### BioFire® FilmArray® Respiratory 2 *plus* (RP2*plus*) Panel Testing for Non-US Customers Only

### Purpose

This procedure provides instructions for testing nasopharyngeal swab (NPS) specimens collected in transport media, using the BioFire RP2*plus* Panel Kit.

### Background

The BioFire RP2*plus* Panel is a multiplexed nucleic acid test intended for use with BioFire® FilmArray® 2.0 or BioFire® FilmArray® Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acid in NPS samples obtained from individuals suspected of respiratory tract infections. The following organism types and subtypes are identified using the BioFire RP2*plus* Panel:

* Adenovirus
* Coronavirus 229E
* Coronavirus HKU1
* Coronavirus NL63
* Coronavirus OC43
* Human Metapneumovirus
* Human Rhinovirus/Enterovirus
* Influenza A, including subtypes H1, H1-2009, and H3
* Influenza B
* Middle East Respiratory Syndrome Coronavirus (MERS-CoV)
* 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*

### Principle of the Procedure

The BioFire® RP2*plus* Panel pouch is a disposable closed system 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 sample. After sample collection, the user injects hydration solution and sample combined with sample buffer into the pouch, places the pouch into a BioFire® FilmArray® Instrument, and starts a run. The entire run process takes about 45 minutes. Additional details can be found in the appropriate BioFire® FilmArray® System Operator’s Manual.

During a run, the BioFire System:

* Lyses the sample by agitation (bead beading).
* Extracts and purifies all nucleic acid 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*plus* Panel array.

### Sample Requirements

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

|  |  |
| --- | --- |
| **Specimen Type** | **NPS** sample collected according to standard technique and immediately placed in 1–3 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*plus* 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) |

***NOTE: NPS specimens should not be centrifuged before testing.***

***NOTE: Bleach can damage organisms/nucleic acid 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** |
| Each kit contains sufficient reagents to test 30 or 6 specimens:   * Individually packaged BioFire RP2*plus* Panel 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 | BioFire® System including:   * BioFire® 2.0 or BioFire® Torch and accompanying software * BioFire® FilmArray® Pouch Loading Station compatible with the use of theInjection Vials * 10% bleach solution or a similar disinfectant |

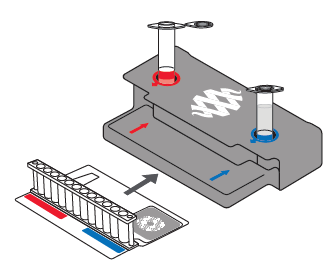
### Procedure

## Use clean gloves and other Personal Protective Equipment (PPE) when handling pouches and samples. Only prepare one BioFire® RP2*plus* Panel 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. Refer to the appropriate BioFire® System training video or operator’s manual for more details.

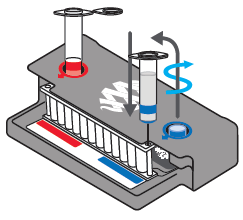
## Step 1: Prepare Pouch

1. Thoroughly clean the work area and the Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse.
2. Obtain the following required materials and place in the clean hood:
   * BioFire RP2*plus* Panel pouch
   * Sample Buffer ampoule
   * Hydration Injection Vial (blue cap)
   * Sample Injection Vial (red cap)
   * Transfer Pipette
3. Remove the pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.

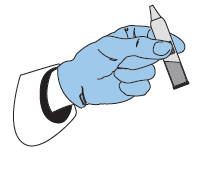
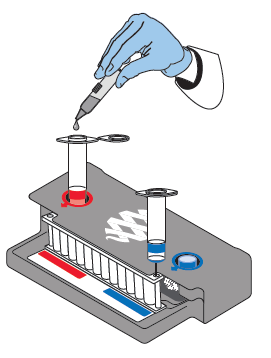
**NOTE:The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.**

1. Check the expiration date on the pouch. Do not use expired pouches.
2. 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.
3. Place a red-capped Sample Injection Vial into the red well of the Pouch Loading Station.
4. Place a blue-capped Hydration Injection Vial into the blue well of the Pouch Loading Station.

## Step 2: Hydrate Pouch

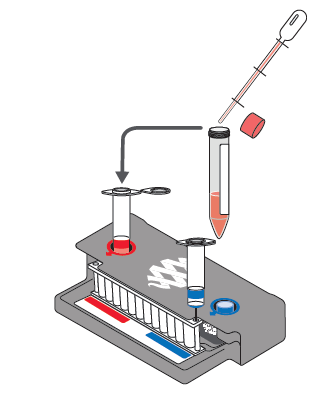
1. Unscrew the Hydration Injection Vial from the blue cap.
2. Remove the Hydration Injection Vial, leaving the blue cap in the Pouch Loading Station.
3. Insert the Hydration Injection Vial’s cannula tip into the pouch hydration port located directly below the blue arrow of the Pouch Loading Station.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

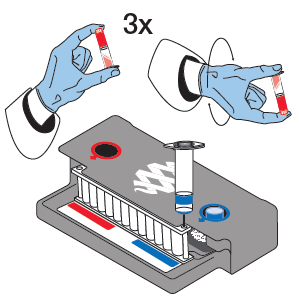
**NOTE: Avoid touching the ampoule tip during handling, as this may introduce contamination.**

* + 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.**

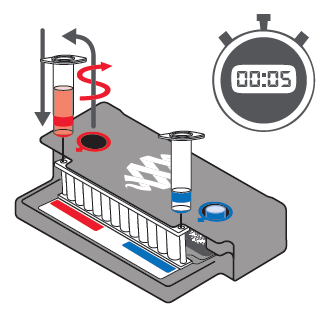
WARNING: The Sample Buffer is harmful if swallowed and can cause serious eye damage and skin irritation.

1. Thoroughly mix the NPS specimen by vortex or inversion.
2. Use the Transfer Pipette provided in the test kit to draw specimen to the third line (approximately 0.3 mL) of the Transfer Pipette.
3. Add the specimen to the Sample Buffer in the Sample Injection Vial.
4. 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.**

1. Remove the Sample Injection Vial from the Pouch Loading Station and invert the vial at least 3 times to mix.
2. 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.***

1. 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.
2. 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.
3. 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*.
4. Discard the Sample Injection Vial and the Hydration Injection Vial in appropriate biohazard sharps container.
5. 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 FilmArray software includes step-by-step on-screen instructions that guide the operator through performing a run. Brief instructions for BioFire® 2.0 and BioFire® Torch systems are given below. Refer to the appropriate BioFire® FilmArray® Operator’s Manual for more detailed instructions.

### BioFire® 2.0

1. Ensure that the BioFire 2.0 system (module 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 an instrument, 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*plus *Panel pouch.***

1. 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.
2. If necessary, select and/or confirm the appropriate protocol for your sample type from the Protocol drop down list. The BioFire RP2*plus* Panel has a single NPS2 protocol available in the drop-down list.
3. Enter a user name and password in the Name and Password fields.

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

1. 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.***

1. When the run is finished, follow the on-screen instructions to remove the pouch, then immediately discard it in a biohazard waste container.
2. 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.

### BioFire® Torch

1. Ensure that the BioFireTorch system is powered on.
2. Select an available module (instrument) 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*plus *Panel pouch.***

1. 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.
2. Insert the pouch into the available Module (instrument).
   * Ensure that the pouch fitment label is lying flat on top of pouch and not folded over. As the pouch is inserted, the Module (instrument) will grab onto the pouch and pull it into the chamber.
3. If necessary, select and/or confirm the appropriate protocol for your sample type from the Protocol drop down list. The BioFire RP2*plus* Panel has a single NPS2 protocol available in the drop-down list.
4. Enter operator user name and password, then select Next.

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

1. 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 (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.***

1. At the end of the run, remove the partially ejected pouch, then immediately discard it in a biohazard waste container.

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.

### Interpretation

When PCR2 is complete, the BioFire® 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® FilmArray® Operator’s Manual). The FilmArray software then performs several analyses and assigns a final assay result. The steps in the analyses are described below.

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

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

### Organism Interpretation

For most organisms detected by the BioFire® RP2*plus* Panel, 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 at least two of the three replicates of the one Human Metapneumovirus assay (hMPV) have similar positive melt peaks with Tm values that are within the assay-specific Tm range. The test results for Adenovirus, Influenza A, and MERS-CoV depend on the interpretation of results from more than one assay. Interpretation and actions for these three multi-assay results are provided below.

### Adenovirus

### The BioFire RP2*plus* Panel pouch contains five different assays (Adeno2, Adeno3, Adeno6, Adeno7.1, and Adeno8) for the detection of Adenovirus. The FilmArray 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 “Detected” for Adenovirus. If all assays are negative, the test report result will be “Not Detected” for Adenovirus.

### Influenza A

### The assays in the BioFire RP2*plus* Panel are designed to both detect Influenza A and to differentiate commonly occurring hemagglutinin subtypes. To accomplish this, the BioFire RP2*plus* Panel uses two Influenza A assays, (FluA-pan-1 and FluApan-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. Retest specimens having “Equivocal” results or multiple Influenza A subtypes detected.

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

| **Assay****Result** | **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 to confirm result b |
| **Influenza A H1-2009****Influenza A H3** | **≥1 positive** | Any result | **Positive** | **Positive** |
| **Influenza A (no subtype detected)** | **2 positives** | Negative | Negative | Negative | Retest(see below) |
| **Influenza A Equivocal** | **1 positive** | Negative | Negative | Negative | Retest |
| **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*plus* Panel can simultaneously detect multiple influenza viruses contained in multivalent vaccines (see Limitations).

bRepeated multiple positives should be further confirmed by other Influenza A subtyping tests that have been cleared for use by your applicable regulatory body.

### Influenza A (no subtype detected)

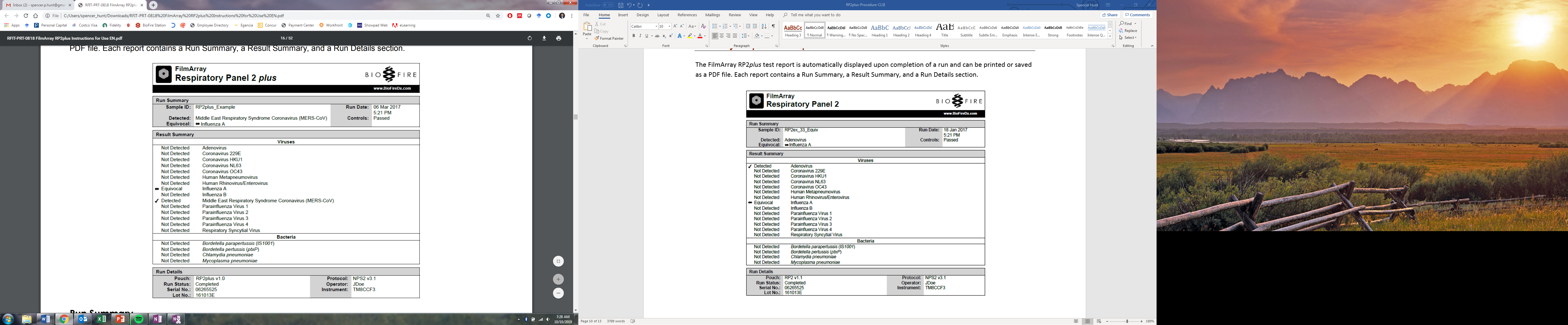
If both FluA-pan assays are positive, but none of the hemagglutinin subtyping assays are positive, then the interpretation is “Influenza A (no subtype detected)”. This result could occur when the titer of the virus in the specimen is low and not detected by the subtyping assays. This result could also 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*plus* Panelpouches 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*plus* Panel accurately identifies the external and negative controls, contact the appropriate public health authorities for confirmatory testing.

**MERS-CoV**

The BioFire RP2*plus* Panel pouch contains two different assays for the detection of MERS-CoV. The FilmArray software interprets each of these assays independently, and the results are combined as a final test result for the virus. Both assays must be positive for the test report result to be Detected. If only one assay is positive, the result is “Equivocal”, and the sample should be retested. If both the assays are negative, the test report result will be “Not Detected”.

### BioFire® RP2*plus* Test Report

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



### Run Summary

The Run Summary section of the test report provides the 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 the organism assays were negative, then “None” will be displayed in the Detected field. Controls are listed as Passed, Failed, or Invalid. Table 2 provides additional information for each of the possible control field results.

#### Table 2. Interpretation of Controls Field on the BioFire® RP2plus Panel Test Report

| **Control Result** | **Explanation** | **Action** |
| --- | --- | --- |
| Passed | The run was successfully completedANDboth pouch controls were successful. | NoneReport the results provided on the test report |
| Failed | The run was successfully completedBUTat 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® FilmArray® Operator’s Manual or contact Technical Support for further instruction.Once the error is resolved, repeat the test or repeat the test using another instrument. |

### Results Summary

The Result Summarysection 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. Table 3 provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

#### Table 3. Reporting of Results and Required Actions

| **Result** | **Explanation** | **Action** |
| --- | --- | --- |
| Detecteda | The run was successfully completedANDThe pouch controls were successful (Passed)ANDThe 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 completedANDThe pouch controls were successful (Passed)ANDThe 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 (Influenza A and MERS-CoV only) | The run was successfully completedANDThe pouch controls were successful (Passed)ANDThe combination of positive and negative assay results for Influenza A and/or MERS-CoV were inconclusive(see Table 1) | Retest the original specimen using a new pouch and report the results of the retest. |
| Invalid | The pouch controls were not successful (Failed)ORThe run was not successful(Run Status displayed as: Aborted, Incomplete, Instrument Error, or Software Error) | See Table 2, Interpretation of Control Field on the BioFire® RP2*plus* Panel Test Report for instruction. |

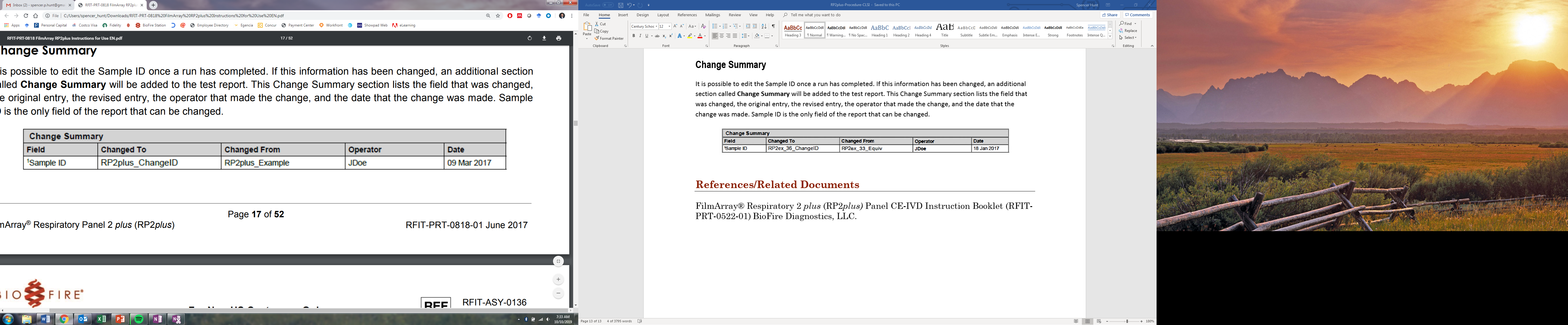
a If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.

### 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, 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.

### 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.



### References/Related Documents

BioFire® FilmArray® Respiratory 2 *plus* (RP2*plus)* Panel CE-IVD Instruction Booklet (RFIT-PRT-0818-01) BioFire Diagnostics, LLC.