EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
FilmArray Respiratory Panel 2 plus (RP2*plus*)

DECISION SUMMARY

A. DEN Number:
DEN170017

B. Purpose for Submission:
De Novo request for evaluation of automatic class III designation for the FilmArray Respiratory Panel 2 *plus* (RP2*plus*).

C. Measurands:
The assay detects and identifies nucleic acids of the following respiratory pathogens: Middle East Respiratory Syndrome Coronavirus (MERS-CoV), 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, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus, *Bordetella parapertussis* (*IS*1001), *Bordetella pertussis* (*ptxP*), *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*.

D. Type of Test:
A multiplexed nucleic acid test intended for use with the FilmArray 2.0 or FilmArray Torch systems for the simultaneous qualitative detection and identification of nucleic acids from Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and multiple common viral and bacterial respiratory pathogens (as identified above) in nasopharyngeal swabs (NPS) obtained from individuals meeting MERS-CoV clinical and/or epidemiological criteria (for example, clinical signs and symptoms associated with MERS-CoV infection, contact with a probable or confirmed MERS-CoV case, history of travel to geographic locations where MERS-CoV cases were detected, or other epidemiological links for which MERS-CoV testing may be indicated.

E. Applicant:
BioFire Diagnostics, LLC

F. Proprietary and Established Names:
FilmArray Respiratory Panel 2 *plus* (RP2*plus*)

G. Regulatory Information:
1. Regulation:
   21 CFR 866.4001

2. Classification:
   Class II (special controls)

3. Product code(s):

   PZF

4. Panel:
   83- Microbiology

H. Indications for Use:

1. Indications for Use:
   The FilmArray Respiratory Panel 2 *plus* (RP2*plus*) is a multiplexed nucleic acid test intended for use with FilmArray 2.0 or FilmArray Torch systems for the simultaneous qualitative detection and identification of nucleic acids from Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and multiple common viral and bacterial respiratory pathogens in nasopharyngeal swabs (NPS) obtained from individuals meeting MERS-CoV clinical and/or epidemiological criteria.

   Testing with the FilmArray RP2*plus* should not be performed unless the patient meets clinical and/or epidemiologic criteria for testing suspected MERS-CoV specimens. This includes: clinical signs and symptoms associated with MERS-CoV infection, contact with a probable or confirmed MERS-CoV case, history of travel to geographic locations where MERS-CoV cases were detected, or other epidemiological links for which MERS-CoV testing may be indicated.

   The FilmArray RP2*plus* identifies:

   - Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

   And the following viral and bacterial respiratory pathogen types and subtypes:

   - 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
   - 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*

The detection and identification of specific viral and bacterial nucleic acids from MERS-CoV and other respiratory pathogens in individuals meeting MERS-CoV clinical and/or epidemiological criteria aids in the differential diagnosis of MERS-CoV infection, if used in conjunction with other clinical and epidemiological information in accordance with the guidelines provided by the appropriate public health authorities.

FilmArray RP2*plus* MERS-CoV positive results are for the presumptive identification of MERS-CoV. The definitive identification of MERS-CoV requires additional testing and confirmation procedures in consultation with the appropriate public health authorities (e.g., local or state public health departments, etc.) for whom reporting is necessary. The diagnosis of MERS-CoV infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the identification of MERS-CoV.

FilmArray RP2*plus* MERS-CoV negative results, even in the context of a FilmArray RP2*plus* positive result for one or more of the common respiratory pathogens, do not preclude MERS-CoV infection and should not be used as the sole basis for patient management decisions. The levels of MERS-CoV that would be present in NPS specimens from individuals with early infection and from asymptomatic MERS-CoV carriers are not well understood. The FilmArray RP2*plus* MERS-CoV negative results may also be due to lower respiratory tract infection with MERS-CoV that may not be detected by an NPS specimen. In this context, collection of lower respiratory and serum specimens (if possible) for MERS-CoV testing using other laboratory tests is highly recommended in addition to testing for MERS-CoV RNA in NPS specimens (i.e., upper respiratory specimens) using the FilmArray RP2*plus*. A negative FilmArray RP2*plus* MERS-CoV result in an asymptomatic individual does not rule out the possibility of future illness and does not demonstrate that the individual is not infectious.

Viral culture should not be attempted in the cases of positive FilmArray RP2*plus* results for MERS-CoV unless a BSL 3 facility is available to receive and culture specimens.

Negative FilmArray RP2*plus* results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or other pathogens that may not be detected by an NPS specimen. Positive FilmArray RP2*plus* results do not rule out co-infection with other organisms: the agent(s) detected by the FilmArray RP2*plus* may not be the definite cause of disease.

Due to the genetic similarity between Human Rhinovirus and Enterovirus, the FilmArray RP2*plus* cannot reliably differentiate them. A positive FilmArray RP2*plus* Rhinovirus/Enterovirus result should be followed up using an alternate method (e.g., cell culture or sequence analysis) if differentiation is required.

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. Performance of detecting Influenza A may vary if other Influenza A strains are circulating or a novel Influenza A virus emerges. If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

2. Special conditions for use statement(s):
   For prescription use only.
   For in vitro diagnostic use.

3. Special instrument requirements:
   FilmArray Respiratory Panel 2 *plus* (RP2*plus*) is performed on the FilmArray 2.0 or the FilmArray Torch systems.

I. Device Description:

The FilmArray Respiratory Panel 2 *plus* (RP2*plus*) is designed to simultaneously detect and identify MERS-CoV and 21 different common pathogens (see the Indications for Use section) of respiratory tract infection from a single NPS specimen in a time frame (~45 minutes) that may allow the test results to be used as an aid in determining appropriate patient treatment and management. FilmArray RP2*plus* is compatible with BioFire Diagnostics' (BioFire) PCR-based *in vitro* diagnostic FilmArray 2.0 and FilmArray Torch systems for infectious disease testing. A specific software module (i.e., FilmArray RP2*plus* pouch module) is used to perform FilmArray RP2*plus* testing on these systems.

A test is initiated by loading Hydration Solution into one port of the FilmArray pouch and a NPS sample (in transport media) mixed with the provided Sample Buffer into the other port of the FilmArray RP2*plus* pouch and placing it in a FilmArray instrument. The FilmArray pouch contains all the reagents required for specimen testing and analysis in a freeze-dried format; the addition of Hydration Solution and Sample/Buffer Mix rehydrates the reagents. After the pouch is prepared, the FilmArray Software guides the user though the steps of placing the pouch into the instrument, scanning the pouch barcode, entering the sample identification, and initiating the run.

The FilmArray instrument contains a coordinated system of inflatable bladders and seal points, which act on the pouch to control the movement of liquid between the pouch blisters. When a bladder is inflated over a reagent blister, it forces liquid from the blister into connecting channels. Alternatively, when a seal is placed over a connecting channel it acts as a valve to open or close a channel. In addition, electronically-controlled pneumatic pistons are positioned over multiple plungers in order to deliver the rehydrated reagents into the blisters at the appropriate times. Two Peltier devices control heating and cooling of the pouch to drive the PCR reactions and the melt curve analysis.

Nucleic acid extraction occurs within the FilmArray pouch using mechanical and chemical lysis followed by purification using standard magnetic bead technology. After extracting and purifying nucleic acids from the unprocessed sample, the FilmArray performs a nested multiplex PCR that is executed in two stages. During the first stage, the FilmArray performs a single, large volume, highly multiplexed reverse transcription PCR (RT-PCR) reaction, PCR1. The products from first stage PCR are then diluted and combined with a fresh, primer-free master mix and a fluorescent double stranded DNA binding dye (LC Green Plus, BioFire Diagnostics, LLC). The solution is then distributed to each well of the array. Array wells contain sets of primers designed specifically to amplify sequences internal to the PCR products generated during the first stage PCR reaction. The 2nd stage PCR, or nested PCR, PCR2, is performed in singleplex fashion in each well of the array. At the conclusion of the 2nd stage PCR, the array is interrogated by melt curve analysis for the detection of signature amplicons denoting the presence of specific targets. A digital camera placed in front of the 2nd stage PCR captures fluorescent images of the PCR reactions and software interprets the data.

The FilmArray Software automatically interprets the results of each DNA melt curve analysis and combines the data with the results of the internal pouch controls to provide a test result for each organism on the panel.

Materials provided in each FilmArray RP2*plus* kit:
Each kit contains sufficient reagents to test 6 samples (6-test kit; RFIT-ASY-0137) or 30 samples (30-test kit; RFIT-ASY-0136):

- Individually-packaged FilmArray RP2*plus* 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

Materials required but not provided:

- 10% bleach solution

FilmArray system including:

- FilmArray 2.0 or FilmArray Touch and accompanying software
- FilmArray Pouch Loading Station

Interpretation of Results
When PCR2 is complete, the FilmArray instrument performs a DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well. 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 (i.e., within 1°C). Assays that do not meet these criteria are called negative.

For the following organisms detected by the FilmArray RP2*plus*, the organism is reported as "Detected" if a single corresponding assay is positive.

- Coronavirus 229E
- Coronavirus HKU1
- Coronavirus NL63
- Coronavirus OC43
- Human Metapneumovirus
- Human Rhinovirus/Enterovirus
- Influenza B
- 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*

The test results for MERS-CoV, Adenovirus, and Influenza A (including subtyping) depend on the interpretation of results from more than one corresponding assay. Interpretation and actions for these results are provided below.

- MERS-CoV
  The FilmArray RP2*plus* 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 MERS-CoV "Detected". If only one assay is positive, the result is MERS-CoV "Equivocal" and the sample should be retested. If both the assays are negative, the test report result will be MERS-CoV "Not Detected".
- Adenovirus
  The FilmArray RP2*plus* 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 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 and Subtyping
  The assays in the FilmArray RP2*plus* are designed to both detect Influenza A and to differentiate commonly occurring hemagglutinin subtypes. To accomplish this, the FilmArray RP2*plus* uses two Influenza A assays, FluA-pan-1 and FluA-pan-2, and three subtyping assays, FluA-H1-2, FluA-H1-2009, and FluA-H3, directed at the respective hemagglutinin gene. Each of the individual assays is interpreted independently and the test result reported for Influenza A is based on the combined results of the five assays as outlined in Table 1.

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 positive	Negative	Negative	Negative	Retest
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	

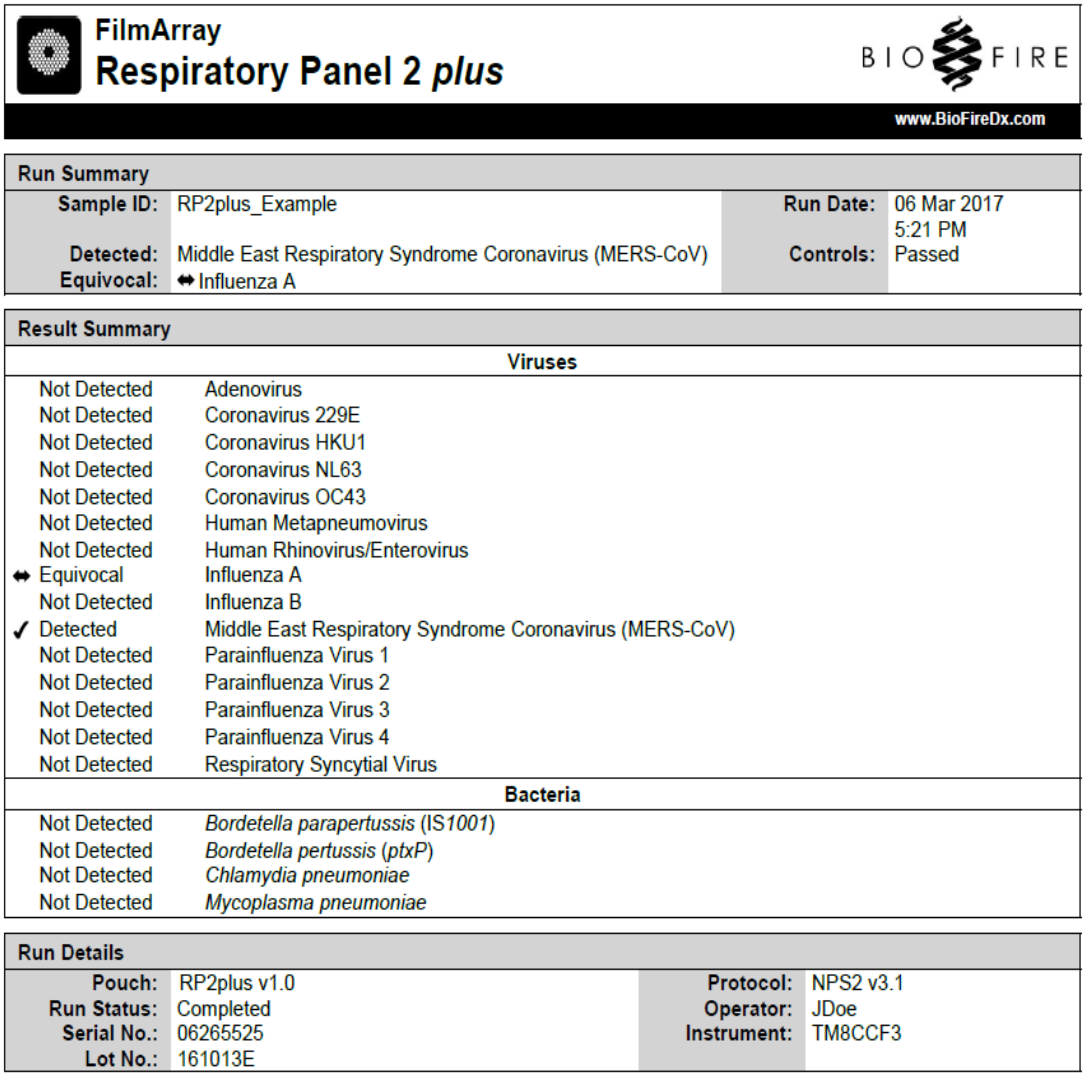
[a] The FilmArray RP2*plus* can simultaneously detect multiple influenza viruses contained in multivalent vaccines.

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

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

FilmArray RP2*plus* Test Report

The FilmArray 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. An example of the test report is presented below:



FilmArray
Respiratory Panel 2 *plus*

BIOFIRE
www.BioFireDx.com

Run Summary

Sample ID:	RP2plus_Example	**Run Date:**	06 Mar 2017 5:21 PM
Detected:	Middle East Respiratory Syndrome Coronavirus (MERS-CoV)	**Controls:**	Passed
Equivocal:	⬌ Influenza A		

Result Summary

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

Run Details

Pouch:	RP2plus v1.0	**Protocol:**	NPS2 v3.1
Run Status:	Completed	**Operator:**	JDoe
Serial No.:	06265525	**Instrument:**	TM8CCF3
Lot No.:	161013E		

- 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 assays for all the organism were negative, then "None" will be displayed in the Detected field. All Influenza A equivocal results (refer to Table 2) will be displayed in the Equivocal field. Controls are listed as "Passed", "Failed" or "Invalid". Table 2 below provides additional information for each of the possible control field results.

Table 2: Interpretation of Controls Field on the FilmArray RP2*plus* Test Report

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

  Table 3 below 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
Detected [a]	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism were POSITIVE (i.e., met the requirements for a positive result described in the Interpretation of Results section above)	Report results.
Not Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism were NEGATIVE (i.e., did not meet the requirements for a positive result described in the Assay Interpretation section above)	Report results.
Equivocal (Influenza A and MERS-CoV only)	The run was successfully completed AND The pouch controls were successful (Passed) AND The combination of positive and negative assay results for Influenza A were inconclusive (see Table 2)	Retest the original specimen using a new pouch and report the results of the retest.
Invalid	The pouch controls were not successful (Failed) OR The run was not successful (Run Status displayed as: Aborted, Incomplete, Instrument Error or Software Error)	See Table 2

[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.

J. Standard/Guidance Document Referenced (if applicable):

- FDA guidance document issued on August 27, 2014, titled *"Highly Multiplexed Microbiological/Medical Countermeasure In Vitro Nucleic Acid Based Diagnostic Devices"*
- FDA guidance document issued on October 9, 2009, titled "*Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay"*
- FDA guidance document issued on October 9, 2009, titled "*Class II Special Controls Guidance Document: Testing for Detection and Differentiation of Influenza A Virus Subtypes Using Multiplex Assays"*
- FDA guidance document issued on October 9, 2009, titled *"Class II Special Controls Guidance Document: Testing for Human Metapneumovirus (hMPV) Using Nucleic Acid Assays"*
- FDA guidance document issued on March 13, 2007, titled *"Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests"*
- FDA guidance document issued on July 15, 2011, titled *"Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses"*
- FDA guidance document issued on April 25, 2005, titled *"Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable"*
- FDA guidance document issued on May 11, 2005, titled *"Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices"*
- FDA guidance document issued on September 9, 1999, titled *"Off-The-Shelf Software Use in Medical Devices"*
- FDA guidance document issued on January 11, 2002, titled *"General Principle of Software Validation"*
- FDA guidance document issued on January 1, 2000, titled *"Guidance for Industry and FDA on Alternative to Certain Prescription Device Labeling Requirements"*
- Interference Testing in Clinical Chemistry; Clinical and Laboratory Standards Institute (CLSI) Approved Guideline – Second Addition, EP7-A2 (2005)
- User Protocol for Evaluation of Qualitative Test Performance; Clinical and Laboratory Standards Institute (CLSI) Approved Guideline – Second Edition, EP12-A2 (January 2008)
- Molecular Diagnostic Methods for Infectious Diseases; Clinical and Laboratory Standards Institute (CLSI) Approved Guideline, MM3-A2 (February 2006)

- Evaluation of Stability of In Vitro Diagnostic Reagents; Clinical and Laboratory Standards Institute (CLSI) Approved Guideline, EP25-A (September 2009)

K. Test Principle:

The FilmArray instrument, software, and pouch work together to perform sample lysis and purification, amplification, and detection of nucleic acid.

One of the primary functions of a FilmArray instrument is to drive the various steps in the testing process by moving liquids to appropriate locations within the pouch. Liquids are moved from the fitment to the blisters of the pouch by means of (b) (4) in the instrument piston block which press on the syringe-like plunging devices in the pouch fitment. There are 12 pistons that operate in a specified sequence to deliver reagents to the appropriate blisters in the pouch when they are needed.

Within the pouch, liquids are moved by using bladders and hard seals to exert pressure on the exterior of the pouch, such that the instrument is never in contact with the liquids contained in the pouch. The bladders are inflatable elastomeric membranes used to 'squish' the pouch blisters, thus forcing the liquid out of the compressed blister and along any connecting channels. The hard-seals are piston driven actuators used to direct the flow of liquids from the blisters by pinching shut the channels and blocking flow while pinched.

Thermal interactions between the pouch and instrument play a crucial role in the amplification of target nucleic acids. Temperature control is driven by a pair of numerically controlled Peltier devices; solid-state thermal control instruments fitted with calibrated temperature sensors and protective circuitry. These Peltier devices heat and cool to perform the first and second stage PCR reactions and carefully control the temperature across the array during DNA melting. The instrument uses a blue LED to illuminate the second stage PCR array and a digital camera to record fluorescence generated in the second stage PCR. The optical system is designed to detect the fluorescence signal generated during DNA melting.

The instrument communicates with the computer and the FilmArray software using Ethernet cables. The software provides instructions to the instrument to control each of the steps described above.

A detailed explanation of specific steps in the testing process is provided below:

1. Sample Lysis and Purification
   Nucleic acid purification occurs in the first four blisters of the pouch using magnetic bead technology.

   a. Sample Lysis
   Prior to loading the sample into the pouch, nucleases are inactivated by mixing the sample with a denaturing buffer (FilmArray Sample Buffer). The sample/sample buffer mixture is then loaded into the pouch via the injection port and pouch vacuum pulls the

liquid into the sample well of the pouch fitment.

During the addition of the sample/sample buffer mixture, the template for the RNA Process Control is rehydrated and introduced into the reaction mixture. The RNA Process Control targets an mRNA of *Schizosaccharomyces pombe*, which is freeze-dried into the sample well of each pouch. The *S. pombe* is processed in parallel with the patient sample through each step of the test including nucleic acid extraction, reverse transcription (RT), PCR1, PCR2, and DNA melting. A positive result for the RNA Process Control indicates that all steps in the test are functioning properly.

The instrument activates a piston located above the fitment to move the sample/sample buffer mixture from the fitment into the trapezoidal sample lysis blister and then heat-seals the fitment to prevent sample loss. The sample lysis blister contains ceramic beads. The instrument then activates the bead beater assembly which rotates a metal bar that strikes the pouch for 60 seconds to lyse organisms in the sample by creating high speed impacts between the sample and beads (bead beating). At the conclusion of the bead beating process, cells and organisms are lysed and the cell contents, including the nucleic acids, are released into the reaction mixture.

b. Nucleic Acid Isolation

The instrument inflates the appropriate bladder and moves the lysed sample into the magnetic bead blister. Here, the liberated nucleic acids are captured by adsorption to silica- magnetic beads.

The instrument then uses a retractable magnet positioned adjacent to the blister to hold the beads against the inside of the blister while they are washed to remove proteins, cell debris, and other potential PCR inhibitors. The instrument moves the wash buffer from the fitment into the appropriate blisters by exerting pressure on pistons located above the fitment.

After the washes are completed, an elution buffer is moved from the fitment to the appropriate blister resulting in the nucleic acids being released from the beads. The instrument then moves the purified nucleic acid solution to the 1st stage PCR blister while the beads and other waste products are pushed back to the trapezoidal blister.

2. Reverse Transcription and 1st Stage Multiplex PCR

In the 1st stage PCR blister, liquid containing the purified nucleic acid rehydrates a freeze-dried reagent pellet containing all the outer primers. A PCR master mix, containing all components needed for PCR and reverse transcription (RT), is moved from the fitment to an adjoining blister. A Peltier device is in contact with these two blisters and the instrument performs a “hot-start” PCR and RT by preheating the blisters containing the purified sample and the PCR master mix. Once the appropriate temperature is reached, the contents of the two blisters are mixed and the RT step and thermo-cycling is initiated.

Since the FilmArray RP2*plus* includes RNA viruses, an RT step is needed to convert the viral RNA into cDNA that can be amplified by PCR. Both the RT and the first stage of the

nested PCR reaction are performed using the same outer primers and master mix.

3. Dilution, 2nd Stage PCR and DNA Melt Analysis
   Following completion of the RT and 1st stage PCR steps, a second singleplex PCR is carried out. To accomplish this, the instrument dilutes the products of the 1st stage PCR with fresh PCR master mix containing a double stranded DNA binding dye (LC Green Plus, BioFire Diagnostics, LLC). This solution is distributed over the 2nd stage PCR array, where it rehydrates the dried primers in each well. The individual wells in the array contain primers for different assays (each assay is present in triplicate wells of the array) that target specific nucleic acid sequences from each of the pathogens or control templates. The primers in the PCR2 array are "nested" or internal to the specific PCR products of the 1st stage multiplex reaction. A second Peltier device is responsible for driving the PCR2 reaction and for controlling temperature during DNA melting. The product of the 2nd stage PCR is visualized with the fluorescent LC Green Plus dye. At the conclusion of PCR2, the temperature of the array is gradually increased and the fluorescence in each well is monitored and analyzed to generate a melt curve. The instrument then transfers images and temperature measurements to the FilmArray software for analysis.

   The PCR2 array also contains a control assay, called the PCR2 Control, which is comprised of a specific set of PCR2 assay primers along with the corresponding template pre-spotted into three specific wells of the array. Failure of the PCR2 Control invalidates the run and indicates a test failure that is specific to the PCR2 step of the testing process.

4. Data Analysis and Result Reporting
   The temperature at which a specific PCR product melts (melting temperature or Tm) is consistent and predictable, and the FilmArray software automatically evaluates the results from replicate wells of each assay for the detection of amplicons with a specific Tm, which denotes the presence of specific bacterial or viral targets. The FilmArray software uses the following steps to interpret the melt curve data generated from each FilmArray RP2*plus* assay:

   a. Analysis of Melt Curves
   First, the FilmArray RP2*plus* Melt Detector performs a set of basic calculations on the melt data to determine if a PCR reaction occurred in each well. If the melt profile indicates that a PCR product is present, then the analysis software calculates one or two Tm values, depending on the number of melt curves present in the data, and the Tm values are compared against an expected melt range for the associated assay. If the software determines that the melt is positive and the melt curve falls inside the assay's specific melt range, then the curve is called positive. If the software determines that the melt is negative or that it is not in the appropriate range, then the curve is called negative.

   b. Analysis of Replicates
   Next, the analysis software evaluates the replicates for each assay (target and control) to determine if the assay is positive or negative. To be called positive, at least two of the three wells associated with an assay must have a positive melt curve and the Tm for

the positive curves must be similar (i.e., within 1°C). Assays with replicates that do not meet these criteria are called negative.

c. Analysis of Controls

Results for control assays are compared to their expected values and assigned a single pass or fail result for each control. Pouch-specific rules define how control failures affect interpretations. The default rule specifies that any control failure invalidates the entire run. For the FilmArray RP2*plus*, failure of the RNA Process Control or the PCR2 Control is interpreted as a control failure and all target assays (regardless of the assay result) are assigned a test result of invalid.

d. Interpretation of Assay Results

Once the results for the individual assays are determined, the software applies interpretation rules to determine the final test results. For many organisms, the target is determined to be present or absent if a single associated assay is positive or negative, respectively. For these organisms, the final test result is either "Detected" (for positive results), "Not Detected" (for negative results) or "Invalid" (when either control fails or the run fails). The FilmArray RP2*plus* also includes test results that rely on the results of multiple assays. See the Interpretation of Results section for more information on interpreting these test results.

L. Performance Characteristics:

1. Analytical performance:

a. Reproducibility

A reproducibility study was conducted at three testing sites on a combination of FilmArray 2.0 and FilmArray Torch systems. The study incorporated a range of potential variation introduced by site (three testing sites), day (five different days), operator (at least two per site), system, instrument or Torch module (at least three per site/sample), and pouch lot (at least three).

A total of four contrived NPS samples containing known quantities of various RP2*plus* analytes (Table 4 below) were prepared in simulated NPS in VTM sample matrix[1]. The contrived samples contained combinations of 12 different FilmArray RP2*plus* analytes[2], each at three different concentrations, Negative, Low Positive (1×LoD), and Moderate Positive (3×LoD). The negative data were acquired from samples not spiked with a particular analyte (i.e., negative data for the analytes in Sample#1 and #2 were obtained from Sample#3 and #4, and vice-versa).

[1] Note: The simulated NPS in VTM sample matrix and the natural NPS in VTM sample matrix were demonstrated to be equivalent to FilmArray RP test detectability of analytes in analytical studies in support of the original FDA-clearance of the FilmArray RP test. Refer to K103175, K110764, and K120267 for additional details of the analytical studies.

[2] Note: Single-spiked and multi-spiked specimens were demonstrated to be equivalent to FilmArray RP test detectability of analytes in analytical studies in support of the original FDA-clearance of the FilmArray RP test. Refer to K103175, K110764, and K120267 for additional details of the analytical studies.

Table 4: Reproducibility Test Panel for the FilmArray RP2*plus*

Organism	Strain/Sero type	Source/ ID	Limit of Detection (LoD) Concentration	Sample #1 Concentration (1xLoD)	Sample #2 Concentration (3xLoD)
Coronavirus OC43	OC43	ATCC VR-759	**5.6E+02 Copies/mL** (3.0E+01 $TCID_{50}$/mL)	**5.6E+02 Copies/mL** (3.0E+01 $TCID_{50}$/mL)	**1.7E+03 Copies/mL** (9.0E+01 $TCID_{50}$/mL)
Parainfluenza virus 2	Type 2	Zeptometrix 0810015CF	**3.0E+01 Copies/mL** (5.0E-01 $TCID_{50}$/mL)	**3.0E+01 Copies/mL** (5.0E-01 $TCID_{50}$/mL)	**9.0E+01 Copies/mL** (1.5E+00 $TCID_{50}$/mL)
Adenovirus C2	Species C Serotype 2	ATCC VR-846	**3.7E+01 Copies/mL** (2.0E+00 $TCID_{50}$/mL)	**3.7E+01 Copies/mL** (2.0E+00 $TCID_{50}$/mL)	**1.1E+02 Copies/mL** (6.0E+00 $TCID_{50}$/mL)
Influenza A H3N2	A/Port Chalmers/1/73	ATCC VR-810	**2.1E+01 Copies/mL** (1.0E-01 $TCID_{50}$/mL)	**2.1E+01 Copies/mL** (1.0E-01 $TCID_{50}$/mL)	**6.3E+01 Copies/mL** (3.0E-01 $TCID_{50}$/mL)
Rhinovirus	Type 1A	Zeptometrix 0810012CFN	**3.8E+01 Copies/mL** (1.0E-01 $TCID_{50}$/mL)	**3.8E+01 Copies/mL** (1.0E-01 $TCID_{50}$/mL)	**1.1E+02 Copies/mL** (3.0E-01 $TCID_{50}$/mL)
Bordetella parapertussis	A747	Zeptometrix 081461	**5.8E+01 *IS1001* Copies/mL** (4.1E+01 CFU/mL)	**5.8E+01 *IS1001* Copies/mL** (4.1E+01 CFU/mL)	**1.7E+02 *IS1001* Copies/mL** (1.2E+02 CFU/mL)
Organism	**Strain/Sero type**	**Source/ ID**	**Limit of Detection (LoD)**	**Sample #3 (1xLoD)**	**Sample #4 (3xLoD)**
Chlamydia pneumoniae	TW183	ATCC VR-92	**6.6E+01 Copies/mL** (1.0E-01 $TCID_{50}$/mL)	**6.6E+01 Copies/mL** (1.0E-01 $TCID_{50}$/mL)	**2.0E+02 Copies/mL** (3.0E-01 $TCID_{50}$/mL)
Influenza B	B/FL/04/06	Zeptometrix 0810255CF [a]	**3.4E+01 Copies/mL** (5.0E+00 $TCID_{50}$/mL)	**3.4E+01 Copies/mL** (5.0E+00 $TCID_{50}$/mL)	**1.0E+02 Copies/mL** (1.5E+01 $TCID_{50}$/mL)
Parainfluenza virus 4	Type 4a	Zeptometrix 0810060CF	**1.6E+03 Copies/mL** (5.0E+01 $TCID_{50}$/mL)	**1.6E+03 Copies/mL** (5.0E+01 $TCID_{50}$/mL)	**4.8E+03 Copies/mL** (1.5E+02 $TCID_{50}$/mL)
Human Metapneumo virus	Type 16, A1 IA10-2003	Zeptometrix 0810161CF	**1.2E+03 Copies/mL** (1.0E+01 $TCID_{50}$/mL)	**1.2E+03 Copies/mL** (1.0E+01 $TCID_{50}$/mL)	**3.6E+03 Copies/mL** (3.0E+01 $TCID_{50}$/mL)
Respiratory Syncytial Virus	Type A	Zeptometrix 0810040ACF	**9.0E+00 Copies/mL** (2.0E-02 $TCID_{50}$/mL)	**9.0E+00 Copies/mL** (2.0E-02 $TCID_{50}$/mL)	**2.7E+01 Copies/mL** (6.0E-02 $TCID_{50}$/mL)
Bordetella pertussis [b]	A639	Zeptometrix 0801459	**1.0E+03 CFU/mL**	**1.0E+03 CFU/mL**	**3.0E+03 CFU/mL**

[a] Formerly Zeptometrix 0810037CF.

[b] For *B. pertussis,* the FilmArray RP2*plus* amplifies a single-copy target and commercially available qPCR assays typically target the multi-copy IS*481* sequences, therefore RP2*plus* testing was performed based on the CFU/mL and an equivalent Copies/mL was not determined for this analyte.

Once prepared, each sample of the reproducibility study panel was tested with the FilmArray RP2*plus* to confirm it contained the intended analytes at the intended concentration and then divided into single-use aliquots (400 µL) and stored frozen (≤ -70˚C) until the day of testing.

After being distributed to the sites, six replicates of each sample were tested on five different days on various FilmArray Torch modules (Site A, Site C) or FilmArray 2.0

instruments (Site B, Site C). Sites A and C were configured with at least three Torch modules per sample, while sites B and C were configured to utilize at least three different FilmArray 2.0 instruments per sample. The Site C tested a total of 12 replicates of each sample per day, with six replicates tested on the Torch system and six replicates tested on the FilmArray 2.0 system.

Daily testing was performed by at least two different operators per system and site, and three different pouch lots were used on rotating days so that data from all variables were distributed between reagent lots. For any required retest per the Instructions for Use, another aliquot of the same sample was tested on the same day by the same operator using the same system, instrument or Torch module, and pouch lot. Results of the valid retest were used as the final test result for the sample aliquot.

In total, 120 data points per sample (over a total of 480 valid runs) were obtained, with 60 data points per sample per system (i.e., FilmArray 2.0 and FilmArray Torch systems), 30 data points per sample per Site A and B, and 60 data points at Site C.

Over the course of the reproducibility study, a total of 15 different FilmArray 2.0 instruments and 19 different FilmArray Torch modules (four Torch bases) were used by seven operators at three sites. Valid results were obtained from 480 out of the 489 runs that were initiated (480/489, 98.2%). The majority of invalid runs (8/9) were associated with a Control failure, while one invalid run was due to an instrument error (Table 5 below).

Table 5: Performance of the FilmArray Systems and RP2*plus* Controls during the Reproducibility Study

	Runs	Control Failure (Percentage)	Instrument Errors (Percentage)	Software Errors (Percentage)
FilmArray 2.0	247	6 (2.4%)	1 (0.4%)	0 (0.0%)
FilmArray Torch	242	2 (0.8%)	0 (0.0%)	0 (0.0%)
Total	489	8 [a] (1.6%)	1 (0.2%)	0 (0.0%)

[a] Seven control failures occurred on pouch lot 349116, while one occurred on pouch lot 347416.

A summary of the reproducibility study results (percent (%) agreement with the expected Detected or Not Detected result) for each analyte (by site and system) is provided in Table 6 below.

Table 6: Reproducibility of FilmArray RP2*plus* Results on FilmArray Torch and FilmArray 2.0 Systems

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result						
			FilmArray Torch			FilmArray 2.0			All Sites/Systems (95% CI)
			Site A	Site C	System Sub-Total	Site B	Site C	System Sub-Total	
Viruses									
MERS-CoV	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480 100%** (99.2%-100%)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result						
			FilmArray Torch			FilmArray 2.0			All Sites/Systems (95% CI)
			Site A	Site C	System Sub-Total	Site B	Site C	System Sub-Total	
Adenovirus	**Moderate Positive (3× LoD) 1.1E+02 Copies/mL** (6.0E+00 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**29/30** 96.7%	**59/60** 98.3%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**118/120 98.3%** (94.1%-99.8%)
	Low Positive (1× LoD) 3.7E+01 Copies/mL (2.0E+00 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**29/30** 96.7%	**59/60** 98.3%	**119/120 99.2%** (95.4%-100%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Coronavirus 229E	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480 100%** (99.2%-100%)
Coronavirus HKU1	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480 100%** (99.2%-100%)
Coronavirus OC43	**Moderate Positive (3× LoD) 1.7E+03 Copies/mL** (9.0E+01 $TCID_{50}$/mL)	**Detected**	**29/30** 96.7%	**29/30** 96.7%	**58/60** 96.7%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**117/120 97.5%** (92.9%-99.5%)
	Low Positive (1× LoD) 5.6E+02 Copies/mL (3.0E+01 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**27/30** 90.0%	**57/60** 95.0%	**117/120 97.5%** (92.9%-99.5%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Coronavirus NL63	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480 100%** (99.2%-100%)
Human Metapneumovirus	**Moderate Positive (3× LoD) 3.6E+03 Copies/mL** (3.0E+01 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120 100%** (97.0%-100%)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result						
			FilmArray Torch			FilmArray 2.0			All Sites/Systems (95% CI)
			Site A	Site C	System Sub-Total	Site B	Site C	System Sub-Total	
	Low Positive (1× LoD) 1.2E+03 Copies/mL (1.0E+01 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**28/30** 93.3%	**30/30** 100%	**58/60** 96.7%	**118/120 98.3%** (94.1%-99.8%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Human Rhinovirus/ Enterovirus	**Moderate Positive (3× LoD) 1.1E+02 Copies/mL** (3.0E-01 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**28/30** 93.3%	**30/30** 100%	**58/60** 96.7%	**118/120 98.3%** (94.1%-99.8%)
	Low Positive (1× LoD) 3.8E+01 Copies/mL (1.0E-01 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120 100%** (97.0%-100%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Influenza A H3	**Moderate Positive (3× LoD) 6.3E+01 Copies/mL** (3.0E-01 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**119/120 99.2%** (95.4%-100%)
	Low Positive (1× LoD) 2.1E+01 Copies/mL (1.0E-01 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120 100%** (97.0%-100%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Influenza A H1-2009	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480 100%** (99.2%-100%)
Influenza A H1	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480 100%** (99.2%-100%)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result						
			FilmArray Torch			FilmArray 2.0			All Sites/Systems (95% CI)
			Site A	Site C	System Sub-Total	Site B	Site C	System Sub-Total	
Influenza B	**Moderate Positive (3× LoD) 1.0E+02 Copies/mL** (1.5E+01 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120 100%** (97.0%-100%)
	Low Positive (1× LoD) 3.4E+01 Copies/mL (5.0E+00 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120 100%** (97.0%-100%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Parainfluenza Virus 1	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480 100%** (99.2%-100%)
Parainfluenza Virus 2	**Moderate Positive (3× LoD) 9.0E+01 Copies/mL** (1.5E+00 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**29/30** 96.7%	**59/60** 98.3%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**118/120 98.3%** (94.1%-99.8%)
	Low Positive (1× LoD) 3.0E+01 Copies/mL (5.0E-01 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**29/30** 96.7%	**59/60** 98.3%	**30/30** 100%	**27/30** 90.0%	**57/60** 95.0%	**116/120 96.7%** (91.7%-99.1%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Parainfluenza Virus 3	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480 100%** (99.2%-100%)
Parainfluenza Virus 4	**Moderate Positive (3× LoD) 4.8E+03 Copies/mL** (1.5E+02 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120 100%** (97.0%-100%)
	Low Positive (1× LoD) 1.6E+03 Copies/mL (5.0E+01 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**29/30** 96.7%	**59/60** 98.3%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**118/120 98.3%** (94.1%-99.8%)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result						
			FilmArray Torch			FilmArray 2.0			All Sites/Systems (95% CI)
			Site A	Site C	System Sub-Total	Site B	Site C	System Sub-Total	
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240** **100%** (98.5%-100%)
Respiratory Syncytial Virus	**Moderate Positive (3× LoD) 2.7E+01 Copies/mL** (6.0E-02 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120** **100%** (97.0%-100%)
	Low Positive (1× LoD) 9.0E+00 Copies/mL (2.0E-02 $TCID_{50}$/mL)	**Detected**	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**30/30** 100%	**29/30** 96.7%	**59/60** 98.3%	**118/120** **98.3%** (94.1%-99.8%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240** **100%** (98.5%-100%)
Bacteria									
Bordetella parapertussis	**Moderate Positive (3× LoD) 1.7E+02 *IS1001* Copies/mL** (1.2E+02 CFU/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**119/120** **99.2%** (95.4%-100%)
	Low Positive (1× LoD) 5.8E+01 *IS1001* Copies/mL (4.1E+01 CFU/mL)	**Detected**	**24/30** [a] 80.0%	**29/30** 96.7%	**53/60**[a] 88.3%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**112/120** **93.3%** (87.3%-97.1%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240** **100%** (98.5%-100%)
Bordetella pertussis	**Moderate Positive (3× LoD) 3.0E+03 CFU/mL**	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120** **100%** (97.0%-100%)
	Low Positive (1× LoD) 1.0E+03 CFU/mL	**Detected**	**28/30** 93.3%	**30/30** 100%	**58/60** 96.7%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**118/120** **98.3%** (94.1%-99.8%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240** **100%** (98.5%-100%)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result						
			FilmArray Torch			FilmArray 2.0			All Sites/Systems (95% CI)
			Site A	Site C	System Sub-Total	Site B	Site C	System Sub-Total	
Chlamydia pneumoniae	**Moderate Positive (3× LoD) 2.0E+02 Copies/mL** (3.0E-01 $TCID_{50}$/mL)	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**60/60** 100%	**120/120 100%** (97.0%-100%)
	Low Positive (1× LoD) 6.6E+01 Copies/mL (1.0E-01 $TCID_{50}$/mL)	**Detected**	**28/30** 93.3%	**30/30** 100%	**58/60** 96.7%	**29/30** 96.7%	**30/30** 100%	**59/60** 98.3%	**117/120 97.5%** (92.9%-99.5%)
	None (no analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**120/120** 100%	**60/60** 100%	**60/60** 100%	**120/120** 100%	**240/240 100%** (98.5%-100%)
Mycoplasma pneumoniae	**None** (no analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** 100%	**120/120** 100%	**120/120** 100%	**240/240** 100%	**480/480 100%** (99.2%-100%)
Overall Agreement with the Expected Result All Analytes and All Test Levels (95% Confidence Interval)			**9,562/9,600 99.6% (99.5% – 99.7%)**						

[a] Data from Site A were further reviewed by the unique site-specific variables including test day, Torch module, and operator. No correlation could be found between the Not Detected results and any one or more of these variables. The Not Detected results at Site A were found to be statistically non-significant ($p>0.05$ by Fisher's exact test) and therefore do not appear to indicate a site- or system-dependent variance in precision of the FilmArray RP2*plus Bordetella parapertussis* (IS*1001*) results.

The reproducibility (standard deviation) of melting temperature (Tm) for the amplification products generated by the FilmArray RP2*plus* was also evaluated, with a Tm standard deviation for each assay of ± 0.5°C [3] or less observed within and between the FilmArray 2.0 and FilmArray Torch systems (Table 7 below).

Table 7: Reproducibility of Tm (°C) For Select FilmArray RP2*plus* Assays on FilmArray Torch and FilmArray 2.0 Systems

Analyte	FilmArray RP2 Assay	Tm Observed (°C)[a]									
		FilmArray Torch				FilmArray 2.0				All Sites/Systems	
		Site A		Site C		Site B		Site C			
		Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev
Controls	Yeast RNA	**82.3**	± 0.3	**82.1**	± 0.2	**82.2**	± 0.3	**82.0**	± 0.2	**82.1**	± 0.3
	PCR2	**76.1**	± 0.2	**75.9**	± 0.2	**76.0**	± 0.2	**75.8**	± 0.2	**75.9**	± 0.2
VIRUSES											
Adenovirus	Adeno2	**89.0**	± 0.2	**88.8**	± 0.1	**88.8**	± 0.3	**88.7**	± 0.2	**88.8**	± 0.2
	Adeno6	**89.6**	± 0.2	**89.4**	± 0.2	**89.4**	± 0.3	**89.2**	± 0.2	**89.4**	± 0.3
Coronavirus OC43	CoV-OC43-	**80.7**	± 0.2	**80.6**	± 0.1	**80.6**	± 0.3	**80.5**	± 0.2	**80.6**	± 0.2

[3] Note: The Tm window for an assay is determined based on a mathematical model of known sequences and empirical data. An observed standard deviation in Tm of 0.5°C or less is believed to be adequately ensure that Tm variability contributed by the system (rather than amplicon sequence) will not lead to inaccurate results.

Analyte	FilmArray RP2 Assay	Tm Observed (°C)[a]									
		FilmArray Torch				FilmArray 2.0				All Sites/Systems	
		Site A		Site C		Site B		Site C			
		Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev
	2										
Human Metapneumovirus	hMPV	**78.2**	± 0.3	**78.0**	± 0.2	**78.0**	± 0.3	**77.8**	± 0.2	**78.0**	± 0.3
Rhinovirus	HRV/EV	**84.3**	± 0.2	**84.2**	± 0.2	**84.3**	± 0.3	**84.1**	± 0.2	**84.2**	± 0.2
Influenza A H3N2	FluA-pan1	**84.2**	± 0.2	**84.0**	± 0.1	**84.0**	± 0.3	**83.8**	± 0.2	**84.0**	± 0.2
	FluA-pan2	**78.9**	± 0.2	**78.9**	± 0.1	**78.9**	± 0.2	**78.8**	± 0.2	**78.9**	± 0.2
	FluA-H3	**82.1**	± 0.2	**81.9**	± 0.2	**82.0**	± 0.3	**81.9**	± 0.2	**82.0**	± 0.2
Influenza B	FluB	**80.4**	± 0.3	**80.3**	± 0.2	**80.4**	± 0.2	**80.2**	± 0.2	**80.3**	± 0.2
Parainfluenza virus 2	PIV2	**83.2**	± 0.2	**83.1**	± 0.2	**83.2**	± 0.2	**83.0**	± 0.2	**83.1**	± 0.2
Parainfluenza virus 4	PIV4	**77.1**	± 0.2	**77.0**	± 0.3	**77.2**	± 0.3	**77.0**	± 0.2	**77.1**	± 0.3
Respiratory Syncytial Virus	RSV	**81.2**	± 0.2	**81.1**	± 0.2	**81.1**	± 0.2	**81.0**	± 0.2	**81.1**	± 0.2
BACTERIA											
Bordetella parapertussis	IS*1001*	**87.7**	± 0.2	**87.6**	± 0.2	**87.6**	± 0.3	**87.5**	± 0.2	**87.6**	± 0.2
Bordetella pertussis	ptxP	**88.6**	± 0.2	**88.5**	± 0.2	**88.5**	± 0.3	**88.2**	± 0.2	**88.4**	± 0.3
Chlamydia pneumoniae	Cpne	**79.6**	± 0.3	**79.5**	± 0.2	**79.5**	± 0.3	**79.3**	± 0.2	**79.5**	± 0.3

[a] Mean Tm values are calculated from a combination of Tm values obtained at the 3× LoD and 1× LoD concentrations.

b. *Linearity/assay Reportable Range:*
Not Applicable

c. *Traceability, Stability, Expected Values (controls, calibrators, or methods):*

Assay Controls
Two process controls are included in each pouch:

RNA Process Control
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, PCR1, dilution, PCR2, and DNA melting. A positive control result indicates that all steps carried out in the FilmArray RP2*plus* pouch were successful.

PCR2 Control
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 PCR2 was successful.

Both control assays must be positive for the test run to pass. If the controls fail, the sample should be retested using a new pouch.

The FilmArray Software automatically fails the run if the melting temperature (Tm) for either the RNA Process Control or the PCR2 Control is outside of an acceptable

range (i.e., 80.3-84.3°C for the RNA Process Control and 73.8-77.8°C for the PCR2 Control). If required by local, state, or accrediting organization quality control requirements, users can monitor the system by trending Tm values for the control assays and maintaining records according to standard laboratory quality control practices.

External Controls

External controls are not provided with the FilmArray RP2*plus*. However, five frozen (-70°C) external control mixes (ECMs) (see Table 8 below) were prepared and provided to the clinical study sites for testing during the prospective clinical trial and the clinical study testing contrived specimens. FilmArray operators were required to complete a valid ECM run (correct results obtained) on each day of clinical specimen testing (tested on a rotating basis).

Table 8: External Control Mixes (ECMs) Utilized in the Clinical Evaluations

External Control Mixes	Expected Calls
ECM 1	Adenovirus, Coronavirus 229E, Human Metapneumovirus, Influenza A H1, Influenza B, and *Mycoplasma pneumoniae*
ECM 2	Coronavirus NL63, Influenza A H3, Parainfluenza Virus 3, Respiratory Syncytial Virus and *Bordetella pertussis (ptxP)*
ECM 3	MERS-CoV, Coronavirus HKU1, Influenza A H1-2009, Parainfluenza Virus 4, and *Bordetella parapertussis* (*IS1001*)
ECM 4	Coronavirus OC43, Human Rhinovirus/Enterovirus, Parainfluenza Virus 1, Parainfluenza Virus 2 and *Chlamydia pneumoniae*
ECM 5	Negative (Not Detected)

Since the completion of the prospective clinical trial and the clinical study testing contrived specimens, Maine Molecular Quality Controls Inc. (MMQCI) has developed an external quality control panel specifically for the FilmArray RP2 and FilmArray RP2*plus* assays that consists of ready-to-use single tubes of negative control (matrix only) and positive control composed of synthetic RNA specific for all target analytes by the FilmArray RP2*plus* assay. Three clinical study sites, previously involved in the FilmArray RP2*plus* clinical evaluation, performed testing with three lots of this control material using three lots of the FilmArray RP2*plus* pouches. The expected results were obtained for all 180 control tests performed (i.e., 90/90 negative controls and 90/90 positive controls). These data were provided to MMQCI for inclusion in a 510(k) submission to the FDA.

The sponsor included the following recommending in the product package insert regarding testing external controls:

Good laboratory practice recommends running external positive and negative controls regularly. Transport media can be used as an external negative control. Previously characterized positive samples or negative samples spiked with well characterized organisms can be used as external positive controls.

Alternatively, Maine Molecular Quality Controls, Inc. provides an external positive

and negative assayed quality control panel designed to monitor the performance of *in vitro* laboratory nucleic acid testing procedures for the qualitative detection of Adenovirus, Coronavirus, Human Metapneumovirus, Human Rhinovirus/ Enterovirus, Influenza A, Influenza A subtype H1, Influenza A subtype H1-2009, Influenza A subtype H3, Influenza B, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Parainfluenza Virus, Respiratory Syncytial Virus, *Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae* by BioFire's FilmArray RP2 and RP2*plus* assays on the FilmArray 2.0 or the FilmArray Torch Systems. The FilmArray RP2/RP2*plus* Control Panel is composed of synthetic nucleic acid specifically designed for and intended to be used solely with the FilmArray RP2 and FilmArray RP2*plus* assays. This material is composed of synthetic nucleic acid specific for all analytes targeted by the FilmArray RP2 and the FilmArray RP2*plus* assays, including a MERS-CoV synthetic nucleic acid of less than 500bp. The material is provided as a liquid in a stabilizing matrix. To use the product, the operator opens the tube and uses the Transfer Pipette to deliver the same volume of material as in the actual test, and otherwise runs the test according to protocol. This control is shipped and stored at -20°C. This product is not intended to replace manufacturer internal controls provided with the test system.

The MMQCI external control material is available for purchase directly from:
Maine Molecular Quality Controls, Inc.
23 Mill Brook Road, Saco, Maine 04072
Phone: (207) 885-1072
http://www.mmqci.com
FilmArray® RP2/RP2*plus* Control Panel M315

External controls should be used in accordance with the appropriate accrediting organization requirements, as applicable. It is ultimately the responsibility of each laboratory to determine the frequency of external control testing with the FilmArray Respiratory Panel 2 *plus* as part of the laboratory's Quality Control program.

Specimen Stability
FilmArray RP2*plus* testing requires approximately 0.3 mL of NPS in Viral Transport Medium (VTM) specimen, collected according to standard technique. Samples in VTM should be tested as soon as possible, though they may be stored at room temperature (approximately 23°C) for up to four hours, under refrigeration (approximately 4°C) for up to three days, or frozen (≤-15 °C or ≤-70°C) for up to 30 days.

Detailed documentation concerning NPS in VTM sample storage and transport was provided in the original FilmArray RP submissions (K103175, K110764, and K120267). The results of the analytical study conducted for the original FilmArray RP submissions supported the claim that NPS specimens in Viral Transport Medium (VTM) can be stored for up to 4 hours at room temperature (18-30°C), 3 days in the refrigerator (2-8°C) or 30 days in the freezer (< -15°C) without affecting the accuracy of FilmArray RP test results. The specimen stability claims for the FilmArray

RP2*plus* remain unchanged.

Fresh vs. Frozen Study
Detailed documentation concerning fresh vs. frozen NPS in VTM specimens was provided in the original FilmArray RP submissions (K103175, K110764, and K120267). The results of the analytical study conducted for the original FilmArray RP submissions demonstrated that preservation of samples (by freezing at ≤-70°C) does not affect the accuracy of test results compared to freshly collected or freshly prepared samples. Therefore, it is appropriate to utilize frozen archived prospective and retrospective clinical samples in the evaluation of FilmArray RP2*plus* to supplement the prospective (fresh) clinical study data, and to use frozen simulated samples in analytical studies for this submission.

Single-Spiked vs. Multi-Spiked Specimen Study
Detailed documentation concerning analytical study results used to determine whether the presence of multiple organisms in a specimen would affect the system LoD was provided in the original FilmArray RP submissions (K103175, K110764, and K120267). The results of the analytical study demonstrated that the LoDs from testing single-spiked and multi-spiked specimens were comparable. Therefore, it is appropriate to utilize the multi-spiked approach in conducting the analytical studies and the clinical study testing contrived clinical specimens in support of this submission.

Simulated vs. Natural NPS in VTM Specimen Study
Detailed documentation concerning analytical study results used to determine whether the simulated NPS in VTM sample matrix and the natural NPS in VTM sample matrix were equivalent regarding FilmArray RP test detectability of analytes was provided in the original FilmArray RP submissions (K103175, K110764, and K120267). The simulated NPS in VTM sample matrix and the natural NPS in VTM sample matrix were demonstrated to be equivalent regarding FilmArray RP test detectability of analytes in an analytical study. Therefore, it is appropriate to utilize the simulated NPS in VTM sample matrix in conducting the analytical studies in support of this submission.

Quantification of Nucleic Acids Derived from Viral and Bacterial Cultures
Most of the FilmArray RP2*plus* analytes require specific technique-dependent methods for culturing and quantification. Consistency in the quantification method of isolates and strains within and between species is important to avoid inconsistencies in molecular detection that may be caused by variability of quantification methods based on infectivity or viability. Therefore, an analytical study was conducted to determine the nucleic acids concentration (Copies/mL) of bacterial and virus stocks used in the analytical and clinical studies of the FilmArray RP2*plus* using commercially available quantitative real-time PCR (qPCR) assay kits.

d. Detection Limit:

Limit of detection (LoD) estimation and confirmation studies were carried out with contrived samples in simulated NPS in VTM (Remel M4 transport medium) matrix designed to resemble a natural clinical NPS in VTM specimen. An equivalence study was performed previously which demonstrated that the simulated matrix was equivalent to the natural clinical NPS matrix and did not impact FilmArray RP test performance. Refer to the "Simulated vs. Natural NPS in VTM Specimen Study" section.

Representative isolates of respiratory viruses and bacteria were selected to make contrived samples in order to obtain positive results for every assay on the panel. In some cases, testing of more than one isolate/strain per analyte was performed to assess LoD for clinically important species or variants when more than one assay was needed to detect the expected diversity of an analyte (e.g., Adenovirus).

An estimated LoD concentration for each analyte was first determined by testing contrived samples serially diluted ten-fold to span at least four concentrations (10× to 0.01×) bracketing the anticipated LoD. Five replicates were tested at each dilution, with additional dilutions tested, if needed, to reach a concentration at which loss in detection could be observed. The estimated LoD concentration was subsequently confirmed by testing 20 replicates on the FilmArray 2.0 and 20 replicates on the FilmArray Torch, for a total of 40 replicates at 1×LoD. An additional 20 replicates per system were tested at a 10-fold lower concentration, for a total of 40 replicates at 0.1×LoD. The required criteria for confirmation of LoD was a detection rate of at least 95% at 1×LoD (≥19/20 per system) and a detection rate of less than 95% below LoD (≤19/20 detected per system at 0.1×LoD).

A multi-spiked approach was employed in both the LoD estimation and the LoD confirmation studies where samples spiked with up to five analytes at various concentrations were tested. An equivalence study was performed previously which demonstrated that the multi-spiked samples were equivalent to the single-spiked samples and employing the multi-spiking approach did not impact FilmArray RP test performance. Refer to the "Single-Spiked vs. Multi-Spiked Specimen Study" section.

LoD confirmation criteria were met for each analyte on both the FilmArray 2.0 and FilmArray Torch systems at the same concentration. The confirmed LoD concentrations are shown in Table 9 below with detected results at the 1× (shaded) and 0.1× concentrations presented separately for each system and combined. The LoD concentration for most analytes is reported in Copies/mL, as determined primarily by commercially-available quantitative PCR and RT-PCR assays. Refer to the "Quantification of Nucleic Acids Derived from Viral and Bacterial Cultures" section**.** In addition**,** the titer of viable units for the specific cultures tested (50% tissue culture infectious dose ($TCID_{50}$/mL) or colony forming unit (CFU/mL)) based on the values obtained from the respective Certificate of Analysis (CoA) were also presented.

Table 9: Limit of Detection Confirmation Testing Results for Each RP2*plus* Analyte at 1× LoD and 0.1× LoD on FilmArray 2.0, FilmArray Torch, and Combined

RP2*plus* Analyte	Isolate/Strain	Concentration	FilmArray 2.0	FilmArray Torch	Total
MERS-CoV	**MERS-CoV** EMC/2012 (heat inactivated) BEI NR-50171	**1.3E+02 Copies/mL** (2.0E+00 $TCID_{50}$/mL)	20/20	20/20	40/40
			100%	100%	100%
		1.3E+01 Copies/mL (2.0E-01 $TCID_{50}$/mL)	14/20	18/20	32/40
			70%	90%	80%
Adenovirus	**Adenovirus** Species A, Serotype 18 ATCC VR-19	**7.7E+02 Copies/mL** (5.0E+00 $TCID_{50}$/mL)	20/20	19/20	39/40
			100%	95%	98%
		7.7E+01 Copies/mL (5.0E-01 $TCID_{50}$/mL)	15/20	17/20	32/40
			75%	85%	80%
	Adenovirus Species B, Serotype 7A Zeptometrix 0810021CF	**3.9E+01 Copies/mL** (5.0E-02 $TCID_{50}$/mL)	20/20	20/20	40/40
			100%	100%	100%
		3.9E+00 Copies/mL (5.0E-03 $TCID_{50}$/mL)	13/20	13/20	26/40
			65%	65%	65%
	Adenovirus Species C, Serotype 2 ATCC VR-846	**3.7E+01 Copies/mL** (2.0E+00 $TCID_{50}$/mL)	19/20	20/20	39/40
			95%	100%	98%
		3.7E+00 Copies/mL (2.0E-01 $TCID_{50}$/mL)	13/20	13/20	26/40
			65%	65%	65%
	Adenovirus Species D, Serotype 37 Zeptometrix 0810119CF	**9.0E+00 Copies/mL** (5.0E-02 $TCID_{50}$/mL)	20/20	20/20	40/40
			100%	100%	100%
		9.0E-01 Copies/mL (5.0E-03 $TCID_{50}$/mL)	13/20	10/20	23/40
			65%	50%	58%
	Adenovirus Species E, Serotype 4a S. Carolina/2004, UIRF	**3.0E+02 Copies/mL** (1.0E+01 $TCID_{50}$/mL)	19/20	19/20	38/40
			95%	95%	95%
		3.0E+01 Copies/mL (1.0E+00 $TCID_{50}$/mL)	1/20	6/20	7/40
			5%	30%	18%
	Adenovirus Species F, Serotype 41 Tak, ATCC VR-930	**1.2E+02 Copies/mL** (1.0E+00 $TCID_{50}$/mL)	20/20	20/20	40/40
			100%	100%	100%
		1.2E+01 Copies/mL (1.0E-01 $TCID_{50}$/mL)	12/20	7/20	19/40
			60%	35%	48%
Coronavirus 229E	**Coronavirus 229E** ATCC VR-740	**6.5E+01 Copies/mL** (4.0E-01 $TCID_{50}$/mL)	20/20	20/20	40/40
			100%	100%	100%
		6.5E+00 Copies/mL (4.0E-02 $TCID_{50}$/mL)	14/20	17/20	31/40
			70%	85%	78%
Coronavirus HKU1	**Coronavirus HKU1** Clinical specimen [a]	**2.0E+03 RNA Copies/mL**	20/20	20/20	40/40
			100%	100%	100%
		2.0E+02 RNA Copies/mL	11/20	13/20	24/40
			55%	65%	60%
Coronavirus NL63	**Coronavirus NL63** BEI NR-470	**5.4E+01 Copies/mL** (2.5E-01 $TCID_{50}$/mL)	20/20	20/20	40/40
			100%	100%	100%
		5.4E+00 Copies/mL (2.5E-02 $TCID_{50}$/mL)	12/20	10/20	22/40
			60%	50%	55%
Coronavirus OC43	**Coronavirus OC43** ATCC VR-759	**5.6E+02 Copies/mL** (3.0E+01 $TCID_{50}$/mL)	19/20	20/20	39/40
			95%	100%	98%
		5.6E+01 Copies/mL (3.0E+00 $TCID_{50}$/mL)	14/20	11/20	25/40
			70%	55%	63%
Human Metapneumovirus	**Human Metapneumovirus** 16, Type A1 IA10-2003 Zeptometrix 0810161CF	**1.2E+03 Copies/mL** (1.0E+01 $TCID_{50}$/mL)	20/20	20/20	40/40
			100%	100%	100%
		1.2E+02 Copies/mL (1.0E+00 $TCID_{50}$/mL)	16/20	17/20	33/40
			80%	85%	83%
			20/20	20/20	40/40

Human Rhinovirus/ Enterovirus	**Enterovirus** Species D, Serotype 68 ATCC VR-1823	**2.6E+01 Copies/mL**	100%	100%	100%
		2.6E+00 Copies/mL	12/20	11/20	23/40
		(3.0E+01 $TCID_{50}$/mL)	60%	55%	58%
	Human Rhinovirus Type 1A Zeptometrix 0810012CFN	**3.8E+01 Copies/mL**	20/20	20/20	40/40
		(1.0E-01 $TCID_{50}$/mL)	100%	100%	100%
		3.8E+00 Copies/mL	11/20	13/20	24/40
		(1.0E-02 $TCID_{50}$/mL)	55%	65%	60%
Influenza A H1	**Influenza A H1N1** A/New Caledonia/20/99 Zeptometrix 0810036CF	**1.4E+02 Copies/mL**	20/20	20/20	40/40
		(1.0E+03 $TCID_{50}$/mL)	100%	100%	100%
		1.4E+01 Copies/mL	9/20	12/20	21/40
		(1.0E+02 $TCID_{50}$/mL)	45%	60%	53%
Influenza A H1-2009	**Influenza A H1N1pdm09** A/Swine/NY/03/2009 Zeptometrix 0810249CF	**3.3E+02 Copies/mL**	20/20	20/20	40/40
		(5.0E-01 $TCID_{50}$/mL)	100%	100%	100%
		3.3E+01 Copies/mL	8/20	15/20	23/40
		(5.0E-02 $TCID_{50}$/mL)	40%	75%	58%
Influenza A H3	**Influenza H3N2** A/Port Chalmers/1/73 ATCC VR-810	**2.1E+01 Copies/mL**	20/20	20/20	40/40
		(1.0E-01 $TCID_{50}$/mL)	100%	100%	100%
		2.1E+00 Copies/mL	8/20	9/20	17/40
		(1.0E-02 $TCID_{50}$/mL)	40%	45%	43%
Influenza B	**Influenza B** B/FL/04/06 Zeptometrix 0810255CF	**3.4E+01 Copies/mL**	20/20	20/20	40/40
		(5.0E+00 $TCID_{50}$/mL)	100%	100%	100%
		3.4E+00 Copies/mL	14/20	9/20	23/40
		(5.0E-01 $TCID_{50}$/mL)	70%	45%	58%
Parainfluenza Virus 1	**Parainfluenza Virus 1** Type 1 Zeptometrix 0810014CF	**1.0E+03 Copies/mL**	20/20	20/20	40/40
		(5.0E+00 $TCID_{50}$/mL)	100%	100%	100%
		1.0E+02 Copies/mL	16/20	15/20	31/40
		(5.0E-01 $TCID_{50}$/mL)	80%	75%	78%
Parainfluenza Virus 2	**Parainfluenza Virus 2** Type 2 Zeptometrix 0810015CF	**3.0E+01 Copies/mL**	20/20	20/20	40/40
		(5.0E-01 $TCID_{50}$/mL)	100%	100%	100%
		3.0E+00 Copies/mL	3/20	9/20	12/40
		(5.0E-02 $TCID_{50}$/mL)	15%	45%	30%
Parainfluenza Virus 3	**Parainfluenza Virus 3** Type 3 Zeptometrix 0810016CF	**3.8E+01 Copies/mL**	19/20	20/20	39/40
		(2.5E+00 $TCID_{50}$/mL)	95%	100%	98%
		3.8E+00 Copies/mL	9/20	10/20	19/40
		(2.5E-01 $TCID_{50}$/mL)	45%	50%	48%
Parainfluenza Virus 4	**Parainfluenza Virus 4** Type 4a Zeptometrix 0810060CF	**1.6E+03 Copies/mL**	20/20	20/20	40/40
		(5.0E+01 $TCID_{50}$/mL)	100%	100%	100%
		1.6E+02 Copies/mL	14/20	12/20	26/40
		(5.0E+00 $TCID_{50}$/mL)	70%	60%	65%
Respiratory Syncytial Virus	**Respiratory Syncytial Virus** Type A Zeptometrix 0810040ACF	**9.0E+00 Copies/mL**	19/20	20/20	39/40
		(2.0E-02 $TCID_{50}$/mL)	95%	100%	98%
		9.0E-01 Copies/mL	7/20	8/20	15/40
		(2.0E-03 $TCID_{50}$/mL) [b]	35%	40%	38%
***Bordetella parapertussis* (IS*1001*)**	***B. parapertussis*** A747 Zeptometrix 0801461	**5.8E+01 *IS1001* Copies/mL** (4.1E+01 CFU/mL)	20/20	19/20	39/40
			100%	95%	98%
		5.8E+00 *IS1001* Copies/mL (4.1E+00 CFU/mL) [b]	9/20	7/20	16/40
			45%	35%	40%
Bordetella pertussis	***B. pertussis***	**1.0E+03 CFU/mL**	20/20	20/20	40/40
			100%	100%	100%

(*ptxP*)	A639 Zeptometrix 0801459	**1.0E+02 CFU/mL**	14/20	10/20	24/40
			70%	50%	60%
Chlamydia pneumoniae	***C. pneumoniae*** TW183 ATCC VR-2282	**6.6E+01 Copies/mL** (1.0E-01 $TCID_{50}$/mL)	20/20	19/20	39/40
			100%	95%	98%
		6.6E+00 Copies/mL (1.0E-02 $TCID_{50}$/mL)	11/20	10/20	21/40
			55%	50%	53%
Mycoplasma pneumoniae	***M. pneumoniae*** M129 Zeptometrix 0801579	**4.6E+02 Copies/mL** (1.0E+01 $TCID_{50}$/mL)	20/20	20/20	40/40
			100%	100%	100%
		4.6E+01 Copies/mL (1.0E+00 $TCID_{50}$/mL)	14/20	10/20	24/40
			70%	50%	60%

[a] A cultured isolate of Coronavirus HKU1 was not available for testing. LoD for Coronavirus HKU1 was therefore determined by testing dilutions of a clinical NPS specimen known to contain the virus. The amount of viral RNA in the specimen (in RNA copies/mL) was determined by real-time RT-PCR against a standard curve.
[b] IS*1001* sequences can be present in more than one copy per cell, so the relationship between CFU/mL and copies/mL may vary from strain to strain and culture to culture. LoD was determined based on the copy number of IS*1001* measured by an independent quantitative real-time PCR assay and for this culture.

e. *Analytical Reactivity (Inclusivity):*

The analytical reactivity/inclusivity of the FilmArray RP2*plus* for each reported analyte was initially assessed by *in silico* analysis and subsequently by testing unique strains or isolates. Isolate/strain selection emphasized variants of known human clinical significance, including relevant species, serotypes, genotypes, isolates collected at various times and from different locations, recently circulating or newly emerging variants, or any strains of particular interest based on sequence alignments, as available. For more genetically diverse analytes a larger collection of isolates was selected to demonstrate and characterize the analytical reactivity of the assay(s) with the expected diversity of the analyte.

In silico Analysis Summary for the Two FilmArray RP2*plus* MERS-CoV Assays (*MERS1* and *MERS2*)

Due to limited availability of well-characterized MERS-CoV strains, empirical testing of MERS-CoV strains in addition to the MERS-CoV strain tested in the LoD studies was not conducted. Analytical reactivity of the FilmArray RP2*plus* MERS-CoV assays was assessed by conducting *in silico* analyses.

Based on an *in silico* analysis of all 184 publicly available MERS-CoV sequences of human host that align with the FilmArray RP2*plus MERS1* assay primers (as of September 12, 2017), there is no evidence of MERS-CoV sequence variants that have been identified from human infections that would contribute to altered or impaired reactivity with the FilmArray RP2*plus MERS1* assay. An *in silico* analysis of all 104 publicly available MERS-CoV sequences from camels (suspected animal host reservoir for MERS-CoV) that align with the FilmArray RP2*plus MERS1* assay primers (as of September 12, 2017) showed that 95% of the sequences analyzed (99/104) are not predicted to contribute to altered or impaired reactivity with the FilmArray RP2*plus MERS1* assay. Five of the 104 sequences analyzed contained one T-C mismatch toward the 3' end of the forward outer primer of the *MERS1*

assay which may result in slightly reduced reactivity at low viral concentrations.

Based on an *in silico* analysis of all 172 publicly available MERS-CoV sequences of human host that align with the FilmArray RP2*plus MERS2* assay primers (as of September 12, 2017), there is no evidence of MERS-CoV sequence variants that have been identified from human infections that would contribute to altered or impaired reactivity with the FilmArray RP2*plus MERS2* assay. An *in silico* analysis of all 105 publicly available MERS-CoV sequences from camels (suspected animal host reservoir for MERS-CoV) that align with the FilmArray RP2*plus MERS2* assay primers (as of September 12, 2017) showed that 100% of the sequences analyzed (105/105) are not predicted to contribute to altered or impaired reactivity with the FilmArray RP2*plus MERS2* assay.

Empirical Testing

Each isolate/strain was prepared and tested as a contrived sample in Remel M4 Transport Medium at a concentration near the LoD (3×LoD) in triplicate, with one replicate on each of three different reagent lots. Most isolates were tested based on molecular quantification in units of copies/mL, though a few were tested in viable units of $TCID_{50}$/mL or CFU/mL. Any isolate that was not reliably detected in 3/3 or 4/5 replicates at the 3×LoD level, was tested again at 10×LoD or a higher concentration, as needed, until detection was observed or a limitation on assay analytical reactivity with that isolate was identified.

Analytical reactivity wet testing results are summarized in Table 10 to Table 21 below.

Table 10: Adenovirus Isolates Tested and Detected by FilmArray RP2*plus*

Species [a]	Serotype	Isolate ID/Source	[Strain/Location/Year]	xLoD Detected	Result
A	12	ATCC VR-863	[Huie/Massachusetts]	3x	Adenovirus Detected
	18	ATCC VR-19	[Washington DC/1954]	1x	
	31	Zeptometrix 0810073CF	-	3x	
B	3	Zeptometrix 0810062CF	-	3x	
	7A	Zeptometrix 0810021CF	-	1x	
	7d/d2	Univ. of Iowa Research Foundation	[Iowa/2001]	3x	
	7h	Univ. of Iowa Research Foundation	[Iowa/1999]	3x	
	11	Univ. of Iowa Research Foundation	[Wisconsin/2005]	3x	
	14	Univ. of Iowa Research Foundation	[Missouri/2005]	3x	
	16	ATCC VR-17	[CH.79/Saudi Arabia/1955]	3x	
	21	Univ. of Iowa Research Foundation	[Missouri/2005]	3x	
	34	ATCC VR-716	[Compton/1972]	3x	
	35	ATCC VR-718	[Holden]	3x	
	50	ATCC VR-1602	[Wan/Amsterdam/1988]	3x	
C	1	Zeptometrix 0810050CF	-	3x	

	2	ATCC VR-846	[Adenoid 6]	1x	
	5	Zeptometrix 0810020CF	-	3x	
	6	ATCC VR-6	[Tonsil 99/Washington DC]	3x	
D	8	Zeptometrix 0810069CF	-	3x	
	20	Zeptometrix 0810115CF	-	3x	
	37	Zeptometrix 0810119CF	-	1x	
E	4a	Univ. of Iowa Research Foundation	[S Carolina/2004]	1x	
	4	Zeptometrix 0810070CF	-	3x	
F	40	Zeptometrix 0810084CF	-	3x	
		NCPV 0101141v	-	3x	
	41	ATCC VR-930	[Tak/73-3544/Netherlands/1973]	1x	
		Zeptometrix 0810085CF	-	3x	

[a] *In silico* analysis of available sequences predicts that the FilmArray RP2*plus* will also react with Adenovirus B55, C57, species D serotypes, and G52.

Table 11: Coronavirus Isolates/Specimens Tested and Detected by FilmArray RP2*plus*

Coronavirus Type	Isolate ID/Source	[Location/Year]	xLoD Detected	Result
MERS-CoV [a]	EMC/2012 BEI NR-50171[b]	[Saudi Arabia/2012]	1x	MERS-CoV Detected
229E	ATCC VR-740	-	1x	Coronavirus 229E Detected
	Zeptometrix 0810229CF	-	3x	
HKU1	Clinical Specimen	[Utah/2015]	1x	Coronavirus HKU1 Detected
	Clinical Specimen	[Utah/2015]	3x	
	Clinical Specimen	[Utah/2015]	3x	
	Clinical Specimen	[S. Carolina/2010]	3x	
	Clinical Specimen	[Detroit/2010]	3x	
NL63	BEI NR-470 [c]	[Amsterdam/2003]	1x	Coronavirus NL63 Detected
	Zeptometrix 0810228CF	-	3x	
OC43	ATCC VR-759 [d]	-	1x	Coronavirus OC43 Detected
	Zeptometrix 0810024CF	-	3x	

[a] Three clinical NPS specimens from three different patients in the 2015 S. Korea MERS-CoV outbreak were tested positive for MERS-CoV by the FilmArray RP2*plus*. Refer to the "Retrospective Clinical Study – MERS-CoV" section of this Decision Memorandum for more details.

[b] BEI Resources, NIAID, NIH: Middle East Respiratory Syndrome Coronavirus (MERS-CoV), EMC/2012, Heat-Inactivated, NR-50171.

[c] Organism obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Human Coronavirus NL63, NR-470.

[d] Discontinued part number; see ATCC VR-1558.

Table 12: Human Metapneumovirus Isolates Tested and Detected by FilmArray RP2*plus*

Genotype	Serotype	Isolate ID/Source	[Location/Year]	xLoD Detected	Result
A1	16	Zeptometrix 0810161CF	[Iowa10/2003]	1x	Human Metapneumovirus Detected
	9	Zeptometrix 0810160CF	[Iowa3/2002]	3x	
A2	20	Zeptometrix 0810163CF	[Iowa14/2003]	3x	
	27	Zeptometrix 0810164CF	[Iowa27/2004]	3x	
B1	3	Zeptometrix 0810156CF	[Peru2/2002]	3x	
	5	Zeptometrix 0810158CF	[Peru3/2003]	3x	
	13	Univ. of Iowa Research Foundation	[Iowa7/2003]	3x	
B2	4	Zeptometrix 0810157CF	[Peru1/2002]	3x	

	8	Zeptometrix 0810159CF	[Peru6/2003]	3x	
	18	Zeptometrix 0810162CF	[Iowa18/2003]	3x	
	22	Univ. of Iowa Research Foundation	[Iowa16/2003]	3x	

Table 13: Human Rhinovirus and Enterovirus Isolates Tested and Detected by FilmArray RP2*plus*

Species [a]	Serotype	Isolate ID/Source	[Strain/Location/Year]	xLoD Detected	Result
Human Rhinovirus					
A	1	Zeptometrix 0810012CFN	[1A]	1x	Human Rhinovirus/ Enterovirus Detected
	2	ATCC VR-482	[HGP]	3x	
	7	ATCC VR-1601	[68-CV11]	3x	
	16	ATCC VR-283	[11757/Washington DC/1960]	3x	
	34	ATCC VR-507 [b]	[137-3]	3x	
	57	ATCC VR-1600	[Ch47]	3x	
	77	ATCC VR-1187	[130-63]	3x	
	85	ATCC VR-1195	[50-525-CV54]	3x	
B	3	ATCC VR-483	[FEB]	3x	
	14	ATCC VR-284	[1059/S Carolina/1959]	3x	
	17	ATCC VR-1663	[33342/N Carolina/1959]	3x	
	27	ATCC VR-1137	[5870]	3x	
	42	ATCC VR-338	[56822]	3x	
	83	ATCC VR-1193	[Baylor 7]	3x	
Enterovirus					
A	Coxsackievirus 10	ATCC VR-168	[NY/1950]	3x	Human Rhinovirus/ Enterovirus Detected
	Enterovirus 71	ATCC VR-1432	[H]	3x	
B	Coxsackievirus A9	Zeptometrix 0810017CF	-	3x	
	Coxsackievirus B3	Zeptometrix 0810074CF	-	3x	
	Coxsackievirus B4	Zeptometrix 0810075CF	-	3x	
	Echovirus 6	Zeptometrix 0810076CF	-	3x	
	Echovirus 9	Zeptometrix 0810077CF	-	3x	
	Echovirus 11	Zeptometrix 0810023CF	-	3x	
C	Coxsackievirus A21	ATCC VR-850	[Kuykendall/California/1952]	3x	
	Coxsackievirus A24	ATCC VR-583	[DN-19/Texas/1963]	3x	
D	68	ATCC VR-1823	[US/MO/2014-18947]	1x	

[a] *In silico* analysis of available sequences predicts that the FilmArray RP2*plus* will react with all currently characterized species and serotypes of Human Rhinovirus and Enterovirus, including Polioviruses.
[b] Discontinued part number; see ATCC VR-1365.

Table 14: Influenza A Isolates Tested and Detected by FilmArray RP2*plus*

Type	Isolate ID/Source		[Strain/Location/Year]	xLoD Detected	Result
H1N1	Human	Zeptometrix 0810036CF	[New Caledonia/20/1999]	1x	Influenza A H1 Detected
		ATCC VR-219	[NWS/1933]	3x	

Type	Isolate ID/Source		[Strain/Location/Year]	xLoD Detected	Result
		ATCC VR-95	[PR/8/1934]	10x [a]	
		ATCC VR-96	[Weiss/1943]	3x	
		ATCC VR-97	[FM/1/1947]	3x	
		ATCC VR-98	[Mal/302/1954]	3x	
		ATCC VR-546	[Denver/1/1957]	3x	
		Zeptometrix 0810036CFN	[Solomon Isl/03/2006]	3x	
		Zeptometrix 0810244CF	[Brisbane/59/2007]	3x	
	Swine	ATCC VR-333	[A/Swine/Iowa/15/1930]	3x	
		ATCC VR-99	[A/Swine/1976/1931]	3x	
		ATCC VR-897	[A/New Jersey/8/76 (Hsw1N1)]	10x [a]	
H1N2	Recombinant	BEI NR-9677 [b]	[Kilbourne F63, A/NWS/1934 (HA) x A/Rockefeller Institute/5/1957 (NA)]	3x	
H1N1 pdm09	Human	Zeptometrix 0810249CFN	[Swine NY/03/2009]	1x	Influenza A H1-2009 Detected
		Zeptometrix 0810248CFN	[Swine NY/01/2009]	3x	
		Zeptometrix 0810109CFN	[Swine NY/02/2009]	3x	
		Zeptometrix 0810109CFJ	[Canada/6294/2009]	3x	
		Zeptometrix 0810165CF	[California/07/2009]	3x	
		Zeptometrix 0810166CF	[Mexico/4108/2009]	3x	
		BEI NR-19823 [c]	[Netherlands/2629/2009]	3x	
		BEI NR-44345 [d]	[Hong Kong/H090-761-V1(0)/2009]	10x [e]	
		BEI NR-42938 [f]	[Georgia/F32551/2012]	3x	
H3N2	Human	ATCC VR-810	[Port Chalmers/1/1973]	1x	Influenza A H3 Detected
		ATCC VR-776	[Alice (live attenuated vaccine)]	3x	
		Zeptometrix 0810238CF	[Texas/50/2012]	3x	
		ATCC VR-547	[Aichi/2/1968]	3x	
		ATCC VR-544	[Hong Kong/8/1968]	3x	
		ATCC VR-822	[Victoria/3/1975]	3x	
		Zeptometrix 0810252CF	[Wisconsin/67/2005]	3x	
		Zeptometrix 0810138CF	[Brisbane/10/2007]	3x	
	Recombinant	ATCC VR-777	[MCR2(A/England/42/72xA/PR8/34)]	3x	
H3N2v	Human	Clinical Specimen	[Ohio/2012]	3x	Influenza A H3 Detected
H2N2	Human	BEI NR-2775 [g]	[Japan/305/1957]	10x [e]	Influenza A (no subtype detected)
	Recombinant	BEI NR-9679 [h]	[Korea/426/1968xPuerto Rico/8/1934]	10x [e]	
H2N3	Avian	MRI Global [i]	Mallard/Alberta/79/2003	3x	Influenza A Equivocal
H5N1		MRI Global [i]	A/Chicken/Yunnan/1251/2003	3x	Influenza A (no subtype detected)
H5N2		MRI Global [i]	Northern pintail/Washington/40964/2014	3x	
H5N3		BEI NR-9682 [j]	A/Duck/Singapore/645/1997	3x	
H5N8		MRI Global [i]	Gyrfalcon/Washington/41088-6/2014	3x	
H7N7		MRI Global [i]	A/Netherlands/219/2003	3x	
H7N9		MRI Global [i]	A/Anhui/01/2013	3x	
H10N7		BEI NR-2765 [k]	Chicken/Germany/N/49	3x	Influenza A

Type	Isolate ID/Source		[Strain/Location/Year]	xLoD Detected	Result
					Equivocal

[a] Reported as Influenza A (no subtype detected) at 3× LoD.
[b] Genomic RNA obtained through the NIH Biodefense and Emerging Infections Research Resources Respiratory NAID, NIH Kilbourne F63: A/NWS/1934 (HA) x A/Rockefeller Institute/5/1957 (NA) (H1N2), Reassortant NWS-F, NR-9677.
[c] Organism obtained through BEI Resources, NIAID, NIH: Influenza A Virus, A/Netherlands/2629/2009 (H1N1)pdm09, NR-19823.
[d] Organism obtained through BEI Resources, NIAID, NIH: Influenza A Virus, A/Hong Kong/H090-761-V1(0)/2009 (H1N1)pdm09, NR-44345.
[e] Reported as Influenza A Equivocal or Influenza A (no subtype detected) at 3× LoD.
[f] Organism obtained through BEI Resources, NIAID, NIH: Influenza A Virus, A/Georgia/F32551/2012 (H1N1)pdm09, NR-42938.
[g] Genomic RNA obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Genomic RNA from Influenza A Virus, A/Japan/305/1957 (H2N2), NR-2775.
[h] Genomic RNA obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Genomic RNA from Kilbourne F38: A/Korea/426/1968 (HA, NA) x A/Puerto Rico/8/1934 (H2N2), NR-9679.
[I] Isolate provided and tested by MRI Global, Kansas City, MO.
[j] Genomic RNA obtained through the NIH Biodefense and Emerging Infections Research Resources Repository NIAID, NIH: Genomic RNA from Kilbourne F181: A/duck/Singapore/645/1997 (H5N3), Wild Type, NR-9682.
[k] Genomic RNA obtained through the NIH Biodefense and Emerging Infections Research Resources Repository NIAID, NIH: Genomic RNA from Influenza A Virus, A/chicken/Germany/N/1949 (H10N7), NR-2765

Table 15: Influenza B Isolates Tested and Detected by FilmArray RP2*plus*

Lineage	Isolate ID/Source	[Strain/Location/Year]	xLoD Detected	Result
N/A	ATCC VR-101	[Lee/1940]	3x	Influenza B Detected
	ATCC VR-102	[Allen/1945]	3x	
	ATCC VR-103	[GL/1739/1954]	3x	
	ATCC VR-296	[1/Maryland/1959]	3x	
	ATCC VR-295	[2/Taiwan/1962]	3x	
	ATCC VR-786	[Brigit/Russia/1969]	3x	
Victoria	ATCC VR-823	[5/Hong Kong/1972]	3x	
	Zeptometrix 0810258CF	[2506/Malaysia/2004]	3x	
	CDC 2005743348	[1/Ohio/2005]	3x	
Yamagata	Zeptometrix 0810256CF	[07/Flordia/2004]	3x	
	Zeptometrix 0810255CF	[04/Flordia/2006]	1x	
	Zeptometrix 0810241CF	[1/Wisconsin/2010]	3x	
	Zeptometrix 0810239CF	[2/Massachusetts/2012]	3x	

Table 16: Parainfluenza Virus Isolates Tested and Detected by FilmArray RP2*plus*

Type	Subtype	Isolate ID/Source	[Strain/Location/Year]	xLoD Detected	Result
1		Zeptometrix 0810014CF	-	1x	Parainfluenza Virus 1 Detected
		ATCC VR-94	[C-35/Washington DC/1957]	3x	
		BEI NR-3226 [a]	[C39]	3x	
		BEI NR-48680 [b]	[FRA/29221106/2009]	3x	
2		Zeptometrix 0810015CF	-	1x	Parainfluenza Virus 2 Detected
		ATCC VR-92	[Greer/Ohio/1955]	3x	
3		Zeptometrix 0810016CF	-	1x	Parainfluenza Virus 3 Detected
		ATCC VR-93	[C-243/Washington DC/1957]	3x	

		BEI NR-3233 [c]	[NIH 47885, Wash/47885/57]	3x	
4	A	Zeptometrix 0810060CF	-	1x	Parainfluenza Virus 4 Detected
		ATCC VR-1378	[M-25/1958]	3x	
	B	Zeptometrix 0810060BCF	-	3x	
		ATCC VR-1377	[CH-19503/Washington DC/1962]	3x	

[a] Discontinued part number.

[b] Obtained through BEI Resources, NIAID, NIH: Human Parainfluenza Virus 1, HPIV1/FRA/29221106/2009, NR-48680.

[c] Obtained through BEI Resources, NIAID, NIH: Human Parainfluenza Virus 3, NIH 47885, NR-3233.

Table 17: Respiratory Syncytial Virus Isolates Tested and Detected by FilmArray RP2*plus*

Type	Source	[Strain/Location/Year]	xLoD Detected	Result
A	Zeptometrix 0810040ACF	[2006]	1x	Respiratory Syncytial Virus Detected
	ATCC VR-26	[Long/Maryland/1956]	3x	
	ATCC VR-1540	[A2/Melbourne/1961]	3x	
B	Zeptometrix 0810040CF	[Ch-93 (18)-18]	3x	
	ATCC VR-1400	[WV/14617/1985]	3x	
	ATCC VR-955	[9320/Massachusetts/1977]	3x	
	ATCC VR-1580	[18537/Washington DC/1962]	10x	

Table 18: *Bordetella parapertussis* (and *Bordetella bronchiseptica*) Isolates Tested and Detected by FilmArray RP2*plus*

Species	Source	[Strain/Location/Year]	xLoD Detected	Result
Bordetella parapertussis	Zeptometrix 0801461	[A747]	1x	*Bordetella parapertussis* (IS*1001*) Detected
	Zeptometrix 0801462	[E595]	3x	
	ATCC 15237	[NCTC 10853]	3x	
	ATCC 15311	[NCTC 5952]	3x	
	ATCC BAA-587	[12822/Germany/1993]	3x	
Bordetella bronchiseptica (containing IS*1001*) [a]	NRRL B-59909	[MBORD849/ Pig/Netherlands]	3x	

[a] Reactivity with IS*1001* sequences in *B. bronchiseptica* represents the intended reactivity of the assay, however the analyte will be inaccurately reported as *B. parapertussis*. The assay does not react with IS*1001*-like sequences in *B. holmesii* (see Analytical Reactivity).

Table 19: *Bordetella pertussis* Isolates Tested and Detected by FilmArray RP2*plus*

Isolate ID/Source	[Strain]	xLoD Detected	Result
Zeptometrix 0801459	[A639]	1x	*Bordetella pertussis* (*ptxP*) Detected
Zeptometrix 0801460	[E431]	3x	
ATCC 8467	[F]	3x	
ATCC 9340	[5,17921]	3x	
ATCC 9797	[18323/NCTC 10739]	3x	
ATCC 10380	[10-536]	3x	
ATCC 51445	[CNCTC Hp 12/63,623]	3x	
ATCC BAA-589	[Tohama]	3x	
ATCC BAA-1335	[MN2531]	3x	

Table 20: *Chlamydia pneumoniae* Isolates Tested and Detected by FilmArray RP2*plus*

Isolate ID/Source	[Strain/Location/Year]	xLoD Detected	Result

ATCC VR-2282	[TW-183/Taiwan/1965]	1x	*Chlamydia pneumoniae* Detected
ATCC VR-1310	[CWL-029]	3x	
ATCC VR-1360	[CM-1/Georgia]	3x	
ATCC 53592	[AR-39/Seattle/1983]	3x	

Table 21: *Mycoplasma pneumoniae* Isolates Tested and Detected by FilmArray RP2*plus*

Type	Isolate ID/Source	[Strain]	xLoD Detected	Result
1	Zeptometrix 0801579	[M129]	1x	*Mycoplasma pneumoniae* Detected
	ATCC 29342	[M129-B7]	3x	
	ATCC 29085	[PI 1428]	3x	
2	ATCC 15531	[FH strain of Eaton Agent [NCTC 10119]	3x	
	ATCC 15492	[Mac]	3x	
unknown	ATCC 15293	[M52]	3x	
	ATCC 15377	[Bru]	3x	
	ATCC 39505	[Mutant 22]	3x	
	ATCC 49894	[UTMB-10P]	3x	

All isolates wet tested in the analytical reactivity study were amplified by the appropriate assay(s) and detected by the FilmArray RP2*plus* at concentrations within 10× LoD.

Analytical reactivity was demonstrated with a wide variety of isolates/strains, including over 25 Adenovirus isolates of different species and serotypes, 25 different Human Rhinovirus or Enterovirus species and serotypes, and several isolates of the major Influenza A subtypes that typically infect humans. Due to the public health concerns related to zoonotic transmission of influenza A viruses to humans (primarily swine and avian lineages), several isolates of swine and avian influenza A viruses were also tested at a concentration equal to 3× the lowest Influenza A LoD.

The expected outcomes of Influenza A H1, Influenza A Equivocal, and Influenza A (no subtype detected) results were reported for influenza A viruses of swine and avian origin, depending on the strain, sequence, and concentration tested. As expected, the majority of zoonotic and recombinant viruses of non-H1, H1-2009, or H3 hemagglutinin subtypes were reported as Influenza A (no subtype detected). One virus (avian H2N3) was reported as Influenza A Equivocal at the lower concentrations of 3× and 10× LoD and Influenza A (no subtype detected) at a higher concentration of 100× LoD. Another avian isolate (H10N7) was reported as Influenza A Equivocal at all concentrations tested, up to 100× LoD.

f. Analytical Specificity/Cross-Reactivity Evaluation:

Analytical specificity (cross-reactivity) of the FilmArray RP2*plus* was evaluated by challenging the system with contrived samples containing a high concentration of organism. On-panel organisms were tested to assess the potential for intra-panel cross-reactivity (e.g., does the Coronavirus OC43 assay

cross-react with Coronavirus HKU1, etc.), while off-panel organisms (those not intended to be detected by the panel) were tested to assess the potential for non-specific amplification of respiratory flora or other respiratory pathogens that may be present in an NPS specimen. Organisms for off-panel testing were selected based on a combination of several factors including 1) relatedness to specific species detected by the FilmArray RP2*plus* (i.e., near-neighbors), 2) clinical relevance (cause respiratory symptoms) or likelihood of being present in NPS specimens, and 3) genetic similarity to FilmArray RP2*plus* assay primers, as determined by *in silico* analyses.

Each organism was tested in Remel M4 transport medium on three different lots of FilmArray RP2*plus* pouches and at the highest concentration possible based on the stock concentration of the cultured isolate. The final concentrations of analytes in the samples were typically ≥1.0E+06 CFU/ml for bacteria and fungi and ≥1.0E+05 $TCID_{50}$/mL for viruses (calculated to be between ~7.0E+01 – 4.0E+05 fold higher than the LoD of the FilmArray RP2*plus* assays).

On-Panel Organisms Testing

A group of 23 on-panel analytes were tested at high concentration to assess the potential for intra-panel cross-reactivity. The organisms tested are shown in Table 22 below and results are discussed below, including discussion of cross-reactivity observed or predicted for three of the on-panel organisms.

Table 22: On-panel Organisms Tested by the FilmArray RP2*plus* for Evaluation of Analytical Specificity (Organisms with the potential for non-specific amplification by another FilmArray RP2*plus* assay are shown in **bold** type)

Viruses			Bacteria
Adenovirus	Human Rhinovirus	Influenza B	***Bordetella parapertussis*** [c,d]
Coronavirus 229 E	Human Metapneumovirus	Parainfluenza Virus 1	***Bordetella pertussis*** [d]
Coronavirus HKU1 [a]	Influenza A H1N1	Parainfluenza Virus 2	*Chlamydia pneumoniae*
Coronavirus NL63	Influenza A H3N2	Parainfluenza Virus 3	*Mycoplasma pneumoniae*
Coronavirus OC43	Influenza A H1N1pdm09	Parainfluenza Virus 4	MERS-CoV [e]
Enterovirus (Echovirus 6)	**Influenza A Hsw1N1** [b]	Respiratory Syncytial Virus	

[a] Two different clinical specimens were tested, containing up to 8.9E+08 RNA copies/mL of Coronavirus HKU1.

[b] Swine origin Hsw1N1 (A/New Jersey/8/1976; ATCC VR-897) was variably detected as either Influenza A H1 or Influenza A H1-2009 at a concentration of 8.9E+06 $CEID_{50}$/mL; indicating the potential for cross-reactivity with the FluA H1-2009 assay at higher concentrations.

[c] Contains pertussis toxin pseudogene sequences that are predicted to be amplified by the *ptxP* assay and reported as *Bordetella pertussis* (*ptxP*) Detected when present at very high concentrations (i.e., >1.2E+09 CFU/mL).

[d] Non-specific amplification by the HRV/EV assay, generating a Human Rhinovirus/Enterovirus Detected result, was observed when testing *B. pertussis* at high concentration (i.e., ≥4.5E+07 CFU/mL). Similar cross-reactivity is predicted to be possible with *B. parapertussis*, but was not observed when tested up to 1.2E+09 CFU/mL.

[e] Heat-inactivated viral culture obtained through BEI Resources, NIAID, NIH: Middle East Respiratory Syndrome Coronavirus (MERS-CoV), EMC/2012, Heat-Inactivated, NR-50171.

Two clinical NPS specimens known to contain high concentrations of

Coronavirus HKU1 (i.e., up to 8.9E+08 Copies/mL or >4.0E+05× LoD) were tested and no inaccurate Coronavirus OC43 Detected results were reported. These data support the conclusion that the intra-panel cross-reactivity observed between the Coronavirus OC43 assay contained in the FilmArray RP and high concentrations of Coronavirus HKU1 (>1.0E+08 Copies/mL) has been eliminated by the redesigned Coronavirus OC43 assay (CoV-OC43-2) contained in the FilmArray RP2*plus*.

Based on sequence analysis and experience with the same assay in the FilmArray RP, cross-reactivity was expected between the assay for the detection of *Bordetella pertussis* (*ptxP*) and non-pertussis *Bordetella* strains that carry a pertussis toxin pseudogene (e.g. *B. parapertussis* and the off-panel species *B. bronchiseptica*) when present at concentrations >1.0E+06 CFU/mL. However, reaction conditions for the FilmArray RP2*plus* were determined to be less permissive for this non-specific interaction and the predicted cross-reactivity was not observed when testing on-panel *B. parapertussis* at a much higher concentration (i.e., >1.2E+09 CFU/mL). (Note: also, see *B. bronchiseptica* testing in off-panel testing section below). Though not observed, the potential for cross-reactivity with *B. parapertussis* will be described in the labeling as possible, but in the FilmArray RP2*plus*, the true organism (*B. parapertussis*) will also be identified.

The FilmArray RP2*plus* includes assays to distinguish classical human Influenza A H1 and the pandemic H1-2009 variant derived from swine. However, due to sequence similarity, some reactivity of the H1-2009 assay may be observed with historical and/or novel H1N1 strains of swine origin. This was demonstrated by testing a swine origin influenza A Hsw1N1 isolate from the 1970s (A/New Jersey/8/1976) which was detected and reported as Influenza A H1 in one replicate and as Influenza A H1-2009 in two replicates when tested at a high concentration of 8.9E+06 $CEID_{50}$/mL.

Finally, the FilmArray RP2*plus* assay for detection of Human Rhinovirus/Enterovirus (HRV/EV) contains a primer pair that, despite several mismatches, is able to amplify sequences of the oxidoreductase gene found in *Bordetella pertussis* as well as selected published *B. parapertussis* and *B. bronchiseptica* sequences. The predicted non-specific amplification was confirmed when testing *B. pertussis* at a concentration of 4.5E+07 CFU/mL and higher. The cross-reactivity was not observed when testing *B. parapertussis* (or the off-panel organism *B. bronchiseptica*, see below) at a concentration of 1.2E+09 CFU/mL, but it may still be possible. *Bordetella* infections are less common than the "common cold' caused by Rhinoviruses (and Enteroviruses), so not only should this cross-reactivity be observed rarely, the presumably more significant *Bordetella pertussis* infection will be appropriately identified for treatment, with possible extra care for a concurrent Rhinovirus infection associated with the non-specific result.

Off-Panel Organisms Testing

In silico analysis of assay specificity was supplemented with wet testing of 50 off-panel bacteria, viruses, and fungi at high concentrations (typically ≥1.0E+06 CFU/ml for bacteria and fungi and ≥1.0E+05 $TCID_{50}$/mL for viruses). The organisms tested are shown in Table 23 and results are discussed below.

Table 23: Off-panel Organisms Tested by the FilmArray RP2*plus* for Evaluation of Analytical Specificity (Organisms with the potential for non-specific amplification by a FilmArray RP2*plus* assay are shown in **bold** type)

Bacteria			Viruses
Acinetobacter calcoaceticus	*Enterobacter aerogenes*	*Neisseria gonorrhoeae*	Bocavirus
Bordetella avium	*Escherichia coli*	*Neisseria meningitidis*	Cytomegalovirus (CMV)
Bordetella bronchiseptica [a,b,c]	*Haemophilus influenzae*	*Proteus mirabilis*	Epstein-Barr Virus (EBV)
Bordetella hinzii	*Klebsiella oxytoca*	*Pseudomonas aeruginosa*	Herpes Simplex Virus 1
Bordetella holmesii	*Klebsiella pneumoniae*	*Serratia marcescens*	Measles Virus
Legionella bozemanii	*Lactobacillus acidophilus*	*Staphylococcus aureus*	Mumps
Legionella dumofii	*Lactobacillus plantarum*	*Staphylococcus epidermidis*	Severe Acute Respiratory Syndrome (SARS) Virus recombinant [d]
Legionella feeleii	*Moraxella catarrhalis*	*Stenotrophomonas maltophilia*	
Legionella longbeacheae	*Mycoplasma genitalium*	*Streptococcus pneumoniae*	**Fungi/Yeast**
Legionella micdadei	*Mycoplasma hominis*	*Streptococcus agalactiae*	*Candida albicans*
Legionella pneumophila	*Mycoplasma orale*	*Streptococcus pyogenes*	*Cryptococcus neoformans*
Chlamydia trachomatis	*Mycobacterium tuberculosis*	*Streptococcus salivarus*	*Aspergillus fumigatus*
Corynebacterium diphteriae	*Neisseria elongata*	*Ureaplasma urealyticum*	*Aspergillus flavus*

[a] Non-specific amplification of pertussis toxin pseudogene sequences by the *ptxP* assay is possible at very high concentrations (≥1.2E+09 CFU/mL), generating a *Bordetella pertussis* (*ptxP*) Detected result.
[b] Non-specific amplification by the HRV/EV assay, generating a Human Rhinovirus/Enterovirus Detected result, is predicted at high concentrations but was not observed when tested up to 1.2E+09 CFU/mL.
[c] Strains of *Bordetella bronchiseptica* that carry IS*1001* insertion sequences will be amplified by the IS*1001* assay as intended, but misidentified by the FilmArray RP2 as *Bordetella parapertussis* (IS*1001*).
[d] SARS NR-18925, Recombinant Infectious Clone of Urbani Strain (icSARS-CoV) from BEI resources.

The only non-specific interaction observed in testing of off-panel organisms was a *Bordetella pertussis* (*ptxP*) Detected result in one of three replicates of *B. bronchiseptica* tested at a concentration of 1.2E+09 CFU/mL. This cross-reactivity has been observed at lower concentrations in the FilmArray RP and was predicted based on the presence of pertussis toxin pseudogene sequences in this species. Though not observed, Human Rhinovirus/Enterovirus assay cross-reactivity with some sequences of *B. bronchiseptica* is predicted by *in silico* analyses. In addition, certain strains of *B. bronchiseptica* are known to carry the IS*1001* insertion sequences that are most common to *B. parapertussis*. In these cases, the FilmArray RP2 assay for detection of *Bordetella parapertussis* (IS*1001*) will amplify the correct targeted sequence, but will misidentify the organism as *Bordetella parapertussis* rather than *B. bronchiseptica*. As *B.*

bronchiseptica is a very rare human pathogen (more commonly associated with veterinary cases of canine "kennel cough"), the probability of inaccurate *Bordetella pertussis* (*ptxP*) results or inaccurate *Bordetella parapertussis* (IS*1001*) results due to cross-reactivity with *B. bronchiseptica* is low.

Analytical Specificity Evaluation Conclusion

Analytical specificity testing has demonstrated that the majority of the FilmArray RP2*plus* assays are highly specific for the organisms they are designed to detect. Cross-reactivity that was identified will occur almost exclusively at high organism levels (i.e., >1.0E+07 units/mL) and most events are associated with near-neighbor species that carry the same genes or highly similar sequences as the targeted organism. Overall, the likelihood, risk, and impact of the FilmArray RP2*plus* non-specific interactions are predicted to be minor. All identified FilmArray RP2*plus* cross-reactivity cases are indicated in the product Instructions for Use, as a precaution to minimize misinterpretation of results. A summary is provided in Table 24 below.

Table 24: Predicted and Observed Cross-Reactivity of the FilmArray RP2*plus*

Cross-reactive Organism	FilmArray RP2*plus* Result	Description
Non-pertussis *Bordetella* species (e.g., *Bordetella parapertussis or B. bronchiseptica*)	***Bordetella pertussis* (*ptxP*) [b,c]**	The *Bordetella pertussis* (*ptxP*) assay can amplify pertussis toxin pseudogene sequences in *B. bronchiseptica* and *B. parapertussis*. Cross-reactivity is observed at high concentration (≥1.2E+09 CFU/mL).
***Bordetella bronchiseptica* [a]** (with IS*1001* sequences)	***Bordetella parapertussis* (IS*1001*)**	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 IS*1001* assay and reported by FilmArray RP2*plus* as *Bordetella parapertussis* (IS*1001*).
***Bordetella pertussis* and other *Bordetella* species**	**Human Rhinovirus/Enterovirus [d,e]**	The Human Rhinovirus/Enterovirus assay may amplify off-target sequences found in strains of *B. pertussis, B. bronchiseptica* and *B. parapertussis*. Cross-reactivity with *B. pertussis* was observed at a concentration of 4.5E+07 CFU/mL or higher.

Influenza A H1N1 (swine origin)	**Influenza A H1-2009** [f]	The Influenza A H1-2009 assay may react with H1 hemagglutinin gene sequences from viruses of swine origin. RP2*plus* results will be either Influenza A H1 or Influenza A H1-2009, depending on the strain and concentration in the sample.

[a] *B. bronchiseptica* infection is rare in humans and more common in domesticated animals ('kennel cough').

[b] Cross-reactivity was observed only when tested at a high concentration (≥1.2E+09 CFU/mL).

[c] Cross-reactivity between the *Bordetella pertussis* (*ptxP*) assay and *B. parapertussis* will be reported as a co-detection (*Bordetella parapertussis* (IS*1001*) Detected and *Bordetella pertussis* (*ptxP*) Detected); while cross-reactivity with most strains of *B. bronchiseptica* (that do not carry IS*1001*) will be reported only as *Bordetella pertussis* (*ptxP*) Detected.

[d] Cross-reactivity with *B. pertussis* was observed when tested at a concentration of 4.5E+07 CFU/mL and higher. Cross-reactivity with *B. parapertussis* and *B. bronchiseptica* is predicted based on *in silico* analysis, but was not observed when tested at a concentration of 1.2E+09 CFU/mL.

[e] Cross-reactivity between the Human Rhinovirus/Enterovirus assays and *B. pertussis* or *B. parapertussis* will be reported as a co-detection (*Bordetella pertussis* (*ptxP*) Detected and Human Rhinovirus/Enterovirus Detected or *Bordetella parapertussis* (IS*1001*) Detected and Human Rhinovirus/Enterovirus Detected); while cross-reactivity with most strains of *B. bronchiseptica* (that do not carry IS*1001*) will be reported (falsely) only as Human Rhinovirus/Enterovirus Detected.

[f] Swine origin Hsw1N1 (A/New Jersey/8/1976 ; ATCC VR-897) was detected as either Influenza A H1 or Influenza A H1-2009 at a concentration of 8.9E+06 $CEID_{50}$/mL.

g. Assay Cut-off:

The FilmArray RP2*plus* is part of BioFire Diagnostics' FilmArray system. The FilmArray system is designed to interpret the test data and automatically report the test results to the operator. The FilmArray system uses the results of the Melt Detector to determine each test result. The Melt Detector is part of the FilmArray Analysis Software and assigns a positive or negative result to each reaction on the array through analysis of the melt data collected during the test. These positive and negative results are combined in the FilmArray Analysis Software (using the replicate, assay and interpretation rules) to report the presence or absence of each pathogen in the panel.

To determine the result for each well, the Melt Detector was developed to analyze the melt data. The Melt Detector analyzes the melt data for each well independently, and utilizes curve-specific characteristics (e.g., shape, signal-to-noise, etc.) to assign a positive or negative call to each curve. To maximize the sensitivity and specificity of the Melt Detector, the algorithm was tuned against a large data set comprising typical and atypical melting curves (i.e., training data set) with expert annotation (positive or negative calls) during the development of the original FilmArray RP.

To evaluate the Melt Detector performance for the FilmArray RP2*plus*, the observed sensitivity and specificity rates for the individual melt curves and assay calls were reported and analyzed. These sensitivity and specificity rates were determined by comparing the FilmArray RP2*plus* test results obtained from well-characterized samples, collected as part of the clinical evaluation and analytic testing of the FilmArray RP2*plus* panel, to expert annotation.

For individual melt curves, the observed sensitivity and specificity, as compared to expert annotation, of the Melt Detector is 99.65% and 99.94%, respectively. For the Analysis Software, the observed sensitivity and specificity, as compared to expert annotation, of the assay calls are greater than 99.80% for sensitivity and 99.96% specificity. These rates are comparable to the observed rates reported in the previous melt detector validation studies.

h. Interfering Substances

An analytical study was performed to assess the potential inhibitory effects of exogenous and endogenous substances and competitive microorganisms that may be commonly found in nasopharyngeal specimens (NPS). This study also assessed the potential inhibitory effects of disinfecting/cleaning substances and specimen collection materials.

For this study, potentially interfering substances were selected for evaluation based upon whether the substance may normally be found in NPS specimens or may be introduced into specimens during NPS collection or subsequent handling and testing. This included endogenous substances that may be found in specimens at normal or elevated levels, such as blood, mucus/mucin, human genomic DNA, and various commensal or infectious microorganisms (both on-panel and off-panel). Exogenous substances that may be present in specimens, such as medications, treatments, or topical applications for soothing symptoms associated with respiratory infections were also included. Lastly, substances such as disinfectants (e.g., bleach and ethanol) and various swabs and transport media that could contact specimens during collection or testing were evaluated as potential technique- specific interfering substances.

Each test substance was added to a contrived sample at a concentration similar to or greater than the level expected to be found in a clinical NPS specimen. The contrived sample contained a mix of five different FilmArray RP2*plus* analytes, each present at a concentration near the limit of detection (LoD). Contrived sample with no substance added served as a positive control (no interference) on each day of testing, and a potentially interfering substance in negative sample matrix served as a negative or substance-only control. Samples containing test substances were evaluated for effects of the substance on the internal pouch control assays as well as effects on the ability of the FilmArray RP2*plus* to provide accurate organism test results compared to the positive control samples.

In total, 39 different endogenous and exogenous substances, potentially competing microorganisms, specimen collection materials (swabs and media), and disinfecting agents were evaluated in this study (see Table 25 below).

Table 25: Results for Potentially Interfering Substances Tested on the FilmArray RP2*plus*

Substance Tested	Concentration Tested	Result
Endogenous Substances		

Human Whole Blood	10% v/v	No Interference
Human Mucin (Sputum)	1 swab/mL sample	No Interference
Human Genomic DNA	20 ng/µL	No Interference
Competitive Microorganisms		
Coronavirus 229E	1.7E+04 $TCID^{50}$/mL	No Interference
Adenovirus A12	8.9E+05 $TCID_{50}$/mL	No Interference
Parainfluenza Virus 3	6.6E+05 $TCID_{50}$/mL	No Interference
Bordetella pertussis	5.8E+08 CFU/mL	No Interference
Enterovirus D68	1.6E+07 $TCID_{50}$/mL [a]	No Interference
Echovirus 6	1.0E+07 $TCID_{50}$/mL	No Interference
Respiratory Syncytial Virus	4.2E+04 $TCID_{50}$/mL	No Interference
Staphylococcus aureus	2.5E+07 CFU/mL	No Interference
Streptococcus pneumoniae	1.7E+07 CFU/mL	No Interference
Haemophilus influenzae	6.2E+07 CFU/mL	No Interference
Candida albicans	1.0E+06 CFU/mL	No Interference
Herpes Simplex Virus 1	1.6E+06 $TCID_{50}$/mL	No Interference
Cytomegalovirus (CMV)	1.2E+06 $TCID_{50}$/mL	No Interference
Exogenous Substances [b]		
Tobramycin (systemic antibiotic)	0.6 mg/mL	No Interference
Mupirocin (active ingredient in anti-bacterial ointment)	2% w/v	No Interference
Saline Nasal Spray with Preservatives (0.65% NaCl, Phenylcarbinol, Benzalkonium chloride)	1% v/v	No Interference
Nasal Decongestant Spray (Oxymetazoline HCl 0.05%, Benzalkonium chloride, phosphate)	1% v/v	No Interference
Analgesic ointment (VicksVapoRub)	1% w/v	No Interference
Petroleum Jelly (Vaseline)	1% w/v	No Interference
Snuff (Tobacco)	1% w/v	No Interference
Disinfecting/Cleaning Substances		
Bleach	1% and 2% v/v [512, 1024 ppm chlorine]	Interference [c]
Disinfecting wipes (ammonium chloride [d])	½ in^2	No Interference
Ethanol	7% v/v	No Interference
DNAZap (Ambion™ AM9891G & AM9892G)	1% v/v	No Interference
RNaseZap (Ambion™ AM9782)	1% v/v	No Interference
Specimen Collection Materials		
Rayon Swabs (Copan 168C)	N/A	No Interference
Nylon Flocked Swabs (Copan 553C)	N/A	No Interference
Polyester Swabs (Copan 175KS01)	N/A	No Interference
Calcium Alginate Swabs (Puritan 25-801 A 50)	N/A	No Interference
M4 Transport Medium (Remel R12500, 3mL/tube)	100%	No Interference
M4-RT Transport Medium (Remel R12506, 3 mL/tube)	100%	No Interference
M5 Transport Medium (Remel R12516, 3 mL/tube)	100%	No Interference

M6™ Transport Medium (Remel R12535, 1.5 mL/tube)	100%	No Interference
Universal Viral Transport vial (BD 220220, 3 mL/tube)	100%	No Interference
Sigma-Virocult™ Viral Collection and Transport System – Swabs and Transport Medium (Medical Wire MW951SENT)	100%	No Interference
ESwab™ Sample Collection and Delivery System – Swabs and Liquid Amies Medium (Copan 482C)	100%	No Interference

[a] A different lot of this isolate was also tested at a lower concentration of 3.2E+04 $TCID_{50}$/mL with no interference observed.

[b] Nasal influenza vaccines (e.g. FluMist) were not evaluated, but are predicted to be reactive with the FilmArray RP2 Influenza A (subtype) and Influenza B assays.

[c] Not Detected results were reported for several analytes after incubation of the sample with 2% bleach for 10 minutes or overnight. It was concluded that interference resulted primarily from damage to the organisms/nucleic acids in the sample, rather than inhibition or interference with pouch function(s).

[d] n-Alkyl (C14, 60%, C16, 30%, C12, 5%, C18, 5%) Dimethyl Benzyl Ammonium Chloride - 0.184% n-Alkyl (C12, 68%, C14, 32%) Dimethyl Ethylbenzyl Ammonium Chloride - 0.184%

Testing showed that none of the substances evaluated had an effect on the FilmArray RP2*plus* control assays and no interference with pouch function was identified. However, it was demonstrated that exposure of samples to bleach prior to testing could damage the organisms/nucleic acids in the sample and generate inaccurate FilmArray RP2*plus* test results (i.e., lack of analyte detection), depending on the concentration and/or length of time the bleach was allowed to interact with the sample.

Interfering Substances Evaluation Conclusion

No interference was observed when testing samples containing potentially inhibitory biological substances (e.g., blood etc.) or high levels of potentially competing microorganisms. Similarly, detection near LoD was robust in samples prepared in a variety of transport media types or when exposed to various swabs that may be used for NPS collection. Saline, decongestants, ointments or other substances that could be introduced into the sample also had no effect on the function of the FilmArray RP2*plus*.

The only limitation is related to potential damage to the organisms in the sample caused by bleach that could lead to false negative results. A warning describing the potential for sample damage caused by bleach will be included in the product Instructions for Use.

Note: Nasal influenza vaccines (e.g., FluMist) were not evaluated in this study, but are predicted to be reactive with the FilmArray RP2*plus* Influenza A (including subtype) and Influenza B assays. Therefore, contamination of specimens with vaccine or recent administration of the vaccine prior to NPS specimen collection could lead to accurate detection by the FilmArray RP2*plus* of the viruses contained in the vaccine, but would not represent infection by those agents.

i. Carry-Over Contamination:

A formal carry-over study in support of this regulatory submission for the FilmArray RP2*plus* was not performed, since carry-over studies with high positive samples followed by negative samples have been performed for other FDA-cleared FilmArray Panels (i.e., FilmArray RP, BCID, and GI) for both the FilmArray 2.0 and the FilmArray Torch systems, and no carry-over has been observed.

j. Comparator Assay for B. parapertussis Analytical Validation

In the prospective clinical trial for the FilmArray RP2*plus*, the FilmArray RP2*plus* results were compared to the results from standard of care testing using the same FDA-cleared multiplexed respiratory pathogens panel performed at the clinical sites for all the FilmArray RP2*plus* analytes except for *Bordetella parapertussis* and MERS-CoV.

Two well-validated PCR assays for *Bordetella parapertussis* (IS*1001*) followed by bi-directional sequencing (designed to give at least 200 base pairs of sequence information) were performed as the comparator method for *Bordetella parapertussis* (IS*1001*). The two comparator PCR assays target the same gene as the FilmArray RP2*plus* assay (i.e., the IS*1001* genetic element), but the primers identify sequences that do not overlap with the FilmArray RP2*plus* assays. The two PCR comparator assays used in the clinical studies were designed by BioFire as published assays could not be found that provided adequate amplicon length or quality for sequencing. Additionally, the assays were nested in order to match the sensitivity of the FilmArray RP2*plus* assay. Replicate assays were run as separate PCR1 reactions so that any cross-contamination events could be detected and resolved via mismatch testing. Each post-PCR1 reaction mixture was then used as template for testing with the appropriate PCR2 assay. PCR plates containing potentially positive amplicons were sent directly to a contract laboratory for ExoSAP clean up and sequencing. A positive result from either assay was considered positive for *Bordetella parapertussis* (IS*1001*).

Validation testing demonstrated that the PCR followed by sequencing assays had similar analytical reactivity performance to the FilmArray RP2*plus* assay, and a LoD that was within 5-fold of the FilmArray RP2*plus* assay (this was considered "equivalent analytical sensitivity").

2. Comparison Studies:

Due to very limited information available on clinical relevant MERS-CoV viral loads in upper respiratory specimens (including NPS in VTM) from MERS patients and asymptomatic MERS-CoV carriers, and the fact that only three natural clinical specimens that are MERS-CoV positive were available for testing, an additional analytical study was conducted. In order to obtain analytical sensitivity information relative to that of established MERS-CoV nucleic acid-based tests authorized under the FDA Emergency Use Authorization (EUA) program, the CDC Novel Coronavirus 2012 Real-time RT-PCR Assay and the RealStar MERS-CoV RT-PCR Kit U.S. from Altona Diagnostics GmbH a MERS-CoV panel was tested.

A MERS-CoV proficiency test panel prepared by the Quality Control for Molecular Diagnostics (QCMD) under a contract with the U.S. government was tested in a blinded fashion utilizing the RealStar MERS-CoV RT-PCR Kit U.S. from Altona Diagnostics GmbH and the FilmArray RP2*plus* in parallel. This MERS-CoV proficiency test panel contains MERS-CoV samples, as well as samples of common coronaviruses, and was characterized by both the QCMD and the CDC. For the purpose of comparative analysis of analytical sensitivity, panel characterization testing data generated at the CDC using the CDC MERS-CoV N2 rRT-PCR (one of the three assays of the CDC Novel Coronavirus 2012 Real-time RT-PCR test under an EUA) was obtained from QCMD after the parallel testing was completed.

The analytical sensitivity of FilmArray RP2*plus* detecting MERS-CoV appeared to be at least equivalent to these two established MERS-CoV nucleic acid-based tests that were authorized under the FDA Emergency Use Authorization (EUA) program. See Table 26 below.

Table 26: QCMD MERS-CoV Proficiency Testing (PT) Panel Results (after un-blinding)

PT Panel Sample #	Sample Description	Target Concentration (Copies/mL [a])	CDC MERS-CoV N2 rRT-PCR (EUA)		Altona RealStar MERS-CoV RT-PCR (EUA)			FilmArray RP2*plus*	FilmArray RP2*plus*
			N2 Ct [b]	MERS-CoV Result	*UpE* Ct	*orf1a* Ct	MERS-CoV Result	MERS-CoV Result	Other Pathogen Detected Result
1	MERS-CoV	1.0E+04	26.46	D	23.66	22.85	D	D	-
2	MERS-CoV	1.0E+04	26.25	D	23.65	22.56	D	D	-
3	MERS-CoV	1.0E+03	30.20	D	26.83	26.03	D	D	-
4	MERS-CoV	1.0E+02	33.01	D	29.65	29.03	D	D	-
5	MERS-CoV	1.0E+01	35.27	D	32.83	32.57	D	D	-
6	CoV-OC43	1.0E+04	No Ct	ND	No Ct	No Ct	ND	ND	CoV-OC43
7	CoV-OC43	1.0E+03	No Ct	ND	No Ct	No Ct	ND	ND	CoV-OC43
8	CoV-NL63	1.0E+04	No Ct	ND	No Ct	No Ct	ND	ND	CoV-NL63
9	CoV-NL63	1.0E+03	No Ct	ND	No Ct	No Ct	ND	ND	CoV-NL63
10	CoV-229E	1.0E+04	No Ct	ND	No Ct	No Ct	ND	ND	CoV-229E
11	CoV-229E	1.0E+03	No Ct	ND	No Ct	No Ct	ND	ND	CoV-229E
12	MERS-CoV	5.0E+04	23.97	D	21.74	20.90	D	D	-

[a] Generated using QCMD in-house assays.
[b] Average Ct of two RT-PCR replicates.
D = Detected; ND = Not Detected

3. Clinical Studies:

Prospective Clinical Study

The clinical performance of the FilmArray RP2*plus* was established during a multi-center study conducted at three geographically distinct U.S. study sites during portions of the 2015-2016 and 2016-2017 respiratory illness seasons. Each study location was representative of the intended use setting (clinical laboratories) and testing was performed by trained clinical laboratory personnel.

Residual NPS specimens in VTM meeting the following eligibility criteria were prospectively collected and tested using the FilmArray RP2*plus* during the clinical study:

Inclusion criteria:

- Specimen is residual NPS in VTM left over from standard of care (SOC) testing under clinician order for respiratory pathogen analysis using the FilmArray RP, an FDA-cleared multiplexed respiratory pathogens panel
- Specimen has been held at room temperature for less than or equal to four hours or 4°C for less than or equal to three days before enrollment (FilmArray RP2*plus* testing or archiving of specimen aliquots at ≤-70°C must be completed within this window)
- At least 1.5 mL of specimen is remaining after standard of care testing and available for use in the study

Exclusion criteria:

- Specimen other than NPS in VTM (e.g., nasopharyngeal aspirate, anterior or mid-turbinate swab, oropharyngeal swab, NPS collected in medium other than VTM)

- Specimen was not tested with the comparator test as part of patient care
- Specimen cannot be tested within the defined storage parameters
- Insufficient specimen volume for testing

A waiver of the informed consent requirement was obtained from the Institutional Review Boards (IRBs) at each study site for the use of residual NPS specimens. Each residual specimen collected for the study was assigned a unique Study Code Number (SCN). The SCN was used to de-identify the specimen aliquots used for FilmArray RP2*plus* testing and comparator PCR/sequencing testing, and to provide select clinical data to the sponsor. The SCN was recorded in a key which linked each SCN to subject identification information to allow collection of subject demographic information, hospitalization status, and the comparator test result(s) provided for subject care. Access to this key was limited to specific site personnel who de-identified study specimens and who had no knowledge of the FilmArray RP2*plus* results.

The following information was recorded on the Case Report Form (CRF) for each subject from whom a specimen was enrolled:

- Age group and sex
- Date of specimen collection
- Subject hospitalization status (Outpatient, Emergency Department, or Hospitalized)
- SOC comparator test result(s)

A total of 1635 residual NPS specimens in VTM were prospectively acquired initially for the clinical study. Between January and March 2016, specimens were prospectively collected from all comers meeting the study eligibility criteria and immediately frozen (N=695 specimens) for later testing as prospective archived/frozen (Category II) specimens. Between September and November 2016, specimens were prospectively collected from all comers meeting the study eligibility criteria and tested fresh (N=940 specimens) as prospective fresh (Category I) specimens. Category II specimens were distributed to sites beginning in September 2016. Study sites also began testing Category I specimens at this time. At each site, Category II specimens were thawed and tested according to the study procedures as time permitted over the remaining duration of the clinical study.

Table 27: Participating Study Sites for the FilmArray RP2*plus* Clinical Evaluation

Site	Study Site Location	Enrolled Population	Category I Specimen Collected (September – November 2016)	Category II Specimen Collected (January – March 2016)	Total Number of Prospective Specimens (Category I and II)
1	Salt Lake City, UT	Predominantly pediatric	350	250	600
2	Chicago, IL	Pediatric and adult	286	244	530
3	Columbus, OH	Predominantly pediatric	304	201	505

Total	**940**	**695**	**1635**

Of the total of 1635 prospective NPS specimens in VTM that were initially acquired for the clinical study, 23 were excluded from the final data analysis (see Table 28 below). The most common reason for specimen exclusion was one instance of a site failing to run an ECM for a given day of testing (10 specimens excluded). The second most common reason for specimen exclusion was the testing of specimens outside the 3-day window (nine specimens). The third most common reason for exclusion was that the specimen was found to not meet the inclusion criteria after the specimen had been enrolled (three specimens).

Table 28: Summary of Specimens Excluded from the Prospective Clinical Study

Reason for Exclusion	Specimens
Did not meet inclusion criteria	3
Specimen of incorrect sample type	2
Comparator test not requested by clinician for standard of care testing	1
FilmArray RP2*plus* test could not be completed within 3-day window	9
No external control run for test date	10
FilmArray RP2*plus* run failure – unable to be retested due to volume limitation	1
Total	**23/1635 (1.4%)**

The final data set of the prospective clinical study consisted of 1612 specimens. Table 29 below provides a summary of demographic information for the 1612 specimens included in the data analysis of the prospective clinical study.

Table 29: Demographic Summary for Prospective FilmArray RP2*plus* Clinical Evaluation

		Overall	Site 1	Site 2	Site 3
Sex	Male	867 (54%)	331 (57%)	271 (51%)	265 (53%)
	Female	745 (46%)	250 (43%)	256 (49%)	239 (47%)
Age	≤ 5 years	885 (55%)	379 (65%)	170 (32%)	336 (67%)
	6 - 21 years	331 (21%)	132 (23%)	89 (17%)	110 (22%)
	22 - 49 years	128 (8%)	27 (5%)	79 (15%)	22 (4%)
	50+ years	268 (17%)	43 (7%)	189 (36%)	36 (7%)
Status	Outpatient	329 (20%)	77 (13%)	66 (13%)	186 (37%)
	Hospitalized	640 (40%)	229 (39%)	197 (37%)	214 (42%)
	Emergency	643 (40%)	275 (47%)	264 (50%)	104 (21%)
Total		**1612**	**581**	**527**	**504**

The performance of the FilmArray RP2*plus* was evaluated by comparing the FilmArray RP2*plus* test results with those from an FDA-cleared multiplexed respiratory pathogens panel as well as with results from two analytically-validated PCR followed by bi-directional sequencing assays for *B. parapertussis* (this analyte is not detected by the FDA-cleared multiplexed respiratory pathogens panel). The *B. parapertussis* comparator assays were designed to amplify a different sequence than that amplified by the

FilmArray RP2*plus*. Any specimen that had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched organism-specific sequences deposited in the NCBI GenBank database (www.ncbi.nlm.nih.gov) with acceptable E-values was considered Positive. Any specimen that tested negative by both comparator assays was considered Negative.

While no MERS-CoV was expected in this study population (although this study population sufficiently simulates the intended use patient population for the FilmArray RP2*plus*), this prospective clinical study was designed to assess clinical specificity of the FilmArray RP2*plus* MERS-CoV assays only. Performance for the FilmArray RP2*plus* MERS-CoV analyte was compared to the expected negative result for MERS-CoV in this study population.

Prospective Clinical Study System Performance

The overall success rate for initial specimen tests in the prospective study was 99.3% (1611/1623) (95% CI: 98.7% - 99.6%); 12 tests were unsuccessful (one due to an incomplete test, one due to an instrument error, and ten due to control failures). Two tests (2/1623; 0.1%) did not complete on the initial run, resulting in an instrument success rate of 99.9% (1621/1623) (95% CI: 99.6% - 100%) for initial specimen tests. Both specimens were able to be retested and valid results were produced after a single retest. Ten tests (10/1621; 0.6%) did not produce valid pouch controls, resulting in a pouch control success rate of 99.4% (1611/1621) (95% CI: 98.9% - 99.7%) for completed runs in the initial specimen tests. Nine of the 10 invalid specimens were retested and produced valid control results after a single retest; one was not retested due to insufficient specimen volume.

Prospective Clinical Study Performance

Positive Percent Agreement (PPA) for each analyte was calculated as 100% x (TP / (TP + FN)). True positive (TP) indicates that both the FilmArray RP2*plus* and the comparator method had a positive result for this specific analyte, and false negative (FN) indicates that the FilmArray RP2*plus* result was negative while the comparator result was positive. Negative Percent Agreement (NPA) was calculated as 100% x (TN / (TN + FP)). True negative (TN) indicates that both the FilmArray RP2*plus* and the comparator method had negative results (or the FilmArray RP2*plus* agrees with the expected negative results for MERS-CoV), and a false positive (FP) indicates that the FilmArray RP2*plus* result was positive but the comparator result was negative (or the MERS-CoV expected result is negative). The exact binomial two-sided 95% confidence interval was calculated.

Samples for which false positive and/or false negative results (i.e., discrepant results) were obtained when comparing the FilmArray RP2*plus* results to the comparator method results (or expected negative results for MERS-CoV) were further investigated. The discrepancy investigation was mainly conducted by performing independent molecular methods with primers that are different from that of the FilmArray RP2*plus* and/or comparator method retesting.

The FilmArray RP2*plus* prospective performance data in positive percent and negative percent agreements against the comparator methods or the expected negative results for MERS-CoV (all sites combined) are presented by analyte in Table 30 below:

Table 30: FilmArray RP2 Prospective Clinical Performance Summary

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Viruses							
MERS-CoV	Fresh	0/0	0	N/A	918/918	100	99.6-100
	Frozen	0/0	0	N/A	694/694	100	99.4-100
	Overall	***0/0***	**0**	**N/A**	***1612/1612***	***100***	***99.8-100***
Adenovirus [a]	Fresh	36/38	94.7	82.7-98.5	850/880	96.6	95.2-97.6
	Frozen	34/36	94.4	81.9-98.5	640/658	97.3	95.7-98.3
	Overall	***70/74***	***94.6***	***86.9-97.9***	***1490/1538***	***96.9***	***95.9-97.6***
CoV-229E [b]	Fresh	5/5	100	56.6-100	909/913	99.6	98.9-99.8
	Frozen	6/7	85.7	48.7-97.4	686/687	99.9	99.2-100
	Overall	***11/12***	***91.7***	***64.6-98.5***	***1595/1600***	***99.7***	***99.3-99.9***
CoV-HKU1 [c]	Fresh	1/1	100	N/A	917/917	100	99.6-100
	Frozen	42/42	100	91.6-100	640/652	98.2	96.8-98.9
	Overall	***43/43***	***100***	***91.8-100***	***1557/1569***	***99.2***	***98.7-99.6***
CoV-NL63 [d]	Fresh	0/0	0	N/A	917/918	99.9	99.4-100
	Frozen	40/40	100	91.2-100	645/654	98.6	97.4-99.3
	Overall	***40/40***	***100***	***91.2-100***	***1562/1572***	***99.4***	***98.8-99.7***
CoV-OC43 [e]	Fresh	11/13	84.6	57.8-95.7	904/905	99.9	99.4-100
	Frozen	22/28	78.6	60.5-89.8	662/666	99.4	98.5-99.8
	Overall	***33/41***	***80.5***	***66.0-89.8***	***1566/1571***	***99.7***	***99.3-99.9***
hMPV [f]	Fresh	5/5	100	56.6-100	913/913	100	99.6-100
	Frozen	68/70	97.1	90.2-99.2	616/624	98.7	97.5-99.3
	Overall	***73/75***	***97.3***	***90.8-99.3***	***1529/1537***	***99.5***	***99.0-99.7***
HRV/EV [g]	Fresh	320/328	97.6	95.3-98.8	532/590	90.2	87.5-92.3
	Frozen	105/108	97.2	92.1-99.1	567/586	96.8	95.0-97.9
	Overall	***425/436***	***97.5***	***95.5-98.6***	***1099/1176***	***93.5***	***91.9-94.7***
FluA [h]	Fresh	3/3	100	43.9-100	915/915	100	99.6-100
	Frozen	75/75	100	95.1-100	616/616	100	99.4-100
	Overall	***78/78***	***100***	***95.3-100***	***1531/1531***	***100***	***99.7-100***
FluA H1	Fresh	0/0	0	N/A	918/918	100	99.6-100
	Frozen	0/0	0	N/A	691/691	100	99.4-100
	Overall	***0/0***	***0***	***N/A***	***1609/1609***	***100***	***99.8-100***
FluA H1-2009	Fresh	0/0	0	N/A	918/918	100	99.6-100
	Frozen	74/74	100	95.1-100	617/617	100	99.4-100
	Overall	***74/74***	***100***	***95.1-100***	***1535/1535***	***100***	***99.8-100***
FluA H3	Fresh	3/3	100	43.9-100	915/915	100	99.6-100
	Frozen	1/1	100	N/A	690/690	100	99.4-100

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
	Overall	***4/4***	***100***	***51.0-100***	***1605/1605***	***100***	***99.8-100***
FluB [i]	Fresh	0/0	0	N/A	918/918	100	99.6-100
	Frozen	14/14	100	78.5-100	678/680	99.7	98.9-99.9
	Overall	***14/14***	***100***	***78.5-100***	***1596/1598***	***99.9***	***99.5-100***
PIV1 [j]	Fresh	5/5	100	56.6-100	913/913	100	99.6-100
	Frozen	4/4	100	51.0-100	689/690	99.9	99.2-100
	Overall	***9/9***	***100***	***70.1-100***	***1602/1603***	***99.9***	***99.6-100***
PIV2 [k]	Fresh	46/47	97.9	88.9-99.6	863/871	99.1	98.2-99.5
	Frozen	0/0	0	N/A	694/694	100	99.4-100
	Overall	***46/47***	***97.9***	***88.9-99.6***	***1557/1565***	***99.5***	***99.0-99.7***
PIV3 [l]	Fresh	40/42	95.2	84.2-98.7	867/876	99.0	98.1-99.5
	Frozen	3/3	100	43.9-100	690/691	99.9	99.2-100
	Overall	***43/45***	***95.6***	***85.2-98.8***	***1557/1567***	***99.4***	***98.8-99.7***
PIV4 [m]	Fresh	6/6	100	61.0-100	910/912	99.8	99.2-99.9
	Frozen	3/3	100	43.9-100	686/691	99.3	98.3-99.7
	Overall	***9/9***	***100***	***70.1-100***	***1596/1603***	***99.6***	***99.1-99.8***
RSV [n]	Fresh	44/45	97.8	88.4-99.6	867/873	99.3	98.5-99.7
	Frozen	131/131	100	97.2-100	545/563	96.8	95.0-98.0
	Overall	***175/176***	***99.4***	***96.9-99.9***	***1412/1436***	***98.3***	***97.5-98.9***
Bacteria							
***B. parapertussis* (IS*1001*)** [o]	Fresh	4/5	80.0	37.6-96.4	913/913	100	99.6-100
	Frozen	2/2	100	34.2-100	692/692	100	99.4-100
	Overall	***6/7***	***85.7***	***48.7-97.4***	***1605/1605***	***100***	***99.8-100***
***B. pertussis* (*ptxP*)** [p]	Fresh	2/2	100	34.2-100	915/916	99.9	99.4-100
	Frozen	0/1	0.0	N/A	693/693	100	99.4-100
	Overall	***2/3***	***66.7***	***20.8-93.9***	***1608/1609***	***99.9***	***99.6-100***
C. pneumoniae [q]	Fresh	2/2	100	34.2-100	915/916	99.9	99.4-100
	Frozen	3/3	100	43.9-100	691/691	100	99.4-100
	Overall	***5/5***	***100***	***56.6-100***	***1606/1607***	***99.9***	***99.6-100***
M. pneumoniae [r]	Fresh	17/17	100	81.6-100	897/901	99.6	98.9-99.8
	Frozen	6/7	85.7	48.7-97.4	686/687	99.9	99.2-100
	Overall	***23/24***	***95.8***	***79.8-99.3***	***1583/1588***	***99.7***	***99.3-99.9***

[a] Adenovirus was detected in 3/4 FN specimens using an independent molecular method. Adenovirus was detected in 38/48 FP specimens using an independent molecular method; an additional two FP specimens were indicated to have been collected from subjects with an acute history of adenovirus infection.

[b] The single FN specimen was negative for CoV-229E when tested using an independent molecular method. All five FP specimens were negative for CoV-229E when tested using an independent molecular method.

[c] CoV-HKU1 was detected in 3/12 FP specimens upon comparator method retest.

[d] CoV-NL63 was detected in 3/10 FP specimens during discrepancy investigation; two were detected using an independent molecular method and one was detected upon comparator method retest.

[e] Of the eight FN specimens, six were TP for CoV-HKU1. They were confirmed to be due to a known cross-reactivity with CoV-HKU1 of the comparator method. All six specimens were negative for CoV-OC43 when tested with two independent PCR assays; the remaining two FN specimens were negative for CoV-OC43 when tested using an independent molecular method. CoV-OC43 was detected in 2/5 FP specimens upon comparator method retest.

[f] Both FN specimens were negative for hMPV when tested using an independent molecular method. hMPV was detected

in 6/8 FP specimens during discrepancy investigation; one was detected using an independent molecular method and five were detected upon comparator method retest.

[g] HRV/EV was detected in 5/11 FN specimens during discrepancy investigation; one was detected using an independent molecular method and four were detected upon FilmArray RP2 retest. HRV/EV was detected in 33/77 FP specimens during discrepancy investigation; four were detected using an independent molecular method and 29 were detected upon comparator method retest.

[h] Three specimens were excluded from influenza A analysis: one with a comparator method result of Influenza A (No Subtype Detected) and two FilmArray RP2 Influenza A (Equivocal) detections.

[i] FluB was detected in both FP specimens during discrepancy investigation; one was detected using an independent molecular method and one was detected upon comparator method retest.

[j] The single FP specimen was negative for PIV1 when tested using an independent molecular method

[k] The single FN specimen was negative for PIV2 when tested using an independent molecular method. PIV2 was detected in 5/8 FP specimens during discrepancy investigation; one was detected using an independent molecular method and four were detected upon comparator retest.

[l] PIV3 was detected in both FN specimens during discrepancy investigation; one was detected using an independent molecular method and one was detected upon FilmArray RP2 retest. PIV3 was detected in 4/10 FP specimens during discrepancy investigation; two were detected using an independent molecular method and two were detected upon comparator method retest.

[m] PIV4 was detected in 1/7 FP specimens using an independent molecular method.

[n] The single FN specimen was negative for RSV when tested using an independent molecular method. RSV was detected in 8/24 FP specimens during discrepancy investigation; three were detected using an independent molecular method and five were detected upon comparator method retest.

[o] *B. parapertussis* was detected in the single FN specimen upon FilmArray RP2 retest.

[p] *B. pertussis* was detected in both the FN and the FP specimens using an independent molecular method.

[q] *C. pneumoniae* was detected in the single FP specimen using an independent molecular method.

[r] *M. pneumoniae* was detected in the single FN specimen upon FilmArray RP2 retest. *M. pneumoniae* was detected in all five FP specimens during discrepancy investigation; three were detected using an independent molecular method and two were detected upon comparator method retest.

Prospective Clinical Study Mixed Infection Analysis

FilmArray RP2*plus* reported a total of 245 specimens with discernible multiple organism detections (15.2% of all specimens, 245/1612; and 24.0% of all positive specimens, 245/1020; Table 31). The majority of multiple detections (190/245; 77.6%) contained two organisms, while 20.0% (49/245) contained three organisms, 1.6% (4/245) contained four organisms, 0.4% (1/245) contained five organisms, and 0.4% (1/245) contained six organisms. Out of the 245 specimens with multiple detections, 124 specimens (50.6%; 124/245) were concordant with the comparator methods. One hundred twenty-one (121) specimens (49.4%; 121/245) contained one or more organisms that had not been detected by the comparator methods (i.e., false positive results).

The three organisms that were most prevalent in multiple detections were also the three most prevalent organisms in the study (i.e. HRV/EV, RSV, and adenovirus).

Table 31: Prevalence of Analytes in Multiple Detections as determined by the FilmArray RP2*plus*

Analyte	Prevalence in Multiple Detections (N=245)
Viruses	

MERS-CoV	0	0%
Adenovirus	85	34.7%
CoV-229E	6	2.4%
CoV-HKU1	41	16.7%
CoV-NL63	31	12.7%
CoV-OC43	19	7.8%
hMPV	33	13.5%
HRV/EV	150	61.2%
FluA H1	0	0%
FluA H1-2009	9	3.7%
FluA H3	2	0.8%
FluB	6	2.4%
PIV1	5	2.0%
PIV2	15	6.1%
PIV3	21	8.6%
PIV4	12	4.9%
RSV	105	42.9%
Bacteria		
B. parapertussis (IS*1001*)	6	2.4%
B. pertussis (*ptxP*)	0	0%
C. pneumoniae	1	0.4%
M. pneumoniae	7	2.9%

All distinct co-infection combinations as detected by the FilmArray RP2*plus* during the prospective clinical study are presented in Table 32 below.

Table 32: Distinct Co-infection Combinations Detected by the FilmArray RP2*plus* in the Prospective Clinical Trial

Distinct Co-infection Combinations Detected by the FilmArray RP2*plus*						Total Co-infections	Number of Discrepant Co-infections [a]	Discrepant Analyte(s)
Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5	Analyte 6			
Adenovirus	HRV/EV					30	15	Adenovirus (15), HRV/EV (1)
HRV/EV	RSV					22	7	HRV/EV (3), RSV (4)
CoV-HKU1	RSV					13	7	CoV-HKU1 (4), RSV (3)
CoV-NL63	RSV					13	3	CoV-NL63 (2), RSV (1)
HRV/EV	PIV2					11	7	HRV/EV (6), PIV2 (2)
HRV/EV	PIV3					11	6	HRV/EV (3), PIV3 (4)
Adenovirus	RSV					10	5	Adenovirus (4), RSV (1)
Adenovirus	HRV/EV	RSV				9	5	Adenovirus (2), HRV/EV (3), RSV (1)
CoV-NL63	HRV/EV					8	2	CoV-NL63 (2)
CoV-HKU1	HRV/EV					5	2	CoV-HKU1 (1), HRV/EV (1)
CoV-OC43	HRV/EV					5	3	HRV/EV (3)

hMPV	HRV/EV					5	1	HRV/EV (1)
Adenovirus	CoV-HKU1	RSV				4	3	Adenovirus (2), RSV (1)
HRV/EV	*M. pneumoniae*					4	1	HRV/EV (1)
Adenovirus	CoV-HKU1					3	2	Adenovirus (1), CoV-HKU1 (1)
Adenovirus	PIV3					3	3	Adenovirus (3), PIV3 (1)
CoV-OC43	RSV					3	0	-
hMPV	PIV4					3	2	hMPV (1), PIV4 (2)
FluA H1-2009	RSV					3	1	RSV (1)
Adenovirus	HRV/EV	PIV3	RSV			2	2	Adenovirus (1), PIV3 (2)
Adenovirus	HRV/EV	PIV3				2	1	HRV/EV (1)
Adenovirus	HRV/EV	*M. pneumoniae*				2	1	Adenovirus (1), *M. pneumoniae* (1)
CoV-HKU1	CoV-OC43	RSV				2	2	CoV-HKU1 (2)
CoV-HKU1	HRV/EV	RSV				2	1	HRV/EV (1)
hMPV	HRV/EV	PIV4				2	0	-
hMPV	HRV/EV	RSV				2	1	HRV/EV (1)
Adenovirus	hMPV					2	1	Adenovirus (1)
Adenovirus	PIV2					2	2	Adenovirus (1), PIV2 (1)
CoV-229E	HRV/EV					2	1	HRV/EV (1)
CoV-229E	RSV					2	1	CoV-229E (1)
CoV-NL63	hMPV					2	1	hMPV (1)
CoV-NL63	FluB					2	2	CoV-NL63 (2)
hMPV	RSV					2	1	RSV (1)
HRV/EV	PIV1					2	0	-
HRV/EV	*B. parapertussis*					2	0	-
Adenovirus	CoV-NL63	hMPV	HRV/EV	PIV4	RSV	1	1	HRV/EV (1), PIV4 (1)
Adenovirus	HRV/EV	PIV3	PIV4	RSV		1	1	PIV4 (1), RSV (1)
Adenovirus	HRV/EV	RSV	*B. parapertussis*			1	1	Adenovirus (1), HRV/EV (1)
CoV-HKU1	CoV-OC43	HRV/EV	RSV			1	0	-
Adenovirus	CoV-HKU1	hMPV				1	1	hMPV (1)
Adenovirus	CoV-OC43	HRV/EV				1	0	-
Adenovirus	CoV-OC43	RSV				1	0	-
Adenovirus	hMPV	FluA H1-2009				1	1	Adenovirus (1)
Adenovirus	HRV/EV	FluA H3				1	1	HRV/EV (1)
CoV-229E	CoV-HKU1	hMPV				1	1	CoV-229E (1)
CoV-HKU1	CoV-NL63	HRV/EV				1	1	CoV-NL63 (1)
CoV-HKU1	CoV-NL63	RSV				1	1	CoV-HKU1 (1)
CoV-HKU1	hMPV	PIV3				1	1	hMPV (1)
CoV-HKU1	hMPV	RSV				1	0	-

CoV-HKU1	PIV1	RSV				1	0	-
CoV-HKU1	PIV4	RSV				1	1	PIV4 (1)
CoV-NL63	CoV-OC43	PIV1				1	0	-
CoV-NL63	RSV	*B.*				1	1	RSV (1)
CoV-OC43	HRV/EV	PIV4				1	1	CoV-OC43
hMPV	HRV/EV	FluB				1	1	hMPV (1), HRV/EV (1), FluB (1)
HRV/EV	FluA H1-2009	RSV				1	0	-
HRV/EV	PIV1	RSV				1	1	PIV1 (1)
HRV/EV	PIV2	RSV				1	1	HRV/EV (1), PIV2 (1)
HRV/EV	RSV	*B.*				1	0	-
Adenovirus	CoV-NL63					1	0	
Adenovirus	CoV-OC43					1	1	Adenovirus (1)
Adenovirus	FluA H1-2009					1	1	Adenovirus (1)
Adenovirus	PIV4					1	1	Adenovirus (1)
CoV-229E	FluA H1-2009					1	0	-
CoV-HKU1	hMPV					1	0	-
CoV-HKU1	FluB					1	0	-
CoV-HKU1	PIV4					1	1	PIV4 (1)
CoV-OC43	hMPV					1	1	CoV-OC43 (1)
CoV-OC43	PIV3					1	0	-
CoV-OC43	*M. pneumoniae*					1	1	CoV-OC43 (1)
hMPV	FluA H1-2009					1	0	-
hMPV	FluB					1	1	FluB (1)
HRV/EV	FluA H1-2009					1	0	-
HRV/EV	FluA H3					1	0	-
HRV/EV	FluB					1	1	HRV/EV (1)
HRV/EV	PIV4					1	1	HRV/EV (1)
HRV/EV	*C. pneumoniae*					1	0	-
PIV2	RSV					1	1	PIV2
RSV	*B. parapertussis*					1	0	-
Total Co-infections						245	121	135/554
Total Double Infections						190	86	91/380
Total Triple Infections						49	30	35/147
Total Quadruple Infections						4	3	5/16
Total Quintuple Infections						1	1	2/5
Total Sextuple Infections						1	1	2/6

[a] A discrepant co-infection or discrepant analyte was defined as one that was detected by FilmArrayRP2*plus* but not detected by the comparator method. Of the 135 discrepant analytes (out of 554 total analytes), 66 (48.9%) were observed as being present in the specimen during discrepancy investigation; 43/135 (31.9%) were observed using an independent molecular method and 27/135 (20.0%) were observed upon comparator method retest.

Additional distinct co-infection combinations detected by the comparator method(s), but

not detected by the FilmArray RP2*plus* in the prospective clinical trial are presented in Table 33 below.

Table 33: Additional Distinct Co-infection Combinations Detected by the Comparator Method(s), but not detected by the FilmArray RP2*plus* in the Prospective Clinical Trial

Distinct Co-infection Combinations Detected by the FilmArray RP2*plus*				Total Specimens with Co-infections	Number of Specimens with Discrepant [a]	Discrepant Analyte(s)
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**			
Adenovirus	FluA H1-2009			1	1	Adenovirus (1)
Adenovirus	PIV3			2	1	Adenovirus (1)
CoV-229E	FluA H1-2009			2	1	CoV-229E (1)
CoV-HKU1	CoV-OC43			3	3	CoV-OC43 (3) [b]
CoV-HKU1	CoV-OC43	RSV		2	2	CoV-OC43 (2) [b]
CoV-HKU1	CoV-OC43	HRV/EV	RSV	2	1	CoV-OC43 (1) [b]
CoV-HKU1	HRV/EV			5	1	HRV/EV (1)
CoV-HKU1	HRV/EV	RSV		1	1	HRV/EV (1)
hMPV	HRV/EV			8	1	HRV/EV (1)
HRV/EV	PIV2			5	1	HRV/EV (1)
HRV/EV	PIV3			6	1	HRV/EV (1)
HRV/EV	*B. parapertussis*			3	1	*B. parapertussis* (1)
PIV2	PIV3			1	1	PIV2 (1), PIV3 (1)
RSV	*M. pneumoniae*			1	1	*M. pneumoniae* (1)
Total Co-infections				42	17	18/91
Total Double Infections				37	13	13/74
Total Triple Infections				3	3	4/9
Total Quadruple Infections				2	1	1/8

[a] This table includes only distinct co-infections that were detected by the comparator method(s) but not by FilmArray RP2*plus*; the remaining co-infections detected by the comparator method(s) are already represented in Table 32 above.
[b] Of the six FilmArray RP2*plus* FN specimens, all were TP for CoV-HKU1. They were confirmed to be due to a known cross-reactivity with CoV-HKU1 of the comparator method. All six specimens were negative for CoV-OC43 when tested with two independent PCR assays.

Retrospective Clinical Study – Common Respiratory Pathogens

Some of the analytes on the FilmArray RP2*plus* were of low prevalence and were not encountered in sufficiently large numbers during the prospective study to adequately demonstrate system performance. To supplement the results of the prospective clinical study, an evaluation of preselected archived retrospective specimens was performed at BioFire Diagnostics. These specimens were archived NPS in VTM specimens that were selected because they had previously tested positive for one of the following analytes at the source laboratory: coronavirus 229E, influenza A H1, influenza A H3, influenza B,

parainfluenza virus 1, parainfluenza virus 4, *Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydia pneumoniae*, parainfluenza virus 2, parainfluenza virus 3, and *Mycoplasma pneumoniae*.

A total of 217 clinical specimens were initially received for testing in this retrospective study (Table 34 below). In preparation for testing, a study code number (SCN) was assigned to each specimen and a key was created in order to randomize them such that operators were blinded to the expected test result.

Prior to testing with the FilmArray RP2*plus*, the composition/integrity of the pre-selected specimens was first confirmed with the same comparator methods employed in the prospective clinical study (i.e., PCR followed by bi-directional sequencing assays for *B. parapertussis* or an FDA-cleared multiplexed respiratory pathogens panel for all other analytes except for MERS-CoV). Specimens were divided into two different groups for testing based on the method of confirmation testing performed: all specimens containing analytes on the FDA-cleared multiplexed respiratory pathogens panel comparator method were tested in Group 1 and specimens containing *B. parapertussis* were tested in Group 2. Negative NPS specimens were included in each group. Table 34 below shows the number of specimens for each analyte (and negatives) that were tested with the FilmArray RP2*plus* in this study.

Table 34: Archived Specimens Used in the Retrospective Clinical Study – Common Respiratory Pathogens

Analyte	Number of Specimens
Group 1	
Coronavirus 229E	19
Influenza A H1	3
Influenza A H3	17
Influenza B	17
Parainfluenza Virus 1 [a]	16
Parainfluenza Virus 2	17
Parainfluenza Virus 3	17
Parainfluenza Virus 4	17
Bordetella pertussis	32
Chlamydia pneumoniae	18
Mycoplasma pneumoniae [a]	21
Negative	4
Total	197 [a]
Group 2	
Bordetella parapertussis	16
Negative	4
Total	20
Total Overall	**217**

[a] One specimen contained two analytes of interest.

The FDA-cleared multiplexed respiratory pathogens panel comparator method was performed on 197 of the 217 achieved specimens only (Group 1). One of the 197 specimens was excluded from performance analysis because of an invalid RP2*plus* run with insufficient volume to retest. Additionally, two of the 197 specimens were also excluded from performance analysis because a valid FDA-cleared multiplexed respiratory pathogens panel comparator method confirmation result was not obtained and there was insufficient specimen volume for retesting: one comparator run was incomplete and the other comparator run had a pouch control failure. FilmArray RP2*plus* results for these specimens are shown below in Table 35. Valid comparator method and FilmArray RP2*plus* results were obtained for 194 of these 197 archived specimens (Group 1).

Table 35: Specimens Excluded Because of Invalid Comparator Test and Insufficient Volume for Retesting

SCN	Analyte Detected at the Source Laboratory	Comparator Method Confirmation Result	FilmArray RP2*plus* Result
026979-ARC-0004	*Bordetella pertussis*	*Bordetella pertussis*	Invalid
026979-ARC-0127	PIV4	Invalid	PIV4
026979-ARC-0170	*C. pneumoniae*	Invalid	*C. pneumoniae*

The *B. parapertussis* PCR followed by bi-directional sequencing comparator assays were performed on 20 of the 217 achieved specimens only (Group 2). The FDA-cleared multiplexed respiratory pathogens panel comparator method was not performed on Group 2 specimens. Valid comparator method and FilmArray RP2*plus* results were obtained for 20 of these 20 archived specimens.

In addition, all Group 1 and Group 2 positive archived specimens (as determined at the source laboratory) not confirmed by the respective comparator method were also excluded from the performance calculation for each of the respective analyte. FilmArray RP2*plus* results for these excluded specimens are shown below in Table 36.

Table 36: Specimens Excluded Because of Unconfirmed Comparator Method Results

SCN	Analyte Detected at the Source Laboratory	Comparator Method Confirmation Result	FilmArray RP2*plus* Result
Samples Excluded from CoV-229E Performance Analysis Because of Unconfirmed Comparator Method CoV-229E			
026979-ARC-0026	CoV-229E	CoV-229E Not Detected	CoV-229E Not Detected
026979-ARC-0049	CoV-229E	CoV-229E Not Detected	CoV-229E
026979-ARC-0055	CoV-229E	CoV-229E Not Detected	CoV-229E Not Detected
026979-ARC-0143	CoV-229E	CoV-229E Not Detected	CoV-229E
Samples Excluded from Flu B Performance Analysis Because of Unconfirmed Comparator Method Flu B Result			
026979-ARC-0199	Influenza B	Influenza B Not Detected	Influenza B
Samples Excluded from PIV 2 Performance Analysis Because of Unconfirmed Comparator Method PIV 2 Result			
026979-ARC-0014	PIV 2	PIV 2 Not Detected	PIV 2 Not Detected
Samples Excluded from *B. pertussis* Performance Analysis Because of Unconfirmed Comparator Method *B. pertussis*			
026979-ARC-0028	*B. pertussis*	*B. pertussis* Not Detected	*B. pertussis* Not Detected
026979-ARC-0036	*B. pertussis*	*B. pertussis* Not Detected	*B. pertussis* Not Detected
026979-ARC-0052	*B. pertussis*	*B. pertussis* Not Detected	*B. pertussis* Not Detected
026979-ARC-0087	*B. pertussis*	*B. pertussis* Not Detected	*B. pertussis* Not Detected
026979-ARC-0097	*B. pertussis*	*B. pertussis* Not Detected	*B. pertussis*
026979-ARC-0131	*B. pertussis*	*B. pertussis* Not Detected	*B. pertussis* Not Detected
Samples Excluded from *C. pneumoniae* Performance Analysis Because of Unconfirmed Comparator Method *C.*			
026979-ARC-0145	*C. pneumoniae*	*C. pneumoniae* Not	*C. pneumoniae*
Samples Excluded from *M. pneumoniae* Performance Analysis Because of Unconfirmed Comparator Method *M.*			
026979-ARC-0094	*M. pneumoniae*	*M. pneumoniae* Not	*M. pneumoniae* Not
026979-ARC-0104	*M. pneumoniae*	*M. pneumoniae* Not	*M. pneumoniae*
026979-ARC-0105	*M. pneumoniae*	*M. pneumoniae* Not	*M. pneumoniae*
026979-ARC-0138	*M. pneumoniae*	*M. pneumoniae* Not	*M. pneumoniae* Not

026979-ARC-0149	*M. pneumoniae*	*M. pneumoniae* Not	*M. pneumoniae*

Table 37: Analyte Confirmation Summary for All Group 1 and Group 2 Specimens

Analyte	Number of Specimens	Number of Specimens Confirmed by the Comparator Method (%)
Group 1		
Coronavirus 229E	19	15 (78.9%)
Influenza A H1	3	3 (100%)
Influenza A H3	17	17 (100%)
Influenza B	17	16 (94.1%)
Parainfluenza Virus 1 [a]	16	16 (100%)
Parainfluenza Virus 2	17	16 (94.1%)
Parainfluenza Virus 3	17	17 (100%)
Parainfluenza Virus 4	16	16 (100%)
Bordetella pertussis	31	25 (80.6%)
Chlamydia pneumoniae	17	16 (94.1%)
Mycoplasma pneumoniae [a]	21	16 (76.2%)
Negative	4	4 (100%)
Total	194 [a]	
Group 2		
Bordetella parapertussis	16	16 (100%)
Negative	4	4 (100%)
Total	20	20 (100%)
Total Overall	**214**	

[a] One specimen contained two analytes of interest.

A summary of the available demographic information of the 214 specimens is provided in Table 38 below.

Table 38: Available Demographic Summary for All Retrospective Specimens (Common Respiratory Pathogens) Included in the Performance Analysis

Total Specimens		214
Sex	Female (%)	75 (35%)
	Male (%)	81 (38%)
	Unknown	58 (27%)
Age Range	≤ 5 years	78 (36%)
	6 - 21 years	46 (21%)
	22 - 49 years	13 (6%)
	50+ years	19 (9%)
	Unknown	58 (27%)

Retrospective Clinical Study (Common Respiratory Pathogens) Performance

The FilmArray RP2*plus* retrospective performance data in positive percent and negative

percent agreements against the comparator methods (or the expected negative results for MERS-CoV) are presented by analyte in Table 39 below:

Table 39: FilmArray RP2*plus* Retrospective Clinical Study Performance Summary

Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses						
MERS-CoV	0/0	0	N/A	214/214	100	98.2-100
Adenovirus	0/0	0	N/A	189/194	97.4	94.1-98.9
CoV- 229E [a]	15/15	100	79.6-100	175/175	100	97.9-100
CoV-HKU1	0/0	0	N/A	194/194	100	98.1-100
CoV-NL63	2/2	100	34.2-100	192/192	100	98.0-100
CoV-OC43	0/0	0	N/A	194/194	100	98.1-100
hMPV	1/1	100	20.7-100	192/193	99.5	97.1-99.9
HRV/EV	18/19	94.7	75.4-99.1	168/175	96.0	92.0-98.0
Influenza A	22/22	100	85.1-100	172/172	100	97.8-100
Influenza A H1	3/3	100	43.9-100	191/191	100	98.0-100
Influenza A 2009-H1	1/1	100	20.7-100	193/193	100	98.0-100
Influenza A H3	18/18	100	82.4-100	176/176	100	97.9-100
Influenza B [b]	16/16	100	80.6-100	177/177	100	97.9-100
Parainfluenza Virus 1	16/16	100	80.6-100	178/178	100	97.9-100
Parainfluenza Virus 2 [c]	16/16	100	80.6-100	177/177	100	97.9-100
Parainfluenza Virus 3	17/17	100	81.6-100	175/177	98.9	96.0-99.7
Parainfluenza Virus 4	17/17	100	81.6-100	174/177	98.3	95.1-99.4
RSV	2/2	100	34.2-100	191/192	99.5	97.1-99.9
Bacteria						
Bordetella parapertussis (IS*1001*) [d]	16/16	100	80.6-100	4/4	100	51.0-100
Bordetella pertussis (*ptx*P) [e]	25/26	96.2	81.1-99.3	160/162	98.8	95.6-99.7
Chlamydia pneumoniae [f]	17/17	100	81.6-100	176/176	100	97.9-100
Mycoplasma pneumoniae [g]	16/16	100	80.6-100	171/173	98.8	95.9-99.7

[a] Four of 19 CoV-229E positive archived specimens by the source laboratory were not confirmed by the comparator method and therefore were excluded from the performance calculation for CoV-229E .

[b] One of the 17 Influenza B positive archived specimens by the source laboratory was not confirmed by the comparator method and therefore was excluded from the performance calculation for Influenza B.

[c] One of the 17 Parainfluenza Virus 2 positive archived specimens the source laboratory was not confirmed by the comparator method and therefore was excluded from the performance calculation for Parainfluenza Virus 2 .

[d] The comparator *B. parapertussis* PCR followed by sequencing assays were performed on 20 achieved specimens only (Group 2). The comparator method for the other analytes was not performed on these 20 specimens.

[e] Six of the 31 *B. pertussis* positive archived specimens by the source laboratory were not confirmed by the comparator method and therefore were excluded from the performance calculation for *B. pertussis*.

[f] One of the 17 *C. pneumoniae* positive archived specimens by the source laboratory was not confirmed by the comparator method and therefore was excluded from the performance calculation for *C. pneumoniae*.

[g] Five of the 21 *M. pneumoniae* positive archived specimens by the source laboratory were not confirmed by the comparator method and therefore were excluded from the performance calculation for *M. pneumoniae*.

Retrospective Clinical Study – MERS-CoV

Three archived retrospective NPS in VTM specimens from three confirmed cases of MERS-CoV infection were evaluated using the FilmArray RP2*plus* in a laboratory in South Korea. Specimens were originally identified as positive for MERS-CoV using the standard of care method, i.e., two rRT-PCR assays targeting upE and ORF1A (based on the primer and probe sequences published by the WHO), during the 2015 MERS-CoV outbreak in South Korea. Due to logistical constraints of working in the BSL3 laboratory, only positive specimens were tested in this study and FilmArray operators could not be blinded to the known analyte content.

Demographic information was provided for all three MERS-CoV positive specimens collected. All three subjects (100%; 3/3) were adult males, 37, 39 and 73 years old. The date of specimen collection was provided for all three specimens: at the time of testing, the average time between specimen collection/archiving and testing for this study was 535 days (median 540 days, range 523 – 546).

Positive Percent Agreement (PPA) of the FilmArray RP2*plus* results with previous test results for MERS-CoV was 100% (See Table 40 below). The FilmArray RP2*plus* did not detect and identify any common respiratory pathogens targeted by the FilmArray RP2*plus* in these three archived retrospective NPS in VTM specimens.

Table 40: FilmArray RP2*plus* MERS-CoV Archived Retrospective Specimens Testing Performance Summary

Analyte	Positive Percent Agreement		
	TP/(TP + FN)	%	95% CI
MERS-CoV	3/3	100	43.8-100

Testing Contrived Clinical Specimens

MERS-CoV and Influenza A H1 is of such rarity that both prospective and retrospective testing efforts were insufficient to demonstrate system performance. To supplement the prospective and retrospective data, an evaluation of contrived specimens was performed at one of the three clinical sites participated in the prospective evaluation. Contrived clinical specimens were prepared using individual unique residual NPS specimens that had previously tested negative by the FDA-cleared multiplexed respiratory pathogens panel (i.e., the same test as the comparator method employed in the prospective and retrospective clinical evaluations) at the source laboratory.

Spiking was performed using multiple quantified isolates of Influenza A H1 and one isolate of MERS-CoV (EMC 2012 from BEI – Inactivated). The spiking scheme for Influenza A H1 was such that at least 25 of the contrived positive specimens had analyte concentrations at 2 × the limit of detection (LoD), while the remaining 25 contrived positive specimens were at additional concentrations that spanned the suspected clinically relevant range. For Influenza A H1, the clinically relevant range was based on FilmArray RP2*plus* Cp observations of influenza A (A H1, A H-2009, and H3) from the prospective

and archived specimen studies.

A clinically relevant range for the concentration of MERS-CoV in NPS specimens could not be readily determined through a search of available literature. The spiking scheme for MERS-CoV was based upon the reported viral load of 9.3E+02 to 1.2E+06 genome copies/mL in non-NPS respiratory samples, such as tracheobronchial secretions, bronchoalveolar lavage (BAL), and flushed suction catheters, collected 11-18 days after the onset of symptoms of MERS-CoV infection, and the results from one publication that showed an approximate five cycle shift to later Ct values (translates into approximately 50 fold lower viral load) for NPS specimens when compared to BAL specimens. In addition to the suspected clinically relevant range, 25 contrived specimens were spiked at 2×LoD.

Contrived positive specimens were prepared and randomized along with 50 un-spiked negative specimens such that the analyte status of each contrived specimen was unknown to the users performing the testing. The results of the FilmArray RP2*plus* testing are presented in Table 41 below.

Table 41: FilmArray RP2*plus* Performance Testing Contrived Specimens

Analyte	Positive Percent Agreement				Negative Percent Agreement		
	× LoD	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
MERS-CoV	0.2	2/2	100%	34.2-100	50/50	100	92.8-100
	2	25/25	100%	86.7-100			
	10	8/8	100%	67.6-100			
	50	5/5	100%	56.6-100			
	100	5/5	100%	56.6-100			
	1000	5/5	100%	56.6-100			
	Combined	**50/50**	**100%**	**92.9-99.6**			
Analyte	**Positive Percent Agreement**				**Negative Percent Agreement**		
	× LoD	**TP/(TP + FN)**	**%**	**95% CI**	**TN/(TN + FP)**	**%**	**95% CI**
Influenza A H1	2	22/23 [a]	95.7%	79.0-99.2	50/50	100	92.8-100
	10	10/10	100%	72.3-100			
	50	5/5	100%	56.6-100			
	200	5/5	100%	56.6-100			
	1000	5/5	100%	56.6-100			
	Combined	**47/48 [a]**	**97.9%**	**89.1-99.6**			

[a] The FN specimen was spiked with influenza A/Weiss/43; this strain was detected at all other concentrations. Two specimens (also spiked with influenza A/Weiss/43) had a result of Influenza A Equivocal or Influenza A H1 Equivocal and were excluded from Influenza A H1 performance calculation.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

   Since its initial discovery in September 2012, as of October 31, 2017, there have been 2,102 laboratory-confirmed cases of MERS-CoV in 27 countries worldwide according the World Health Organization (WHO). Of these, at least 733 cases have led to MERS-CoV-related deaths (representing a 35% case fatality rate). Starting in May of 2015, an outbreak began in the Republic of Korea with the index case being a 68-year-old South Korean man who had recently traveled to Saudi Arabia. The outbreak concluded in early July 2015. During that time South Korea reported 186 laboratory-confirmed cases with 38 MERS-CoV related deaths (20.4% case fatality rate).

   According to the CDC, the MERS-CoV situation in the U.S. represents a very low risk to the general public in this country at this time. Only two patients in the U.S. have ever tested positive for MERS-CoV infection (both in May 2014), while more than 800 have tested negative. In May 2014, CDC confirmed two unlinked imported cases of MERS-CoV infection in the United States, one in Indiana, the other in Florida. Both cases were among healthcare providers who lived and worked in Saudi Arabia. Both traveled to the U.S. from Saudi Arabia, where they are believed to have been infected. Both were hospitalized in the U.S. and later discharged after fully recovering.

   CDC continues to closely monitor the MERS-CoV situation.

M. Instrument Name

FilmArray 2.0 or FilmArray Torch System

N. System Descriptions:

1. Modes of Operation:

   After samples and hydration reagent have been placed in the reagent pouch, the remaining processing steps are executed under control of the instrument.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes __X__ or No _____

3. Specimen Identification:

   Specimen identification can be entered manually or via barcode.

4. Specimen Sampling and Handling:

The FilmArray RP2*plus* is intended for use with nasopharyngeal swab (NPS) collected in VTM specimens. The operator places a Hydration Injection Vial and a Sample Injection Vial into the FilmArray Pouch Loading Station. The operator first hydrates the test pouch with the Hydration Injection Vial and then adds Sample Buffer into the Sample Injection Vial using the provided Sample Buffer ampoule. Using a transfer pipette provided in the kit, the operator adds ~300 µl of specimen into the Sample Injection Vial, closes the Sample Injection Vial, removes the Sample Injection Vial containing the sample mixture from the Loading Station, inverts the vial at least three times to mix, and then inserts it into the Loading Station port where the proper amount of specimen is pulled into the FilmArray RP2*plus* pouch by vacuum. The FilmArray RP2*plus* pouch is then placed in the FilmArray 2.0 instrument or the available module of a FilmArray Torch system for testing.

5. Calibration:

   Not applicable

6. Quality Control:

   See Quality Control Section above (L.1.c "Traceability, Stability, Expected Values (controls, calibrators, or methods)")

O. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

Not Applicable

P. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809 and the specials controls for this device type.

Q. Identified Risks to Health and Identified Mitigations:

Identified Risks to Health	Identified Mitigations
Incorrect identification or lack of identification of the emerging respiratory pathogen and other common respiratory pathogens by the device can lead to improper patient management and public health response	General Controls and Special Controls

R. Benefit/Risk Analysis:

Summary

Summary of the Benefit(s)	When used for the proposed intended use, the probable benefits to the clinician, the patient and indirectly to public health authorities include: 1) establishment of the device performance in a manner that demonstrates consistent accurate test results; and, 2) ability to use a well validated device to detect and concurrently distinguish emerging respiratory pathogens, such as MERS-CoV, in human clinical specimens to aid in the differential diagnosis of the emerging respiratory pathogen infection, such as MERS-CoV infection, in conjunction with other clinical, epidemiologic, and laboratory data.
Summary of the Risk(s)	The probable risks associated with the device, when used as intended, are those related to the risk of inaccurate test results, which could lead to error in diagnosis and error or delay in treatment, delay in diagnosing the patient's true illness, and delay or failure to implement inappropriate infection/outbreak control measures. These risks are mitigated by the general and special controls.
Summary of Other Factors	The FilmArray RP2*plus* is the first device granted marketing authorization by FDA for the detection of MERS-CoV, and the first device that concurrently distinguishes MERS-CoV from common viral and bacterial respiratory pathogens. MERS-CoV infection is a life-threatening disease. Most people identified as infected with MERS-CoV developed severe acute respiratory illness, and for many people with MERS, more severe complications followed. There is no specific antiviral treatment recommended for MERS-CoV infection. The analytical studies conducted by the sponsor were robust while the clinical studies were limited by the number of specimens available for the device evaluation. The data provided is adequate to demonstrate the device's performance characteristics. No post-market information was available.
Conclusions Do the probable benefits outweigh the probable risks?	The probable benefits of the FilmArray RP2*plus* outweigh the probable risks associated with its use when mitigated by the proposed special controls and general controls. There are no substantial clinical concerns with the classification of this device in Class II, given the combination of general and special controls.

S. Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

T. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.4001.

FDA believes that special controls, along with the applicable general controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: PZF

Device Type: A multiplex respiratory panel to detect and identify emerging respiratory pathogen(s) and common respiratory pathogens in human clinical specimens

Class: II (special controls)

Regulation: 21 CFR 866.4001

(a) *Identification*. A multiplex respiratory panel to detect and identify emerging respiratory pathogen(s) and common respiratory pathogens in human clinical specimens is identified as an *in vitro* diagnostic device intended for the qualitative detection and identification of both emerging and common respiratory pathogens from individuals meeting specific emerging respiratory pathogen clinical and/or epidemiological criteria. For example, clinical signs and symptoms associated with infection of the emerging respiratory pathogen, contact with a probable or confirmed emerging respiratory pathogen case, history of travel to geographic locations where cases of the emerging respiratory pathogen were detected, or other epidemiological links for which testing of the emerging respiratory pathogen may be indicated. A device to detect and identify emerging respiratory pathogen(s) and common respiratory pathogens in human clinical specimens, and in turn to distinguish emerging respiratory pathogen(s) from common respiratory pathogens, is intended to aid in the differential diagnosis of the emerging respiratory pathogen infection, in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the appropriate public health authorities.

(b) *Classification*. Class II (special controls). A multiplex respiratory panel to detect and identify emerging respiratory pathogen(s) and common respiratory pathogens in human clinical specimens must comply with the following special controls:

(1) The intended use for the 21 CFR 809.10 compliant labeling must include a description of what the device detects and measures, the specimen types, the results provided to the user, the clinical indications for which the test is to be used, the specific intended population(s), the testing location(s) where the device is to be used (if applicable), and other conditions of use as appropriate.

(2) The 21 CFR 809.10 compliant labeling must include:

(i) A device description, including device components, ancillary reagents required but not provided, and an explanation of the methodology.

(ii) Performance characteristics from analytical studies, including but not limited to, cut-off (if applicable), analytical sensitivity (i.e., Limit of

Detection), inclusivity, reproducibility, interference, cross reactivity, instrument carry-over/cross-contamination (if applicable), and specimen stability.

(iii) Detailed instructions for minimizing the risk of potential users' exposure to the emerging respiratory pathogen(s) that may be present in test specimens and those used as control materials.

(iv) Detailed instructions for minimizing the risk of generating false positive test results due to carry-over contamination from positive test specimens and/or positive control materials.

(v) A warning statement that the interpretation of test results requires experienced healthcare professionals who have training in principles and use of infectious disease diagnostics and reporting of results, in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.

(vi) A warning statement that culture should not be attempted in cases of positive results for an emerging respiratory pathogen unless a facility with an appropriate level of laboratory biosafety (e.g., BSL 3 and BSL 3+, etc.) is available to receive and culture specimens.

(vii) A warning statement that device positive results for one or more common respiratory pathogens do not rule out bacterial infection, or co-infection with other common respiratory pathogens.

(viii) A warning statement that respiratory pathogen(s) detected may not be the definite cause of disease.

(ix) A warning statement that the use of additional laboratory testing (e.g. bacterial culture, immunofluorescence, x-ray findings, etc.) and clinical presentation must be taken into consideration in order to obtain the final diagnosis of a respiratory infection.

(x) A limiting statement that device negative results for the common respiratory pathogens do not preclude infection of a respiratory pathogen and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

(xi) A limiting statement that analyte targets (e.g., pathogen nucleic acid sequences or other molecular signatures) may persist in vivo, independent of organism viability. Detection of analyte target(s) does not imply that the corresponding pathogen(s) is infectious, nor is the causative agent(s) for clinical symptoms.

(xii) A limiting statement that detection of pathogen nucleic acid sequences or other molecular signatures 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 negative values resulting from improperly collected, transported, or handled specimens.

(xiii) A limiting statement that 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.

(xiv) A limiting statement that there is a risk of false negative results due to the presence of nucleic acid sequence variants in the pathogen targets of the device.

(xv) A limiting statement that Device performance was not established in immunocompromised patients.

(xvi) A limiting statement that positive and negative predictive values are highly dependent on prevalence. The device performance was established during one or more specific respiratory seasons. The performance for some respiratory pathogens may vary depending on the prevalence and patient population tested. False positive test results are likely when prevalence of disease due to a particular respiratory pathogen is low or non-existent in a community.

(xvii) In situations where the performance of the device was estimated based largely on testing pre-selected banked retrospective clinical specimens and/or contrived clinical specimen, a limiting statement that the estimated device performance of that specific pathogen or pathogen subtype may not reflect the performance or prevalence in the intended use population.

(xviii) For devices with an intended use that includes detection of emerging respiratory pathogen(s), a limiting statement that testing with the device should not be performed unless the patient meets clinical and/or epidemiologic criteria for testing suspected specimens of the emerging respiratory pathogen.

(xix) For devices with an intended use that includes detection of emerging respiratory pathogen(s), a limiting statement that positive results obtained with the device for the emerging respiratory pathogen are for the presumptive identification of that pathogen and that the definitive identification of the emerging respiratory pathogen requires additional testing and confirmation procedures in consultation with the

appropriate public health authorities (e.g., local or state public health departments, etc.) for whom reporting is necessary.

(xx) For devices with an intended use that includes detection of emerging respiratory pathogen(s), a limiting statement that negative results for the emerging respiratory pathogen, even in the context of device positive results for one or more of the common respiratory pathogens, do not preclude infection with the emerging respiratory pathogen and should not be used as the sole basis for patient management decisions.

(xxi) For devices with an intended use that includes detection of emerging respiratory pathogen(s), a limiting statement that negative results for the emerging respiratory pathogen may be due to infection of the emerging respiratory pathogen at a specific respiratory tract location that may not be detected by a particular clinical specimen type. A negative result for the emerging respiratory pathogen in an asymptomatic individual does not rule out the possibility of future illness and does not demonstrate that the individual is not infectious.

(xxii) For devices with an intended use that includes detection of emerging respiratory pathogen(s), a limiting statement that a nationally notifiable Rare Disease of Public Health Significance caused by an emerging respiratory pathogen must be reported, as appropriate, to public health authorities in accordance with local, state, and federal law.

(3) The compliant design controls must include:

(i) Performance results of an appropriate clinical study (e.g., a prospective clinical study) for each specimen type, and, if appropriate, results from additional characterized samples. The clinical study must be performed on a study population consistent with the intended use population and must compare the device performance to results obtained using FDA-accepted comparator methods or to expected negative results if the infection is not generally expected in the intended use population. Clinical specimens evaluated in the study must contain relevant organism concentrations applicable to the specimen type(s) and the targeted analyte(s). Detailed documentation must be kept of that study and its results, including the study protocol, study report for the proposed intended use, testing results, and results of all statistical analyses.

(ii) For devices with an intended use that includes detection of emerging respiratory pathogen(s) for which an FDA recommended panel is available, design controls must include the performance results of an analytical study testing an FDA recommended reference panel of characterized samples that contain the emerging respiratory pathogen. Detailed documentation must be kept of that study and its results,

including the study protocol, study report for the proposed intended use, testing results, and results of all statistical analyses.

(iii) An appropriate risk mitigation strategy, including a detailed description of all procedures and methods, for the post-market identification of genetic mutations and/or novel respiratory pathogen isolates or strains (e.g., regular review of published literature and annual in silico analysis of target sequences to detect possible mismatches to the device). The compliant design controls for this device must also include all of the results, including any findings, from the application of this post-market mitigation strategy.

(iv) For devices with an intended use that includes detection of multiple common respiratory pathogens, in addition to detecting emerging respiratory pathogen(s) in human clinical specimens, a detailed description of the identity, phylogenetic relationship, or other recognized characterization of the common respiratory pathogens that the device is designed to detect is addressed. Also, address in detail how the device results might be used in a diagnostic algorithm and other measures that might be needed for a laboratory diagnosis of respiratory tract infection. Perform an evaluation of the device compared to a currently appropriate and FDA accepted comparator method. Detailed documentation must be kept of that study and its results, including the study protocol, study report for the proposed intended use, testing results, and results of all statistical analyses.

(v) A detailed device description, including device components, ancillary reagents required but not provided, and a detailed explanation of the methodology, including molecular target(s) for each analyte, design of target detection reagents, rationale for target selection, limiting factors of the device (e.g., saturation level of hybridization and maximum amplification and detection cycle number, etc.), internal and external controls, and computational path from collected raw data to reported result (e.g., how collected raw signals are converted into a reported signal and result), as applicable and appropriate.

(vi) A detailed description of the device software, including, but not limited to, software applications and hardware-based devices that incorporate software.

(vii) For devices with an intended use that includes detection of Influenza A and Influenza B viruses and/or detection and differentiate between the Influenza A virus subtypes in human clinical specimens, in addition to detecting emerging respiratory pathogen(s), a detailed description of the identity, phylogenetic relationship, or other recognized characterization of the Influenza A and B viruses that the device is designed to detect, a description of how the device results might be

used in a diagnostic algorithm and other measures that might be needed for a laboratory identification of Influenza A or B virus and of specific Influenza A virus subtypes, and a description of the clinical and epidemiological parameters that are relevant to a patient case diagnosis of Influenza A or B and of specific Influenza A virus subtypes. Perform an evaluation of the device compared to a currently appropriate and FDA accepted comparator method. Detailed documentation must be kept of that study and its results, including the study protocol, study report for the proposed intended use, testing results, and results of all statistical analyses.

(4) For devices with an intended use that includes detection of Influenza A and Influenza B viruses and/or detection and differentiate between the Influenza A virus subtypes in human clinical specimens, in addition to detecting emerging respiratory pathogen(s), the 21 CFR 809.10 compliant labeling must include the following:

(i) Where applicable, a limiting statement that performance characteristics for Influenza A were established when Influenza A/H3 and A/H1-2009 (or other pertinent Influenza A subtypes) were the predominant Influenza A viruses in circulation. When other Influenza A viruses are emerging, performance characteristics may vary.

(ii) Where applicable, a warning statement that reads if infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

(iii) Where the device results interpretation involves combining the outputs of several targets to get the final results, such as a device that both detects Influenza A and differentiates all known Influenza A subtypes that are currently circulating, the device's 21 CFR 809.10(b)(9) compliant labeling must include a clear interpretation instruction for all valid and invalid output combinations, and recommendations for any required follow up actions or retesting in the case of an unusual or unexpected device result.

(iv) A limiting statement that if a specimen yields a positive result for Influenza A, but produces negative test results for all specific influenza A subtypes intended to be differentiated (i.e., H1-2009 and H3), this result requires notification of appropriate local, state, or federal public health authorities to determine necessary measures for

verification and to further determine whether the specimen represents a novel strain of Influenza A.

(5) The manufacturer must perform annual analytical reactivity testing of the device with contemporary influenza strains. This annual analytical reactivity testing must meet the following criteria:

(i) The appropriate strains to be tested will be identified by FDA in consultation with the Centers for Disease Control and Prevention (CDC) and sourced from CDC or an FDA designated source. If the annual strains are not available from CDC, FDA will identify an alternative source for obtaining the requisite strains.

(ii) The testing must be conducted according to a standardized protocol considered and determined by FDA to be acceptable and appropriate.

(iii) By July 31 of each calendar year, the results of the last 3 years of annual analytical reactivity testing must be included as part of the device's labeling. If a device has not been on the market long enough for 3 years of annual analytical reactivity testing to have been conducted since the device received marketing authorization from FDA, then the results of every annual analytical reactivity testing since the device received marketing authorization from FDA must be included. The results must be presented as part of the device's labeling in a tabular format, which includes the detailed information for each virus tested as described in the certificate of authentication, either by:

(A) Placing the results directly in the device's 21 CFR 809.10(b) compliant labeling that physically accompanies the device in a separate section of the labeling where the analytical reactivity testing data can be found; or

(B) In the device's label or in other labeling that physically accompanies the device, prominently providing a hyperlink to the manufacturer's public Web site where the analytical reactivity testing data can be found. The manufacturer's home page, as well as the primary part of the manufacturer's Web site that discusses the device, must provide a prominently placed hyperlink to the Web page containing this information and must allow unrestricted viewing access.

(6) If one of the actions listed at section 564(b)(1)(A)–(D) of the Federal Food, Drug, and Cosmetic Act occurs with respect to an influenza viral strain, or if the Secretary of Health and Human Services (HHS) determines, under section 319(a) of the Public Health Service Act, that a disease or disorder presents a

public health emergency, or that a public health emergency otherwise exists, with respect to an influenza viral strain:

(i) Within 30 days from the date that FDA notifies manufacturers that characterized viral samples are available for test evaluation, the manufacturer must have testing performed on the device with those viral samples in accordance with a standardized protocol considered and determined by FDA to be acceptable and appropriate. The procedure and location of testing may depend on the nature of the emerging virus.

(ii) Within 60 days from the date that FDA notifies manufacturers that characterized viral samples are available for test evaluation and continuing until 3 years from that date, the results of the influenza emergency analytical reactivity testing, including the detailed information for the virus tested as described in the certificate of authentication, must be included as part of the device's labeling in a tabular format, either by:

(A) Placing the results directly in the device's 21 CFR 809.10(b) compliant labeling that physically accompanies the device in a separate section of the labeling where analytical reactivity testing data can be found, but separate from the annual analytical reactivity testing results; or

(B) In a section of the device's label or in other labeling that physically accompanies the device, prominently providing a hyperlink to the manufacturer's public Web site where the analytical reactivity testing data can be found. The manufacturer's home page, as well as the primary part of the manufacturer's Web site that discusses the device, must provide a prominently placed hyperlink to the Web page containing this information and must allow unrestricted viewing access.