BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Mini

Instructions for Use	https://www.biofiredx.com/e-labeling/ITI0205
Quick Guide	https://www.biofiredx.com/e-labeling/ITI0217
Safety Data Sheet (SDS)	https://www.biofiredx.com/e-labeling/ITI0229

IVD

Rx Only

Customer and Technical Support Information *For more information on how to contact Customer and Technical Support, refer to Appendix B.	Phone: 1-800-682-2666 (toll free) E-mail: BioFireSupport@biomerieux.com Website: www.biofiredx.com Or contact the local bioMérieux sales representative or an authorized distributor.

INTENDED PURPOSE

Intended Use

The BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Mini is a multiplexed polymerase chain reaction (PCR) test intended for use with the BIOFIRE® SPOTFIRE® System for the simultaneous, qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swab (NPS) specimens obtained from **individuals with signs and symptoms of respiratory tract infection**, **including COVID-19**; (**Respiratory menu**) or in throat swab (TS) specimens from **individuals with signs and symptoms of pharyngitis** (**Sore Throat menu**).

The following analytes are identified and differentiated using the SPOTFIRE R/ST Panel Mini:

Respiratory Menu	**Sore Throat Menu**
Viruses	Viruses
Coronavirus SARS-CoV-2	Human rhinovirus
Human rhinovirus	Influenza A virus
Influenza A virus	Influenza B virus
Influenza B virus	Respiratory syncytial virus
Respiratory syncytial virus	Bacteria
	Streptococcus pyogenes (group A Strep)

Nucleic acids from the viral and bacterial organisms identified by this test are generally detectable in NPS/TS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of respiratory infection and/or pharyngitis is indicative of the presence of the identified microorganism and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. 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 and/or pharyngitis may be due to infection with pathogens that are not detected by this test, or a respiratory tract infection that may not be detected by an NPS or TS specimen. Positive results do not rule out co-infection with other organisms. The agent(s) detected by the SPOTFIRE R/ST Panel Mini 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 and/or pharyngitis.

Intended User and Use Environment

The SPOTFIRE R/ST Panel is intended for use by trained medical professionals proficient in using the SPOTFIRE System at the point of care (POC) or for laboratory professionals in a laboratory setting.

Refer to the SPOTFIRE System Operator's Manual for the appropriate physical environmental specifications and/or conditions for performing a SPOTFIRE R/ST Panel test.

SUMMARY AND EXPLANATION OF THE TEST

The SPOTFIRE R/ST Panel Mini, designed for use with the SPOTFIRE System, is an automated polymerase chain reaction (PCR)-based sample-to-answer diagnostic test that simultaneously identifies nucleic acids from 5 different bacterial and viral organisms from nasopharyngeal swab (NPS) specimens, or 5 different bacterial and viral organisms from throat swab (TS) specimens, in transport media collected from individuals with signs and symptoms of respiratory infection or pharyngitis, respectively. The SPOTFIRE R/ST Panel Mini uses a single instrument protocol with different reporting of analytes for the two sample types. Sample type is selected at the time of testing and the system's software controls the analyte reporting based on the selected sample type.

Summary of Detected Organisms

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is an RNA virus in the family *Coronaviridae*. Human coronaviruses were established as respiratory pathogens in the 1960s and are a cause of common colds[1,2]. 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.[3,4] 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 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.[5]

Note: MERS-CoV, SARS-CoV, and seasonal coronaviruses (coronaviruses 229E, OC43, HKU1, and NL63) are not detected by the SPOTFIRE R/ST Panel Mini.

Influenza A and B are RNA viruses in the *Orthomyxoviridae* family. The dominant type of influenza virus varies often due to antigenic drift.[6] 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.[7] 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).[8] 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.[9] Complications with viral or bacterial pneumonia increase mortality from influenza infections.[10]

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

Flu Season	Influenza A	% of Subtyped Influenza A			Influenza B
		H1	H1-2009	H3[2]	
2023-2024[3]	82.7%	0.0	79.2	20.8	17.3%
2022-2023[4]	95.2%	0.0	30.3	69.7	4.8%
2021-2022	94.7%	0.0	12.7	99.9	5.3%
2020-2021[4]	63.6%	0.0	40.3	59.7[4]	36.4%
2019-2020	58.7%	0.0	92.8	7.2	41.3%

[1] CDC FluView data accessed on February 5, 2024.

[2] Includes H3N2 and H3N2v subtypes

[3] Cumulative results through January 27, 2024.

[4] Season during which SPOTFIRE R/ST Panel Mini prospective clinical data described in this submission were accumulated.

Respiratory syncytial virus (RSV) is an RNA virus in the *Paramyxoviridae* family and is related to human metapneumoviruses and parainfluenza viruses.[11] RSV has two major subtypes (A and B), which vary annually in their prevalence.[12] RSV is the most common cause of severe respiratory disease in infants, with acute bronchiolitis as the major

cause of hospitalization.[11] RSV is now also recognized as an important pathogen in adults, although adult infections are in generally less severe and limited to the upper respiratory tract.[13] Peak activity of RSV is typically in January and February.[14]

Rhinoviruses and enteroviruses are related RNA viruses in the *Picornaviridae* family.[15] There are more than 100 serotypes of human rhinovirus based on the serology of the capsid protein.[15] Rhinovirus is noted as causing the "common cold", but may also be involved in precipitating asthma attacks and severe complications.[15] 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.[16] Both rhinoviruses and enterovirus are prevalent year round.[17,18]

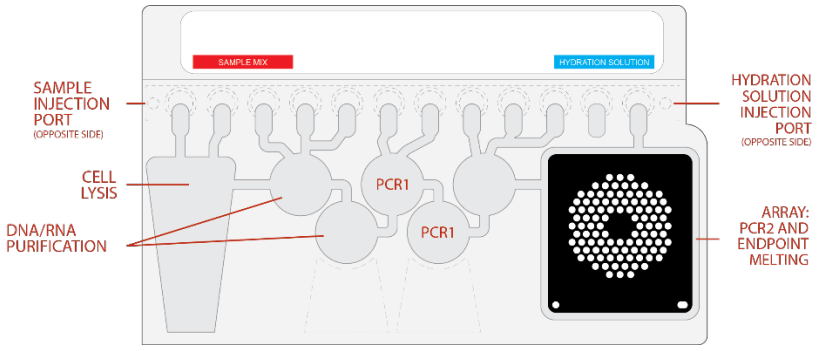
Streptococcus pyogenes or Group A Streptococcus (GAS) is a gram-positive beta-hemolytic streptococcus that causes acute pharyngitis, commonly known as strep throat. Patients experience sudden onset of sore throat, painful swallowing and fever. GAS causes pharyngitis in people of all ages but is most common in children and adolescents[19]. GAS is the most common bacterial cause of pharyngitis in both children[20] and adults[21]. GAS is spread person-to-person through saliva or nasal secretions and is most common in schools, daycare centers, and military training facilities. *S. pyogenes* infections occur most commonly in winter and spring[19].

PRINCIPLE OF THE PROCEDURE

The SPOTFIRE R/ST Panel Mini pouch is a closed system disposable that stores all the necessary reagents for sample preparation, reverse transcription, polymerase chain reaction (PCR), and detection to isolate, amplify, and detect nucleic acid from multiple respiratory pathogens within a single NPS or TS specimen. After sample collection, the user injects hydration solution and sample combined with Sample Buffer into the pouch, places the pouch into the SPOTFIRE System, and starts a run. The entire run process takes about 15 minutes. Additional details can be found in the SPOTFIRE System Operator's Manual.

During a run, the SPOTFIRE 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 analyzes the data to generate a result for each analyte.



MATERIALS PROVIDED

The SPOTFIRE R/ST Panel contains materials consisting of primers, buffers, dNTPs, polymerase, molecular grade water, guanidinium chloride (50 - < 60%), Triton-X 100 (10 - < 20%), and LCGreen® Plus.

Each kit contains sufficient reagents to test 30 samples

- Individually packaged SPOTFIRE R/ST Panel Mini pouches
- Sample Preparation Reagent Kits (SPRKs)
    - Single-use Sample Buffer ampoule
    - Single-use pre-filled Hydration Injection Vial (blue)
    - Single-use Sample Injection Vial (red)
    - Individually packaged fixed-volume Transfer Pipette
- BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Mini Software
  This software is required to run the SPOTFIRE R/ST Panel Mini on the SPOTFIRE System.

MATERIALS REQUIRED BUT NOT PROVIDED

- SPOTFIRE System
    - BIOFIRE® SPOTFIRE® Control Station
    - BIOFIRE® SPOTFIRE® Module(s)
    - BIOFIRE® Pouch Loading Station
- 10% bleach solution or a similar disinfectant

The following materials are examples of those that are compatible with the requirements listed in the Sample Requirements section:

Sample Type	Collection Material	Part Number
Nasopharyngeal Swab (NPS)	Flexible Minitip Size Nylon® flocked swab with 100mm breakpoint	BD 220252 **OR** Copan 553C/503CS01
	BD™ 3 mL Universal Viral Transport Media	BD 220220 **OR** Copan 3C047N
	BD™ 3 mL Universal Viral Transport Media with Flexible Minitip Size Nylon® flocked swab with 100mm breakpoint	BD 220531 **OR** Copan 3C057N
Throat Swab (TS)	Flexible Regular Size Nylon® flocked swab with 80mm breakpoint with 1 mL Amies Medium	Copan Eswab™ 480C

- Additional acceptable collection materials for NPS :
    - Remel Microtext^{s} M4RT® Multi-Microbe Media (ThermoFisher part number R12700)

- Remel MicroTest™ M4RT® Multi-Microbe Media with MicroTip Flocked Swab (ThermoFisher part number R12566)

Note: Compatibility of the SPOTFIRE R/ST Panel Mini with the above-named alternative transport media has been demonstrated analytically, however, clinical performance has not been established.

WARNINGS AND PRECAUTIONS

General Precautions

1. A trained healthcare professional should carefully interpret the results from the SPOTFIRE R/ST Panel Mini in conjunction with a patient's signs and symptoms, results from other diagnostic tests, and relevant epidemiological information.
2. The BIOFIRE System Software displays step-by-step on-screen instructions for the test procedure, including selection of the applicable sample type for the test. Failure to select the correct sample type leads to incomplete reporting of results for all the analytes applicable to the sample type and to reporting of results for some analytes that are not appropriate to the sample type. If the incorrect sample type is selected, do not report the results; repeat the test from the same sample using a new reagent pouch and by selecting the correct sample type from the SPOTFIRE R/ST Panel Mini menu.
3. SPOTFIRE R/ST Panel Mini pouches are only for use with the SPOTFIRE System.
4. Always check the expiration date on the kit. Do not use kit components or a pouch after its expiration date.
5. A desiccant packet is included in each pouch canister to preserve the stability of the SPOTFIRE R/ST Panel Mini. Do not use a pouch if a desiccant packet is not present in the can.
6. Performance characteristics of the SPOTFIRE R/ST Panel Mini have only been determined with nasopharyngeal swab (NPS) and throat swab (TS) specimens in transport medium.
7. SPOTFIRE R/ST Panel Mini pouches are stored under vacuum in individually wrapped canisters. To preserve the integrity of the pouch vacuum for proper operation, be sure that a Module will be available and operational before unwrapping any pouches for loading.
8. If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.

Safety Precautions

1. Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable clean powder-free gloves and lab coats (if available). 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.
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 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 local regulations.
7. Sample Buffer contains Guanidinium chloride and Triton X100. 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
        - P260 – Do not breathe vapor.
        - 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.
    - Disposal
        - Dispose of waste and residues in accordance with local authority requirements.

    Please refer to the SPOTFIRE R/ST Panel Mini Safety Data Sheet (SDS) for more information: [https://www.biofiredx.com/e-labeling/ITI0229]
8. Sample Buffer will form hazardous compounds and fumes when mixed with bleach or other disinfectants.

⚠CAUTION: 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 SPOTFIRE R/ST Panel Mini, it is important to guard against contamination of the sample and work area by carefully following the testing process outlined in this instruction document, including these guidelines:

- Personnel collecting and/or testing specimens may carry or shed common respiratory pathogens asymptomatically and can inadvertently contaminate the specimen while it is being processed. Careful adherence to the sample processing steps described in this document is recommended to avoid possible contamination. Samples may be processed in a clean biosafety cabinet (if available) or according to local/laboratory guidelines. If a biosafety cabinet is not used, a dead air box, a splash shield, or a face shield may be used when preparing samples.
- Personnel with active respiratory symptoms (runny nose, cough) should wear a standard surgical mask (or equivalent) and should avoid touching the mask while handling specimens.
- It is recommended to avoid handling specimens or pouches in an area used to routinely process respiratory pathogen culture, and/or immunofluorescence testing, unless the area is thoroughly cleaned first.
- Prior to processing specimens, thoroughly clean both the work area and the 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 the SPRK package.
- Avoid collecting or handling specimens in areas that are exposed to vaccine material for pathogens detected by the SPOTFIRE R/ST Panel Mini (e.g., influenza, SARS-CoV-2, and poliovirus (Human rhinovirus)). Vaccines may contain PCR-detectable DNA or RNA. If possible, particular care should be taken to avoid contamination of the specimen or testing areas. Contamination of specimens or testing materials with vaccine can cause false-positive results.

2. Preventing amplicon contamination

A common concern with PCR-based assays is false positive results caused by contamination of the work area with PCR amplicon. Because the SPOTFIRE R/ST Panel Mini 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.

⚠**CAUTION:** 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 SPOTFIRE 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 SPOTFIRE R/ST Panel Mini.**

   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 regulations for notification of reportable disease are continually updated and include a number of organisms for surveillance and outbreak investigations. Laboratories are responsible for following their local regulations and should consult their local public health laboratories for isolate and/or clinical sample submission guidelines.

Positive results for Coronavirus SARS-CoV-2 or suspected novel influenza should be reported to local health departments according to local reporting requirements. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Precaution Related to REACH Regulation (EC 1907/2006)

This statement only applies to countries within the European Union (EU) with regard to the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation (EC 1907/2006):

It is recommended that all material associated with the test, including the material used to clean up spills, contaminated packaging, and/or unused and expired IVD tests, is incinerated. Please ensure that you follow local regulations regarding disposal.

REAGENT STORAGE, HANDLING, AND STABILITY

1. Store the test kit, including reagent pouches and buffers, at room temperature (15–25 ºC).
2. Avoid storage of any materials near heating or cooling vents or in direct sunlight.
3. Always check the expiration date on the kit. Do not use reagents beyond the expiration date printed on the pouch or kit.
4. A desiccant packet is included in each pouch canister to preserve the stability of the SPOTFIRE R/ST Panel Mini. Do not use a pouch if a desiccant packet is not present in the can.
5. 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.
6. 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).
7. 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 3 mL of transport media. **Throat swab (TS)** collected according to standard technique and immediately placed in 1 mL of transport media. Detailed NPS and TS specimen collection instructions can be found in the SPOTFIRE R/ST Panel Mini Quick Guide.
Minimum Sample Volume	0.3 mL (300 µL)
Transport and Storage	Specimens should be tested with the SPOTFIRE R/ST Panel Mini 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) (for up to 30 days)[a]

[a] Frozen storage for up to 30 days was evaluated for NPS and TS sample types. 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.

QUALITY CONTROL

Internal 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 SPOTFIRE R/ST Panel Mini 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.

External Controls

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

PROCEDURE

Refer to the SPOTFIRE R/ST Panel Mini Quick Guide or the SPOTFIRE System Operator's Manual for more detail and pictorial representations of these instructions.

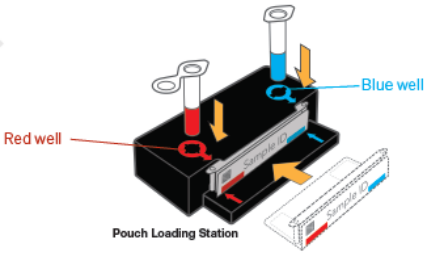
Use clean gloves and other Personal Protective Equipment (PPE) when handling pouches and samples. Only prepare one SPOTFIRE R/ST Panel Mini pouch at a time and change gloves between samples and pouches. Once sample is added to the pouch, promptly transfer to the SPOTFIRE System to start the run. After the run is complete, discard the pouch in a biohazard container.

Step 1: Prepare Pouch

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

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

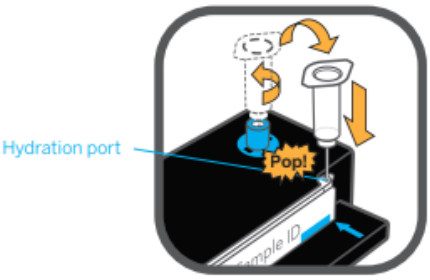
3. Check the expiration date on the pouch. Do not use expired pouches.
4. Label the SPOTFIRE R/ST Panel Mini pouch with the Sample ID and confirm the pouch and Sample IDs match.



Note: Do not cover the pouch barcode located on the left side of the label with the Sample ID.

5. Insert the pouch into the Pouch Loading Station, aligning the red and blue labels on the pouch with the red and blue arrows on the Pouch Loading Station.
6. Remove injection vials and Sample Buffer ampoule from the SPRK.
7. Place the injection vials into the Pouch Loading Station as follows:
   - Place a red-capped Sample Injection Vial into the red well of the Pouch Loading Station.
   - Place a blue-capped Hydration Injection Vial into the blue well of the Pouch Loading Station.
8. Reserve Transfer Pipette and Sample Buffer ampoule for use in Step 3.

Step 2: Hydrate Pouch



1. Unscrew Hydration Injection Vial, leaving the blue plastic cover in well of the Pouch Loading Station.
2. Insert the tip of the Hydration Injection Vial's blunt needle into the pouch hydration port located directly below the blue arrow of the Pouch Loading Station.
3. 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. The correct volume of liquid will be pulled into the pouch automatically.
4. Leave Hydration Injection Vial in pouch.

Note: Some hydration solution may remain in the Hydration Injection Vial

- 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*.

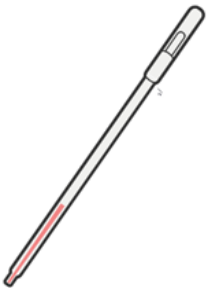
5. Verify that the pouch has been hydrated.
   - Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen in some wells.
   - If the pouch fails to hydrate (dry reagents appear as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

Step 3: Prepare Patient or QC Sample

1. Remove the lid from the sample or QC material tube.

Note: Positive QC materials are a PCR contamination risk.

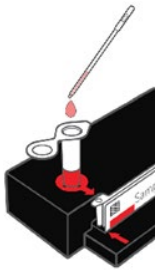
2. Transfer sample to the Sample Injection Vial using the following steps:
   - Remove the fixed-volume Transfer Pipette from its individual packaging within the SPRK, being careful not to touch the tip.
   - Fully squeeze the bulb of the fixed-volume Transfer Pipette and then lower the tip into the sample tube below the surface of the liquid.
   - Slowly release the bulb completely, drawing up liquid into the fixed-volume Transfer Pipette.
   - Visually confirm that liquid was drawn approximately one-third up the length of the stem of the fixed-volume Transfer Pipette, as shown in the image to the right.



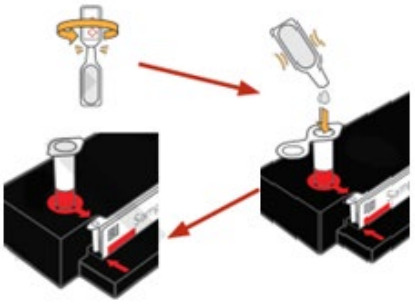
Note: In rare cases, the fixed-volume Transfer Pipette may not draw the correct amount of liquid. If the liquid volume is not drawn approximately one-third up the length of the stem of the fixed-volume Transfer Pipette, discard it and retrieve a new fixed-volume Transfer Pipette from another SPRK package.

Note: When performing QC testing, some liquid may be leftover in the QC material tube.

- Raise the fixed-volume Transfer Pipette out of the sample liquid.
- Dispense the sample into the Sample Injection Vial by again fully squeezing the bulb of the fixed-volume Transfer Pipette.
- After dispensing the sample, discard the fixed-volume Transfer Pipette in an appropriate biohazard container.



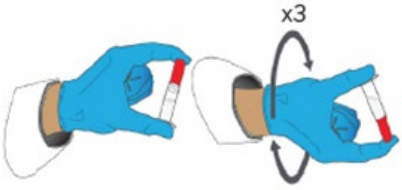
3. Add Sample Buffer to the Sample Injection Vial:
   - Open the Sample Buffer ampoule by twisting the tab off the tip.
   - Dispense full volume of Sample Buffer by squeezing it into the Sample Injection Vial.
   - Discard Sample Buffer ampoule in an appropriate biohazard container.
   - Tightly close the lid of the Sample Injection Vial.



Note: To avoid contamination, do not touch the open tip of the Sample Buffer ampoule.

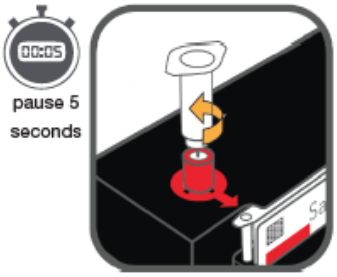
CAUTION—The Sample Buffer is harmful if swallowed and can cause serious eye damage and/or skin irritation. Use appropriate PPE.

4. Lift entire Sample Injection Vial out of the Pouch Loading Station and mix sample by gently inverting 3 times.
5. Return Sample Injection Vial to red well of the Pouch Loading Station.



Step 4: Load Patient or QC Sample

1. Slowly twist to unscrew the Sample Injection Vial and pause for 5 seconds with the blunt needle remaining in cap to avoid dripping.



Note: Pausing 5 seconds decreases the risk of dripping and contamination from the sample.

2. Remove the Sample Injection Vial, leaving red plastic cover in the well of the Pouch Loading Station.

Note: To avoid contamination, do not touch the tip of blunt needle

3. Insert the tip of the blunt needle into the pouch sample port located directly below the red arrow of the Pouch Loading Station.
4. 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.
5. Wait 5 seconds as the sample mix is pulled into the pouch.

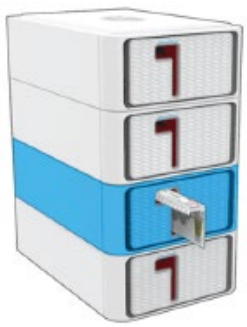
Note: Some sample mix may remain in the Sample Injection Vial

6. 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.
7. Remove Hydration Injection Vial and Sample Injection Vial from SPOTFIRE R/ST Panel Mini pouch. Screw each vial back into its plastic cover in the Pouch Loading Station before disposing of the vials in an appropriate biohazard container.
8. Remove the pouch from the Pouch Loading Station.

Step 5: Start Run

The BIOFIRE® SPOTFIRE® Software includes step-by-step, on-screen instructions that guide the operator through performing a run. Brief instructions for the SPOTFIRE System are given below. Refer to the SPOTFIRE System Operator's Manual for more detailed instructions.

1. Ensure that the SPOTFIRE System is powered on and the software is launched.
2. Select an available Module on the Home Screen and follow on-screen instructions to run test.



Note: If running QC samples, select the QC icon at the top of the screen and follow on-screen instructions.

⚠ **Caution:** QC Testing should only be performed after selecting the QC icon. Failure to do so may lead to erroneous QC results.

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

- Pouch identification (Lot Number and Serial Number), Pouch Type, and Protocol information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Expiration Date, and Pouch Type can be manually entered from the information provided on the pouch label into the appropriate fields.

4. Scan 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. Select the appropriate sample type (e.g. Nasopharyngeal Swab or Throat Swab).

Note: Selecting the appropriate sample type is not required when testing QC samples.

6. Review the entered run information on the screen before inserting the pouch.

Note: The selected Module's front panel LED will blink blue, indicating it is ready to accept a pouch.

7. Insert the pouch into the Module that is blinking blue. The Module will grab onto the pouch and pull it into the chamber and automatically start the run.
    - Once the run has started, the screen displays the panel name and Sample ID, and the minutes remaining on the run.

Note: The selected Module's front panel LED will turn solid green to indicate that the run is in progress.

- When the run is finished, the pouch will automatically eject from the SPOTFIRE System.

8. Use gloves to remove pouch from System and discard the pouch into an appropriate biohazard container.
9. Results are automatically created upon completion of a run. The test report can be viewed by clicking the Complete tile on the Home Screen or by clicking the appropriate icon for Patient Test Results or QC. The run file is automatically saved in the system database, and the test report can be viewed, printed, and/or saved as a PDF file.

INTERPRETATION OF RESULTS

Assay Interpretation

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

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

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

Organism Interpretation

For most organisms detected by the SPOTFIRE R/ST Panel Mini, the organism is reported as Positive if a single corresponding assay is positive (at least two of the three assay wells on the array have similar positive melt peaks with Tm values that are within the assay-specific Tm range). The test results for Coronavirus SARS-CoV-2 and Influenza A virus depend on the interpretation results from more than one assay. Interpretation and actions for the multi-assay results are provided below.

Coronavirus SARS-CoV-2

Two assays are included for the detection of SARS-CoV-2. One assay targets the spike protein (S) gene and the other targets the membrane protein (M) gene. If either one or both assays are positive, the test report result will be Coronavirus SARS-CoV-2 Positive. If both assays are negative, the test report result will be Coronavirus SARS-CoV-2 Negative.

Influenza A virus

The Influenza A Virus result is determined by two groups of two assays each. Group 1 assays (N=2) target the matrix (M) and non-structural (NS) genes. Group 2 assays (N=2) target the hemagglutinin gene. Results from all four assays are evaluated to determine the reported Influenza A Virus result; the algorithm for results reporting is shown in Table 2.

Table 2. Possible Influenza A Virus Assay Results, Interpretations, and Actions Required

Influenza A Virus Result	Assay Result Combinations		Action Required
	Group 1 Assays (N=2)	Group 2 Assays (N=2)	
POSITIVE	**≥1 Positive**	**≥1 Positive**	None
UNCERTAIN	**≥1 Positive**	Negative	Retest
	Negative	**≥1 Positive**	
NEGATIVE	Negative	Negative	None

Human rhinovirus

A single assay is used for the detection of Human rhinovirus. Due to the genetic similarity between human rhinovirus and enterovirus, this assay cannot distinguish between them (See Limitations and Analytical Reactivity (Inclusivity) Both organisms will be reported as Human rhinovirus.

SPOTFIRE R/ST Panel Mini Test Report

The SPOTFIRE R/ST Panel Mini 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.

Run Summary

The Run Summary section of the test report provides the test type (Respiratory Menu or Sore Throat Menu), time and date of the run, Sample ID, and the identity of the operator that performed the test.

Results Summary

The Result Summary section of the test report lists the overall results of the test. Possible test results include Negative, Positive, Uncertain (Influenza A virus only), and Invalid. An Action Bar will appear underneath the test results only when further action is necessary.

The result for each organism tested by the panel is also shown. A check mark next to an organism indicates a positive result, while a question mark next to an organism indicates an uncertain result (Influenza A virus only). When no symbol is present, the result was negative. If the run result is Invalid, this section is not displayed.

Table 3 provides an explanation for each interpretation of patient test results and any follow-up necessary to obtain a final result. Table 4 provides an explanation for each interpretation of a QC test result and any follow-up necessary to obtain a final result. Table 5 provides an explanation for Invalid results.

Table 3. Interpretation of Results

Result	Explanation	Action
NEGATIVE	Test controls Pass **AND** Test is negative for all organisms	Report the results
POSITIVE: [Organism name(s)]	Test controls Pass **AND** Test is positive for the organism(s) listed	Report the results
POSITIVE: Multiple Organisms (4+) *	Test controls Pass **AND** Test is positive for four or more organisms	Detection of four or more organisms is possible but rare. If contamination is suspected, clean the area and retest the sample, then report the results of the retest. If additional guidance is needed, contact your local bioMérieux subsidiary or distributor.
UNCERTAIN: Influenza A virus	Test controls Pass **AND** Results for Influenza A virus are inconclusive	**Retest the original sample ONCE** and report the results of the retest.

* In rare cases, POSITIVE: Multiple Organisms (4+) and UNCERTAIN: Influenza A virus may both appear in the Result Summary section when exactly three organisms (each indicated by a checkmark) in addition to an Influenza A virus uncertain result (indicated by a question mark) are shown in the results section.

Table 4. Interpretation of QC Results

QC Result	Explanation	Action
PASS	Test controls Pass **AND** A Positive QC Test or Negative QC Test has Pass results	Report the results
FAIL	Test controls Pass **AND** A Positive QC Test has negative results	**Retest the Positive QC material ONCE.** If the failure persists, contact your local bioMérieux subsidiary or distributor for further instruction.
	Test controls Pass **AND** A Negative QC Test has positive results	If contamination is suspected, clean the area according to instructions on p.1 of the SPOTFIRE R/ST Panel Mini Quick Guide and retest using a new Negative QC vial. If the failure persists, contact your local bioMérieux subsidiary or distributor for further instruction.

Table 5. Interpretation of Invalid Results

QC Result	Explanation	Action
INVALID: [Failure Reason]	Run does not complete due to any of the following failures: • Instrument error • Aborted run • Run incomplete • Software error • Operational conditions out of range • Internal control failure	Follow on-screen instructions. If the failure persists, contact your local bioMérieux subsidiary or distributor..

Run Details Summary

The Run Details section provides additional information about the run including pouch information (pouch type, lot number, and serial number), control results, sample type, and the Module used to perform the test.

The Run Details will automatically be shown when printed and can also be accessed from the on-screen report by selecting the “Show Details” button.

The “Controls” field displays Pass only if the run completed successfully (no instrument or software errors) and both of the pouch control assays (RNA Process Control and PCR2 Control) were successful. The Control field will display Fail if the run was completed successfully (no instrument or software errors) but one or both pouch control assays failed. If the control result is Fail, then the results for all the tests on the panel are not displayed and the specimen will need to be retested with a new pouch. Table 6 provides additional information for each of the possible control field results.

Table 6. Interpretation of Controls Field on the SPOTFIRE R/ST Panel Mini Test Report

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

LIMITATIONS

1. For prescription use only.
2. This product can be used only with the SPOTFIRE System.
3. The SPOTFIRE R/ST Panel Mini is a qualitative test and does not provide a quantitative value for the virus(es) and/or bacteria detected in the specimen.
4. Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
5. The SPOTFIRE R/ST Panel Mini has been evaluated for use with human specimen material only.
6. The SPOTFIRE R/ST Panel Mini has not been validated for testing of specimens other than nasopharyngeal swab (NPS) or throat swab (TS) specimens in transport medium.
7. The SPOTFIRE R/ST Panel Mini has not been validated for the testing of pooled specimens or the screening of specimens from asymptomatic individuals that are not suspected of COVID-19 infection.
8. The performance of SPOTFIRE R/ST Panel Mini has not been established for specimens collected from individuals without signs or symptoms of respiratory and/or pharyngitis infection.
9. The performance of the SPOTFIRE R/ST Panel Mini has not been specifically evaluated for immunocompromised individuals.
10. The performance of the SPOTFIRE R/ST Panel Mini has not been specifically evaluated in a population known to be vaccinated against illnesses caused by any of the SPOTFIRE R/ST Panel Mini analytes (e.g., SARS-CoV-2 (COVID-19), influenza, or RSV).
11. The effect of antibiotic or antiviral treatment on test performance has not been evaluated.
12. The performance of the SPOTFIRE R/ST Panel Mini has not been specifically evaluated for specimens in patients with Multisystem Inflammatory Syndrome in Children (MIS-C) or similar syndromes.
13. The performance of the SPOTFIRE R/ST Panel Mini 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.
14. The performance of the SPOTFIRE R/ST Panel Mini has not been established for monitoring treatment of infection with any of the panel organisms.
15. The performance of SPOTFIRE R/ST Panel Mini has not been established for screening of blood or blood products.
16. 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.
17. 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.
18. A negative SPOTFIRE R/ST Panel Mini 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 or throat 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.

19. If four or more organisms are reported as positive in a specimen, retesting is recommended to confirm the polymicrobial result.
20. 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.
21. Positive and negative predictive values are highly dependent on prevalence. The likelihood of a negative result being false is higher during peak activity when prevalence of disease is high. The likelihood of a positive result being false is higher during periods when prevalence is moderate to low.
22. Performance characteristics for influenza A virus were established when influenza A/H1-2009 and A/H3 were predominant. When other influenza A viruses are emerging, performance characteristics may differ.
23. Due to the small number of positive results observed for certain organisms during the prospective clinical study, performance characteristics for influenza A virus and influenza B virus were established primarily with retrospective clinical specimens.
24. The SPOTFIRE R/ST Panel Mini may not be able to distinguish between existing viral strains and new variants as they emerge. For example, the SPOTFIRE R/ST Panel Mini can detect influenza A H3N2v (first recognized in August 2011) but will not be able to distinguish this variant from other seasonal influenza A viruses. If variant virus infection is suspected, clinicians should contact their local health department to arrange specimen transport and request a timely diagnosis at a public health laboratory.
25. Recent administration of nasal vaccines prior to NPS or TS specimen collection could lead to accurate virus detection by the SPOTFIRE R/ST Panel Mini of the viruses contained in the vaccine but would not represent infection by those agents.
26. Due to the genetic similarity between human rhinovirus and enterovirus, the SPOTFIRE R/ST Panel Mini cannot reliably differentiate them and will report both as Human rhinovirus. A SPOTFIRE R/ST Panel Mini Human rhinovirus Positive result should be followed-up using an alternate method (e.g. cell culture or sequence analysis) if differentiation between the viruses is required. Refer to Analytical Reactivity (Inclusivity).
27. The SPOTFIRE R/ST Panel Mini Human rhinovirus assay may amplify off-target sequences found in strains of *B. pertussis*, *B. bronchiseptica* and *B. parapertussis*. Bovine and canine picornaviruses may also be detected and reported as Human rhinovirus when present at high concentration. Refer to Analytical Specificity (Cross-Reactivity & Exclusivity).
28. There is a risk of false positive results due to contamination with organisms, nucleic acids, vaccine material, amplified products, or from non-specific signals in the assay. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.
29. Transport media may contain non-viable organisms and/or nucleic acid at levels that can be detected by the SPOTFIRE R/ST Panel Mini.
30. There is a risk of false positive results due to non-specific amplification and cross-reactivity with organisms found in the respiratory tract. Observed and predicted cross-reactivity for the SPOTFIRE R/ST Panel Mini is described in the Analytical Specificity (Cross-Reactivity & Exclusivity) section. Erroneous results due to cross-reactivity with organisms that were not evaluated or new variant sequences that emerge is also possible.
31. Primers for both SPOTFIRE R/ST Panel Mini SARS-CoV-2 assays share substantial sequence homology with non-human coronaviruses and cross-reactivity with these closely related viral sequences is predicted. Refer to Analytical Specificity (Cross-Reactivity & Exclusivity) for more information. It is unlikely that these viruses would be found in

human clinical specimens; but if present, the cross-reactive product(s) produced by the SPOTFIRE R Panel Mini will be reported as Coronavirus SARS-CoV-2.

32. The clinical performance has not been established for all circulating variants of SARS-CoV-2 but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time. Refer to the *In Silico* Reactivity Predictions for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Assays section for more information.
33. The SPOTFIRE R/ST Panel Mini influenza A and influenza B assays are based on those included in other previously BIOFIRE panels. Positive agreement with the SPOTFIRE R/ST Panel Mini for detection of influenza A and influenza B was established using retrospective, archived specimens. The performance observed with prospectively collected specimens may differ.
34. Additional follow-up testing by culture is required if the SPOTFIRE R/ST Panel Mini *Streptococcus pyogenes* assay result is negative and clinical symptoms persist, or in the event of an outbreak of acute rheumatic fever (ARF).

EXPECTED VALUES

In the prospective clinical evaluation of the SPOTFIRE R/ST Panel Mini, 1120 nasopharyngeal swab (NPS) specimens and 877 throat swab (TS) specimens were collected from consented volunteers or obtained as residual, leftover specimens from subjects of all ages and tested at five study sites across the United States and one site in the United Kingdom over approximately 19 months (December 2020 to September 2021 for NPS and TS specimens, and September 2022 to May 2023 for TS specimens only). Expected value summaries (as determined by the SPOTFIRE R/ST Panel Mini) are stratified by specimen enrollment site in Table 7 and Table 8 for NPS and TS specimens, respectively. Expected values are stratified by subject age group in Table 9 and Table 10 for NPS and TS specimens, respectively.

Table 7. Expected Value (EV; as determined by the number of SPOTFIRE R/ST Panel Mini positives / specimens analyzed) Summary by Site for NPS Specimens Collected During the SPOTFIRE R/ST Panel Mini Prospective Clinical Evaluation (December 2020 to June 2021)

SPOTFIRE R/ST Panel Mini R Menu Result	Overall		Site 1		Site 2		Site 3		Site 4		Site 5	
	#/SA[a]	EV[b]	#/SA	EV	#/SA	EV	#/SA	EV	#/SA	EV	#/SA	EV
Viruses												
Coronavirus SARS-CoV-2	***77/1110***	***6.9%***	27/361	7.5%	7/36	19.4%	8/502	1.6%	8/147	5.4%	27/64	42.2%
Human rhinovirus	***417/1115***	***37.4%***	59/361	16.3%	7/36	19.4%	265/508	52.2%	85/147	57.8%	1/63	1.6%
Influenza A virus	***0/1115***	***0%***	0/361	0%	0/36	0%	0/508	0%	0/147	0%	0/63	0%
Influenza B virus	***0/1110***	***0%***	0/361	0%	0/36	0%	0/502	0%	0/147	0%	0/64	0%
Respiratory syncytial virus	***28/1115***	***2.5%***	7/361	1.9%	0/36	0%	16/508	3.1%	5/147	3.4%	0/63	0%

[a] #/SA = number of SPOTFIRE R/ST Panel Mini positives divided by the number of specimens analyzed (i.e., with valid comparator results) for that analyte
[b] EV = Expected Value

Table 8. Expected Value (EV; as determined by the number of SPOTFIRE R/ST Panel Mini positives / specimens analyzed) Summary by Site for TS Specimens Collected During the SPOTFIRE R/ST Panel Mini Prospective Clinical Evaluation (December 2020 to September 2021 and September 2022 to May 2023)

SPOTFIRE R/ST Panel Mini ST Menu Result	Overall		Site 1		Site 2		Site 3		Site 4		Site 5		Site 6	
	#/SA[a]	EV[b]	#/SA	EV	#/SA	EV	#/SA	EV	#/SA	EV	#/SA	EV	#/SA	EV
Viruses														
Human rhinovirus	***245/875***	***28.0%***	17/152	11.2%	15/97	15.5%	125/324	38.6%	77/253	30.4%	1/22	4.5%	10/27	37.0%
Influenza A virus	***35/875***	***4.0%***	18/152	11.8%	6/97	6.2%	8/324	2.5%	3/253	1.2%	0/22	0%	0/27	0%
Influenza B virus	***4/876***	***0.5%***	1/152	0.7%	0/97	0%	3/324	0.9%	0/253	0%	0/22	0%	0/28	0%
Respiratory syncytial virus	***23/875***	***2.6%***	4/152	2.6%	0/97	0%	11/324	3.4%	8/253	3.2%	0/22	0%	0/27	0%
Bacteria														
Streptococcus pyogenes (group A Strep)	***212/869***	***24.4%***	15/152	9.9%	27/97	27.8%	92/320	28.8%	68/251	27.1%	6/21	28.6%	4/28	14.3%

[a] #/SA = number of SPOTFIRE R/ST Panel Mini positives divided by the number of specimens analyzed (i.e., with valid comparator results) for that analyte
[b] EV = Expected Value

Table 9. Expected Value (EV; as determined by the SPOTFIRE R/ST Panel Mini positives / specimens analyzed) Summary by Age Group for NPS Specimens Collected During the SPOTFIRE R/ST Panel Mini Prospective Clinical Evaluation (December 2020 to June 2021)

SPOTFIRE R/ST Panel Mini R Menu Result	Overall		≤5 years		6-18 years		19-40 years		41-60 years		61+ years	
	#/SA[a]	EV[b]	#/SA	EV	#/SA	EV	#/SA	EV	#/SA	EV	#/SA	EV
Viruses												
Coronavirus SARS-CoV-2	***77/1110***	***6.9%***	9/450	2.0%	7/257	2.7%	27/158	17.1%	21/147	14.3%	13/98	13.3%
Human rhinovirus	***417/1115***	***37.4%***	236/455	51.9%	139/257	54.1%	23/159	14.5%	14/146	9.6%	5/98	5.1%
Influenza A virus	***0/1115***	***0%***	0/455	0%	0/257	0%	0/159	0%	0/146	0%	0/98	0%
Influenza B virus	***0/1110***	***0%***	0/450	0%	0/257	0%	0/158	0%	0/147	0%	0/98	0%
Respiratory syncytial virus	***28/1115***	***2.5%***	21/455	4.6%	6/257	2.3%	0/159	0%	1/146	0.7%	0/98	0%

[a] #/SA = number of SPOTFIRE R/ST Panel Mini positives divided by the number of specimens analyzed (i.e., with valid comparator results) for that analyte
[b] EV = Expected Value

Table 10. Expected Value (EV; as determined by the SPOTFIRE R/ST Panel Mini positives / specimens analyzed) Summary by Age Group for TS Specimens Collected During the SPOTFIRE R/ST Panel Mini Prospective Clinical Evaluation (December 2020 to September 2021 and September 2022 to May 2023)

SPOTFIRE R/ST Panel Mini ST Menu Result	Overall		≤5 years		6-18 years		19-40 years		41-60 years		61+ years	
	#/SA[a]	EV[b]	#/SA	EV	#/SA	EV	#/SA	EV	#/SA	EV	#/SA	EV
Viruses												
Human rhinovirus	***245/875***	***28.0%***	58/137	42.3%	152/455	33.4%	23/186	12.4%	9/72	12.5%	3/25	12.0%
Influenza A virus	***35/875***	***4.0%***	2/137	1.5%	10/455	2.2%	15/186	8.1%	5/72	6.9%	3/25	12.0%
Influenza B virus	***4/876***	***0.5%***	1/138	0.7%	2/455	0.4%	1/186	0.5%	0/72	0%	0/25	0%
Respiratory syncytial virus	***23/875***	***2.6%***	6/137	4.4%	13/455	2.9%	0/186	0%	3/72	4.2%	1/25	4.0%
Bacteria												
Streptococcus pyogenes (group A Strep)	***212/869***	***24.4%***	35/136	25.7%	130/451	28.8%	39/185	21.1%	8/72	11.1%	0/25	0%

[a] #/SA = number of SPOTFIRE R/ST Panel Mini positives divided by the number of specimens analyzed (i.e., with valid comparator results) for that analyte
[b] EV = Expected Value

Observed multiple detections (as determined by the SPOTFIRE R/ST Panel Mini) during the prospective clinical evaluation are presented in Table 11. At least one analyte was detected in a total of 501 NPS specimens (44.7% positivity rate; 501/1120); at least one analyte was detected in a total of 446 TS specimens (50.9% positivity rate; 446/877). Polymicrobial detections of up to two organisms were observed in NPS specimens and polymicrobial detections of up to three organisms were observed in TS specimens.

Table 11. Expected Values (multiple detections as determined by the SPOTFIRE R/ST Panel Mini) for the SPOTFIRE R/ST Panel Mini Prospective Clinical Evaluation (December 2020 to September 2021 for NPS and TS specimens, and September 2022 to May 2023 for TS specimens only)

SPOTFIRE R/ST Panel Mini Result	Expected Value (as Determined by Testing of 1120 Prospective NPS Specimens)		Expected Value (as Determined by Testing of 877 Prospective TS Specimens)	
	Number Detected and Reported	% of Total (% of Positives)	Number Detected and Reported	% of Total (% of Positives)
Detected (at least one organism result)	501	44.7% (100%)	446	50.9% (100%)
One organism result	480	42.9% (95.8%)	374	42.6% (83.9%)
Two organism results	21	1.9% (4.2%)	71	8.1% (15.9%)
Three organism results	0	0% (0%)	1	0.1% (0.2%)

The SPOTFIRE R/ST Panel Mini reported a total of 21 NPS specimens with discernable multiple organism detections (1.9% of all NPS specimens, 21/1120; 4.2% of positive NPS specimens, 21/501) and a total of 72 TS specimens with discernable multiple organism detections (8.2% of all TS specimens, 72/877; 16.1% of positive TS specimens, 72/446). The resulting co-detection combinations as reported by the SPOTFIRE R/ST Panel Mini are presented in Table 12 and Table 13. These tables also indicate the number of specimens with false positive (FP) results for each co-detection combination, as well as the specific analyte(s) that were discrepant.

Table 12. Multiple Detection Combinations in NPS Specimens as Reported by the SPOTFIRE R/ST Panel Mini

Distinct Co-Detection Combinations		Total Specimens with Co-Detections	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s)
Analyte 1	Analyte 2			
Coronavirus SARS-CoV-2	Human rhinovirus	10	5	Coronavirus SARS-CoV-2 (1), Human rhinovirus (5)
Coronavirus SARS-CoV-2	Respiratory syncytial virus	1	0	-
Human rhinovirus	Respiratory syncytial virus	10	7	Human rhinovirus (6), Respiratory syncytial virus (2)
Total Co-Detections		**21**	**12**	**14/42[a]**

[a] Of the 14 discrepant analytes (out of 42 total analytes), nine (64.3%) were confirmed as being present in the specimen during discrepancy investigation.

Table 13. Multiple Detection Combinations in TS Specimens as Reported by the SPOTFIRE R/ST Panel Mini

Distinct Co-Detection Combinations			Total Specimens with Co-Detections	Number of Specimens with False Positive Co-Detections	False Positive Organism(s)[a]
Analyte 1	**Analyte 2**	**Analyte 3**			
Human rhinovirus	Respiratory syncytial virus	*S. pyogenes*	1	0	-
Human rhinovirus	Influenza A virus		2	1	Human rhinovirus
Human rhinovirus	Respiratory syncytial virus		6	1	Respiratory syncytial virus
Human rhinovirus	*S. pyogenes*		57	19	Human rhinovirus (15), *S. pyogenes* (6)
Influenza A virus	Respiratory syncytial virus		1	0	-
Influenza A virus	*S. pyogenes*		2	1	*S. pyogenes*
Influenza B virus	*S. pyogenes*		2	0	-
Respiratory syncytial virus	*S. pyogenes*		1	1	*S. pyogenes*
Total Co-Detections			**72**	**23**	**25/145[b]**
Total Double Detections			**71**	**23**	**25/142**
Total Triple Detections			**1**	**0**	**0/3**

[a] FP *Streptococcus* results based on culture comparator method

[b] Of the 25 discrepant analytes (out of 145 total analytes), 12 (48.0%) were confirmed as being present in the specimen during discrepancy investigation

PERFORMANCE CHARACTERISTICS

Clinical Performance

Prospective Clinical Evaluation

The clinical performance (encompassing both accuracy and ease of use) of the SPOTFIRE R/ST Panel Mini was established during a prospective multi-center study that was further supplemented with archived and contrived specimens. Six geographically distinct urgent care or emergency department study sites representative of the intended use setting (five in the US and one in the UK) participated in these studies from December 2020 to September 2021 (NPS and TS specimens) and from September 2022 to May 2023 (TS specimens only). All SPOTFIRE R/ST Panel Mini testing was performed according to the manufacturer's instructions by operators with training and educational backgrounds representative of those in the CLIA-waived or near-patient testing setting. No hands-on training was provided to the SPOTFIRE R/ST Panel Mini test operators; rather, training was limited to written materials (i.e. quick reference guides) that were intended to be included with the BIOFIRE SPOTFIRE System.

A total of 1215 NPS specimens and 1165 TS specimens were enrolled from consented volunteers or obtained as residual leftover specimens from subjects of all ages for the prospective clinical study; 95 of these NPS specimens and 288 of these TS specimens were excluded. The most common reasons for specimen exclusion were that the SPOTFIRE R/ST Panel Mini pouch was later determined to be expired or the specimen was unable to be tested within the designated timeframe. The final data set consisted of 1120 NPS specimens and 877 TS specimens. Across the six study sites, 352 specimens (259 NPS specimens and 93 TS specimens) were initially collected and immediately frozen for later testing at the source study site. The remaining 1645 specimens (861 NPS specimens and 784 TS specimens) were collected and tested fresh (without freezing). No difference in performance was observed when fresh and frozen specimen results were compared. Therefore, the data collected from 352 valid frozen specimens are combined with data from the valid 1645 fresh specimens for all analyses.

Table 14 provides a summary of demographic information for the specimens included in the study.

Table 14. Demographic Summary for the SPOTFIRE R/ST Panel Mini Prospective Clinical Evaluation

Category		Prospective NPS Specimens	Prospective TS Specimens
Sex	Male	587 (52.4%)	361 (41.2%)
	Female	533 (47.6%)	516 (58.8%)
Age	≤5 years	457 (40.8%)	138 (15.7%)
	6-18 years	258 (23.0%)	456 (52.0%)
	19-40 years	160 (14.3%)	186 (21.2%)
	41-60 years	147 (13.1%)	72 (8.2%)
	61+ years	98 (8.8%)	25 (2.9%)
Total		**1120**	**877**

For most analytes, the performance of the SPOTFIRE R/ST Panel Mini was evaluated by comparing the test results with those from FDA-cleared multiplexed respiratory pathogen panels. The comparator method for *Streptococcus pyogenes* (group A Strep) was two analytically validated PCR assays followed by bidirectional sequencing (this analyte was not detected by the FDA-cleared panels). Additionally, *Streptococcus pyogenes* was also evaluated by culture isolation of beta-hemolytic streptococci followed by molecular species identification using PCR confirmed by bidirectional sequencing (performed on cultured isolate).

The performance for the prospective study is summarized in Table 15 and Table 16 for NPS and TS specimens, respectively. Sensitivity or positive percent agreement (PPA) for each analyte was calculated as 100% × (TP / (TP + FN)). True positive

(TP) indicates that both the SPOTFIRE R/ST Panel Mini and the comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the SPOTFIRE R/ST Panel Mini was negative while the comparator result was positive. Specificity or negative percent agreement (NPA) was calculated as 100% × (TN / TN + FP)). True negative (TN) indicates that both the SPOTFIRE R/ST Panel Mini and the comparator method had negative results, and false positive (FP) indicates that the SPOTFIRE R/ST Panel Mini was positive while the comparator method was negative. The exact binomial two-sided 95% confidence interval (95%CI) was calculated. Investigations of discrepant results are summarized in the footnotes.

Table 15. SPOTFIRE R/ST Panel Mini Prospective Clinical Performance Summary for NPS Specimens

SPOTFIRE R/ST Panel Mini R Menu Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Viruses						
Coronavirus SARS-CoV-2[a]	71/73	97.3	90.5-99.2%	1031/1037	99.4	98.7-99.7%
Human rhinovirus[b]	345/348	99.1	97.5-99.7%	695/767	90.6	88.3-92.5%
Influenza A virus	0/0	-	-	1115/1115	100	99.7-100%
Influenza B virus	0/0	-	-	1110/1110	100	99.7-100%
Respiratory syncytial virus[c]	26/27	96.3	81.7-99.3%	1086/1088	99.8	99.3-99.9%

[a] SARS-CoV-2 was detected in 1/2 FN specimens upon SPOTFIRE R/ST Panel Mini retest. SARS-CoV-2 was detected in 2/6 FP specimens using an additional molecular method.
[b] Human rhinovirus/enterovirus was detected in 1/3 FN specimens upon SPOTFIRE R/ST Panel Mini retest. Human rhinovirus/enterovirus was detected in 48/72 FP specimens using an additional molecular method.
[c] Respiratory syncytial virus was detected in the single FN specimen upon SPOTFIRE R/ST Panel Mini retest. Respiratory syncytial virus was detected in 1/2 FP specimens using an additional molecular method.

Table 16. SPOTFIRE R/ST Panel Mini Prospective Clinical Performance Summary for TS Specimens[a]

SPOTFIRE R/ST Panel Mini ST Menu Analyte		Sensitivity/PPA			Specificity/NPA		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Viruses							
Human rhinovirus[b]		202/213	94.8	91.0-97.1%	619/662	93.5	91.4-95.1%
Influenza A virus		35/35	100	90.1-100%	840/840	100	99.5-100%
Influenza B virus		4/4	100	51.0-100%	872/872	100	99.6-100%
Respiratory syncytial virus[c]		21/24	87.5	69.0-95.7%	849/851	99.8	99.1-99.9%
Bacteria							
Streptococcus pyogenes (group A Strep)	PCR[d]	209/217	96.3	92.9-98.1%	654/660	99.1	98.0-99.6%
	Culture[e]	174/177	98.3	95.1-99.4%	654/692	94.5	92.6-96.0%

[a] The performance measures of sensitivity and specificity only refer to the *Streptococcus* analyte for which culture was used as the reference method. Performance measures of PPA and NPA refer to all other analytes, for which molecular assays were used as comparator methods.
[b] Human rhinovirus was detected in 7/11 FN specimens using an additional molecular method. Human rhinovirus was detected in 14/43 FP specimens using an additional molecular method.
[c] Respiratory syncytial virus was detected in all three FN specimens upon SPOTFIRE R/ST Panel Mini retest. Respiratory syncytial virus was detected in 1/2 FP specimens using an additional molecular method.
[d] *S. pyogenes* was detected in 7/8 FN specimens during discrepancy investigation: four using an additional molecular method and three upon SPOTFIRE R/ST Panel Mini retest. *S. pyogenes* was detected in 2/6 FP specimens using an additional molecular method.
[e] *S. pyogenes* was detected in all three FN specimens during discrepancy investigation: one using an additional molecular method and two upon SPOTFIRE R/ST Panel Mini retest. *S. pyogenes* was detected in 34/38 FP specimens using an additional molecular method.

The overall success rate for initial specimen tests was 96.4% (2044/2120). Fourteen (14) tests (14/2120; 0.7%) did not complete on the initial test attempt, resulting in an instrument success rate of 99.3% (2106/2120) for initial specimen tests. Retests were not possible due to insufficient specimen volume. Of the 2106 tests that successfully produced a completed run on the initial test, 2044 had valid internal pouch controls. This represents a 97.1% (2044/2106) success rate for internal pouch controls in completed runs in the initial specimen tests.

Testing of Preselected Archived Specimens

A number of analytes on the SPOTFIRE R/ST Panel Mini were of low prevalence during the prospective study and were not encountered in large enough numbers to adequately demonstrate system performance. To supplement the results of the prospective clinical study, an evaluation of preselected archived retrospective NPS and TS specimens was performed.

A total of 562 frozen archived NPS specimens and 136 frozen archived TS specimens were obtained from 15 external laboratories world-wide and retrospectively tested at the four US clinical sites. Of these, 542 NPS specimens and 128 TS specimens had valid results that were included in performance analysis. The analyte composition of the archived specimens was confirmed using the same comparator methods as the prospective study (described above) for the analyte result to be included in the performance analysis.

The specimens were randomized such that the users performing both the confirmation and the SPOTFIRE R/ST Panel Mini testing were blinded to the expected test result. A summary of the available demographic information of the tested specimens is provided in Table 17, and the results of the SPOTFIRE R/ST Panel Mini performance for these archived NPS and TS specimens are shown in Table 18 and Table 19.

Table 17. Demographic Summary for Valid Archived NPS Specimens

Category		Archived NPS Specimens	Archived TS Specimens
Sex	Male	254 (46.9%)	59 (46.1%)
	Female	185 (34.1%)	69 (53.9%)
	Unknown	103 (19.0%)	0 (0%)
Age	≤5 years	234 (43.2%)	22 (17.2%)
	6-18 years	98 (18.1%)	85 (66.4%)
	19-40 years	36 (6.6%)	16 (12.5%)
	41-60 years	35 (6.5%)	5 (3.9%)
	61+ years	39 (7.2%)	0 (0%)
	Unknown	100 (18.5%)	0 (0%)
Total		**542**	**128**

Table 18. SPOTFIRE R/ST Panel Mini Archived Performance Summary for NPS Specimens

SPOTFIRE R/ST Panel Mini R Menu Result	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses						
Coronavirus SARS-CoV-2	0/0	-	-	0/0	-	-
Human rhinovirus[a]	29/30	96.7	83.3-99.4%	439/454	96.7	94.6-98.0%
Influenza A virus[b]	58/59	98.3	91.0-99.7%	423/423	100	99.1-100%
Influenza B virus	30/30	100	88.6-100%	28/28	100	87.9-100%
Respiratory syncytial virus[c]	37/37	100	90.6-100%	440/447	98.4	96.8-99.2%

[a] The single FN specimen was unable to be investigated. Human rhinovirus was detected in 4/14 FP specimens during discrepancy investigation using an additional molecular method; one additional FP specimen was unable to be investigated.
[b] Influenza A virus was detected in the single FN specimen by standard of care.
[c] Respiratory syncytial virus was detected in 4/6 FP specimens during discrepancy investigation using an additional molecular method; one additional FP specimen was unable to be investigated.

Table 19. SPOTFIRE R/ST Panel Mini Archived Performance Summary for TS Specimens

SPOTFIRE R/ST Panel Mini ST Menu Result	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses						
Human rhinovirus[a]	2/2	100	34.2-100%	55/57	96.5	88.1-99.0%

SPOTFIRE R/ST Panel Mini ST Menu Result	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Influenza A virus	11/11	100	74.1-100%	44/44	100	92.0-100%
Influenza B virus	20/20	100	83.9-100%	0/0	-	-
Respiratory syncytial virus[b]	2/2	100	34.2-100%	56/57	98.2	90.7-99.7%
Bacteria						
Streptococcus pyogenes (group A Strep)[c]	38/39	97.4	86.8-99.5%	10/10	100	72.2-100%

[a] Human rhinovirus both FP specimens were unable to be investigated.
[b] Respiratory syncytial virus was detected in the single FP specimen by standard of care.
[c] *Streptococcus pyogenes* was detected in the single FN by standard of care.

Contrived Testing

Several analytes were not observed in TS specimens in sufficient numbers to demonstrate SPOTFIRE R/ST Panel Mini performance in the prospective and archived specimen studies. Therefore, contrived specimens (N=431; at least 50 for each analyte) were made from unique, analyte-negative clinical TS specimens that were spiked with a variety of different isolates/strains for each organism at concentrations that spanned the detection range of each assay. At least half (50%) of the contrived specimens had analyte concentrations at 2 × the limit of detection (LoD). Contrived specimens were randomized and coded along with 29 negative (unspiked) specimens such that the analyte status of each specimen was unknown to the users performing the testing. The coded contrived specimens were distributed to prospective clinical study sites for testing.

The results of the SPOTFIRE R/ST Panel Mini performance for contrived TS specimens are shown in Table 20.

Table 20. BIOFIRE SPOTFIRE R/ST Panel Mini Contrived TS Specimen Performance Summary

SPOTFIRE R/ST Panel Mini ST Menu Result	PPA			NPA		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses						
Influenza A virus	93/93	100	96.0-100%	332/332	100	98.9-100%
Influenza B virus	47/49	95.9	86.3-98.9%	333/333	100	98.9-100%
Respiratory syncytial virus	49/50	98.0	89.5-99.6%	381/381	100	99.0-100%

ANALYTICAL PERFORMANCE CHARACTERISTICS

Limit of Detection

The limit of detection (LoD) for SPOTFIRE R/ST Panel Mini analytes was estimated by testing dilutions of contrived samples containing known concentrations of organism in both VTM and Amies media. The LoD concentrations were confirmed by testing at least 20 replicates at the estimated LoD. Confirmation of LoD required detection in at least 95% of replicates tested. The confirmed LoD concentrations for the SPOTFIRE R/ST Panel Mini are listed in Table 21.

Table 21. Limit of Detection (LoD) for the SPOTFIRE Respiratory/Sore Throat (R/ST) Panel Mini Analytes

Analyte	Isolate Source ID	LoD Concentration[a]
Viruses		
Coronavirus SARS-CoV-2[c]	USA-WA1/2020 (heat inactivated) ATCC VR-1986HK	**1.1E-01 $TCID_{50}$/mL** (2.5E+02 copies/mL)
Human rhinovirus	Human rhinovirus 1A ZeptoMetrix 0810012CFN	**2.1E-01 $TCID_{50}$/mL** (1.1E+00 copies/mL)
	Enterovirus D68 US/MO/14-18947 ATCC VR-1823[b]	**1.1E+01 $TCID_{50}$/mL** (5.4E+01 copies/mL)
Influenza A virus	Influenza A H1N1 pdm A/Michigan/45/15 ZeptoMetrix 0810538CF	**8.2E-01 $TCID_{50}$/mL** (9.2E+03 copies/mL)
	Influenza A H3N2 A/Hong Kong/4801/14 ZeptoMetrix 0810526CF	**8.6E-01 $TCID_{50}$/mL** (3.4E+02 copies/mL)
Influenza B virus	B/Florida/02/06 (Victoria Lineage) ZeptoMetrix 0810037CF	**3.3E-02 $TCID_{50}$/mL** (1.6E+02 copies/mL)
	B/Nevada/03/2011 (Victoria Lineage) BEI NR-44023	**1.6E+00 $CEID_{50}$/mL** (4.3E+00 copies/mL)
	B/Florida/04/06 (Yamagata Lineage) ZeptoMetrix 0810255CF	**4.0E-01 $TCID_{50}$/mL** (3.2E+01 copies/mL)
Respiratory syncytial virus	Type A 2006 ZeptoMetrix 0810040ACF	**6.2E-02 $TCID_{50}$/mL** (2.2E+01 copies/mL)
	Type B 3/2015 Isolate #1 ZeptoMetrix 0810479CF	**2.8E-02 $TCID_{50}$/mL** (2.4E+01 copies/mL)
***Streptococcus pyogenes* (group A Strep)[d]**	SF-130 T1 ATCC 12344	**4.5E+02 cells/mL** (2.9E+03 copies/mL)

[a] LoD concentration may vary from what is listed based on the accuracy and precision of the quantification method.
[b] Due to the genetic similarity between human rhinovirus and enterovirus, the SPOTFIRE R/ST Panel Mini cannot differentiate them and will report both as Human rhinovirus.
[c] Results only reported when the Nasopharyngeal Swab sample type is selected.
[d] Results only reported when the Throat Swab sample type is selected.

NOTE: LoD concentrations in copies/mL in Table 21 above are based on extraction of nucleic acids from isolate cultures followed by quantitative real-time PCR (qPCR) or digital PCR (dPCR). The accuracy of concentrations may be affected by extraction efficiency, standard curve accuracy (qPCR only), assay conditions, inhibitors, and/or sequence variance. The 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) or $CEID_{50}$ (50% Chicken Embryo 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 and $CEID_{50}$/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 or $CEID_{50}$/mL.

Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the SPOTFIRE R/ST Panel Mini assays was assessed by testing viral and bacterial isolates that represented clinical and genetic diversity and included the available phylogenetic, geographic, and temporal diversity of each SPOTFIRE R/ST Panel Mini analyte. Isolates were tested in triplicate at concentrations near the LoD.

A summary of analytical reactivity is included in Table 22 to Table 27.

NOTE: Influenza A assays will react variably with non-human influenza A viruses and rarely encountered human influenza A viruses (that are not H1-2009 or H3), generally producing an Uncertain: Influenza A virus result.

NOTE: The SPOTFIRE R/ST Panel Mini 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, influenza B, and poliovirus (Human rhinovirus). 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 22. Summary of Reactivity to Coronavirus SARS-CoV-2 Isolates

Type	Source/Isolate ID	Strain/Location/Year	Result
SARS-CoV-2 [a]	ATCC VR-1986HK	[USA-WA1/2020]	**Coronavirus SARS-CoV-2 Positive**
	ATCC VR-1991D	[Hong Kong/VM20001061/2020]	
	ATCC VR-1992D	[2019-nCoV/Italy-INMI1]	
	ATCC VR-1994D	[Germany/BavPat1/2020]	
	ATCC VR-3326D	[USA/CA_CDC_5574/2020]	
	BEI NR-52499 [b]	[England/02/2020]	
	BEI NR-52501 [c]	[Singapore/2/2020]	
	BEI NR-52503 [d]	[USA-IL1/2020]	
	BEI NR-52505 [e]	[USA-AZ1/2020]	
	BEI NR-52507 [f]	[USA-CA3/2020]	
	BEI NR-52510 [g]	[Chile/Santiago_op4d1/2020]	
	BEI NR-53518 [h]	[New York-PV08410/2020]	
	LGC SeraCare AccuPlex™ 0505-0298 [i]	[Omicron B.1.1.529 Variant]	

[a] See Table 28 for additional SARS-CoV-2 reactivity predictions based on *in silico* analysis.
[b] The following reagent was deposited by Professor Maria Zambon and obtained through BEI Resources, NIAID, NIH: Genomic RNA from SARS-Related Coronavirus 2, Isolate England/02/2020, NR-52499.
[c] The following reagent was contributed by Duke-National University of Singapore, Programme in Emerging Infectious Diseases for distribution through BEI Resources, NIAID, NIH: Genomic RNA from SARS-Related Coronavirus 2, Isolate Singapore/2/2020, NR-52501.
[d] The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-IL1/2020, NR-52503.
[e] The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-AZ1/2020, NR-52505.
[f] The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-CA3/2020, NR-52507.
[g] The following reagent was obtained through BEI Resources, NIAID, NIH: Genomic RNA from SARS-Related Coronavirus 2, Isolate Chile/Santiago_op4d1/2020, NR-52510.
[h] The following reagent was obtained through BEI Resources, NIAID, NIH: Genomic RNA from SARS-Related Coronavirus 2, Isolate New York-PV08410/2020, NR-53518.
[i] Recombinant alphavirus that contains the full SARS-CoV-2 genome with mutations identified in the S and N gene of the SARS-CoV-2 Omicron variant B.1.1.529.

Table 23. Summary of Reactivity to Human Rhinovirus and Enterovirus Isolates

Species	Serotype	Source/Isolate ID	[Strain/Location/Year]	Result
Human rhinovirus				
A	1	ZeptoMetrix 0810012CFN	[1A]	**Human rhinovirus Positive**
	77	ATCC VR-1187	[130-63]	
	85	ATCC VR-1195	[50-525-CV54]	
	34	ATCC VR-1365	[137-3]	
	57	ATCC VR-1600	[Ch47]	

Species	Serotype	Source/Isolate ID	[Strain/Location/Year]	Result
	7	ATCC VR-1601	[68-CV11]	
	16	ATCC VR-283	[11757]	
	2	ATCC VR-482	[HGP]	
B	17	ATCC VR-1663	[33342]	
	14	ATCC VR-284	[1059]	
	42	ATCC VR-1950	[56822]	
	3	ATCC VR-483	[FEB]	
	27	ATCC VR-1137	[5870]	
	83	ATCC VR-1193	[Baylor 7]	
Enterovirus				
A	Enterovirus 71	ATCC VR-1432	[71 H]	
	Coxsackievirus 10	ATCC VR-168	[NY/1950]	
B	Coxsackievirus 9	ZeptoMetrix 0810017CF	-	
	Echovirus 11	ZeptoMetrix 0810023CF	-	
	Coxsackievirus B3	ZeptoMetrix 0810074CF	-	
	Coxsackievirus B4	ZeptoMetrix 0810075CF	-	
	Echovirus 6	ZeptoMetrix 0810076CF	-	
	Echovirus 9	ZeptoMetrix 0810077CF	-	
C	Coxsackievirus A24	ATCC VR-583	[DN-19/TX/1963]	
	Coxsackievirus A21	ATCC VR-850	[Kuykendall/CA/1952]	
D	Enterovirus D68	ATCC VR-1823	[US/MO/14-18947]	

Human rhinovirus Positive

Table 24. Summary of Reactivity to Influenza A Isolates

Type	Host	Source/Isolate ID	Strain/Location/Year	Result
H1N1pdm09	Human	ZeptoMetrix 0810538CF	[Michigan/45/15]	
		BEI NR-19823	[Netherlands/2629/2009]	
		BEI NR-42938	[Georgia/F32551/2012]	
		BEI NR-44345	[Hong Kong/H090-761-V1(0)/2009]	
		ZeptoMetrix 0810109CFJ	[Canada/6294/2009]	
		ZeptoMetrix 0810165CF	[California/07/2009]	
		ZeptoMetrix 0810166CF	[Mexico/4108/2009]	
		ZeptoMetrix 0810249CF	[SwineNY/03/2009]	
H3N2	Human	ATCC VR-544	[Hong Kong/8/1968]	
		ATCC VR-547	[Aichi/2/1968]	
		ATCC VR-776	[Alice]	
		ATCC VR-810	[Port Chalmers/1/1973]	
		ATCC VR-822	[Victoria/3/1975]	
		ZeptoMetrix 0810138CF	[Brisbane/10/2007]	
		ZeptoMetrix 0810238CF	[Texas/50/2012]	
		ZeptoMetrix 0810252CF	[Wisconsin/67/2005]	
		ZeptoMetrix 0810526CF	[Hong Kong/4801/14]	
H1N1	Human	ZeptoMetrix 0810036CF	[New Caledonia/20/1999]	
		ZeptoMetrix 0810036CFN	[Solomon Islands/3/2006]	
		ZeptoMetrix 0810244CF	[Brisbane/59/2007]	
	Swine	ATCC VR-333	[Swine/Iowa/15/1930]	
		ATCC VR-897	[A/New Jersey/8/76]	
		ATCC VR-99	[Swine/1976/1931]	
H2N2	Human	BEI NR-2775 [a]	[A/Japan/305/1957]	
H5N3	Avian	BEI NR-9682 [b]	[A/Duck/Singapore/645/97]	
H1N2	Recombinant	BEI NR-3478 [c]	[Kilbourne F63 A/NWS/1934 (HA) x A/Rockefeller Institute/5/1957 (NA)]	
H10N7	Avian	BEI NR-2765 [d]	[A/Chicken/Germany/N/49]	

Influenza A virus Positive

Influenza A virus Uncertain

[a] The following reagent was obtained through BEI Resources, NIAID, NIH: Genomic RNA from Influenza A Virus, A/Japan/305/1957 (H2N2), NR-2775.
[b] The following reagent was obtained through BEI Resources, NIAID, NIH: Genomic RNA from Kilbourne F181: A/duck/Singapore/645/1997 (H5N3), Wild Type, NR-9682.
[c] The following reagent was obtained through BEI Resources, NIAID, NIH: Kilbourne F63: A/NWS/1934 (HA) x A/Rockefeller Institute/5/1957 (NA) (H1N2), Reassortant NWS-F, NR-3478.
[d] The following reagent was obtained through BEI Resources, NIAID, NIH: Genomic RNA from Influenza A Virus, A/chicken/Germany/N/1949 (H10N7), NR-2765.

Table 25. Summary of Reactivity to Influenza B Isolates

Lineage	Source/Isolate ID	[Strain/Location/Year]	Result
Yamagata	ZeptoMetrix 0810255CF	[Florida/04/06]	
	ZeptoMetrix 0810239CF	[2/Massachusetts/2012]	
	ZeptoMetrix 0810241CF	[1/Wisconsin/2010]	
	ZeptoMetrix 0810256CF	[07/Florida/2004]	
Victoria	ZeptoMetrix 0810037CF	[B/Florida/02/06]	

Influenza B virus Positive

Lineage	Source/Isolate ID	[Strain/Location/Year]	Result
	BEI NR-44023	[B/Nevada/03/2011]	
	ATCC VR-823	[5/Hong Kong/1972]	
	CDC 2005743348	[1/Ohio/2005]	
	ZeptoMetrix 0810258CF	[2506/Malaysia/2004]	
Unknown	ATCC VR-101	[Lee/1940]	
	ATCC VR-102	[Allen/1945]	
	ATCC VR-103	[GL/1739/1954]	
	ATCC VR-295	[2/Taiwan/1962]	
	ATCC VR-296	[1/Maryland/1959]	
	ATCC VR-786	[Brigit/Russia/1969]	

Table 26. Summary of Reactivity to Respiratory Syncytial Virus Isolates

Type	Source/Isolate ID	Strain/Location/Year	Result
A	ZeptoMetrix 0810040ACF	[2006]	**Respiratory syncytial virus Positive**
	ATCC VR-26	[Long/Maryland/1956]	
	ATCC VR-1540	[A2/Melbourne/1961]	
	ZeptoMetrix 0810474CF	[2/2015 Isolate #2]	
	ZeptoMetrix 0810452CF	[12/2014 Isolate #2]	
B	ZeptoMetrix 0810479CF	[3/2015 Isolate #1]	
	ZeptoMetrix 0810040CF	[Ch-93 (18)-18]	
	ATCC VR-1400	[WV/14617/1985]	
	ATCC VR-955	[9320/Massachusetts/1977]	
	ATCC VR-1580	[18537/WashingtonDC/1962]	
	ZeptoMetrix 0810451CF	[11/2014 Isolate #2]	

Table 27. Summary of Reactivity to Streptococcus pyogenes Isolates

Source ID	[Strain/Location/Year]	Result
ATCC 12344	[SF-130 T-type 1]	***Streptococcus pyogenes*** **(group A Strep) Positive**
ATCC BAA-947	[MGAS 5005 M-type 1]	
ATCC 700294	[SF370 M1 GAS M-type 1]	
ATCC 12384	[C203 T-type 3]	
ATCC BAA-595	[MGAS 315, M type 3]	
ATCC 51500	[DLS 88002, Weller], M type 3]	
NCTC 8193	[T5 B, M type 5]	
ATCC 12348	[S43 T-type 6]	
ATCC BAA-1065	[MGAS 2096 [A374], M type 12]	
ATCC 12356	[Typing strain J17E [A. Coburn R9], M type 17]	
ATCC BAA-572	[MGAS 8232, M type 18]	
ATCC 8133	[Typing strain T23 [F. Griffith strain Barts 102], M type 23]	
ATCC 12360	[Typing strain J17F [A. Coburn R17], M type 26]	
NCTC 8310	[Coggins, M type 29]	
NCTC 8195	[Quinn (D24/46), M type 30]	
NCTC 8229	[C 95/12B, M type 39]	
ATCC 12372	[Typing strain C143 [C143], M type 40]	
ATCC 12377	[C105 [20RS14], M type 46]	
NCTC 10880	[R66/3489 (378), M type 62]	
ATCC 19615	[Bruno]	
ATCC 49399	[AC A62]	
ATCC 700466	CDC-SS-872 [R67/3884]	
ATCC 25663	[P20080]	
ATCC 49117	[397]	
ATCC 21060 [a]	[Su]	***Streptococcus pyogenes*** **(group A Strep) Negative**

[a] A deletion in the gene target was identified that prevents amplification/detection of this isolate.

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

Evaluation of analytical reactivity for the SPOTFIRE R/ST Panel Mini SARS-CoV-2 assays (SARS-CoV2-1 and SARS-CoV2-2) was based on *in silico* analysis of all available sequences in the GISAID database as of January 21, 2024.

This analysis determined that the >99.99% of 13,722,765 sequences will be detected by one or both SPOTFIRE R/ST Panel Mini SARS-CoV-2 assays based on homology and mismatch location with one or both sets of primers. A limitation on

detection (both assays impaired) is predicted for less than 0.006% of the sequences evaluated (828/13,722,765) (Table 28).

The sequences evaluated include lineages and variants of concern (VOC) or variants under investigation (VUI) that may have important epidemiological, immunological, or pathogenic properties from a public health perspective, such as Delta and Omicron variants. Variants evaluated are listed in the *BioFire® Respiratory Panels SARS-CoV-2 Reactivity Tech Note* technical note at www.biofiredx.com/support/documents.

All lineages and variants of public health interest identified as of January 2024 are predicted to be detected; new sequences and variants will continue to be monitored for impacts on detection by the SPOTFIRE R/ST Panel Mini assays.

Table 28. *In silico* Prediction of SARS-CoV-2 Detection by SPOTFIRE R/ST Panel Mini 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

***In silico* prediction**				
Predicted Assay Result		**SARS-CoV2-1**		**Total Sequences**
Number of Sequences		**+**	**-**	
SARS-CoV2-2	**+**	13,463,727	203,941	13,721,937/13,722,765
	-	54,269	828	99.994%

Periodic updates to the *in silico* analysis are performed based on the most currently available GISAID sequences and can also be accessed in the BioFire® Respiratory Panels SARS-CoV-2 Reactivity Tech Note at www.biofiredx.com/support/documents.

Analytical Specificity (Cross-Reactivity & Exclusivity)

The potential for non-specific amplification and detection by the SPOTFIRE R/ST Panel Mini assays was evaluated by in silico analysis of available sequences and by testing of high concentrations of on-panel and off-panel organisms. The organisms evaluated included relevant bacteria, fungi, and viruses that are either phylogenetically related to organisms detected by the SPOTFIRE R/ST Panel Mini or pathogenic/commensal organisms that may be present in NPS or TS specimens. Each organism was tested in triplicate at the highest possible concentration (generally ≥1.0E+07 units/mL for bacteria and ≥1.0E+05 units/mL for viruses).

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 cross-reactivity of the Human rhinovirus assay with *Bordetella* and picornavirus species. A summary of potential cross-reactivity is provided in Table 29. The on-panel and off-panel isolates and concentrations tested are listed in Table 30 and Table 31, respectively.

Table 29. Predicted and Observed Cross-Reactivity of the SPOTFIRE R/ST Panel Mini

Cross-Reactive Organism/Sequence	SPOTFIRE R Panel Mini Analyte Result	Description
Bat coronavirus **Pangolin coronavirus** **Bat SARS-like coronavirus**	**Coronavirus SARS-CoV-2**[a]	The SARS-CoV-2 assays can amplify select sequences from closely related sarbecoviruses isolated from bats and pangolin.
Bordetella bronchiseptica[b] ***Bordetella parapertussis***[b] ***Bordetella pertussis***	**Human rhinovirus**	The Human rhinovirus assay may amplify the oxidoreductase gene from *Bordetella* species (*B. pertussis, B. parapertussis,* and *B. bronchiseptica*) when organisms are present at a high concentration. Cross-reactivity with *B. pertussis* was observed at a concentration of ≥1.3E+10 CFU/mL.
Bovine picornavirus **Canine picornavirus**	**Human rhinovirus**[a]	Bovine and canine picornaviruses may be detected and reported as Human rhinovirus when present at high concentration.

[a] Indicated cross-reactivity is predicted based on *in silico* analysis.
[b] Cross-reactivity between the HRV/EV assay and *B. bronchiseptica* and *B. parapertussis* is predicted based on *in silico* analysis but was not observed when testing organisms at the highest possible concentrations (8.3E+09 cells/mL for *B. bronchiseptica* and 4.6E+09 CFU/mL for *B. parapertussis*).

Table 30. Summary of Results for On-Panel Organisms Tested During Evaluation of Analytical Specificity of the SPOTFIRE R/ST Panel Mini

Organism	Isolate ID	Concentration Tested	Observed Cross-Reactivity
Bacteria			
Streptococcus pyogenes	ATCC 12344	3.4E+08 cells/mL	None
Viruses			
Coronavirus SARS-CoV-2 (heat-inactivated)	ATCC VR-1986HK	7.6E+07 copies/mL	None
Enterovirus D68	ATCC VR-1823	1.6E+07 $TCID_{50}$/mL	None
Human rhinovirus A1	ZeptoMetrix 0810012CFN	1.3E+06 $TCID_{50}$/mL	None
Influenza A H1N1pdm09	ZeptoMetrix 0810538CF	1.4E+05 $TCID_{50}$/mL	None
Influenza A H3N2	ZeptoMetrix 0810526CF	7.2E+05 $TCID_{50}$/mL	None
Influenza B (Victoria Lineage)	BEI NR-44023	2.8E+08 $CEID_{50}$/mL	None
	ZeptoMetrix 0810037CF	2.5E+05 $TCID_{50}$/mL	None
Influenza B (Yamagata Lineage)	ZeptoMetrix 0810256CF	2.1E+04 $TCID_{50}$/mL	None
Respiratory syncytial virus A	ZeptoMetrix 0810040ACF	4.2E+05 $TCID_{50}$/mL	None
Respiratory syncytial virus B	ZeptoMetrix 0810479CF	4.2E+05 $TCID_{50}$/mL	None

Table 31. Summary of Results for Off-Panel Organisms Tested During Evaluation of Analytical Specificity of the SPOTFIRE R/ST Panel Mini

Organism	Isolate ID	Concentration Tested	Observed Cross-Reactivity
Bacteria			
Arcanobacterium bernardiae	ATCC BAA-441	1.6E+09 cells/mL	None
Arcanobacterium haemolyticum	ATCC 9345	1.5E+08 cells/mL	None
Arcanobacterium pyogenes	ATCC 49698	6.7E+09 cells/mL	None
Bacillus cereus	ATCC 7064	8.3E+09 cells/mL	None
Bordetella bronchiseptica	ATCC 10580	8.3E+09 cells/mL	None
	ATCC 4617	7.9E+09 cells/mL	None
	ATCC 19395	7.9E+09 cells/mL	None
	NRRL B-59914	7.1E+09 cells/mL	None
Bordetella holmesii	ATCC 700052	8.3E+09 cells/mL	None
Bordetella parapertussis	ZeptoMetrix 0801462	4.6E+09 CFU/mL	None
Bordetella pertussis	ZeptoMetrix 0801459	1.3E+10 CFU/mL	**Human rhinovirus**[a]
		1.3E+09 CFU/mL	None
Burkholderia cepacia	ATCC 51671	1.3E+09 CFU/mL	None
Campylobacter rectus	ATCC 33238	7.6E+07 cells/mL	None
Chlamydia pneumoniae	ATCC 53592	2.9E+07 IFU/mL	None
Chlamydia trachomatis	ZeptoMetrix 0801775	1.3E+08 IFU/mL	None
Corynebacterium diptheriae	ATCC 27010	8.0E+09 cells/mL	None
Corynebacterium pseudodiphtheriticum	ATCC 10700	8.7E+09 cells/mL	None
Enterococcus casseliflavus	ATCC 49605	8.0E+09 cells/mL	None
Enterococcus faecalis	ZeptoMetrix 0801637	8.0E+08 CFU/mL	None
Escherichia coli	ATCC BAA-2196	7.2E+09 cells/mL	None
Fusobacterium necrophorum ssp. *funduliforme*	ATCC 51357	4.4E+08 cells/mL	None
Fusobacterium nucleatum	ATCC 25586	4.9E+08 cells/mL	None
Fusobacterium varium	ATCC 27725	1.6E+08 cells/mL	None
Gemella haemolysans	ATCC 10379	4.0E+09 cells/mL	None
Gemella morbillorum	ATCC 27824	1.0E+08 cells/mL	None
Granulicatella adiacens	ATCC 49175	1.3E+09 cells/mL	None
Haemophilus influenzae	ATCC 10211	8.3E+09 cells/mL	None
Haemophilus parahaemolyticus	ATCC 49700	8.7E+09 cells/mL	None

Organism	Isolate ID	Concentration Tested	Observed Cross-Reactivity
Klebsiella pneumoniae	CDC AR#0115	7.3E+09 CFU/mL	None
Lactobacillus rhamnosus	ATCC 7469	7.9E+09 cells/mL	None
Lactococcus lactis	ATCC 29146	6.2E+09 cells/mL	None
Legionella pneumophila	ATCC 33215	7.0E+09 cells/mL	None
Leptotrichia buccalis	ATCC 14201	4.4E+08 cells/mL	None
Moraxella catarrhalis	ATCC 43627	7.2E+09 cells/mL	None
Mycobacterium tuberculosis	ZeptoMetrix 0801660	6.1E+06 CFU/mL	None
Mycoplasma buccale	Mycoplasma Experience NC10136	1.4E+07 CFU/mL	None
Mycoplasma faucium	Mycoplasma Experience NC10174	1.4E+06 CFU/mL	None
Mycoplasma fermentans	Mycoplasma Experience NC10117	2.8E+07 CFU/mL	None
Mycoplasma genitalium	Mycoplasma Experience NC10195	1.8E+06 CFU/mL	None
Mycoplasma hominis	Mycoplasma Experience NC10111	1.2E+07 CFU/ml	None
Mycoplasma lipophilum	Mycoplasma Experience NC10173	1.5E+06 CFU/mL	None
Mycoplasma orale	Mycoplasma Experience NC10112	2.2E+07 CFU/mL	None
Mycoplasma pneumoniae	ZeptoMetrix 0801579	2.5E+07 CCU/mL	None
Mycoplasma salivarium	Mycoplasma Experience NC10113	4.4E+06 CFU/mL	None
Neisseria elongata	ATCC 25295	8.5E+09 cells/mL	None
Neisseria gonorrhoeae	ZeptoMetrix 0801482	4.9E+07 CFU/mL	None
Neisseria lactamica	ATCC 23971	2.7E+09 cells/mL	None
Neisseria meningitidis	ATCC 13113	7.4E+09 cells/mL	None
Mycoplasma pneumoniae	ZeptoMetrix 0801579	2.5E+07 CCU/mL	None
Neisseria sicca	ATCC 9913	7.2E+09 cells/mL	None
Neisseria subflava	ATCC 49275	8.0E+09 cells/mL	None
Parvimonas micra[b]	ATCC 33270	6.0E+07 cells/mL	None
Pneumocystis carinii	ATCC PRA-159	1.0E+07 nuclei/mL	None
Porphyromonas endodontalis	ATCC 35406	1.6E+07 cells/mL	None
Porphyromonas gingivalis	ATCC BAA-308	5.0E+08 cells/mL	None
Prevotella histicola	BEI HM-471	9.0E+08 cell/mL	None
Prevotella melaninogenica	ATCC 25845	6.9E+08 cells/mL	None
Prevotella oralis	ATCC 33322	6.2E+08 cells/mL	None
Pseudomonas aeruginosa	CDC AR#0092	8.3E+09 cells/mL	None
Rhodococcus equi	ATCC 33706	7.3E+09 cells/mL	None
Serratia marcescens	ATCC 27137	8.9E+09 cells/mL	None
Staphylococcus aureus	ATCC BAA-1700	7.4E+09 cells/mL	None
Staphylococcus epidermidis	ATCC 12228	8.0E+09 cells/mL	None
Staphylococcus haemolyticus	ATCC 29968	8.0E+09 cells/mL	None
Staphylococcus intermedius	ATCC 29663	8.2E+09 cells/mL	None
Staphylococcus saprophyticus	ATCC 15305	8.1E+09 cells/mL	None
Streptococcus agalactiae	ATCC 13813	6.0E+09 cells/mL	None
Streptococcus anginosus	ATCC 700231	7.1E+09 cells/mL	None
Streptococcus constellatus ssp. *pharyngis*	NCTC 13122	5.6E+08 cells/mL	None
Streptococcus dysgalactiae ssp. *dysgalactiae*	ATCC 43078	6.7E+09 cells/mL	None
	NCTC 4669	7.4E+09 cells/mL	None
	NCTC 4335	8.4E+09 cells/mL	None
	NCTC 4670	6.6E+09 cells/mL	None
	CCUG 27665	7.4E+09 cells/mL	None
	CCUG 28112	6.7E+09 cells/mL	None
	CCUG 28114	7.5E+09 cells/mL	None
Streptococcus dysgalactiae ssp. *equisimilis*	ZeptoMetrix 0801516	7.8E+08 CFU/mL	None
	CCUG 28117	7.1E+09 cells/mL	None
	CCUG 27664	7.5E+09 cells/mL	None
	ATCC 10009	6.9E+09 cells/mL	None
Streptococcus gallolyticus	ATCC 43143	2.8E+09 cells/mL	None
Streptococcus gordonii	ATCC 10558	4.5E+09 cells/mL	None
Streptococcus intermedius	ATCC 27335	2.9E+09 cells/mL	None
Streptococcus mitis	ATCC 15914	3.2E+09 cells/mL	None
Streptococcus mutans	ATCC 25175	2.3E+09 cells/mL	None
Streptococcus oralis	ATCC 10557	1.1E+09 cells/mL	None
Streptococcus parasanguinis	ATCC 15912	7.8E+09 cells/mL	None

Organism	Isolate ID	Concentration Tested	Observed Cross-Reactivity
Streptococcus pneumoniae	ATCC 49619	2.5E+08 cells/mL	None
Streptococcus salivarius	ATCC 13419	6.6E+09 cells/mL	None
Streptococcus sanguinis	ATCC 10556	1.1E+09 cells/mL	None
Tannerella forsythia	ATCC BAA-2717	2.6E+08 cells/mL	None
Treponema denticola	ATCC 33520	2.2E+08 cells/mL	None
Ureaplasma urealyticum	ATCC 27618	5.7E+07 cells/mL	None
Veillonella parvula	ATCC 10790	4.7E+08 cells/mL	None
Fungi			
Candida albicans	ATCC MYA-2876	2.8E+08 cells/mL	None
Saccharomyces cerevisiae	ATCC 18824	1.9E+08 cells/mL	None
Viruses			
Adenovirus A	ZeptoMetrix 0810073CF	1.4E+05 $TCID_{50}$/mL	None
Adenovirus B	ZeptoMetrix 0810062CF	1.2E+07 $TCID_{50}$/mL	None
Adenovirus C	ZeptoMetrix 0810110CF	2.2E+06 $TCID_{50}$/mL	None
Adenovirus D	ZeptoMetrix 0810119CF	1.7E+05 $TCID_{50}$/mL	None
Adenovirus E	ZeptoMetrix 0810070CF	1.4E+05 $TCID_{50}$/mL	None
Adenovirus F	ZeptoMetrix 0810085CF	1.1E+06 $TCID_{50}$/mL	None
Coronavirus 229E	ATCC VR-740	8.9E+06 $TCID_{50}$/mL	None
Coronavirus HKU1	Clinical Specimens	4.5E+07 copies/mL	None
Coronavirus NL63	ZeptoMetrix 0810228CF	5.0E+05 $TCID_{50}$/mL	None
Coronavirus OC43	ZeptoMetrix 0810024CF	3.6E+05 $TCID_{50}$/mL	None
Cytomegalovirus	ZeptoMetrix 0810003CF	1.9E+05 $TCID_{50}$/mL	None
Epstein-Barr virus	ZeptoMetrix 0810008CF	5.9E+06 copies/mL	None
Human herpes simplex virus 1	ATCC VR-260	8.9E+06 $TCID_{50}$/mL	None
Human metapneumovirus A1	ZeptoMetrix 0810161CF	2.5E+05 $TCID_{50}$/mL	None
Human metapneumovirus A2	ZeptoMetrix 0810164CF	3.6E+05 $TCID_{50}$/mL	None
Human metapneumovirus B1	ZeptoMetrix 0810156CF	1.6E+04 $TCID_{50}$/mL	None
Human metapneumovirus B2	ZeptoMetrix 0810162CF	1.3E+06 $TCID_{50}$/mL	None
Measles virus	ZeptoMetrix 0810025CF	2.5E+05 $TCID_{50}$/mL	None
Middle east respiratory syndrome coronavirus (heat-inactivated)	ZeptoMetrix 0810575CFHI	1.2E+05 $TCID_{50}$/mL	None
Mumps virus	ZeptoMetrix 0810079CF	2.0E+06 $TCID_{50}$/mL	None
Parainfluenza virus 1	ZeptoMetrix 0810014CF	4.2E+05 $TCID_{50}$/mL	None
Parainfluenza virus 2	ZeptoMetrix 0810015CF	1.2E+07 $TCID_{50}$/mL	None
Parainfluenza virus 3	ZeptoMetrix 0810016CF	3.4E+07 $TCID_{50}$/mL	None
Parainfluenza virus 4	ZeptoMetrix 0810060CF	3.4E+07 $TCID_{50}$/mL	None
Severe acute respiratory syndrome coronavirus (purified genomic RNA)	BEI NR-52346	5.3E+05 genomes/mL	None

[a] The Human rhinovirus assay may amplify off-target sequences found in strains of *Bordetella* species (*B. pertussis, B. parapertussis,* and *B. bronchiseptica*) when present at a concentration ≥1.3E+10 CFU/mL.
[b] *Parvimonas micra* was formerly classified as *Micromonas micros* and *Peptostreptococcus micro*.

Near-LoD/Reproducibility Evaluation

A near-LoD/reproducibility evaluation was performed to demonstrate that the SPOTFIRE R/ST Panel Mini could reproducibly provide accurate results for weak-positive and negative samples when used by minimally trained operators. Contrived samples were tested at three of the prospective clinical study sites and additionally on three unique SPOTFIRE Systems at BioFire Diagnostics (BioFire) by trained BioFire personnel. The contrived samples contained combinations of SPOTFIRE R/ST Panel Mini analytes prepared at or near (1× to 3×) the LoD. For testing performed at clinical sites, samples were tested over five testing events (non-consecutive days) by two operators during the course of their normal workday routine. Each site was equipped with a single SPOTFIRE System. Testing at all three sites was performed with a single reagent lot. For each testing event, each operator ran two replicate pouches for a total of 20 replicates per site and 60 total replicates across all three sites. For testing performed at BioFire, samples were tested over five consecutive days, by two operators per system, using three different reagent lots. Each day of testing, the two operators each tested three replicates on each system for a total of 30 replicates per system and 90 total replicates across all systems. When combined, each analyte was tested in a total of 150 replicates by at least 12 different operators across six different SPOTFIRE Systems.

A summary of results (percent (%) agreement with the expected positive or negative result) for each analyte (by site and system) is provided in Table 32. The SPOTFIRE R/ST Panel Mini reported the expected positive results for panel analytes in 98% -100% of samples and the expected negative results for all analytes in 100% of samples. Comparison of the positive percent agreement between user groups (99.8% for trained operators at BioFire versus 99.0% for minimally trained operators) demonstrates that the accuracy of the SPOTFIRE R/ST Panel Mini is not dependent upon the specific expertise of the user.

Table 32. Reproducibility of Results for SPOTFIRE R/ST Panel Mini and SPOTFIRE System

Analyte Isolate (Source ID)		Concentration Tested (test level)	Expected Result	SpotFire System testing								All Sites /Systems [95% Confidence Interval]
				BioFire Dx				Clinical				
				System A	System B	System C	Total	Site 1	Site 2	Site 3	Total	
Coronavirus SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus 2 (ATCC VR-1986HK)		**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600 100%** [99.4-100%]
		2.5E+02 copies/mL (1× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**150/150 100%** [97.6-100%]
Human rhinovirus Enterovirus D68 US/MO/14-18947 (ATCC VR-1823)		**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600 100%** [99.4-100%]
		1.1E+01 $TCID_{50}$/mL (1× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**150/150 100%** [97.6-100%]
Influenza A virus	**No Analyte**		**Negative**	90/90 (100%)	90/90 (100%)	90/90 (100%)	270/270 (100%)	60/60 (100%)	60/60 (100%)	60/60 (100%)	180/180 (100%)	**450/450 100%** [99.2-100%]
	Influenza A H1N1pdm (ZeptoMetrix 0810538CF)	2.5E+00 $TCID_{50}$/mL (3× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**150/150 100%** [97.6-100%]
	Influenza A H3N2 (ZeptoMetrix 0810526CF)	2.6E+00 $TCID_{50}$/mL (3× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90 (100%)**	19/20 (95.0%)	19/20 (95.0%)	20/20 (100%)	**58/60 (96.7%)**	**148/150 98.7%** [95.3-99.8%]
Influenza B virus (ZeptoMetrix 0810037CF)		**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600 100%** [99.4-100%]
		9.9E-02 $TCID_{50}$/mL (3× LoD)	**Positive**	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	**89/90 (98.9%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**149/150 99.3%** [96.3-100%]

Analyte Isolate (Source ID)	Concentration Tested (test level)	Expected Result	SpotFire System testing								All Sites /Systems [95% Confidence Interval]
			BioFire Dx				Clinical				
			System A	System B	System C	Total	Site 1	Site 2	Site 3	Total	
Respiratory syncytial virus (ZeptoMetrix 0810040ACF)	**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600 100%** [99.4-100%]
	6.2E-02 $TCID_{50}$/mL (1× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90 (100%)**	20/20 (100%)	19/20 (95.0%)	20/20 (100%)	**59/60 (98.3%)**	**149/150 99.3%** [96.3-100%]
Streptococcus pyogenes **(group A Strep)** (ATCC 12344)	**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	**240/240 (100%)**	**600/600 100% [99.4-100%]**
	1.4E+03 cells/mL (3× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90 (100%)**	20/20 (100%)	19/20 (95.0%)	20/20 (100%)	**59/60 (98.3%)**	**149/150 99.3% [96.3-100%]**
Total positive agreement (%) by system/user group			209/210 99.5%	210/210 100%	210/210 100%	**629/630 99.8%**	139/140 99.3%	137/140 97.9%	140/140 100%	**416/420 99.0%**	**1045/1050 99.5%** [98.9-99.9%]
Overall positive agreement (%) [95% Confidence Interval]											

Interference

Potentially interfering substances that could be present in NPS or TS specimens or may be introduced into specimens during collection or subsequent handling and testing were evaluated for their effect on SPOTFIRE R/ST Panel Mini performance. The substances tested included endogenous substances that may be found at normal or elevated levels in clinical specimens (e.g. blood, mucus/mucin, human genomic DNA), various commensal or infectious microorganisms, medications, washes or topical applications for the nasal passage or throat, 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 (3×) the LoD. The concentration of substance added to the samples was equal to or greater than the highest level expected to be in NPS or TS specimens.

Valid and accurate results were obtained for each sample containing substances and microorganisms at the concentrations listed in Table 33.

Table 33. Substances Tested on the SPOTFIRE R/ST Panel Mini - No Interference Observed

Substance Tested	Concentration Tested
Endogenous Substances	
Human Whole Blood (with Na Citrate)	10% (v/v)
Human Sputum/Mucus	1% (v/v)
Human Genomic DNA	20 ng/µL
Exogenous Substances[a]	
Promethazine hydrochloride	1.04 µmol/L (3.34E-04 mg/mL)
Acetaminophen (paracetamol)	1.0E+03 µmol/L (1.5E-01 mg/mL)
Acetylsalicylic acid (Aspirin)	167 µmol/L (3.0E-02 mg/mL)
Ibuprofen	1060 µmol/L (2.2E-01 mg/mL)
Albuterol sulfate (common ingredient in rescue inhalers)	0.188 µmol/L (5.4E-05 mg/mL)
Triple antibiotic ointment (neomycin/polymyxin B/bacitracin)	2% w/v
Mucinex® Severe Nasal Congestion Relief Clear & Cool Nasal Spray (Oxymetazoline hydrochloride 0.05%)	1% v/v
Saline nasal spray (sodium chloride 0.65%, disodium phosphate, phenylcarbinol, monosodium phosphate and benzalkonium chloride solution)	1% v/v
Vicks® VapoRub® Cough Suppressant Topical Analgesic (Camphor 4.8%, eucalyptus oil 1.2%, and menthol 2.6%)	1% w/v
Vaseline® Petroleum Jelly (100% white petrolatum)	1% w/v
Orajel™ (benzalkonium chloride 0.13%, benzocaine 20%, menthol 0.5%, zinc chloride 0.15%)	2% w/v
Chloraseptic® Sore Throat Spray (Phenol 1.4%)	1% v/v
Vicks® Formula 44™ DM (dextromethorphan hydrobromide 0.67 mg/mL, guaifenesin 13 mg/mL) (cough syrup)	1% v/v
Phenylephrine hydrochloride (common ingredient in nasal decongestants)	1% w/v
Nasal spray (fluticasone propionate 50 mcg)	1% v/v
Sucrets® Sore Throat (dyclonine hydrochloride 2.0 mg/lozenge)	1% w/v
Benadryl® Allergy Liqui-gels® (diphenhydramine hydrochloride 25 mg/capsule)	1% v/v
Zicam® Cold Remedy (Galphimia Glauca 4x, Luffa Operculata 4x, Sabadilla 4x)	1% v/v
Cold-eeze® (zinc gluconate 2.3%)	1% w/v
HALLS lozenge (menthol 5 mg/lozenge)	1% w/v
Listerine® Cool Mint® (menthol 0.042%, thymol 0.064%, methyl salicylate 0.06%, eucalyptol 0.092%)	6.5% v/v
Copenhagen® Snuff (Tobacco)	1% w/v
JUICE HEAD (30% propylene glycol, 70% vegetable glycerin) (e-juice)	1% v/v
Technique-Specific Substances	
Rayon swab (COPAN Diagnostics Inc.)	1 swab
Nylon flocked swab (COPAN Diagnostics Inc.)	1 swab
Polyester swab (COPAN Diagnostics Inc.)	1 swab
Calcium Alginate swab (Puritan®)	1 swab
Cary-Blair	90% v/v
Dulbecco's Modified Eagles-Medium (DMEM)	90% v/v
Hanks Balanced Salt Solution	100% v/v
0.9% Normal Saline	100% v/v
BD™ Universal Viral Transport	100% v/v
Remel MicroTest™ M4RT Tube w/o beads	100% v/v
Remel MicroTest™ M4 Tube w/o beads	90% v/v
Viral Preservative Media (VPM)	90% v/v

Substance Tested	Concentration Tested
Phosphate Buffered Saline (PBS)	90% v/v
PrimeStore® MTM Molecular Transport Media	90% v/v
Stuart Transport Medium	90% v/v
eNAT™ Molecular Transport Medium	90% v/v
Bleach	1% v/v, 2% v/v[b]
Ethanol	7% v/v
Disinfecting wipes (ammonium chloride)	0.25 – 0.5 inch square/sample
DNAZap™	1% v/v
RNaseZap™	1% v/v
Competing Microorganisms	
On-Panel	
Enterovirus D68	7.8 E+07 copies/mL
Respiratory syncytial virus A	1.5E+07 copies/mL
Streptococcus pyogenes	2.2E+08 copies/mL
Off-Panel	
Adenovirus A31	1.6E+07 copies/mL
Coronavirus 229E	1.5E+07 copies/mL
Cytomegalovirus (CMV)	4.2E+04 $TCID_{50}$/mL
Herpes simplex virus 1	9.0E+06 $TCID_{50}$/mL
Parainfluenza virus 3	8.0E+06 copies/mL
Bordetella pertussis	1.6E+09 copies/mL
Staphylococcus aureus	7.4E+08 CFU/mL
Streptococcus pneumoniae	2.5E+07 CFU/mL
Haemophilus influenzae	8.3E+08 CFU/mL
Candida albicans	2.8E+07 CFU/mL

[a] Nasal influenza vaccines (e.g. FluMist®) were not evaluated but are predicted to be reactive with the Influenza A and Influenza B assays.
[b] Incubation of sample with 1% (v/v) bleach for 15 minutes, 4 hours, or ~18.5-hour (overnight) or 2% (v/v) bleach for 15 minutes did not result in interference.

NOTE: Avoid contact between samples and bleach prior to testing (bleach can damage nucleic acids and prevent amplification and detection by the panel).

NOTE: Compatibility of the SPOTFIRE R/ST Panel Mini with NPS in PrimeStore® MTM has not been evaluated in the intended use setting. PrimeStore® MTM and 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 or TS specimens.

Carry-Over

When using sensitive molecular tests to analyze patient samples, cross-contamination, or carry-over, of analyte material between samples is a concern. This study evaluated the risk of sample-to-sample carry-over during pouch loading and testing for contrived liquid samples.

To simulate a high-risk scenario, sample loading alternated between samples with high levels of organism (N=5 for each organism; refer to Table 34 for test concentrations) and negative samples. All samples were loaded using the same Pouch Loading Station. Analyte positivity was evaluated for all pouches to determine the risk of false positive results due to sample-to-sample carry-over. No unexpected positive results were observed in this study.

The data support that sample-to-sample carry-over poses a negligible risk to the accuracy of the SPOTFIRE R/ST Panel Mini test results when the test is used according to the provided instructions.

Table 34: Test Concentration for Evaluation of Carry-over

Organism	Source ID	Concentration Tested (multiple of LoD)
Enterovirus	ATCC VR-1823	1.6E+07 $TCID_{50}$/mL (1,500,000×)
Influenza A H3N2	ZeptoMetrix 0810526CF	7.2E+04 $TCID_{50}$/mL (84,000×)
Coronavirus SARS-CoV-2	ATCC VR-1986HK	8.4E+02 $TCID_{50}$/mL (7,600×)

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
5.7.10 	Unique Device Identifier				
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
	A BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel Mini		The NOTE symbols explain how to perform the SPOTFIRE R/ST Panel Mini test more efficiently.		

APPENDIX B

Contact and Legal Information

Customer and Technical Support	
Reach Us on the Web http://www.BioFireDX.com **Reach Us by E-mail** BioFireSupport@biomerieux.com **Reach Us by Fax** (801) 588-0507	**Reach Us by Phone** 1-844-815-0363 (toll free) **Reach Us by Mail** 515 Colorow Drive Salt Lake City, UT 84108 USA
Or contact the local bioMérieux sales representative or an authorized distributor.	

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

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23. Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline M29.

REVISION HISTORY

Version	Revision Date	Description of Revision(s)
01	June 2024	Initial release.

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