TRAINING UPDATE

Lab Location:	SGMC & WOMC	**Date Distributed:**	8/24/2020
Department:	Core Lab	**Due Date:**	9/17/2020
		Implementation:	**8/31/2020**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

BioFire® FilmArray® Respiratory Panel 2.1 (RP2.1)

SGMC.M1013 v2

Description of change(s):

Note:

- The resulting changes **are already in effect** and are the same as reporting for QIAstat results

Section	Reason
10.4	Deleted reporting steps & added reference to addenda B
19	Added addenda B with current reporting process

This revised SOP will be implemented August 31, 2020

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	**BioFire® FilmArray® Respiratory Panel 2.1 (RP2.1)**
Prepared by	Ron Master Date: 5/29/2020
Owner	Ron Master Date: 5/29/2020

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
BioFire® Respiratory Panel 2.1 (RP2.1)	Multiplexed PCR / BioFire Torch Instrument	RVPNLS

Synonyms/Abbreviations
Respiratory pathogen panel, respiratory viral panel, RP2

Department
Microbiology

2. ANALYTICAL PRINCIPLE

The BioFire Respiratory Panel 2.1 (RP2.1) is a multiplexed nucleic acid test intended for use with the FilmArray® Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections. The following organism types and subtypes are identified using the BioFire RP2.1:

- Adenovirus
- Coronavirus 229E
- Coronavirus HKU1
- Coronavirus NL63
- Coronavirus OC43
- Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)
- Human Metapneumovirus
- Human Rhinovirus/Enterovirus
- Influenza A, including subtypes H1, H1-2009, and H3
- Influenza B
- Parainfluenza Virus 1
- Parainfluenza Virus 2
- Parainfluenza Virus 3
- Parainfluenza Virus 4
- Respiratory Syncytial Virus
- *Bordetella parapertussis* (IS*1001*)
- *Bordetella pertussis* (ptxP)
- *Chlamydia pneumoniae*
- *Mycoplasma pneumoniae*

The BioFire RP2.1 pouch contains two different assays for the detections of the SARS-CoV-2, the spike protein (S) gene and the membrane protein (M) gene. The BioFire FilmArray if either one or both assays is positive, the test report will show Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) as Detected. If both assays are negative, the test

report will show Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) as Not Detected.

The BioFire RP 2.1 pouch is a closed system disposable that stores all the necessary reagents for sample preparation, reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from multiple respiratory pathogens within a single NPS specimen. After sample collection, the user injects hydration solution and sample combined with sample buffer into the pouch, places the pouch into a FilmArray instrument, and starts a run. The entire run process takes about 45 minutes. Additional detail can be found in the appropriate FilmArray Operator's Manual.

During a run, the FilmArray® system:

- Lyses the sample by agitation (bead beading).
- Extracts and purifies all nucleic acids from the sample using magnetic bead technology.
- Performs nested multiplex PCR by:
- First performing reverse transcription and a single, large volume, massively-multiplexed reaction (PCR1)
    - Then performing multiple singleplex second-stage PCR reactions (PCR2) to amplify sequences within the PCR1 products
    - Uses endpoint melting curve data to detect and generate a result for each target on the BioFire RP2 array.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Nasopharyngeal swab (NPS) collected according to standard technique and immediately placed in Universal Transport Medium (UTM) or Viral Culture Medium (VCM).
Special Collection Procedures	Specimens should be sent to the laboratory immediately after collection.
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred	Nasopharyngeal swab (NPS) collected according to standard technique and immediately placed in 1-3 mL of transport medium
-Other Acceptable	None
Collection Container	Swab in transport medium

Criteria	
Volume - Optimum - Minimum	3 mL (3000 µL) 0.3 mL (300 µL)
Transport Container and Temperature	NP swab in transport medium (UTM or VCM) at room temperature
Stability & Storage Requirements	Room Temperature: 4 hours (15-25°C)
	Refrigerated: 3 days (2-8°C)
	Frozen: ≤ -15°C or ≤ -70°C for up to 30 days
Timing Considerations	Must test within 4 hours
Unacceptable Specimens & Actions to Take	• Any specimen, which does not meet the above criteria • Follow specimen rejection process
Compromising Physical Characteristics	Specimen leaking
Other Considerations	Nasopharyngeal specimens should not be centrifuged before testing.

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

4. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
BioFire® Respiratory Panel 2.1 (RP2.1) pouches	BioFire RP2.1 423738 – 30 test kit

4.2 Reagent Preparation and Storage

Assay Kit	
Kit Contents	Each kit contains sufficient reagents to test 30 specimens: Individually-packaged BioFire RP2.1 pouches • Single-use (1.0 mL) Sample Buffer ampoules • Single-use pre-filled (1.5 mL) Hydration Injection Vials (blue) • Single-use Sample Injection Vials (red) • Individually-packaged Transfer Pipettes All kit components should be stored and used together. Do not use components from one kit with those of another kit.

Storage	Store the test kit, including reagent pouches and buffers, at room temperature (15–25ºC). DO NOT REFRIGERATE. Avoid storage of any materials near heating or cooling vents or in direct sunlight
Stability	Unopened material is stable through the expiration date when stored at room temperature (15–25ºC). 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 60 minutes).
Preparation	See Section 8.1

5. CALIBRATORS/STANDARDS

N/A

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Maine Molecular RP2.1/RP2.1 *plus* Control Panel	Maine Molecular Quality Controls, Inc. / M441
External positive control	6 single use tubes
External negative control	6 single use tubes

6.2 Control Preparation and Storage

External Controls	Maine Molecular M441
Preparation	Allow to come completely to room temperature (18-25°C) Use the control as provided. **DO NOT DILUTE**. Immediately before use mix the control by first inverting several times followed by vortexing the tube for 3-5 seconds. Tap the tube several times on the bench to remove any control caught in the cap before opening the tube.
Storage/Stability	Frozen at -20°C or colder Unopened material is stable through the expiration date when stored frozen. Each control vial is single use, discard after use.

6.3 Frequency

External Controls are performed with each new kit lot number and shipment or reagents or every 31 days, whichever is more frequent.

Internal QC results are checked and recorded with patient test results. The FilmArray instrument will not report a patient result unless all internal controls yield acceptable results

6.4 Tolerance Limits and Criteria for Acceptable QC

A. Tolerance Limits

Tolerance Limits	
External Positive Control	Detected
External Negative Control	Not Detected
Internal Controls	
DNA Process Control	Passes if Meets the Assigned Acceptance Criteria
PCR2 Control	Passes if Meets the Assigned Acceptance Criteria

- **If the internal control result is Failed, then the results for all of the tests on the panel are displayed as Invalid and the sample will need to be retested with a new pouch.**

B. Criteria for Acceptable QC
- All controls must yield acceptable results.
- Controls and patient data must be reviewed for acceptability and for atypical or unexpected results or trends prior to reporting patient results.
- DO NOT release results from runs with unacceptable controls or with unusual patterns, trends or distribution in patient values.

C. Corrective Action
- All rejected runs must be effectively addressed and include the following documentation:
  - Control(s) that failed (e.g., positive control with negative result) and/or atypical or unexpected patient results
  - Actions taken
  - Statement of what was done with the patient samples from the affected run/batch,
  - Date and initials of the person recording the information.
- Patient samples in failed analytical runs must be reanalyzed.

NOTE: The laboratory director or designee may override rejection of partial or complete runs. Justification for the override must be documented in detail.

6.5 Documentation

- Record external quality control results on the BioFire FilmArray Respiratory Panel 2 (RP2) External QC Form.
- Record internal QC for each patient test on the BioFire FilmArray Respiratory Panel 2 (RP2) Internal QC Log.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

The laboratory participates in CAP proficiency testing.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

BioFire® FilmArray® Torch Systems
BioFire FilmArray Software

7.2 Equipment

Pouch Loading Station compatible with the use of the Injection Vials
Biosafety Cabinet

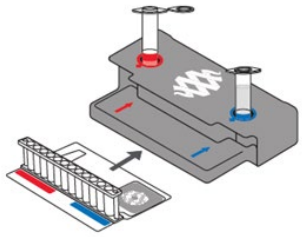
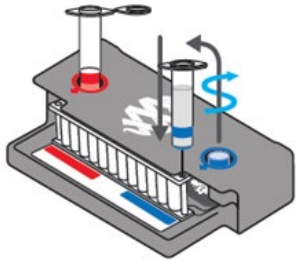
7.3 Supplies

Individually packaged Transfer Pipettes
10% bleach solution
70% ethanol
Distilled water
Gauze squares 4” x 4”
Sharps container
Gloves

8. PROCEDURE

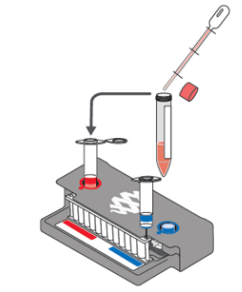
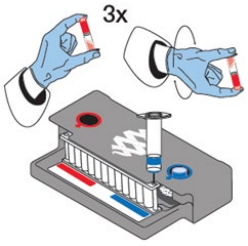
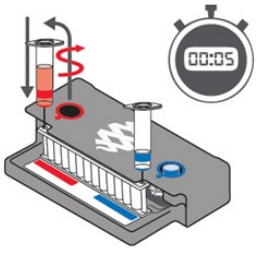
NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

Refer to addendum A for required maintenance.

8.1	Specimen / Reagent Preparation
Prepare Pouch	
1.	Thoroughly clean the work area and the BioFire Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse.
2.	Change gloves.
3.	Remove 1 pouch canister, 1 blue hydration fluid vial, 1 red sample injection vial, 1 sample buffer ampoule, and 1 sterile pipette from the RP2.1 kit and place in the hood.
4.	Remove the pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister. **NOTE: If the vacuum seal of the pouch is not intact, the pouch may still be used. 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.**
5.	Check the expiration date on the pouch. Do not use expired pouches.
6.	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 
7.	Place a red-capped Sample Injection Vial into the red well of the Pouch Loading Station.
8.	Place a blue-capped Hydration Injection Vial in the blue well of the Pouch Loading Station.
Hydrate Pouch	
1.	Unscrew the Hydration Injection Vial from the blue cap by turning counter-clockwise.
2.	Remove the Hydration Injection Vial, leaving the blue cap in the BioFire Pouch Loading Station
3.	Insert the Hydration Injection Vial's cannula tip into the pouch hydration port located directly below the blue arrow of the BioFire Pouch Loading Station. 

8.1	Specimen / Reagent Preparation
4.	Hold the pouch in place and forcefully push down on the blue Hydration Solution vial with the palm of your hand in a firm and quick motion to puncture the seal until a faint "pop" is heard and there is an ease in resistance. Wait as the correct volume of Hydration Solution is pulled into the pouch by vacuum. If the hydration solution is not automatically drawn into the pouch, repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is again not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.
5.	Verify that the pouch has been hydrated. Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen. If the pouch fails to hydrate (dry reagents appear as white pellets), repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is still not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.

8.2	Test Run
Prepare Sample Mix	
1.	Add Sample Buffer to the red-capped Sample Injection Vial. Hold the Sample Buffer ampoule with the tip facing up.  **Note: Avoid touching the ampoule tip during handling, as this may introduce contamination.** Firmly pinch at textured plastic tab on the side of the ampoule until the seal snaps. Invert the ampoule over the red-capped Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.  **Note: Avoid squeezing the ampoule additional times. This will generate foaming, which should be avoided.**

8.2	Test Run
	Warning: The Sample Buffer is harmful if swallowed and can cause serious eye damage and skin irritation.
2.	Thoroughly mix the Nasopharyngeal Specimen (NP swab in UTM or VCM) by vortexing for 5 seconds or inversion.
3.	Use the transfer pipette provided in the test kit to draw specimen to the third line (approximately 0.3 mL) of the transfer pipette.
4.	Add the specimen to the Sample Buffer in the red-capped Sample Injection Vial. 
5.	Tightly close the lid of the Sample Injection Vial and discard the transfer pipette in a biohazard waste container. **NOTE: DO NOT use the Transfer Pipette to mix the sample once it is loaded into the Sample Injection Vial.**
6.	Remove the Sample Injection Vial from the FilmArray Pouch Loading Station and invert the vial 3 to 5 times to mix. 
7.	Return the Sample Injection Vial to the red well of the FilmArray Pouch Loading Station.
Load Sample Mix	
1.	Slowly twist the Sample Injection Vial counter-clockwise to unscrew the Sample Injection Vial from the red cap and wait for 5 seconds with the vial resting in the cap. **NOTE: Waiting 5 seconds decreases the risk of dripping and contamination from the sample.** 

8.2	Test Run
2.	Lift the Sample Injection Vial, leaving red cap in the well of the Pouch Loading Station, and insert the Sample Injection Vial cannula tip into the pouch sample port located directly below the red arrow of the Pouch Loading Station.
3.	Hold the pouch in place and forcefully push down on the red Sample Injection Vial with the palm of your hand in a firm and quick motion to puncture seal (a faint "pop" is heard) and sample is pulled into the pouch by vacuum.
4.	Verify that the sample has been loaded. Flip the barcode label down and check to see that fluid has entered the reagent well next to the sample loading port. If the pouch fails to pull sample from the Sample Injection Vial, the pouch should be discarded. Retrieve a new pouch and repeat from *Step 1: Prepare Pouch*.
5.	Discard the Sample Injection Vial and the Hydration Injection Vial in an appropriate biohazard sharps container.
6.	Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the Pouch Loading Station.
7.	Discard gloves and don clean gloves before removing the pouch from the BSC.
Run Pouch - FilmArray® Torch	
1.	Ensure that the FilmArray Torch system is turned on.
2.	With clean gloves remove the pouch from the BSC. Select an available Module on the touch screen. Scan the barcode on the BioFire pouch using the barcode scanner on the front left of the Torch instrument.
3.	Pouch identification (Lot Number and Serial Number), Pouch Type, and Protocol are preprogrammed in the rectangular barcode located on the pouch. The information will be automatically entered when the barcode is scanned. **Note: When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the BioFire RP2.1 pouch.**
4.	Enter the Sample ID. • Scan the Sunquest specimen label. The BioFire will translate to a numeric accession number with an alpha prefix and suffix. • If unable to scan Sunquest specimen label, then manually key in the numeric accession number. You do not have to enter the alpha prefix and suffix.
5.	Insert the pouch into the available Module. Ensure that the pouch fitment label is lying flat on top of pouch and not folded over. As the pouch is inserted, the Module will grab onto the pouch and pull it into the chamber.
6.	If necessary, select and/or confirm the appropriate protocol for your sample type from the Protocol drop down list. The BioFire RP2.1 has a single protocol available in the drop down list.

8.2	Test Run
7.	Enter operator username and password, then select Next. **Note: The font color of the username is red until the username is recognized by the software.**
8.	Review the entered run information on the screen. If correct, select **Start Run**. Once the run has started, the screen displays a list of the steps being performed by the instrument and the number of minutes remaining in the run. **Note: The bead-beater apparatus can be heard as a high-pitched noise (whine) during the first minute of operation.**
9.	At the end of the run, remove the partially ejected pouch, then immediately discard it in a biohazard waste container.
10.	The report will print automatically. The run file is automatically saved in the FilmArray database, and the test report can be viewed, printed, and/or saved as a PDF file. Select the **Finished Module** on the Dashboard if the report did not print to view the report on the screen. Select **Print** to print the report.

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

N/A

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

The FilmArray Software automatically analyzes and interprets the assay results and displays the final results in a test report.

The test report will list Detected or Not Detected for each organism. Multiple organisms can be detected. If 3 or more organisms are detected in a specimen, a retest of the specimen is recommended to confirm the polymicrobial result

The Controls field on the test report will display Passed, Failed, or Invalid. The Controls field will display **Passed** only if the run completed successfully (no instrument or software errors) and both of the pouch control assays (DNA Process Control and PCR2 Control) were successful. If the control result is **Failed,** then the result for all of the tests on the panel are displayed as Invalid and the sample will need to be retested with a new pouch.

The table below provides a summary and explanation of the possible control results and follow-up actions.

Control Result	Explanation	Action Required	Outcome
Passed	The run was successfully completed AND Both pouch controls were successful.	None	Report the results provided on the test report.
Failed	The run was successfully completed BUT At least one of the pouch controls (RNA Process Control and/or PCR2 Control) failed.	Repeat the test using a new pouch.	Accept the results of the repeat testing. If the error persists, contact technical support for further instruction.
Invalid	The controls are invalid because the run did not complete. (Typically this indicates a software or hardware error.)	Note any error codes displayed during the run and the Run Status field in the Run Details section of the report. Refer to the Operator's Manual or contact Technical Support for further instruction. Once the error is resolved, repeat the test or repeat the test using another instrument. If the error occurred in the first 30 seconds of the run, the same pouch may be used for the repeat test (within 60 minutes of pouch loading) using the same instrument or another instrument, as available. If the error occurred later in the run or you are unsure when the error occurred, return to the original sample to load a new pouch. Repeat the test with the new pouch on the same instrument or another instrument, as available.	Accept the valid results of the repeat testing. If the error persists, contact Technical Support for further instruction.

BioFire Respiratory Panel 2.1 (RP2.1) Test Report
The test report is printed and retained.

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

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

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

10.2 Rounding / Units of Measure / Clinically Reportable Range (CRR)

N/A

10.3 Review Patient Data

- Review patient results for unusual patterns, trends or distribution.
- Report atypical or unexpected results or trends for this test to appropriate supervisory personnel, prior to releasing results.

10.4 Repeat Criteria and Resulting

IF the result is …	THEN…
If the control result is Failed	The results for all the tests on the panel are displayed as Invalid and the sample will need to be retested with a new pouch.
If Detected results are reported for 3 or more organisms in a sample	Retest the sample to confirm the polymicrobial result.
Detected	Report result as "Detected"
Not Detected	Report result as "Not Detected"
Invalid	Do not report and contact supervisor. Typically this indicates a software or hardware error

IF the result is …	THEN…
Equivocal	Retest the original specimen using a new pouch and report the results of the retest using the appropriate code below. If the result of the retest is again "Equivocal", the final result should be reported as "Detected".

Message Code	Message
Detected	DET
Not Detected	NTD
Equivocal	EQU

Result Reporting – refer to addenda B

11. EXPECTED VALUES

11.1 Reference Ranges

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

11.2 Critical Values

SARS-CoV-2 Detected *(inpatients only)*

11.3 Standard Required Messages

N/A

12. CLINICAL SIGNIFICANCE

The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of a respiratory infection aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

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

Due to the genetic similarity between Human Rhinovirus and Enterovirus, the BioFire RP2.1 cannot reliably differentiate them. A positive BioFire RP2.1 Rhinovirus/Enterovirus result should be followed up using an alternate method (e.g., cell culture or sequence analysis) if differentiation is required.

Performance characteristics for Influenza A were established when Influenza A H1-2009, A H1, and A H3 were the predominant Influenza A viruses in circulation. Performance of detecting Influenza A may vary if other Influenza A strains are circulating or a novel Influenza A virus emerges. 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 state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal swabs in transport media during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

14. LIMITATIONS OF METHOD

- The use of this assay as an *in vitro* diagnostic under US FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the CLIA 1998, 42 U.S.C. §263a, to perform high and moderate complexity tests.
- BioFire Respiratory Panel 2.1 (RP2.1) performance has only been established on the FilmArray 2.0 and FilmArray Torch systems.
- The BioFire RP2.1 is a qualitative test and does not provide a quantitative value for the organism(s) in the specimen.
- Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- The performance of the BioFire RP2.1 has been evaluated for use with human specimen material only.
- The BioFire RP2.1 has not been validated for testing of specimens other than nasopharyngeal swab (NPS) specimens in transport medium.
- The performance of BioFire RP2.1 has not been established for specimens collected from individuals without signs or symptoms of respiratory infection.
- The performance of the BioFire RP2.1 has not been specifically evaluated for NPS specimens from immunocompromised individuals.
- The effect of antibiotic treatment on test performance has not been evaluated.
- The performance of the BioFire RP2.1 has not been established with potentially interfering medications for the treatment of influenza or cold viruses. The effect of interfering substances has only been evaluated for those listed in the Interference section. Interference from substances that were not evaluated could lead to erroneous results.
- The performance of the BioFire RP2.1 has not been established for monitoring treatment of infection with any of the panel organisms.
- The performance of BioFire RP2.1 has not been established for screening of blood or blood products.
- 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.
- A negative BioFire RP2.1 result does not exclude the possibility of viral or bacterial infection. Negative test results may occur from the presence of sequence variants in the region targeted by the assay, the presence of inhibitors, technical error, sample mix-up or an infection caused by an organism not detected by the panel. 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.
- If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.
- 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.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods when prevalence is moderate to low.
- Performance characteristics for Influenza A were established when Influenza A H1-2009, A H1 and A H3 were the predominant Influenza A viruses in circulation. Performance of detecting

Influenza A may vary if other Influenza A strains are circulating or a novel Influenza A virus emerges.

- Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Bordetella parapertussis, Bordetella pertussis, Chlamydia pneumoniae*, Coronavirus 229E, Influenza A H1, Influenza A H3, Influenza B, Parainfluenza Virus 1, and Parainfluenza Virus 4 were established primarily with retrospective clinical specimens. Performance characteristics for Influenza A H1 was established primarily using contrived clinical specimens.
- The BioFire RP2.1 influenza A subtyping assays target the influenza A hemagglutinin (H) gene only. The BioFire RP2.1 does not detect or differentiate the influenza A neuraminidase (N) subtypes.
- The BioFire RP2.1 may not be able to distinguish between existing viral strains and new variants as they emerge. For example, the BioFire RP2.1 can detect Influenza A H3N2v (first recognized in August, 2011), but will not be able to distinguish this variant from Influenza A H3N2 seasonal. If variant virus infection is suspected, clinicians should contact their state or local health department to arrange specimen transport and request a timely diagnosis at a state public health laboratory.
- Recent administration of nasal influenza vaccines (e.g. FluMist) prior to NPS specimen collection could lead to accurate virus detection by the BioFire RP2.1 of the viruses contained in the vaccine, but would not represent infection by those agents.
- Due to the genetic similarity between Human Rhinovirus and Enterovirus, the BioFire RP2.1 cannot reliably differentiate them. A BioFire RP2.1 Rhinovirus/Enterovirus Detected result should be followed-up using an alternate method (e.g. cell culture or sequence analysis) if differentiation between the viruses is required.
- BioFire RP2.1 detects a single-copy Pertussis Toxin promoter target (ptxP, present at one copy per cell) in *B. pertussis*. Other PCR tests for *B. pertussis* target the multi-copy IS481 insertion sequence (present in both *B. pertussis* and *B. holmesii*) and are therefore capable of detecting lower levels of *B. pertussis* (i.e. more sensitive).
    - BioFire RP2.1 should not be used if *B. pertussis* infection is specifically suspected; a *B. pertussis* molecular test that is FDA-cleared for use on patients suspected of having a respiratory tract infection attributable to *B. pertussis* only should be used instead.
    - Due to lower sensitivity, the BioFire RP2.1 *B. pertussis* assay is less susceptible than IS481 assays to the detection of very low levels of contaminating *B. pertussis* vaccine material. However, care must always be taken to avoid contamination of specimens with vaccine material as higher levels may still lead to false positive results with the BioFire RP2.1 test (see contamination prevention guidelines).
    - The IS481 sequence is also present in *B. holmesii* and to a lesser extent in *B. bronchiseptica*, whereas the BioFire RP2.1 assay (ptxP) was designed to be specific for *B. pertussis*. However, the BioFire RP2.1 *Bordetella pertussis* (ptxP) assay can also amplify pertussis toxin pseudogene sequences when present in *B. bronchiseptica* and *B. parapertussis*. Cross-reactivity was observed at high concentration (e.g., ≥1.2E+09 CFU/mL).
- There is a risk of false positive results due to cross-contamination with organisms, nucleic acids or amplified products. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.
- Primers for both BioFire RP2.1 SARS-CoV-2 assays share substantial sequence homology with the RaTG13 (accession: MN996532) and cross-reactivity with this closely-related viral sequence is predicted. In addition, the SARS-CoV-2 assay may cross-react with the Pangolin coronavirus isolate (accession: MT084071) and two other bat SARS-like coronavirus sequences (accession MG772933 and MG772934). It is unlikely that these viruses would be found in a human clinical nasopharyngeal swab; but if present, the cross-

reactive product(s) produced by the BioFire RP2.1 will be detected as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).

- There is a risk of false positive results for *Bordetella* species and Human Rhinovirus/Enterovirus due to non-specific amplification and cross-reactivity with organisms that can be found in the respiratory tract. Observed and predicted cross-reactivity for BioFire RP2.1 is described in the *Analytical Specificity* section of the IFU. Erroneous results due to cross-reactivity with organisms that were not evaluated or new variant sequences that emerge is also possible.
- Some strains of *B. bronchiseptica* (rarely isolated from humans) do carry IS*1001* insertion sequences identical to those carried by most strains of *B. parapertussis.* These sequences will be amplified by the IS*1001* assay and reported by BioFire RP2.1 as *B. parapertussis* (IS*1001*).
- The BioFire RP2.1 Human Rhinovirus/Enterovirus assay may amplify off-target sequences found in strains of *B. pertussis, B. bronchiseptica* and *B. parapertussis*. Cross-reactivity with *B. pertussis* was observed at a concentration of 4.5E+07 CFU/mL or higher.

14.1 Analytical Measurement Range (AMR)

N/A

14.2 Precision

N/A

14.3 Interfering Substances

Potentially interfering substances that could be present in NPS specimens or introduced during specimen collection and testing were evaluated for their effect on BioFire RP2.1 performance. Substances included endogenous substances that may be found in specimens at normal or elevated levels (e.g. blood, mucus/mucin, human genomic DNA), various commensal or infectious microorganisms, medications, washes or topical applications for the nasal passage, various swabs and transport media for specimen collection, and substances used to clean, decontaminate, or disinfect work areas.

Each substance was added to contrived samples containing representative organisms at concentrations near (2-3×) LoD. The concentration of substance added to the samples was equal to or greater than the highest level expected to be in NPS specimens.

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

See manufacturer's Product Insert for list of substances evaluated.

14.4 Clinical Sensitivity/Specificity/Predictive Values

The performance of the BioFire RP2.1 was evaluated by comparing the BioFire RP2.1 test results with those from an FDA-cleared multiplexed respiratory pathogen panel (the main comparator method) as well as with results from two analytically-validated PCR assays followed by bi-directional sequencing for *B. parapertussis* (this analyte is not detected by the FDA-cleared multiplexed respiratory pathogen panel). The *B.*

parapertussis comparator assays were designed to amplify a different sequence than that amplified by the BioFire RP2.1. Any specimen that had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched organism-specific sequences deposited in the NCBI GenBank. database (www.ncbi.nlm.nih.gov) with acceptable E-values was considered Positive.

See manufacturer's Product Insert for data.

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

BioFire FilmArray Respiratory Panel 2.1 (RP2.1) External QC Form (AG.F524)
BioFire FilmArray Respiratory Panel 2.1 (RP2.1) Internal QC Log (AG.F525)
FilmArray Torch Maintenance Record (AG.F516)

17. REFERENCES

- BioFire Respiratory Panel 2.1 (RP2.1) Instruction Booklet (BFR0000-8303-01) May 2020 BioFire Diagnostics, LLC.
- FilmArray® Torch Specification Sheet, HTFA-PRT-0058-01, QS-339B-01

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
1	8/21/20	10.4	Deleted reporting steps	M Sabonis	R Master
1	8/21/20	19	Added addenda B	M Sabonis	R Master

19. ADDENDA

A. FilmArray® Torch Systems Maintenance
B. Result Reporting

Addenda A

FilmArray® Torch Systems Maintenance

Weekly:

1. Decontaminate Loading Station: soak loading station in 10% bleach 15 min, soak in water twice to remove bleach.
2. Decontaminate Instrument surfaces: wipe instrument surfaces and touch screen with 10% bleach followed by wiping with water to remove bleach. Do not drip bleach or water inside instrument when cleaning surfaces.

Monthly:

1. Check filters: clean or replace as needed
2. Shut down and reboot system: Turn off the power switch on the Torch instrument by first pressing the button switch on the back, right panel. Wait until the screen turns off (black). Then turn off the instrument toggle switch on back, left panel. Wait at least 1 minute before turning the instrument on with the toggle switch on back, left panel.

Addenda B

Result Reporting

General information:

- The BioFire is interfaced with Sunquest.
- Upon completion of testing, the results will print dynamically to the BioFire printer and transmit to Sunquest.
- If ALL results are negative then the results will autofile into Sunquest and transmit to Cerner.
- If any of the tests are Positive, then ALL the tests for that accession number are held in Sunquest. (**Refer to section 10.4 if multiple results are positive and retest as needed**)
- Review results in Sunquest OEM as described below.

Reviewing and releasing results:

1. Access OEM
2. At DEVICE: prompt, type in Method code **WOBF** (WOMC) or **SGBF** (SGMC).
3. Results will display cup by cup.
   - If ALL are negative, then those results auto-filed and require no action. Proceed to next cup.
   - For positive results that were held, continue with step 4 below.
   - Refer to *OEM - On Line Result Entry Method* procedure (LIS SOP) for additional information about review and release of results.

4. Positive (Detected) Results
   a. For all organisms except COVID, review and release results.
   b. For positive SARS CoV2 (COVID):
      - **Results are not called to the ED** (release results without call documentation).
      - **Non ED patients** will be tagged with CALL in Sunquest to indicate the result must be called and documented using proper format.
        Append CBACK documentation to results including who you called, date, time and tech code; then click Accept to release. Required format is:

        -CBACK-;full name of person called DATE TIME Tech code
        Example -CBACK-;Sue Smith 032420 1420 4568

5. Perform an OFC (Online File Cleanup) at least once per shift. This process cleans up the online data that was sent to Sunquest.
   a. In Sunquest (SmarTerm) access function OFC
   b. Type in the method code (WOBF or SGBF).
   c. At the Start at Cup Number prompt, type in 1 and then press ENTER.
   d. At the Stop at Cup Number prompt, press ENTER.

Notes:

Fort Washington Medical Center (MR# starts with FWMC-)

- Call all results and document call in Sunquest.
- Fax results to FWMC (see FWMC requisition for specific details)

Howard University Hospital (MR# starts with HUH-)

- Call all results and document call in Sunquest.
- Fax results to HUH (see HUH requisition for specifics)