# *BioFire® Respiratory Panel 2.1-EZ (RP2.1-EZ)*

IVD

**For use with the BioFire® FilmArray® 2.0 EZ Configuration System**

***Rx Only***

***For Emergency Use Authorization (EUA) only***

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|---|---|
| **Instructions for Use** | https://www.biofiredx.com/e-labeling/ITI0129 |
| **Quick Guide** | https://www.biofiredx.com/e-labeling/ITI0108 |
| **Safety Data Sheet (SDS)** | https://www.biofiredx.com/e-labeling/ITI0142 |
| **Software Pouch Module** | https://www.biofiredx.com/e-labeling/ITIFA20RP21EZ10 |
| **Patient Fact Sheet** | https://www.biofiredx.com/e-labeling/ITIRP21EZ05 |
| **Provider Fact Sheet** | https://www.biofiredx.com/e-labeling/ITIRP21EZ06 |
| **EUA Authorization Letter** | https://www.biofiredx.com/e-labeling/ITIRP21EZ07 |

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| **Customer and Technical Support Information**<br>*For more information on how to contact Support, refer to Appendix B. | Phone: 1-844-815-0363 (toll free)<br>1-801-582-0636 (in Utah)<br>E-mail: support@BioFireDX.com<br>Website: www.biofiredx.com |

# INTENDED USE

The BioFire Respiratory Panel 2.1-EZ (RP2.1-EZ) is a multiplexed polymerase chain reaction (PCR) 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 respiratory infection consistent with 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 the requirements to perform high, moderate, or waived complexity tests. The BioFire RP2.1-EZ is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

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

| Viruses | Bacteria |
|---|---|
| Adenovirus<br>Coronavirus 229E<br>Coronavirus HKU1<br>Coronavirus NL63<br>Coronavirus OC43<br>Coronavirus SARS-CoV-2<br>Human Metapneumovirus<br>Human Rhinovirus/Enterovirus<br>Influenza A, including subtypes H1, H3 and H1-2009<br>Influenza B<br>Parainfluenza Virus[a]<br>Respiratory Syncytial Virus | *Bordetella parapertussis*<br>*Bordetella pertussis*<br>*Chlamydia pneumoniae*<br>*Mycoplasma pneumoniae* |

[a] Four types of Parainfluenza Virus (PIV1, PIV2, PIV3 and PIV4) can be detected and will be reported as Parainfluenza Virus Detected (type information is not reported).

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. Positive results from individuals exhibiting signs and/or symptoms of respiratory infection are indicative of the presence of the identified microorganism(s); clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. 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 and do not rule out co-infection with other viruses. The agent detected by the BioFire RP2.1-EZ may not be the definite cause of disease.

Laboratories and patient care settings 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 SARS-CoV-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 (e.g., bacterial and viral culture, immunofluorescence and radiography) when evaluating a patient with possible respiratory tract infection.

The BioFire RP2.1-EZ is intended for use by trained operators who are proficient in performing tests using the BioFire® FilmArray® 2.0 EZ Configuration (BioFire 2.0 EZ) System. The BioFire RP2.1-EZ is only for use under the Food and Drug Administration's Emergency Use Authorization.

# SUMMARY AND EXPLANATION OF THE TEST

The BioFire RP2.1-EZ is a real-time, nested multiplexed polymerase chain reaction test designed to simultaneously identify nucleic acids from 15 different viruses, including SARS-CoV-2, and 4 bacteria associated with respiratory tract infection, from a single nasopharyngeal swab (NPS) specimen in transport media. The SARS-CoV-2 primers contained in the BioFire RP2.1-EZ are designed to detect RNA from the SARS-CoV-2 virus in nasopharyngeal swabs from patients who are suspected of COVID-19 by their healthcare provider. Internal controls are used to monitor all stages of the test process.

The BioFire RP2.1-EZ test is a simplified version of the BioFire® Respiratory Panel 2.1 (RP2.1). The de novo request for the BioFire RP2.1 test was granted by the FDA on March 17, 2021 (DEN200031) for use in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity tests. The BioFire RP2.1-EZ kit includes the same reagent pouch and other components of the BioFire RP2.1 kit however, the BioFire RP2.1-EZ Sample Buffer ampoule is easier to handle and dispense and testing instructions are designed for patient care settings.

The BioFire RP2.1-EZ is run on a the BioFire 2.0 EZ which provides a simple results report with recommended actions for operators in a patient care setting outside of the clinical laboratory environment. The BioFire RP2.1-EZ results differ from those provided by the BioFire RP2.1 in that a single Parainfluenza Virus result is provided when any of the four Parainfluenza Virus subtypes are detected.

# PRINCIPLE OF THE PROCEDURE

The BioFire RP2.1-EZ 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 BioFire® FilmArray® Sample Buffer into the pouch, places the pouch into an instrument, and starts a run. The entire run process takes about 45 minutes and a results report is provided upon completion. Additional detail can be found in the BioFire 2.0 EZ Operator's Manual.

During a run, the system:

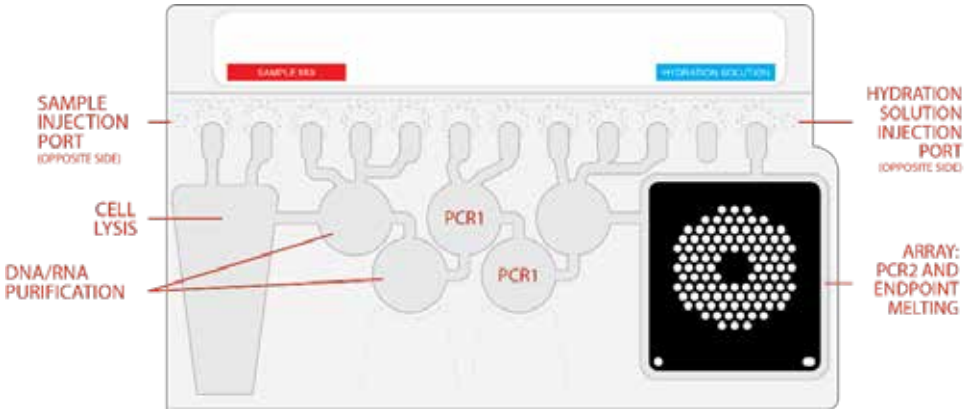
Lyses the sample by agitation (bead beating) in addition to chemical lysis mediated by the Sample Buffer.

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.




# MATERIALS PROVIDED

Each kit contains sufficient reagents to test 30 samples (30-test kit – REF# 423883):

Individually packaged BioFire RP2.1 pouches

Single-use Sample Buffer ampoules

Single-use pre-filled BioFire® FilmArray® Hydration Injection Vials (blue)

Single-use BioFire® FilmArray® Sample Injection Vials (red)

Individually packaged Transfer Pipettes

BioFire RP2.1-EZ Pouch Module Software
This software is required to run the BioFire RP2.1 pouch on the BioFire 2.0 EZ System and can be downloaded at https://www.biofiredx.com/e-labeling/ITIFA20RP21EZ10 if not already installed on the BioFire 2.0 EZ System.

# MATERIALS REQUIRED BUT NOT PROVIDED

BioFire® FilmArray® 2.0 EZ Configuration System (BioFire 2.0 EZ):

- BioFire® FilmArray® 2.0 instrument (Part Number: FLM2-ASY-0001)
- BioFire® FilmArray® EZ Configuration Computer (Part Number: FLM2-ASY-0006)
- BioFire® FilmArray® EZ Configuration-specific software (included with EZ Configuration)
- BioFire® FilmArray® EZ Training Video (included with EZ Configuration)
- BioFire® FilmArray® Pouch Loading Station (included with BioFire FilmArray 2.0 instrument)

10% bleach solution or a similar disinfectant

# MATERIALS AVAILABLE BUT NOT PROVIDED

Nasopharyngeal swab (NPS) collection materials (or equivalent):

- Flexible nasopharyngeal flocked swab, Nylon® tip
- Up to 3 mL of universal transport media or saline

External Control Material

- Maine Molecular Quality Controls, Inc. BioFire RP2.1/RP2.1*plus* Control Panel M441, Part number M441 (or equivalent)

  BioFire recommends using the BioFire RP2.1/RP2.1*plus* Control Panel M441. However, other commercial external control materials may be appropriate. Please follow state and local regulations and refer to the External Controls section for more information on running external controls with the BioFire RP2.1-EZ.

# WARNINGS AND PRECAUTIONS

## General Precautions

1. For *in vitro* diagnostic use under Emergency Use Authorization only.
2. This product has not been FDA cleared or approved, but has been authorized for emergency use 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 requirements to perform high, moderate, or waived complexity tests. This product is for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.
3. This product has been authorized only for the detection and differentiation of nucleic acid of SARS-CoV-2 from multiple respiratory viral and bacterial organisms.
4. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics 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-EZ in conjunction with a patient's signs and symptoms, results from other diagnostic tests, and relevant epidemiological information.
6. This product is only for use with the BioFire 2.0 EZ System.

7. Performance characteristics of the BioFire RP2.1-EZ have only been determined with nasopharyngeal swab (NPS) specimens in transport medium or saline.
8. Always check the expiration date on the pouch. Do not use a pouch after its expiration date.
9. BioFire RP2.1-EZ pouches are stored under vacuum in individually wrapped canisters. To preserve the integrity of the pouch vacuum for proper operation, be sure that the instrument will be available and operational before unwrapping any pouches for loading.
10. 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. Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) for more information. https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html.

## 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 or more current guidelines specific for SARS-CoV-2.
3. Follow your institution's safety procedures for handling biological samples.
4. 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. Viral culture should not be attempted in cases of positive results for SARS-CoV-2 and/or any similar microbial agents unless a facility with an appropriate level of laboratory biosafety (e.g., BSL 3 and BSL 3+, etc.) is available to receive and culture specimens.
5. 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.
6. Dispose of materials used in this assay, including reagents, samples, and used buffer vials, according to federal, state, and local regulations.
7. Sample Buffer contains Guanidinium chloride and Triton X100. The following statements apply:

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Health Hazards

- Acute Toxicity, oral (Category 4)
  - H302 – Harmful if swallowed.
- Skin corrosion/irritation (Category 2)

- § H315 - Causes skin irritation.
- o Serious eye damage/eye irritation (Category 1)
  - § H318 - Causes serious eye damage.

Environment Hazards

- o Hazardous to the aquatic environment, acute aquatic hazard (Category 1)
  - § H400 - Very toxic to aquatic life.
- o Hazardous to the aquatic environment, long-term aquatic hazard (Category 1)
  - § H410 - Very toxic to aquatic life with long lasting effects.

Precautionary Statements

- o Prevention
  - § P273 – Avoid release to the environment.
  - § P280 – Wear protective gloves/protective clothing/eye protections/face protection.
- o Response
  - § P391 - Collect spillage.
  - § P332 + P313 - If skin irritation occurs: Get medical advice/attention.
  - § P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  - § P301 + P312 - IF SWALLOWED: Call a POISON CENTRE/doctor if you feel unwell.
  - § P337 + P313 - If eye irritation persists: Get medical advice/attention.

Please refer to the BioFire RP2.1-EZ Safety Data Sheet (SDS) for more information: https://www.biofiredx.com/e-labeling/ITI0142.

8. 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.**

9. 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-EZ, 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:

Personnel collecting and/or testing specimens 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 may 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 may be used when preparing samples.

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, unless the area is thoroughly cleaned first.

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-EZ (*e.g.* influenza, SARS-CoV-2, *Bordetella pertussis*, and poliovirus (Human Rhinovirus/Enterovirus)). Vaccines may contain PCR-detectable DNA or RNA. If possible, particular care should be taken to avoid contamination of the specimen or testing areas (especially with nasal spray vaccines such as FluMist® and *B. pertussis* acellular vaccines such as Pentacel®, Daptacel®, and Adacel®; http://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-pcr-bestpractices.html). Contamination of specimens or testing materials with vaccine can cause false-positive results.

**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-EZ 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 BioFire 2.0 EZ Operator's Manual.**

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

3. Transport media or saline may contain non-viable organisms and/or nucleic acids at levels that can be detected by the BioFire RP2.1-EZ.

The presence of non-viable organisms and/or nucleic acids in transport media or saline may lead to false positive test results.

## 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 and patient care settings in the U.S. are required to report all 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. Always check the expiration date and do not use reagents beyond the expiration date printed on the pouch or kit.
4. 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.
5. 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).
6. 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. If COVID-19 infection is suspected, refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19): https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html.

| | |
|---|---|
| **Specimen Type** | **Nasopharyngeal Swab** (NPS) collected according to standard technique and immediately placed in up to 3 mL of transport media or saline.<br>Detailed NPS specimen collection instructions can be found in the BioFire RP2.1-EZ Quick Guide. |
| **Minimum Sample Volume** | 0.3 mL (300 µL) |
| **Transport and Storage** | Specimens should be tested with the BioFire RP2.1-EZ as soon as possible.<br>If storage is required, specimens can be held:<br>At room temperature for up to 4 hours (15-25 °C)<br>Refrigerated for up to 3 days (2-8 °C)<br>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 at -70°C or lower may be acceptable. Please follow your institution's rules and protocols regarding sample storage validation.

***NOTE: 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

Refer to the BioFire RP2.1-EZ Quick Guide, the BioFire 2.0 EZ Training Video, or the BioFire 2.0 EZ Operator's Manual for more detail and pictorial representations of these instructions.

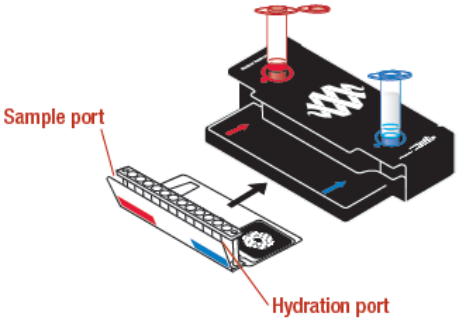
Use clean gloves and other Personal Protective Equipment (PPE) when handling pouches and samples. Only prepare one BioFire RP2.1 pouch at a time and change gloves between samples and pouches. Once sample is added to the pouch, promptly transfer to the instrument to start the run. After the run is complete,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

## Step 1: Prepare Pouch

1. Thoroughly clean the work area and the 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.***

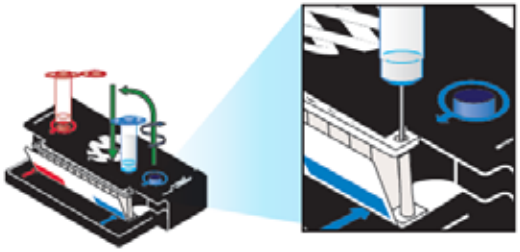
3. Check the expiration date on the pouch. Do not use expired pouches.
4. Label the BioFire RP2.1 pouch with the patient's ID.
5. Insert the pouch into the Pouch Loading Station, aligning the red and blue labels on the pouch with the red and blue arrows on the Pouch Loading Station.
6. Place a red-capped Sample Injection Vial into the red well of the Pouch Loading Station.




7. 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. Allow ~10 seconds for the pouch reagents to fully hydrate.

   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.

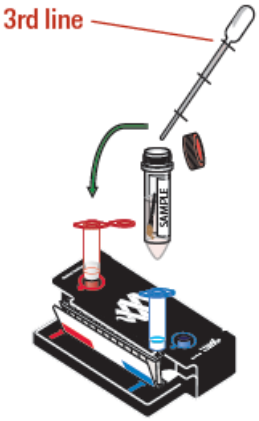
   Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen.

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*.

6. Discard the Hydration vial in an appropriate biohazard container.

## Step 3: Prepare Sample Mix

1. Gently twist and remove the lid from the NPS sample tube.
2. Transfer sample to the red-capped Sample Injection Vial using the transfer pipette as follows:




Check the Sample ID on the sample tube against the Sample ID on the pouch to ensure they match.

Unwrap the transfer pipette, being careful not to touch the tip.

Squeeze the bulb of the transfer pipette and lower it into the sample.

Gently release the bulb and draw up liquid until it reaches the 3rd line of the pipette (approximately 0.3 mL).

Raise the pipette out of the sample liquid and then release the bulb fully to ensure the sample stays in the pipette.

Dispense the sample into the red-capped Sample Injection Vial by squeezing the bulb of the transfer pipette.

Discard the transfer pipette in a suitable biohazard container.

***NOTE: DO NOT use the transfer pipette to mix the sample once it has been added to the Sample Injection Vial.***

3. Gently twist and remove tab at the tip of the Sample Buffer ampoule (tube).
4. Add Sample Buffer to the Sample Injection Vial as follows:


Invert the ampoule over the red-capped Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze.

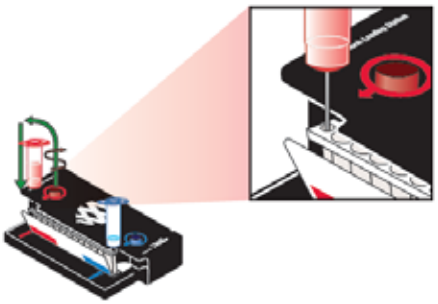
***NOTE: Avoid touching the ampoule tip during handling, as this may introduce contamination.***

***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.**

5. Once the Sample Buffer has been added, tightly close the lid of the Sample Injection Vial.
6. Remove the entire Sample Injection Vial from the Pouch Loading Station and gently 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 container.
6. Remove the pouch from the FilmArray Pouch Loading Station.

## Step 5: Run Pouch

The system software includes step-by-step, on-screen instructions that guide the operator through performing a run. Refer to the BioFire 2.0 EZ Operator’s Manual for more detailed instructions.

1. Ensure that the system (instrument and computer) is powered on and the software is launched.
2. Open the lid of the instrument (if not already open).

***NOTE: A green light on the front of the instrument indicates the instrument is ready for use.***

3. Insert the pouch into the instrument.

   Position the pouch so that the array is on the right with the film directed downward into the instrument. The red and blue labels on the pouch should align with the red and blue arrows on the instrument. The pouch will click into place.

   If inserted correctly, the barcode is visible and the label is readable on the top of the pouch.

   The instrument and software must detect that the pouch has been inserted correctly before continuing to the next step.

***NOTE: If pouch does not slide into the instrument easily, gently push the lid of the instrument back to ensure that it is completely open.***

4. Scan the barcode on the pouch using the barcode scanner

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 (RP2.1v1.0), and Protocol (NPS2v3.2) can be manually entered from the information provided on the pouch label into the appropriate fields.

***NOTE: The barcode cannot be scanned prior to placing the pouch in the instrument. A "Cannot scan now" message will display. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.***

5. 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.

6. If necessary, select and/or confirm the correct protocol (NPS2 v3.2) from the Protocol drop down list.
7. 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.***

8. Review the entered run information on the screen. If correct, close the instrument lid.
9. Select Start Run on the computer screen.

   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.***

10. When the run is finished, follow the on-screen instructions to remove the pouch.
11. Immediately discard the pouch in a biohazard container.
12. Results are automatically displayed on the report section of the screen. The run file is automatically saved in the system 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-EZ Panel 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, all test results will be listed as Invalid and the sample should be retested using a new pouch.

## External Controls

Recommended external control material is available from Maine Molecular Quality Controls, Inc. (MMQCI) (online at www.mmqci.com). The MMQCI control panel (Table 1) consists of a set of ready-to-use positive and negative control solutions for all viruses and bacteria detected by the BioFire RP21.-EZ, along with an insert explaining the expected results.

**Table 1. Control Materials Compatible with all Analytes on the BioFire RP2.1-EZ**

| Vendor | Product Name | Part Number |
|---|---|---|
| Maine Molecular Quality Controls, Inc. (MMQCI) | BioFire RP2.1/RP2.1plus Control Panel M441 | M441 (Negative Control M44221 and Positive Control M44321) |

***NOTE: MMQCI control test solutions should be stored at -20°C or colder. Follow manufacturer's instructions and allow solutions to thaw to room temperature before testing.***

It is recommended that when using the BioFire RP2.1-EZ under Emergency Use Authorization (EUA), external controls be tested at minimum:

When receiving a new shipment of pouches

When training a new user

Two pouches will need to be used, one for the positive control material and one for the negative control material. The negative control test should be run before the positive control test. Prior to loading, invert the tube three times to mix the material and tap the bottom of the tube on the counter three times to remove any liquid from the lid. Run each test following the same steps used for a patient test. If all analytes are detected for the positive control test, disregard instructions to retest.

**Expected positive test result**

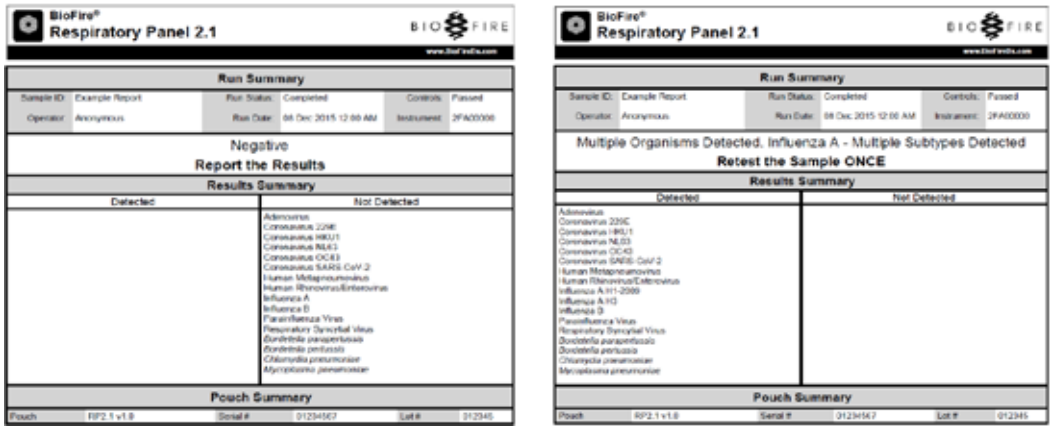



**Figure 1. Expected Negative and Positive RP2.1-EZ External Control Report Examples**

If the expected results for the external control materials are not obtained, contact BioFire Technical Support prior to running patient samples.

***NOTE: Other commercial external control materials may be available and appropriate for use with the BioFire RP2.1-EZ. Use in accordance with the manufacturers' instructions and appropriate accrediting organization requirements, as applicable***.

***NOTE: Some commercial control materials for Coronavirus SARS-CoV-2 are not compatible with the BioFire RP2.1-EZ test.***

# 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 refer to the BioFire 2.0 EZ Operator's Manual). The software then performs several analyses and assigns a final assay result. The steps in the analyses are described below.

**Analysis of melt curves.** The 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-EZ, 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 Coronavirus SARS-CoV-2, Adenovirus, Influenza A, and Parainfluenza Virus depend on the interpretation of results from more than one assay. Interpretation and actions for the multi-assay results are provided below.

### Coronavirus SARS-CoV-2

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

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

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

### Adenovirus

The BioFire RP2.1-EZ uses five assays (Adeno2, Adeno3, Adeno6, Adeno7.1, and Adeno8) for the detection of Adenovirus. The 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

Assays in the BioFire RP2.1-EZ are designed to both detect Influenza A and to differentiate the common hemagglutinin subtypes. To accomplish this, the BioFire RP2.1-EZ 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 and the test result reported for Influenza A is based on the combined results of the individual assays.

In general, Influenza A is determined to be Detected if at least one of the two FluA-pan assays is positive and a subtyping assay is also positive. If neither of the FluA-pan assays is positive, but a subtyping assay is positive, then the result is considered Uncertain for that specific subtype. If one of the FluA-pan assays is positive and none of the subtyping assays are positive, the result is Uncertain for Influenza A. All Uncertain results should be retested once. If the result of the retest is again 'Uncertain', the final result should be considered 'Detected'.

### Influenza A (no subtype detected)

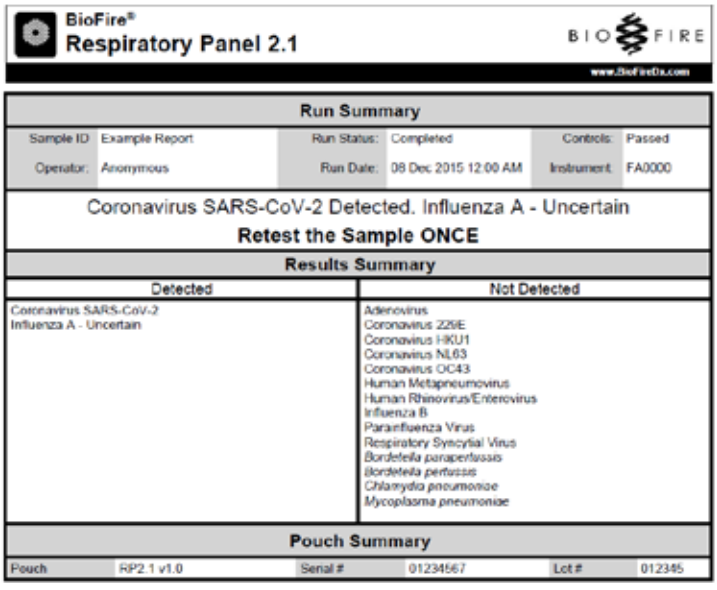
If both FluA-pan assays are positive, but none of the hemagglutinin subtyping assays are positive, then the interpretation is Influenza A (no subtype detected). This result could occur when the level of the virus in the specimen is low and not detected by the subtyping assays. This result could also indicate the presence of a novel Influenza A strain. In both cases, the sample in question should be retested. If the retest provides the same Influenza A – no subtype results, contact the appropriate public health authorities for confirmatory testing.

### Parainfluenza Virus

The BioFire RP2.1-EZ uses four assays (PIV1, PIV2, PIV3 and PIV4) for the detection of parainfluenza viruses. The 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 Parainfluenza Virus Detected. If all assays are negative, the test report result will be Parainfluenza Virus Not Detected.

## BioFire RP2.1-EZ Test Report

The BioFire RP2.1-EZ 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 Pouch Summary section. The test report can be saved as a file or printed.



BioFire®
Respiratory Panel 2.1

**Run Summary**

| | | | | | |
|---|---|---|---|---|---|
| Sample ID: | Example Report | Run Status: | Completed | Controls: | Passed |
| Operator: | Anonymous | Run Date: | 08 Dec 2015 12:00 AM | Instrument: | FA0000 |

Coronavirus SARS-CoV-2 Detected. Influenza A - Uncertain
**Retest the Sample ONCE**

**Results Summary**

| Detected | Not Detected |
|---|---|
| Coronavirus SARS-CoV-2<br>Influenza A - Uncertain | Adenovirus<br>Coronavirus 229E<br>Coronavirus HKU1<br>Coronavirus NL63<br>Coronavirus OC43<br>Human Metapneumovirus<br>Human Rhinovirus/Enterovirus<br>Influenza B<br>Parainfluenza Virus<br>Respiratory Syncytial Virus<br>*Bordetella parapertussis*<br>*Bordetella pertussis*<br>*Chlamydia pneumoniae*<br>*Mycoplasma pneumoniae* |

**Pouch Summary**

| | | | | | |
|---|---|---|---|---|---|
| Pouch | RP2.1 v1.0 | Serial # | 01234567 | Lot # | 012345 |

## Run Summary

The Run Summary section of the test report provides the Sample ID, (Completed, Incomplete, Aborted, Instrument Error, or Software Error), Control results (Passed, Failed, or Invalid), identity of the operator that performed the test, Run Data (and time), the identity of the instrument used to perform the test, and an overall summary of the test results.

The Control field displays Pass only if the run completed successfully (no instrument or software errors) and both of the pouch control assays (RNA Process Control and PCR2 Control) were successful. The Control field will display Failed if the run was completed successfully (no instrument or software errors) but one or both of the pouch control assays failed. If the control result is Failed, then the result for all of the tests on the panel are displayed as Invalid and the specimen will need to be retested with a new pouch. Table 3 provides additional information for each of the possible control field results.

In the test result field, the overall test result (Detected (with a list of detected targets), Multiple Organisms Detected, Negative, Uncertain (Influenza A only), or Invalid) will be listed as well as the required action for that result (e.g., Report the results or Retest the sample). See Table 4 for complete result interpretation and required actions.

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

| Control Result | Explanation |
|---|---|
| Passed | The run was successfully completed<br>AND<br>Both pouch controls were successful. |
| Failed | The run was successfully completed<br>BUT<br>At least one of the pouch controls (RNA Process Control and/or PCR2 Control) failed. |
| Invalid | The controls are invalid because the run did not complete.<br>(Typically this indicates a software or hardware error). |

## Results 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, Uncertain (Influenza A only), or Invalid. Table 4 provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

## Pouch Summary

The **Pouch Summary** section provides additional pouch information including the pouch type, lot number, and serial number.

## 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 |

## Results Explanation and Required Actions

Test results for the organisms included in the BioFire RP2.1-EZ are provided in two locations on the report. The Result Summary section provides a complete list of the test results. Possible results include Detected, Not Detected, Uncertain, and Failed/Invalid. Positive (Detected) and Uncertain results are also displayed in the Run Summary section. 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 |
|---|---|---|
| Negative | The run was successfully completed<br>AND<br>The pouch controls were successful (Passed)<br>AND<br>The assay(s) for the organism were NEGATIVE | Report results. |
| <Organism Name> Detected | The run was successfully completed<br>AND<br>The pouch controls were successful (Passed)<br>AND<br>The assay(s) for the organism were POSITIVE | Report results. |
| Multiple Organisms Detected | The run was successfully completed<br>AND<br>The pouch controls were successful (Passed)<br>AND<br>The assay(s) for the organisms were POSITIVE<br><br>- more than one non-Influenza A organism is detected<br>OR<br>- exactly one Influenza A subtype is detected and one or more non-influenza A organisms is detected. | Report the results.<br>**Note:** Detection of four or more pathogens may indicate a possible contamination event. If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result. If results are not duplicated, contact BioFire Technical Support and discontinue testing until the area has been decontaminated. |
| Influenza A - Uncertain | The run was successfully completed<br>AND<br>The pouch controls were successful (Passed)<br>AND<br>The combination of positive and negative assay results for Influenza A was inconclusive. | Retest the original sample ONCE and report the result of the retest.<br>Uncertain results can occur when the titer of the virus in the specimen is low (below LoD). Uncertain results could also indicate the presence of a novel Influenza A strain or reactivity with non-human influenza A viruses or rare human influenza A viruses that are not H1, H1-2009 or H3. Such strains generally produce Influenza A Uncertain or Influenza A (no subtype detected) results. |
| Influenza A – No Subtype Detected | The run was successfully completed<br>AND<br>The pouch controls were successful (Passed)<br>AND<br>The combination of positive assay results for Influenza A was did not detect a specific subtype. | Retest the sample ONCE<br>Report the results of the Retest<br>If the retest provides the same Influenza A – No Subtype results, contact the appropriate public health authorities for confirmatory testing. |
| Influenza A – Multiple Subtypes Detected | The run was successfully completed<br>AND<br>The pouch controls were successful (Passed)<br>AND<br>The combination of positive assay results indicates the presence of more than one Influenza A subtype. | Multiple Influenza A infections are possible but rare. This result can be caused by a recent FluMist® nasal Influenza vaccination (see “Interference” section below).<br>Retest the Sample ONCE<br>Report the results of the Retest |
| Failed | Run completes and the controls fail<br>OR<br>The run does not complete. | All results are invalid because the run failed. Note any error codes displayed and refer to the BioFire 2.0 EZ Operator’s Manual for more information. If the error persists, contact Technical Support for further instruction.<br>Retest the sample and if valid, report the results of the retest. |

# LIMITATIONS

1. For prescription use only.
2. This product can be used only with the BioFire® FilmArray® 2.0 EZ Configuration System.
3. The BioFire RP2.1-EZ is a qualitative test and does not provide a quantitative value for the virus(es) and/or bacteria detected in the specimen.
4. Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
5. The BioFire RP2.1-EZ has been evaluated for use with human specimen material only.
6. The BioFire RP2.1-EZ has not been validated for testing of specimens other than nasopharyngeal swab (NPS) specimens in transport medium or saline.
7. The BioFire RP2.1-EZ has not been validated for the testing of pooled specimens or the screening of specimens from asymptomatic individuals that are not suspected of COVID-19 infection.
8. The performance of BioFire RP2.1-EZ has not been established for specimens collected from individuals without signs or symptoms of respiratory infection.
9. The performance of the BioFire RP2.1-EZ has not been specifically evaluated for NPS specimens from immunocompromised individuals.
10. The performance of this device has not been assessed in a population vaccinated against COVID-19.
11. The effect of antibiotic treatment on test performance has not been evaluated.
12. The performance of the BioFire RP2.1-EZ 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.
13. The performance of the BioFire RP2.1-EZ has not been established for monitoring treatment of infection with any of the panel organisms.
14. The performance of BioFire RP2.1-EZ has not been established for screening of blood or blood products.
15. False positive and false negative results can be the result of a variety of sources and causes, it is important that these results be used in conjunction with other clinical, epidemiological, or laboratory information.
16. 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.
17. A negative BioFire RP2.1-EZ result does not exclude the possibility of viral or bacterial infection. Negative test results may occur due to 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.
18. If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.

19. 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.
20. 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.
21. 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.
22. 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.
23. The BioFire RP2.1-EZ influenza A subtyping assays target the influenza A hemagglutinin (H) gene only. The BioFire RP2.1-EZ does not detect or differentiate the influenza A neuraminidase (N) subtypes.
24. The BioFire RP2.1-EZ may not be able to distinguish between existing viral strains and new variants as they emerge. For example, the BioFire RP2.1-EZ 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.
25. Recent administration of nasal vaccines (e.g. FluMist) prior to NPS specimen collection could lead to accurate virus detection by the BioFire RP2.1-EZ of the viruses contained in the vaccine, but would not represent infection by those agents.
26. Due to the genetic similarity between Human Rhinovirus and Enterovirus, the BioFire RP2.1-EZ cannot reliably differentiate them. A BioFire RP2.1-EZ 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.
27. BioFire RP2.1-EZ 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).

    The BioFire RP2.1-EZ 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-EZ *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-EZ 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-EZ assay (*ptxP*) was designed to be specific for *B. pertussis*. However, the BioFire RP2.1-EZ *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).

28. There is a risk of false positive results due to contamination with organisms, nucleic acids, vaccine material, amplified products, or from non-specific signals in the assay. Particular attention should be given to the *Laboratory Precautions* noted under the *Warnings and Precautions* section.
29. Transport media or saline may contain non-viable organisms and/or nucleic acid at levels that can be detected by the BioFire RP2.1-EZ.
30. There is a risk of false positive results due to non-specific amplification and cross-reactivity with organisms found in the respiratory tract. Observed and predicted cross-reactivity for BioFire RP2.1-EZ is described in the *Analytical Specificity (Cross-Reactivity)* section. Erroneous results due to cross-reactivity with organisms that were not evaluated or new variant sequences that emerge is also possible.
31. Primers for both BioFire RP2.1-EZ 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-EZ will be detected as Coronavirus SARS-CoV-2.
32. The clinical performance has not been established in all circulating variants of SARS-CoV-2 but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
33. 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-EZ as *Bordetella parapertussis*.
34. The BioFire RP2.1-EZ 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-EZ 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/in-vitro-diagnostics-euas.

However, to assist clinical laboratories and/or patient care settings using the BioFire RP2.1-EZ, the relevant Conditions of Authorization are listed below:

Authorized laboratories* using the BioFire RP2.1-EZ must include with test result reports 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 must use the BioFire RP2.1-EZ as outlined in the authorized labeling. 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 the BioFire RP2.1-EZ are not permitted.

Authorized laboratories that receive the BioFire RP2.1-EZ must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

Authorized laboratories using the BioFire RP2.1-EZ must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

Authorized laboratories must collect information on the performance of the BioFire RP2.1-EZ and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and BioFire (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 the BioFire RP2.1-EZ, 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 the BioFire RP2.1-EZ must 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, that meet the requirements to perform high, moderate, or waived complexity tests. This product is for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation" as "authorized laboratories."

# PERFORMANCE CHARACTERISTICS OF THE BIOFIRE RP2.1-EZ (BASED ON BIOFIRE RP2.1 AND BIOFIRE RP2)

The Expected Values and Clinical and Analytical Performance data were obtained from studies using the BioFire RP2.1 (Ref #: 423742; De Novo DEN200031 request granted) and is cleared for use in moderate complexity labs) and/or the BioFire® FilmArray® Respiratory Panel 2 (RP2) (Ref #: RFIT-ASY-0129, RFIT-ASY-0130; cleared for use in moderate complexity labs) and are applicable to the BioFire RP2.1-EZ.

The BioFire RP2.1-EZ kit contains the same reagent pouch as the BioFire RP2.1, which is also the same reagent pouch as in the BioFire RP2, except for the addition of assays to detect Coronavirus SARS-CoV-2. Studies were performed with the BioFire RP2.1 to establish the performance of the SARS-CoV-2 assays and to demonstrate that the addition of the SARS-CoV-2 assays did not alter performance for the other viruses and bacteria that are detected by the panels. The combination of the original studies of the BioFire RP2 and new studies with the BioFire RP2.1 demonstrate the performance of the BioFire RP2.1 pouch; additional studies have demonstrated that BioFire 2.0 EZ System is robust for testing NPS specimens in a near patient or CLIA-waived setting. The performance characteristics for the BioFire RP2.1-EZ are the same as presented below for the BioFire RP2.1 and/or BioFire RP2.

# EXPECTED VALUES

In the prospective clinical evaluation of the original 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.

**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)**

| BioFire RP2 Result | 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% |
| **Coronavirus 229E** | 9 | 1.0% | 4 | 1.2% | 5 | 1.8% | 0 | 0% |
| **Coronavirus HKU1** | 1 | 0.1% | 0 | 0% | 1 | 0.4% | 0 | 0% |
| **Coronavirus NL63** | 1 | 0.1% | 0 | 0% | 0 | 0% | 1 | 0.3% |
| **Coronavirus OC43** | 12 | 1.3% | 4 | 1.2% | 1 | 0.4% | 7 | 2.3% |
| **Human Metapneumovirus** | 5 | 0.5% | 2 | 0.6% | 2 | 0.7% | 1 | 0.3% |
| **Human Rhinovirus/Enterovirus** | 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 H1-2009** | 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** | 115 | 12.5% | 34 | 10.3% | 29 | 10.2% | 52 | 17.2% |
| **Respiratory Syncytial Virus** | 50 | 5.4% | 9 | 2.7% | 5 | 1.8% | 36 | 11.9% |
| **Bacteria** | | | | | | | | |
| ***Bordetella parapertussis*** | 4 | 0.4% | 0 | 0% | 0 | 0% | 4 | 1.3% |
| ***Bordetella pertussis*** | 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)**

| BioFire RP2 Result | 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% |
| **Coronavirus 229E** | 7 | 1.0% | 2 | 0.8% | 3 | 1.2% | 2 | 1.0% |
| **Coronavirus HKU1** | 54 | 7.8% | 28 | 11.2% | 16 | 6.6% | 10 | 5.0% |
| **Coronavirus NL63** | 49 | 7.1% | 24 | 9.6% | 17 | 7.0% | 8 | 4.0% |
| **Coronavirus OC43** | 26 | 3.7% | 8 | 3.2% | 10 | 4.1% | 8 | 4.0% |
| **Human Metapneumovirus** | 76 | 11.0% | 26 | 10.4% | 25 | 10.3% | 25 | 12.4% |

| BioFire RP2 Result | 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 (%) |
| **Human Rhinovirus/Enterovirus** | 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 H1-2009** | 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** | 17 | 2.4% | 8 | 3.2% | 4 | 1.6% | 5 | 2.5% |
| **Respiratory Syncytial Virus** | 149 | 21.5% | 59 | 23.6% | 51 | 21.0% | 39 | 19.4% |
| **Bacteria** | | | | | | | | |
| ***Bordetella parapertussis*** | 2 | 0.3% | 1 | 0.4% | 1 | 0.4% | 0 | 0% |
| ***Bordetella pertussis*** | 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% |

In the prospective clinical evaluation of the BioFire RP2.1, 524 eligible specimens (NPS) were collected and tested at three study sites across the United States over approximately four months (July – October 2020). The expected value (as determined by BioFire RP2.1) summary for the three observed analytes during this study stratified by specimen collection site is presented in Table 7.

**Table 7. Expected Value (As Determined by BioFire RP2.1) Summary by Collection Site for the BioFire RP2.1 Prospective Clinical Evaluation (July – October 2020)**

| BioFire RP2.1 Result | Overall (n 524) | | Site 1 (n 309) Tampa Bay, FL | | Site 2 (n 110) Lake Success, NY | | Site 3 (n 105) Chicago, IL | |
|---|---|---|---|---|---|---|---|---|
| | No. | Expected Value (%) | No. | Expected Value (%) | No. | Expected Value (%) | No. | Expected Value (%) |
| **Adenovirus** | 3 | 0.6% | 3 | 1.0% | 0 | 0% | 0 | 0% |
| **Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)** | 66 | 12.6% | 46 | 14.9% | 12 | 10.9% | 8 | 7.6% |
| **Human Rhinovirus/Enterovirus** | 33 | 6.3% | 12 | 3.9% | 11 | 10.0% | 10 | 9.5% |

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 8. Expected value (as determined by BioFire RP2.1) summary by age group for the three observed analytes in the BioFire RP2.1 prospective clinical evaluation (July – October 2020) is presented in Table 9.

**Table 8. 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)**

| BioFire RP2 Result | Overall (N=1612) | | ≤5 years (N=885) | | 6 21 years (N=331) | | 22 49 years (N=128) | | 50+ years (N=268) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. | Expected Value (%) | No. | Expected Value (%) | No. | Expected Value (%) | No. | Expected Value (%) | No. | Expected Value (%) |
| **Viruses** | | | | | | | | | | |
| **Adenovirus** | 118 | 7.3% | 96 | 10.8% | 18 | 5.4% | 2 | 1.6% | 2 | 0.7% |
| **Coronavirus 229E** | 16 | 1.0% | 3 | 0.3% | 7 | 2.1% | 1 | 0.8% | 5 | 1.9% |
| **Coronavirus HKU1** | 55 | 3.4% | 37 | 4.2% | 9 | 2.7% | 2 | 1.6% | 7 | 2.6% |
| **Coronavirus NL63** | 50 | 3.1% | 41 | 4.6% | 6 | 1.8% | 2 | 1.6% | 1 | 0.4% |
| **Coronavirus OC43** | 38 | 2.4% | 28 | 3.2% | 7 | 2.1% | 0 | 0% | 3 | 1.1% |

| BioFire RP2 Result | Overall (N=1612) | | ≤5 years (N=885) | | 6 21 years (N=331) | | 22 49 years (N=128) | | 50+ years (N=268) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. | Expected Value (%) | No. | Expected Value (%) | No. | Expected Value (%) | No. | Expected Value (%) | No. | Expected Value (%) |
| **Human Metapneumovirus** | 81 | 5.0% | 60 | 6.8% | 12 | 3.6% | 3 | 2.3% | 6 | 2.2% |
| **Human Rhinovirus/Enterovirus** | 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 H1-2009** | 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** | 132 | 8.2% | 104 | 11.8% | 17 | 5.1% | 4 | 3.1% | 7 | 2.6% |
| **Respiratory Syncytial Virus** | 199 | 12.3% | 168 | 19.0% | 10 | 3.0% | 8 | 6.3% | 13 | 4.9% |
| **Bacteria** | | | | | | | | | | |
| ***Bordetella parapertussis*** | 6 | 0.4% | 4 | 0.5% | 2 | 0.6% | 0 | 0% | 0 | 0% |
| ***Bordetella pertussis*** | 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% |

**Table 9. Expected Value (As Determined by BioFire RP2.1) Summary by Age Group for the BioFire RP2.1 Prospective Clinical Evaluation (July – October 2020)**

| BioFire RP2.1 Result | Overall (N=524) | | 0 18 years (N=55) | | 19 40 years (N=170) | | 41 60 years (N=146) | | 61+ years (N=153) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. | Expected Value (%) | No. | Expected Value (%) | No. | Expected Value (%) | No. | Expected Value (%) | No. | Expected Value (%) |
| **Adenovirus** | 3 | 0.6% | 1 | 1.8% | 2 | 1.2% | 0 | 0% | 0 | 0% |
| **Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)** | 66 | 12.6% | 5 | 9.1% | 24 | 14.1% | 22 | 15.1% | 15 | 9.8% |
| **Human Rhinovirus/Enterovirus** | 33 | 6.3% | 19 | 34.5% | 5 | 2.9% | 7 | 4.8% | 2 | 1.3% |

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 10. 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). The single polymicrobial detection (as determined by BioFire RP2.1) during the BioFire RP2.1 prospective clinical evaluation (July – October 2020) stratified by age group is presented in Table 11. Overall, the BioFire RP2.1 detected at least one organism in a total of 101 specimens (19.3% positivity rate; 101/524). Two organisms were detected by the BioFire RP2.1 in 1.0% of positive specimens (1/101; 0.2% of all tested specimens, 1/524).

**Table 10. Expected Value (Multiple Detections with ≥ 5 occurrences as Determined by the BioFire RP2) Summary by Age Group for the BioFire RP2 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 + Parainfluenza Virus | 25 (1.6%) | 22 (2.5%) | 2 (0.6%) | 0 (0%) | 1 (0.4%) |
| 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%) |
| Adenovirus + RSV | 10 (0.6%) | 8 (0.9%) | 2 (0.6%) | 0 (0%) | 0 (0%) |

| 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 + 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%) |
| Adenovirus + Parainfluenza Virus | 6 (0.4%) | 6 (0.7%) | 0 (0%) | 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%) |

**Table 11. Expected Value (Multiple Detections as Determined by the BioFire RP2.1) Summary by Age Group for the BioFire RP2.1 Prospective Clinical Evaluation (July – October 2020)**

| Multiple Detection Combination | Overall (N=524) | 0 18 years (N=55) | 19 40 years (N=170) | 41 60 years (N=146) | 61+ years (N=153) |
|---|---|---|---|---|---|
| Adenovirus + SARS-CoV-2 | 1 (0.2%) | 0 (0%) | 1 (0.6%) | 0 (0%) | 0 (0%) |

# PERFORMANCE CHARACTERISTICS

## Clinical Performance

### Prospective Clinical Evaluation of BioFire RP2 (2015-2017)

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 12 provides a summary of demographic information for the 1612 specimens included in the prospective study.

**Table 12. 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 13.

**Table 13. 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*** |
| **CoV-NL63**[d] | Fresh | 0/0 | - | - | 917/918 | 99.9 | 99.4-100 |

| Analyte | | Positive Percent Agreement | | | Negative Percent Agreement | | |
|---|---|---|---|---|---|---|---|
| | | TP/(TP + FN) | % | 95%CI | TN/(TN + FP) | % | 95%CI |
| | 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*** |
| **PIV**[j] | Fresh | 97/99 | 98.0 | 92.9-99.4 | 801/819 | 97.8 | 96.6-98.6 |
| | Frozen | 10/10 | 100 | 72.2-100 | 677/684 | 99.0 | 97.9-99.5 |
| | ***Overall*** | ***107/109*** | ***98.2*** | ***93.6-99.5*** | ***1478/1503*** | ***98.3*** | ***97.6-98.9*** |
| **RSV**[k] | 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***[l] | 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 |
| | ***Overall*** | ***6/7*** | ***85.7*** | ***48.7-97.4*** | ***1605/1605*** | ***100*** | ***99.8-100*** |
| ***B. pertussis***[m] | 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***[n] | 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***[o] | 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 PIV 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. PIV was detected in 10/25 FP specimens during discrepancy investigation; four were detected using an independent molecular method and six were detected upon comparator method retest.
[k] 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.
[l] *B. parapertussis* was detected in the single FN specimen upon BioFire RP2 retest.
[m] *B. pertussis* was detected in the both the FN and FP specimens using an independent molecular method.
[n] *C. pneumoniae* was detected in the single FP specimen using an independent molecular method.
[o] *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 14). The majority of multiple detections (190/245; 77.6%) contained two organisms, while 20.0% (49/245) contained three organisms, 2.0% (5/245) contained four 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 15.

**Table 14. 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% |
| PIV | 52 | 21.2% |
| RSV | 105 | 42.9% |
| **Bacteria** | | |

| Analyte | Prevalence in Multiple Detections (N=245) | |
|---|---|---|
| *B. parapertussis* | 6 | 2.4% |
| *B. pertussis* | 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 15. 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 | PIV | | 25 | 14 | HRV/EV (10), PIV (6) |
| 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) |
| 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) |
| 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 68 discrepant analytes (out of 299 total analytes), 32 (47.1%) were observed as being present in the specimen during discrepancy investigation; 22/68 (32.4%) were observed using an independent molecular method and 13/68 (19.1%) 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 the original BioFire RP2 (2015-2017)

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 16.

**Table 16. 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 17 by analyte.

**Table 17. 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 |

| Analyte | Positive Percent Agreement | | | Negative Percent Agreement | | |
|---|---|---|---|---|---|---|
| | TP/(TP + FN) | % | 95% CI | TN/(TN + FP) | % | 95% CI |
| 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 H1-2009 | 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 [c] | 65/65 | 100 | 94.4-100 | 123/127 | 96.9 | 92.2-98.8 |
| RSV | 2/2 | 100 | 34.2-100 | 191/192 | 99.5 | 97.1-99.9 |
| **Bacteria** | | | | | | |
| *Bordetella parapertussis* [d] | 16/16 | 100 | 80.6-100 | 4/4 | 100 | 51.0-100 |
| *Bordetella pertussis* [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 positive archived specimens the source laboratory was not confirmed by the comparator method and therefore was excluded from the performance calculation for Parainfluenza Virus .

[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 Preselected SARS-CoV-2 Archived Specimens with the BioFire RP2.1 (2020)

Preselected archived SARS-CoV-2 specimens were used to evaluate the performance of the new BioFire RP2.1 SARS-CoV-2 assays when testing clinical specimens. This involved testing of 50 natural retrospective (archived) NPS specimens that had previously been characterized as positive for SARS-CoV-2 by different assays with EUA designation. Specimens were obtained from three geographically distinct laboratories in the United States (Table 18) and had been collected in March and April, 2020.

**Table 18. Archived Source and Number of Positive Speicmens Identfiied**

| Site | Location | Positive Samples Tested |
|---|---|---|
| Site 1 | Salt Lake City, Utah | 15 |
| Site 2 | Seattle, Washington | 15 |
| Site 3 | Omaha, Nebraska | 20 |

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 19). For SARS-CoV-2 archived specimens the PPA was 98.0% (48/49) and NPA was 100%.

**Table 19. BioFire RP2.1 SARS-CoV-2 Archived NPS Specimen Performance Data Summary**

| Agreement with known analyte composition | | | | | | |
|---|---|---|---|---|---|---|
| **Authorized Molecular Comparator Method** | **PPA:** TP/(TP+FN) | **%** | **95% CI** | **NPA:** TN/(TN+FP) | **%** | **95% CI** |
| EUA 1 | 14/15[1] | 93.3 | [70.2-98.8%] | N/A | N/A | N/A |
| EUA 2 | 15/15 | 100 | [79.6-100%] | N/A | N/A | N/A |
| EUA 3 | 19/19 | 100 | [83.2-100%] | N/A | N/A | N/A |
| Negative Specimens | N/A | N/A | N/A | 49/49 | 100 | [92.7 – 100%] |
| **Overall Agreement** | **48/49[1]** | **98.0** | **[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 20).

**Table 20. 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%) |

## Testing of Contrived Specimens with the original BioFire RP2 (2015-2017)

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 H1-2009, 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 | | | |

| Analyte | Positive Percent Agreement | | | | Negative Percent Agreement | | |
|---|---|---|---|---|---|---|---|
| | × LoD | TP/(TP + FN) | % | 95% CI | TN/(TN + FP) | % | 95% CI |
| | 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.

## Testing of Contrived SARS-CoV-2 Specimens with the BioFire RP2.1 (2020)

Archived clinical specimen testing was complemented with testing of 50 contrived clinical NPS specimens spiked with inactivated SARS-CoV-2 isolate USA-WA1/2020 at various multiples of the LoD concentration (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 natural 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 result. PPA and NPA are shown in Table 22. For SARS-CoV-2 contrived testing, both the PPA and NPA were 100%.

**Table 22. 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%]** | |

## Clinical Comparison to the BioFire RP2 (2020)

A clinical comparison study between the BioFire RP2 and modified BioFire RP2.1 was conducted to demonstrate equivalent performance of all non-SARS-CoV-2 assays. This was performed using 220 natural retrospective (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 23).

**Table 23. 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** | 41 | 0 | 100% | 179 | 0 | 100% |
| **Respiratory Syncytial Virus** | 10 | 0 | 100% | 210 | 0 | 100% |

| Analyte | RP2.1+ RP2+ | RP2.1- RP2+ | PPA | RP2.1- RP2- | RP2.1+ RP2- | NPA |
|---|---|---|---|---|---|---|
| **Bacteria** | | | | | | |
| ***Bordetella parapertussis* (IS*1001*)** | 10 | 0 | 100% | 210 | 0 | 100% |
| ***Bordetella pertussis* (*ptxP*)** | 10 | 0 | 100% | 210 | 0 | 100% |
| ***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 24 along with the specificity values from the other studies. Overall NPA (specificity) for all three studies in individual, natural clinical specimens was 279/279 (100%; Table 24).

**Table 24. 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%]** |

# Supplemental Clinical Data Generated Post-Authorization to Support BioFire RP2.1 De Novo

## Prospective Clinical Evaluation of the BioFire RP2.1 (2020)

The clinical performance of the BioFire RP2.1 was established during a multi-center study conducted at three geographically distinct U.S. study sites between July and October 2020. A total of 534 NPS specimens were acquired for the clinical study; 10 of these were excluded from the final data analysis. The reasons for specimen exclusion were: the specimen was found not to meet the inclusion criteria after the specimen had been enrolled (insufficient volume, N=1; specimen stored at incorrect temperature, N=6), a BioFire RP2.1 run failure with insufficient volume for retesting (N=1), and the inability to determine a composite comparator interpretation for a specimen due to invalid comparator results (Rule #3, Table 26, N=2). The final data set consisted of 524 specimens. Table 25 provides a summary of demographic information for the 524 specimens included in the study.

**Table 25. Demographic Summary for Prospective BioFire RP2.1 Clinical Evaluation**

| | | Overall | Site 1 | Site 2 | Site 3 |
|---|---|---|---|---|---|
| Sex | Male | 270 (52%) | 170 (55%) | 53 (48%) | 47 (45%) |
| | Female | 251 (48%) | 139 (45%) | 54 (49%) | 58 (55%) |
| | Unknown | 3 (<1%) | 0 (0%) | 3 (3%) | 0 (0%) |
| Age | 0-18 years | 55 (10%) | 24 (8%) | 18 (16%) | 13 (12%) |
| | 19-40 years | 170 (32%) | 102 (33%) | 45 (41%) | 23 (22%) |
| | 41-60 years | 146 (28%) | 93 (30%) | 33 (30%) | 20 (19%) |
| | 61+ years | 153 (29%) | 90 (29%) | 14 (13%) | 49 (47%) |
| **Total** | | **524** | **309** | **110** | **105** |

The performance of the BioFire RP2.1 was evaluated by comparing the test results for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) with a composite comparator of three moleculartests with US FDA Emergency Use Authorization (EUA). The interpretation rules to determine the composite EUA comparator result are shown in Table 26.

**Table 26. BioFire RP2.1 Clinical Evaluation Composite Comparator Interpretations Rules[a]**

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

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

The performance for the BioFire RP2.1 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) result is summarized in Table 27. Positive Percent Agreement (PPA) was calculated as 100% × (TP / (TP + FN)). True positive (TP) indicates that both the BioFire RP2.1 and the comparator method had a positive result for the specific analyte, and false

negative (FN) indicates that the BioFire RP2.1 was negative while the comparator result was positive. Negative Percent Agreement (NPA) was calculated as 100% × (TN / (TN + FP)). True negative (TN) indicates that both the BioFire RP2.1 and the comparator method had negative results, and false positive (FP) indicates that the BioFire RP2.1 was positive while the comparator result was negative. The exact binomial two-sided 95% confidence interval (95%CI) was calculated. PPA was 98.4% (61/62) and NPA was 98.9% (457/462). SARS-CoV-2 was detected in the single FN specimen by all three comparator EUA tests. Among the five FP specimens, SARS-CoV-2 was detected by one of the three comparator EUA tests in four of the specimens, leading to a negative composite EUA interpretation (Rule #2, Table 26); SARS-CoV-2 was detected in the remaining FP specimen using an additional independent molecular method.

**Table 27. BioFire RP2.1 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Performance**

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

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

A single polymicrobial detection of two organisms was observed (0.2% of all specimens, 1/524; and 1.0% of positive specimens, 1/101; Table 28).

**Table 28. Multiple Detection Combinations as Determined by the BioFire RP2.1, Prospective Study**

| Distinct Co Detection Combinations (Performance[a]) | | Total Specimens with Co Detection |
|---|---|---|
| Analyte 1 | Analyte 2 | |
| Adenovirus | Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (TP) | 1 |
| | **Total Co-Detections** | **1** |

[a] Performance only determined for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

The overall success rate for initial specimen tests was 99.6% (525/527). Two tests (2/527; 0.4%) did not complete on the initial run, resulting in an instrument success rate of 99.6% (525/527) for initial specimen tests. One specimen was able to be retested and valid results were produced after a single retest. Of the 525 tests that successfully produced a completed run on the initial test, all had valid pouch controls. This represents a 100% (525/525) success rate for pouch controls in completed runs in the initial specimen tests.

# ANALYTICAL PERFORMANCE CHARACTERISTICS

## Limit of Detection (LoD)

The Limit of Detection (LoD) refers to the lowest concentration of an organism that can be reliably detected by the test. BioFire RP2.1/RP2.1-EZ LoD concentrations were estimated by serial dilution and confirmed by detection in at least 19 of 20 replicates (≥95%) tested. All LoD confirmation testing was performed with contrived samples containing one or multiple organisms spiked in transport medium or saline NPS matrix. The BioFire RP2.1-EZ LoD concentration is provided in nucleic acid copies/mL and/or viable or infectious units (e.g. CFU/mL or $TCID_{50}$/mL) (Table 29). Nucleic acid copies/mL concentrations were determined by quantitative real-time PCR or digital droplet PCR.

**Table 29. Summary of Limit of Detection (LoD) for BioFire RP2.1-EZ Analytes**

| BioFire RP2.1 EZ Analyte | Isolate | LoD Concentration |
|---|---|---|
| **Viruses** | | |
| **Adenovirus** | Species C Serotype 2<br>WHO International Standard<br>NIBSC 16/324 | **3.0E+03 IU/mL** [a,b]<br>(3.0E+03 copies/mL) |
| **Coronavirus 229E** | ATCC VR-740 | **6.5E+01 copies/mL**<br>(4.0E-01 $TCID_{50}$/mL) |
| **Coronavirus HKU1** | Clinical specimen | **2.0E+03 copies/mL** |
| **Coronavirus NL63** | BEI NR-470 | **5.4E+01 copies/mL**<br>(2.5E-01 $TCID_{50}$/mL) |
| **Coronavirus OC43** | ATCC VR-759 | **5.6E+02 copies/mL**<br>(3.0E+01 $TCID_{50}$/mL) |
| **Coronavirus SARS-CoV-2** | USA-WA1/2020 | **5.0E+02 copies/mL** |
| | ATCC VR-1986HK (heat inactivated) | (6.9E-02 $TCID_{50}$/mL) |
| **Human Metapneumovirus** | 16, Type A1 IA10-2003<br>Zeptometrix 0810161CF | **1.0E+01 $TCID_{50}$/mL**[c] |
| **Human Rhinovirus/Enterovirus** | Human Rhinovirus<br>Type 1A<br>Zeptometrix 0810012CFN | **3.8E+01 copies/mL** |
| | Enterovirus D68<br>ATCC VR-1823 | **2.6E+01 copies/mL**<br>(3.0E+02 $TCID_{50}$/mL) |
| **Influenza A H1** | Influenza A H1N1<br>A/New Caledonia/20/99<br>Zeptometrix 0810036CF | **1.4E+02 copies/mL**<br>(1.0E+03 $TCID_{50}$/mL) |
| **Influenza A H1-2009** | Influenza A H1N1pdm09<br>A/Swine/NY/03/2009<br>Zeptometrix 0810249CF | **3.3E+02 copies/mL**<br>(5.0E-01 $TCID_{50}$/mL) |
| **Influenza A H3** | | **2.1E+01 copies/mL**<br>(1.0E-01 $TCID_{50}$/mL) |

| BioFire RP2.1 EZ Analyte | Isolate | LoD Concentration |
|---|---|---|
| | Influenza H3N2<br>A/Port Chalmers/1/73<br>ATCC VR-810 | |
| **Parainfluenza Virus** | Type 4a<br>Zeptometrix 0810060CF | **1.6E+03 copies/mL**[d]<br>(5.0E+01 $TCID_{50}$/mL) |
| **Respiratory Syncytial Virus** | Type A<br>Zeptometrix 0810040ACF | **9.0E+00 copies/mL**<br>(2.0E-02 $TCID_{50}$/mL) |
| **Bacteria** | | |
| ***Bordetella parapertussis*** | A747<br>Zeptometrix 0801461 | **6.0E+01 IS*1001* copies/mL**[e]<br>4.1E+01 CFU/mL |
| ***Bordetella pertussis*** | A639<br>Zeptometrix 0801459 | **1.0E+03 CFU/mL** |
| ***Chlamydia pneumoniae*** | TW183<br>ATCC VR-2282 | **1.3E+02 copies/mL** |
| ***Mycoplasma pneumoniae*** | M129<br>Zeptometrix 0801579 | **4.6E+02 copies/mL** |

[a] IU = International Units. 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] Detection in ≥95% of 20 replicates was confirmed for two other adenovirus serotypes (B7 and F41) at concentrations similar to or less than 3.0E+03 copies/mL.

[c] The isolate culture used for LoD testing of Human Metapneumovirus was quantified only in units of $TCID_{50}$/mL. A copies/mL value has not been determined.

[d] Detection in ≥95% of 20 replicates was confirmed for three other Parainfluenza Virus types (PIV1, PIV2 and PIV3) at concentrations similar to or less than 1.6E+03 copies/mL.

[e] 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.

***NOTE: LoD concentrations in copies/mL in Table 29 above are based on extraction of nucleic acids from isolate cultures followed by quantitative real-time PCR (qPCR). The accuracy of qPCR concentrations may be affected by extraction efficiency, standard curve accuracy, assay conditions, inhibitors, and/or sequence variance. The qPCR quantification has not been compared to a reference material or other quantification methods.***

***NOTE: LoD concentrations of cultured viruses provided in units of $TCID_{50}$ (50% Tissue Culture Infectious Dose) are not a direct count of viral particles or nucleic acid, but an indirect measure of viral concentration based on infectivity and cytotoxicity. $TCID_{50}$/mL will therefore vary 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 detection for different cultures and/or different molecular assays based on LoD values measured in $TCID_{50}$/mL.***

## 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 30.

**Table 30. 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

## Coronavirus SARS-CoV-2 Detection - Comparison with Other EUA Tests

Detection of Coronavirus SARS-CoV-2 by the BioFire RP2.1/RP2.1-EZ was compared to detection by two other moderate or high complexity tests that have received Emergency Use Authorization from the U.S. Food and Drug Administration (BioFire® COVID-19 Test from BioFire Defense, LLC and another FDA authorized molecular test)

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/RP2.1-EZ 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 31).

**Table 31. Inactivated SARS-CoV-2 Detection Comparison Between BioFire RP2.1-EZ 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-EZ | COVID-19 | RP2.1-EZ | COVID-19 | RP2.1-EZ | COVID-19 | RP2.1-EZ | COVID-19 | RP2.1-EZ | 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 FDA authorized molecular tests, 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/RP2.1-EZ and another FDA authorized molecular test according to the manufacturer's instructions for use. SARS-CoV-2 was detected by both panels (BioFire RP2.1/RP2.1-EZ) equivalently at each concentration (Table 32).

**Table 32. Infectious SARS-CoV-2 Detection Comparison Between BioFire RP2.1-EZ 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-EZ | FDA authorized test | BioFire RP2.1-EZ | FDA authorized test | BioFire RP2.1-EZ | FDA authorized test | BioFire RP2.1-EZ | | BioFire RP2.1-EZ | FDA authorized test |
| 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 |

## Analytical Reactivity (Inclusivity)

Analytical reactivity (inclusivity) of BioFire RP2.1 assays was evaluated by *in silico* analysis and testing of different viral and bacterial isolates or specimens. The isolates tested represent the temporal and geographic diversity of the analytes, including relevant species, strains, serotypes, or genotypes. Each isolate was tested in triplicate at a concentration near LoD with either the BioFire RP2.1/RP2.1-EZ or the BioFire RP2 pouches. Any isolates that were not reliably detected near LoD were re-tested at a higher concentration (10× LoD) and detected.

Testing data were supplemented with *in silico* analysis of sequence data retrieved from public databases, such as NCBI (National Center for Biotechnology Information). Sequence analysis was used to identify specific strains or sequences from an organism that might not be detected by the panel assays, or to identify serotypes, genotypes etc. that were not available for testing but that are predicted to be detected based on the sequence data.

Table 33-Table 44 provide information about each of the isolates tested, including the concentration tested and detection results. Notes are also provided for specific predictions about reactivity based on *in silico* analyses. Due to limited access to characterized isolates of Coronavirus SARS-CoV-2, the analytical reactivity for this virus was established almost exclusively based on the *in silico* analysis of the sequence data (Table 45).

***NOTE: BioFire RP2.1-EZ 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 Uncertain or Influenza A (no subtype detected) results.***

***NOTE: The BioFire RP2.1-EZ assays may react with vaccines that contain specific segments of the pathogen genome or full genome or vaccines containing attenuated/inactivated pathogen, including vaccines for SARS-CoV-2, Influenza A (various subtypes), Influenza B, poliovirus (Human Rhinovirus/Enterovirus), and Bordetella pertussis. Care should be taken to minimize contamination of samples with vaccines, and clinical history of vaccine administration should be considered in the interpretation of results, particularly for vaccines administered by nasal spray.***

**Table 33. Adenovirus Assay Reactivity (Isolates Tested and Detected)**

| 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] | 3x | |
| | 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 | ATCC VR-12 | [Slobitski] | 3x | |
| | 14 | ATCC VR-15 | [De Wit/Netherlands/1955] | 3x | |
| | 16 | ATCC VR-17 | [CH.79/Saudia Arabia/1955] | 3x | |
| | 21 | ATCC VR-1833 | [128/Saudi Arabia/1956] | 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] | 3x | |
| | | NIBSC 16/324 | - | 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 | - | 3x | |
| E | 4a | Univ of Iowa Research Foundation | [S Carolina/2004] | 3x | |
| | 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 RP2.1 adenovirus assays will also react with Adenovirus B55, C57, species D serotypes, and G52.

[b] All adenovirus isolates were tested on BioFire RP2.1/RP2.1-EZ pouches at 3x the LoD established with the WHO International Standard (3.0E+03 IU or copies/mL) or less.

**Table 34. Coronavirus Assay Reactivity (Isolates/Specimens Tested and Detected)**

| Coronavirus Type | Isolate ID/Source | [Location/Year] | xLoD[a] 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 | [Amsterdam/2003] | 1x | Coronavirus NL63 |
| | Zeptometrix 0810228CF | - | 3x | |
| OC43 | ATCC VR-759[b] | - | 1x | Coronavirus OC43 |
| | Zeptometrix 0810024CF | - | 3x | |
| SARS-CoV-2[c] | ATCC VR-1986HK (heat inactivated) World Reference Center for Emerging Viruses and Arboviruses | USA-WA1/2020 | 1x | Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) |

[a] 1x LoD samples were tested and detected with BioFire RP2.1/RP2.1-EZ pouches; 3x LoD samples were tested and detected with BioFire RP2 pouches.

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

[c] See Table 45 for additional SARS-CoV-2 reactivity predictions based on *in silico* analysis.

**Table 35. Human Metapneumovirus Assay Reactivity (Isolates Tested and Detected)**

| Genotype | Serotype | Isolate ID/Source | [Location/Year] | xLoD[a] 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 | |
| | 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 | |

[a] 1x LoD sample was tested and detected with BioFire RP2.1/RP2.1-EZ pouches; 3x LoD samples were tested and detected with BioFire RP2 pouches.

**Table 36. Human Rhinovirus/Enterovirus Reactivity (Isolates Tested and Detected)[a]**

| Species | Serotype | Isolate ID/Source | [Strain/Location/Year] | xLoD[b] 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[c] | [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 | |
| | Enterovirus 71 | ATCC VR-1432 | [H] | 3x | |

| Species | Serotype | Isolate ID/Source | [Strain/Location/Year] | xLoD[b] Detected | Result |
|---|---|---|---|---|---|
| **Human Rhinovirus** | | | | | |
| B | Coxsackievirus A9 | Zeptometrix 0810017CF | - | 3x | Human Rhinovirus/ Enterovirus |
| | 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] Sequence analysis predicts that the HRV/EV assay can react with nucleic acids in polio vaccines.

[b] 1x LoD samples were tested and detected with BioFire RP2.1/RP2.1-EZ pouches; 3x LoD samples were tested and detected with BioFire RP2 pouches.

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

**Table 37. Influenza A and Influenza A H1/H1-2009/H3 Reactivity (Isolates Tested and Detected)**

| Type | Isolate ID/Source | | [Strain/Location/Year] | xLoD[a] 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[b] | |
| | | 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[b] | |
| H1N2 | Recombinant | BEI NR-9677[c] | [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 | [Netherlands/2629/2009] | 3x | |
| | | BEI NR-44345 | [Hong Kong/H090-761-V1(0)/2009] | 10x[d] | |
| | | BEI NR-42938 | [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[e] | Human | Clinical Specimen | [Ohio/2012] | 3x | |
| H2N2 | Human | BEI NR-2775[f] | [Japan/305/1957] | 10x[d] | Influenza A (no subtype detected) |
| | Recombinant | BEI NR-9679[g] | [Korea/426/1968xPuerto Rico/8/1934 ] | 10x[d] | |
| H2N3 | Avian | MRI Global[h] | [Mallard/Alberta/79/2003] | 3x | Influenza A Uncertain |
| H5N1 | | MRI Global[h] | [A/Chicken/Yunnan/1251/2003] | 3x | Influenza A (no subtype detected) |
| H5N2 | | MRI Global[h] | [A/Northern pintail/Washington/40964/2014] | 3x | |
| H5N3 | | BEI NR-9682[i] | [A/Duck/Singapore/645/1997] | 3x | |
| H5N8 | | MRI Global[h] | [Gyrfalcon/Washington/41088-6/2014] | 3x | |
| H7N7 | | MRI Global[h] | [A/Netherlands/219/2003] | 3x | |
| H7N9 | | MRI Global[h] | [A/Anhui/01/2013] | 3x | |
| H10N7 | | BEI NR-2765[j] | [Chicken/Germany/N/49] | 3x | Influenza A Uncertain |

[a] 1x LoD samples were tested and detected with BioFire RP2.1/RP2.1-EZ pouches; 3x LoD samples were tested and detected with BioFire RP2 pouches.

[b] Reported as Influenza A (no subtype detected) at 3× LoD, Inflnenza A H1 Detected at 10× LoD.

[c] 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.
[d] Reported as Influenza A Uncertain or Influenza A (no subtype detected) at 3× LoD.
[e] Human isolate of recent swine variant H3N2 virus.
[f] Genomic RNA obtained through BEI Resources, NIAID, NIH: Genomic RNA from Influenza A Virus, A/Japan/305/1957 (H2N2), NR-2775.
[g] 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.
[h] Isolate provided and tested by MRI Global, Kansas City, MO.
[i] Genomic RNA obtained through BEI Resources, NIAID, NIH: Genomic RNA from Kilbourne F181: A/duck/Singapore/645/1997 (H5N3), Wild Type, NR-9682.
[j] Genomic RNA obtained through BEI Resources, NIAID, NIH: Genomic RNA from Influenza A Virus, A/chicken/Germany/N/1949 (H10N7), NR-2765.

**Table 38. Influenza B Reactivity (Isolates Tested and Detected)**

| Lineage | Isolate ID/Source | [Strain/Location/Year] | xLoD[a] 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 | |

[a] 1x LoD sample was tested and detected with BioFire RP2.1/RP2.1-EZ pouches; 3x LoD samples were tested and detected with BioFire RP2 pouches.

**Table 39. Parainfluenza Virus Reactivity (Isolates Tested and Detected)**

| Type | Subtype | Isolate ID/Source | [Strain/Location/Year] | xLoD[a] Detected | Result |
|---|---|---|---|---|---|
| 1 | | Zeptometrix 0810014CF | - | 1x | Parainfluenza Virus |
| | | ATCC VR-94 | [C-35/Washington DC/1957] | 3x | |
| | | BEI NR-3226[b] | [C39] | 3x | |
| | | BEI NR-48680 | [FRA/29221106/2009] | 3x | |
| 2 | | Zeptometrix 0810015CF | - | 1x | |
| | | ATCC VR-92 | [Greer/Ohio/1955] | 3x | |
| 3 | | Zeptometrix 0810016CF | - | 1x | |
| | | ATCC VR-93 | [C-243/Washington DC/1957] | 3x | |
| | | BEI NR-3233 | [NIH 47885, Wash/47885/57] | 3x | |
| 4 | A | Zeptometrix 0810060CF | - | 1x | |
| | | ATCC VR-1378 | [M-25/1958] | 3x | |
| | B | Zeptometrix 0810060BCF | - | 3x | |
| | | ATCC VR-1377 | [CH-19503/Washington DC/1962] | 3x | |

[a] 1x LoD samples were tested and detected with BioFire RP2.1-EZ pouches; 3x LoD samples were tested and detected with BioFire RP2 pouches.
[b] Discontinued part number.

**Table 40. Respiratory Syncytial Virus Reactivity (Isolates Tested and Detected)**

| Type | Source | [Strain/Location/Year] | xLoD[a] 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 | |

[a] 1x LoD sample was tested and detected with BioFire RP2.1/RP2.1-EZ pouches; 3x LoD samples were tested and detected with BioFire RP2 pouches.

**Table 41. *Bordetella parapertussis* Reactivity (Isolates Tested and Detected)**

| Species | Source | [Strain/Location/Year] | xLoD[a] Detected | Result |
|---|---|---|---|---|
| *Bordetella parapertussis* | Zeptometrix 0801461 | [A747] | 1x | *Bordetella parapertussis* |
| | Zeptometrix 0801462 | [E595] | 3x | |
| | ATCC 15237 | [NCTC 10853] | 3x | |

| Species | Source | [Strain/Location/Year] | xLoD[a] Detected | Result |
|---|---|---|---|---|
| | ATCC 15311 | [NCTC 5952] | 3x | |
| | ATCC BAA-587 | [12822/Germany/1993] | 3x | |
| *Bordetella bronchiseptica*[b] (containing IS*1001*) | NRRL B-59909 | [MBORD849/ Pig/Netherlands] | 3x | |

[a] 1x LoD sample was tested and detected with BioFire RP2.1/RP2.1-EZ pouches; 3x LoD samples were tested and detected with BioFire RP2 pouches.

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

**Table 42. *Bordetella pertussis* Reactivity (Isolates Tested and Detected)[a]**

| Isolate ID/Source | [Strain] | xLoD Detected | Result |
|---|---|---|---|
| Zeptometrix 0801459 | [A639] | 1x | *Bordetella pertussis* |
| 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 | |

[a] The *Bordetella pertussis* assay can react with pertussis vaccines.

[b] 1x LoD sample was tested and detected with BioFire RP2.1/RP2.1-EZ pouches; 3x LoD samples were tested and detected with BioFire RP2 pouches.

**Table 43. *Chlamydia pneumoniae* Reactivity (Isolates Tested and Detected)**

| 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] All *C. pneumoniae* isolates were tested and detected with BioFire RP2.1/RP2.1-EZ pouches

**Table 44. M*ycoplasma pneumoniae* Reactivity (Isolates Tested and Detected)**

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

[a] 1x LoD sample was tested and detected with BioFire RP2.1/RP2.1-EZ pouches; 3x LoD samples were tested and detected with BioFire RP2 pouches.

## *In Silico* Reactivity Predictions for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Assays

Evaluation of analytical reactivity for the BioFire RP2.1-EZ SARS-CoV-2 assays (SARSCoV2-1 and SARSCoV2-2) was based on *in silico* sequence analysis of all available sequences in the GISAID database as of February 21, 2022. In total, 7,023,069 sequences from around the globe were aligned to the assay primers.

This analysis determined that >99.99% of 7,023,069 sequences will be detected by one or both BioFire RP2.1-EZ SARS-CoV-2 assays based on homology and mismatch location with one or both sets of primers. A limitation on detection (both assays impaired) is predicted for only 0.005% of the sequences evaluated (327/ 7,023,069 ) (Table 45).

The sequences evaluated include lineages and variants of concern (VOC) or variants under investigation (VUI) that may have important epidemiological, immunological, or pathogenic properties from a public health perspective, such as Delta and Omicron variants. Variants evaluated are listed in the *BioFire® FilmArray® Respiratory Panels (RP2.1, RP2.1plus*

*and RP2.1-EZ) SARS-CoV-2 Reactivity (SARS-CoV-2) Tech Note* technical note at www.biofiredx.com/support/documents.

All lineages and variants of public health interest identified as of February 2022 are predicted to be detected; new sequences and variants will continue to be monitored for impacts on detection by the BioFire RP2.1-EZ assays.

**Table 45. *In silico* Prediction of SARS-CoV-2 Detection by the BioFire RP2.1-EZ 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 + | SARSCoV2 1 - | # (%) sequences predicted to be detected (one or both assays positive) |
|---|---|---|---|---|
| SARSCoV2 2 | + | 6,849,554 | 139,867[a] | 7,022,742 / 7,023,069[b] (99.995%) |
| | - | 33,321 | 327 | |

[a] Includes 7,634 sequences of lineage B.1.525 (VUI-202102/03) with a mutation that is predicted to impair detection by the SARS-CoV2-1 assay, but detection by the SARSCoV2-2 assay is predicted to be unaffected (explanation in addendum 04).

[b] Three-hundred-twenty-seven deposited sequences (0.005%; representing 125 unique sequences) have mismatches in the 3' half of primer(s), or a 5' deletion, for both the SARSCoV2-1 and SARSCoV2-2 assays. Various degrees of impaired detection are predicted for these variant sequences.

## Analytical Specificity (Cross-Reactivity)

The potential for non-specific amplification and detection by the BioFire RP2.1-EZ assays was evaluated by *in silico* analysis of available sequences and also by testing of high concentrations of organisms in BioFire RP2.1/RP2.1-EZ pouches. 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-EZ. Each organism was tested in triplicate, with bacteria and fungi generally tested at ≥1.0E+07 units/mL and viruses tested at ≥1.0E+05 units/mL.

*In silico* analysis and testing identified a risk of SARS-CoV-2 assay cross-reactivity with a few sequences of SARS-like viruses isolated from animals (bats and pangolin). 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 assay(s) will be detected Coronavirus SARS-CoV-2. In addition, intra-panel cross-reactivity was identified for select *Bordetella* species and Influenza A subtypes of swine origin. A summary of potential cross-reactivity is provided Table 46 and the on-panel or off-panel isolates and concentrations tested are listed in Table 47 and Table 48 respectively.

**Table 46. Predicted and Observed Cross-Reactivity of the BioFire RP2.1-EZ**

| Cross-reactive Organism(s)/Sequence(s) | BioFire RP2.1 EZ Result | Description |
|---|---|---|
| **Bat coronavirus_RaTG13** (accession# MN996532) **Pangolin coronavirus** (accession# MT084071) **Bat SARS-like coronavirus** (accession# MG772933 and MG772934) | **Coronavirus 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 one sequence (bat coronavirus_RaTG13). |
| **Non-pertussis *Bordetella* species** *(e.g. Bordetella parapertussis, Bordetella bronchiseptica*[a]) | ***Bordetella pertussis*** [b] | The *Bordetella pertussis* assay can non-specifically amplify and detect pertussis toxin pseudogene sequences in *B. bronchiseptica* and *B. parapertussis*, but only when present in a sample at a very high concentration (≥1.2E+09 CFU/mL). |
| ***Bordetella bronchiseptica***[a] (with IS*1001* sequences) | ***Bordetella parapertussis*** | Some strains of *B. bronchiseptica* carry IS*1001* insertion sequences identical to those carried by *B. parapertussis*. These sequences will be efficiently amplified and detected by the *B. parapertussis* assay. |
| ***Bordetella pertussis*** ***Bordetella parapertussis***[c] ***Bordetella bronchiseptica***[c] | **Human Rhinovirus/Enterovirus**[d] | The Human Rhinovirus/Enterovirus assay may amplify off-target sequences found in strains of *B. pertussis*, *B. bronchiseptica*, and *B. parapertussis* when present at high concentration. Cross-reactivity with *B. pertussis* was observed at a concentration of 4.5E+07 CFU/mL or higher. |
| **Influenza A H1N1** (swine origin) | **Influenza A H1-2009**[e] | The Influenza A H1-2009 assay may react with H1 hemagglutinin gene sequences from viruses of swine origin. BioFire RP2.1-EZ 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* assay and *B. parapertussis* will be reported as a co-detection (*Bordetella parapertussis* Detected and *Bordetella pertussis* Detected); while cross-reactivity with most strains of *B. bronchiseptica* (that do not carry IS*1001*) will be reported only as *Bordetella pertussis* Detected.

[c] Cross-reactivity with *B. parapertussis* and *B. bronchiseptica* is predicted based on *in silico* analysis but cross-reactivity was not observed when isolates were tested at concentrations >2.0E+09 CFU/mL.

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

[e] 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 high concentration (≥8.9E+06 $CEID_{50}$/mL).

**Table 47. On-Panel Organisms Tested for Evaluation of BioFire RP2.1-EZ Analytical Specificity**

| Organism | | Isolate ID | Concentration Tested | Cross Reactivity Detected |
|---|---|---|---|---|
| **Bacteria** | | | | |
| *Bordetella parapertussis* | | Zeptometrix 0801462 | 6.43E+09 CFU/mL | ***Bordetella pertussis***[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 |
| Coronavirus SARS-CoV-2 | | USA-WA1/2020 | 2.4E+09 copies/mL | None |
| Human Metapneumovirus | | Zeptometrix 0810159CF | 1.05E+06 $TCID_{50}$/mL | None |
| Human Rhinovirus (Type 1A) | | Zeptometrix 0810012CFN | 8.40E+05 $TCID_{50}$/mL | None |
| Enterovirus (D68) | | ATCC VR-1823 | 1.58E+07 $TCID_{50}$/mL | None |
| Influenza A H1N1 (A1/FM/1/47) | | ATCC VR-97 | 1.58E+08 $CEID_{50}$/mL | None |
| Influenza A Hsw N1 (A/NewJersey/8/76) | | ATCC VR-897 | 8.89E+06 $CEID_{50}$/mL | **Influenza A H1-2009**[c] |
| Influenza A (H1N1) pdm09 (Michigan/45/15) | | Zeptometrix 0810538CF | 9.40E+04 $TCID_{50}$/mL | None |
| Influenza A H3N2 (A/Alice) | | ATCC VR-776 | 3.33E+08 $CEID_{50}$/mL | None |
| Influenza B (Massachusetts/2/12) | | Zeptometrix 0810239CF | 9.55E+05 $TCID_{50}$/mL | None |
| Parainfluenza Virus 1 | | Zeptometrix 0810014CF | 6.80E+07 $TCID_{50}$/mL | None |
| 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] *In silico* analysis and testing support that the *Bordetella pertussis* assay may amplify pertussis toxin pseudogene sequences from some strains of *B. parapertussis* at high concentration (>1.2E+09 CFU/mL).

[b] *In silico* analysis and testing support that the 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] 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 48. Off-Panel Organisms Tested for Evaluation of BioFire RP2.1-EZ 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*** |
| *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 |
| *Chlamydia 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 |

| Organism | Isolate ID | Concentration Tested | Cross Reactivity Detected/Predicted |
|---|---|---|---|
| *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 |
| *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 (recombinant) | BEI NR-44009 | 3.15E+06 $TCID_{50}$/mL | None |
| Bat SARS-like Coronavirus HKU5 (recombinant) | BEI NR-48814 | 1.95E+06 $TCID_{50}$/mL | 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 |

## Reproducibility

Reproducibility testing of contrived samples was performed over five days at three test sites, with different instruments and operators at each site. Negative data for all analytes were collected from one or more unspiked samples tested with the BioFire RP2.1/RP2.1-EZ. Positive data were collected from samples containing a subset of representative organisms (RNA viruses, DNA virus, and bacteria) spiked at Low Positive (1x LoD) and Moderate Positive (3x LoD) concentrations. Testing incorporated a range of potential variation introduced by site, operator (at least two per site), instrument/module (at least three per site/system), and kit lot (three).

A summary of results (percent (%) agreement with the expected Detected or Not Detected result) for each analyte (by site) is provided in Table 49.

**Table 49. Reproducibility of BioFire RP2.1-EZ Detection Results**

| Analyte (Isolate Source ID) | Concentration Tested | Expected Result | Agreement with Expected Result | | | |
|---|---|---|---|---|---|---|
| | | | Site A | Site B | Site C | Total [95% Confidence Interval] |
| **Adenovirus (NIBSC 16/324) WHO International Standard** | **Negative (no analyte)** | **Not Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| | **Moderate Positive (3× LoD) 9.0E+03 IU/mL** | **Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| | **Low Positive (1× LoD) 3.0E+03 IU/mL** | **Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| **Coronavirus 229E** | **Negative (no analyte)** | **Not Detected** | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | **180/180 (100%) [98.0 – 100%]** |
| **Coronavirus HKU1** | **Negative (no analyte)** | **Not Detected** | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | **180/180 (100%) [98.0 – 100%]** |
| **Coronavirus NL63 (BEI NR-470)** | **Negative (no analyte)** | **Not Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| | **Moderate Positive (3× LoD) 7.5E-01 $TCID_{50}$/mL 1.6E+02 copies/mL** | **Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| | **Low Positive (1× LoD) 2.5E-01 $TCID_{50}$/mL 5.4E+01 copies/mL** | **Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| **Coronavirus OC43** | **Negative (no analyte)** | **Not Detected** | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | **180/180 (100%) [98.0 – 100%]** |
| **Coronavirus SARS-CoV-2 (ATCC VR-1986HK)** | **Negative (no analyte)** | **Not Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| | **Moderate Positive (3× LoD) 1.5E+03 copies/mL** | **Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| | **Low Positive (1× LoD) 5.0E+02 copies/mL** | **Detected** | 20/20 (100%) | 19/20 (95%) | 19/20 (95%) | **58/60 (96.7%) [88.5 – 99.6%]** |
| **Human Metapneumovirus** | **Negative (no analyte)** | **Not Detected** | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | **180/180 (100%) [98.0 – 100%]** |
| **Human Rhinovirus/ Enterovirus** | **Negative (no analyte)** | **Not Detected** | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | **180/180 (100%) [98.0 – 100%]** |

| Analyte (Isolate Source ID) | Concentration Tested | Expected Result | Agreement with Expected Result | | | |
|---|---|---|---|---|---|---|
| | | | Site A | Site B | Site C | Total [95% Confidence Interval] |
| **Influenza A H1** | **Negative (no analyte)** | **Not Detected** | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | **180/180 (100%) [98.0 – 100%]** |
| **Influenza A H1-2009 (Zeptometrix 0810109CFN)** | **Negative (no analyte)** | **Not Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| | **Moderate Positive (3× LoD) 1.5E+00 $TCID_{50}$/mL 9.9E+02 copies/mL** | **Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| | **Low Positive (1× LoD) 5.0E-01 $TCID_{50}$/mL 3.3E+02 copies/mL** | **Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| **Influenza A H3** | **Negative (no analyte)** | **Not Detected** | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | **180/180 (100%) [98.0 – 100%]** |
| **Influenza B** | **Negative (no analyte)** | **Not Detected** | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | **180/180 (100%) [98.0 – 100%]** |
| **Parainfluenza Virus** | **Negative (no analyte)** | **Not Detected** | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | **180/180 (100%) [98.0 – 100%]** |
| **Respiratory Syncytial Virus (Zeptometrix 0810040ACF)** | **Negative (no analyte)** | **Not Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| | **Moderate Positive (3× LoD) 6.0E-02 $TCID_{50}$/mL 2.7E+01 copies/mL** | **Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| | **Low Positive (1× LoD) 2.0E-02 $TCID_{50}$/mL 9.0E+00 copies/mL** | **Detected** | 19/20 (95%) | 20/20 (100%) | 18/20 (90%) | **57/60 (95%) [86.1 – 99.0%]** |
| ***Bordetella parapertussis* (Zeptometrix 0801461)** | **Negative (no analyte)** | **Not Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| | **Moderate Positive (3× LoD) 1.8E+02 IS*1001* copies/mL** | **Detected** | 19/20 (95%) | 20/20 (100%) | 20/20 (100%) | **59/60 (98.3%) [91.1 – 100%]** |
| | **Low Positive (1× LoD) 6.0E+01 IS*1001* copies/m** | **Detected** | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **60/60 (100%) [94.0 – 100%]** |
| ***Bordetella pertussis*** | **Negative (no analyte)** | **Not Detected** | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | **180/180 (100%) [98.0 – 100%]** |
| ***Chlamydia pneumoniae*** | **Negative (no analyte)** | **Not Detected** | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | **180/180 (100%) [98.0 – 100%]** |
| ***Mycoplasma pneumoniae*** | **Negative (no analyte)** | **Not Detected** | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | **180/180 (100%) [98.0 – 100%]** |

# Interference

Potentially interfering substances that could be present in NPS specimens or introduced during specimen collection and testing were evaluated for their effect on the performance of the test. Results from samples containing a substance were compared to results from control samples without substance. Substances 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 (see Table 50) was equal to or greater than the highest level expected to be in NPS specimens.

None of the substances were shown to interfere with the pouch/test 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 test results (lack of analyte detection). The effect of bleach was dependent on the concentration and/or length of time the bleach interacted with the sample.

**Table 50. Evaluation of Potentially Interfering Substances for NPS Specimens**

Substances in **bold** font were tested with BioFire RP2.1/RP2.1-EZ pouches on samples containing Coronavirus SARS-CoV-2 as well as other viruses and bacteria. All other substances were tested in the BioFire RP2 interference study.

| 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<br>(active ingredient in anti-bacterial ointment) | 2% w/v | No Interference |
| Saline Nasal Spray with Preservatives<br>(0.65% NaCl, Phenylcarbinol, Benzalkonium chloride) | 1% v/v | No Interference |
| Nasal Decongestant Spray<br>(Oxymetazoline HCl 0.05%, Benzalkonium chloride, phosphate) | 1% v/v | No Interference |
| Analgesic ointment (Vicks®VapoRub®) | 1% w/v | No Interference |
| Petroleum Jelly (Vaseline®) | 1% w/v | No Interference |
| Snuff (Tobacco) | 1% w/v | No Interference |
| **Disinfecting/Cleaning Substances** | | |
| Bleach | 1% and 2% v/v<br>[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 |

| Substance Tested | Concentration Tested | Result |
|---|---|---|
| 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 Influenza A (subtype) and Influenza B assays.

[b] Several analytes were not detected in samples that had been incubated 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-EZ 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<br>Medical devices - Symbols to be used with medical devices labels, labeling and information to be supplied | | | | | |
|---|---|---|---|---|---|
| 5.1.1  | Manufacturer | 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, Category 1 |  | Acute toxicity, oral, Category 4 & Skin corrosion, irritation, Category 2 |  | Acute aquatic hazard, Category 1 & Long-term aquatic hazard, Category 1 |
| **Manufacturer Symbols (BioFire Diagnostics, LLC)** | | | | | |
|  | The NOTE symbols explain how to perform the BioFire RP2.1-EZ test more efficiently. | | | | |
|  | A panel in the BioFire Respiratory Panel product family | | | | |

# APPENDIX B

## Contact and Legal Information

| Customer and Technical Support for U.S. Customers | |
|---|---|
| **Reach Us on the Web**<br>http://www.BioFireDX.com<br>**Reach Us by E-mail**<br>support@BioFireDX.com<br>**Reach Us by Mail**<br>515 Colorow Drive<br>Salt Lake City, UT 84108<br>USA | **Reach Us by Phone**<br>1-844-815-0363 – Toll Free<br>1-801-582-0636 – Utah<br>**Reach Us by Fax**<br>(801) 588-0507 |

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/

# APPENDIX C

## References


1. Biosafety in Microbiological and Biomedical Laboratories. (2009).
2. Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline M29.
3. Adams, D. A. *et al.* Summary of Notifiable Diseases - United States, 2011. *MMWR Morb. Mortal. Wkly. Rep.* **60**, 1–117 (2013).
4. *CIFOR Analysis of State Legal Authorities*. http://www.cifor.us/.
5. Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; NCCLS Approved Guideline. (2006).
6. User Protocol for Evaluation of Qualitative Test Performance; NCCLS Approved Guideline. (2008).
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 | September 2020 | Initial release. |
| 02 | December 2020 | Addition of FDA SARS-CoV-2 Reference Panel testing data to the Limit of Detection (LoD) section. |
| 03 | April 2021 | Addition of:<br>Warnings related to SARS-CoV-2 potential vaccine contamination risks.<br>Saline as an acceptable transport medium for NPS specimens<br>Limitations:<br>o The performance of this device has not been assessed in a population vaccinated against COVID-19.<br>o False positive and false negative results can be the result of a variety of sources and causes, it is important that these results be used in conjunction with other clinical, epidemiological, or laboratory information.<br>o Transport media or saline may contain non-viable organisms and/or nucleic acid at levels that can be detected by the BioFire RP2.1-EZ.<br>o There is a risk of false positive results due to non-specific amplification and cross-reactivity with organisms found in the respiratory tract. Observed and predicted cross-reactivity for BioFire RP2.1-EZ is described in the Analytical Specificity (Cross-Reactivity) section. Erroneous results due to cross-reactivity with organisms that were not evaluated or new variant sequences that emerge is also possible.<br>Data from 2020 SARS-CoV-2 clinical trial to Clinical Performance section.<br>Applicable health hazard information for the BioFire Sample Buffer.<br>Update to:<br>Reporting of Uncertain results (Table 4) to specify that the original sample should be retested once, and the result reported.<br>Clinical Performance section – reorganization of data from original BioFIre RP2 study (2016) to integrate with new data.<br>*In silico* reactivity data based on analysis of available sequences through February 21, 2021; includes specific mention of variants of concern. |
| 04 | August 2021 | Update to:<br>*In silico* reactivity data based on analysis of available sequences through July 21, 2021; includes specific mention of variants of concern.<br>Minor edits requested by FDA and to align content of RP2.1 family IFUs |
| 05 | March 2022 | Update to:<br>SARS-CoV-2 i*n silico* reactivity data based on analysis of available sequences through February 21, 2022.<br>Customer support phone number to refer to outpatient support number rather than general support number |

# INDEX

# BioFire® Respiratory Panel 2.1-EZ (RP2.1-EZ)


**For use with the BioFire® FilmArray® 2.0 EZ Configuration System**

**For Emergency Use Authorization (EUA) Only**

**For Prescription Use**

**For In Vitro Diagnostic Use**

BIOMÉRIEUX BIO FIRE®

## Syndromic Testing:
## The Right Test, The First Time.

# BioFire® Respiratory Panel 2.1-EZ (RP2.1-EZ) Quick Guide

For use with the BioFire® FilmArray® 2.0 EZ Configuration System. For Emergency Use Authorization (EUA) Only

**This product has not been FDA cleared or approved, but has been authorized for emergency use 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 requirements to perform high, moderate or waived complexity tests. This product is for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.**

**This product has been authorized only for the detection and differentiation of nucleic acid of SARS-CoV-2 from multiple respiratory viral and bacterial organisms.**

**The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics 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.**

**These instructions only apply to to the BioFire Respiratory Panel 2.1-EZ (RP2.1-EZ). Read the BioFire RP2.1-EZ Instructions for Use, including quality control procedures, before performing a test.**

**The Quick Guide (this document) is provided in paper format in the BioFire RP2.1-EZ kit. It, along with all other test related documentation, can be accessed online at the links below. Paper copies of these documents are available upon request by contacting technical support via phone, fax, email, or regular mail. A training video is provided with the BioFire® FilmArray® 2.0 System EZ Configuration instrument.**

| Document | URL with KEY-CODE |
|---|---|
| BioFire RP2.1-EZ Instructions for Use EUA | https://www.biofiredx.com/e-labeling/ITI0129 |
| BioFire RP2.1-EZ Reagent Quick EUA Guide | https://www.biofiredx.com/e-labeling/ITI0108 |
| BioFire RP2.1-EZ Reagent EUA SDS | https://www.biofiredx.com/e-labeling/ITI0142 |
| RP2.1-EZ EUA Patient Fact Sheet | https://www.biofiredx.com/e-labeling/ITIRP21EZ05 |
| RP2.1-EZ EUA Provider Fact Sheet | https://www.biofiredx.com/e-labeling/ITIRP21EZ06 |
| RP2.1-EZ EUA Authorization Letter | https://www.biofiredx.com/e-labeling/ITIRP21EZ07 |

## CONTAMINATION PRECAUTIONS

Due to the sensitive nature of the BioFire RP2.1-EZ, it is important to guard against contamination of the work area, specimen, and test by following these guidelines:

### 1. Preventing organism contamination

- Do not prepare specimen or load pouch in areas used to prepare vaccine material and/or perform viral and bacterial culture.
- Avoid collecting or handling specimens in areas that are exposed to vaccine material for pathogens detected by the BioFire RP2.1-EZ (e.g. influenza, *Bordetella pertussis*, and poliovirus (Human Rhinovirus/Enterovirus)). Vaccines may contain PCR-detectable DNA or RNA and particular care should be taken to avoid contamination of the specimen or testing areas (especially with nasal spray vaccines such as FluMist® and *B. pertussis* acellular vaccines such as Pentacel®, Daptacel®, and Adacel®). Contamination of specimens or testing materials with vaccine can cause false-positive results.
- Handle specimens and pouches one at a time.
- Change gloves and clean the work area between each patient specimen.
- Laboratory workers with active respiratory symptoms (e.g., runny nose, cough) should wear a standard surgical mask, face shield, or equivalent, and avoid touching their face while preparing specimens and/or performing the test procedure.

### 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 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 to prevent amplicon contamination:

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

**If a pouch leak or other contamination is suspected, contact BioFire technical support. Testing should be discontinued until the area has been decontaminated.**

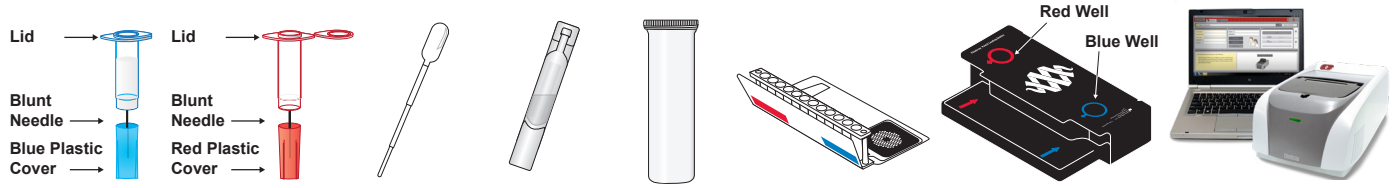
## BEFORE PREPARING A TEST SAMPLE

Watch the provided training video before performing first test.

### ⚠ Warning—to avoid contamination always wear gloves and PPE.

**Note**—clean pouch loading station and loading area with 10% bleach solution followed by a water wipe between samples and at the end of each testing day. Change gloves after cleaning.

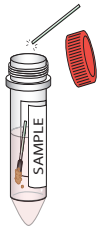
## PROVIDED MATERIALS




BioFire® FilmArray® Hydration Injection Vial | BioFire® FilmArray® Sample Injection Vial | Transfer Pipette | Sample Buffer Tube | Canister | Pouch | BioFire® FilmArray® Pouch Loading Station | BioFire 2.0 EZ Configuration

## STEP 1: COLLECT NASOPHARYNGEAL SWAB (NPS) SAMPLE

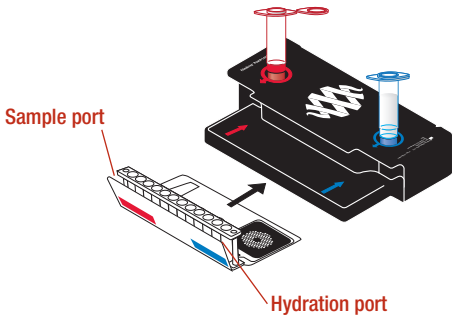
Follow Nasopharyngeal Swab Collection Quick Guide section on page 9 to collect an NPS specimen in transport media or saline.




## STEP 2: PREPARE POUCH

**Note**—ensure area has been cleaned.

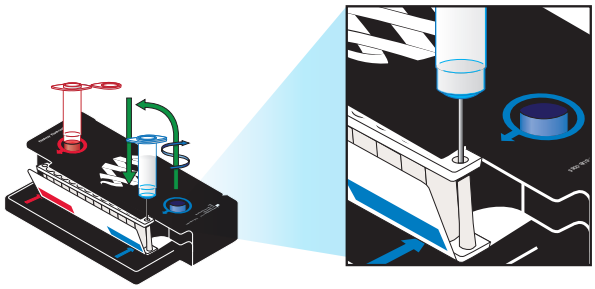
- Change gloves.
- Remove the BioFire RP2.1 pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.
- Check the expiration date on the pouch. Do not use expired pouches.
- Label the BioFire RP2.1 pouch with the patient's ID.
- Orient BioFire RP2.1 pouch as shown in the picture and insert into the Pouch Loading Station.
- Place Sample Injection Vial into red well.
- Place Hydration Injection Vial into blue well.



## STEP 3: HYDRATE POUCH

- Unscrew Hydration Injection Vial, leaving blue plastic cover in the well of pouch loading station.
- Insert the tip of the blunt needle into pouch hydration port.
- Forcefully push down all the way until you hear a popping sound to puncture internal seal with blunt needle.
- Wait 10 seconds as the hydration solution is pulled into the BioFire® RP2.1 pouch.

**Note**—some hydration solution may remain in the Hydration Injection Vial.



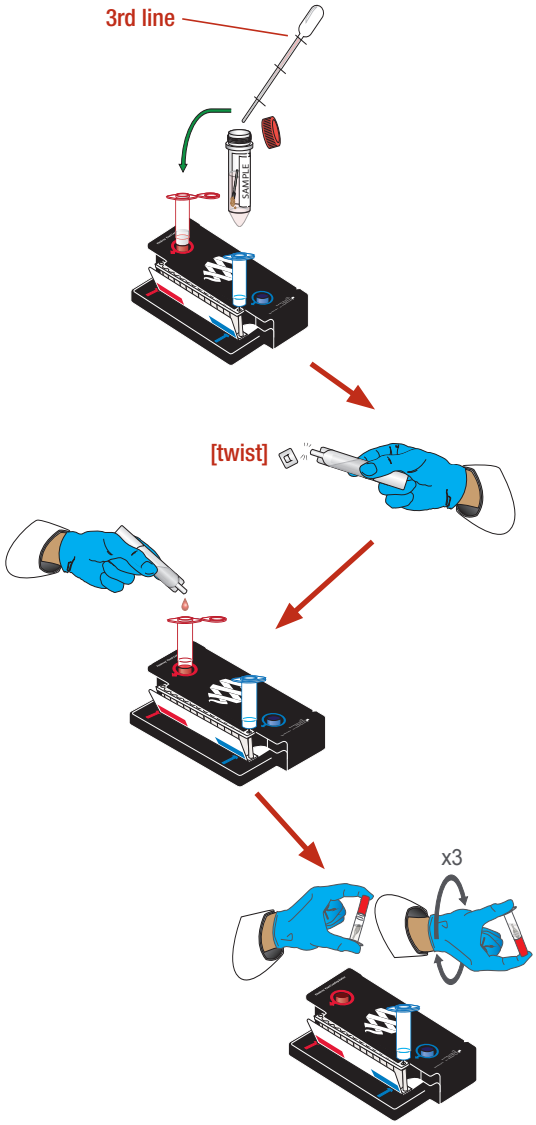
## STEP 4: PREPARE SAMPLE MIX

- Remove lid from the NPS sample tube, leaving sample swab inside.
- Transfer sample to the Sample Injection Vial using the following steps:
  - Unwrap the transfer pipette, being careful not to touch the tip.
  - Squeeze the bulb of the transfer pipette and lower it into the NPS sample tube.
  - Gently start releasing the bulb and drawing up liquid until it reaches the third line of the pipette.
  - Raise the pipette out of the sample liquid and then release the bulb fully to ensure the sample stays in the pipette.
  - Dispense the sample into the Sample Injection Vial by again squeezing the bulb of the transfer pipette.
  - Discard the transfer pipette in a suitable biohazard container.
- Add sample buffer to Sample Injection Vial:
  - Open sample buffer tube by twisting tab off of the tip.
  - Dispense full volume of sample buffer by squeezing it into the Sample Injection Vial.

**Note**—do not touch the open tip of the sample buffer tube.

- Discard sample buffer tube.
- Tightly close lid of Sample Injection Vial.
- Lift entire Sample Injection Vial out of pouch loading station and mix sample by gently inverting 3 times.
- Return Sample Injection Vial to red well.

⚠ Warning—***the sample buffer is harmful if swallowed, can cause serious eye damage and/or skin irritation.***



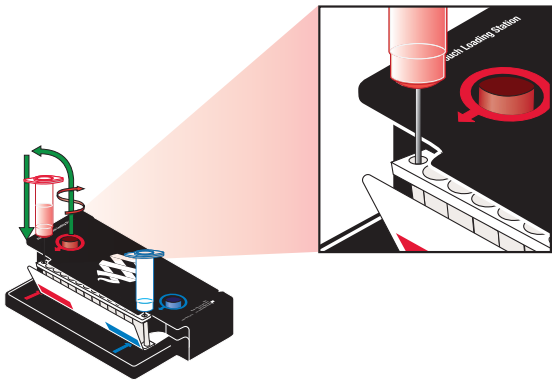
## STEP 5: LOAD SAMPLE MIX

- Unscrew Sample Injection Vial from red plastic cover and pause for 5 seconds before removing Sample Injection Vial.
- Remove Sample Injection Vial, leaving red plastic cover in the well of pouch loading station.

**Note**—do not touch the tip of blunt needle with your hands.

- Insert the tip of the blunt needle into pouch sample port.
- Forcefully push down all the way until you hear a popping sound to puncture internal seal with blunt needle.
- Wait 10 seconds as the sample mix is pulled into the BioFire® RP2.1 pouch.

**Note**—some sample mix may remain in the Sample Injection Vial.



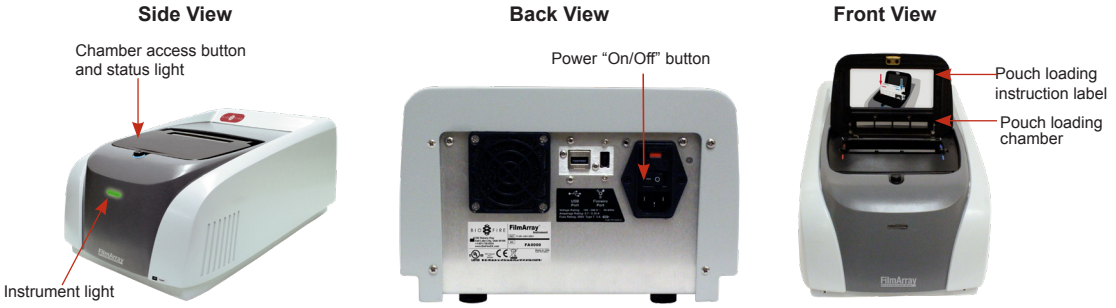
## STEP 6: COMPLETE SAMPLE PREPARATION

- Remove Hydration Injection Vial and Sample Injection Vial from the pouch. Screw each vial back into its plastic cover in the pouch loading station before disposing of the vials in a suitable biohazard container.
- Change gloves.
- Remove the pouch from the pouch loading station.
- Continue to step 7 for instructions on inserting a pouch into the BioFire instrument and starting a run.
- Run pouch immediately. If the instrument is in use, run pouch within 60 minutes of loading. The pouch should be held at room temperature, but ensure that the pouch is not exposed to temperatures above 40° C (104° F).
- Clean area before preparing next sample(s).

## BEFORE STARTING a RUN on BioFire EZ System

**Note**—refer to BioFire 2.0 EZ Configuration Operator's Manual for complete instructions.

- Ensure that the BioFire instrument is turned on.
- Check the instrument light on the front of the instrument. A solid green light indicates that the instrument is ready for use.

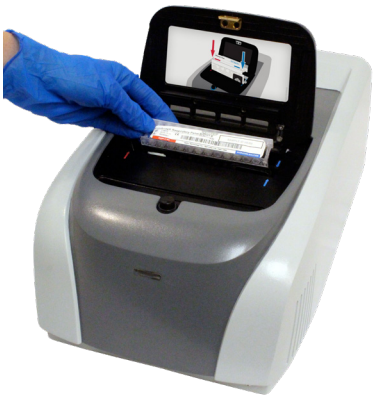



- If not already open, launch the BioFire® FilmArray® Software by double clicking on the icon on the laptop screen.

## STEP 7: INSERT POUCH

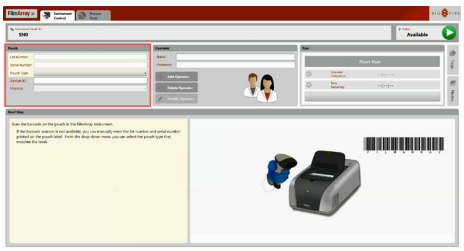
- Open instrument lid (if not already open).
- Load the pouch into the BioFire instrument as shown in the figure.
- Position pouch with black array on the right side and the film portion of the pouch entering the instrument first. The pouch will lock into place when properly inserted.
- Ensure that the red and blue labels on the pouch align with the red and blue arrows on the BioFire instrument. If inserted correctly, the bar code label is visible on the top of the pouch. There is an audible click when the BioFire pouch is securely in place.
- If the pouch is not completely in place, the instrument and software will not continue to the next step.

**Note**—The instrument lid must be opened completely. If the pouch cannot be easily inserted into the pouch chamber of the instrument, gently push the instrument lid back completely and try again.



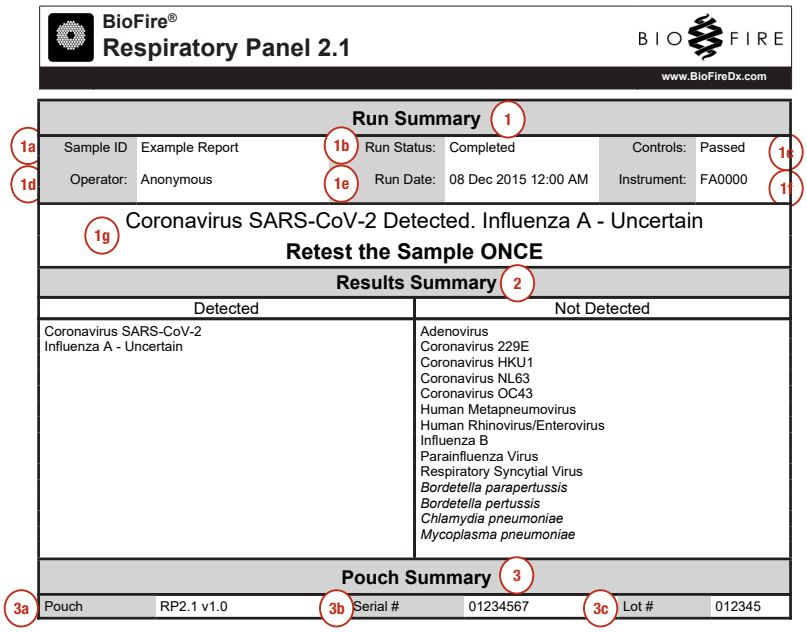
## STEP 8: COMPLETE RUN

- Follow on screen instructions on the laptop to start a run.
- A flashing blue light on the instrument indicates that the test is complete. The lid my be opened and the pouch removed.
- At the end of the run, put on gloves, remove the pouch from the instrument, and dispose in a suitable biohazard container; then, discard gloves. Removing the pouch clears all of the fields on the screen and prepares the instrument for another test.



## VIEWING RESULTS

The BioFire RP2.1-EZ Panel test report is automatically displayed upon completion of a run and contains three sections—Run Summary, Results Summary, and Pouch Summary. The test report can be saved as a file or printed. Refer to BioFire 2.0 EZ Configuration Operator's Manual for complete instructions on viewing, printing, and saving a report.



BioFire®
Respiratory Panel 2.1

BIOFIRE
www.BioFireDx.com

**Run Summary** (1)

| | | | | | |
|---|---|---|---|---|---|
| Sample ID (1a) | Example Report | Run Status: (1b) | Completed | Controls: | Passed (1c) |
| Operator: (1d) | Anonymous | Run Date: (1e) | 08 Dec 2015 12:00 AM | Instrument: | FA0000 (1f) |

Coronavirus SARS-CoV-2 Detected. Influenza A - Uncertain (1g)
**Retest the Sample ONCE**

**Results Summary** (2)

| Detected | Not Detected |
|---|---|
| Coronavirus SARS-CoV-2<br>Influenza A - Uncertain | Adenovirus<br>Coronavirus 229E<br>Coronavirus HKU1<br>Coronavirus NL63<br>Coronavirus OC43<br>Human Metapneumovirus<br>Human Rhinovirus/Enterovirus<br>Influenza B<br>Parainfluenza Virus<br>Respiratory Syncytial Virus<br>*Bordetella parapertussis*<br>*Bordetella pertussis*<br>*Chlamydia pneumoniae*<br>*Mycoplasma pneumoniae* |

**Pouch Summary** (3)

| | | | | | |
|---|---|---|---|---|---|
| Pouch (3a) | RP2.1 v1.0 | Serial # (3b) | 01234567 | Lot # (3c) | 012345 |

1. The **Run** Summary section displays
   a. **Sample ID;**
   b. **Run Status;**
   c. **Controls** results listed as
      - Pass, Fail, or Invalid;

   ( See *BioFire RP2.1-EZ Test Report* section in the BioFire Respiratory Panel 2.1-EZ (RP2.1-EZ) Instructions for Use.)

   d. Run **Operator** name;
   e. **Run Date** and time;
   f. identifying designation of the **Instrument** that was used;
   g. an overall summary of the test results that presents an overall result including
      - **Detected** with a list of detected targets;
      - **Multiple Organisms Detected**;
      - **Negative**;
      - **Uncertain** (Influenza A only); **or**
      - **Invalid**—as well as required actions (e.g., report the results or retest the sample).

2. The **Results Summary** section lists the result for each target tested by the panel. Possible results include
   - **Detected;**
   - **Not Detected;**
   - **Uncertain** (Influenza A only); or
   - **Invalid.**

   Targets that are detected are listed in the left-hand column; targets that are not detected are listed in the right-hand column. See Table 1 for detailed information about interpretation of test results and appropriate follow-up for Invalid and Uncertain results.

3. The **Pouch Summary** section provides additional information about the pouch including
   a. **Pouch** type;
   b. **Serial #**
   c. **Lot #** (number).

**Table 1. Results explanations and required actions**

| Result | Explanation | Required Action |
|---|---|---|
| Negative | The run was successfully completed AND<br>The pouch controls were successful (Passed) AND<br>The assay(s) for the organism were NEGATIVE | Report the results. |
| <Organism Name> Detected | The run was successfully completed AND<br>The pouch controls were successful (Passed) AND<br>The assay(s) for the organism were POSITIVE | Report the results. |
| Multiple Organisms Detected | The run was successfully completed AND<br>The pouch controls were successful (Passed) AND<br>The assay(s) for the organisms were POSITIVE<br>- more than one non-Influenza A organism is detected OR<br>- exactly one Influenza A subtype is detected and one or more non-influenza A organisms is detected. | Report the results.<br>Note: Detection of four or more pathogens may indicate a possible contamination event. If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result. If results are not duplicated, contact BioFire Technical Support and discontinue testing until the area has been decontaminated. |
| Influenza A—Uncertain | The run was successfully completed AND<br>The pouch controls were successful (Passed) AND<br>The combination of positive and negative assay results for Influenza A was inconclusive. | Retest the original sample ONCE and report the result of the retest.<br>If the result of the retest is again 'Uncertain', the final result should be considered 'Detected'. |
| Influenza A—No Subtype Detected | The run was successfully completed AND<br>The pouch controls were successful (Passed) AND<br>The combination of positive assay results for Influenza A was did not detect a specific subtype. | Retest the sample ONCE<br>Report the results of the Retest<br>If the retest provides the same Influenza A – No Subtype results, contact the appropriate public health authorities for confirmatory testing. |
| Influenza A—Multiple Subtypes Detected | The run was successfully completed AND<br>The pouch controls were successful (Passed) AND<br>The combination of positive assay results indicates the presence of more than one Influenza A subtype. | Multiple Influenza A infections are possible but rare. This result can be caused by a recent FluMist® nasal Influenza vaccination (see "Interference" section below).<br>Retest the Sample ONCE<br>Report the results of the Retest |
| Failed | Run completes and the controls fail OR<br>The run does not complete. | All results are invalid because the run failed. Note any error codes displayed and refer to the FilmArray® 2.0 EZ Configuration Operator's Manual for more information. If the error persists, contact Technical Support for further instruction.<br>Retest the sample and if valid, report the results of the retest. |

⚠ **Warning - Detection of four or more pathogens may indicate a possible contamination event. If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result. If results are not duplicated, contact BioFire Technical Support and discontinue testing until the area has been decontaminated.**

## QUALITY CONTROL TESTING

Recommended external quality control material is available from Maine Molecular Quality Controls, Inc., www.mmqci.com. The MMQCI control panel consists of a set of ready-to-use positive and negative control solutions for all viruses and bacteria detected by the BioFire RP21.-EZ, along with an insert explaining the expected results.

**BioFire RP2.1/RP2.1plus Control Panel M441, Part Number M441**
Negative Control Part Number M44221
Positive Control Part Number M44321

**Note—**control test solutions should be stored at -20° C or colder. Follow manufacturer's instructions—allow solutions to thaw to room temperature before testing.

It is recommended that when using the BioFire RP2.1-EZ under Emergency Use Authorization (EUA), external controls be tested at minimum:

- When receiving a new shipment of pouches
- When training a new user

Two pouches will need to be used, one for the positive control material and one for the negative control material. The negative control test should be run before the positive control test. Prior to loading, invert the tube three times to mix the material and tap the bottom of the tube on the counter three times to remove any liquid from the lid. Run each test following the same steps used for a patient test. If all analytes are detected for the positive control test, disregard instructions to retest.

If the expected results for the external control materials are not obtained, contact BioFire technical support prior to running patient samples.

### Expected negative test result

BioFire® Respiratory Panel 2.1 — BIOFIRE — www.BioFireDx.com

**Run Summary**

| | | | | | |
|---|---|---|---|---|---|
| Sample ID: | Example Report | Run Status: | Completed | Controls: | Passed |
| Operator: | Anonymous | Run Date: | 08 Dec 2015 12:00 AM | Instrument: | 2FA00000 |

Negative
**Report the Results**

**Results Summary**

| Detected | Not Detected |
|---|---|
| | Adenovirus<br>Coronavirus 229E<br>Coronavirus HKU1<br>Coronavirus NL63<br>Coronavirus OC43<br>Coronavirus SARS-CoV-2<br>Human Metapneumovirus<br>Human Rhinovirus/Enterovirus<br>Influenza A<br>Influenza B<br>Parainfluenza Virus<br>Respiratory Syncytial Virus<br>*Bordetella parapertussis*<br>*Bordetella pertussis*<br>*Chlamydia pneumoniae*<br>*Mycoplasma pneumoniae* |

**Pouch Summary**

| Pouch | RP2.1 v1.0 | Serial # | 01234567 | Lot # | 012345 |
|---|---|---|---|---|---|

### Expected positive test result

BioFire® Respiratory Panel 2.1 — BIOFIRE — www.BioFireDx.com

**Run Summary**

| | | | | | |
|---|---|---|---|---|---|
| Sample ID: | Example Report | Run Status: | Completed | Controls: | Passed |
| Operator: | Anonymous | Run Date: | 08 Dec 2015 12:00 AM | Instrument: | 2FA00000 |

Multiple Organisms Detected. Influenza A - Multiple Subtypes Detected
**Retest the Sample ONCE**

**Results Summary**

| Detected | Not Detected |
|---|---|
| Adenovirus<br>Coronavirus 229E<br>Coronavirus HKU1<br>Coronavirus NL63<br>Coronavirus OC43<br>Coronavirus SARS-CoV-2<br>Human Metapneumovirus<br>Human Rhinovirus/Enterovirus<br>Influenza A H1-2009<br>Influenza A H3<br>Influenza B<br>Parainfluenza Virus<br>Respiratory Syncytial Virus<br>*Bordetella parapertussis*<br>*Bordetella pertussis*<br>*Chlamydia pneumoniae*<br>*Mycoplasma pneumoniae* | |

**Pouch Summary**

| Pouch | RP2.1 v1.0 | Serial # | 01234567 | Lot # | 012345 |
|---|---|---|---|---|---|

Note—Other commercial external control materials may be available and appropriate for use with the BioFire RP2.1-EZ. Use in accordance with the manufacturers' instructions and appropriate accrediting organization requirements, as applicable.

Note—Some commercial control materials for Coronavirus SARS-CoV-2 are not compatible with the BioFire RP2.1-EZ test.

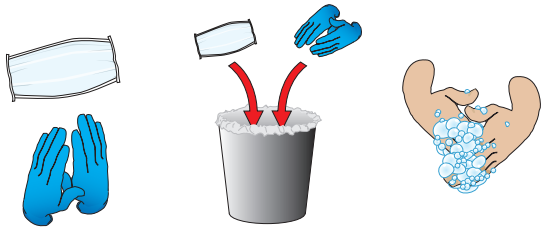
## NASOPHARYNGEAL SWAB COLLECTION QUICK GUIDE

Transport media, saline, and swabs are not provided as part of the BioFire RP2.1-EZ kit. BioFire recommends the following or an equivalent.

- Universal transport media or saline, 3 mL
- Flexible nasopharyngeal flocked swab, Nylon® tip

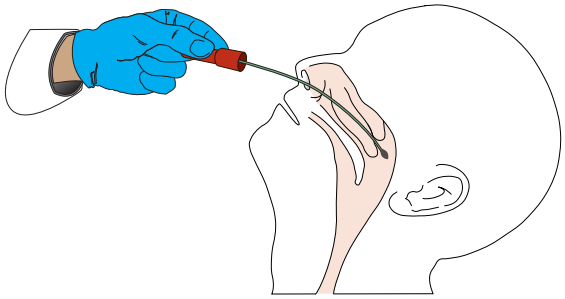
## STEP 1: USE INFECTION-CONTROL PRECAUTIONS

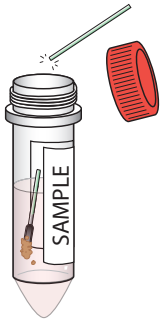
- Wear personal protective equipment (PPE)—a surgical mask and disposable gloves.
- When completed, dispose of all PPE and other contaminated materials in the trash.
- Wash hands thoroughly with soap and water or alcohol based hand gel before and after the procedure.



## STEP 2: COLLECT NASOPHARYNGEAL SWAB

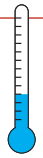
- If the patient is wearing a surgical mask remove the mask to perform the procedure and replace when done.
- Use a flexible fine-shafted swab with scored break lines and a polyester tip (DACRON®, Nylon®, or rayon, not cotton or calcium alginate).
- The distance from the patient's nose to the ear gives an estimate of the distance the swab should be inserted.
- If possible, instruct the patient to sit with their head against a wall; an adult may hold a small child with their head resting against the adult's chest.
- Insert swab into one nostril straight back, not upwards, to the nasopharynx and leave in place for a few seconds.
- Slowly withdraw swab with a rotating motion.
- Place tip of the swab into a vial containing 3mL of transport media or saline and break the shaft.
  Discard the shaft in a suitable biohazard container, leaving the tip of the swab in the tube.
- Prepare sample for use or storage keeping swab tip in vial. No further manipulation is required.






## STEP 3: STORAGE

- NPS specimen(s) can be kept refrigerated at 4° C for up to 72 hours.



4° C for up to 72 hr.

## SERVICE AND SUPPORT

Contact BioFire technical support for specific guidance to complete a BioFire RP2.1-EZ test..


**Email**
support@biofiredx.com

**Phone**
1-844-815-0363 (toll free)
1-801-582-0636 (in Utah)





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