)	BEI NR-48814	Unknown[b] (undiluted culture)	None
Middle East Respiratory Syndrome Coronavirus (MERS-CoV)	BEI NR-44260 EMC/2012	2.7E+09 copies/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

[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] Each coronavirus was cultured in a contracted biosafety level 3 laboratory and tested as undiluted culture. No quantification of the Bat SARS-like coronaviruses was available at the time of testing.

Reproducibility

The reproducibility of SARS-CoV-2 detection by the BioFire RP2.1 has not been specifically evaluated for the Emergency Use Authorization of the panel. Reproducibility results for the BioFire RP2 (presented below) are applicable to the BioFire RP2.1, as the panel tests the same sample type, by the same methods, on the same systems.

Reproducibility testing of contrived samples was performed with the BioFire RP2 at three test sites on a combination of FilmArray 2.0 and FilmArray Torch systems. The study incorporated a range of potential variation introduced by site, day, operator (at least two per site), system, instrument or Torch module (at least three per site/sample), and pouch lot (at least three). The samples contained various combinations of twelve different BioFire RP2 analytes, each at three different concentrations (Negative, Low Positive (1×LoD), and Moderate Positive (3×LoD)). Frozen samples were repeatedly tested on five different days for 120 data points per sample (60 per system) from 480 total valid runs.

A summary of results (percent (%) agreement with the expected Detected or Not Detected result) for each analyte (by site and system) is provided in Table 43.

Table 43. Reproducibility of BioFire RP2 Results on FilmArray Torch and FilmArray 2.0 Systems

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result						
			FilmArray Torch			FilmArray 2.0			All Sites/Systems (95% CI)
			Site A	Site C	System Sub-Total	Site B	Site C	System Sub-Total	
Viruses									
Adenovirus C2 ATCC VR-846	**Moderate Positive** **3× LoD** 6.0E+00 $TCID_{50}$/mL (1.1E+02 copies/mL)	**Detected**	**30/30** 100%	**29/30** 96.7%	**59/60** 98.3%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**118/120** **98.3%** (94.1%-99.8%)
	Low Positive **1× LoD** 2.1E+00 $TCID_{50}$/mL (3.7E+01 copies/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**29/30** 96.7%	**59/60** 98.3%	**119/120** **99.2%** (95.4%-100%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240** **100%** (98.5%-100%)
Coronavirus 229E	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480** **100%** (99.2%-100%)
Coronavirus HKU1	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480** **100%** (99.2%-100%)
Coronavirus OC43 ATCC VR-759	**Moderate Positive** **3× LoD** 9.0E+01 $TCID_{50}$/mL (1.7E+03 copies/mL)	**Detected**	**29/30** 96.7%	**29/30** 96.7%	**58/60** 96.7%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**117/120** **97.5%** (92.9%-99.5%)
	Low Positive **1× LoD** 3.0E+01 $TCID_{50}$/mL (5.6E+02 copies/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**27/30** 90.0%	**57/60** 95.0%	**117/120** **97.5%** (92.9%-99.5%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240** **100%** (98.5%-100%)
Coronavirus NL63	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480** **100%** (99.2%-100%)
Human Metapneumovirus Type 16, A1 IA10-2003 Zeptometrix 0810161CF	**Moderate Positive** **3× LoD** 3.0E+01 $TCID_{50}$/mL (3.6E+03 copies/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120** **100%** (97.0%-100%)
	Low Positive **1× LoD** 1.0E+01 $TCID_{50}$/mL (1.2E+03 copies/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**28/30** 93.3%	**30/30** 100%	**58/60** 96.7%	**118/120** **98.3%** (94.1%-99.8%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240** **100%** (98.5%-100%)
Human Rhinovirus/ Enterovirus Rhinovirus 1A Zeptometrix 0810012CFN	**Moderate Positive** **3× LoD** 3.0E-01 $TCID_{50}$/mL (1.1E+02 copies/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**28/30** 93.3%	**30/30** 100%	**58/60** 96.7%	**118/120** **98.3%** (94.1%-99.8%)
	Low Positive **1× LoD** 1.0E-01 $TCID_{50}$/mL (3.8E+01 copies/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120** **100%** (97.0%-100%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240** **100%** (98.5%-100%)
Influenza A H3 Influenza A H3N2 A/Port	**Moderate Positive** **3× LoD** 3.0E-01 $TCID_{50}$/mL (6.3E+01 copies/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**119/120** **99.2%** (95.4%-100%)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result						
			FilmArray Torch			FilmArray 2.0			All Sites/Systems (95% CI)
			Site A	Site C	System Sub-Total	Site B	Site C	System Sub-Total	
Chalmers/1/73 ATCC VR-810	**Low Positive 1× LoD** 1.0E-01 $TCID_{50}$/mL (2.1E+01 copies/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120 100%** (97.0%-100%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Influenza A H1-2009	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480 100%** (99.2%-100%)
Influenza A H1	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480 100%** (99.2%-100%)
Influenza B B/FL/04/06 Zeptometrix 0810255CF	**Moderate Positive 3× LoD** 1.5E+01 $TCID_{50}$/mL (1.0E+02 copies/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120 100%** (97.0%-100%)
	Low Positive 1× LoD 5.0E+00 $TCID_{50}$/mL (3.4E+01 copies/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120 100%** (97.0%-100%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Parainfluenza Virus 1	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480 100%** (99.2%-100%)
Parainfluenza Virus 2 Type 2 Zeptometrix 0810015CF	**Moderate Positive 3× LoD** 1.5E+00 $TCID_{50}$/mL (9.0E+01 copies/mL)	**Detected**	**30/30** 100%	**29/30** 96.7%	**59/60** 98.3%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**118/120 98.3%** (94.1%-99.8%)
	Low Positive 1× LoD 5.0E-01 $TCID_{50}$/mL (3.0E+01 copies/mL)	**Detected**	**30/30** 100%	**29/30** 96.7%	**59/60** 98.3%	**30/30** 100%	**27/30** 90.0%	**57/60** 95.0%	**116/120 96.7%** (91.7%-99.1%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Parainfluenza Virus 3	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480 100%** (99.2%-100%)
Parainfluenza Virus 4 Type 4a Zeptometrix 0810060CF	**Moderate Positive 3× LoD** 1.5E+02 $TCID_{50}$/mL (4.8E+03 copies/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120 100%** (97.0%-100%)
	Low Positive 1× LoD 5.0E+01 $TCID_{50}$/mL (1.6E+03 copies/mL)	**Detected**	**30/30** 100%	**29/30** 96.7%	**59/60** 98.3%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**118/120 98.3%** (94.1%-99.8%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Respiratory Syncytial Virus Type A	**Moderate Positive 3× LoD** 6.0E-02 $TCID_{50}$/mL (2.7E+01 copies/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120 100%** (97.0%-100%)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result						
			FilmArray Torch			FilmArray 2.0			All Sites/Systems (95% CI)
			Site A	Site C	System Sub-Total	Site B	Site C	System Sub-Total	
Zeptometrix 0810040ACF	**Low Positive 1× LoD** 2.0E-02 $TCID_{50}$/mL (9.0E+00 copies/mL)	**Detected**	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**30/30** 100%	**29/30** 96.7%	**59/60** 98.3%	**118/120 98.3%** (94.1%-99.8%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Bacteria									
Bordetella parapertussis (IS*1001*) A747 Zeptometrix 0801461	**Moderate Positive 3× LoD** 1.8E+02 IS*1001* copies/mL (1.2E+02 CFU/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**119/120 99.2%** (95.4%-100%)
	Low Positive 1× LoD 6.0E+01 IS*1001* copies/mL (4.1E+01 CFU/mL)	**Detected**	**24/30**[a] 80.0%	**29/30** 96.7%	**53/60**[a] 88.3%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**112/120 93.3%** (87.3%-97.1%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Bordetella pertussis (ptxP) A639 Zeptometrix 0801459	**Moderate Positive 3× LoD** 3.0E+03 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120 100%** (97.0%-100%)
	Low Positive 1× LoD 1.0E+03 CFU/mL	**Detected**	**28/30** 93.3%	**30/30** 100%	**58/60** 96.7%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**118/120 98.3%** (94.1%-99.8%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Chlamydia (Chlamydophila) pneumoniae TW183 ATCC VR-2282	**Moderate Positive 3× LoD** 3.0E-01 $TCID_{50}$/mL (2.0E+02 copies/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120 100%** (97.0%-100%)
	Low Positive 1× LoD 1.0E-01 $TCID_{50}$/mL (6.6E+01 copies/ml)	**Detected**	**28/30** 93.3%	**30/30** 100%	**58/60** 96.7%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**117/120 97.5%** (92.9%-99.5%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Mycoplasma pneumoniae	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480 100%** (99.2%-100%)
Overall Agreement with the Expected Result All Analytes and All Test Levels (95% Confidence Interval)			**9,562/9,600 99.6% (99.5% – 99.7%)**						

[a] Data from Site A were further reviewed by the unique site-specific variables including test day, Torch module, and operator. No correlation could be found between the Not Detected results and any one or more of these variables. The Not Detected results at Site A were found to be statistically non-significant (p>0.05 by Fisher's exact test) and therefore do not indicate a site- or system-dependent variance in precision of the BioFire RP2 *Bordetella parapertussis* (IS*1001*) results.

Interference

The ability of endogenous or exogenous substances that could be present in NPS specimens (or introduced during specimen collection and handling) to interfere with accurate detection of SARS-CoV-2 and other analytes was evaluated with select direct testing on the BioFire RP2.1 and extrapolated from the interference evaluation of the BioFire RP2. Results from testing using the BioFire RP2 are applicable to the BioFire RP2.1, because the panel tests the same sample type, by the same methods, on the same systems, with no change to primers or reaction chemistry and conditions.

Potentially interfering substances were evaluated using contrived samples spiked with substance. Results from samples containing a substance were compared to results from control samples without substance. The substances tested included endogenous substances that may be found in specimens at normal or elevated levels (e.g. blood, mucus/mucin, human genomic DNA), various commensal or infectious microorganisms, medications, washes or topical applications for the nasal passage, various swabs and transport media for specimen collection, and substances used to clean, decontaminate, or disinfect work areas. Each substance was added to contrived samples containing representative organisms at concentrations near (2-3×) LoD. The concentration of substance added to the samples was equal to or greater than the highest level expected to be in NPS specimens and each sample was tested in triplicate.

None of the substances were shown to interfere with the BioFire RP2 or BioFire RP2.1 function. However, it was observed that exposure of samples to bleach prior to testing could damage the organisms/nucleic acids in the sample, leading to inaccurate BioFire RP2/RP2.1 test results (lack of analyte detection). The effect of bleach was dependent on the concentration and/or length of time the bleach was allowed to interact with the sample.

Table 44. Evaluation of Potentially Interfering Substances for NPS Specimens on the BioFire RP2 and BioFire RP2.1

Substances in **bold** font were tested with the BioFire RP2.1 on samples containing SARS-CoV-2 and other organisms at 3× LoD. All other substances were tested with the BioFire RP2.

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 cell/µL**	**No Interference**
Competitive Microorganisms		
Coronavirus 229E	1.7E+04 $TCID_{50}$/mL	No Interference
Coronavirus OC43	**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		

Substance Tested	Concentration Tested	Result
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 (MTM)	**70% v/v**	**No Interference**
Sigma-Virocult™ Viral Collection and Transport System (Swab and Transport Medium)	100%	No Interference
Copan ESwab™ Sample Collection and Delivery System (Swab and Liquid Amies Medium)	100%	No Interference

[a] Nasal influenza vaccines (e.g. FluMist) were not evaluated, but are predicted to be reactive with the 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).

NOTE: Compatibility of the BioFire RP2.1 with NPS in PrimeStore® MTM has not been evaluated in the intended use setting. PrimeStore® MTM and BioFire FilmArray Sample Buffer contain guanidine salts that will react with bleach to form a toxic gas. Use caution if using bleach for disinfection purposes when collecting or testing NPS specimens.

APPENDIX A

Symbols Glossary

ISO 15223-1 Medical devices - Symbols to be used with medical devices labels, labeling and information to be supplied					
5.1.1 	Manufacturer	5.1.2 	Authorized representative in the European Community	5.1.4 	Use-By date (YYYY-MM-DD)
5.1.5 	Batch Code (Lot Number)	5.1.6 	Catalog Number	5.1.7 	Serial Number
5.2.8 	Do Not Use if Package Is Damaged	5.3.2 	Keep Away from Sunlight	5.3.7 	Temperature Limit
5.4.2 	Do Not Reuse	5.4.3 	Consult Instructions for Use	5.5.1 	In vitro Diagnostic Medical Device
5.5.5 	Contains Sufficient For <n> Tests				
Use of Symbols in Labeling – 81 FR 38911, Docket No. (FDA-2013-N-0125)					
Rx Only	Prescription Use Only				
United Nations Globally Harmonized System of Classification and Labeling of chemicals (GHS) (ST/SG/AC.10/30)					
	Serious eye damage, cat. 1		Acute toxicity, cat. 4 & Skin irritation, cat. 2		Acute aquatic hazard, cat.1 & Long-term aquatic hazard, cat.1
European Union Directive 98/79/EC of the European Parliament and of the Council on in vitro Diagnostic Medical Device					
CE	European Union Conformity				
Manufacturer Symbols (BioFire Diagnostics, LLC)					
	The NOTE symbols explains how to perform the BioFire RP2.1 test more efficiently.				
	A BioFire RP2.1 Panel				

APPENDIX B

Contact and Legal Information

Customer and Technical Support for U.S. Customers	
Reach Us on the Web http://www.BioFireDX.com **Reach Us by E-mail** support@BioFireDX.com **Reach Us by Mail** 515 Colorow Drive Salt Lake City, UT 84108 USA	**Reach Us by Phone** 1-800-735-6544 – Toll Free (801) 736-6354 – Utah **Reach Us by Fax** (801) 588-0507
Customer and Technical Support outside of the U.S.	
Contact the local bioMérieux sales representative or an authorized distributor for technical support.	

BioFire Diagnostics, LLC
515 Colorow Drive
Salt Lake City, UT 84108
USA

Warranty Information

Product warranty information is available online at:

http://www.biofiredx.com/support/documents/

For warranty information for customers outside the United States, contact the local bioMérieux sales representative or an authorized distributor.

APPENDIX C

References

1. Biosafety in Microbiological and Biomedical Laboratories. (2009).

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7. Zhou, P. *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **579**, 270–273 (2020).

8. Cagliani, R., Forni, D., Clerici, M. & Sironi, M. Computational inference of selection underlying the evolution of the novel coronavirus, SARS-CoV-2. *J. Virol.* (2020) doi:10.1128/JVI.00411-20.

REVISION HISTORY

Version	Revision Date	Description of Revision(s)
01	May 2020	Initial release
02	December 2020	• Addition of FDA SARS-CoV-2 Reference Panel testing data to the Limit of Detection section. • Addition of the following to Intended Use: “that meet requirements”. • Addition of FDA EUA language to General Precautions section. • Update to FDA EUA website link in Conditions of Authorization for the Laboratory section.

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