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| FilmArray Respiratory Panel 2.1 (RP2.1) | | | | | | | | |
| **Purpose** | This procedure provides instructions for performing the Respiratory Panel 2 on the BioFire FilmArray system. | | | | | | | |
| **Policy Statements** | This procedure applies to all technical staff performing testing on the BioFire FilmArray. | | | | | | | |
| **Principle and Clinical Significance** | 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 Nasopharyngeal (NP/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.  Respiratory pathogens cause acute local and systemic disease, with the most severe cases occurring in children, the elderly, and immunocompromised individuals. Respiratory symptoms can include coughing, nasal discharge, congestion, fever, wheezing, headache, and myalgia. Due to the similarity of diseases caused by many viruses and bacteria, diagnosis based on clinical symptoms alone is difficult. Organisms identified by this test are generally detectable during the acute phase of infection. Identification of potential causative agents provides data to aid the physician in determining appropriate patient treatment and public health response for disease containment. The BioFire RP2.1 is designed for simultaneous detection and identification of the respiratory viruses and bacteria listed below:  **Viruses:**  **Adenoviruses (AdV)**  **Coronaviruses (CoV) - 229E**, **OC43**, **HKU1**, **NL63**  **Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)**  **Human Metapneumovirus (hMPV)**  **Influenza A – H1, H1-2009, H3**  **Influenza B**  **Parainfluenza Viruses (PIVs) – PIV 1-4**  **Respiratory Syncytial Virus (RSV)**  **Rhinoviruses (HRV)** and **Enteroviruses (EV)**  **Bacteria:**  ***Bordetella pertussis***  ***Bordetella parapertussis***  ***Chlamydia pneumoniae***  ***Mycoplasma pneumoniae***  During a run, the FilmArray 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 on the BioFire RP2.1 Panel (EUA).   + 2 of 3 melt curves must show similar results for an analyte to be reported as detected   Refer to the BioFire RP2.1 Instruction for Use (IFU) for a detailed description on the clinical significance of each target. | | | | | | | |
| **Materials** |  | |  |  | | |  | |
|  | **Reagents** | | **Supplies** | **Equipment** | | | **Reagent Storage** | |
|  | * Individually packaged BioFire RP2.1 panel pouches (REF 523738) – store at room temp * Single-use Sample Buffer ampoules * Single-use pre-filled Hydration Injection Vials (blue) * Single-use Sample Injection vials (red) * Household bleach or Bleach wipes (0.65%) | | * Individually packaged Transfer Pipettes * UTM * Maine Molecular Controls (Cat. No. M441) – store at -70 °C | * FilmArray Torch and software * FilmArray Pouch Loading Station * Biosafety Hood | | | * Store kit at room temperature-Do Not Freeze. * Avoid storage near heating or cooling vents. * All kit components should be stored and used together. Do not mix components from one kit with another kit. | |
| **Sample** | Flocked NP swab in UTM (CHC# 32788).  Specimens should be processed and tested 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 | | | | | | | |
| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology* and *Virology Policy Manual*:   1. *Biohazard Containment* 2. *Safety in the Microbiology/Virology Laboratory*  * *Biohazardous Spills*  1. Wear appropriate personal protective equipment (PPE) including disposable gloves and lab coats.    1. If you have signs or any symptoms of a respiratory illness a mask must be worn to set up the test. 2. Change gloves often when handling reagents or samples. 3. Dispose of materials used in this assay, including reagents, used buffer vials in biohazdardous waste. 4. Sample buffer will form hazardous compounds and fumes when mixed with bleach or other disinfectants. | | | | | | | |
| **Quality Control** | There are two process controls included in each pouch.   1. RNA Process Control-The RNA Process Control assay targets RNA transcript from the yeast *Schizosaccharomyces pombe*. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, reverse transcription, 1st stage PCR, dilution, 2nd stage PCR, and DNA melting. A positive control result indicated that all steps carried out in the pouch were successful. 2. PCR2 Control-The PCR2 Control assay detects a DNA target that is dried into the wells of the array along with the corresponding primers. A positive result indicated that 2nd stage PCR was successful.   Both control assays must be positive for the test run to pass. If either control fails, the Controls field of the test report will display FAILED and all results will be listed as INVALID. The sample should be retested using a new pouch.  **External Quality Controls**  Perform QC using external positive and negative controls every 30 days using Maine Molecular Positive and Negative QC material (M441)   * Rotate use of torch modules for testing  1. Allow control to come to room temperature. 2. Invert control 5 – 10 times, then vortex for 3 – 5 seconds. 3. Tap the tube several times (or quick spin) to remove any control caught in the cape before opening the tube. 4. Run as would a patient sample.   **Acceptable results:**  **Positive:** all organisms and resistance markers detected  **Negative:** all organisms and resistance markers NOT detected  **New Lot/Shipment Quality Control:**  Perform QC using external positive and negative controls with each new lot or shipment before putting into service.  Record and file results on the BioFire RP2.1 Quality Control Log  **Acceptable results:**  **Positive:** all organisms and resistance markers detected  **Negative:** all organisms and resistance markers NOT detected  Notify Supervisor, Technical Specialist or Technical Director with unacceptable or undesirable results.  **Wipe Testing:**  To be performed every 30 days to monitor for contamination.   1. Clean the hood and supplies with 10% bleach dilution followed by water 2. Prepare a sterile cryovial or conical by aliquoting approximately 500µL nuclease free water. 3. Set up the loading block as if testing a patient specimen 4. Soak a culturette swab in the nuclease free water for approximately 1 minute. 5. Swab working areas including processing hood surface, vortex and any other high touch surfaces in the **sample prep** area. 6. Using a biohazard pad as a barrier, break swab off into the red sample injection vial, add the sample buffer to the vial and test as a patient specimen. 7. Positive results are cause for alert and decontamination. Stop reporting patient results, and consult Technical Specialist to discuss contaminant testing. 8. See [the FilmArray Torch Operator’s Manual](file:///G:\LAB\Microbiology\BioFire%20FilmArray\htfa-prt-0001_filmarray_torch_operator_s_manual_ivd_en.pdf) for decontamination instructions 9. Record and file results on the wipe testing log in the FilmArray binder   **Desirable results:**  All organisms and resistance markers NOT detected  Notify Supervisor, Technical Specialist or Technical Director with unacceptable or undesirable results. | | | | | | | |
| **Laboratory Precautions** | 1. Prevent organism contamination 2. Samples that contain high concentrations of organisms should be processed in a biosafety hood. 3. Prior to processing a sample, thoroughly clean both the work area and FilmAray Pouch Loading Station using freshly prepared 10% bleach dilution. Wipe disinfected surfaces with water. 4. Use clean gloves to remove materials from bulk packaging bags and reseal bulk packaging bags when not in use. 5. Samples and pouches should be handled one at a time 6. Change gloves and clean work area between each sample 7. Prevent amplicon contamination 8. Discard pouches in biohazard container immediately after the run has completed. 9. Avoid excessive handling of pouches after test runs. 10. Avoid exposing pouches to sharp edges or anything that might cause a puncture. 11. If liquid is observed on the exterior of a pouch, immediately contain and discard in a biohazard container. The instrument/Module and work space must be decontaminated. | | | | | | | |
| **Procedure-Prepare the Pouch** | **NOTE: \*\*\*All users with respiratory illness symptoms must wear a face mask when performing this assay\*\*\***   1. Thoroughly clean the work area and the FilmArray Pouch Loading Station with freshly prepared 10% bleach dilution (or suitable disinfectant) followed by a water rinse. 2. Change gloves. 3. Remove FilmArray Pouch, Sample Injection Vial (RED), Hydration Injection Vial (BLUE), Sample Buffer ampoule and a transfer pipette from the box. Avoid touching the open well of the Sample Injection vial and the tip of the Sample Buffer ampoule as this may introduce contamination. 4. Place the blue-capped hydration injection vial in the blue well of the FilmArray Pouch Loading Station. 5. Place the red-capped sample injection vial in the red well of the FilmArray pouch loading station. 6. Obtain patient sample and place into hood. 7. Remove the pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister. 8. Label the pouch with the small CID label. Do not cover the bar code. Slide the pouch into the FilmArray pouch loading station. | | | | | | | |
| **Procedure-Hydrate the Pouch** | 1. Twist counterclockwise and lift the hydration injection vial, leaving blue cap in the well of the FilmArray pouch loading station. 2. Insert the cannula tip into the port in the pouch located directly below the blue arrow of the FilmArray pouch loading station. Push down forcefully in a firm and quick motion until you hear a faint “pop” and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum. 3. Discard tip into the sharps container. 4. 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 port was broken or retrieve a new pouch and repeat from Step 2 of the prepare pouch section. | | | | | | | |
| **Procedure-Prepare Sample Mix** | 1. Hold the Sample Buffer ampoule so that the tip is facing up. 2. Gently pinch the textured plastic tab on the side of the ampoule until the seal snaps. 3. Re-position thumb and forefinger to grip between the textured plastic tab and the bottom of the ampoule, then invert the ampoule over the red-capped sample injection vial and re-position thumb and forefinger to grip the bottom of the ampoule. Dispense sample buffer using a slow, forceful squeeze, followed by a second squeeze. Squeezing the ampoule additional times will generate excessive bubbles, which should be avoided. 4. Vortex the patient specimen for 10 seconds. 5. Using the transfer pipette provided in the test kit, draw sample to the third line (approximately 0.3 mL). Add sample to the red sample injection vial. 6. Tightly close the lid of the sample injection vial and mix by gently inverting at least three times. | | | | | | | |
| **Procedure-Load Sample Mix** | 1. Slowly unscrew the sample injection vial so it loosens from its red cap and pause for 3-5 seconds.   **NOTE:** If any droplets form at the end of the needle, carefully wipe the tip off the on inner edge of the red screw cap from the vial (still in the block)   1. Remove the sample injection vial leaving cap in pouch loading station and insert the cannula tip into the port in the pouch fitment located directly below the red arrow of the FilmArray pouch loading station. Push down forcefully in a firm and quick motion until you hear a faint “pop” and feel an ease in resistance. The correct volume of the liquid will be pulled into the pouch by vacuum. 2. 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 the prepare pouch section. 3. Discard the sample injection vial and the hydration injection vial in an appropriate biohazard sharps container. 4. Record the sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the FilmArray pouch loading station. 5. Change gloves. | | | | | | | |
| **Procedure-Run Pouch** | 1. Ensure that the FilmArray torch system is on. 2. Select an available module on the touch screen. 3. Scan the barcode on the FilmArray pouch using the barcode scanner. If the barcode cannot be scanned the required information can be manually entered into the appropriate fields. 4. Enter the sample ID (CID). This can be done manually or scanned in by the using the barcode scanner when a barcoded sample ID is used. 5. Insert the pouch into the module. 6. If necessary, select and/or confirm a protocol from the protocol drop down list. 7. Enter the operator user name and password (micro and micro), then select next. 8. Review the entered run information on the screen. If correct, select start run. 9. Thoroughly clean the work area and the FilmArray Pouch Loading Station with freshly prepared 10% bleach dilution (or suitable disinfectant) followed by a water rinse. 10. At the end of the run, the status of the module changes to finished and the pouch is partially ejected. 11. Select the finished module on the dashboard to view the report. 12. Wearing gloves, remove the pouch from the module, place in a biohazard bag, seal and immediately discard the pouch in the biohazard container under the O&P bench. 13. Change gloves. | | | | | | | |
| **Interpretation/ Results** | 1. The FilmArray Software automatically analyzes and interprets the assay results and displays the final results in a test report. 2. 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.   **NOTE:** Any organism with a Detected result will be listed in the corresponding field of the summary.   1. Controls are listed as Passed, Failed or Invalid. See **Table 1** below for Internal Control Result Interpretation. 2. The **Result Summary** section of the test report lists the result for each target tested by the panel. See **Table 2** below for Result Interpretation.   **NOTE:** If **four** or more distinct organisms are detected repeat testing from the original sample. Only report results if both runs match. Consult with the Technical Director or Technical Director if results do **NOT** match upon repeat.   1. The **Run Details** section provides additional information about the run.   **Table 1: Internal Control Result Interpretation Table**   | 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 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. |   **Table 2 Sample Result Interpretation Table**   | Result | Explanation | Action | | --- | --- | --- | | Detected\*\* | 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 | The run was successfully completedANDThe pouch controls were successful (Passed)ANDThe combination of positive and negative assay results for Influenza A were inconclusive(See Table 3) | 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 1, Control Result Interpretation for instruction. |   \*\***NOTE:** If four or more organisms are detected in a specimen, retest to confirm. Consult with the Technical Director or Technical Director if results do **NOT** match upon repeat.  **\*\*NOTE:** If Influenza A and Influenza B are detected (dual positive). Repeat testing to confirm. If results do not match consult the Technical Specialists or Technical Director. | | | | | | | |
| **Organism Interpretation** | 1. For most organisms detected by the BioFire RP2.1, the organism is reported as Detected if a single corresponding assay is positive if at least two of the three replicates have similar positive melt peaks with Tm values that are within the assay-specific Tm range. 2. The test results for Adenovirus and Influenza A depend on the interpretation of results from more than one assay. Interpretation and actions for these two multi-assay results are provided below.   **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 [3](#_bookmark0) 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 3 Gene Targets for SARS-CoV-2 Assays on the BioFire RP2.1 Panel (EUA)**   |  |  | | --- | --- | | Assay Name | Gene Target | | SARSCoV2-1 | Spike protein (S) gene | | SARSCoV2-2 | Membrane protein (M) gene |   **Adenovirus**  The BioFire 2.1 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 Adenovirus Detected. If all assays are negative, the test report result will be Adenovirus Not Detected.  **Influenza A**  The targets in the BioFire RP2.1 panel are designed to both detect Influenza A and to differentiate commonly occurring 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 4** below.  **NOTE:** Retest specimens having Equivocal results or multiple Influenza A subtypes detected.  **Influenza A (no subtype detected)**  If both of the 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. See Troubleshooting procedure below.  **Table 4: Possible Assay Results and Interpretations for Influenza A**   | AssayResult | 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 H1Influenza A H3 | ≥1 positive | Positive | Negative | Positive | Multiple infections are possible but rare, retest to confirm result | | Influenza A H1-2009Influenza 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 | | 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 | | | | | | | | |
| **Results Reporting** | 1. Results will automatically transmit to the LIS. 2. Log into Sunquest to release results. 3. Select Result Entry from Menu options 4. In the Configuration field select TORCH from the dropdown box.      1. Click on the  button located in the lower right corner to populate the transmitted results. 2. Ensure the correct specimen ID (accession number) is shown. Review messages located on the top and results. Compare results to the FilmArray report.    1. If you have a positive **Alert Value**: Click on the analyte, press tab and add the code **RP**, press tab, enter semi-colon, and record who the result was relayed to with the time/date.   **NOTE:** All samples will automatically have the following result comment attached: “The BioFire Respiratory Panel 2.1 was issued an Emergency Use Authorization (EUA) by the FDA on May 1, 2020.”     1. If all results match, click  button located on the lower left corner. Click  when the “Verify Release Destination” window opens. 2. Open Lab Inquiry, search for the report by accession, review results.    1. Print results if positive or invalid. Attach to instrument report. 3. Verify accession, CID, and patient name match on print out and label. Place in the FilmArray result binder. | | | | | | | |
| **Alert values** | **Alert Values:** Report ***Bordetella pertussis*** and **SARS-CoV-2** by telephone to the physician or patient’s nurse.   1. Add the code **RP**, press tab, enter semi-colon, and record who the result was relayed to with the time/date. See example below in **Figure 1**. | | | | | | | |
| **Troubleshooting** | **Invalid results:**   1. Repeat test. 2. If results are invalid on repeat, call provider to notify them of invalid FilmArray results. 3. See reporting instructions below.   **Broken or leaked pouch:**   1. Follow the decontamination procedure outline in the instrument manual. 2. Perform wipe testing before patient testing 3. If wipe test is negative proceed with testing   **Influenza A Equivocal Results**   1. Retest the sample 2. If the result is resolved, report. 3. If results do not resolve, report as Equivocal (see below).   **Influenza A Multiple Subtypes Detected**   1. Retest the sample   **NOTE:** Retest specimens having Equivocal results or multiple Influenza A subtypes detected.  **Influenza A (no subtype detected):**   1. If both of the FluA-pan assays are positive, but none of the hemagglutinin subtyping assays are positive, then the interpretation is Influenza A (no subtype detected). Retest the sample to verify accuracy. 2. If the retest provides a subtype, report the result. 3. If the retest provides the same result (no subtype), then the function of the RP2 pouches should be verified by testing positive external QC (known positive samples for Influenza A H1, Influenza A H3 and Influenza A H1-2009), and a negative control (UTM). 4. If the BioFire RP2.1 accurately identifies the external and negative controls, report the result (see below) and contact the appropriate public health authorities for confirmatory testing.   **NOTE:** See the FilmArray Torch User manual for additional scenarios that may require Troubleshooting. | | | | | | | |
| **Reporting Invalid Results** | **NOTE:** If Invalid results are obtained after testing the original sample twice, the results will be reported as unresolved.  **NOTE:** Invalid results will NOT be transmitted to the LIS, the report must be generated manually.   1. Call invalid results to the patient’s provider or RN. 2. Click on **Result Entry** and switch to manual resulting mode. 3. Under configuration select **RESP2**. 4. Click on the first analyte and enter **unresolved** (UNRE), tab and enter the code **SIA,** and the following comment will append: “This sample is inhibitory to amplification and the results are inconclusive. Consider repeat collection if clinically indicated.” 5. Press tab and add the code **RP**, press tab, enter semi-colon, and record who the result was relayed to with the time/date. 6. Click on each additional analyte and enter the code **HIDE.** See example below. 7. Click  button located on the lower left corner. Click  when the “Verify Release Destination” window opens. See the example below in **Figure 2**.   **Figure 2: Reporting Invalid Results**     1. Record invalid results on the problem log. | | | | | | | |
| **Reporting Influenza A Equivocal Results** | **NOTE:** If equivocal Influenza A results are obtained after testing the original sample twice, the results will be reported as equivocal and a comment will need to be appended.   1. Results will automatically transmit to the LIS. 2. Call results to the patient’s provider or RN. 3. Log into Sunquest to release results. 4. Select Result Entry from Menu options 5. In the Configuration field select TORCH from the dropdown box.      1. Click on the  button located in the lower right corner to populate the transmitted results. 2. Ensure the correct specimen ID (accession number) is shown. Review messages located on the top and results. Compare results to the FilmArray report. 3. Click on the Influenza A analyte equivocal result, tab to the box below and enter the code **EQU,** and the following comment will append: “The combination of positive and negative assay results for Influenza A were inconclusive. Consider submission of a new sample for testing.” 4. Press tab and add the code **RP**, press tab, enter semi-colon, and record who the result was relayed to with the time/date. 5. Click  button located on the lower left corner. Click and  when the “Verify Release Destination” window opens. 6. Record equivocal results on the problem log. 7. File paperwork in results binder. | | | | | | | |
| **Reporting Influenza A results with a subtype detected on secondary run** | Initial results will appear as shown in **Figure 3:**  **Figure 3: No Influenza A Subtype Detected**     1. Results will automatically transmit to the LIS 2. Log into Sunquest to release results. 3. Select Result Entry from Menu options 4. In the Configuration field select TORCH from the dropdown box.      1. Click on the  button located in the lower right corner to populate the transmitted results. 2. Ensure the correct CID and Results with the subtype detected are shown. Review messages located on the top and results. Compare results to the FilmArray report.   **NOTE:** Be sure you select the results with the suptype detected, as shown in **Figure 4:**  **Figure 4: Influenza Subtype detected on repeat**     1. Click  button located on the lower left corner. Click and  when the “Verify Release Destination” window opens. (See **Figure 5)**      1. File paperwork in results binder. | | | | | | | |
| **Reporting Influenza A results with no subtype detected** | **NOTE:** If Influenza A with no subtype results are obtained after testing the original sample twice and verifying function of the instrument through performing QC, the results will be reported, and an aliquot of the the sample is to be send to MDH for confirmatory testing.   1. Results will automatically transmit to the LIS. 2. Call results to the patient’s provider or RN. 3. Log into Sunquest to release results. 4. Select Result Entry from Menu options 5. In the Configuration field select TORCH from the dropdown box.      1. Click on the  button located in the lower right corner to populate the transmitted results. 2. Ensure the correct CID is shown. Review messages located on the top and results. Compare results to the FilmArray report. 3. Click on the Influenza A result, tab and in the box under the positive result enter the code **NOSUB,** and the following comment will append: “No Influenza A subtype detected. Confirmation to be performed by a reference laboratory.” 4. Press tab and add the code **RP**, press tab, enter semi-colon, and record who the result was relayed to with the time/date. 5. Click  button located on the lower left corner. Click and  when the “Verify Release Destination” window opens. 6. File paperwork in results binder. | | | | | | | |
| **Resulting reports from MDH** | 1. When results are sent back: 2. Open SmarTerm and enter function “MEM”. 3. Return past worksheet (leave empty). 4. Under the “Test” prompt, enter test code RESP2 and accept. 5. Under the “Acc. No.” prompt, enter “M-accession number”. 6. Underneath result, add -SCAND and accept. 7. Make a copy of the MDH report to keep (attach to original patient printouts to be filed away) and put original in filing stack to be scanned into Cerner. | | | | | | | |
| **Sample Storage** | 1. Mark positive samples on top and write results on label. 2. Store sample in fridge for 7 days. 3. Save label in bin. | | | | | | | |
| **Limitations** | 1. The use of this assay an an in vitro diagnostics under US FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), to perform high and moderate complexity tests. 2. FilmArray Respiratory Panel 2.1 (RP2) performance has only been established on the FilmArray 2.0 and FilmArray Torch systems. 3. The BioFire RP2.1 is a qualitative test and does not provide a quantitative value for the organism(s) in the specimen. 4. Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient. 5. The performance of the BioFire RP2.1 has been evaluated for use with human specimen material only. 6. The BioFire RP2.1 has not been validated for testing of specimens other than nasopharyngeal swab (NP/NPS) specimens in transport medium. 7. The performance of BioFire RP2.1 has not been established for specimens collected from individuals without signs or symptoms of respiratory infection. 8. The performance of the BioFire RP2.1 has not been specifically evaluated for NPS specimens from immunocompromised individuals. 9. The effect of antibiotic treatment on test performance has not been evaluated. 10. 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. 11. The performance of the BioFire RP2.1 has not been established for monitoring treatment of infection with any of the panel organisms. 12. The performance of BioFire RP2.1 has not been established for screening of blood or blood products. 13. 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. 14. 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. 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 is not detected by a nasopharyngeal swab specimen. 15. There is a risk of false positive results due to cross-contamination by target organisms, their nucleic acids or amplified product. Particular attention should be given to the Laboratory Precautions noted under the *Warnings and Precautions* section of the RP2 Package Insert. 16. There is a risk of false positive results due to non-specific amplification and cross-reactivity with organisms found in the respiratory tract. Observed and predicted cross-reactivity for BioFire RP2.1 is described in the *Analytical Specificity* section of the RP2 Package Insert. Erroneous results due to cross-reactivity with organisms that were not evaluated or new variant sequences that emerge is also possible. 17. If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result. 18. 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. 19. 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. 20. Clinical performance was established when Influenza A H1-2009 (H1N1pdm09) was the predominant Influenza A virus in circulation. When other Influenza A viruses are emerging, performance may vary. 21. 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. 22. 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. 23. 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. 24. Recent administration of nasal influenza vaccines (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. 25. 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. 26. 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 IS*481* 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). 27. 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. 28. Due to lower sensitivity, the BioFire RP2.1 *B. pertussis* assay is less susceptible than IS*481* 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. 29. The IS*481* 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). 30. 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 IS1001 assay and reported by BioFire RP2.1 as *Bordetella parapertussis* (IS*1001*). 31. There is a risk of false positive results for *Bordetella* species and Human Rhinovirus/Enterovirus due to non-specific amplification and cross-reactivity for BioFire RP2.1. Erroneous results due to cross-reactivity with organisms that were not evaluated or new variant sequences that emerge is also possible. 32. There is a risk of false positive results due to cross-contamination with organisms, nucleic acids or amplified products. 33. Primers for both BioFire RP2.1 SARS-CoV-2 assays share substantial sequence homology with the Bat coronavirus RaTG12 (Accession: MN996532) and cross-reactivity with this closely-related viral sequence is predicted. In addition, the SARS-CoV-2 assay may cross-react with Pangolin coronavirus (accession: MT084071) and two other bat SARS-like coronavirus sequences (accession MG772933 and MG772934). It is unlikely tha these viruses would be found in a human clinical nasopharyngeal swab; but if present, the cross-reactive product(s) produced by the BioFire RP 2.1 will be detected as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). | | | | | | | |
| **Method Performance Specifications** | See the [FilmArray Respiratory Panel 2 (RP2) Instructions for Use](file:///G:\LAB\Microbiology\BioFire%20FilmArray\FilmArray%20-%20RP2\procedure,%20PI,%20info\filmarray_rp2_instructions_for_use_en.pdf) document for detailed illustrations of the performance characteristics of the assay. | | | | | | | |
| **References** | 1. FilmArray Respiratory Panel (RP2.1) Instructions for Use, REF 423738, May 2020, BioFire Diagnostics. 2. Ramanan P, Bryson AL, Binnicker MJ, Pritt BS, et al: Syndromic panel-based testing in clinical microbiology. Clin Microbiol 2018 Rev 31:e00024-17. Available at: <https://cmr.asm.org/content/31/1/e00024-17> | | | | | | | |
| **Alternate Methods** | 1. Respiratory pathogen panel sendout (Mayo) 2. Viral respiratory culture – send out 3. Bacterial respiratory culture | | | | | | | |
| **Customer and Technical Support** | Web information at [www.biofiredx.com](http://www.biofiredx.com). Email at [support@biofiredx.com](mailto:support@biofiredx.com). Call at 1-800-735-6544 or fax to 801-588-0507. | | | | | | | |
| **Training Plan/ Competency Assessment** | **Training Plan** | | | | | **Initial Competency Assessment** | | |
| 1. Employee must read the procedure. 2. Employee will demonstrate the ability to perform procedure, record results, and document corrective action after instruction by the trainer. | | | | | 1. Direct observation | | |
|  |  | | | | | | | |
| **Proficiency Testing** | API Respiratory Panel (370): 5 samples x 3 shipments | | | | | | | |
| **Historical Record** |  |  | | |  | | |  |
|  | **Version** | **Written/Revised by:** | | | **Effective Date:** | | | **Summary of Revisions** |
| 1 | Julie Laramie | | | 01/21/2020 | | | Initial Version |
| 2 | Julie Laramie | | | 02/17/2020 | | | Added repeat testing when Flu A and Flu B are positive |
| 3 | Julie Laramie | | | 9/7/2020 | | | -Updated from RP2 to RP2.1 to include SARS-CoV-2  -Changed QC material  -Added resulting reports from MDH |
|  | 4 | Julie Laramie | | | 2/15/2021 | | | -Eliminated printing neg results from lab inquiry |  |  |
|  |  | | |  | | |  |
| **Archived by:** |  | | | **Archived Date:** | | |  |