DATE: _____

BioFire® Respiratory 2.1 (RP2.1) Panel Training Checklist

For Emergency Use Authorization (EUA) only

Trainee Name: _____ Trainer Name: _____

INTENDED USE

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

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

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

SARS-CoV-2 RNA and nucleic acids from the respiratory viral and bacterial organisms identified by this test are generally detectable in nasopharyngeal swabs (NPS) during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection is indicative of the presence of the identified microorganism and aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results are indicative of the presence of the identified organism, but do not rule out co-infection with other pathogens. The agent(s) detected by the BioFire RP2.1 Panel (EUA) may not be the definite cause of disease.

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

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

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

REAGENT STORAGE, HANDLING, AND STABILITY

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

SAMPLE REQUIREMENTS

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

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

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

NOTE: NPS specimens should not be centrifuged before testing.

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

PROCEDURE

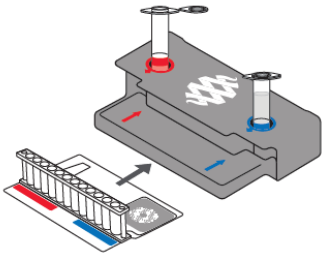
Use clean gloves and other Personal Protective Equipment (PPE) when handling pouches and samples. Only prepare one BioFire RP2.1 Panel (EUA) pouch at a time, and change gloves between samples and pouches. Once sample is added to the pouch, promptly transfer to the instrument to start the run. After the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

Step 1: Prepare Pouch

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

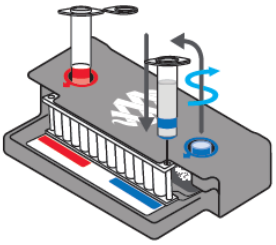
NOTE: The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.

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



Step 2: Hydrate Pouch

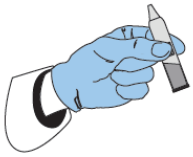
1. Unscrew the Hydration Injection Vial from the blue cap.
2. Remove the Hydration Injection Vial, leaving the blue cap in the Pouch Loading Station.
3. Insert the Hydration Injection Vial's cannula tip into the pouch hydration port located directly below the blue arrow of the Pouch Loading Station.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



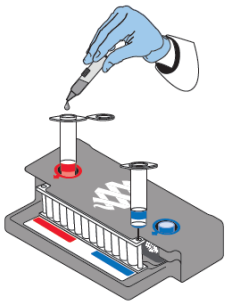
   - If the hydration solution is not automatically drawn into the pouch, repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is again not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.
5. Verify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

Step 3: Prepare Sample Mix

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

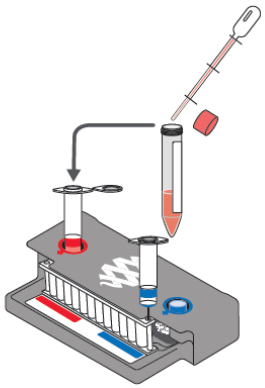


NOTE: Avoid touching the ampoule tip during handling, as this may introduce contamination.



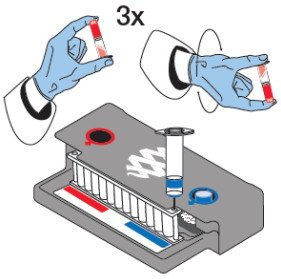
- Firmly pinch at textured plastic tab on the side of the ampoule until the seal snaps.
- Invert the ampoule over the red-capped Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

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



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

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

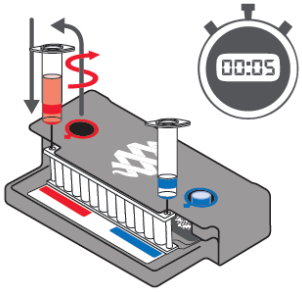


NOTE: DO NOT use the Transfer Pipette to mix the sample once it is loaded into the Sample Injection Vial.

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

Step 4: Load Sample Mix

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



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

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

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

Step 5: Run Pouch

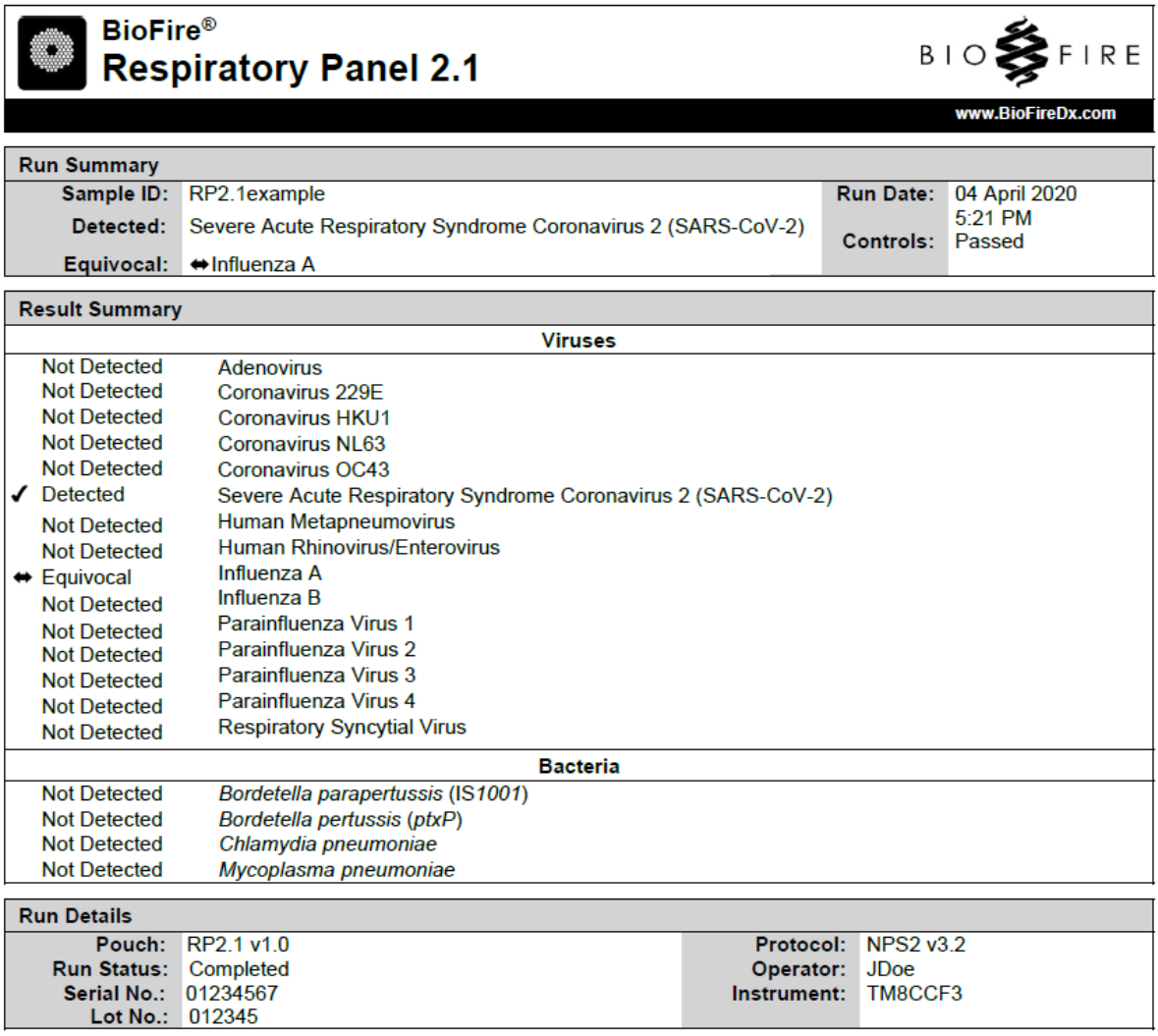
The BioFire® FilmArray® Software includes step-by-step on-screen instructions that guide the operator through performing a run.

For the BioFire® 2.0: Refer to BioFire® FilmArray® 2.0 Instrument Operating Instructions Training Checklist.

For the BioFire® Torch: Refer to the BioFire® FilmArray® Operating Instructions Training Checklist.

Step 6: BioFire RP2.1 Panel (EUA) Test Report

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



BioFire® Respiratory Panel 2.1

www.BioFireDx.com

Run Summary

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

Result Summary

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

Run Details

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

Run Summary

The Run Summary section of the test report provides the Sample ID, time and date of the run, control results, and an overall summary of the test results. Any organism with a Detected result will be listed in the corresponding field of the summary. If all of the organism assays were negative then 'None' will be displayed in the Detected field. Controls are listed as Passed, Failed, or Invalid. Table 1 provides additional information for each of the possible control field results.

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

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

Result Summary

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

Table 2. Reporting of Results and Required Actions

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

Result	Explanation	Action
Detected[a]	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism were POSITIVE (i.e., met the requirements for a positive result described in the Assay Interpretation section above)	Report results.
Not Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism were NEGATIVE (i.e., did not meet the requirements for a positive result described in the Assay Interpretation section above)	Report results.
Equivocal	The run was successfully completed AND The pouch controls were successful (Passed) AND The combination of positive and negative assay results for Influenza A were inconclusive (see **Error! Reference source not found.**)	Retest the original specimen and report the result. If the result of the retest is again 'Equivocal', the final result should be considered 'Detected'.

Result	Explanation	Action
Invalid	The pouch controls were not successful (Failed) OR The run was not successful (Run Status displayed as: Aborted, Incomplete, Instrument Error, or Software Error)	See **Run Summary** The Run Summary section of the test report provides the Sample ID, time and date of the run, control results, and an overall summary of the test results. Any organism with a Detected result will be listed in the corresponding field of the summary. If all of the organism assays were negative then 'None' will be displayed in the Detected field. Controls are listed as Passed, Failed, or Invalid. Table 1 provides additional information for each of the possible control field results. Table 1, Interpretation of Control Field on the BioFire Test Report for instruction.

Run Details

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

Change Summary

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

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

QUALITY CONTROL

External Controls

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

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

RELATED DOCUMENTS

Laboratory Reference Documents

The following documents are available for hospitals and laboratories use and can be found on the BioFire Diagnostics website: https://www.biofiredx.com/covid-19/

- The BioFire RP2.1 Panel (EUA) Letter of Authorization
- Authorized Fact Sheet for Healthcare Providers-provide with test results
- Authorized Fact Sheet for Patients-provide with test results
- Authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd

Trainer Signature: _____ Trainee Signature: _____