

BioFire® Respiratory Panel 2.1 (RP2.1) Testing

Purpose

This procedure provides instructions for testing nasopharyngeal swabs (NPS) collected in transport media or saline, using the BioFire® Respiratory Panel 2.1 (RP2.1) Kit (423742).

Background

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

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

- 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* (IS1001)
- *Bordetella pertussis* (*ptxP*)
- *Chlamydia pneumoniae*
- *Mycoplasma pneumoniae*

Principle of the Procedure

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

During a run, the BioFire system:

- Lyses the sample by agitation (bead beading) in addition to chemical lysis mediated by the Sample Buffer.
- Extracts and purifies all nucleic acids from the sample using magnetic bead technology.
- Performs nested multiplex PCR by:
 - First performing reverse transcription, followed by a multiplexed first stage PCR reaction (PCR1).
 - Then performing multiple simultaneous second-stage PCR reactions (PCR2) in the array to amplify sequences within the PCR1 products
- Uses endpoint melting curve data to detect target-specific amplicons and analyses the data to generate a result for each analyte.

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 or saline.
Minimum Sample Volume	0.3 mL (300 µL)
Transport and Storage	Specimens should be tested with the BioFire RP2.1 as soon as possible. If storage is required, specimens can be held: <ul style="list-style-type: none"> • At room temperature for up to 4 hours (15-25 °C) • Refrigerated for up to 3 days (2-8 °C) • Frozen (≤ -15 °C or ≤ -70 °C) (for up to 30 days)^a

^a Frozen storage for up to 30 days was evaluated for this sample type. However, longer frozen storage at -70°C or lower may be acceptable. Please follow your 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.

Materials

Materials Provided	Materials Required But Not Provided
<p>Each kit contains sufficient reagents to test 30 samples:</p> <ul style="list-style-type: none"> • Individually packaged BioFire RP2.1 pouches • Single-use (1.0 mL) Sample Buffer ampoules • Single-use pre-filled (1.5 mL) BioFire® Hydration Injection Vials (blue) • Single-use Sample Injection Vials (red) • Individually packaged Transfer Pipettes • BioFire RP2.1 Pouch Module Software This software is required to run the BioFire RP2.1 and can be downloaded at https://www.biofire.com/e-labeling/ITIFA20RP2110 if not already installed on the BioFire 2.0 or BioFire Torch Systems. 	<p>BioFire system including:</p> <ul style="list-style-type: none"> • BioFire2.0 or BioFire Torch Systems and accompanying system specific core software • BioFire® Pouch Loading Station[†] • 10% bleach solution or a similar disinfectant

Procedure

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

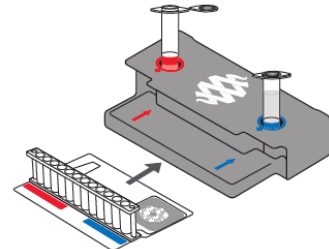
Step 1: Prepare Pouch

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



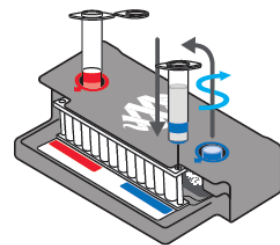
NOTE: The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.

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 BioFire Pouch Loading Station.
6. Place a blue-capped **Hydration Injection Vial** into the **blue well** of the BioFire Pouch Loading Station.



Step 2: Hydrate Pouch

1. Unscrew the **Hydration Injection Vial** from the blue cap.
2. Remove the **Hydration Injection Vial**, leaving the blue cap in the BioFire Pouch Loading Station.
3. Insert the **Hydration Injection Vial's** cannula tip into the **pouch hydration port** located directly below the blue arrow of the BioFire 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.

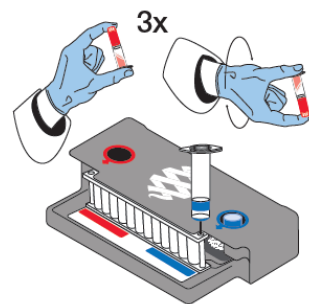
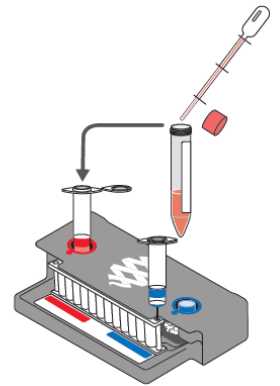
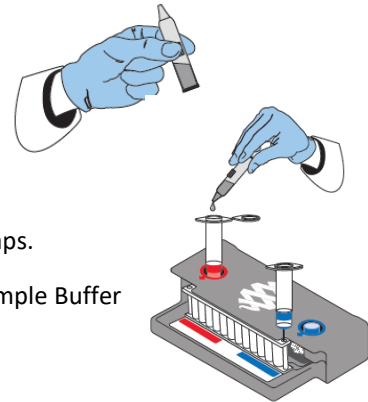
 **NOTE: Avoid squeezing the ampoule additional times. This will generate foaming, which should be avoided.**

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.



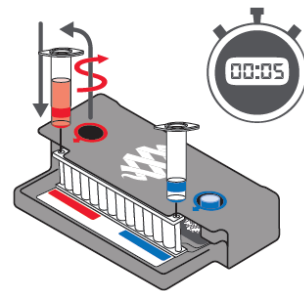
Step 4: Load Sample Mix

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 BioFire Pouch Loading Station.



Step 5: Run Pouch

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

BioFire 2.0

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



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

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



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

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

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



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

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

BioFire Torch

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



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

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

7. Enter operator 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 module and the number of minutes remaining in the run.



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

9. At the end of the run, remove the partially ejected pouch, then immediately discard it in a biohazard waste container.
10. The run file is automatically saved in the BioFire database, and the test report can be viewed, printed, and/or saved as a PDF file.

Assay Interpretation

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

Analysis of melt curves. The BioFire Software evaluates the DNA melt curve for each well of the PCR2 array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (T_m) of the curve and compares it against the expected T_m range for the assay. If the software determines that the T_m falls inside the assay-specific T_m range, the melt curve is called positive. If the software determines that the melt curve is not in the appropriate T_m 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 T_m for at least two of the three positive melt curves must be similar (within 1°C). Assays that do not meet these criteria are called negative.

Organism Interpretation

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

Adenovirus

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

Influenza A

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

A strain. Specimens with an Equivocal result or multiple Influenza A subtypes detected should be retested once.

Table 1. Possible Assay Results for Influenza A and the Corresponding Interpretation

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

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

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

Influenza A (no subtype detected)

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

negative controls, contact the appropriate public health authorities for confirmatory testing.

SARS-CoV-2



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

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

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

BioFire RP2.1 Test Report

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

 BioFire® Respiratory Panel 2.1			
www.BioFireDx.com			
Run Summary			
Sample ID:	RP2.1example	Run Date:	04 April 2020
Detected:	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	Controls:	5:21 PM 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	<i>Bordetella parapertussis</i> (IS1001)		
Not Detected	<i>Bordetella pertussis</i> (ptxP)		
Not Detected	<i>Chlamydia pneumoniae</i>		
Not Detected	<i>Mycoplasma pneumoniae</i>		
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 provides additional information for each of the possible control field results.

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

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

Results Summary

The **Result Summary** section of the test report lists the result for each target tested by the panel. Possible results for each organism are Detected, Not Detected, 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 4. Reporting of Results and Required Actions

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

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

^b Equivocal results can occur when the titer of the virus in the specimen is low (below LoD). Equivocal results could also indicate the presence of a novel Influenza A strain or reactivity with non-human influenza A viruses or rare human influenza A viruses that are not H1, H1-2009 or H3. Such strains generally produce Influenza A Equivocal or Influenza A (no subtype detected) results.

Run Details

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

Change Summary

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

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

References/Related Documents

BioFire RP2.1 De Novo Instructions for Use, (BFR0000-8579-01) BioFire Diagnostics, LLC.