BioFire® Respiratory Panel 2.1 (RP2.1)

IVD

Rx Only

Instructions for Use	https://www.biofiredx.com/e-labeling/ITI0105
Quick Guide	https://www.biofiredx.com/e-labeling/ITI0111
Safety Data Sheet (SDS)	https://www.biofiredx.com/e-labeling/ITI0119
Pouch Module Software	https://www.biofiredx.com/e-labeling/ITIFA20RP2110

Customer and Technical Support Information *For more information on how to contact Customer and Technical Support, refer to Appendix B.	Contact the local bioMérieux sales representative or an authorized distributor.

INTENDED PURPOSE

Intended Use

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

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

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

Nucleic acids from the respiratory viral and bacterial organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection is indicative of the presence of the identified

microorganism and aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

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

Intended User and Use Environment

The BioFire RP2.1 is intended for use by trained medical and laboratory professionals in a laboratory setting or under the supervision of a trained laboratory professional.

SUMMARY AND EXPLANATION OF THE TEST

Respiratory pathogens cause acute local and systemic disease, with the most severe cases occurring in children, the elderly, and immunocompromised individuals. Respiratory symptoms can include coughing, nasal discharge, congestion, fever, wheezing, shortness of breath, headache, and myalgia. Due to the similarity of diseases caused by many viruses and bacteria, diagnosis based on clinical symptoms alone is difficult. Identification of potential causative agents provides data to aid the physician in determining appropriate patient treatment and public health response for disease containment. The BioFire RP2.1 is a real-time, nested multiplexed polymerase chain reaction test designed to simultaneously identify nucleic acids from 22 different viruses and bacteria associated with respiratory tract infection from a single nasopharyngeal swab (NPS) specimen.

Summary of Detected Organisms

Adenoviruses (AdV) are a diverse group of non-enveloped DNA viruses with seven species (A to G).[1] Adenovirus species B, C, and E cause acute respiratory disease, but all types have been associated with human disease.[2] Other Adenovirus species (A, D, F and G) can cause a variety of illnesses, including cystitis, gastroenteritis, and conjunctivitis [3], and may also be found in respiratory specimens. Outbreaks often occur in institutional settings such as military training, long-term care facilities, and pediatric tertiary-care hospitals, due to high rates of transmission in closed populations.[4–6] Adenoviruses are shed for long periods of time and persist on surfaces in an infective state.[6]

Coronaviruses (CoV) - Human coronaviruses were established as respiratory pathogens in the 1960s and seven serological variants associated with human disease have been characterized to date: four types (**coronaviruses 229E, OC43, HKU1, NL63**) that regularly circulate in human populations, constituting about 15% of common colds[7,8], and three strains (Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), and **Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)**) that have spread from animal to human populations since 2002[8] and represent the ongoing public health threat posed by emerging zoonotic pathogens. SARS-CoV was declared contained by the WHO in 2003, less than 12 months after its emergence, and no new cases have been reported since 2004. MERS-CoV, which was first described in 2012,[9] continues to cause occasional outbreaks, which are characterized by animal to human transmissions followed by person-to-person transmission.[10] SARS-CoV-2 is the novel coronavirus that causes COVID-19, an illness that reached a level of pandemic spread in the short time since its emergence in late 2019.[11,12] Coronaviruses have been linked to croup and exacerbation of asthma.[13,14] Infections with coronavirus 229E, OC43, HKU1, and NL63 occur more often in the winter, and there appears to be a periodicity of circulation.[15] Illnesses caused by these coronaviruses are generally self-limiting.[16] Though coronaviruses as a group are most commonly associated with upper respiratory tract infections; all human coronaviruses are also associated with lower respiratory tract infection and MERS-CoV, SARS-CoV, and SARS-CoV-2 can cause Acute Respiratory Distress Syndrome (ARDS), as well as significant rates of hospitalization, complications, and death, especially in patients with underlying health conditions.[17]

Note: MERS-CoV and SARS-CoV are not detected by the BioFire RP2.1.

Human Metapneumovirus (hMPV) is in the family *Paramyxoviridae*.[18] HMPV was discovered in 2001 as a respiratory pathogen in children.[19] Further studies confirmed hMPV infections in persons of all ages.[20] The two genotypes, A and B, can circulate at the same time and do not appear to differ in the severity of illness.[18] HMPV is the second leading cause of bronchiolitis in young children.[18] Additionally, infection can result in a broad range of upper and lower respiratory symptoms: cough, rhinorrhea, wheeze, dyspnea, and fever.[21] HMPV is estimated to be responsible for 5-7% of respiratory tract infections in children and 3% among individuals of all ages.[21] The seasonal peak of hMPV is winter and early spring and often co-occurs with the seasonal peak of Respiratory Syncytial Virus (RSV).[22]

Influenza A and B are RNA viruses in the *Orthomyxoviridae* family. During annual influenza epidemics, 5-20% of the population is affected with upper respiratory tract infections with rapid onset of fever.[23] The dominant type of influenza virus varies often due to antigenic drift and shift.[24] Influenza A can be subtyped by the hemagglutinin (H) and neuraminidase (N) genes; influenza A subtypes **H1**N1 and **H3**N2 are the strains that most commonly infect humans. More severe disease and increased mortality are associated with H3N2 subtype.[24] During the 2009-10 Influenza season, influenza A (H1N1)pdm09

(H1- 2009,also known as “swine flu”) became the dominant circulating influenza virus, accounting for approximately 99% of reported influenza infections and has since replaced pre-2009 H1N1 strains (Table 1).[25] Currently, at least four antiviral medications are available for influenza treatment – amantadine, rimantadine, zanamivir and oseltamivir – with type-specific efficacy and drug resistance arising with the spread of new strains of the virus.[26] Complications with viral or bacterial pneumonia increase mortality from influenza infections.[27]

Table 1. Proportions of Influenza Subtype Infections in the United States (as reported by the US Centers for Disease Control)

Flu Season[1]	Influenza A	% of Subtyped Influenza A			Influenza B
		H1	H1-2009	H3	
2020-2021[2,3]	53.8%	0.0	47.1	52.9	46.2%
2019-2020	58.7%	0.0	92.8	7.2	41.3%
2018-2019	94.3%	0.0	55.5	44.5	5.7%
2017-2018	71.0%	0.0	15.5	84.5	29.0%
2016-2017[4]	77.1%	0.0	3.1	96.9	22.9%
2015-2016[4]	70.3%	0.0	78.4	21.6	29.7%
2014-2015	83.1%	0.0	0.5	99.5	16.9%
2013-2014	85.2%	0.0	87.3	12.7	14.8%
2012-2013	70.4%	0.0	5.2	94.8	29.6%

[1] CDC FluView data accessed on December 16, 2020.

[2] Cumulative results through December 5, 2020.

[3] Season during which BioFire RP2.1 prospective clinical data described in this submission were accumulated.

[4] Season during which BioFire RP2 prospective clinical data described in this submission were accumulated.

Parainfluenza Viruses (PIVs) are RNA viruses in the *Paramyxoviridae* family. In the 1950s, parainfluenza viruses were determined to be respiratory pathogens different from influenza viruses.[28] Parainfluenza viruses are divided into four types (**parainfluenza viruses 1, 2, 3, and 4**). Parainfluenza virus 1 causes biennial epidemics in the fall, with 50% of croup cases attributed to this virus.[28] Parainfluenza virus 2 causes of epidemics every one to two years, which may alternate with parainfluenza virus 1 circulation.[28] Children less than six months old are particularly susceptible to parainfluenza virus 3 infection, with outbreaks occurring in neonatal intensive care units. PIV3 is associated with the highest mortality and morbidity of all strains[29] and epidemics are most common in the spring and summer.[28] Parainfluenza virus 4 infection affects all age groups but because of infrequent detection periodicity of infection has not been established.[30,31]

Respiratory Syncytial Virus (RSV) is a member of the RNA viruses in the *Paramyxoviridae* family, related to human metapneumoviruses and parainfluenza viruses.[32] RSV has two major subtypes (A and B), which vary annually in their prevalence.[33] RSV is the most common cause of severe respiratory disease in infants, with acute bronchiolitis as the major cause of hospitalization.[32] RSV is now also recognized as an important pathogen in adults, although adult infections are in general less severe and limited to the upper respiratory tract.[34] Peak activity of RSV is typically in January and February.[35]

Rhinoviruses and **Enteroviruses** are related RNA viruses in the *Picornaviridae* family.[36] There are more than 100 serotypes of human rhinovirus based on the serology of the capsid protein.[36] Rhinovirus is noted as causing the “common cold”, but may also be involved in precipitating asthma attacks and severe complications.[36] Enteroviruses are divided into four species that include a total of at least 89 distinct types. Individual types can be associated with different clinical manifestations, including nonspecific respiratory illnesses in infants or adults.[37] Both rhinoviruses and enterovirus are prevalent year round.[38,39]

Bordetella pertussis, a gram-negative bacterium, is the predominant causative agent of whooping cough or pertussis, a vaccine-preventable, highly infectious disease that is reportable to public health organizations.[40–42] Pertussis occurs most commonly in children but also occurs in adolescents and adults and outbreaks have been documented in fully vaccinated populations due to waning immunity (immunity has been shown to decrease 5-10 years after vaccination).[42,43] Early (catarrhal) pertussis disease is non-specific, and classic signs of pertussis (paroxysmal coughing, inspiratory ‘whoop’, post-tussive emesis, as well as apnea or cyanosis in infants) do not arise until approximately two weeks after the initial onset of symptoms. ***Bordetella parapertussis*** is known to cause a milder pertussis-like disease.[42] No peak season has been defined for *Bordetella* infections.

Chlamydia pneumoniae (previously known as *Chlamydophila pneumoniae*) is an obligate intracellular bacterium that causes acute respiratory infections and is a common cause of community-acquired atypical (walking) pneumonia and bronchitis.[44–46] *C. pneumoniae* has an incubation period of approximately three weeks and can be transmitted from asymptomatic carriers.[46] Outbreaks occur in schools, military barracks, and nursing homes.[47] No peak season has been identified for *C. pneumoniae* infections.

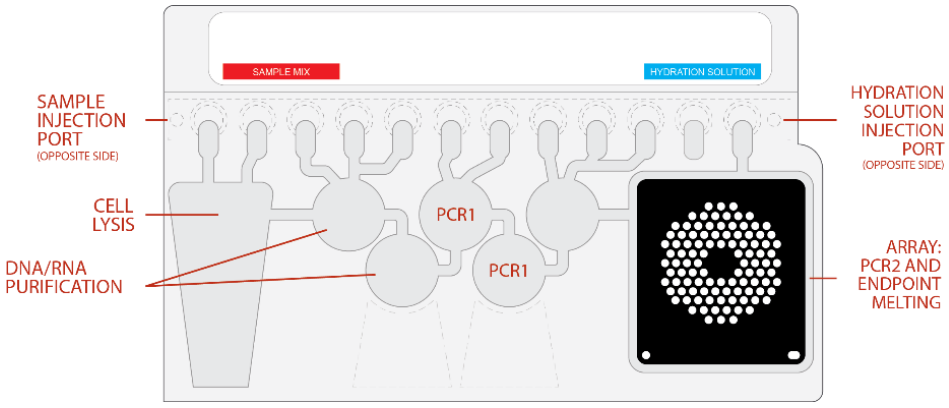
Mycoplasma pneumoniae is another bacterial agent of community-acquired atypical pneumonia, occurring frequently in outbreak situations.[48,49] Incubation time for *M. pneumoniae* infection is approximately 1 to 4 weeks.[50] *M. pneumoniae* respiratory disease does not have a defined season of highest incidence but epidemics have a periodicity of 3-7 years.[49]

PRINCIPLE OF THE PROCEDURE

The BioFire RP2.1 pouch is a closed system disposable that stores all the necessary reagents for sample preparation, reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from multiple respiratory pathogens within a single NPS specimen. After sample collection, the user injects hydration solution and sample combined with BioFire® FilmArray® Sample Buffer into the pouch, places the pouch into a BioFire® FilmArray® System instrument module, and starts a run. The entire run process takes about 45 minutes. Additional detail can be found in the appropriate BioFire System Operator's Manual.

During a run, the BioFire 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# 423742):

- Individually packaged BioFire RP2.1 pouches
- Single-use (1.0 mL) Sample Buffer ampoules
- Single-use pre-filled (1.5 mL) BioFire® FilmArray® Hydration Injection Vials (blue)
- Single-use BioFire® FilmArray® Sample Injection Vials (red)
- Individually packaged Transfer Pipettes
- BioFire RP2.1 Pouch Module Software
  This software is required to run the BioFire RP2.1 and can be downloaded at https://www.biofiredx.com/e-labeling/ITIFA20RP2110 if not already installed on the BioFire 2.0 or BioFire Torch Systems.

MATERIALS REQUIRED BUT NOT PROVIDED

- BioFire System including:
  - BioFire2.0 or BioFire Torch Systems including accompanying system-specific core software
  - BioFire® FilmArray® Pouch Loading Station
- 10% bleach solution or a similar disinfectant

WARNINGS AND PRECAUTIONS

General Precautions

1. A trained healthcare professional should carefully interpret the results from the BioFire RP2.1 in conjunction with a patient's signs and symptoms, results from other diagnostic tests, and relevant epidemiological information.
2. BioFire RP2.1 pouches are only for use with BioFire 2.0 and BioFire Torch Systems.
3. Always check the expiration date on the pouch. Do not use a pouch after its expiration date.
4. BioFire RP2.1 pouches are stored under vacuum in individually wrapped canisters. To preserve the integrity of the pouch vacuum for proper operation, be sure that an instrument/module will be available and operational before unwrapping any pouches for loading.

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*[52]
   - CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*[53]

- 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:

The following statements apply.

- Health Hazards
  - Acute Toxicity, oral (Category 4)
    - H302 – Harmful if swallowed.
  - Skin corrosion/irritation (Category 2)
    - H315 - Causes skin irritation.
  - Serious eye damage/eye irritation (Category 1)
    - H318 - Causes serious eye damage.
- Environment Hazards
  - Hazardous to the aquatic environment, acute aquatic hazard (Category 1)
    - H400 - Very toxic to aquatic life.
  - Hazardous to the aquatic environment, long-term aquatic hazard (Category 1)
    - H410 - Very toxic to aquatic life with long lasting effects.
- Precautionary Statements
  - Prevention
    - P273 – Avoid release to the environment.
    - P280 – Wear protective gloves/protective clothing/eye protections/face protection.
  - 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 Safety Data Sheet (SDS) for more information: https://www.biofiredx.com/e-labeling/ITI0119.

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

- Laboratory personnel may carry or shed common respiratory pathogens asymptomatically and can inadvertently contaminate the specimen while it is being processed. Careful adherence to the sample processing steps described in this document is recommended to avoid possible contamination. Samples should be processed in a clean biosafety cabinet if available, or according to local laboratory guidelines. If a biosafety cabinet is not used, a dead air box (e.g., AirClean PCR workstation), a splash shield (e.g., Bel-Art Scienceware Splash Shields), or a face shield can be used when preparing samples instead.
- Laboratory personnel with active respiratory symptoms (runny nose, cough) should wear a standard surgical mask (or equivalent) and should avoid touching the mask while handling specimens.
- It is recommended to avoid handling specimens or pouches in an area used to routinely process respiratory pathogen culture, and/or immunofluorescence testing, 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 (*e.g.* influenza, poliovirus, SARS-CoV-2, and *Bordetella pertussis*), if possible. Particular care should be taken during these processes to avoid contamination. Some *B. pertussis* acellular vaccines (i.e. Pentacel®, Daptacel®, and Adacel®) contain PCR-detectable DNA. Contamination of specimens or testing materials with vaccine can cause false-positive *B. pertussis* results (http://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-pcr-bestpractices.html).

2. **Preventing amplicon contamination**

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

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

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

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

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

   The presence of non-viable organisms and/or nucleic acids in transport media 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. [51,52] Additionally, the Centers for Disease Control and Prevention (CDC) recommends that when pathogens from reportable diseases are detected by a culture independent diagnostic test (CIDT), the laboratory should facilitate obtaining the isolate or clinical materials for submission to the appropriate public health laboratory to aid in outbreak detection and epidemiological investigations. Laboratories are responsible for following their state and/or local regulations and should consult their local and/or state public health laboratories for isolate and/or clinical sample submission guidelines.

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

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

REAGENT STORAGE, HANDLING, AND STABILITY

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

SAMPLE REQUIREMENTS

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

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

[a] Frozen storage for up to 30 days was evaluated for this sample type. However, longer frozen storage 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

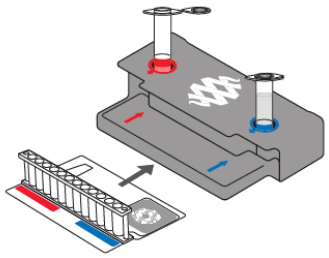
Use clean gloves and other Personal Protective Equipment (PPE) when handling pouches and samples. Only prepare one BioFire RP2.1 pouch at a time and change gloves between samples and pouches. Once sample is added to the pouch, promptly transfer to the instrument to start the run. After the run is complete,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

Step 1: Prepare Pouch

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

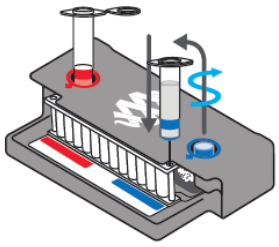
NOTE: The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.

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



Step 2: Hydrate Pouch

1. Unscrew the Hydration Injection Vial from the blue cap.
2. Remove the Hydration Injection Vial, leaving the blue cap in the BioFire Pouch Loading Station.
3. Insert the Hydration Injection Vial's cannula tip into the pouch hydration port located directly below the blue arrow of the Pouch Loading Station.
4. Forcefully push down in a firm and quick motion to puncture seal until a faint "pop" is heard and there is an ease in resistance. Wait as the correct volume of Hydration Solution is pulled into the pouch by vacuum.

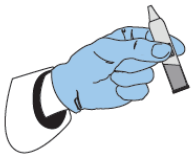


   - If the hydration solution is not automatically drawn into the pouch, repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is again not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.

5. Verify that the pouch has been hydrated.
   - Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen.
   - If the pouch fails to hydrate (dry reagents appear as white pellets), repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is still not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.

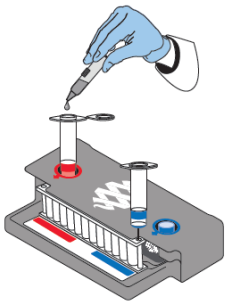
Step 3: Prepare Sample Mix

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



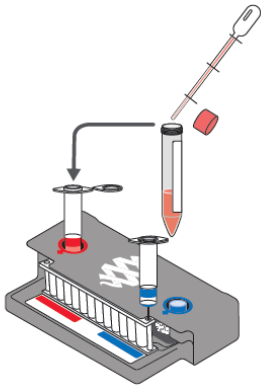
NOTE: Avoid touching the ampoule tip during handling, as this may introduce contamination.

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

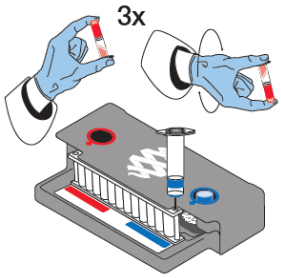


NOTE: Avoid squeezing the ampoule additional times. This will generate foaming, which should be avoided.

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



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

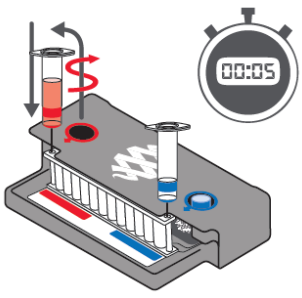


NOTE: DO NOT use the Transfer Pipette to mix the sample once it is loaded into the Sample Injection Vial.

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

Step 4: Load Sample Mix

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



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

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

- Flip the barcode label down and check to see that fluid has entered the reagent well next to the sample loading port.
- If the pouch fails to pull sample from the Sample Injection Vial, the pouch should be discarded. Retrieve a new pouch and repeat from *Step 1: Prepare Pouch*.

5. Discard the Sample Injection Vial and the Hydration Injection Vial in appropriate biohazard sharps container.
6. Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the FilmArray Pouch Loading Station.

Step 5: Run Pouch

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

BioFire 2.0

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

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

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

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

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

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

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

8. When the run is finished, follow the on-screen instructions to remove the pouch, then immediately discard it in a biohazard waste container.
9. The run file is automatically saved in the BioFire Software database, and the test report can be viewed, printed, and/or saved as a PDF file.

BioFire Torch

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

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

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

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

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

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

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

9. At the end of the run, remove the partially ejected pouch, then immediately discard it in a biohazard waste container.
10. The run file is automatically saved in the BioFire Software 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 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, the sample should be retested using a new pouch.

Monitoring Test System Performance

The software will automatically fail the run if the melting temperature (Tm) for either the RNA Process Control or the PCR2 Control is outside of an acceptable range (80.3-84.3°C for the RNA Process Control and 73.8-77.8°C for the PCR2 Control). If required by local, state, or accrediting organization quality control requirements, users can monitor the system by trending Tm values for the control assays and maintaining records according to standard laboratory quality control practices.[56,57] Refer to the appropriate BioFire System Operator's Manual for instructions on obtaining control assay Tm values. The PCR2 Control is used in several BioFire pouch types and can, therefore, be used to monitor the system when multiple pouch types are used on the same BioFire System.

External Controls

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

NOTE: Contamination can cause unexpected positive results in negative or positive external controls. If unexpected positive results are observed, thoroughly clean and decontaminate the work space and contact customer support if the unexpected results persist.

INTERPRETATION OF RESULTS

Assay Interpretation

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

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

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

Organism Interpretation

For most organisms detected by the BioFire RP2.1, the organism is reported as Detected if a single corresponding assay is positive. For example, Human Metapneumovirus will have a test report result of Human Metapneumovirus Detected if the hMPV assay is positive (at least two of the three hMPV assay wells on the array have similar positive melt peaks with Tm values that are within the assay-specific Tm range). The test results for Adenovirus, and Influenza A, and SARS-CoV-2 depend on the interpretation of results from more than one assay. Interpretation and actions for the multi-assay results are provided below.

Adenovirus

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

Influenza A

The assays in the BioFire RP2.1 are designed to both detect Influenza A and to differentiate the common hemagglutinin subtypes. To accomplish this, the BioFire RP2.1 uses two Influenza A assays, (FluA-pan-1 and FluA-pan-2) and three subtyping assays directed at the hemagglutinin gene (FluA-H1-2, FluA-H1-2009, and FluA-H3). Each of the individual assays is interpreted independently (as described above) and the test result reported for Influenza A is based on the combined results of the five assays as outlined in Table 2. An Influenza A (or subtyping) Equivocal result could occur when the titer of the virus in the specimen is low and not detected by one or more required assays (Table 2). An Influenza A Equivocal result could also indicate the presence of an atypical Influenza A subtype (e.g. avian H7N9 or H5N1 types), or a novel Influenza A strain. Specimens with an Equivocal result or multiple Influenza A subtypes detected should be retested once.

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

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

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

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

Influenza A (no subtype detected)

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

SARS-CoV-2

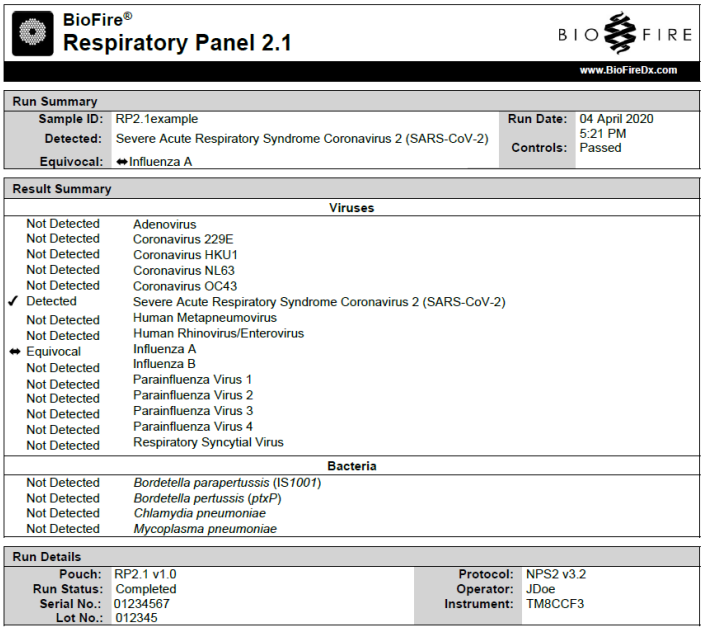
The BioFire RP2.1 pouch contains two different assays for the detection of the SARS-CoV-2. The target of each assay is shown in Table 3 below. The BioFire Software interprets each assay independently and if either one or both of the assays is positive, the test report will show Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) as Detected. If both assays are negative, the test report result will be Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Not Detected.

Table 3. Gene Targets for SARS-CoV-2 Assays on the BioFire RP2.1

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

BioFire RP2.1 Test Report

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



BioFire® Respiratory Panel 2.1

www.BioFireDx.com

Run Summary

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

Result Summary

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

Run Details

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

Run Summary

The Run Summary section of the test report provides the Sample ID, time and date of the run, control results and an overall summary of the test results. Any organism with a Detected result will be listed in the corresponding field of the summary. If all of the organism assays were negative then 'None' will be displayed in the Detected field. Controls are listed as Passed, Failed, or Invalid. Table 4 provides additional information for each of the possible control field results.

Table 4. Interpretation of Controls Field on the BioFire RP2.1 Test Report

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

Result Summary

The Result Summary section of the test report lists the result for each target tested by the panel. Possible results for each organism are Detected, Not Detected, or Invalid (Equivocal is also a possible result for Influenza A and its subtypes). Table 5 provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

Table 5. Reporting of Results and Required Actions

Result	Explanation	Action
Detected[a]	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism were POSITIVE (i.e., met the requirements for a positive result described in the Assay Interpretation section above)	Report results.
Not Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism were NEGATIVE (i.e., did not meet the requirements for a positive result described in the Assay Interpretation section above)	Report results.
Equivocal	The run was successfully completed AND The pouch controls were successful (Passed) AND The combination of positive and negative assay results for Influenza A were inconclusive (see Table 2)	Retest the original sample ONCE and report the result of the retest[b].
Invalid	The pouch controls were not successful (Failed) OR The run was not successful (Run Status displayed as: Aborted, Incomplete, Instrument Error or Software Error)	See Table 4 , Interpretation of Control Field on the FilmArray Test Report for instruction.

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

[b] Equivocal results can occur when the titer of the virus in the specimen is low (below LoD). Equivocal 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 Equivocal or Influenza A (no subtype detected) results.

Run Details

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

Change Summary

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

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

LIMITATIONS

1. For prescription use only.
2. BioFire RP2.1 performance has only been established on the BioFire 2.0 and BioFire Torch Systems.
3. The BioFire RP2.1 is a qualitative test and does not provide a quantitative value for the organism(s) 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 performance of the BioFire RP2.1 has been evaluated for use with human specimen material only.
6. The BioFire RP2.1 has not been validated for testing of specimens other than nasopharyngeal swab (NPS) specimens in transport medium.
7. The performance of BioFire RP2.1 has not been established for specimens collected from individuals without signs or symptoms of respiratory infection.
8. The performance of the BioFire RP2.1 has not been specifically evaluated for NPS specimens from immunocompromised individuals.
9. The performance of this device has not been assessed in a population vaccinated against COVID-19.
10. The effect of antibiotic treatment on test performance has not been evaluated.
11. The performance of the BioFire RP2.1 has not been established with potentially interfering medications for the treatment of influenza or cold viruses. The effect of interfering substances has only been evaluated for those listed in the Interference section. Interference from substances that were not evaluated could lead to erroneous results.
12. The performance of the BioFire RP2.1 has not been established for monitoring treatment of infection with any of the panel organisms.
13. The performance of BioFire RP2.1 has not been established for screening of blood or blood products.
14. 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.
15. 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.
16. A negative BioFire RP2.1 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.
17. If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.
18. 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.
19. 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.

20. 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.
21. 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.
22. The BioFire RP2.1 influenza A subtyping assays target the influenza A hemagglutinin (H) gene only. The BioFire RP2.1 does not detect or differentiate the influenza A neuraminidase (N) subtypes.
23. The BioFire RP2.1 may not be able to distinguish between existing viral strains and new variants as they emerge. For example, the BioFire RP2.1 can detect influenza A H3N2v (first recognized in August, 2011), but will not be able to distinguish this variant from influenza A H3N2 seasonal. If variant virus infection is suspected, clinicians should contact their state or local health department to arrange specimen transport and request a timely diagnosis at a state public health laboratory.
24. Recent administration of nasal vaccines (e.g. FluMist) prior to NPS specimen collection could lead to accurate virus detection by the BioFire RP2.1 of the viruses contained in the vaccine, but would not represent infection by those agents.
25. Due to the genetic similarity between Human Rhinovirus and Enterovirus, the BioFire RP2.1 cannot reliably differentiate them. A BioFire RP2.1 Rhinovirus/Enterovirus Detected result should be followed-up using an alternate method (e.g. cell culture or sequence analysis) if differentiation between the viruses is required.
26. BioFire RP2.1 detects a single-copy Pertussis Toxin promoter target (*ptxP*, present at one copy per cell) in *B. pertussis*. Other PCR tests for *B. pertussis* target the multi-copy IS*481* insertion sequence (present in both *B. pertussis* and *B. holmesii*) and are therefore capable of detecting lower levels of *B. pertussis* (i.e. more sensitive).
    - The BioFire RP2.1 should not be used if *B. pertussis* infection is specifically suspected; a *B. pertussis* molecular test that is FDA-cleared for use on patients suspected of having a respiratory tract infection attributable to *B. pertussis* only should be used instead.
    - Due to lower sensitivity, the BioFire RP2.1 *B. pertussis* assay is less susceptible than IS*481* assays to the detection of very low levels of contaminating *B. pertussis* vaccine material. However, care must always be taken to avoid contamination of specimens with vaccine material as higher levels may still lead to false positive results with the BioFire RP2.1 test (see contamination prevention guidelines).
    - The IS*481* sequence is also present in *B. holmesii* and to a lesser extent in *B. bronchiseptica*, whereas the BioFire RP2.1 assay (*ptxP*) was designed to be specific for *B. pertussis*. However, the BioFire RP2.1 *Bordetella pertussis* (*ptxP*) assay can also amplify pertussis toxin pseudogene sequences when present in *B. bronchiseptica* and *B. parapertussis*. Cross-reactivity was observed only at high concentration (e.g. ≥1.2E+09 CFU/mL).
27. 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.
28. Transport media may contain non-viable organisms and/or nucleic acid at levels that can be detected by the BioFire RP2.1.
29. 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 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.

30. Primers for both BioFire RP2.1 SARS-CoV-2 assays share substantial sequence homology with the Bat coronavirus RaTG13 (accession: MN996532) and cross-reactivity with this closely-related viral sequence is predicted. In addition, the SARSCoV2-2 assay may cross-react with Pangolin coronavirus (accession: MT084071) and two other bat SARS-like coronavirus sequences (accession MG772933 and MG772934). It is unlikely that these viruses would be found in a human clinical nasopharyngeal swab; but if present, the cross-reactive product(s) produced by the BioFire RP2.1 will be detected as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (see Analytical Specificity (Cross-Reactivity) section).
31. Some strains of *B. bronchiseptica* (rarely isolated from humans) do carry IS*1001* insertion sequences identical to those carried by most strains of *B. parapertussis*. These sequences will be amplified by the IS1001 assay and reported by BioFire RP2.1 as *Bordetella parapertussis* (IS*1001*).
32. The BioFire RP2.1 Human Rhinovirus/Enterovirus assay may amplify off-target sequences found in strains of *B. pertussis, B. bronchiseptica* and *B. parapertussis*. Cross-reactivity with *B. pertussis* was observed at a concentration of ≥4.5E+07 CFU/mL.

BIOFIRE RP2.1 AND BIOFIRE RP2

The BioFire RP2.1 (Ref #: 423742) was developed by adding the reagents required to detect the SARS-CoV-2 targets into the existing BioFire® FilmArray® Respiratory Panel 2 (RP2) (Ref #: RFIT-ASY-0129, RFIT-ASY-0130). Assays for all analytes shared between the two panels and reaction conditions of the test were unchanged from BioFire RP2. Studies were performed to demonstrate the performance of the new SARS-CoV-2 assays and to demonstrate that the performance characteristics of the assays from BioFire RP2 are unaffected by the panel modification. The original studies of the BioFire RP2 remain relevant for the performance of the BioFire RP2.1.

EXPECTED VALUES

In the prospective clinical evaluation of the 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 6 and

Table 7.

Table 6. 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 1	5	0.5%	3	0.9%	2	0.7%	0	0%
Parainfluenza Virus 2	54	5.9%	8	2.4%	13	4.6%	33	10.9%
Parainfluenza Virus 3	49	5.3%	20	6.0%	13	4.6%	16	5.3%
Parainfluenza Virus 4	8	0.9%	3	0.9%	1	0.4%	4	1.3%
Respiratory Syncytial Virus	50	5.4%	9	2.7%	5	1.8%	36	11.9%
Bacteria								
***Bordetella parapertussis* (IS*1001*)**	4	0.4%	0	0%	0	0%	4	1.3%
***Bordetella pertussis* (*ptxP*)**	3	0.3%	1	0.3%	0	0%	2	0.7%
Chlamydia pneumoniae	3	0.3%	1	0.3%	0	0%	2	0.7%
Mycoplasma pneumoniae	21	2.3%	2	0.6%	7	2.5%	12	4.0%

Table 7. 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%
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 1	5	0.7%	2	0.8%	2	0.8%	1	0.5%
Parainfluenza Virus 2	0	0%	0	0	0	0%	0	0%
Parainfluenza Virus 3	4	0.6%	2	0.8%	0	0%	2	1.0%
Parainfluenza Virus 4	8	1.2%	4	1.6%	2	0.8%	2	1.0%
Respiratory Syncytial Virus	149	21.5%	59	23.6%	51	21.0%	39	19.4%
Bacteria								
***Bordetella parapertussis* (IS*1001*)**	2	0.3%	1	0.4%	1	0.4%	0	0%
***Bordetella pertussis* (*ptxP*)**	0	0%	0	0%	0	0%	0	0%
Chlamydia pneumoniae	3	0.4%	0	0%	2	0.8%	1	0.5%
Mycoplasma pneumoniae	7	1.0%	3	1.2%	4	1.6%	0	0%

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 8.

Table 8. 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) is presented in Table 9. 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 10.

Table 9. 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%
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 1	10	0.6%	9	1.0%	0	0%	1	0.8%	0	0%
Parainfluenza Virus 2	54	3.3%	39	4.4%	10	3.0%	1	0.8%	4	1.5%
Parainfluenza Virus 3	53	3.3%	44	5.0%	6	1.8%	2	1.6%	1	0.4%
Parainfluenza Virus 4	16	1.0%	13	1.5%	1	0.3%	0	0%	2	0.7%
Respiratory Syncytial Virus	199	12.3%	168	19.0%	10	3.0%	8	6.3%	13	4.9%
Bacteria										
***Bordetella parapertussis* (IS*1001*)**	6	0.4%	4	0.5%	2	0.6%	0	0%	0	0%
***Bordetella pertussis* (*ptxP*)**	3	0.2%	0	0%	3	0.9%	0	0%	0	0%
Chlamydia pneumoniae	6	0.4%	1	0.1%	4	1.2%	1	0.8%	0	0%
Mycoplasma pneumoniae	28	1.7%	10	1.1%	14	4.2%	3	2.3%	1	0.4%

Table 10. 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), stratified by age group, is presented in Table 11. 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 12. 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 11. 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 + RSV	22 (1.4%)	22 (2.5%)	0 (0%)	0 (0%)	0 (0%)
CoV-HKU1 + RSV	13 (0.8%)	12 (1.4%)	0 (0%)	0 (0%)	1 (0.4%)
CoV-NL63 + RSV	13 (0.8%)	12 (1.4%)	0 (0%)	0 (0%)	1 (0.4%)
HRV/EV + PIV2	11 (0.7%)	9 (1.0%)	1 (0.3%)	0 (0%)	1 (0.4%)
HRV/EV + PIV3	11 (0.7%)	10 (1.1%)	1 (0.3%)	0 (0%)	0 (0%)
Adenovirus + RSV	10 (0.6%)	8 (0.9%)	2 (0.6%)	0 (0%)	0 (0%)
Adenovirus + HRV/EV + RSV	9 (0.6%)	9 (1.0%)	0 (0%)	0 (0%)	0 (0%)
CoV-NL63 + HRV/EV	8 (0.5%)	7 (0.8%)	1 (0.3%)	0 (0%)	0 (0%)
CoV-HKU1 + HRV/EV	5 (0.3%)	3 (0.3%)	2 (0.6%)	0 (0%)	0 (0%)
CoV-OC43 + HRV/EV	5 (0.3%)	5 (0.6%)	0 (0%)	0 (0%)	0 (0%)
hMPV + HRV/EV	5 (0.3%)	5 (0.6%)	0 (0%)	0 (0%)	0 (0%)

Table 12. 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 original 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 in Table 13 provides a summary of demographic information for the 1612 specimens included in the prospective study.

Table 13. 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 14.

Table 14. 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
	Frozen	40/40	100	91.2-100	645/654	98.6	97.4-99.3
	Overall	***40/40***	***100***	***91.2-100***	***1562/1572***	***99.4***	***98.8-99.7***
CoV-OC43[e]	Fresh	11/13	84.6	57.8-95.7	904/905	99.9	99.4-100
	Frozen	22/28	78.6	60.5-89.8	662/666	99.4	98.5-99.8
	Overall	***33/41***	***80.5***	***66.0-89.8***	***1566/1571***	***99.7***	***99.3-99.9***
hMPV[f]	Fresh	5/5	100	56.6-100	913/913	100	99.6-100
	Frozen	68/70	97.1	90.2-99.2	616/624	98.7	97.5-99.3
	Overall	***73/75***	***97.3***	***90.8-99.3***	***1529/1537***	***99.5***	***99.0-99.7***
HRV/EV[g]	Fresh	320/328	97.6	95.3-98.8	532/590	90.2	87.5-92.3
	Frozen	105/108	97.2	92.1-99.1	567/586	96.8	95.0-97.9
	Overall	***425/436***	***97.5***	***95.5-98.6***	***1099/1176***	***93.5***	***91.9-94.7***
FluA[h]	Fresh	3/3	100	43.9-100	915/915	100	99.6-100
	Frozen	75/75	100	95.1-100	616/616	100	99.4-100
	Overall	***78/78***	***100***	***95.3-100***	***1531/1531***	***100***	***99.7-100***
FluA H1	Fresh	0/0	-	-	918/918	100	99.6-100
	Frozen	0/0	-	-	691/691	100	99.4-100
	Overall	***0/0***	***-***	***-***	***1609/1609***	***100***	***99.8-100***
FluA H1-2009	Fresh	0/0	-	-	918/918	100	99.6-100
	Frozen	74/74	100	95.1-100	617/617	100	99.4-100
	Overall	***74/74***	***100***	***95.1-100***	***1535/1535***	***100***	***99.8-100***
FluA H3	Fresh	3/3	100	43.9-100	915/915	100	99.6-100
	Frozen	1/1	100	-	690/690	100	99.4-100
	Overall	***4/4***	***100***	***51.0-100***	***1605/1605***	***100***	***99.8-100***
FluB[i]	Fresh	0/0	-	-	918/918	100	99.6-100
	Frozen	14/14	100	78.5-100	678/680	99.7	98.9-99.9
	Overall	***14/14***	***100***	***78.5-100***	***1596/1598***	***99.9***	***99.5-100***
PIV1[j]	Fresh	5/5	100	56.6-100	913/913	100	99.6-100
	Frozen	4/4	100	51.0-100	689/690	99.9	99.2-100

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
	Overall	***9/9***	***100***	***70.1-100***	***1602/1603***	***99.9***	***99.6-100***
PIV2[k]	Fresh	46/47	97.9	88.9-99.6	863/871	99.1	98.2-99.5
	Frozen	0/0	-	-	694/694	100	99.4-100
	Overall	***46/47***	***97.9***	***88.9-99.6***	***1557/1565***	***99.5***	***99.0-99.7***
PIV3[l]	Fresh	40/42	95.2	84.2-98.7	867/876	99.0	98.1-99.5
	Frozen	3/3	100	43.9-100	690/691	99.9	99.2-100
	Overall	***43/45***	***95.6***	***85.2-98.8***	***1557/1567***	***99.4***	***98.8-99.7***
PIV4[m]	Fresh	6/6	100	61.0-100	910/912	99.8	99.2-99.9
	Frozen	3/3	100	43.9-100	686/691	99.3	98.3-99.7
	Overall	***9/9***	***100***	***70.1-100***	***1596/1603***	***99.6***	***99.1-99.8***
RSV[n]	Fresh	44/45	97.8	88.4-99.6	867/873	99.3	98.5-99.7
	Frozen	131/131	100	97.2-100	545/563	96.8	95.0-98.0
	Overall	***175/176***	***99.4***	***96.9-99.9***	***1412/1436***	***98.3***	***97.5-98.9***
Bacteria							
***B. parapertussis* (IS*1001*)**[o]	Fresh	4/5	80.0	37.6-96.4	913/913	100	99.6-100
	Frozen	2/2	100	34.2-100	692/692	100	99.4-100
	Overall	***6/7***	***85.7***	***48.7-97.4***	***1605/1605***	***100***	***99.8-100***
***B. pertussis* (*ptxP*)**[p]	Fresh	2/2	100	34.2-100	915/916	99.9	99.4-100
	Frozen	0/1	0.0	-	693/693	100	99.4-100
	Overall	***2/3***	***66.7***	***20.8-93.9***	***1608/1609***	***99.9***	***99.6-100***
C. pneumoniae[q]	Fresh	2/2	100	34.2-100	915/916	99.9	99.4-100
	Frozen	3/3	100	43.9-100	691/691	100	99.4-100
	Overall	***5/5***	***100***	***56.6-100***	***1606/1607***	***99.9***	***99.6-100***
M. pneumoniae[r]	Fresh	17/17	100	81.6-100	897/901	99.6	98.9-99.8
	Frozen	6/7	85.7	48.7-97.4	686/687	99.9	99.2-100
	Overall	***23/24***	***95.8***	***79.8-99.3***	***1583/1588***	***99.7***	***99.3-99.9***

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

BioFire RP2 reported a total of 245 specimens with discernible multiple organism detections (15.2% of all specimens, 245/1612; and 24.0% of positive specimens, 245/1020; Table 15). The majority of multiple detections (190/245; 77.6%) contained two organisms, while 20.0% (49/245) contained three organisms, 1.6% (4/245) contained four organisms, 0.4% (1/245) contained five organisms, and 0.4% (1/245) contained six organisms. Out of the 245 specimens with multiple detections, 124 specimens (50.6%; 124/245) were concordant with the comparator methods. One hundred twenty-one (121) specimens (49.4%; 121/245) contained one or more organisms that had not been detected by the comparator methods (i.e. false positive results).

The three organisms that were most prevalent in multiple detections were also the three most prevalent organisms in the study as a whole (i.e. HRV/EV, RSV, and adenovirus). The most prevalent multiple detections (≥5 instances) are shown in Table 16.

Table 15. Prevalence of Analytes in Multiple Detections as determined by the BioFire RP2

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

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

Table 16. Multiple Detection Combinations (≥5 instances) as Determined by the BioFire RP2

Distinct Multiple Detection Combinations			Total Multiple Detections	Number of Specimens with False Positive Detections	False Positive Analyte(s)[a]
Analyte 1	Analyte 2	Analyte 3			
Adenovirus	HRV/EV		30	15	Adenovirus (15), HRV/EV (1)
HRV/EV	RSV		22	7	HRV/EV (3), RSV (4)
CoV-HKU1	RSV		13	7	CoV-HKU1 (4), RSV (3)
CoV-NL63	RSV		13	3	CoV-NL63 (2), RSV (1)
HRV/EV	PIV2		11	7	HRV/EV (6), PIV2 (2)
HRV/EV	PIV3		11	6	HRV/EV (3), PIV3 (4)
Adenovirus	RSV		10	5	Adenovirus (4), RSV (1)
Adenovirus	HRV/EV	RSV	9	5	Adenovirus (2), HRV/EV (3), RSV (1)
CoV-NL63	HRV/EV		8	2	CoV-NL63 (2)
CoV-HKU1	HRV/EV		5	2	CoV-HKU1 (1), HRV/EV (1)
CoV-OC43	HRV/EV		5	3	HRV/EV (3)
hMPV	HRV/EV		5	1	HRV/EV

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

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

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 18, N=2). The final data set consisted of 524 specimens. Table 17 provides a summary of demographic information for the 524 specimens included in the study.

Table 17. 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 tests with US FDA Emergency Use Authorization (EUA). The interpretation rules to determine the composite EUA comparator result are shown in Table 18.

Table 18. 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 19. 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 18); SARS-CoV-2 was detected in the remaining FP specimen using an additional independent molecular method.

Table 19. 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 20).

Table 20. 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.

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 21.

Table 21. 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 22 by analyte.

Table 22. BioFire RP2 Archived Specimen Performance Data Summary

Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses						
Adenovirus	0/0	0	N/A	189/194	97.4	94.1-98.9
CoV- 229E [a]	15/15	100	79.6-100	175/175	100	97.9-100
CoV-HKU1	0/0	0	N/A	194/194	100	98.1-100
CoV-NL63	2/2	100	34.2-100	192/192	100	98.0-100
CoV-OC43	0/0	0	N/A	194/194	100	98.1-100
hMPV	1/1	100	20.7-100	192/193	99.5	97.1-99.9
HRV/EV	18/19	94.7	75.4-99.1	168/175	96.0	92.0-98.0
Influenza A	22/22	100	85.1-100	172/172	100	97.8-100
Influenza A H1	3/3	100	43.9-100	191/191	100	98.0-100
Influenza A 2009-H1	1/1	100	20.7-100	193/193	100	98.0-100
Influenza A H3	18/18	100	82.4-100	176/176	100	97.9-100
Influenza B [b]	16/16	100	80.6-100	177/177	100	97.9-100
Parainfluenza Virus 1	16/16	100	80.6-100	178/178	100	97.9-100
Parainfluenza Virus 2 [c]	16/16	100	80.6-100	177/177	100	97.9-100
Parainfluenza Virus 3	17/17	100	81.6-100	175/177	98.9	96.0-99.7
Parainfluenza Virus 4	17/17	100	81.6-100	174/177	98.3	95.1-99.4
RSV	2/2	100	34.2-100	191/192	99.5	97.1-99.9
Bacteria						
Bordetella parapertussis (IS*1001*) [d]	16/16	100	80.6-100	4/4	100	51.0-100
Bordetella pertussis (*ptx*P) [e]	25/26	96.2	81.1-99.3	160/162	98.8	95.6-99.7
Chlamydia pneumoniae [f]	17/17	100	81.6-100	176/176	100	97.9-100
Mycoplasma pneumoniae [g]	16/16	100	80.6-100	171/173	98.8	95.9-99.7

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

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

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

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

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

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

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

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

An evaluation of preselected archived SARS-CoV-2 specimens was conducted 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 23) and had been collected in March and April, 2020.

Table 23. Archived Source and Identification Methods

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

Table 24. BioFire RP2.1 SARS-CoV-2 Archived NPS Specimen Performance Data Summary

Agreement with known analyte composition						
Comparator Method	**PPA:** TP/(TP+FN)	**%**	**95% CI**	**NPA:** TN/(TN+FP)	**%**	**95% CI**
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	NA	N/A	49/49	100	[92.7 – 100%]
Overall Agreement	**48/49[1]**	**98**	**[89.3 – 99.6%]**	**49/49**	**100**	**[92.7 – 100%]**

[1] One FN specimen was positive upon retest

Notably, of the 48 specimens with SARS-CoV-2 Detected results, 10.4% (5/48) had other analytes identified by the BioFire RP2.1 (Table 25).

Table 25. 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 H-2009, and H3) from the prospective and archived specimen studies. Contrived positive specimens were prepared and randomized along with 50 un-spiked influenza A H1 negative specimens such that the analyte status of each contrived specimen was unknown to the users performing the testing. The results of the BioFire RP2 testing contrived specimens are presented in Table 26.

Table 26. BioFire RP2 Performance Using Contrived Specimens

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

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

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 specimens spiked with inactivated SARS-CoV-2 isolate USA-WA1/2020 at various levels of LoD (25 at 2× LoD, 15 at 3× LoD, and 10 at 5× LoD) and randomized with ten non-spiked specimens. Each specimen was a unique 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 Detected result. PPA and NPA are shown in Table 27. For SARS-CoV-2 contrived testing, both the PPA and NPA were 100%.

Table 27. 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 28).

Table 28. 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%

Analyte	RP2.1+ RP2+	RP2.1- RP2+	PPA	RP2.1- RP2-	RP2.1+ RP2-	NPA
Coronavirus NL63	10	0	100%	210	0	100%
Coronavirus OC43	10	0	100%	210	0	100%
Human Metapneumovirus	12	0	100%	208	0	100%
Human Rhinovirus/Enterovirus	19	3	86.4%	195	3	98.5%
Influenza A	30	0	100%	180	0	100%
Influenza A H1	5	0	100%	215	0	100%
Influenza A H1-2009	12	0	100%	208	0	100%
Influenza A H3	13	0	100%	207	0	100%
Influenza B	10	0	100%	210	0	100%
Parainfluenza Virus 1	9	0	100%	211	0	100%
Parainfluenza Virus 2	11	0	100%	209	0	100%
Parainfluenza Virus 3	10	1	90.9%	208	1	99.5%
Parainfluenza Virus 4	11	0	100%	209	0	100%
Respiratory Syncytial Virus	10	0	100%	210	0	100%
Bacteria						
***Bordetella parapertussis* (IS*1001*)**	10	0	100%	210	0	100%
***Bordetella pertussis* (*ptxP*)**	10	0	100%	210	0	100%
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 29 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 29).

Table 29. 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%]**

Limit of Detection

The limit of detection (LoD) for BioFire RP2.1 analytes was estimated by testing dilutions of contrived samples containing known concentrations of organisms. Confirmation of the BioFire RP2.1 LoD was achieved by testing 20 replicates on BioFire 2.0 and BioFire Torch Systems. LoD was confirmed when the organism was detected in at least 19 of 20 replicates tested (19/20 = 95%). The confirmed LoD for each BioFire RP2.1 analyte is listed in Table 30 and LoD is equivalent when testing on BioFire 2.0 and BioFire Torch Systems.

Table 30. Limit of Detection (LoD) for BioFire Respiratory Panel 2.1 (RP2.1) Analytes

Analyte	Isolate		LoD Concentration
Viruses			
Adenovirus [a]	Species C Serotype 2 WHO International Standard NIBSC 16/324		**3.0E+03 IU/mL**[b] 3.0E+03 copies/mL[b]
Coronavirus 229E	ATCC VR-740		**6.5E+01 copies/mL** 4.0E-01 $TCID_{50}$/mL
Coronavirus HKU1	Clinical specimen		**2.0E+03 RNA copies/mL**
Coronavirus NL63	BEI NR-470		**5.4E+01 copies/mL** 2.5 E-01 $TCID_{50}$/mL
Coronavirus OC43	ATCC VR-759		**5.6E+02 copies/mL** 3.0E+01 $TCID_{50}$/mL
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	USA-WA1/2020	Heat-inactivated virus[c] ATCC VR-1986HK	**5.0E+02 copies/mL**[c] 6.9E-02 $TCID_{50}$/mL
		Infectious virus[c]	**1.6E+02 copies/mL**[c] 1.1E-02 $TCID_{50}$/mL
Human Metapneumovirus	16, Type A1 IA10-2003 Zeptometrix 0810161CF		**1.0E+01 $TCID_{50}$/mL**[d]
Human Rhinovirus/ Enterovirus	Human Rhinovirus Type 1A Zeptometrix 0810012CFN		**3.8E+01 copies/mL**
	Enterovirus D68 ATCC VR-1823		**2.6E+01 copies/mL** 3.0E+02 $TCID_{50}$/mL
Influenza A H1	Influenza A H1N1 A/New Caledonia/20/99 Zeptometrix 0810036CF		**1.4E+02 copies/mL** 1.0E+03 $TCID_{50}$/mL
Influenza A H1-2009	Influenza A H1N1pdm09 A/Swine/NY/03/2009 Zeptometrix 0810249CF		**3.3E+02 copies/mL** 5.0E-01 $TCID_{50}$/mL
Influenza A H3	Influenza H3N2 A/Port Chalmers/1/73 ATCC VR-810		**2.1E+01 copies/mL** 1.0E-01 $TCID_{50}$/mL
Influenza B	B/FL/04/06 Zeptometrix 0810255CF		**3.4E+01 copies/mL** 5.0E+00 $TCID_{50}$/mL
Parainfluenza Virus 1	Type 1 Zeptometrix 0810014CF		**1.0E+03 copies/mL** 5.0E+00 $TCID_{50}$/mL
Parainfluenza Virus 2	Type 2 Zeptometrix 0810015CF		**3.0E+01 copies/mL** 5.0E-01 $TCID_{50}$/mL
Parainfluenza Virus 3	Type 3 Zeptometrix 0810016CF		**3.8E+01 copies/mL** 2.5E+00 $TCID_{50}$/mL
Parainfluenza Virus 4	Type 4a Zeptometrix 0810060CF		**1.6E+03 copies/mL** 5.0E+01 $TCID_{50}$/mL
Respiratory Syncytial Virus	Type A Zeptometrix 0810040ACF		**9.0E+00 copies/mL** 2.0E-02 $TCID_{50}$/mL
Bacteria			
***Bordetella parapertussis* (IS*1001*)**	A747 Zeptometrix 0801461		**6.0E+01 IS*1001* copies/mL** 4.1E+01 CFU/mL[e]
***Bordetella pertussis* (*ptxP*)**	A639 Zeptometrix 0801459		**1.0E+03 CFU/mL**
Chlamydia pneumoniae	TW183 ATCC VR-2282		**1.3E+02 copies/mL**[f]
Mycoplasma pneumoniae	M129 Zeptometrix 0801579		**4.6E+02 copies/mL**

[a] LoD testing for Adenovirus also included detection in ≥19/20 replicates tested for Adenovirus B7 at 8.7E+02 copies/mL and Adenovirus F41 at 1.1E+03 copies/mL.

[b] 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.

[c] LoD testing for SARS-CoV-2 was performed with inactivated and infectious virus in pooled NPS specimen matrix (and viral transport medium). The concentration of viral RNA for the inactivated virus was determined by digital droplet PCR (as indicated on the Certificate of Analysis from ATCC). The infectious virus (obtained from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA; contributed by the U.S. Centers for Disease Control (CDC)) was cultured, quantified and tested in a biosafety level 3 laboratory and the concentration of viral RNA was determined by quantitative real-time PCR using *E* gene primers and probe as described on the World Health Organization (WHO) website: https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf.

[d] No corresponding copies/mL concentration available. Human Metapneumovirus LoD testing for BioFire RP2.1 was performed with a culture that has only been quantified in units of $TCID_{50}$/mL. The confirmed Human Metapneumovirus LoD for the same assay in a previous version of the panel (BioFire RP2) was established with a different culture of the same isolate and is also 1.0E+01 $TCID_{50}$/mL, with a corresponding molecular concentration of 1.2E+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 quantitative real-time PCR.

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

NOTE: LoD concentrations in copies/mL in Table 30 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 31.

Table 31. 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

Analytical Reactivity (Inclusivity)

Analytical reactivity (inclusivity) of BioFire RP2.1 assays was evaluated by *in silico* analysis and testing of 180 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 or the BioFire RP2. All isolates were detected at concentrations within 10× LoD (Table 32 –Table 43).

In silico analysis of sequence data was used to make predictions of reactivity for all assays and *in silico* predictions are noted for less common strains or serotypes that were not tested and/or when characterized isolates were not available for testing (e.g. SARS-CoV-2, Table 44).

NOTE: BioFire RP2.1 Influenza A assays will react variably with non-human influenza A viruses and rarely encountered human influenza A viruses that are not H1, H1-2009 or H3; generally producing Influenza A Equivocal or Influenza A (no subtype detected) results.

NOTE: The BioFire RP2.1 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 32. 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 the BioFire RP2.1 at 3x the LoD established with the WHO International Standard (3.0E+03 IU or copies/mL) or less.

Table 33. 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

Coronavirus Type	Isolate ID/Source	[Location/Year]	xLoD[a] Detected	Result
	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-1986 HK (heat inactivated)	[USA-WA1/2020]	1x	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)
	World Reference Center for Emerging Viruses and Arboviruses			

[a] 1x LoD samples were tested and detected with the BioFire RP2.1.

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

[c] See Table 44 for additional SARS-CoV-2 reactivity predictions based on *in silico* analysis.

Table 34. Human Metapneumovirus 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 the BioFire RP2.1.

Table 35. 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	Human Rhinovirus/ Enterovirus
	Enterovirus 71	ATCC VR-1432	[H]	3x	
B	Coxsackievirus A9	Zeptometrix 0810017CF	-	3x	
	Coxsackievirus B3	Zeptometrix 0810074CF	-	3x	
	Coxsackievirus B4	Zeptometrix 0810075CF	-	3x	
	Echovirus 6	Zeptometrix 0810076CF	-	3x	
	Echovirus 9	Zeptometrix 0810077CF	-	3x	
	Echovirus 11	Zeptometrix 0810023CF	-	3x	
C	Coxsackievirus A21	ATCC VR-850	[Kuykendall/California/1952]	3x	
	Coxsackievirus A24	ATCC VR-583	[DN-19/Texas/1963]	3x	
D	68	ATCC VR-1823	[US/MO/2014-18947]	1x	

[a] 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 the BioFire RP2.1.

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

Table 36. 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 Equivocal
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 Equivocal

[a] 1x LoD samples were tested and detected with the BioFire RP2.1.

[b] Reported as Influenza A (no subtype detected) at 3× 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 Equivocal 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 37. 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 the BioFire RP2.1.

Table 38. 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 1
		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	Parainfluenza Virus 2
		ATCC VR-92	[Greer/Ohio/1955]	3x	
3		Zeptometrix 0810016CF	-	1x	Parainfluenza Virus 3
		ATCC VR-93	[C-243/Washington DC/1957]	3x	
		BEI NR-3233	[NIH 47885, Wash/47885/57]	3x	
4	A	Zeptometrix 0810060CF	-	1x	Parainfluenza Virus 4
		ATCC VR-1378	[M-25/1958]	3x	
	B	Zeptometrix 0810060BCF	-	3x	
		ATCC VR-1377	[CH-19503/Washington DC/1962]	3x	

[a] 1x LoD samples were tested and detected with the BioFire RP2.1.

[b] Discontinued part number.

Table 39. 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 the BioFire RP2.1.

Table 40. *Bordetella parapertussis* (IS*1001*) Reactivity (Isolates Tested and Detected)

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

[a] 1x LoD sample was tested and detected with the BioFire RP2.1.

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

Table 41. *Bordetella pertussis* (*ptxP*) Reactivity (Isolates Tested and Detected)[a]

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

[a] The ptxP assay can react with pertussis vaccines.

[b] 1x LoD sample was tested and detected with the BioFire RP2.1.

Table 42. *Chlamydia pneumoniae* Reactivity (Isolates Tested and Detected)

Isolate ID/Source	[Strain/Location/Year]	xLoD[a] Detected	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 the BioFire RP2.1.

Table 43. 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 the BioFire RP2.1.

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

Evaluation of analytical reactivity for the BioFire RP2.1 SARS-CoV-2 assays (SARSCoV2-1 and SARSCoV2-2) was based on *in silico* sequence analysis of all available sequences in the NCBI and GISAID databases as of February 21, 2021. In total, 467,066 sequences from around the globe were aligned to the assay primers.

This analysis determined that 99.998% of 467,066 sequences will be detected by one or both BioFire RP2.1 SARS-CoV-2 assays with no limitations. Approximately 1.2% of the sequences (5,405/467,066) have a mismatched base within the 3' half of a primer that may affect one assay but will be detected by the second assay. A limitation on detection (both assays impaired) is predicted for only 0.002% of the sequences evaluated (9/467,066) (Table 44).

The sequences evaluated include the following lineages and variants of concern (VOC) or variants under investigation (VUI) that may have important epidemiological, immunological, or pathogenic properties from a public health perspective:

- A.23 lineage (Uganda)
  - VUI-202102/01 (A.23.1 with E484K in Spike)
- B.1.1, B1.1.7, B.1.258 lineages (United Kingdom; Δ69-70 and N501Y in Spike)
  - VOC-202012/01 (B.1.1.7)
  - VOC-202102/02 (B.1.1.7 with E484K in Spike)
- B.1.1.28 lineage (Brazil)

  - VOC-202101/02 - P1 variant (Brazil/Japan)
  - VUI-202101/01 - P2 variant (Brazil)
- B.1.1.318 (United Kingdom)
  - VUI-202102/04
- B.1.351 lineage (South Africa)
  - VOC-202012/02 (501Y.V2 in Spike)
- B.1.429 lineage (United States)
  - CAL.20C variant
- B.1.525 lineage (United Kingdom)
  - VUI-202102/03 or UK1188
- B.1.526 (United States)

All lineages and variants of public health interest identified as of February 2021 are predicted to be detected; new sequences and variants will continue to be monitored for impacts on detection by the BioFire RP2.1 assays.

Table 44. *In silico* Prediction of SARS-CoV-2 Detection by the BioFire RP2.1 Assays

+/+ indicates detected by both assays with no impairment, +/- indicates detection by one assay with no impairment and potential for impaired detection by the other assay, -/- indicates potential for impaired detection by both assays

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

[a] Includes sequences of lineage B.1.525 (VUI-202102/03), which has a mutation in the Spike gene that is predicted to impair detection by the SARS-CoV2-1 assay, but detection by the SARSCoV2-2 (Membrane gene) assay is predicted to be unaffected.

[b] Nine deposited sequences (0.002%; representing five unique sequences) have mismatches in the 3' half of primer(s) for both the SARSCoV2-1 and SARSCoV2-2 assays. Impaired detection is predicted for these sequences, which have not been linked to specific lineages or variants of public health concern.

Analytical Specificity (Cross-Reactivity)

The potential for non-specific amplification and detection by the BioFire RP2.1 assays was evaluated by *in silico* analysis of available sequences and also by testing of high concentrations of organisms. 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. 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 bats and pangolin as well as intra-panel cross-reactivity with *Bordetella* species and Influenza A subtypes of swine origin. A summary of potential cross-reactivity is provided in Table 45 and the on-panel or off-panel isolates and concentrations tested are listed in Table 46 and Table 47, respectively.

Table 45. Predicted and Observed Cross-Reactivity of the BioFire RP2.1

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

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

[b] Cross-reactivity between the *Bordetella pertussis* (ptxP) assay and *B. parapertussis* will be reported as a co-detection (*Bordetella parapertussis* (IS*1001*) Detected and *Bordetella pertussis* (*ptx*P) Detected); while cross-reactivity with most strains of *B. bronchiseptica* (that do not carry IS*1001*) will be reported only as *Bordetella pertussis* (*ptx*P) 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* (*ptx*P) Detected and Human Rhinovirus/Enterovirus Detected or *Bordetella parapertussis* (IS*1001*) Detected and Human Rhinovirus/Enterovirus Detected); while cross-reactivity with most strains of *B. bronchiseptica* (that do not carry 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 46. On-Panel Organisms Tested for Evaluation of BioFire RP2.1 Analytical Specificity

Organism		Isolate ID	Concentration Tested	Cross-Reactivity Detected
Bacteria				
Bordetella parapertussis		Zeptometrix 0801462	6.43E+09 CFU/mL	***Bordetella pertussis* (*ptxp*)**[a]
Bordetella pertussis		ATCC 9797	5.50E+09 CFU/mL	**Human Rhinovirus/Enterovirus**[b]
Chlamydia pneumoniae		ATCC 53592	1.93E+07 IFU/mL	None
Mycoplasma pneumoniae		Zeptometrix 0801579	2.65E+07 CCU/mL	None
Viruses				
Adenovirus	7A (species B)	Zeptometrix 0810021CF	1.02E+07 $TCID_{50}$/mL	None
	1 (species C)	Zeptometrix 0810050CF	2.26E+07 $TCID_{50}$/mL	None
	4 (species E)	ATCC VR-1572	1.58E+06 $TCID_{50}$/mL	None
Coronavirus 229E		Zeptometrix 0810229CF	1.13E+05 $TCID_{50}$/mL	None
Coronavirus HKU1		Clinical specimen	8.94E+06 RNA copies/mL	None
Coronavirus NL63		Zeptometrix 0810228CF	2.34E+05 $TCID_{50}$/mL	None
Coronavirus OC43		Zeptometrix 0810024CF	6.37E+06 $TCID_{50}$/mL	None
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)		USA-WA1/2020	2.4E+09 copies/mL	None
Human Metapneumovirus		Zeptometrix 0810159CF	1.05E+06 $TCID_{50}$/mL	None
Human Rhinovirus (Type 1A)		Zeptometrix 0810012CFN	8.40E+05 $TCID_{50}$/mL	None
Enterovirus (D68)		ATCC VR-1823	1.58E+07 $TCID_{50}$/mL	None
Influenza A H1N1 (A1/FM/1/47)		ATCC VR-97	1.58E+08 $CEID_{50}$/mL	None
Influenza A Hsw N1 (A/NewJersey/8/76)		ATCC VR-897	8.89E+06 $CEID_{50}$/mL	**Influenza A H1-2009**[c]
Influenza A (H1N1) pdm09 (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* (*ptxP*) 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 47. Off-Panel Organisms Tested for Evaluation of BioFire RP2.1 Analytical Specificity

Organism	Isolate ID	Concentration Tested	Cross-Reactivity Detected/Predicted
Bacteria			
Acinetobacter calcoaceticus	ATCC 23055	5.15E+09 CFU/mL	None
Arcanobacterium haemolyticum	ATCC 9345	5.70E+09 CFU/mL	None
Bacillus anthracis	Evaluated *in silico*		None
Bordetella avium	ATCC 35086	1.88E+09 cells/mL	None
Bordetella bronchiseptica	ATCC 10580	2.09E+09 cells/mL	***Bordetella pertussis* (*ptxp*)**
Bordetella hinzii	ATCC 51783	4.30E+06 CFU/mL	None
Bordetella holmesii	ATCC 700052	3.15E+07 CFU/mL	None
Burkholderia cepacia	ATCC 17762	5.04E+09 CFU/mL	None
Chlamydia trachomatis	Zeptometrix 0801775	1.67E+08 IFU/mL	None
Chlaymdia psittaci	Evaluated *in silico*		None
Corynebacterium diphtheriae	Zeptometrix 0801882	7.47E+08 CFU/mL	None
Corynebacterium striatum	ATCC BAA-1293	5.20E+09 CFU/mL	None
Coxiella burnetii	Evaluated *in silico*		None
Escherichia coli	AR Bank #0538	5.53E+09 CFU/mL	None
Fusobacterium necrophorum	ATCC 27852	1.33E+08 cells/mL	None
Haemophilus influenzae	ATCC 33391	5.85E+09 CFU/mL	None
Klebsiella (Enterobacter) aerogenes	AR Bank #0074	6.83E+09 CFU/mL	None
Klebsiella oxytoca	JMI 7818	5.60E+09 CFU/mL	None
Klebsiella pneumoniae	NCTC 13465	1.75E+08 CFU/mL	None

Organism	Isolate ID	Concentration Tested	Cross-Reactivity Detected/Predicted
Lactobacillus acidophilus	Zeptometrix 0801540	1.60E+08 CFU/mL	None
Lactobacillus plantarum	Zeptometrix 0801507	1.20E+09 CFU/mL	None
Legionella (Fluoribacter) bozemanae	ATCC 33217	3.24E+09 cells/mL	None
Legionella (Fluoribacter) dumoffii	ATCC 33279	2.65E+09 cells/mL	None
Legionella feeleii	ATCC 35849	1.49E+09 cells/mL	None
Legionella longbeachae	Zeptometrix 0801577	1.93E+08 CFU/mL	None
Legionella (Tatlockia) micdadei	Zeptometrix 0801576	1.80E+09 CFU/mL	None
Legionella pneumophila	Zeptometrix 0801530	1.75E+09 CFU/mL	None
Leptospira interrogans	ATCC BAA-1198D-5 (genomic DNA)	7.89E+08 GE/mL	None
Moraxella catarrhalis	ATCC 8176	5.73E+09 CFU/mL	None
Mycobacterium tuberculosis	Zeptometrix 0801660 (avirulent strain)	9.07E+06 CFU/mL	None
Mycoplasma genitalium	ATCC 33530D (genomic DNA)	8.40E+07 GE/mL	None
Mycoplasma hominis	Zeptometrix 0804011	2.11E+09 CCU/mL	None
Mycoplasma orale	ATCC 19524	1.00E+07 CCU/mL	None
Neisseria elongata	Zeptometrix 0801510	1.99E+08 CFU/mL	None
Neisseria gonorrhoeae	ATCC 19424	2.31E+09 CFU/mL	None
Neisseria meningitidis	ATCC 13090	1.99E+09 CFU/mL	None
Proteus mirabilis	ATCC 12453	5.60E+09 CFU/mL	None
Pseudomonas aeruginosa	ATCC 27853	4.33E+09 CFU/mL	None
Serratia marcescens	JMI 697	4.75E+09 CFU/mL	None
Staphylococcus aureus (MRSA)	ATCC 10832	1.88E+08 CFU/mL	None
Staphylococcus epidermidis	ATCC 29887	4.95E+09 CFU/mL	None
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 at three test sites on a combination of BioFire 2.0 and BioFire Torch Systems. Negative data for all analytes were collected from one or more unspiked samples tested with the BioFire RP2.1. 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 tested on the BioFire RP2.1 or the BioFire RP2. Testing incorporated a range of potential variation introduced by site, operator (at least two per site), system, instrument/module (at least three per site/system), and kit lot (three). Frozen samples were repeatedly tested on five different days for 120 data points per sample (60 per system).

A summary of results (percent (%) agreement with the expected Detected or Not Detected result) for each analyte (by site and system) is provided in Table 48.

Table 48. Reproducibility of Results on BioFire 2.0 and BioFire Torch Systems

Data in *italics* were collected in the BioFire RP2 reproducibility evaluation. The same number of replicates (120) were tested per sample on the BioFire RP2.1 and the BioFire RP2, but testing was distributed differently between sites and systems.

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result								
			BioFire 2.0				BioFire Torch				All Sites/Systems [95% Confidence Interval]
			Site A	Site B	Site C	System Total	Site A	Site B	Site C	System Total	
Adenovirus (NIBSC 16/324) WHO International Standard	**Negative** (no analyte)	**Not Detected**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**120/120 100%** [97.0-100.0%]
	Moderate Positive 3× LoD 9.0E+03 IU/mL	**Detected**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**120/120 100%** [97.0-100.0%]
	Low Positive 1× LoD 3.0E+03 IU/mL	**Detected**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**120/120 100%** [97.0-100.0%]
Coronavirus 229E	**Negative** (no analyte)	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]
Coronavirus HKU1	**Negative** (no analyte)	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]
Coronavirus NL63 (BEI NR-470)	**Negative** (no analyte)	**Not Detected**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**120/120 100%** [97.0-100.0%]
	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%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**120/120 100%** [97.0-100.0%]
	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%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**120/120 100%** [97.0-100.0%]
Coronavirus OC43 (ATCC VR-759)	**Negative (no analyte)**	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]
	Moderate Positive 3× LoD *9.0E+01 $TCID_{50}$/mL*	**Detected**	-	*29/30 (96.7%)*	*30/30 (100%)*	***59/60 (98.3%)***	*29/30 (96.7%)*	-	*29/30 (96.7%)*	***58/60 (96.7%)***	***117/120 97.5%*** *[92.9-99.5%]*
	Low Positive 1× LoD *3.0E+01 $TCID_{50}$/mL*	**Detected**	-	*30/30 (100%)*	*27/30 (90.0%)*	***57/60 (95.0%)***	*30/30 (100%)*	-	*30/30 (100%)*	***60/60 (100%)***	***117/120 97.5%*** *[92.9-99.5%]*

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result								
			BioFire 2.0				BioFire Torch				All Sites/Systems [95% Confidence Interval]
			Site A	Site B	Site C	System Total	Site A	Site B	Site C	System Total	
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (ATCC VR-1986HK)	**Negative (no analyte)**	**Not Detected**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**120/120 100%** [97.0-100.0%]
	Moderate Positive 3× LoD 1.5E+03 copies/mL	**Detected**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**120/120 100%** [97.0-100.0%]
	Low Positive 1× LoD 5.0E+02 copies/mL	**Detected**	20/20 (100%)	19/20 (95%)	19/20 (95%)	**58/60 (96.7%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**118/120 98.3%** [94.1-99.8%]
Human Metapneumovirus (Zeptometrix 0810161CF)	**Negative (no analyte)**	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]
	Moderate Positive 3× LoD *3.0E+01 $TCID_{50}$/mL*	**Detected**	-	*30/30 (100%)*	*30/30 (100%)*	***60/60 (100%)***	*30/30 (100%)*	-	*30/30 (100%)*	***60/60 (100%)***	***120/120 100%*** *[97.0-100.0%]*
	Low Positive 1× LoD *1.0E+01 $TCID_{50}$/mL*	**Detected**	-	*28/30 (93.3%)*	*30/30 (100%)*	***58/60 (96.7%)***	*30/30 (100%)*	-	*30/30 (100%)*	***60/60 (100%)***	***118/120 98.3%*** *[94.1-99.8%]*
Human Rhinovirus/ Enterovirus **Human Rhinovirus** (Zeptometrix 0810012CFN)	**Negative (no analyte)**	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]
	Moderate Positive 3× LoD *3.0E-01 $TCID_{50}$/mL*	**Detected**	-	*28/30 (93.3%)*	*30/30 (100%)*	***58/60 (96.7%)***	*30/30 (100%)*	-	*30/30 (100%)*	***60/60 (100%)***	***118/120 98.3%*** *[94.1-99.8%]*
	Low Positive 1× LoD *1.0E-01 $TCID_{50}$/mL*	**Detected**	-	*30/30 (100%)*	*30/30 (100%)*	***60/60 (100%)***	*30/30 (100%)*	-	*30/30 (100%)*	***60/60 (100%)***	***120/120 100%*** *[97.0-100.0%]*
Influenza A H1	**Negative (no analyte)**	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]
Influenza A H1-2009 (Zeptometrix 0810109CFN)	**Negative (no analyte)**	**Not Detected**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**120/120 100%** [97.0-100.0%]
	Moderate Positive 3× LoD 1.5E+00 $TCID_{50}$/mL 1.0E+03 copies/mL	**Detected**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**120/120 100%** [97.0-100.0%]
	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%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**120/120 100%** [97.0-100.0%]
Influenza A H3 (ATCC VR-810)	**Negative (no analyte)**	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]
	Moderate Positive 3× LoD *3.0E-01 $TCID_{50}$/mL*	**Detected**	-	*29/30 (96.7%)*	*30/30 (100%)*	***59/60 (98.3%)***	*30/30 (100%)*	-	*30/30 (100%)*	***60/60 (100%)***	***119/120 99.2%*** *[95.4-99.9%]*

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result								
			BioFire 2.0				BioFire Torch				All Sites/Systems [95% Confidence Interval]
			Site A	Site B	Site C	System Total	Site A	Site B	Site C	System Total	
	Low Positive 1× LoD *1.0E-01 $TCID_{50}$/mL*	**Detected**	-	*30/30 (100%)*	*30/30 (100%)*	***60/60 (100%)***	*30/30 (100%)*	-	*30/30 (100%)*	***60/60 (100%)***	***120/120 100%*** *[97.0-100.0%]*
Influenza B (Zeptometrix 0810037CF)	**Negative (no analyte)**	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]
	Moderate Positive 3× LoD *1.5E+01 $TCID_{50}$/mL*	**Detected**	-	*30/30 (100%)*	*30/30 (100%)*	***60/60 (100%)***	*30/30 (100%)*	-	*30/30 (100%)*	***60/60 (100%)***	***120/120 100%*** *[97.0-100.0%]*
	Low Positive 1× LoD *5.0E+00 $TCID_{50}$/mL*	**Detected**	-	*30/30 (100%)*	*30/30 (100%)*	***60/60 (100%)***	*30/30 (100%)*	-	*30/30 (100%)*	***60/60 (100%)***	***120/120 100%*** *[97.0-100.0%]*
Parainfluenza Virus 1	**Negative (no analyte)**	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]
Parainfluenza Virus 2 (Zeptometrix 0810015CF)	**Negative (no analyte)**	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]
	Moderate Positive 3× LoD *1.5E+00 $TCID_{50}$/mL*	**Detected**	-	*29/30 (96.7%)*	*30/30 (100%)*	***59/60 (98.3%)***	*30/30 (100%)*	-	*29/30 (96.7%)*	***59/60 (98.3%)***	***118/120 98.3%*** *[94.1-99.8%]*
	Low Positive 1× LoD *5.0E-01 $TCID_{50}$/mL*	**Detected**	-	*30/30 (100%)*	*27/30 (90.0%)*	***57/60 (95.0%)***	*30/30 (100%)*	-	*29/30 (96.7%)*	***59/60 (98.3%)***	***116/120 96.7%*** *[91.7-99.1%]*
Parainfluenza Virus 3	**Negative (no analyte)**	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]
Parainfluenza Virus 4 (Zeptometrix 0810060CF)	**Negative (no analyte)**	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]
	Moderate Positive 3× LoD *1.5E+02 $TCID_{50}$/mL*	**Detected**	-	*30/30 (100%)*	*30/30 (100%)*	***60/60 (100%)***	*30/30 (100%)*	-	*30/30 (100%)*	***60/60 (100%)***	***120/120 100%*** *[97.0-100.0%]*
	Low Positive 1× LoD *5.0E+01 $TCID_{50}$/mL*	**Detected**	-	*29/30 (96.7%)*	*30/30 (100%)*	***59/60 (98.3%)***	*30/30 (100%)*	-	*29/30 (96.7%)*	***59/60 (98.3%)***	***118/120 98.3%*** *[94.1-99.8%]*
Respiratory Syncytial Virus (Zeptometrix 0810040ACF)	**Negative (no analyte)**	**Not Detected**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**120/120 100%** [97.0-100.0%]
	Moderate Positive 3× LoD 6.0E-02 $TCID_{50}$/mL 1.1E+02 copies/mL	**Detected**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**120/120 100%** [97.0-100.0%]
	Low Positive 1× LoD 2.0E-02 $TCID_{50}$/mL 3.6E+01 copies/mL	**Detected**	19/20 (95%)	20/20 (100%)	18/20 (90%)	**57/60 (95%)**	20/20 (100%)	20/20 (100%)	19/20 (95%)	**59/60 (98.3%)**	**116/120 96.7%** [91.7-99.1%]
	Negative (no analyte)	**Not Detected**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**120/120 100%** [97.0-100.0%]

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result								
			BioFire 2.0				BioFire Torch				All Sites/Systems [95% Confidence Interval]
			Site A	Site B	Site C	System Total	Site A	Site B	Site C	System Total	
***Bordetella parapertussis* (IS*1001*)** (Zeptometrix 0801461)	**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%)**	19/20 (95%)	19/20 (95%)	20/20 (100%)	**58/60 (96.7%)**	**117/120 97.5%** [92.9-99.5%]
	Low Positive 1× LoD 6.0E+01 IS*1001* copies/mL	**Detected**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	20/20 (100%)	19/20 (95%)	20/20 (100%)	**59/60 (98.3%)**	**119/120 99.2%** [95.4-99.9%]
Bordetella pertussis (*ptxP*) (Zeptometrix 0801459)	**Negative (no analyte)**	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]
	Moderate Positive 3× LoD *3.0E+03 CFU/mL*	**Detected**	-	*30/30 (100%)*	*30/30 (100%)*	***60/60 (100%)***	*30/30 (100%)*	-	*30/30 (100%)*	***60/60 (100%)***	***120/120 100%*** *[97.0-100.0%]*
	Low Positive 1× LoD *1.0E+03 CFU/mL*	**Detected**	-	*30/30 (100%)*	*30/30 (100%)*	***60/60 (100%)***	*28/30 (93.3%)*	-	*30/30 (100%)*	***58/60 (96.7%)***	***118/120 98.3%*** *[94.1-99.8%]*
Chlamydia pneumoniae	**Negative (no analyte)**	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]
Mycoplasma pneumoniae	**Negative (no analyte)**	**Not Detected**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	60/60 (100%)	60/60 (100%)	60/60 (100%)	**180/180 (100%)**	**360/360 100%** [99.0-100.0%]

Interference

Potentially interfering substances that could be present in NPS specimens or introduced during specimen collection and testing were evaluated for their effect on BioFire RP2.1 performance. 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 (Table 49) 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 BioFire RP2.1 function. However, it was observed that exposure of samples to bleach prior to testing could damage the organisms/nucleic acids in the sample, leading to inaccurate 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 49. Evaluation of Potentially Interfering Substances for NPS Specimens on the BioFire RP2 and BioFire RP2.1

Substances in **bold** font were tested with the BioFire RP2.1 on samples containing SARS-CoV-2 and other analytes near LoD.
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 (active ingredient in anti-bacterial ointment)	2% w/v	No Interference
Saline Nasal Spray with Preservatives (0.65% NaCl, Phenylcarbinol, Benzalkonium chloride)	1% v/v	No Interference
Nasal Decongestant Spray (Oxymetazoline HCl 0.05%, Benzalkonium chloride, phosphate)	1% v/v	No Interference
Analgesic ointment (Vicks®VapoRub®)	1% w/v	No Interference
Petroleum Jelly (Vaseline®)	1% w/v	No Interference
Snuff (Tobacco)	1% w/v	No Interference
Disinfecting/Cleaning Substances		
Bleach	1% and 2% v/v [up to 1024 ppm chlorine]	Interference[b]
Disinfecting wipes (ammonium chloride)	½ in^2	No Interference
Ethanol	7% v/v	No Interference
DNA*Zap* (Ambion™ AM9891G & AM9892G)	1% v/v	No Interference
RNase*Zap* (Ambion™ AM9782)	1% v/v	No Interference
Specimen Collection Materials		
Rayon Swabs (Copan 168C)	N/A	No Interference
Nylon Flocked Swabs (Copan 553C)	N/A	No Interference

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

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

APPENDIX A

Symbols Glossary

ISO 15223-1 Medical devices - Symbols to be used with medical devices labels, labeling and information to be supplied					
5.1.1 	Manufacturer	5.1.4 	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 explains how to perform the BioFire RP2.1 test more efficiently.				
	A panel in the BioFire RP Panel product family that includes SARS-CoV-2.				

APPENDIX B

Contact and Legal Information

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

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

Warranty Information

Product warranty information is available online at:

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

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

APPENDIX C

References

1. Jones, M. S. *et al.* New adenovirus species found in a patient presenting with gastroenteritis. *J. Virol.* **81**, 5978–5984 (2007).
2. Lenaerts, L., De Clercq, E. & Naesens, L. Clinical features and treatment of adenovirus infections. *Rev. Med. Virol.* **18**, 357–374 (2008).
3. Centers for Disease Control and Prevention. Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases (DVD) Web site. http://www.cdc.gov/ncidod/dvrd/revb/respiratory/eadfeat.htm.
4. Calder, J. A. M. *et al.* Adenovirus type 7 genomic-type variant, New York City, 1999. *Emerg. Infect. Dis.* **10**, 149–152 (2004).
5. Metzgar, D. *et al.* Abrupt emergence of diverse species B adenoviruses at US military recruit training centers. *J. Infect. Dis.* **196**, 1465–1473 (2007).
6. Russell, K. L. *et al.* Transmission dynamics and prospective environmental sampling of adenovirus in a military recruit setting. *J. Infect. Dis.* **194**, 877–885 (2006).
7. Corman, V. M., Muth, D., Niemeyer, D. & Drosten, C. Hosts and Sources of Endemic Human Coronaviruses. in *Advances in Virus Research* vol. Advances in Virus Research 163–188 (Elsevier, 2018).
8. Yang, Y. *et al.* The deadly coronaviruses: The 2003 SARS pandemic and the 2020 novel coronavirus epidemic in China. *J. Autoimmun.* **109**, 102434 (2020).
9. Zaki, A. M., Van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D. & Fouchier, R. A. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N. Engl. J. Med.* **367**, 1814–1820 (2012).
10. Chan, J. F. *et al.* Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. *Clin. Microbiol. Rev.* **28**, 465–522 (2015).
11. Wu, Y. *et al.* SARS-CoV-2 is an appropriate name for the new coronavirus. *The Lancet* **395**, 949–950 (2020).
12. Cucinotta, D. & Vanelli, M. WHO Declares COVID-19 a Pandemic. *Acta Bio Medica Atenei Parm.* **91**, 157–160 (2020).
13. van der Hoek, L. *et al.* Croup is associated with the novel coronavirus NL63. *PLoS Med.* **2**, e240 (2005).
14. Kahn, J. S. & McIntosh, K. History and recent advances in coronavirus discovery. *Pediatr. Infect. Dis. J.* **24**, S223-227, discussion S226 (2005).
15. Kuypers, J. *et al.* Clinical disease in children associated with newly described coronavirus subtypes. *Pediatrics* **119**, e70-76 (2007).
16. Yuen, K.-S., Ye, Z.-W., Fung, S.-Y., Chan, C.-P. & Jin, D.-Y. SARS-CoV-2 and COVID-19: The most important research questions. *Cell Biosci.* **10**, 40 (2020).
17. Petrosillo, N., Viceconte, G., Ergonul, O., Ippolito, G. & Petersen, E. COVID-19, SARS and MERS: are they closely related? *Clin. Microbiol. Infect.* S1198743X20301713 (2020) doi:10.1016/j.cmi.2020.03.026.
18. Kahn, J. S. Epidemiology of human metapneumovirus. *Clin. Microbiol. Rev.* **19**, 546–557 (2006).
19. van den Hoogen, B. G. *et al.* A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* **7**, 719–24 (2001).
20. Falsey, A. R., Erdman, D., Anderson, L. J. & Walsh, E. E. Human metapneumovirus infections in young and elderly adults. *J. Infect. Dis.* **187**, 785–790 (2003).
21. VAN DEN HOOGEN, B. G., OSTERHAUS, D. M. E. & FOUCHIER, R. A. M. Clinical impact and diagnosis of human metapneumovirus infection. *Pediatr. Infect. Dis. J.* **23**, S25–S32 (2004).
22. Esper, F. *et al.* A 1-year experience with human metapneumovirus in children aged< 5 years. *J. Infect. Dis.* **189**, 1388–1396 (2004).
23. World Health Organization. WHO Fact Sheet No. 221, April, 2009. Influenza (Seasonal).
24. Bammer, L., Fukuda, A., Klimov, and N. Cox. Influenza. in *VPD Surveillence Manual* (2002).
25. Update: influenza activity - United States, August 30, 2009-March 27, 2010, and composition of the 2010-11 influenza vaccine. *MMWR Morb. Mortal. Wkly. Rep.* **59**, 423–430 (2010).
26. Bammer, L., Fukuda, A., Klimov, and N. Cox. Influenza. in *VPD Surveillence Manual* (2002).
27. Morens, D. M., Taubenberger, J. K. & Fauci, A. S. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *J Infect Dis* **198**, 962–70 (2008).
28. Henrickson, K. J. Parainfluenza viruses. *Clin. Microbiol. Rev.* **16**, 242–264 (2003).
29. Senchi, K., Matsunaga, S., Hasegawa, H., Kimura, H. & Ryo, A. Development of oligomannose-coated liposome-based nasal vaccine against human parainfluenza virus type 3. *Front Microbiol* **4**, 346 (2013).
30. Lau, S. K. P. *et al.* Human parainfluenza virus 4 outbreak and the role of diagnostic tests. *J. Clin. Microbiol.* **43**, 4515–4521 (2005).
31. Fry, A. M. *et al.* Seasonal trends of human parainfluenza viral infections: United States, 1990–2004. *Clin. Infect. Dis.* **43**, 1016–1022 (2006).
32. Mohapatra, S. S. & Boyapalle, S. Epidemiologic, experimental, and clinical links between respiratory syncytial virus infection and asthma. *Clin. Microbiol. Rev.* **21**, 495–504 (2008).

33. Anderson, L. J., Hendry, R. M., Pierik, L. T., Tsou, C. & McIntosh, K. Multicenter study of strains of respiratory syncytial virus. *J. Infect. Dis.* **163**, 687–692 (1991).
34. Falsey, A. R. & Walsh, E. E. Respiratory syncytial virus infection in adults. *Clin. Microbiol. Rev.* **13**, 371–384 (2000).
35. Hall, C. B. Respiratory syncytial virus and parainfluenza virus. *N. Engl. J. Med.* **344**, 1917–1928 (2001).
36. Anzueto, A. & Niederman, M. S. Diagnosis and treatment of rhinovirus respiratory infections. *Chest* **123**, 1664–1672 (2003).
37. Jacques, J. *et al.* Epidemiological, molecular, and clinical features of enterovirus respiratory infections in French children between 1999 and 2005. *J. Clin. Microbiol.* **46**, 206–213 (2008).
38. Jacobs, S. E., Lamson, D. M., George, K. S. & Walsh, T. J. Human rhinoviruses. *Clin. Microbiol. Rev.* **26**, 135–162 (2013).
39. Sawyer, M. H. Enterovirus infections: diagnosis and treatment. *Curr. Opin. Pediatr.* **13**, 65–69 (2001).
40. Centers for Disease Control and Prevention. Centers for Disease Control and Prevention, Pertussis (Whooping Cough) Web Site.
41. World Health Organization. WHO Immunization, Vaccines, and Biologics; Pertussis Web Site.
42. Mattoo, S. & Cherry, J. D. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to Bordetella pertussis and other Bordetella subspecies. *Clin. Microbiol. Rev.* **18**, 326–382 (2005).
43. Srugo, I. *et al.* Pertussis infection in fully vaccinated children in day-care centers, Israel. *Emerg. Infect. Dis.* **6**, 526–529 (2000).
44. Hahn, D. L., Azenabor, A. A., Beatty, W. L. & Byrne, G. I. Chlamydia pneumoniae as a respiratory pathogen. *Front. Biosci. J. Virtual Libr.* **7**, e66-76 (2002).
45. Grayston, J. T. Chlamydia pneumoniae, strain TWAR pneumonia. *Annu. Rev. Med.* **43**, 317–323 (1992).
46. Kuo, C. C., Jackson, L. A., Campbell, L. A. & Grayston, J. T. Chlamydia pneumoniae (TWAR). *Clin. Microbiol. Rev.* **8**, 451–461 (1995).
47. Peeling, R. W. & Brunham, R. C. Chlamydiae as pathogens: new species and new issues. *Emerg. Infect. Dis.* **2**, 307–319 (1996).
48. Outbreak of community-acquired pneumonia caused by Mycoplasma pneumoniae--Colorado, 2000. *Can. Commun. Dis. Rep. Relevé Mal. Transm. Au Can.* **27**, 104–107 (2001).
49. Klement, E. *et al.* Identification of risk factors for infection in an outbreak of Mycoplasma pneumoniae respiratory tract disease. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **43**, 1239–1245 (2006).
50. Centers for Disease Control and Prevention. Centers for Disease Control and Prevention, Disease Listing: Mycoplasma pneumoniae Web Site.
51. Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; NCCLS Approved Guideline. (2006).
52. User Protocol for Evaluation of Qualitative Test Performance; NCCLS Approved Guideline. (2008).

REVISION HISTORY

Version	Revision Date	Description of Revision(s)
01	January 2022	Initial release

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