BioFire Respiratory Panel Procedure #_1243__

Background

FilmArray Respiratory Panel (RP) is a multiplexed nucleic acid test intended for use with the FilmArray Instrument for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections. The following organism types and subtypes are identified using the FilmArray RP: Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype H1-2009, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Human Rhinovirus/Enterovirus, Respiratory Syncytial Virus, *Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*.

The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of a respiratory infection aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other 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. Positive results do not rule out co-infection with other organisms: the agent(s) detected by the FilmArray RP may not be the definite cause of disease. Additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.

Principle of Operation

The FilmArray RP pouch is a closed system disposable that houses all the chemistry required to isolate, amplify and detect nucleic acid from multiple respiratory pathogens within a single NPS specimen. The rigid plastic component (fitment) of the FilmArray RP pouch contains reagents in freeze-dried form. The flexible plastic portion of the pouch is divided into discrete segments (blisters) which, through interactions with actuators and sensors in the FilmArray Instrument, are where the required chemical processes are carried out. The user of the FilmArray RP system loads the sample into the FilmArray RP pouch, places the pouch into the FilmArray Instrument, and starts the run. All other operations are automated.

Materials and supplies

BioFire RP kit [RPIT-ASY-0104] contains sufficient reagents to test 30 specimens:

- Individually packaged FilmArray RP pouches
- Single-use (0.5 mL) Sample Buffer vials (red cap
- Single-use (1.5 mL) Hydration Solution Vials (blue cap)
- Individually packaged Transfer Pipettes
- Single use Sample Buffer Ampoules

Kits are maintained at room temperature

Open pouches must be used within 30 minutes

Do not use pouches if the outer packaging has been damaged or if the vacuum is not intact.
WARNING: If liquid is observed on the exterior of a pouch, the liquid and pouch should be immediately contained and discarded in a biohazard container. The instrument and work space must be decontaminated as described in the FilmArray Operator’ **s Manual.**
DO NOT PERFORM ADDITIONAL TESTING UNTIL THE AREA HAS BEEN DECONTAMINATED.

FilmArray System including:

- FilmArray Instrument, related PC and software
- FilmArray Pouch Loading Station

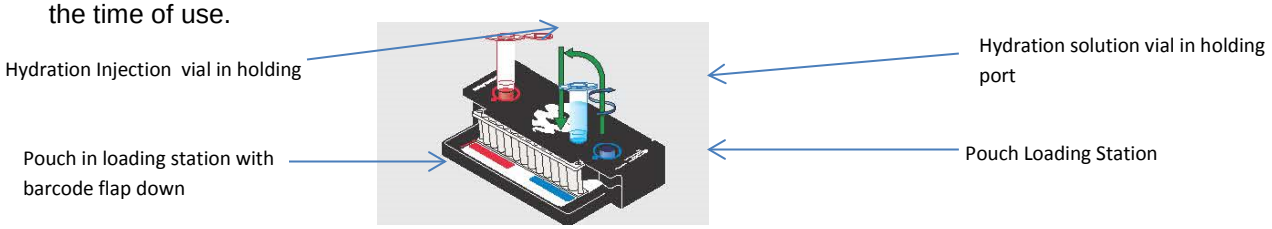
Specimen requirements

Nasopharyngeal swab (NPS) placed into viral transport media (VTM). Other specimen types or media have not been evaluated for performance and should be rejected or results interpreted with caution. Minimum volume is 300µL.

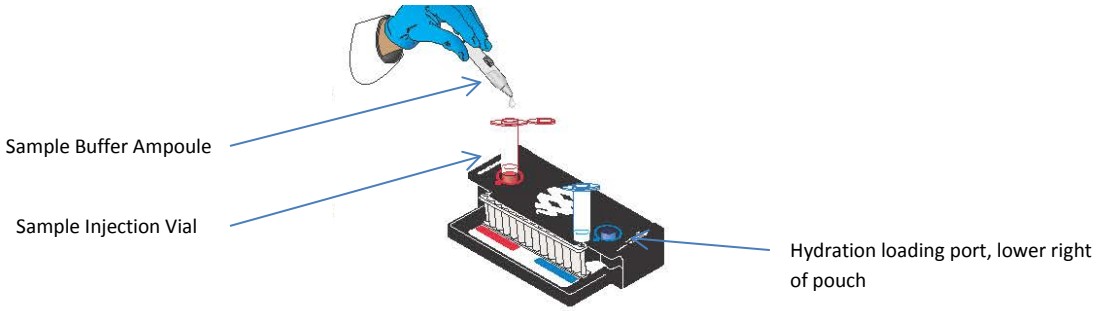
Specimens in VTM should be processed and tested as soon as possible. If storage is required, specimens in VTM can be held at room temperature (18–30 ºC) for up to 4 hours, at refrigerator temperature (2-8 °C) for up to 3 days, or at freezer temperature (<-15 °C) for up to 30 days.

Procedure:

1. Turn on power to instrument and PC. From PC desktop, select FilmArray Instrument Control icon.
2. Handling of specimen must follow departmental guidelines for infectious material. Sample loading procedures must be performed within appropriate biosafety cabinet.
3. Vortex sample to assure adequate suspension.
4. Assure work area is clean to avoid contamination of sample: clean loading station and surrounding work space using 10% bleach, especially the sample loading area around red port, followed by an alcohol wipe.
5. Remove the FilmArray pouch from its vacuum-sealed package. Since solutions are drawn into the FilmArray RP pouch by vacuum, it is important to keep pouches in their protective packaging until the time of use.



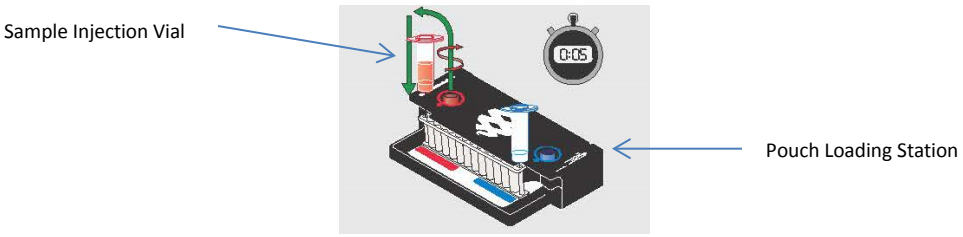
6. Place the hydration injection vial (blue cap vial) in right (blue) holder and the sample injection vial (red cap vial) in the left (red) sample holder.
7. Place the FilmArray RP pouch into the FilmArray Pouch Loading Station, label facing operator. Flip the pouch label downward to observe pouch sections containing tablets.
8. Unscrew the Pouch Hydration Injection Vial leaving cap in Pouch Loading Station, and insert into the pouch hydration port



9. Add Sample Buffer to Sample Injection Vial. Invert Sample Buffer Ampoule so that rip is facing up. Do not touch the tip of the ampoule. Firmly pinch textured plastic tab on side of ampoule until seal snaps. With the tip facing down, dispense Sample Buffer into Sample Injection Vial

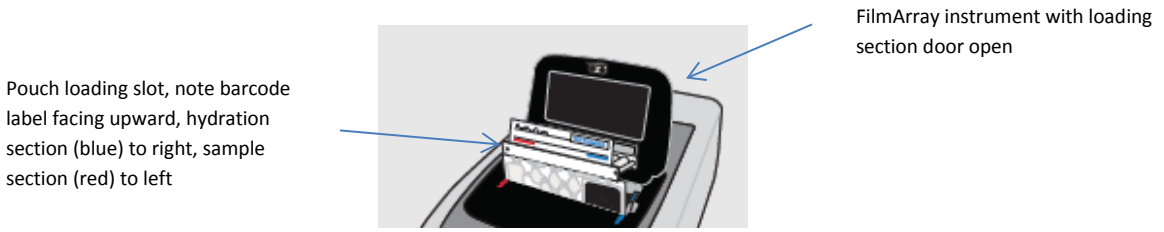
(red) using a slow, forceful squeeze, followed by a second squeeze. Avoid generating excessive bubbles.

10. Using the provided transfer pipette, add 300uL (third line on pipette) of NPS sample to the Sample Injection Vial (red) while positioned in pouch loading station red port. Close lid of Sample Injection Vial.
11. Mix sample by gently inverting Sample Injection Vial three times. Return to red well of Pouch Loading Station.
12. Unscrew Sample Injection Vial (red) from cap. Pause for 3-5 seconds, then remove Sample Injection Vial, leaving red cap in Pouch Loading Station.
13. Insert Sample Injection Vial into pouch sample port. Forcefully push down to puncture seal. Wait as Sample Mix is drawn into pouch.



14. Transfer the pouch to the instrument and initiate a run.

    a. With pouch barcode label facing upwards and holding the hard plastic edges of the pouch, glide the soft place portion of the pouch into the loading area (be sure that door is all the way open to allow access to pouch slot).



    b. Gently press the hard plastic portion of the pouch into place (will hear a snap when properly loaded)
    c. Using barcode reader, scan pouch lot (move cursor to pouch lot number area if necessary).
    d. Using barcode reader or PC keyboard, enter sample ID and/or patient name
    e. Close loading area door.
    f. Using mouse, move cursor to Operator ID, enter ID and appropriate password
    g. Using mouse, locate cursor over “Start run” icon and right click or hit enter.

NOTE: If the pouch does not slide into the instrument easily, gently push the lid of the instrument back to be sure that it is completely open.

NOTE: The barcode cannot be scanned prior to placing the pouch in the instrument. A “Cannot scan now” message will be displayed.

NOTE: To reduce the risk of exposure to hazardous or potentially infectious material, DO NOT re-cap the syringes.

15. To aid in proper insertion of the pouch in the instrument, the FilmArray Instrument Control application provides on-screen animations illustrating the steps needed to start the run. The clock on the right side of PC screen will provide estimated time of completion as both a count-down timer and time of day completion
16. View results on the test report at the completion of the run.
17. Once testing is complete, a visual cartoon will illustrate the removal of pouch from loading section.
    a. A pop-up save menu will appear, select Cancel
    b. Using PC mouse, move cursor to folder tab located on right side of screen to open up View Report.
    c. Select "Print All" icon located atop the result page.
    d. Assure that internal pouch control processes (PCR2 control and RNA Process Control) have passed and record results under RVP sample QC located in Daily Log binder. If any of the two control processes do not pass, then the test failed and needs to be repeated.
18. Record appropriate actions in BioFire Daily Log and report results in LIS.
19. Discard all syringes into red biohazard sharps containers. Discard all vials and transfer pipettes into biohazard waste containers.
20. Clean loading station and surrounding work space using 10% bleach, followed by an alcohol wipe.
21. If no additional testing is anticipated (e.g. E-Threat), then power off instrument and PC.

FilmArray RP Test Report

The FilmArray RP test report is automatically displayed upon completion of a run and contains three sections, the Run Summary, the Results Summary, and the Run Details (see the FilmArray Respiratory Panel Quick Guide to view an example of a test report). The test report can be saved as a file or printed. 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 target with a Detected or Equivocal result will be listed in the corresponding field of the summary. If all of the tests were negative then None will be displayed in the Detected field. Controls are listed as Passed, Failed or Invalid. See Control Field section below for detailed information about the interpretation of controls and appropriate follow-up in the case of control failures.

The **Results Summary** section of the test report lists the result for each target tested by the panel. Possible results are Detected, Not Detected, Equivocal or Invalid. See Results Summary section below for detailed information about interpretation of test results and appropriate follow-up for Invalid and Equivocal results. The assay-by-assay results for each target are available in an optional 2nd page of the report. To access the 2nd page of the report, select the Details button at the bottom of the report screen or Print All for a printed report. The 2nd page of the report provides the results of each assay regardless of the pouch control results.

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 protocols that were used to perform the test, the identity of the operator that performed the test, and the instrument used to perform the test. Once a run has completed, it is possible to edit the Sample ID. If this information has been changed, an additional

section called **Change History** will be added to the test report. This Change History 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.

Control Field

The Control field on the test report will display Passed, Failed, or Invalid. The Control field will display Passed only if the run completed successfully (no instrument or software errors) and both of the pouch control assays (RNA Process Control and PCR2 Control) were successful. The Control field will display Failed if the run was completed successfully (no instrument or software errors) but one or both of the pouch control assays failed (0 or 1 positive replicates for either of the controls, each of which is tested in triplicate). If the control result is Failed, then the result for all of the tests on the panel are displayed as Invalid and the specimen will need to be retested with a new pouch. The FilmArray Instrument monitors each run to ensure that the instrument is working within specification and to detect hardware or software errors that might compromise the accuracy of the test result. If the instrument detects an out-of specification condition, or a significant error, it will automatically abort the run. If this happens, or if a run is aborted by the user, then the Control field on the report will display Invalid and all test results in the Result Summary of the report will also be displayed as Invalid. To determine why a run failed to complete, note any specific error codes that are displayed on the screen and refer to the Run Status in the Run Details section of the report. The Run Status will display Incomplete, Aborted, Software Error, Instrument Error, or Instrument Communication Error. Refer to the FilmArray Operator's Manual or call Technical Support for further instruction. The specimen should be retested after the error is corrected or by using an alternate FilmArray Instrument.

Interpretation of results

The FilmArray Software automatically analyzes and interprets the assay results and displays the final results in a test report (see the FilmArray Respiratory Panel Quick Guide to view an example of a test report). The analyses performed by the FilmArray Software and details of the test report are described below.

Assay Interpretation

The FilmArray 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 FilmArray Operator's Manual).

Analysis of melting curves. The FilmArray Software calculates the melting temperature (Tm) of the curve. The Tm value is then compared against the expected Tm range for the assay. If the software determines that the melt is positive and the melt peak falls inside the assay-specific Tm range, the curve is called positive. If the software determines that the melt is negative or is not in the appropriate Tm range, the 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 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 FilmArray RP, the organism is considered to be "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 have similar positive melt peaks with Tm values that are within the assay-specific Tm range. The test results for Adenovirus, the Human Rhinovirus/Enterovirus group, and Influenza A depend on the

interpretation of results from several assays. Interpretation and follow-up testing for these three results are provided below.

Rhinovirus/Enterovirus Group
The FilmArray RP pouch contains six different assays (HRV1, HRV2, HRV3, HRV4, Entero 1, Entero 2) for the detection of Rhinoviruses and Enteroviruses. Though these viruses are both very diverse, they are also closely related. Therefore, the six assays are not able to reliably differentiate Rhinovirus and Enterovirus. The FilmArray Software interprets each of the six assays independently (as described above) and the results are combined as a final test result for the virus(es). If any of the six assays are positive, the test report result will be Human Rhinovirus/Enterovirus Detected. If all six assays are negative, the test report result will be Human Rhinovirus/Enterovirus Not Detected. A positive FilmArray RP Human Rhinovirus/Enterovirus result should be followed-up using an alternate method (e.g., viral culture or sequence analysis).

NOTE: Despite the names, the HRV (1-4) and Entero (1-2) assays are not specific for detection of Human Rhinovirus or Enterovirus, respectively. Individual assay results cannot be used to differentiate these two viruses.

Adenovirus
The FilmArray RP pouch contains two different assays (Adeno, Adeno2) 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 either or both assays are positive, the test report result will be Adenovirus Detected. If both the Adeno and Adeno2 assays are negative, the test report result will be Adenovirus Not Detected.

Influenza A
The assays in the FilmArray RP are designed to both detect Influenza A and to differentiate commonly occurring hemagglutinin subtypes. To accomplish this, the FilmArray RP uses two Influenza A assays, (FluApan-1 and FluA-pan-2) and three subtyping assays directed at the hemagglutinin gene (FluA-H1-pan, FluAH1-2009 and FluA-H3). The FluA-H1-pan assay is designed to detect both Influenza A H1 and the Influenza AH1-2009 variant. 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.

In general, Influenza A is determined to be Detected if at least one of the two FluA-pan assays is positive and a subtyping assay is also positive. If neither of the FluA-pan assays is positive, but a subtyping assay is positive, then the result is considered Equivocal for that specific subtype and the sample should be retested. If one of the FluA-pan assays is positive and none of the subtyping assays are positive, the result is Equivocal for Influenza A and the specimen should be retested. All Equivocal results should be retested.

Result Reporting
Results are reported as either “Negative” or “Positive”, with positive samples indicating subtypes if applicable (for Coronavirus and Inflluenza A)

NOTE: Pertussis is a nationally notifiable infectious condition in the U.S. If *Bordetella pertussis* is detected, notify the state and/or local health departments.

Laboratory Precautions
Preventing organism contamination:

Due to the sensitive nature of the FilmArray RP system, it is important to guard against contamination of the work area by following these guidelines:

- Laboratory workers can be infected with common respiratory pathogens and can inadvertently contaminate the sample while it is being processed. To avoid this, specimens should be processed and pouches should be loaded in a biosafety cabinet.
- Prior to processing specimens, thoroughly clean both the work area and the FilmArray Pouch Loading Station using a suitable cleaner such as freshly prepared 10% bleach or a similar disinfectant. To avoid residue build-up and potential PCR inhibition, wipe disinfected surfaces with water.
- Some *Bordetella pertussis* acellular vaccines (i.e. Pentacel®, Daptacel®, and Adacel®) contain PCRdetectable DNA. Contamination of specimens or testing materials with vaccine can cause false-positive *B. pertussis* results. Specimens should not be collected or processed in areas that are exposed to *B. pertussis* vaccine material and particular care should be taken during specimen collection and handling to avoid contamination (http://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-pcrbestpractices.html).
- Specimens and pouches should be handled one-at-a-time.
- Change gloves and clean the work area between preparation of each patient specimen.
- Laboratory workers with active respiratory symptoms (runny nose, cough) should wear a standard surgical mask (or equivalent) and should avoid touching the mask while preparing specimens.

Preventing amplicon contamination:

A common concern with PCR-based assays is false positive results caused by contamination of the work area with PCR amplicon. Because the FilmArray RP pouch is a closed system, the risk of amplicon contamination is low provided that pouches remain intact after the test is completed. Adhere to the following guidelines to prevent amplicon contamination:

- Discard used pouches in an appropriate biohazard container immediately after the run has completed.
- Avoid excessive handling of pouches after test runs.
- Avoid exposing pouches to sharp edges or anything that might cause a puncture.

.

Quality and Process Controls

A. Internal pouch control consists of two processes:

1. RNA Process Control
    a. The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and becomes rehydrated when sample is loaded. 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 indicates that all steps carried out in the FilmArray RP pouch were successful.
2. PCR2 Control
    a. The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that 2nd stage PCR was successful. Both control assays must be positive for the test run to pass. When either control fails, the Controls field of the test report (upper right hand corner) will display Failed and all results will be listed as Invalid. If the controls fail, the sample should be retested using a new pouch.
    b. Record raw data for each QC process control
        i. From Browse run tab, select Quick Search to load last 100 samples
        ii. Using mouse, place cursor over desired sample, and right click mouse

1. Select View Control Results
2. Record TM (melting temperature) for the sample on QC log sheet

B. External Quality Control

a. Two levels of commercially available control material will be run on the first non-holiday Monday of each month.

b. Two level commercial controls are available from Maine Molecular Quality Controls, Inc (MMQCI) product: FilmArray® RP Control Panel M210 v1.1. Material is stored at -20°C or colder. To run QC:

i. Allow the control to be tested to come to room temperature (18° – 25°C).
ii. Prepare and hydrate a pouch according to FilmArray® Instructions.
iii. Immediately before use, mix the control by flicking the tube several times and then shake the tube down firmly to remove any control caught in the cap.
iv. Aspirate the control using the FilmArray® transfer pipette.
v. Analyze the FilmArray® RP Control M211 v1.1 and FilmArray® RP Control M212 v1.1 as you would a patient sample.
vi. Discard after completion of test.
vii. Record results onto QC worksheet located in PM/QC binder. An example of a package insert for two level MMQCI QC expected results:

Assay		M211v1.1	M212v1.1
Adenovirus		Positive	Negative
Adeno2		Positive	Negative
Coronavirus 229E		Negative	Positive
Coronavirus HKU1		Negative	Positive
Coronavirus NL63		Negative	Positive
Coronavirus OC43		Negative	Positive
Human Metapneumovirus		Positive	Negative
Human Rhinovirus/ Enterovirus	Entero 1	Positive	Negative
	Entero 2	Positive	Negative
	HRV1	Positive	Negative
	HRV2	Positive	Negative
	HRV3	Positive	Negative
	HRV4	Positive	Negative
Influenza A H1-2009	FluA-H1-2009	Positive	Negative
Influenza A H3	FluA-H1-pan	Positive	Positive
	FluA-H3	Positive	Negative
	FluA-pan1	Negative	Positive
	FluA-pan2	Positive	Negative
Influenza B		Negative	Positive
Parainfluenza Virus 1		Positive	Negative
Parainfluenza Virus 2		Negative	Positive
Parainfluenza Virus 3		Negative	Positive
Parainfluenza Virus 4		Positive	Negative
Respiratory Syncytial Virus		Negative	Positive
Bordetella pertussis		Negative	Positive
Chlamydophila pneumoniae		Negative	Positive
Mycoplasma pneumoniae		Negative	Positive

Lot Performance Verification

Prior to implementing a new lot or new shipment of same lot material, the material must be tested using the external QC material as described above. The new lot or new shipment pouch QC must be within acceptable limits prior to clinical use.

Limitations of procedure

- This test is a qualitative test and does not provide a quantitative value for the virus(es) and/or bacteria detected in the specimen.
- The performance of the test has been evaluated for use with human specimen material only.
- This test has not been validated for testing specimens other than nasopharyngeal swab (NPS) specimens.
- Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- 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.
- The detection of viral and bacterial nucleic acid is dependent upon proper specimen collection, handling, transportation, storage and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive or false negative values resulting from improperly collected, transported or handled specimens.
- A negative FilmArray RP 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 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.
- 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.
- Organism and amplicon contamination may produce erroneous results for this test. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.
- There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.
- Cross-reactivity with respiratory tract organisms other than those listed in the Analytical Specificity section below may lead to erroneous results.
- The FilmArray RP Adenovirus assay may show variable detection with non-respiratory serotypes within species A, D, F and G.
- The FilmArray RP Influenza A subtyping assays target the Influenza A hemagglutinin gene only. The FilmArray RP does not detect or differentiate the Influenza A neuraminidase gene.
- Clinical specificity was established when Influenza A H1-2009 was the predominant Influenza A virus in circulation. When other Influenza A viruses are emerging, clinical specificity may vary.
- Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Bordetella pertussis,* Coronavirus 229E, Coronavirus OC43, Influenza A H1, Influenza A H3, Influenza A H1-2009, Influenza B, *Mycoplasma pneumoniae*, Parainfluenza Virus 1, Parainfluenza Virus 2, and Parainfluenza Virus 4, were established primarily with retrospective clinical specimens. Performance characteristics

for *Chlamydophila pneumoniae* were established primarily using contrived clinical specimens. The performance of this test has not been established for monitoring treatment of seasonal Influenza A H1, A H3, A H1-2009 or RSV infections.

- The performance of this test has not been established for screening of blood or blood product for the presence of seasonal Influenza A H1, A H3 or A H1-2009.
- The performance of this test 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 labeling. Interference by substances other than those described in the "Interference" section below could lead to erroneous results.
- The performance of the FilmArray RP has not been established in individuals who received influenza vaccine.
- Recent administration of a nasal influenza vaccine may cause false positive results for Influenza A and/or Influenza B.
- Due to the genetic similarity between Human Rhinovirus and Enterovirus, the FilmArray RP cannot reliably differentiate them. A positive FilmArray RP Rhinovirus/Enterovirus result should be followed-up using an alternate method (e.g. cell culture or sequence analysis).
- The Coronavirus OC43 assay may cross-react with Coronavirus HKU1. As a result, when both HKU1 and OC43 are detected in the same patient specimen, the result may be due to assay cross-reactivity. A coinfection with these two viruses is also possible.
- The FilmArray RP may not be able to distinguish between existing viral strains and new variants as they emerge. For example, the FilmArray RP can detect Influenza A H3N2v (first recognized in August, 2011), but will not be able to distinguish this variant from Influenza A H3N2 seasonal.
- Results of the FilmArray RP *B. pertussis* assay may not be concordant with the results of commonly used *Bordetella* PCR assays that target the multi-copy insertion sequence (IS481) due to differences in sensitivity and specificity. IS481 is a multi-copy target and is present in several *Bordetella* species (*B. pertussis, B. holmesii and B. bronchiseptica*). The FilmArray RP *B. pertussis* assay targets the single-copy promoter region of the pertussis toxin gene and is designed to be highly specific for detection of *B. pertussis.* Crossreactivity with other closely related *Bordetella* species (e.g. *B. parapertussis, B. holmesii, and B.bronchiseptica*) has not been observed with the FilmArray *B. pertussis* assay. However, cross reactivity has been observed when testing at concentrations above $1x10^6$ CFU/mL.

NOTE: Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Bordetella pertussis,* Coronavirus 229E, Coronavirus OC43, Influenza A H1, Influenza A H3, Influenza A H1-2009, Influenza B, *Mycoplasma pneumoniae*, Parainfluenza Virus 1, Parainfluenza Virus 2, and Parainfluenza Virus 4 were established primarily with retrospective clinical specimens. Performance characteristics for *Chlamydophila pneumoniae* were established primarily using contrived clinical specimens.

NOTE: Due to the genetic similarity between Human Rhinovirus and Enterovirus, the FilmArray RP cannot reliably differentiate them. A positive FilmArray RP Rhinovirus/Enterovirus result should be followed-up using an alternate method (e.g., cell culture or sequence analysis). The FilmArray RP assay for Coronavirus OC43 may cross-react with some isolates of Coronavirus HKU1. A dual positive result may be due to cross-reactivity or may indicate a co-infection. Performance characteristics for Influenza A were established when Influenza A H1-2009, A H1, and A H3 were the predominant Influenza A viruses in circulation.

Validation of performance characteristics

Imprecision: Imprecision performed for within-day (N=3) and day-to-day for (N=3) pooled samlpes demonstrated 100% agreement for suspected organisms. Imprecision performed on (N=4) pooled samples for between testing personnel demonstrated 100% agreement for suspected organisms.

Accuracy: A total of 32 BioFire RP panels were performed on 15 UCDHS molecular samples and 7 pooled samples from Zeptometrix were analyzed over a 5 days period. There was 100% agreement between reported/expected results and BioFire RP panel results. All negative NPS performed in molecular pathology (N=5) were also negative by BioFire method.

References

BioFire Diagnostics, LLC FilmArray Respiratory Panel (RP) CE-IVD Instruction Booklet 0088 BioFire Diagnostics, LLC | 390 Wakara Way, Salt Lake City, Utah 84108, USA | 1-801-736-6354

BioFire Respiratory Panel Procedure #_1243__

Procedure History

Date	Written By	Revision	Approved Date	Approved By
12/2014	R Gosselin	New	12/10/2014	L Howell MD
1/2015	R Gosselin	Monthly QC Daily Log revision	1/23/2015	C Polage MD
9/2015	L Gandy	New kit		
