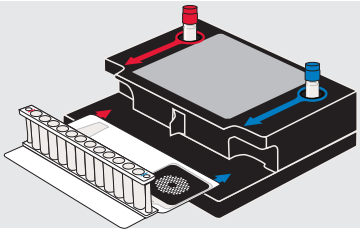
FilmArray® Respiratory Panel Quick Guide

To avoid contamination always wear gloves and work behind a protective shield.

Step 1: Prepare Pouch

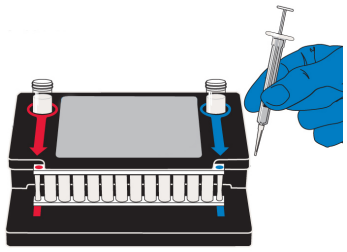
- ❑ Remove pouch from packaging. You should hear a "whooshing" sound.
- ❑ Insert pouch into Pouch Loading Station.
- ❑ Place Sample Buffer vial into red well.
- ❑ Place Hydration Solution vial into blue well.



Note: Do not remove pouch from packaging until ready to test sample.

Step 2: Hydrate Pouch

- ❑ Uncap Hydration Solution vial.
- ❑ Unwrap Pouch Hydration Syringe.
- ❑ Uncap syringe and draw 1 mL of Hydration Solution.
- ❑ Insert syringe tip into hydration port of pouch located directly below blue arrow.
- ❑ Holding the barrel of the syringe, forcefully push down to puncture port seal.
  DO NOT PUSH THE SYRINGE PLUNGER.
- ❑ Wait as Hydration Solution is drawn into pouch.

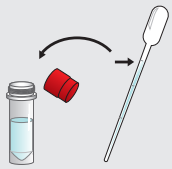


Note: See FilmArray Manual Troubleshooting section if pouch fails to hydrate.

Warning: If bubbles are present in liquid, leave syringe tip in vial and gently tap side of syringe, releasing bubbles to float to surface.

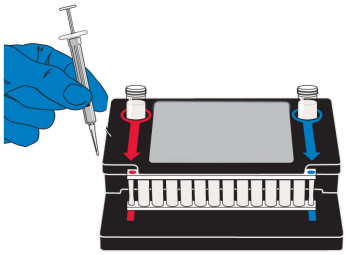
Step 3: Prepare Sample Mix

- ❑ Uncap Sample Buffer vial.
- ❑ Using transfer pipette, draw sample to 3rd line of pipette.
- ❑ Add sample to Sample Buffer vial. Gently mix up and down.



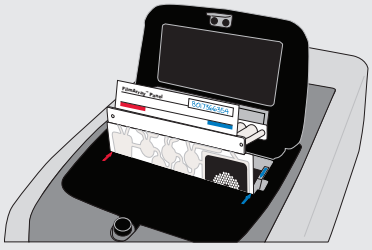
Step 4: Load Sample Mix

- ❑ Unwrap Sample Loading Syringe.
- ❑ Uncap syringe and draw 0.3 mL of Sample Mix.
- ❑ Insert syringe tip into sample port of pouch located directly below red arrow.
- ❑ Holding the barrel of the syringe, forcefully push down to puncture port seal.
  DO NOT PUSH THE SYRINGE PLUNGER.
- ❑ Wait as Sample Mix is drawn into pouch.



Step 5: Load and Run Pouch

- ❑ Remove pouch from Pouch Loading Station.
- ❑ With system software running, open instrument lid.
- ❑ Insert larger film portion of pouch into instrument's pouch loading slot.
- ❑ Snap pouch into place.
- ❑ Scan pouch barcode.
- ❑ Follow instructions on software screen to:
  - Enter Sample ID.
  - Enter your username and password.
  - Click "Start Run."
- ❑ Read results. (See back.)



Note: You will hear an audible "click" when pouch is seated properly.

Warning: If the pouch does not load easily ensure that lid is opened completely.

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FilmArray® Respiratory Panel Quick Guide

FilmArray™ Respiratory Panel

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www.BioFireDx.com

Run Summary Section

1. Detected:
   - Names of any detected pathogens
   - If "None", no pathogens were detected
   - If "⚠Invalid", RETEST SAMPLE
2. Equivocal (↔):
   - If "None", no pathogens were equivocal
   - If a pathogen is listed, RETEST SAMPLE
   - If "⚠Invalid", RETEST SAMPLE
3. Controls:
   - If "Passed", results are valid
   - If "⚠Failed", RETEST SAMPLE
   - If "⚠Invalid", RETEST SAMPLE

The Run Summary Section displays information about the sample and a summary of the control and test results.

Run Summary			
Sample ID:	1-214	**Run Date:**	23 Jan 2013 11:30 AM
1. **Detected:**	Human Rhinovirus/Enterovirus	3. **Controls:**	Passed
2. **Equivocal:**	↔ Influenza A H1-2009		

Results Summary Section

4. "Not Detected", pathogen was not detected
5. "✓Detected", pathogen was detected
6. " ↔ Equivocal", result could not be determined, RETEST SAMPLE

If "⚠Invalid", RETEST SAMPLE

Note: If repeated "Invalid" results are obtained, contact BioFire Diagnostics, the local bioMérieux sales representative, or an authorized distributor.

The Results Summary Section lists the test results for each pathogen targeted by the Respiratory Panel.

Result Summary		
4.	Not Detected	Adenovirus
	Not Detected	Coronavirus 229E
	Not Detected	Coronavirus HKU1
	Not Detected	Coronavirus NL63
	Not Detected	Coronavirus OC43
5.	Not Detected	Human Metapneumovirus
✓	Detected	Human Rhinovirus /Enterovirus
6. ↔	Equivocal	Influenza A H1 2009
	Not Detected	Influenza B
	Not Detected	Parainfluenza Virus 1
	Not Detected	Parainfluenza Virus 2
	Not Detected	Parainfluenza Virus 3
	Not Detected	Parainfluenza Virus 4
	Not Detected	Respiratory Syncytial Virus
	Not Detected	*Bordetella pertussis*
	Not Detected	*Chlamydophila pneumoniae*
	Not Detected	*Mycoplasma pneumoniae*

Run Details Section

7. If "Completed", run is complete

If "Incomplete", "Aborted", "Instrument Communication Error", "Instrument Error", or "Software Error", RETEST SAMPLE

Note: If repeated 'Error' messages are obtained, contact BioFire Diagnostics, the local bioMérieux sales representative, or an authorized distributor.

The Run Details section displays information about the pouch, instrument, run status, and operator.

Run Details			
Pouch	Respiratory Panel	**Protocol:**	NPS v.2.0
7. **Run Status:**	Completed	**Operator:**	Ashley Hunter (ah)
Serial No.:	00026577	**Instrument:**	*ITI FA "FA1115"*
Lot No.:	100302A		

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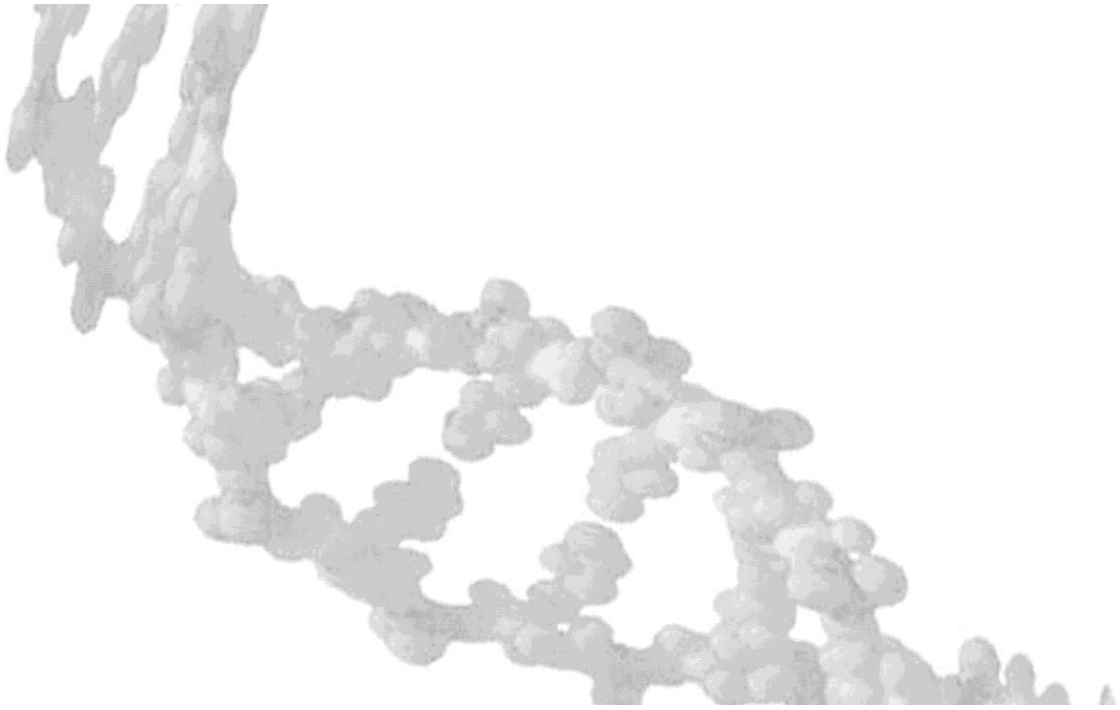
RFIT-PRT-0156-04

REF RFIT-ASY-0105
RFIT-ASY-0107



FilmArray® Respiratory Panel (RP)

Instruction Booklet

For use with the syringe-based loading system

IVD

CE 0088

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Customer and Technical Support for U.S. Customers

Reach Us on the Web
http://www.BioFireDX.com

Reach Us by E-mail
support@BioFireDX.com

Reach Us by Mail

390 Wakara Way
Salt Lake City, UT 84108
USA

Reach Us by Phone
1-800-735-6544 - Toll Free
(801) 736-6354 - Utah

Reach Us by Fax
(801) 588-0507

Customer and Technical Support outside of the U.S.

Contact your local sales representative or distributor for technical support

BioFire Diagnostics, LLC
390 Wakara Way
Salt Lake City, UT 84108
USA

EC REP

Qarad b.v.b.a
Cipalstraat 3
B-2440 Geel, Belgium

 RFIT-PRT-0103-04 February 2015

TABLE OF SYMBOLS

The following symbols can be found on FilmArray Respiratory Panel Kit components or throughout this Instruction Booklet. Use the definitions below as a guideline to interpreting the symbols.

Table of Symbols					
	Manufacturer		Catalog Number		Expiry Date YYYY-MM-DD
	Consult Instructions for Use		Lot Number		Storage Temperature Limitations
	European Union Conformity		Serial Number		Contains Sufficient For <n> Tests
	*In vitro* Diagnostic Medical Device		Keep Away from Sunlight		Do Not Reuse
	Serious eye damage, cat. 1		Acute toxicity, cat. 4 & Skin irritation, cat. 2		Do Not Use if Package is Damaged

TABLE OF CONTENTS

NAME AND INTENDED USE

FilmArray Respiratory Panel

FilmArray Respiratory Panel (RP) is a multiplexed nucleic acid test intended for use with FilmArray systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections. The following organism types and subtypes are identified using the FilmArray RP: Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype H1-2009, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Human Rhinovirus/Enterovirus, Respiratory Syncytial Virus, *Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of a respiratory infection aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test or, lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen. Positive results do not rule out co-infection with other organisms: the agent(s) detected by the FilmArray RP may not be the definite cause of disease. Additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.

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

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

The FilmArray RP assay for Coronavirus OC43 may cross-react with some isolates of Coronavirus HKU1. A dual positive result may be due to cross-reactivity or may indicate a co-infection.

Performance characteristics for Influenza A were established when Influenza A H1-2009, A H1, and A H3 were the predominant Influenza A viruses in circulation. 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.

SUMMARY AND EXPLANATION OF THE TEST

Respiratory pathogens cause acute local and systemic disease of varying severity, with the most severe cases occurring in children, the elderly and immunocompromised individuals. Respiratory symptoms can include coughing, nasal discharge, congestion, fever, wheezing, headache and myalgia. Due to the similarity of diseases caused by many viruses and bacteria, diagnosis based on clinical symptoms alone is difficult. Identification of potential causative agents provides data to aid the physician in determining appropriate patient treatment and public health response for disease containment. The FilmArray RP pouch is designed for simultaneous detection and identification of the following upper respiratory viruses and bacteria: Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Influenza A (with subtyping for hemagglutinin genes H1, H1-2009 and H3), Influenza B, Human Metapneumovirus, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus, Rhinovirus/Enterovirus, *Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*. A summary of characteristics for these organisms is provided in Table 1.

Table 1. Respiratory Pathogens Detected in the FilmArray Respiratory Panel

Organism (abbreviation)	Classification (Genome type)	Season of Highest Incidence[a]	Most Commonly Infected Demographic
Adenovirus (AdV)	Adenovirus (DNA)	Late winter to early summer[1]	All ages, immunocompromised[1]
Coronavirus (CoV) 229E, HKU1, NL63, OC43	Coronavirus (RNA)	Winter, spring[2-3]	Children, adults[2-3]
Enterovirus (EV)	Picornavirus(RNA)	Summer, early fall [4]	All ages[5]
Human Rhinovirus (HRV)	Picornavirus (RNA)	Fall, spring[6]	All ages[6]
Human Metapneumovirus (hMPV)	Paramyxovirus (RNA)	Winter, early spring[7]	Children[7]
Influenza A (Flu A) (subtypes H1, H1-2009, and H3)	Orthomyxovirus (RNA)	Winter [8]	All ages [8], 5-20 % of US population[9]
Influenza B (Flu B)	Orthomyxovirus (RNA)	Winter [8]	All ages [8], 5-20 % of US population[9]
Parainfluenza Virus 1 (PIV1)	Paramyxovirus (RNA)	Fall, periodicity of 1-2 years[10]	Infants, young children, immunocompromised[10]
Parainfluenza Virus 2 (PIV2)	Paramyxovirus (RNA)	Fall, periodicity of 1-2 years[10]	Infants, young children, immunocompromised[10]
Parainfluenza Virus 3 (PIV3)	Paramyxovirus (RNA)	Spring, summer[10]	Infants, young children, immunocompromised [10]
Parainfluenza Virus 4 (PIV4)	Paramyxovirus (RNA)	Unknown	All ages[11]
Respiratory Syncytial Virus (RSV)	Paramyxovirus (RNA)	Winter, varies by location[12-13]	Children, older adults[12-13]
Bordetella pertussis	Bacterium (DNA)	No peak season	All ages[14]
Chlamydophila pneumoniae	Bacterium (DNA)	No peak season	Older children, young adults, immunocompromised[15]
Mycoplasma pneumoniae	Bacterium (DNA)	Outbreaks most common in summer, outbreak periodicity 4 – 7 years	Older children, young adults[16-17]

[a] based on North American seasons

Summary of Detected Organisms

Adenoviruses are a diverse group of non-enveloped DNA viruses with seven species (A to G) categorized by hemagglutination and approximately 55 serotypes. All serotypes have been associated with human disease. Adenovirus species B, C, and E cause acute respiratory disease. Outbreaks occur in institutional settings such as military training, long-term care facilities, and pediatric tertiary-care hospitals, due to high rates of transmission in closed populations [18-20]. Adenoviruses (species A, D, F and G) can cause a variety of illnesses, including cystitis, gastroenteritis, and conjunctivitis [1]. Adenoviruses are shed for long periods of time and persist on surfaces in an infective state [20].

Coronaviruses 229E, HKU1, NL63, and OC43. Human Coronaviruses were established as respiratory pathogens in the 1960's. Initially, two serologic variants were characterized (229E and OC43) and recently, two additional human coronaviruses (HKU1 and NL63) have been identified [2, 21-23]. These viruses are most commonly associated with upper respiratory tract infections; however, they have also been detected in individuals with lower respiratory tract infections [2-3, 24]. Coronaviruses have been associated with croup and exacerbation of asthma [2, 22]. Coronavirus infection occurs more often in the winter and there appears to be a periodicity of epidemics for strains 229E and OC43 of every two to three years [3].

Human Metapneumovirus was discovered in 2001 as a respiratory pathogen in children [25]. Further studies confirmed hMPV infections in persons of all ages [26]. Human Metapneumoviruses are in the family *Paramyxoviridae* [7]. Infection in infants and young children is commonly associated with bronchiolitis [7]. The two genotypes, A and B, can circulate at the same time and do not appear to differ in the severity of illness [7].

Influenza A and B are RNA viruses in the *Orthomyxoviridae* family. During annual Influenza epidemics, 5-20% of the population is affected with upper respiratory tract infections with rapid onset of fever [8]. The dominant type of Influenza virus varies often due to antigenic drift and shift [27]. During the 2009-10 Influenza season, Influenza A H1-2009 was the dominant circulating Influenza virus, accounting for approximately 99% of reported Influenza infections [28] (Table 2). Influenza A can be subtyped by the hemagglutinin (H) and neuraminidase (N) genes; subtypes H1N1 and H3N2 are the strains that most commonly infect humans. More severe disease and increased mortality are associated with H3N2 subtype [27]. Currently, at least four antiviral medications are available for Influenza treatment – amantadine, rimantadine, zanamivir and oseltamivir – with type-specific efficacy and drug resistance arising with the spread of new strains of the virus [27, 29]. Complications with viral or bacterial pneumonia increase mortality from Influenza infections [30].

Table 2. Proportions of Influenza Subtype Infections in the United States (as reported by the US Centers for Disease Control)

Flu Season	Influenza A	% of Influenza A			Influenza B
		H1	H1-2009	H3	
2009-2010[1,2]	99.6%	0.1	99.8	0.1	0.4%
2008-2009	89.4%	13.2	79.9	6.9	10.6%
2007-2008	71%	26.2	0.0	73.8	29%
2006-2007	79.2%	62.3	0.0	37.7	20.8%
2005-2006	79.7%	8.1	0.0	91.9	20.3%

[1] Season during which prospective clinical data described in this package insert were accumulated
[2] Cumulative results from August 30, 2009 to May 22, 2010

Parainfluenza Viruses. Parainfluenza viruses are RNA viruses in the *Paramyxoviridae* family. In the 1950's, Parainfluenza viruses were determined to be respiratory pathogens different from influenza viruses [10]. Parainfluenza viruses are divided into four types antigenically and genetically, with type 4 further subtyped as A and B

[10]. Parainfluenza Virus 1 causes biennial epidemics in the fall, with 50% of croup cases attributed to this virus [10]. Parainfluenza Virus 2 has a periodicity of epidemics of one to two years that may alternate with Parainfluenza 1 outbreaks [10]. Children less than six months old are particularly susceptible to Parainfluenza Virus 3 infection, with outbreaks occurring in neonatal intensive care units and epidemics are most common in the spring and summer [10]. Parainfluenza Virus 4 infection affects all age groups and a periodicity of infection has not been established [11].

Respiratory Syncytial Virus is a member of the RNA viruses in the *Paramyxoviridae* family, related to human metapneumoviruses and parainfluenza viruses [12]. RSV is the most common cause of severe respiratory disease in infants, with acute bronchiolitis as the major cause of hospitalization [12]. An association of asthma with RSV infection is being investigated [12]. Treatment or prophylaxis with a humanized monoclonal antibody against the fusion protein of RSV has shown a reduction in disease for high risk infants [12].

Rhinoviruses and **Enteroviruses** are related RNA viruses in the *Picornavirus* family [6]. There are more than 100 serotypes of Human Rhinovirus based on the serology of the capsid protein [6]. Rhinovirus is noted as causing the "common cold", but may also be involved in precipitating asthma attacks and severe complications [6]. Enteroviruses are divided into four species that include a total of 89 serotypes. Individual serotypes can be associated with different clinical manifestations, including nonspecific respiratory illnesses in infants or adults [5].

Bordetella pertussis is the causative agent of whooping cough or pertussis, a vaccine-preventable disease that is reportable to public health organizations [31-32]. *B. pertussis* is a gram-negative bacterium with a high infectivity rate that is treatable with several antibiotics [14]. Vaccine-induced immunity has been shown to decrease after 5-10 years. Pertussis occurs most commonly in children but also occurs in adolescents and adults and outbreaks have been documented in fully vaccinated populations due to waning immunity [14, 33]. The highest mortality from pertussis occurs with infants and elderly [14]. Early (catarrhal) pertussis disease is non-specific, and classic signs of pertussis (paroxysmal coughing, inspiratory 'whoop', post-tussive emesis, as well as apnea or cyanosis in infants) do not arise until approximately 2 weeks after the initial onset of symptoms. Symptoms of *B. pertussis* infection are known to vary due to a number of factors including: age, previous immunization or infection, passively acquired antibody, and antibiotic treatment [14]. No peak season has been defined for *B. pertussis* infection.

Chlamydophila pneumoniae is an obligate intracellular bacterium that causes acute respiratory infections and is a common cause of community-acquired pneumonia [34-35]. Outbreaks occur in schools, military barracks, and nursing homes [36]. No peak season has been identified for *C. pneumoniae* infections.

Mycoplasma pneumoniae is a causative agent of community-acquired atypical pneumonia, frequently in outbreak situations [17, 37]. Incubation time for *M. pneumoniae* infection is approximately 1 to 4 weeks [16]. *M. pneumoniae* respiratory disease does not have a defined season of highest incidence but epidemics have a periodicity of 3-7 years [37].

Principle of the Procedure

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

The following is an overview of the testing procedure:

1. Remove the FilmArray pouch from its vacuum-sealed package. Since solutions are drawn into the FilmArray RP pouch by vacuum, it is important to keep pouches in their protective packaging until the time of use.

2. Place the FilmArray RP pouch into the FilmArray Pouch Loading Station. The FilmArray Pouch Loading Station has been designed to prevent error by providing instructions and visual cues in the form of color-coded arrows to ensure that the pouch is properly loaded.

3. Load Hydration Solution into the FilmArray RP pouch using the Pouch Hydration Syringe provided in the FilmArray RP Kit. The syringe is fitted with a blunt stainless steel cannula, which is used to deliver the solution into the pouch. Loading the pouch with Hydration Solution rehydrates the freeze-dried reagents contained in the pouch fitment.

4. Mix NPS specimen with Sample Buffer using a provided Transfer Pipette. The Sample Buffer contains reagents that inactivate RNases in the sample and promote binding of nucleic acids to magnetic beads for isolation.

5. Load the sample/buffer mixture into the FilmArray RP pouch using the Sample Loading Syringe provided. When the sample mixture is loaded, a process control contained in the fitment of the pouch is introduced into the sample. The process control monitors all of the critical processes that occur in the pouch.

6. Transfer the pouch to the instrument and initiate a run. To aid in proper insertion of the pouch in the instrument, the FilmArray software provides on-screen animations illustrating the steps needed to start the run.

7. View results on the test report at the completion of the run.

The following is an overview of the operations and processes that occur during a FilmArray run:

1. **Nucleic Acid Purification** - Nucleic acid purification occurs in the first three blisters of the pouch. The sample is lysed by agitation (bead beating) and the liberated nucleic acid is captured, washed and eluted using magnetic bead technology. These steps require about ten minutes and the bead-beater apparatus can be heard as a high-pitched whine during the first minute of operation.

2. **Reverse Transcription and 1st Stage Multiplex PCR** - Since many pathogens identified by the FilmArray RP pouch are RNA viruses, a reverse transcription (RT) step is performed to convert the viral RNA into cDNA prior to amplification. The purified nucleic acid solution is combined with a preheated master mix to initiate the RT step and subsequent thermocycling for multiplex PCR. The effect of 1st stage PCR is to enrich for the target nucleic acids present in the sample.

3. **2nd Stage PCR** - The products of 1st stage PCR are diluted and mixed with fresh PCR reagents containing an intercalating fluorescent DNA dye (LCGreen® Plus, BioFire Diagnostics, LLC). This solution is distributed over the 2nd stage PCR array. The individual wells of the array contain primers for different assays (each present in triplicate) that target specific nucleic acid sequences from each of the pathogens detected, as well as control template material. These primers are 'nested' or internal to the specific products of the 1st stage multiplex reaction, which enhances both the sensitivity and specificity of the reactions.

4. **DNA Melting Analysis** – After 2nd stage PCR, the temperature is slowly increased and fluorescence in each well of the array is monitored and analyzed to generate a melting curve. The temperature at which a specific PCR product melts (melting temperature or Tm) is consistent and predictable and the FilmArray software automatically evaluates the data from replicate wells for each assay to report results. For a description of data interpretation and reporting see the Interpretation of Results section of this booklet.

The FilmArray software controls the operation of the instrument, collects and analyzes data and automatically generates a test report at the end of the run. The entire process takes about an hour. Additional detail can be found in the FilmArray Operator's Manual.

MATERIALS PROVIDED

Each kit contains sufficient reagents to test 30 or 6 specimens:

- Individually packaged FilmArray RP pouches
- Single-use (0.5 mL) Sample Buffer vials (red lid)
- Single-use (1.5 mL) Hydration Solution Vials (blue lid)
- Individually packaged Transfer Pipettes
- Individually packaged Sample Loading Syringes with attached cannula (red cap)
- Individually packaged Pouch Hydration Syringes with attached cannula (blue cap)

MATERIALS REQUIRED BUT NOT PROVIDED

FilmArray System including:

- FilmArray or FilmArray 2.0 instrument and software
- FilmArray Pouch Loading Station

WARNINGS AND PRECAUTIONS

General Precautions

1. For *in vitro* diagnostic use only.
2. This device is restricted to sale by or on the order of a physician, or to a clinical laboratory; its use is restricted to, by, or on the order of a physician.
3. A trained healthcare professional should carefully interpret the results from the FilmArray RP in conjunction with a patient's signs and symptoms and results from other diagnostic tests.
4. FilmArray RP pouches are only for use with FilmArray systems.
5. Performance characteristics of the FilmArray RP have only been determined with nasopharyngeal swab (NPS) specimens.
6. Always check the expiration date on the pouch and do not use a pouch after its expiration date.
7. Pertussis is a nationally notifiable infectious condition in the U.S. If *Bordetella pertussis* is detected, notify the state and/or local health departments.

Safety Precautions

1. Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable powder-free gloves and lab coats. Protect skin, eyes and mucus membranes. Change gloves often when handling reagents or specimens.
2. Handle all specimens and waste materials as if they were capable of transmitting infectious agents. Observe safety guidelines such as those outlined in CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*

[38], the CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections* [39], or other appropriate guidelines.

3. Follow your institution's safety procedures for handling biological samples.
4. 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 department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
5. Dispose of materials used in this assay, including reagents, specimens, and used buffer vials, according to federal, state, and local regulations.
6. Sample Buffer is assigned the following classifications: Acute toxicity (Category 4), Serious Eye damage (Category 1), and Skin irritation (Category 2). Accordingly, the Sample Buffer is harmful if swallowed, causes serious eye damage, and causes skin irritation. The following precautions should be observed:

   Wear protective gloves/eye protection/face protection.

   IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Seek medical attention.

   IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.

   Please refer to the FilmArray Respiratory Panel Safety Data Sheet (SDS) for more information.

7. Sample Buffer will form hazardous compounds and fumes when mixed with bleach or other disinfectants.

   WARNING: Bleach should never be added to Sample Buffer or sample waste.

8. Bleach, a recommended disinfectant, is corrosive and may cause severe irritation or damage to eyes and skin. Vapor or mist may irritate the respiratory tract. Bleach is harmful if swallowed or inhaled. The following first aid measures are recommended.

   Eye contact: Hold eye open and rinse with water for 15-20 minutes. Remove contact lenses after the first 5 minutes and continue rinsing eye. Seek medical attention.

   Skin contact: Immediately flush skin with plenty of water for at least 15 minutes. If irritation develops, seek medical attention.

   Ingestion: Do not induce vomiting. Drink a glassful of water. If irritation develops, seek medical attention.

   Please refer to the appropriate Safety Data Sheet (SDS) for more information.

Laboratory Precautions

1. **Preventing organism contamination**

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

   - Laboratory workers can be infected with common respiratory pathogens and can inadvertently contaminate the sample while it is being processed. To avoid this, specimens should be processed and pouches should be loaded in a biosafety cabinet. If a biosafety cabinet is not used, a dead air box (e.g., AirClean PCR workstation), a splash shield (e.g., Bel-Art Scienceware Splash Shields), or a face shield should be used when preparing specimens.

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

2. **Preventing amplicon contamination**

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

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

WARNING: If liquid is observed on the exterior of a pouch, the liquid and pouch should be immediately contained and discarded in a biohazard container. The instrument and work space must be decontaminated as described in the FilmArray Operator's Manual.

DO NOT PERFORM ADDITIONAL TESTING UNTIL THE AREA HAS BEEN DECONTAMINATED.

Precaution Related to Public Health Reporting in the United States

Local, state, and federal regulations for notification of reportable disease are continually updated and include a number of organisms for surveillance and outbreak investigations [40-41]. Additionally, the Centers for Disease Control (CDC) recommends that when pathogens from reportable diseases are detected by a culture independent diagnostic test (CIDT), the laboratory should facilitate obtaining the isolate or clinical materials for submission to the appropriate public health laboratory to aid in outbreak detection and epidemiological investigations. Laboratories are responsible for following their state and/or local regulations and should consult their local and/or state public health laboratories for isolate and/or clinical sample submission guidelines.

REAGENT STORAGE, HANDLING AND STABILITY

1. Store the test kit, including reagent pouches and buffers, at room temperature (15–25 ºC). **DO NOT REFRIGERATE.**
2. Avoid storage of any materials near heating or cooling vents or in direct sunlight.
3. Always check the expiration date and do not use reagents beyond the expiration date printed on the pouch or kit.
4. All kit components should be stored and used together. Do not use components from one kit with those of another kit.
5. Do not remove pouches from their packaging until a sample is ready to be tested. Once the pouch packaging has been opened, the pouch should be loaded as soon as possible (within approximately 30 minutes).
6. Once a pouch has been loaded, the test run should be started as soon as possible (within 60 minutes).

SPECIMEN COLLECTION AND PREPARATION

This section describes the requirements for specimen collection, preparation, and handling that will help ensure accurate test results.

Nasopharyngeal Swab Collection - NPS specimens should be collected according to standard technique and immediately placed in viral transport media (VTM).

Minimum Sample Volume - 300 µL of sample is required for testing.

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

PROCEDURE

Refer to the FilmArray Respiratory Panel Quick Guide, the FilmArray Training Video or the FilmArray Operator's Manual for more detail and pictorial representations of these instructions.

Gloves and other Personal Protective Equipment (PPE) should be used when handling pouches and specimens. Only one FilmArray RP pouch should be loaded at a time. Once the pouch is loaded, it should be promptly transferred to the instrument to start the run. After the run is complete, the pouch should be discarded in a biohazard container.

Pouch Preparation

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

NOTE: If the vacuum seal of the pouch packaging is not intact, the pouch may still be used. Attempt to hydrate the pouch using the steps below. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test

3. Place the FilmArray Pouch into the FilmArray Pouch Loading Station. To do so, hold the pouch so that the barcoded label is upright and readable, and then slide the flexible film portion of the pouch into the slot at the base of the loading station. In the correct configuration, the inlet ports on both ends of the rigid plastic part of the pouch will point up, and the red and blue labels on the pouch will align with the red and blue arrows on the FilmArray Pouch Loading Station.
4. Place a blue-capped Hydration Solution vial in the blue well of the FilmArray Pouch Loading Station.
5. Place a red-capped Sample Buffer vial in the red well of the FilmArray Pouch Loading Station.

Pouch Hydration

1. Remove the blue-labeled Pouch Hydration Syringe from the packaging. If the cannula/tip is not firmly attached to the syringe, hold the capped tip and rotate the syringe to tighten.
2. Using the Pouch Hydration Syringe (blue cap), draw Hydration Solution to the 1 mL mark on the syringe, taking care to avoid the formation of bubbles. If you notice bubbles at the base of the syringe, leave the tip of the cannula in the Hydration Solution vial and dislodge the bubbles by gently tapping the side of the syringe with your finger. The bubbles will float up to the plunger.

   NOTE: DO NOT remove air bubbles by inverting the syringe and expelling liquid.

3. Insert the cannula tip into the port in the pouch fitment located directly below the blue arrow of the FilmArray Pouch Loading Station. While holding the body of the syringe, push down forcefully in a firm and quick motion until you hear a faint “pop” and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum; there is no need to use the plunger.

   NOTE: DO NOT push the syringe plunger. Injecting liquid will cause the pouch to overfill.

4. Verify that the pouch has been hydrated.

   Most of the liquid will have been drawn out of the syringe. Also, check to see that fluid has entered and hydrated reagents in the reagent wells (eleven wells located at the base of the rigid plastic part of the pouch). Flip the barcode label down to see the reagent wells. Small air bubbles may be seen. If the pouch fails to hydrate (dry reagents appear as white pellets), repeat Step 3 to verify that the seal of the port was broken or retrieve a new pouch and repeat from Step 2 of the Pouch Preparation section.

Sample Loading

1. Remove the cap from the Sample Buffer vial.
2. Using the Transfer Pipette provided in the test kit, draw sample (NPS in VTM) to the third line (approximately 0.3 mL). Add sample to the red-capped Sample Buffer vial and gently pipette up and down to mix. Discard the Transfer Pipette in a biohazard waste container.
3. Remove the red-labeled Sample Loading Syringe from the packaging. If the cannula/tip is not firmly attached to the syringe, hold the capped tip and rotate the syringe to tighten.
4. Using the Sample Loading Syringe, draw approximately 0.3 mL of sample/sample buffer mix (to the 0.3 mL/cc mark on the syringe), taking care to avoid the formation of bubbles. If you notice bubbles at the base of the syringe, leave the tip of the cannula in the Sample Buffer vial and dislodge the bubbles by gently tapping the side of the syringe with your finger. The bubbles will float up to the plunger.

NOTE: To avoid contaminating the work area, DO NOT remove air bubbles by inverting the syringe and expressing liquid.

5. Insert the cannula tip into the port in the pouch fitment located directly below the red arrow of the FilmArray Pouch Loading Station. While holding the body of the syringe, push down forcefully in a firm and quick motion until you hear a faint “pop” and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum; there is no need to use the plunger.

NOTE: DO NOT push the syringe plunger. Injecting liquid will cause the pouch to overfill.

6. Verify that the sample has been loaded.

   Most of the liquid will have been drawn out of the syringe. Flip the barcode label down and check to see that fluid has entered the reagent well next to the sample loading port. If the pouch fails to pull sample from the Sample Loading Syringe, the pouch should be discarded. Retrieve a new pouch and repeat from Step 2 of the Pouch Preparation section.

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

7. Dispose of syringes in an appropriate biohazard sharps container.
8. Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the FilmArray Pouch Loading Station.

Using the FilmArray Instrument to Perform the Test

The FilmArray software includes a step-by-step on-screen tutor that shows each step of the test.

1. Ensure that the computer and FilmArray instrument(s) are on and the FilmArray software is launched.
2. Open the lid of an available instrument (if not already open).

NOTE: An available instrument is indicated by a constant green light on the front of the instrument.

3. Insert the FilmArray pouch into the instrument.

   Position the pouch so that the array is on the right with the film directed downward into the instrument. The red and blue labels on the FilmArray pouch should align with the red and blue arrows on the instrument. The pouch will click into place when inserted correctly.

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

4. Scan the barcode on the FilmArray pouch using the barcode scanner.

   Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol are preprogrammed in the rectangular barcode located on the FilmArray pouch. The information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type and Protocol can be manually entered from the information provided on the pouch label into the

appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

NOTE: The barcode cannot be scanned prior to placing the pouch in the instrument.

5. Enter the Sample ID.

   The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.

6. If necessary, select a protocol from the Protocol drop down list.
7. Enter a user name and password in the Name and Password fields.
8. Close the FilmArray instrument lid.
9. Click the Start Run button on the screen.

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

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

10. When the run is finished, follow the on-screen instructions to open the instrument and remove the pouch.
11. Immediately discard the pouch in a biohazard container.
12. Results are automatically displayed in the report section of the screen. The run file is automatically saved in the FilmArray database and the report can be printed and/or saved as a PDF file.

QUALITY CONTROL

Process Controls

Two process controls are included in each pouch:

1. **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, 1st stage PCR, dilution, 2nd stage PCR and DNA melting. A positive control result indicates that all steps carried out in the FilmArray RP pouch were successful.
2. **PCR2 Control**
   The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that 2nd stage PCR was successful.

Both control assays must be positive for the test run to pass. When either control fails, the Controls field of the test report (upper right hand corner) will display Failed and all results will be listed as Invalid. If the controls fail, the sample should be retested using a new pouch.

Monitoring Test System Performance

It is possible to trend Tm values for the RNA Process Control and/or PCR2 Control assays and maintain records according to standard laboratory quality control practices [40-41]. The FilmArray software will automatically fail the run if the Tm for either the RNA Process Control or the PCR2 Control is outside of the acceptable range (80.6-84.6 for the RNA Process Control and 74.5-78.5 for the PCR2 Control). Any trend variations should be investigated. Contact Technical Support for help with out-of-range controls.

NOTE: Trending for the two control assays (RNA Process Control and PCR2 Control) should be tracked on separate control charts as the expected Tm values for these two controls are different.

Good laboratory practice recommends running external positive and negative controls regularly. Use viral transport medium as the external negative control, and previously characterized positive samples or negative samples spiked with well characterized organisms as external positive controls. External controls should be used in accordance with the appropriate accrediting organizations, as applicable.

INTERPRETATION OF RESULTS

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

Assay Interpretation

When 2nd stage PCR is complete, the FilmArray instrument performs a high resolution DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well (for more information see FilmArray Operator's Manual). The FilmArray software then performs several analyses and assigns a final assay result.

Analysis of melting curves. The FilmArray software evaluates the DNA melting curve for each well of the 2nd stage PCR 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. The Tm value is then compared against the expected Tm range for the assay. If the software determines that the melt is positive and the melt peak falls inside the assay-specific Tm range, the curve is called positive. If the software determines that the melt is negative or is not in the appropriate Tm range, the curve is called negative.

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

Organism Interpretation. For most organisms detected by the FilmArray RP, the organism is considered to be Detected if a single corresponding assay is positive. For example, Human Metapneumovirus will have a test report result of Human Metapneumovirus Detected if at least two of the three replicates of the one Human Metapneumovirus assay have similar positive melt peaks with Tm values that are within the assay-specific Tm range. The test results for Adenovirus, the Human Rhinovirus/Enterovirus group, and Influenza A depend on the interpretation of results from several assays. Interpretation and follow-up testing for these three results are provided below.

Rhinovirus/Enterovirus Group

The FilmArray RP pouch contains six different assays (HRV1, HRV2, HRV3, HRV4, Entero 1, Entero 2) for the detection of Rhinoviruses and Enteroviruses. Though these viruses are both very diverse, they are also closely related. Therefore, the six assays are not able to reliably differentiate Rhinovirus and Enterovirus. The FilmArray software interprets each of the six assays independently (as described above) and the results are

combined as a final test result for the virus(es). If any of the six assays are positive, the test report result will be Human Rhinovirus/Enterovirus Detected. If all six assays are negative, the test report result will be Human Rhinovirus/Enterovirus Not Detected. A positive FilmArray RP Human Rhinovirus/Enterovirus result should be followed-up using an alternate method (e.g., viral culture or sequence analysis).

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

Adenovirus

The FilmArray RP pouch contains two different assays (Adeno, Adeno2) for the detection of Adenovirus. The FilmArray software interprets each of these assays independently (as described above) and the results are combined as a final test result for the virus. If either or both assays are positive, the test report result will be Adenovirus Detected. If both the Adeno and Adeno2 assays are negative, the test report result will be Adenovirus Not Detected.

Influenza A

The assays in the FilmArray RP are designed to both detect Influenza A and to differentiate commonly occurring hemagglutinin subtypes. To accomplish this, the FilmArray RP uses two Influenza A assays, (FluA-pan-1 and FluA-pan-2) and three subtyping assays directed at the hemagglutinin gene (FluA-H1-pan, FluA-H1-2009 and FluA-H3). The FluA-H1-pan assay is designed to detect both Influenza A H1 and the Influenza A H1-2009 variant. Each of the individual assays is interpreted independently (as described above) and the test result reported for Influenza A is based on the combined results of the five assays as outlined in Table 3.

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

Table 3. Possible Assay Results for Influenza A and the Corresponding Interpretation

Assay / Final Result	FluA-pan Assays (n=2)	FluA-H1-pan	FluA-H1-2009	FluA-H3	Required Follow-up
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 and Influenza A H3	**≥1 positive**	**Positive**	Negative	**Positive**	Multiple infections are possible but rare [a], retest to confirm result [b]
Influenza A H1-2009 and Influenza A H3	**≥1 positive**	Any result	**Positive**	**Positive**	
Influenza A (no subtype detected)	**2 positive**	Negative	Negative	Negative	See below
Influenza A Equivocal	**1 positive**	Negative	Negative	Negative	Retest
Influenza A H1 Equivocal	Negative	**Positive**	Negative	Negative	
Influenza A H3 Equivocal	Negative	Negative	Negative	**Positive**	

Assay / Final Result	FluA-pan Assays (n=2)	FluA-H1-pan	FluA-H1-2009	FluA-H3	Required Follow-up
Influenza A H1-2009 Equivocal	Negative	Any result	**Positive**	Negative	

[a] The FilmArray RP can simultaneously detect multiple Influenza viruses contained in the FluMist® nasal Influenza vaccine (see "Interference" section below).

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

Influenza A (no subtype detected)

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

FilmArray RP Test Report

The FilmArray RP test report is automatically displayed upon completion of a run and contains three sections, the Run Summary, the Results Summary, and the Run Details (see the FilmArray Respiratory Panel Quick Guide to view an example of a test report). The test report can be saved as a file or printed.

The **Run Summary** section of the test report provides the Sample ID, time and date of the run, control results and an overall summary of the test results. Any target with a Detected or Equivocal result will be listed in the corresponding field of the summary. If all of the tests were negative then None will be displayed in the Detected field. Controls are listed as Passed, Failed or Invalid. See Control Field section below for detailed information about the interpretation of controls and appropriate follow-up in the case of control failures.

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

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

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

Control Field

The Control field on the test report will display Passed, Failed, or Invalid. The Control field will display Passed only if the run completed successfully (no instrument or software errors) and both of the pouch control assays (RNA Process Control and PCR2 Control) were successful. The Control field will display Failed if the run was completed successfully (no instrument or software errors) but one or both of the pouch control assays failed (0 or 1 positive replicates for either of the controls, each of which is tested in triplicate). If the control result is Failed, then the result for all of the tests on the panel are displayed as Invalid and the specimen will need to be retested with a new pouch.

Table 4 provides a summary and explanation of the possible control results and follow-up actions.

The FilmArray instrument monitors each run to ensure that the instrument is working within specification and to detect hardware or software errors that might compromise the accuracy of the test result. If the instrument detects an out-of-specification condition, or a significant error, it will automatically abort the run. If this happens, or if a run is aborted by the user, then the Control field on the report will display Invalid and all test results in the Result Summary of the report will also be displayed as Invalid. To determine why a run failed to complete, note any specific error codes that are displayed on the screen and refer to the Run Status in the Run Details section of the report. The Run Status will display Incomplete, Aborted, Software Error, Instrument Error, or Instrument Communication Error. Refer to the FilmArray Operator's Manual or call Technical Support for further instruction. The specimen should be retested after the error is corrected or by using an alternate FilmArray instrument.

Table 4. Interpretation of Control Field on the FilmArray RP Test Report

Control Result	Explanation	Action Required	Outcome
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 failed.	Repeat the test using a new pouch.	Accept the results of the repeat testing. If the error persists, contact Technical Support for further instruction.

Control Result	Explanation	Action Required	Outcome
Invalid	The controls are invalid because the run failed. (typically 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 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. If the error occurred in the first 30 seconds of the run, the same pouch may be used for the repeat test (within 60 minutes of pouch loading) using the same instrument or another instrument, as available. If the error occurred later in the run or you are unsure when the error occurred, return to the original sample to load a new pouch. Repeat the test with the new pouch on the same instrument or another instrument, as available.	Accept the valid results of the repeat testing. If the error persists, contact Technical Support for further instruction.

Result Summary

Test results for the organisms included in the FilmArray RP are provided in two locations on the report. The Result Summary section provides a complete list of the test results. Possible results include Detected, Not Detected, Equivocal, and Invalid. Positive (Detected) and Equivocal results are also displayed in the Run Summary section. Table 5 provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

Table 5. Interpretation of Results on the FilmArray RP Test Report

Organism Result	Explanation	Action
Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism were POSITIVE (i.e., met the requirements for a positive result described in the Assay Interpretation section above)	Report results.
Not Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism were NEGATIVE (i.e., did not meet the requirements for a positive result described in the Assay Interpretation section above)	Report results.
Equivocal (Influenza A 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 was inconclusive (see Table 3)	Retest the original specimen using a new pouch and report the results of the retest.
Invalid	The pouch controls were not successful (Failed) OR The run was not successful (Run Status displayed as: Aborted, Incomplete, Instrument Error, Software Error, or Instrument Communication Error)	See Table 4 , Interpretation of Control Field on the FilmArray Test Report for instruction.

LIMITATIONS OF THE PROCEDURE

- For prescription use only
- FilmArray Respiratory Panel performance has only been established on the FilmArray and FilmArray 2.0 systems.
- This test is a qualitative test and does not provide a quantitative value for the virus(es) and/or bacteria detected in the specimen.
- The performance of the test has been evaluated for use with human specimen material only.
- This test has not been validated for testing specimens other than nasopharyngeal swab (NPS) specimens.
- The performance of this test has not been established for immunocompromised individuals.
- The performance of this test has not been established for patients without signs and symptoms of respiratory infection.
- Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- Viral and bacterial nucleic acids may persist *in vivo* independent of organism viability. Detection of organism target(s) does not imply that the corresponding organisms are infectious or are the causative agents for clinical symptoms.
- The detection of viral and bacterial nucleic acid is dependent upon proper specimen collection, handling, transportation, storage and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive or false negative values resulting from improperly collected, transported or handled specimens.
- A negative FilmArray RP result does not exclude the possibility of viral or bacterial infection. Negative test results may occur from the presence of sequence variants in the region targeted by the assay, the presence of inhibitors, technical error, sample mix-up or an infection caused by an organism not detected by the panel. Test results may also be affected by concurrent antiviral/antibacterial therapy or levels of organism in the specimen that are below the limit of detection for the test. Negative results should not be used as the sole basis for diagnosis, treatment, or other management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test or lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods when prevalence is moderate to low.
- Organism and amplicon contamination may produce erroneous results for this test. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.
- There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.
- Cross-reactivity with respiratory tract organisms other than those listed in the Analytical Specificity section below may lead to erroneous results.
- The FilmArray RP Adenovirus assay may show variable detection with non-respiratory serotypes within species A, D, F and G.
- The FilmArray RP Influenza A subtyping assays target the Influenza A hemagglutinin gene only. The FilmArray RP does not detect or differentiate the Influenza A neuraminidase gene.
- Clinical specificity was established when Influenza A H1-2009 was the predominant Influenza A virus in circulation. When other Influenza A viruses are emerging, clinical specificity may vary.

- Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Bordetella pertussis,* Coronavirus 229E, Coronavirus OC43, Influenza A H1, Influenza A H3, Influenza A H1-2009, Influenza B, *Mycoplasma pneumoniae*, Parainfluenza Virus 1, Parainfluenza Virus 2, and Parainfluenza Virus 4, were established primarily with retrospective clinical specimens. Performance characteristics for *Chlamydophila pneumoniae* were established primarily using contrived clinical specimens. The performance of this test has not been established for monitoring treatment of seasonal Influenza A H1, A H3, A H1-2009 or RSV infections.
- The performance of this test has not been established for screening of blood or blood product for the presence of seasonal Influenza A H1, A H3 or A H1-2009.
- The performance of this test has not been established with potentially interfering medications for the treatment of influenza or cold viruses. The effect of interfering substances has only been evaluated for those listed in the labeling. Interference by substances other than those described in the "Interference" section below could lead to erroneous results.
- The performance of the FilmArray RP has not been established in individuals who received influenza vaccine. Recent administration of a nasal influenza vaccine may cause false positive results for Influenza A and/or Influenza B.
- Due to the genetic similarity between Human Rhinovirus and Enterovirus, the FilmArray RP cannot reliably differentiate them. A positive FilmArray RP Rhinovirus/Enterovirus result should be followed-up using an alternate method (e.g. cell culture or sequence analysis).
- The Coronavirus OC43 assay may cross-react with Coronavirus HKU1. As a result, when both HKU1 and OC43 are detected in the same patient specimen, the result may be due to assay cross-reactivity. A co-infection with these two viruses is also possible.
- The FilmArray RP may not be able to distinguish between existing viral strains and new variants as they emerge. For example, the FilmArray RP can detect Influenza A H3N2v (first recognized in August, 2011), but will not be able to distinguish this variant from Influenza A H3N2 seasonal.
- Results of the FilmArray RP *B. pertussis* assay may not be concordant with the results of commonly used *Bordetella* PCR assays that target the multi-copy insertion sequence (IS481) due to differences in sensitivity and specificity. IS481 is a multi-copy target and is present in several *Bordetella* species (*B. pertussis, B. holmesii and B. bronchiseptica*). The FilmArray RP *B. pertussis* assay targets the single-copy promoter region of the pertussis toxin gene and is designed to be highly specific for detection of *B. pertussis.* Although cross-reactivity with closely-related *Bordetella* species was not observed in the clinical study or Analytical Specificity testing of a variety of strains at 1 x 10^6 CFU/mL, instances of cross-reactivity can occur with high levels (above 1 x 10^6 CFU/mL) or with rare sequence variants of other *Bordetella* species, such as *B. bronchiseptica* and *B. parapertussis*.

EXPECTED VALUES

NOTE: The expected values presented in this section were obtained prior to the addition of a second Adenovirus assay to FilmArray RP.

In the first phase of the prospective FilmArray RP clinical study, 853 eligible prospective nasopharyngeal (NPS) swab specimens were collected and tested at three sites across the U.S. from December 2009 thru May 2010. A second phase of the prospective FilmArray RP clinical study was conducted to obtain additional positive specimens for Parainfluenza Viruses 1, 2, and 4. During the anticipated peak season for Parainfluenza Viruses 1 and 2 (September 2010 thru January 2011), another 264 eligible specimens were collected and tested at two of the three sites. The number and percentage of positive cases as determined by the FilmArray RP calculated by testing site or by age group are presented in the following tables:

Table 6. Expected Value (as Determined by the FilmArray RP) Summary by Site for the First Phase Prospective Clinical Evaluation (December 2009 – May 2010)

Organism	Overall (n=853)		Site 1 (n=275)		Site 2 (n=333)		Site 3 (n=245)	
	Number	Expected Value	Number	Expected Value	Number	Expected Value	Number	Expected Value
Adenovirus	38	4.5%	5	1.8%	11	3.3%	22	9.0%
Influenza A	11	1.3%	10	3.6%	1	0.3%	0	0%
Influenza A H1	0	0%	0	0%	0	0%	0	0%
Influenza A H3	0	0%	0	0%	0	0%	0	0%
Influenza A H1-2009	11	1.3%	10	3.6%	1	0.3%	0	0%
Influenza B	0	0%	0	0%	0	0%	0	0%
Parainfluenza Virus 1	1	0.1%	0	0%	0	0%	1	0.4%
Parainfluenza Virus 2	0	0%	0	0%	0	0%	0	0%
Parainfluenza Virus 3	33	3.9%	1	0.4%	1	0.3%	31	12.7%
Parainfluenza Virus 4	8	0.9%	0	0%	4	1.2%	4	1.6%
Respiratory Syncytial Virus	139	16.3%	4	1.5%	86	25.8%	49	20.0%
Coronavirus 229E	14	1.6%	5	1.8%	3	0.9%	6	2.4%
Coronavirus HKU1	25	2.9%	9	3.3%	13	3.9%	3	1.2%
Coronavirus NL63	23	2.7%	4	1.5%	9	2.7%	10	4.1%
Coronavirus OC43	8	0.9%	2	0.7%	6	1.8%	0	0%
Human Metapneumovirus	94	11.0%	12	4.4%	41	12.3%	41	16.7%
Human Rhinovirus/Entero	225	26.4%	36	13.1%	92	27.6%	97	39.6%
Bordetella pertussis	4	0.4%	2	0.7%	1	0.3%	1	0.4%
Chlamydophila pneumoniae	1	0.1%	0	0%	0	0%	1	0.4%
Mycoplasma pneumoniae	2	0.2%	2	0.2%	0	0%	0	0%

Table 7. Expected Value (as Determined by the FilmArray RP) Summary by Site for the Second Phase Prospective Clinical Evaluation (September 2010 – January 2011)

Organism	Overall (n=264)		Site 2 (n=180)		Site 3 (n=84)	
	Number	Expected Value	Number	Expected Value	Number	Expected Value
Adenovirus	15	5.7%	11	6.1%	4	4.6%
Influenza A	5	1.9%	0	0%	5	6.0%
Influenza A H1	0	0%	0	0%	0	0%

Organism	Overall (n=264)		Site 2 (n=180)		Site 3 (n=84)	
	Number	Expected Value	Number	Expected Value	Number	Expected Value
Influenza A H3	5	1.9%	0	0%	5	6.0%
Influenza A H1-2009	0	0%	0	0%	0	0%
Influenza B	1	0.4%	1	0.6%	0	0%
Parainfluenza Virus 1	1	0.4%	0	0%	1	1.1%
Parainfluenza Virus 2	9	3.4%	4	2.2%	5	5.7%
Parainfluenza Virus 3	5	1.9%	0	0%	5	5.7%
Parainfluenza Virus 4	2	0.7%	1	0.6%	1	1.1%
Respiratory Syncytial Virus	31	11.7%	11	6.1%	20	23.8%
Coronavirus 229E	0	0%	0	0%	0	0%
Coronavirus HKU1	0	0%	0	0%	0	0%
Coronavirus NL63	1	0.4%	1	0.6%	0	0%
Coronavirus OC43	11	4.2%	1	0.5%	10	11.9%
Human Metapneumovirus	4	1.5%	0	0%	4	4.6%
Human Rhinovirus/Entero	125	47.3%	91	50.6%	34	39.1%
Bordetella pertussis	3	1.1%	3	1.6%	0	0%
Chlamydophila pneumoniae	0	0%	0	0%	0	0%
Mycoplasma pneumoniae	2	0.8%	1	0.5%	1	1.2%

Table 8. Expected Value (as Determined by FilmArray RP) Summary by Age Group for First Phase Prospective Clinical Evaluation (December 2009 – May 2010)

Organism	Total (Expected Value)	≤ 5 years	6-21 years	22-49 years	≥ 50 years
Adenovirus	38 (4.5%)	32	2	3	1
Influenza A	11 (1.3%)	1	1	7	2
Influenza A H1	0 (0%)	0	0	0	0
Influenza A H3	0 (0%)	0	0	0	0
Influenza A H1-2009	11 (1.3%)	1	1	7	2
Influenza B	0 (0%)	0	0	0	0
Parainfluenza Virus 1	1 (0.1%)	0	1	0	0
Parainfluenza Virus 2	0 (0%)	0	0	0	0
Parainfluenza Virus 3	33 (3.9%)	31	1	0	1
Parainfluenza Virus 4	8 (0.9%)	7	1	0	0
Respiratory Syncytial Virus	139 (16.3%)	127	3	4	5
Coronavirus 229E	14 (1.6%)	6	2	5	1
Coronavirus HKU1	25 (2.9%)	12	1	8	4
Coronavirus NL63	23 (2.7%)	17	2	2	2
Coronavirus OC43	8 (0.9%)	4	0	2	2
Human Metapneumovirus	94 (11.0%)	76	4	10	4
Human Rhinovirus/Entero	225 (26.4%)	161	24	29	11
Bordetella pertussis	4 (0.4%)	2	1	0	1
Chlamydophila pneumoniae	1 (0.1%)	1	0	0	0
Mycoplasma pneumoniae	2 (0.2%)	0	0	2	0

Table 9. Expected Value (as Determined by FilmArray RP) Summary by Age Group for Second Phase Prospective Clinical Evaluation (September 2010-January 2011)

Organism	Total (Expected Value)	≤ 5 years	6-21 years	22-49 years	≥ 50 years
Adenovirus	15 (5.7%)	15	0	n/a	n/a
Influenza A	5 (1.9%)	4	1	n/a	n/a
Influenza A H1	0 (0%)	0	0	n/a	n/a
Influenza A H3	5 (1.9%)	4	1	n/a	n/a
Influenza A H1-2009	0 (0%)	0	0	n/a	n/a
Influenza B	1 (0.4%)	1	0	n/a	n/a
Parainfluenza Virus 1	1 (0.4%)	1	0	n/a	n/a
Parainfluenza Virus 2	9 (3.4%)	9	0	n/a	n/a
Parainfluenza Virus 3	5 (1.9%)	5	0	n/a	n/a
Parainfluenza Virus 4	2 (0.7%)	2	0	n/a	n/a
Respiratory Syncytial Virus	31 (11.7%)	30	1	n/a	n/a
Coronavirus 229E	0 (0%)	0	0	n/a	n/a
Coronavirus HKU1	0 (0%)	0	0	n/a	n/a
Coronavirus NL63	1 (0.4%)	1	0	n/a	n/a
Coronavirus OC43	11 (4.2%)	9	2	n/a	n/a
Human Metapneumovirus	4 (1.5%)	4	0	n/a	n/a
Human Rhinovirus/Entero	125 (47.3%)	118	7	n/a	n/a
Bordetella pertussis	3 (1.1%)	3	0	n/a	n/a
Chlamydophila pneumoniae	0 (0%)	0	0	n/a	n/a
Mycoplasma pneumoniae	2 (0.8%)	2	0	n/a	n/a

The number and percentage of co-infection cases as determined by the FilmArray RP calculated by age group are presented in the following tables:

Table 10. Expected Value (Co-infections as Determined by FilmArray RP) Summary by Age Group for First Phase Prospective Clinical Evaluation (December 2009 – May 2010)

Co-Infection	Total (Expected Value)	≤ 5 Years	6-21 Years	22-49 Years	≥ 50 Years
HRV/EV + RSV	21 (2.46%)	20	0	1	0
HRV/EV + AdV	8 (0.94%)	8	0	0	0
HRV/EV + PIV3	8 (0.94%)	7	1	0	0
HRV/EV + hMPV	7 (0.82%)	7	0	0	0
hMPV + RSV	4 (0.47%)	4	0	0	0
HRV/EV + CoV NL63	4 (0.47%)	3	0	1	0
CoV HKU1 + hMPV	3 (0.35%)	3	0	0	0
CoV HKU1 + HRV/EV	3 (0.35%)	1	0	2	0
CoV HKU1 + RSV	3 (0.35%)	3	0	0	0
CoV NL63 + hMPV	3 (0.35%)	3	0	0	0
CoV NL63 + RSV	3 (0.35%)	3	0	0	0
hMPV + PIV3	3 (0.35%)	3	0	0	0

Co-Infection	Total (Expected Value)	≤ 5 Years	6-21 Years	22-49 Years	≥ 50 Years
AdV + HRV/EV + PIV3	2 (0.23%)	2	0	0	0
CoV OC43 + RSV	2 (0.23%)	2	0	0	0
CoV HKU1 + CoV OC43	2 (0.23%)	0	0	1	1
HRV/EV + PIV4	2 (0.23%)	2	0	0	0
AdV + hMPV	1 (0.12%)	1	0	0	0
AdV + PIV3	1 (0.12%)	1	0	0	0
AdV + RSV	1 (0.12%)	1	0	0	0
AdV + CoV NL63	1 (0.12%)	1	0	0	0
AdV + RSV + CoV 229E	1 (0.12%)	1	0	0	0
AdV + HRV/EV + *B. pertussis*	1 (0.12%)	1	0	0	0
AdV + *C. pneumoniae*	1 (0.12%)	1	0	0	0
CoV 229E + RSV	1 (0.12%)	1	0	0	0
CoV 229E + CoV NL63 + HRV/EV+RSV	1 (0.12%)	1	0	0	0
CoV 229E + HRV/EV	1 (0.12%)	1	0	0	0
CoV HKU1 + HRV/EV + RSV	1 (0.12%)	1	0	0	0
CoV NL63 + hMPV + RSV	1 (0.12%)	1	0	0	0
HRV/EV + *B. pertussis*	1 (0.12%)	1	0	0	0
HRV/EV + PIV1	1 (0.12%)	0	1	0	0
hMPV + PIV4	1 (0.12%)	1	0	0	0
PIV4 + RSV	1 (0.12%)	1	0	0	0

Table 11. Expected Value (Co-infections as Determined by FilmArray RP) Summary by Age Group for Second Phase Prospective Clinical Evaluation (September 2010 – January 2011)

Co-Infection	Total (Expected Value)	≤ 5 Years	6-21 Years	22-49 Years	≥ 50 Years
HRV/EV + RSV	6 (2.2%)	6	0	n/a	n/a
HRV/EV + AdV	6 (2.2%)	6	0	n/a	n/a
CoV OC43 + HRV/EV	5 (1.9%)	5	0	n/a	n/a
HRV/EV + PIV2	1 (0.4%)	1	0	n/a	n/a
HRV/EV + PIV3	1 (0.4%)	1	0	n/a	n/a
HRV/EV + PIV4	1 (0.4%)	1	0	n/a	n/a
hMPV + RSV	1 (0.4%)	1	0	n/a	n/a
Flu B + RSV	1 (0.4%)	1	0	n/a	n/a
AdV + CoV OC43	1 (0.4%)	1	0	n/a	n/a
CoV OC43 + hMPV	1 (0.4%)	1	0	n/a	n/a
CoV OC43 + RSV	1 (0.4%)	1	0	n/a	n/a
HRV/EV + *B. pertussis*	1 (0.4%)	1	0	n/a	n/a
PIV2 + *M. pneumoniae*	1 (0.4%)	1	0	n/a	n/a

PERFORMANCE CHARACTERISTICS

Clinical Performance

NOTE: The clinical performance data presented in the first part of this section were obtained prior to the addition of a second Adenovirus assay to FilmArray RP. Data from a clinical comparison study demonstrating performance of a modified FilmArray RP (containing a second Adenovirus assay) as compared to the original FilmArray RP are presented starting on page 34.

The clinical performance of the FilmArray RP was established during two phases of a prospective clinical study. The first phase of the prospective clinical study was conducted at 3 U.S. clinical sites over a 6 month time period (December 2009 thru May 2010). The second phase of the prospective clinical study was carried out at 2 of the 3 sites for an additional 5 month time period (September 2010 thru January 2011) in an attempt to achieve increased detection of several low prevalence organisms (i.e. PIV1, PIV2, and PIV4) during the anticipated peak season for PIV1 and PIV2. Subjects with signs and symptoms of respiratory infection were invited to participate. Upon obtaining informed consent, NPS samples were collected for FilmArray and comparator testing. A total of 857 subjects were initially enrolled in the 2009/2010 respiratory season (Phase 1) and 4 were withdrawn. Table 12A provides a summary of demographic information for the 853 subjects that participated in the first phase of the prospective study. Another 287 subjects were enrolled in the 2010/2011 respiratory season (Phase 2) and 20 specimens were omitted from analysis due to improper specimen storage prior to testing. Another 3 specimens were also excluded from analysis due to the lack of clinical protocol-required associated valid external control mix results. Table 12B provides a summary of the remaining 264 subjects.

Table 12. Demographic Summary for FilmArray RP Prospective Clinical Study

A. December 2009 thru May 2010

Sex	Number of Subjects
Male	449 (53%)
Female	404 (47%)
Age	**Number of Subjects**
≤5	484 (57%)
6-21	95 (11%)
22-49	190 (22%)
≥50	84 (10%)

B. September 2010 thru January 2011

Sex	Number of Subjects
Male	151 (57%)
Female	113 (43%)
Age	**Number of Subjects**
≤5	240 (91%)
6-21	24 (9%)
22-49	0 (0%)
≥50	0 (0%)

A NPS specimen from each subject was tested with the FilmArray RP. The performance of the FilmArray RP was evaluated by comparing the FilmArray RP test result for each member of the panel with the appropriate comparator/reference methods shown in Table 13.

Table 13. Reference/Comparator Methods Used to Assess FilmArray RP Performance

Virus	Reference/Comparator Method(s)
Adenovirus	Viral culture followed by DFA identification[a]
Influenza A	
Influenza B	

Virus	Reference/Comparator Method(s)
Parainfluenza Virus 1	
Parainfluenza Virus 2	
Parainfluenza Virus 3	
Respiratory Syncytial Virus	
Influenza A H1 subtyping	Viral Culture followed by 1 PCR test of viral culture with bi-directional sequence confirmation[b]
Influenza A H3 subtyping	
Influenza A H1-2009 subtyping	
Parainfluenza Virus 4	
Human Rhinovirus	2 PCR tests of patient specimens with bi-directional sequence confirmation[c]
Human Enterovirus	
Coronavirus 229E	
Coronavirus HKU1	
Coronavirus NL63	
Coronavirus OC43	
Human Metapneumovirus	
Bordetella pertussis	
Chlamydophila pneumoniae	
Mycoplasma pneumoniae	

[a] Performance of the FilmArray RP detecting AdV, Flu A, Flu B, PIV1, PIV2, PIV3, or RSV, respectively, was compared to viral culture followed by fluorescent antibody identification. "True" positives were considered as any sample that tested positive by viral culture followed by DFA testing. "True" negatives were considered as any sample that tested negative by viral culture followed by DFA testing.

[b] Performance of the FilmArray RP detecting Flu A H1, A H3, A H1-2009, or PIV4, respectively, was compared to viral culture followed by one analytically validated PCR assay with bi-directional sequence confirmation. The comparator assays were designed to amplify a different sequence from that amplified by the FilmArray assay(s). None of the comparator PCR assays overlapped any FilmArray amplicon sequence even if the same gene was targeted. "True" Flu A H1, A H3, or A H1-2009 positives, respectively, were considered as any sample that tested positive for Flu A by viral culture, and had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched the respective subtype sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov) with acceptable E-values. "True" Flu A H1, A H3, or A H1-2009 negatives, respectively, were considered as any sample that tested negative for Flu A by viral culture, or any sample that tested positive for Flu A virus by viral culture, but tested negative by the respective subtype specific PCR assay. "True" PIV4 positives were considered as any sample for which bi-directional sequencing data matching PIV4 sequences deposited in the NCBI GenBank database was obtained from testing of viral culture material. "True" PIV4 negatives were considered as any sample where testing of viral culture material with the analytically validated PIV4 specific PCR assay was negative.

[c] Performance of the FilmArray RP detecting HRV, EV, CoV 229E, CoV HKU1, CoV NL63, CoV OC43, hMPV, *B. pertussis, C. pneumoniae,* or *M. pneumoniae* respectively, was compared to a predetermined algorithm that used composite comparator methods. The methods consist of two analytically validated PCR assays followed by bi-directional sequencing. The comparator assays were designed to amplify a different sequence from that amplified by the FilmArray assay(s). None of the comparator PCR assays overlapped any FilmArray amplicon sequence even if the same gene was targeted. "True positives were considered as any sample 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. "True" negatives were considered as any sample that tested negative by both of the comparator PCR assays.

A total of 853 specimens were evaluated for Adenovirus, Influenza A, Influenza B, Parainfluenza Virus 3, Respiratory Syncytial Virus, Coronavirus HKU1, Coronavirus NL63, Human Metapneumovirus, and Human Rhinovirus/Enterovirus in the first phase of the study. A total of 1117 specimens were evaluated for Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 4, Coronavirus 229E, Coronavirus OC43, *B. pertussis, C. pneumoniae,* and *M. pneumoniae* in the first and second phases of the study. (During the second phase of the prospective clinical study, only the reference/comparator methods for Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 4, Coronavirus 229E, Coronavirus OC43, *B. pertussis, C. pneumoniae,* and *M. pneumoniae* were performed on all specimens and used to calculate the performance.) Clinical sensitivity or positive percent agreement (PPA) was calculated as 100% x (TP / TP + FN). True positive (TP) indicates that both the FilmArray RP and comparator method had a positive result for this specific organism, and false negative (FN) indicates that the FilmArray result was negative while the comparator result was positive. Specificity or negative percent agreement (NPA) was calculated as 100% x (TN / TN + FP). True negative (TN) indicates that both the FilmArray RP and the comparator method had negative results, and a false positive (FP) indicates that the FilmArray RP result was positive but the comparator result was negative. The exact binomial two-sided 95% confidence interval was calculated. The results are summarized in Table 14.

Table 14. Clinical Sensitivity and Specificity or Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for the FilmArray RP Prospective Clinical Study

Organism	Sensitivity		95% CI	Specificity		95% CI
Adenovirus	24/27[a]	88.9%	70.8 - 97.7%	812/826[b]	98.3%	97.2 - 99.1%
Influenza A	9/10	90.0%	55.5 – 99.8%	841/843[c]	99.8%	99.2 -100%
Influenza A H1	0/0	n/a	n/a	853/853	100%	99.6 - 100%
Influenza A H3	0/0	n/a	n/a	853/853	100%	99.6 - 100%
Influenza A H1-2009	8/9	88.9%	51.8 - 99.7%	841/844[c]	99.6%	99.0 - 99.9%
Influenza B	0/0	n/a	n/a	853/853	100%	99.6 - 100%
Parainfluenza Virus 1	1/1	100%	n/a	1115/1116[d]	99.9%	99.5 – 100%
Parainfluenza Virus 2	7/8[e,g]	87.4%	47.4 – 99.7%	1107/1109[f,g]	99.8%	99.4 – 100%
Parainfluenza Virus 3	23/24[h]	95.8%	78.9 – 99.9%	819/829[i]	98.8%	97.8 - 99.4%
Parainfluenza Virus 4	9/9	100%	66.4 – 100%	1107/1108[j]	99.9%	99.5 – 100%
Respiratory Syncytial Virus	52/52	100%	93.2 - 100%	714/801[k]	89.1%	86.8 - 91.2%
Organism	**PPA**		**95% CI**	**NPA**		**95% CI**
Coronavirus 229E	12/12	100%	73.5 - 100%	1103/1105[l]	99.8%	99.4 - 100%
Coronavirus HKU1	23/24	95.8%	78.9 - 99.9%	827/829[m]	99.8%	99.1 - 100%
Coronavirus NL63	23/24	95.8%	78.9 - 99.9%	829/829	100%	99.6 - 100%
Coronavirus OC43	14/14	100%	76.8 - 100%	1098/1103[n,o]	99.6%	99.0 - 99.9%
Human Metapneumovirus	88/93	94.6%	87.9 - 98.2%	754/760	99.2%	98.3 - 99.7%
Human Rhinovirus/Enterovirus	190/205	92.7%	88.2 - 95.8%	613/648	94.6%	92.6 - 96.2%
Bordetella pertussis	6/6	100%	54.1 - 100%	1110/1111	99.9%	99.5 - 100%
Chlamydophila pneumoniae	1/1	100%	n/a	1116/1116	100%	99.7 - 100%
Mycoplasma pneumoniae	4/4	100%	39.8 - 100%	1113/1113	100%	99.7 - 100%

[a] The FilmArray RP detected Adenovirus in 1/3 false negative specimens when retested. The Adenovirus in the retested specimen was identified as species C by bi-directional sequence analysis. The Adenoviruses in the remaining two false negative specimens were identified as species C and species B.

[b] Adenoviruses were identified in 13/14 false positive specimens using bi-directional sequence analysis. Ten were identified as species C, two as species B, and one as species E.

[c] Influenza A viruses (H1-2009 subtype) were identified in 3/3 false positive specimens (2/2 false positive compared to influenza A culture alone) using sequence analysis with an alternate assay.
[d] PIV1 was identified in this specimen using bi-directional sequence analysis.
[e] PIV2 virus was not detected in the single false negative specimen using PCR analysis.
[f] PIV2 virus was detected in both false positive specimens using bi-directional sequencing analysis.
[g] Two adjacent specimens (one false positive and one false negative) may have been switched during the viral culture reference method testing as is evidenced by bi-directional sequence analysis of these specimens.
[h] The FilmArray RP detected PIV3 in the single false negative specimen when retested.
[i]. PIV3 viruses were identified in 10/10 false positive specimens using bi-directional sequence analysis.
[j] A PIV4 virus was detected in this false positive NPS specimen by bi-directional sequence analysis although it was not detected from viral culture.
[k] RSV viruses were identified in 83/87 false positive specimens using bi-directional sequence analysis.
[l] CoV 229E was identified by bi-directional sequence analysis in 1/2 false positive specimens using an alternate assay.
[m] CoV HKU1 viruses were identified in 2/2 false positive specimens using bi-directional sequence analysis with an alternate assay.
[n] CoV OC43 was detected in 2/5 false positive specimens using an alternate assay with bi-directional sequence analysis.
[o] 2/5 false positives were determined to be cross-reactive products derived from amplification of CoV HKU1 virus with the CoV OC43 assay primers.

The FilmArray RP detected a total of 94 mixed infections in the first phase of the prospective clinical evaluation performed from December 2009 thru May 2010 (853 tested and analyzed specimens). This represents 18% of the total positive specimens (94/524). Eighty-seven (87/94; 92.6%) were double infections, 6 (6/94; 6.3%) were triple infections, and one was a quadruple infection (1/94; 1.1%). The total number of test results comprising these co-infections was 189. The single most common co-infection was Human Rhinovirus/Enterovirus with Respiratory Syncytial Virus (20/94; 21.1%). These viruses were the most prevalent in the tested population. Out of the 94 co-infections, 54 contained one or more organisms that had not been detected with the reference/comparator methods, i.e. discrepant co-infection.

Table 15. Distinct Co-infection Combinations Detected by the FilmArray RP in the First Phase of the Prospective Clinical Trial (December 2009 to May 2010)

Distinct Co-infection Combinations Detected by FilmArray RP				Total Co-infections	Number of Discrepant Co-infections[a]	Discrepant Organism(s) [a]
Organism 1	**Organism 2**	**Organism 3**	**Organism 4**			
AdV	*B. pertussis*	HRV/EV		1	1	AdV/*B. pertussis*
AdV	HRV/EV	PIV3		2	2	AdV (2)
AdV	CoV 229E	RSV		1	1	AdV; RSV
AdV	*C. pneumoniae*			1	0	
AdV	CoV NL63			1	1	AdV
AdV	hMPV			1	1	AdV; hMPV
AdV	HRV/EV			8	2	AdV; HRV/EV
AdV	PIV3			1	1	PIV3
AdV	RSV			1	1	RSV
CoV 229E	RSV			1	0	
B. pertussis	HRV/EV			1	1	HRV/EV
CoV 229E	CoV NL63	HRV/EV	RSV	1	1	RSV; CoV 229E
CoV 229E	HRV/EV			1	0	

Distinct Co-infection Combinations Detected by FilmArray RP				Total Co-infections	Number of Discrepant Co-infections[a]	Discrepant Organism(s) [a]
Organism 1	**Organism 2**	**Organism 3**	**Organism 4**			
CoV HKU1	HRV/EV	RSV		1	1	RSV
CoV HKU1	CoV OC43			2	2	CoV OC43 (2)[b]
CoV HKU1	hMPV			3	0	
CoV HKU1	HRV/EV			3	0	
CoV HKU1	RSV			3	1	RSV
CoV NL63	hMPV	RSV		1	1	RSV
CoV NL63	hMPV			3	0	
CoV NL63	HRV/EV			4	1	HRV/EV
CoV NL63	RSV			3	2	RSV (2)
CoV OC43	RSV			2	2	RSV (2); CoV OC43 (2)
hMPV	HRV/EV			7	3	hMPV (2); HRV/EV (1)
hMPV	PIV3			3	1	PIV3
hMPV	PIV4			1	1	hMPV
hMPV	RSV			4	4	hMPV (1); RSV (3)
HRV/EV	PIV1			1	1	PIV1
HRV/EV	PIV3			8	6	HRV/EV (3); PIV3 (3)
HRV/EV	PIV4			2	0	
HRV/EV	RSV			21	16	HRV/EV (8); RSV (13)
PIV4	RSV			1	0	
Total Co-infections				**94**	**54**	**65/200(32.5%)**
Total Double Infections				**87**	**47**	**55/178 (30.9%)**
Total Triple Infections				**6**	**6**	**8/18 (44.4%)**
Total Quadruple Infections				**1**	**1**	**2/4 (50%)**

[a] A discrepant co-infection or discrepant organism was defined as one that was detected by FilmArray RP but not detected by the reference/comparator methods or by discrepancy investigation using bi-directional sequence analysis. Sixty-five (65) organisms from 54 of the 94 co-infections were not detected by the reference/comparator methods. Forty-four (44) were investigated using bi-directional sequence analysis with an alternate assay; in 37 cases the investigative assay successfully identified the organism in the specimen.

[b] Both discrepant CoV O43 results were false-positive due to cross-reactivity of the CoV OC43 assay with CoV HKU1 viruses in the specimens.

Table 16. Additional Distinct Co-infection Combinations Detected by Reference/Comparator Methods, but not by the FilmArray RP in the First Phase of the Prospective Clinical Trial (December 2009 to May 2010)

Distinct Co-infection Combinations[a]		Total Co-infections	Number of Discrepant Co-infections	Discrepant Organism(s)
Organism 1	**Organism 2**			
CoV 229E	HRV/EV	1	1	HRV/EV (1)
hMPV	HRV/EV	3	3	hMPV (2); HRV/EV (1)
HRV/EV	RSV	1	1	HRV/EV (1)
Total Co-infections		**5**	**5**	
Total Double Infections		**5**	**5**	

[a]This table includes only distinct co-infections that were detected by the reference/comparator method but not by FilmArray RP; the remaining co-infections detected by the reference/comparator methods are already represented in the table above.

Table 17. Mixed Infections Detected by FilmArray RP in the First Phase (December 2009 to May 2010) and the Second Phase (September 2010 to January 2011) of the Prospective Clinical Trial and Prevalence of Individual Organisms in Mixed Infections

Organism Combinations	Number of Positive Samples	Percentage of Total Samples Tested (n/1117)	Organism	Number of Mixed Infections	Prevalence in Mixed Infections (n/121)
HRV/EV + RSV	27	2.40%	HRV/EV	82	68%
AdV + HRV/EV	14	1.30%	RSV	59	49%
HRV/EV + PIV3	9	0.80%	AdV	24	20%
hMPV + HRV/EV	7	0.60%	hMPV	25	21%
CoV OC43 + HRV/EV	5	0.50%	PIV3	15	15%
hMPV + RSV	5	0.40%	CoV NL63	13	11%
CoV NL63 + HRV/EV	4	0.40%	CoV OC43	12	10%
CoV HKU1 + hMPV	3	0.30%	CoV HKU1	12	10%
CoV HKU1 + HRV/EV	3	0.30%	PIV4	5	4%
CoV HKU1 + RSV	3	0.30%	CoV 229E	4	3%
CoV NL63 + hMPV	3	0.30%	*B. pertussis*	3	2%
CoV NL63 + RSV	3	0.40%	PIV2	2	2%
CoV OC43 + RSV	3	0.30%	*C. pneumoniae*	1	1%
hMPV + PIV3	3	0.30%	Flu B	1	1%
HRV/EV + PIV4	3	0.30%	*M. pneumoniae*	1	1%
AdV + HRV/EV + PIV3	2	0.20%	PIV1	1	1%
AdV + RSV	1	0.20%			
CoV HKU1 + CoV OC43	2	0.20%			
HRV/EV + *B. pertussis*	2	0.10%			
AdV + *B. pertussis* + HRV/Entero	1	0.10%			

Organism Combinations	Number of Positive Samples	Percentage of Total Samples Tested (n/1117)	Organism	Number of Mixed Infections	Prevalence in Mixed Infections (n/121)
AdV + *C. pneumoniae*	1	0.10%			
AdV + CoV 229E + RSV	1	0.10%			
Adenovirus + CoV NL63	1	0.10%			
Adenovirus + CoV OC43	1	0.10%			
Adenovirus + hMPV	1	0.10%			
Adenovirus + PIV3	1	0.10%			
CoV 229E + CoV NL63 + HRV/EV + RSV	1	0.10%			
CoV 229E + HRV/EV	1	0.10%			
CoV 229E + RSV	1	0.10%			
CoV HKU1 + HRV/EV + RSV	1	0.10%			
CoV NL63 + hMPV + RSV	1	0.10%			
CoV OC43 + hMPV	1	0.10%			
hMPV + PIV4	1	0.10%			
HRV/EV + PIV1	1	0.10%			
HRV/EV + PIV2	1	0.10%			
Flu B + RSV	1	0.10%			
PIV4 + RSV	1	0.10%			
PIV2 + *M. pneumoniae*	1	0.10%			

A total of 1117 prospective clinical specimens were tested and analyzed during the first phase and the second phase of the prospective clinical evaluation (2009/2010 respiratory season and 2010/2011 respiratory season). Of the 1117 analyzed prospective clinical specimens, 94.6% (1057/1117) of these specimens yielded valid results on the first attempt (i.e. first loaded pouch). Invalid results or no results were obtained for the remaining 60 (5.4%) specimens (no results for 24 specimens due to incomplete runs; 36 specimens were Invalid due to pouch control failures). Of the 24 incomplete runs, 3 were aborted by users (0.3%); 6 were due to software-related errors (0.6%) and 15 were due to instrument errors (1.3%). Fifty-seven (57) of the 60 initially failed (no results or invalid) specimens yielded valid results after a single retest using a new pouch/sample. The remaining three (3) specimens failed on the second attempt (2 due to failed pouch controls, 1 due to an instrument error), but yielded valid results following a second retest using another pouch/sample.

A single pouch leak (1/1244 loaded pouches; 0.08%) was observed during the first phase of the prospective clinical evaluation. Following discovery of the leak, the operator followed recommended decontamination procedures and performed contamination surveillance swab testing of the area surrounding the instrument. No contamination was detected. No pouch leaks were observed in the second phase of the prospective clinical study.

Testing of Preselected Archived Specimens

Several organisms, such as Influenza, were either not encountered in the prospective clinical study or had a low prevalence. To supplement the results of the prospective clinical study, an evaluation of preselected archived specimens was performed. The specimens were archived clinical NPS that were selected because they had previously tested positive for one of the following organisms: Adenovirus, *Bordetella pertussis*, Coronaviruses 229E and OC43, Enterovirus, Influenzas A H1, H1-2009, and H3, Influenza B, *Mycoplasma pneumoniae*, and Parainfluenza Viruses 1-4. Prior to testing with the FilmArray RP, the presence (or absence for negative specimens) of the expected

organisms was confirmed in each specimen using validated organism-specific PCR assays followed by bi-directional sequencing. The specimens were organized into "test panels" and randomized such that the users performing the FilmArray RP testing were blinded as to the expected test result. Each panel contained specimens known to be positive and specimens known to be negative for the specific organism being evaluated, which allows for the calculation of a positive percent agreement (PPA) and a negative percent agreement (NPA). A summary of the available demographic information of the tested samples is provided in Table 18 and the results of the FilmArray testing are presented in Table 19.

Table 18. Demographic Summary of FilmArray RP Archived Specimen Study

Total Specimens		**668**
Sex	Female (%)	217 (32.5%)
	Male (%)	217 (32.5%)
	Unknown[a]	234 (35%)
Age	Avg	13
	Median	7
	Min	0.5
	Max	91
Age Range	≤5	239 (35.8%)
	6-21	112 (16.8%)
	22-49	42 (6.3%)
	≥50	47 (7%)
	≥5[b]	3 (0.4%)
	Unknown[a]	225 (33.7%)

[a] Demographic information was not provided for specimens from one source. Because the specimens were provided by a pediatric hospital, it is understood that the age range of specimens was from <1 yrs to 21 yrs.
[b] One source provided age category "less than 5 years of age or equal to/greater than 5 years of age"

Table 19. Summary of FilmArray RP Archived Specimen Performance Data

Organism	**Positive Percent Agreement (PPA)**			**Negative Percent Agreement (NPA)**		
	TP/TP +FN	**Percent**	**95% CI**	**TN/TN+FP**	**Percent**	**95% CI**
Adenovirus	27/27[a]	100%	87.2 - 100%	28/28	100%	87.7 - 100%
B. pertussis	53/56[b]	94.6%	85.1 - 98.9%	56/58[c]	96.5%	88.1 - 99.6%
Coronavirus 229E	13/13	100%	75.3 - 100%	45/47[d]	95.7%	85.5 - 99.5%
Coronavirus OC43	24/24	100%	85.8 - 100%	33/36[e]	91.7%	77.5 - 98.2%
Enterovirus	22/23[f]	95.7%	78.0 - 99.9%	90/90	100%	96.0 - 100%
Influenza A H1	32/32[g]	100%	89.1 - 100%	127/127	100%	97.1 - 100%
Influenza A H1-2009	34/34[h]	100%	89.7 - 100%	125/125	100%	97.1 - 100%
Influenza A H3	54/54[i]	100%	93.4 - 100%	105/105	100%	96.5 - 100%
Influenza B	30/30[j]	100%	88.4 - 100%	129/129	100%	97.2 - 100%
M. pneumoniae	54/64[k]	84.4%	73.1 - 92.2%	58/65[l]	89.2%	79.1 - 95.6%
Parainfluenza Virus 1	34/35[m]	97.1%	85.1 - 99.9%	94/94	100%	96.2 - 100%
Parainfluenza Virus 2	28/28[n]	100%	87.6 - 100%	101/101	100%	96.4 - 100%
Parainfluenza Virus 3	36/36	100%	90.3 - 100%	93/93	100%	96.1 - 100%
Parainfluenza Virus 4	11/11[o]	100%	71.5 - 100%	6/6	100%	54.1 - 100%

[a] Out of 28 AdV specimens received for testing, the organism status of one (1) specimen could not be confirmed by organism specific PCR followed by bidirectional sequencing and was excluded from further analysis.
[b] Two (2) *B. pertussis*-positive specimens were originally identified by the source lab as negative for *B. pertussis* but were unexpectedly found to be positive by organism specific PCR followed by bidirectional sequencing. Both of these specimens were negative when tested with the FilmArray, and both were found to be negative for *B. pertussis* during discrepancy investigation of confirmation testing.
[c] Ten (10) *B. pertussis*-negative specimens were originally identified by the source lab as positive for *B. pertussis* but this could not be confirmed by organism specific PCR followed by bidirectional sequencing. Two (2) of these specimens were positive when tested with the FilmArray and both of these specimens were found to be positive for *B. pertussis* during discrepancy investigation of confirmation testing. Two (2) source laboratory positive samples were found to contain *B. holmesii;* both of these samples gave the expected negative result when tested with the FilmArray RP.
[d] One (1) CoV 229E-negative specimen was originally identified by the source lab as positive for CoV 229E but this could not be confirmed by organism specific PCR followed by bidirectional sequencing. This specimen was positive when tested with the FilmArray RP.
[e] Four (4) CoV OC43-negative specimens were originally identified by the source lab as positive for CoV OC43 but this could not be confirmed by organism specific PCR followed by bidirectional sequencing. Two (2) of these specimens were positive when tested with the FilmArray RP.
[f] Out of 30 Enterovirus specimens received for testing, the organism status of 7 specimens could not be confirmed with organism-specific PCR and bi-directional sequencing and were excluded from further analysis.
[g] Out of 37 Flu A H1 specimens received for testing, the organism status of 5 specimens could not be confirmed with organism-specific PCR and bi-directional sequencing and were excluded from further analysis.
[h] Out of 37 Flu A H1-2009 specimens received for testing, the organism status of 2 specimens could not be confirmed with organism-specific PCR and bi-directional sequencing and were excluded from further analysis. One additional sample was excluded due to an invalid FilmArray result.
[i] Out of 58 FluA A H3 specimens received for testing, the organism status of 4 specimens could not be confirmed with organism-specific PCR and bi-directional sequencing and were excluded from further analysis.
[j] Out of 36 Flu B specimens received for testing, the organism status of 5 specimens could not be confirmed with organism-specific PCR and bi-directional sequencing and were excluded from further analysis. One additional sample was excluded due to an invalid FilmArray result.
[k] The Ct results obtained during confirmation PCR testing for the 10 samples that were not detected by FilmArray indicated low organism levels in the sample (Ct range 34.3-38.7) possible resulting from sample degradation during storage of these archived specimens.
[l] Twenty-two (22) *M. pneumoniae*-negative specimens were originally identified by the source lab as positive for *M. pneumoniae* but this could not be confirmed by organism specific PCR followed by bidirectional sequencing. Seven (7) of these specimens were positive when tested with the FilmArray RP.
[m] Out of 38 PIV1 specimens received for testing, the organism status of 3 specimens could not be confirmed with organism-specific PCR and bi-directional sequencing and were excluded from further analysis.
[n] Out of 29 PIV2 specimens received for testing, the organism status of 1 specimen could not be confirmed with organism-specific PCR and bi-directional sequencing and was excluded from further analysis.
[o] Out of 13 PIV4 specimens received for testing, the organism status of 2 specimens could not be confirmed with organism-specific PCR and bi-directional sequencing and were excluded from further analysis.

Testing of Contrived *C. pneumoniae* Specimens

Archived NPS specimens that had previously tested positive for *C. pneumoniae* were unavailable for testing. Therefore, contrived *C. pneumoniae* specimens were used as surrogate clinical specimens to test the sensitivity and specificity of the FilmArray RP *C. pneumoniae* assay. Residual specimens that had been collected during the prospective clinical evaluation were spiked with *C. pneumoniae* at clinically relevant levels (or unspiked; 50 of each). The organism status of each contrived specimen was blinded to the users testing the specimens.

Table 20. FilmArray RP Contrived *C. pneumoniae* Specimen Performance Data

Organism	Positive Percent Agreement (PPA)	Negative Percent Agreement (NPA)

	TP/TP +FN	Percent	95% CI	TN/TN+FP	Percent	95% CI
C. pneumoniae	50/50	100%	92.9 – 100%	50/50	100%	92.9 – 100%

Clinical Comparison Study (Modified FilmArray RP as Compared to Original FilmArray RP)

The original FilmArray RP was modified by the addition of a second Adenovirus assay to improve Adenovirus detection by the device. To demonstrate performance of the modified FilmArray RP, a comparison study was performed by testing 222 de-identified archived nasopharyngeal swab specimens collected between 2008 and 2011 throughout the U.S. (at least 8 geographically distinct locations) and Scotland (at least 1 location) with both the original FilmArray RP and modified FilmArray RP. A total of 26 Adenovirus specimens were detected by the modified FilmArray RP, of these only 15 were detected by the original FilmArray RP, demonstrating a 73% greater detection rate by the modified FilmArray RP in this clinical comparison study. For the other 19 organisms on the panel, performance appeared to be equivalent between the original and modified FilmArray RP versions.

Table 21. Performance Comparison of the Modified FilmArray RP to the Original FilmArray RP using Archived Specimens

Analyte	Positive Agreement				Negative Agreement			
	orig + mod +	orig + mod -	PPA	95% CI	orig - mod -	orig - mod +	NPA	95% CI
Adenovirus	15	0	100% (15/15)	78.2 – 100%	196	11 [a]	94.7% (196/207)	90.7 – 97.3%
CoV 229E	6	0	100% (6/6)	54.1 – 100%	216	0	100% (216/216)	98.3 – 100%
CoV HKU1	8	0	100% (8/8)	63.1 – 100%	214	0	100% (214/214)	98.3 – 100%
CoV NL63	15	1 [b]	93.8% (15/16)	69.8 – 99.8%	206	0	100% (206/206)	98.2 – 100%
CoV OC43	13	0	100% (13/13)	75.3 – 100%	208	1 [c]	99.5% (208/209)	97.4– 100%
hMPV	10	0	100% (10/10)	69.2 – 100%	209	3 [d]	98.6% (209/212)	95.9 – 99.7%
HRV/EV	57	4 [e]	93.4% (57/61)	84.0 – 98.2%	158	3 [e]	98.1% (158/161)	94.6 – 99.6%
Flu A	36	0	100% (36/36)	90.3 – 100%	184	1 [f]	99.5% (184/185)	97.0 – 100%
Flu A H1	9	0	100% (9/9)	66.4 – 100%	213	0	100% (213/213)	98.3 – 100%
Flu A H1-2009	15	0	100% (15/15)	78.2 – 100%	205	1 [f]	99.5% (205/206)	97.3 – 100%
Flu A H3	13	0	100% (13/13)	75.3 – 100%	209	0	100% (209/209)	98.3 – 100%
Flu B	10	0	100% (10/10)	69.2 – 100%	212	0	100% (212/212)	98.3 – 100%
RSV	21	0	100% (21/21)	83.9 – 100%	201	0	100% (201/201)	98.2 – 100%
PIV1	11	0	100% (11/11)	71.5 – 100%	211	0	100% (211/211)	98.3 – 100%
PIV2	8	0	100% (8/8)	63.1 – 100%	214	0	100% (214/214)	98.3 – 100%
PIV3	18	0	100% (18/18)	81.5 – 100%	204	0	100% (204/204)	98.2 – 100%
PIV4	6	0	100% (6/6)	54.1 – 100%	214	2 [g]	99.1% (214/216)	96.7 – 99.9%
B. pertussis	25	1 [h]	96.2% (25/26)	80.4 – 99.9%	196	0	100% (196/196)	98.1 – 100%

Analyte	Positive Agreement				Negative Agreement			
	orig + mod +	orig + mod -	PPA	95% CI	orig - mod -	orig - mod +	NPA	95% CI
C. pneumoniae	1	0	100% (1/1)	n/a	221	0	100% (221/221)	98.3 – 100%
M. pneumoniae	0	0	n/a	n/a	222	0	100% (222/222)	98.4 – 100%

orig = original FilmArray RP, mod = modified FilmArray RP, PPA = positive percent agreement, NPA = negative percent agreement, CI = confidence interval

[a] 10/11 of the additional AdV detections by the modified FilmArray RP were confirmed to contain AdV by bi-directional sequence analysis; these AdV were identified by sequencing as AdVC2, AdVC5, AdVC6, AdVE4, and one undetermined serotype. One specimen could not be sequenced due to low analyte levels.

[b] A single specimen was found to be positive for CoV NL63 when tested with the original RP pouch but not the modified RP pouch. The specimen had previously been identified as positive for *B. pertussis* and had not been tested for CoV NL63 by the source laboratory. This specimen previously tested using the original pouch was negative for CoV NL63 . There was insufficient specimen for discrepancy investigation. Low viral load is suspected to have caused this spurious result.

[c] The Coronavirus OC43 discrepancy was due to cross-reactivity between the OC43 assay and HKU1 virus that was detected in the RP modified pouch and not in the original RP pouch.

[d] 2/3 human Metapneumovirus discrepant specimens were confirmed by bi-directional sequence analysis. Low viral load is suspected to have prevented detection by sequencing for the other specimen.

[e] 0/7 Human Rhinovirus/Enterovirus discrepant specimens were confirmed by bi-directional sequence analysis. Low viral load is suspected to have prevented detection by sequencing.

[f] A single specimen containing Influenza A H1-2009 provided repeated equivocal results on the original RP pouch, but was detected by the modified pouch.

[g] Parainfluenza Virus 4 was confirmed in both discrepant specimens by bi-directional sequence analysis.

[h] *B. pertussis* was confirmed in the discrepant specimen by bi-directional sequence analysis.

To supplement the archived specimen data for low prevalence organisms and to provide additional Adenovirus performance data specifically for AdVC2 and AdVC6, a set of 44 contrived (spiked NPS) specimens (10 AdVC2, 10 AdVC6, 10 *C. pneumoniae*, and 14 *M. pneumoniae*) was tested with both the modified FilmArray RP and the original FilmArray RP. The organism status of each contrived specimen was blinded to the users analyzing the specimens. Modified FilmArray RP detected all 20 Adenovirus-spiked specimens, while original FilmArray RP detected none of the Adenovirus-spiked specimens. Modified FilmArray RP also detected another Adenovirus in the background of a specimen spiked with *C. pneumoniae*. Performance appeared to be equivalent between modified FilmArray RP and original FilmArray RP for *C. pneumoniae* and *M. pneumoniae*.

Table 22. Clinical Comparison Data of the Modified FilmArray RP to the Original FilmArray RP for 44 Contrived Specimens

Organism	Positive Agreement				Negative Agreement			
	orig + mod +	orig + mod -	PPA	95% CI	orig - mod -	orig - mod +	NPA	95% CI
Adenovirus (AdVC2 and AdVC6)	0	0	n/a	n/a	23	21[a]	52.3% (23/44)	36.7 – 67.5%
C. pneumoniae	8	1	88.9% (8/9)	51.8 – 99.7%	35	0	100% (35/35)	90.0 – 100%
M. pneumoniae	14	0	100% (14/14)	76.8 – 100%	30	0	100% (30/30)	88.4 – 100%

orig = original FilmArray RP, mod = modified FilmArray RP, PPA = positive percent agreement, NPA = negative percent agreement, CI = confidence interval

[a] In addition to ten specimens spiked with AdVC6 and ten specimens spiked with AdVC2, modified FilmArray RP also detected an Adenovirus in the background of one specimen that had been spiked with *C. pneumoniae*. This detection was confirmed by bi-directional sequence analysis to be AdVC2.

The Adenoviruses detected in archived specimens were categorized into serotype groups using bi-directional sequence analysis. Combined, the archived and contrived specimen comparison data demonstrates improved detection of AdVC2, AdVC5, AdVC6, AdVE4 by modified FilmArray RP as compared to original FilmArray RP.

Table 23. Adenovirus Serotype Detections by the Modified FilmArray RP and the Original FilmArray RP in Archived and Contrived Specimens

Adenovirus (Serotyped by PCR)	Number of Adenovirus-positive Specimens (as Detected by Modified FilmArray RP)	Detections by Original FilmArray RP
AdVC1	8	8/8 (100%)
AdVC2	15 [a]	2/15 (13%)
AdVC5	2	1/2 (50%)
AdVC6	16 [a]	1/16 (6%)
AdVB3	1	1/1 (100%)
AdVE4	3	2/3 (67%)
AdV serotype unknown	2	0/2 (0%)

[a] Ten AdVC2 specimens and ten AdVC6 specimens were contrived by spiking these viruses into NPS specimens. One AdVC2 was also detected and sequence confirmed in the background of a specimen spiked with *C. pneumoniae.*

Limit of Detection

The analytical sensitivity or Limit of Detection (LoD) for FilmArray RP organisms was determined by testing limiting dilutions of quantified cultures or clinical specimens. LoD is defined as the lowest concentration at which the organism is consistently detected (detection in ≥95% of samples tested). Simulated NPS sample matrix (cultured human cells in VTM) was spiked with one or more organisms and at least 20 replicates were tested at the estimated LoD concentration. The confirmed LoD for each FilmArray RP organism (determined with the original FilmArray RP unless otherwise noted) is listed in Table 24.

Table 24. LoD for Organisms Detected by FilmArray RP

Organism	Strain	LoD Concentration
Adenovirus[a]	AdVC1	100 $TCID_{50}$/mL
	AdVC2	
	AdVE4	
	AdVC6	
Coronavirus 229E	ATCC VR-740	4 $TCID_{50}$/mL
Coronavirus HKU1[b]	Clinical Specimen [b]	1.9 x 10^6 RNA copies/mL
Coronavirus NL63	NR-470	5 $TCID_{50}$/mL
Coronavirus OC43	ATCC VR-759	600 $TCID_{50}$/mL
Enterovirus	Echovirus 6	30,000 $TCID_{50}$/mL[c]
Human Metapneumovirus	hMPV-16, IA10-2003 (Type A1)	2 $TCID_{50}$/mL
Human Rhinovirus	Type 1A	1 $TCID_{50}$/mL
Influenza A H1N1	A/Brisbane/59/07	200 $TCID_{50}$/mL
	A/New Caledonia/20/99	2,000 $TCID_{50}$/mL
Influenza A H1-2009	A/SwineNY/03/2009	100 $TCID_{50}$/mL

Organism	Strain	LoD Concentration
Influenza A H3N2	A/Wisconsin/67/2005	5 $TCID_{50}$/mL
	A/Port Chalmers/1/73	50 $TCID_{50}$/mL
Influenza B	B/FL/04/06	60 $TCID_{50}$/mL
	B/Taiwan/2/62	
Parainfluenza Virus 1	Type 1	500 $TCID_{50}$/mL
Parainfluenza Virus 2	Type 2	10 $TCID_{50}$/mL
Parainfluenza Virus 3	Type 3	10 $TCID_{50}$/mL
Parainfluenza Virus 4	Type 4 (subtype A)	5,000 $TCID_{50}$/mL
Respiratory Syncytial Virus	Type A	2 $TCID_{50}$/mL
Bordetella pertussis	A639	4,000 CFU/mL
Chlamydophila pneumoniae	TW183	3,000 DNA copies/mL
Mycoplasma pneumoniae	M129 (Type 1)	30 $TCID_{50}$/mL

[a] LoD for Adenovirus serotypes was confirmed with 20 replicates using the modified FilmArray RP. LoD was decreased from 300 $TCID_{50}$/mL with the original panel to 100 $TCID_{50}$/mL with the modified panel. Analytical sensitivity for all other organisms was determined to be equivalent between the original and modified FilmArray RP.

[b] Coronavirus HKU1 was evaluated using a clinical specimen containing a high load of Coronavirus HKU1. The specimen was quantified by real-time PCR against a standard curve of synthetic Coronavirus HKU1 RNA transcript to obtain quantification of the viral nucleic acid in specimen (RNA copies/mL).

[c] The Enterovirus LoD (30,000 $TCID_{50}$/mL) represents the sensitivity of the Entero1 and Entero2 assays. A positive Human Rhinovirus/Enterovirus result can be obtained at 100-fold lower concentrations (300 $TCID_{50}$/mL) based on detection of the virus with a combination of 6 relevant assays (HRV1-4, Entero1, and Entero2).

NOTE: Most viruses were re-grown and quantified in $TCID_{50}$ (50% Tissue Culture Infectious Dose) for LoD determination. The unit $TCID_{50}$ is a measure of infectivity or cytotoxicity rather than number of organisms or copies of nucleic acid. Variability in $TCID_{50}$/mL may not accurately reflect differences in the relative sensitivity of detection between different organisms or different strains of the same organism.

Analytical Reactivity (Inclusivity)

The analytical reactivity of the FilmArray RP assays was evaluated with an inclusivity panel consisting of strains/isolates that represent the genetic, temporal, and geographic diversity of the FilmArray RP: 33 Adenovirus, 6 Coronavirus (1 229E, 3 HKU1, 1 OC43, and 1 NL63), 10 human Metapneumovirus, 12 Enterovirus, 14 Rhinovirus, 22 Influenza A (including 10 Influenza A H1, 3 Influenza A H1-2009, and 9 Influenza A H3), 11 Influenza B, 12 Parainfluenza Virus (3 PIV1, 2 PIV2, 3 PIV3, and 4 PIV4), 6 Respiratory Syncytial Virus, 9 *Bordetella pertussis*, 4 *Chlamydophila pneumoniae*, and 9 *Mycoplasma pneumoniae*. Each strain was initially tested in a simulated NPS sample matrix at or near the LoD (using the original FilmArray RP unless otherwise noted). Higher concentrations were tested if the organism was not detected at LoD. Each of the 148 strains tested in this study were detected by the FilmArray RP.

For some organisms, additional analysis of clinical data and *in silico* analysis of NCBI database sequences was carried out to supplement the testing of the inclusivity panel.

Adenovirus

Adenovirus inclusivity testing was performed on 33 Adenovirus isolates representing 22 different serotypes within species A-F. Each isolate was tested at LoD concentration (100 $TCID_{50}$/mL) using the modified FilmArray RP. If an Adenovirus isolate was not detected at LoD, testing was repeated at 10x LoD. Isolates not detected at 10x

LoD (1,000 $TCID_{50}$/mL) are listed as Not Detected, however, some reactivity may be observed with these serotypes if present in a sample at high levels.

Table 25 provides inclusivity results for respiratory serotypes belonging to species B, C, and E. The FilmArray RP was designed to detect all respiratory species/serotypes of Adenovirus.

AdVB55 and AdVC57 are the only respiratory serotypes that were not evaluated for inclusivity. Available sequence information for these serotypes indicates a perfect match to FilmArray Adenovirus assay primers and efficient detection at 1x LoD is predicted.

Table 25. Results of Inclusivity Testing for Adenovirus - Respiratory Serotypes

Species	Serotype	Isolate	Concentration Detected	Multiple of LoD Detected
B	3	Zeptometrix #0810062CF	100 $TCID_{50}$/mL	1x
	7a	Zeptometrix #0810021CF	100 $TCID_{50}$/mL	1x
	7d2	Iowa/2001	100 $TCID_{50}$/mL	1x
	7h	Iowa/1999	100 $TCID_{50}$/mL	1x
	11	Wisconsin/2005	100 $TCID_{50}$/mL	1x
	14	Missouri/2005	100 $TCID_{50}$/mL	1x
	16	ATCC VR-17	100 $TCID_{50}$/mL	1x
	21	Missouri/2005	100 $TCID_{50}$/mL	1x
	34	Texas/2005	100 $TCID_{50}$/mL	1x
	35	ATCC VR-718	100 $TCID_{50}$/mL	1x
	50	ATCC VR-1602	100 $TCID_{50}$/mL	1x
C	1	Zeptometrix #0810050CF	100 $TCID_{50}$/mL	1x
	2	New York/2004	100 $TCID_{50}$/mL	1x
		ATCC VR-846	100 $TCID_{50}$/mL	1x
		Clinical isolate #266153	100 $TCID_{50}$/mL	1x
		Clinical isolate #266161	100 $TCID_{50}$/mL	1x
		Clinical isolate #266213	100 $TCID_{50}$/mL	1x
	5	Zeptometrix #0810020CF	100 $TCID_{50}$/mL	1x
	6	Colorado/2005	100 $TCID_{50}$/mL	1x
		ATCC VR-6	100 $TCID_{50}$/mL	1x
		Clinical isolate #274924	100 $TCID_{50}$/mL	1x
		Clinical isolate #274948	100 $TCID_{50}$/mL	1x
		Clinical isolate #275032	100 $TCID_{50}$/mL	1x
E	4a	South Carolina/2004	100 $TCID_{50}$/mL	1x
	4p3	New Jersey/2005	100 $TCID_{50}$/mL	1x

Table 26 provides inclusivity results for non-respiratory serotypes belonging to species A, D, and F. The sensitivity of detection for non-respiratory adenoviruses will vary.

Table 26. Results of Inclusivity Testing for Adenovirus - Non-Respiratory Serotypes

Species	Serotype	Isolate	Concentration Detected	Multiple of LoD Detected
A	12	ATCC VR-863	Not Detected[a]	
	18	ATCC VR-19	Not Detected[a]	
	31	Zeptometrix #0810073CF	1000 $TCID_{50}$/mL	10x

D[b]	8	Zeptometrix #0810069CF	100 $TCID_{50}$/mL	1x
	20	Zeptometrix #0810115CF	100 $TCID_{50}$/mL	1x
	37	Zeptometrix #0810119CF	100 $TCID_{50}$/mL	1x
F	40	Zeptometrix #0810084CF	Not Detected[a]	
	41	Indiana/2004	100 $TCID_{50}$/mL	1x

[a] Adenovirus serotypes AdVA12, AdVA18 and AdVF40 were not detected when tested at 10x LoD ($TCID_{50}$/mL).
[b] Adenovirus species D is comprised of over 30 different serotypes.

Supplemental Adenovirus Reactivity Information (clinical data from original and modified FilmArray RP):

A subset of prospective and retrospective archived clinical specimens that were FilmArray RP positive for Adenovirus was subjected to PCR and bi-directional sequence analysis. BLAST analysis of the sequence data obtained for a region of the Adenovirus hexon gene identified species B (serotypes 3, 3+11p, 7, 16 and 21), species C (serotypes 1, 2, 5, and 6), and species E (serotype 4) in these clinical specimens. Species A, D, F, and G (often associated with conjunctivitis and gastroenteritis) were not identified in clinical specimens.

Coronaviruses 229E, HKU1, OC43 and NL63

Table 27. Results of Inclusivity Testing for Coronaviruses

Coronavirus	Strain / Isolate	Concentration Detected	Multiple of LoD Detected
229E	ATCC VR-740	4 $TCID_{50}$/mL	1x
HKU1	Clinical Specimen #1120	2.08×10^6 RNA copies/mL[b]	1.1x
	Clinical Specimen #6123	1.41×10^4 $TCID_{50}$/mL[a] ~1.9×10^6 RNA copies/mL[b]	1x
	Clinical Specimen #6213 (Type B)[c]	1.9×10^6 RNA copies/mL[b]	1x
NL63	BEI Resources NR-470	50 $TCID_{50}$/mL	1x
OC43	ATCC VR-759	600 $TCID_{50}$/mL	1x

[a] Virus contained in Clinical Specimen #6123 was grown in culture and quantified ($TCID_{50}$/mL) by infectivity assay.
[b] Quantification of the viral RNA contained in clinical specimens containing Coronavirus HKU1 was performed using real-time RT-PCR against a standard curve generated from a synthetic RNA template.
[c] Classification of Coronavirus HKU1 Clinical Specimen #6213 (Type B) was determined by bi-directional sequence analysis of the nucleocapsid (N) gene.

Human Metapneumovirus

Table 28. Results of Inclusivity Testing for Human Metapneumovirus

Subtype	Strain ID		Concentration Detected	Multiple of LoD Detected
A1	**9**	IA3-2002	2 $TCID_{50}$/mL	1x
	16	IA10-2003	2 $TCID_{50}$/mL	1x

Subtype	Strain ID		Concentration Detected	Multiple of LoD Detected
A2	**20**	IA14-2003	2 $TCID_{50}$/mL	1x
	27	IA27-2004	2 $TCID_{50}$/mL	1x
B1	**3**	Peru2-2002	2 $TCID_{50}$/mL	1x
	5	Peru3-2003	2 $TCID_{50}$/mL	1x
	13	IA7-2003	2 $TCID_{50}$/mL	1x
	18	IA18-2003	2 $TCID_{50}$/mL	1x
B2	**8**	Peru6-2003	2 $TCID_{50}$/mL	1x
	22	IA16-2003	2 $TCID_{50}$/mL	1x

Human Rhinovirus and Enterovirus

Analytical inclusivity testing evaluated the reactivity of the FilmArray RP HRV and Entero assays with 25 individual serotypes representing various species, including Enterovirus species A-D and Human Rhinovirus species A and B.

Table 29. Results of inclusivity Testing for Human Rhinovirus / Enterovirus

Species	Strain / Serotype	Concentration Detected	Multiple of LoD[a] Detected
Enterovirus A	Coxsackievirus A10 ATCC VR-168	30,000 $TCID_{50}$/mL	1x
	Enterovirus 71 ATCC VR-1432	1:30,000 dilution of stock	n/a
	Enterovirus 71	9,400 $TCID_{50}$/mL [b]	<1x
Enterovirus B	Coxsackievirus A9	9,400 $TCID_{50}$/mL [b]	<1x
	Coxsackievirus B3	30,000 $TCID_{50}$/mL	1x
	Coxsackievirus B4	30,000 $TCID_{50}$/mL	1x
	Echovirus 6	30,000 $TCID_{50}$/mL	1x
	Echovirus 9	9,400 $TCID_{50}$/mL [b]	<1x
	Echovirus 11	300,000 $TCID_{50}$/mL	10x
Enterovirus C	Coxsackievirus A21 /Kuykendall ATCC VR-850	30,000 $TCID_{50}$/mL	1x
	Coxsackievirus A24 DN-19 ATCC VR-583	30,000 $TCID_{50}$/mL	1x
Enterovirus D	Enterovirus 68 (F02-3607 corn) ATCC VR-1197	30,000 $TCID_{50}$/mL	1x
Rhinovirus A	**A1**	1 $TCID_{50}$/mL	1x
	A2 (HGP) ATCC VR-482	10 $TCID_{50}$/mL	10x
	A7 (68-CV11) ATCC VR-1601	1 $TCID_{50}$/mL	1x
	A16 (11757) ATCC VR-283	10 $TCID_{50}$/mL	10x

Species	Strain / Serotype	Concentration Detected	Multiple of LoD[a] Detected
	A34 (137-3) ATCC VR-507	1 $TCID_{50}$/mL	1x
	A57 (Ch47) ATCC VR-1600	100 $TCID_{50}$/mL	100x
	A77 (130-63) ATCC VR-1187	1 $TCID_{50}$/mL	1x
	A85 (50-525-CV54) ATCC VR-1195	10 $TCID_{50}$/mL	10x
Rhinovirus B	**B3** (FEB) ATCC VR-483	1 $TCID_{50}$/mL	1x
	B14 (1059) ATCC VR-284	1 $TCID_{50}$/mL	1x
	B17 (33342) ATCC-282	100 $TCID_{50}$/mL	100x
	B27 (5870) ATCC VR-1137	1 $TCID_{50}$/mL	1x
	B42 (56822) ATCC VR-338	10 $TCID_{50}$/mL	10x
	B83 (Baylor 7) ATCC VR-1193	1 $TCID_{50}$/mL	1x

[a] The LoD for Enterovirus used for this study was 30,000 $TCID_{50}$/mL. The LoD for Rhinovirus was 1 $TCID_{50}$/mL.
[b] Strains were tested below the Enterovirus LoD concentration due to a lesser concentration of virus in the culture fluid.

Supplemental Human Rhinovirus/Enterovirus Reactivity Information (clinical data and *in silico* analyses):

In addition to the analytical inclusivity testing, BLAST analysis was performed on sequence data obtained from prospective and retrospective archived clinical specimens that were FilmArray positive for Human Rhinovirus/Enterovirus. The following species and subtypes were identified in clinical specimens:

Enterovirus Species A:	Enterovirus serotype 71
	Coxsackievirus A2 and A6
Enterovirus Species B:	Echovirus serotypes 3, 6, 7, 11, 15, 21 and 30
	Coxsackievirus B1, B3 and A9
	Enterovirus serotypes 81 and 88
Rhinovirus Species A:	Human Rhinovirus serotypes 1B, 8, 9,10, 13, 19, 21, 22, 23, 28, 30, 32, 34, 38, 39, 40, 46, 47, 49, 51, 54, 56, 58, 59, 61, 62, 66, 68, 75, 77, 78, 80, 82, 98 and 100
Rhinovirus Species B:	Human Rhinovirus serotypes 27, 69, 83 and 91
Rhinovirus Species C:	at least 3 individual strains and 12 distinct isolates[a]

[a]Human Rhinovirus species C (also known as Enterovirus species D) has not been classified into serotypes.

Enterovirus species C includes Poliovirus types 1-3. Simulated reactivity of the FilmArray RP Human Rhinovirus/Enterovirus assays with Enterovirus Species C Poliovirus sequences was generated using a

bioinformatics approach. Alignment of assay primer sequences with the listed GenBank sequences indicates the FilmArray RP assay can react with Poliovirus types 1, 2 and 3, giving a Human Rhinovirus/Enterovirus result.

Table 30. Simulated Reactivity of the FilmArray RP HRV and Entero Assays with Poliovirus Sequences

Strain	GenBank ID	Simulated FilmArray RP Result
Human poliovirus 1 strain CHN8264c/GZ/CHN/2004	FJ769385	Human Rhinovirus/Enterovirus
Human poliovirus 2, complete genome	AY177685	Human Rhinovirus/Enterovirus
Human poliovirus 3 strain IRA10853, complete genome	EU684056	Human Rhinovirus/Enterovirus

Influenza A

Table 31. Results of Inclusivity Testing for Human Isolates of Influenza A

Type	Strain	Concentration Detected	Multiple of LoD Detected
Influenza A H1	A/Brisbane/59/07	200 $TCID_{50}$/mL	1x
	A/Solomon Islands/3/2006	200 $TCID_{50}$/mL	1x
	A/Hawaii/15/01 CDC#2001701117	1:300[a]	n/a
	A/New Caledonia/20/99	200 $TCID_{50}$/mL	1x
	A1/Denver/1/57	200 $TCID_{50}$/mL	1x
	A/Mal/302/54	200 $TCID_{50}$/mL	1x
	A1/FM/1/47	200 $TCID_{50}$/mL	1x
	A/Weiss/43	200 $TCID_{50}$/mL	1x
	A/PR/8/34	2000 $TCID_{50}$/mL	10x
	A/NWS/33	200 $TCID_{50}$/mL	1x
Influenza A H1-2009	A/SwineNY/01/2009	100 $TCID_{50}$/mL	1x
	A/SwineNY/02/2009	100 $TCID_{50}$/mL	1x
	A/SwineNY/03/2009	100 $TCID_{50}$/mL	1x
Influenza A H3	A/Brisbane/10/07	5 $TCID_{50}$/mL	1x
	A/Wisconsin/67/2005	5 $TCID_{50}$/mL	1x
	A/NewYork/55/2005 CDC#2005705561	1:300,000 [a]	n/a
	A/Victoria/3/75	5 $TCID_{50}$/mL	1x
	A/Port Chalmers/1/73	5 $TCID_{50}$/mL	1x
	A/Aichi/2/68	50 $TCID_{50}$/mL	10x
	A/Hong Kong/8/68	5 $TCID_{50}$/mL	1x
	Alice (vaccine) A/England/42/72 ATCC VR-776	5 $TCID_{50}$/mL	1x

Type	Strain	Concentration Detected	Multiple of LoD Detected
	MRC-2 Recombinant strain ATCC VR-777	5 $TCID_{50}$/mL	1x

[a] Strain was not re-cultured and titered. Dilutions of the original culture/titer from the CDC were used. The HA titer for A/Hawaii/15/01 is unknown and the HA titer for A/NewYork/55/2005 was 256.

Supplemental Reactivity Information for Influenza Strains of Human, Swine and Avian Origin (analytical testing data and *in silico* analyses):

Influenza A viruses are classified by host and subtype, a combination of H (hemagglutinin) and N (neuraminidase) types. Occasionally, non-human Influenza strains are transferred to human hosts. Several strains of swine and avian influenzas were tested or analyzed using bioinformatics for reactivity with the Influenza A and subtyping assays contained in the FilmArray RP. The Influenza A assays (FluA-pan1 and FluA-pan2) were designed to react with all strains of Influenza A. FilmArray RP Influenza A subtyping assays (FluA-H1-pan, FluA-H1-2009 and FluA-H3) were designed to react with specific HA subtypes, with a focus on human host strains. The FluA-H1-2009 assay was designed to be specific for the human pandemic variant of swine origin H1N1 that emerged in 2009.

Testing of viral isolates or nucleic acid from viral culture indicate that the FilmArray RP Influenza A and subtyping assays react with some strains of swine and avian origin as expected (Table 32)

Table 32. Results of Inclusivity Testing with Swine and Avian Isolates of Influenza A

Host	Subtype	Isolate / Strain	Test Concentration	FilmArray Result
Swine	H1N1	Influenza A/Swine/1976/31	~1 x 10^6 EID_{50}/mL	Influenza A H1[a]
	H1N1	Influenza A/Swine/Iowa/15/30	~1 x 10^7 EID_{50}/mL	
Avian	H1N2	Kilbourne F63 A/NWS/34 (HA) x A/Rockefeller Institute/5/57 (NA)[b]	14.8 ng RNA	Influenza A H1

[a] No reactivity was observed with the H1-2009 subtyping assay or other FilmArray RP assays.
[b] Purified and quantified RNA from avian influenza culture was obtained from BEI Resources.

Laboratory testing of Influenza A strains was supplemented with *in silico* predictions of reactivity using bioinformatics and sequence alignments between FilmArray RP assay primers and sequences for Influenza A strains of human, swine, and avian origin. For each strain, multiple (3) GenBank IDs were evaluated, corresponding to the gene segments targeted by the FilmArray RP assays (matrix (MA), non-structural (NS) and hemagglutinin (HA)). Simulated reactivity was determined based on the number and location of mismatches in the targeted region.

Based on this analysis, the strains listed in Table 33 are predicted to react with the FilmArray RP Influenza A and H1 or H3 subtyping assays as indicated.

Table 33. Simulated Reactivity of FilmArray Influenza A assays with Human, Swine, and Avian Influenza Strains

Host	Subtype	Isolate / Strain	GenBank ID	Simulated FilmArray Result
Human	H1N1	A/California/UR06-0393/2007(H1N1)	CY026540	Influenza A H1
			CY026543	
			CY026539	
	H1N2	A/New York/297/2003(H1N2)	CY002668	
			CY002665	
			CY002664	
	H1-2009	A/Aalborg/INS133/2009(H1N1)	CY063606	Influenza A H1-2009
			CY063610	
			CY063607	
	H3N2	A/Boston/38/2008(H3N2)	CY044581	Influenza A H3
			CY044584	
			CY044580	
	A/(H3N2)v[a]	A/Pennsylvania/09/2011(H3N2)	JN655537	
			JN655534	
			JN655538	
Swine	H1N1	A/swine/Wisconsin/1/1971(H1N1)	CY022414	Influenza A H1
			CY022417	
			CY022413	
	H1N2	A/swine/Hong Kong/NS857/2001(H1N2)	GQ229348	
			GQ229350	
			GQ229347	
Avian	H3N1	A/blue-winged teal/ALB/452/1983(H3N1)	CY004635	Influenza A H3
			CY004638	
			CY005940	
	H3N5	A/mallard/Netherlands/2/1999(H3N5)	CY060261	
			CY060264	
			CY060265	
	H3N7	A/northernshoveler/California/HKWF1367/2007(H3N7)	CY033372	
			CY033375	
			CY033376	
	H3N8	A/American black duck/Washington/699/1978(H3N8)	GU052299	
			GU052302	
			GU052300	

[a]Swine-origin variant H3N2 virus identified in human clinical specimens in 2011. All available GenBank sequences (as of Dec. 12, 2011) were evaluated and a single representative sequence is provided in this table. All isolates for which sequences are available are predicted to generate an Influenza A H3 result when tested with the FilmArray RP.

The strains of human, swine or avian origin listed in Table 34 are predicted to react only with the FilmArray RP Influenza A (FluA-pan1 and FluA-pan2) assays, giving an Influenza A (no subtype detected) result.

Table 34. Simulated Reactivity of FilmArray Influenza A assays with Human, Swine, and Avian Influenza Strains

Host	Subtype	Strain	GenBank ID	Simulated FilmArray RP Reactivity
Human	H2N2	A/Albany/20/1957(H2N2)	CY022013	Influenza A (no subtype detected)
			CY022014	
			CY022017	
	H5N1	A/Cambodia/R0405050/2007(H5N1)	HQ200572	
			HQ200573	
			FJ225472	
		A/Hong Kong/486/97(H5N1)	AF084281	
			AF255368	
			AF115289	
	H7N2	A/New York/107/2003(H7N2)	EU587368	
			EU587373	
			EU587374	
	H7N3	A/Canada/rv504/2004(H7N3)	CY015006	
			CY015007	
			CY015010	
	H7N7	A/Netherlands/219/03(H7N7)	AY340089	
			AY342422	
			AY338459	
	H9N2	A/Hong Kong/1073/99(H9N2)	AJ404626	
			AJ278647	
			AJ278649	
Swine	H1N2	A/swine/Sweden/1021/2009(H1N2)	GQ495135	
			GQ495136	
			GQ495132	
	H5N1	A/swine/East Java/UT6010/2007(H5N1)	HM440124	
			HM440111	
			HM440123	
Avian	H2N2	A/chicken/New York/13828-3/1995(H2N2)	CY014822	
			CY014825	
			CY014821	
		A/Japan/305/1957(H2N2)	CY045804	
			CY014977	
			CY014980	
		A/Korea/426/1968(H2N2)	CY031595	
			CY031596	
			CY031599	
	H3N2	A/American black duck/North Carolina/675-075/2004(H3N2)	GU051135	
			GU051136	
			GU051137	
	H3N6	A/American black duck/New Brunswick/25182/2007(H3N6)	CY047696	
			CY047697	
			CY047700	
	H4N6	A/blue-winged teal/Minnesota/Sg-00043/2007(H4N6)	CY063977	
			CY063978	
			CY063981	
	H5N1	A/rook/Rostov-on-Don/26/2007(H5N1)	EU814503	
			EU814504	
			EU814507	
		A/turkey/VA/505477-18/2007(H5N1)	GU186509	
			GU186510	

Host	Subtype	Strain	GenBank ID	Simulated FilmArray RP Reactivity
			GU186513	
		A/chicken/Bangladesh/1151-10/2010(H5N1)	HQ156765	
			HQ156766	
			HQ156764	
	H5N2	A/duck/Pennsylvania/10218/1984(H5N2)	AB295603	
			AB286120	
			AB286652	
	H5N3	A/duck/Singapore/F119/3/1997(H5N3)	GU052802	
			GU052803	
			GU052805	
	H6N1	A/duck/PA/486/1969(H6N1)	EU743286	
			EU743287	
			EU743289	
	H6N2	A/mallard/Czech Republic/15902-17K/2009(H6N2)	HQ244430	
			HQ244433	
			HQ244434	
	H7N7	A/mallard/Korea/GH171/2007(H7N7)	FJ750872	
			FJ959087	
			FJ959090	
	H9N2	A/turkey/Wisconsin/1/1966(H9N2)	CY014663	
			CY014664	
			CY014667	
	H10N7	A/chicken/Germany/N/1949(H10N7)	GQ176136	
			GQ176135	
			GQ176132	
	H11N9	A/duck/Memphis/546/1974(H11N9)	CY014691	
			GQ257441	
			CY014687	

Influenza B

Table 35. Results of Inclusivity Testing for Influenza B

Strain	Concentration Detected	Multiple of LoD Detected
B/FL/04/06	60 $TCID_{50}$/mL	1x
B/Ohio/01/2005 CDC#2005743348	1:3,000,000[a]	n/a
B/Florida/07/04	60 $TCID_{50}$/mL	1x
B/Malaysia/2506/04	600 $TCID_{50}$/mL	10x
B/Hong Kong/5/72 ATCC VR-823	60 $TCID_{50}$/mL	1x
B/Taiwan/2/62 ATCC VR-295	60 $TCID_{50}$/mL	1x
B/Maryland/1/59 ATCC VR-296	600 $TCID_{50}$/mL	10x
B/GL/1739/54 ATCC VR-103	60 $TCID_{50}$/mL	1x
B/Allen/45 ATCC VR-102	6,000 EID_{50}/mL	n/a
B/Lee/40ATCC VR-101	60 $TCID_{50}$/mL	1x
B/Brigit Recombinant ATCC VR-786	60 $TCID_{50}$/mL	1x

[a] Strain was not re-cultured and titered. Dilutions of the original culture/titer from the CDC were used. The HA titer for B/Ohio/01/2005 was 128.

Parainfluenza Viruses

Table 36. Results of Inclusivity Testing for Parainfluenza Viruses

Type		Strain or Source	Concentration	Multiple of LoD Detected
PIV1		Zeptometrix #0810014CF	500 $TCID_{50}$/mL	1x
		C-35 ATCC VR-94	500 $TCID_{50}$/mL	1x
		C39 BEI NR-3226	500 $TCID_{50}$/mL	1x
PIV2		Zeptometrix #0810015CF	10 $TCID_{50}$/mL	1x
		Greer ATCC VR-92	10 $TCID_{50}$/mL	1x
PIV3		Zeptometrix #0810016CF	10 $TCID_{50}$/mL	1x
		C-243 ATCC VR-93	500 $TCID_{50}$/mL	50x
		NIH 47885 BEI NR-3233	100 $TCID_{50}$/mL	10x
PIV4	**A**	M25 ATCC VR-1378	5000 $TCID_{50}$/mL	1x
		Zeptometrix #0810060CF	5000 $TCID_{50}$/mL	1x
	B	CH-19503 ATCC VR-1377	5000 $TCID_{50}$/mL	1x
		Zeptometrix #08010060BCF	5000 $TCID_{50}$/mL	1x

Respiratory Syncytial Virus

Table 37. Results of Inclusivity Testing for Respiratory Syncytial Virus

Type	Strain or Source	Concentration Detected	Multiple of LoD Detected
A	Zeptometrix #0810040ACF	2 $TCID_{50}$/mL	1x
	A/A2 ATCC VR-1540	2 $TCID_{50}$/mL	1x
	A/Long ATCC VR-26	2 $TCID_{50}$/mL	1x
B	B/9320 ATCC VR-955	2 $TCID_{50}$/mL	1x
	B/Wash18537/62 ATCC VR-1580	2 $TCID_{50}$/mL	1x
	B/WV/14617/85 ATCC VR-1400	2 $TCID_{50}$/mL	1x

Bordetella pertussis

Table 38. Results of Inclusivity Testing for *Bordetella pertussis*

Strain	Concentration Detected	Multiple of LoD Detected
A639	4000 CFU/mL	1x
E431	4000 CFU/mL	1x
F ATCC 8467	4000 CFU/mL	1x
5 [17921] ATCC 9340	4000 CFU/mL	1x
18323 [NCTC 10739] ATCC 9797	4000 CFU/mL	1x
10-536 ATCC 10380	4000 CFU/mL	1x
CNCTC Hp 12/63 [623] ATCC 51445	4000 CFU/mL	1x
Tohama I ATCC BAA-589 (vaccine strain)	4000 CFU/mL	1x
MN2531 ATCC BAA-1335	4000 CFU/mL	1x

Chlamydophila pneumoniae

Table 39. Results of Inclusivity Testing for *Chlamydophila pneumoniae*

Strain	Concentration Detected	Multiple of LoD Detected
AR-39 ATCC 53592	3000 copies/mL	1x
CDC/CWL-029 VR-1310	3000 copies/mL	1x
CM-1 ATCC VR-1360	3000 copies/mL	1x
TW183 ATCC VR-2282	3000 copies/mL	1x

Mycoplasma pneumoniae

Table 40. Results of Inclusivity Testing for *Mycoplasma pneumoniae*

Type	Strain	Concentration Detected	Multiple of LoD Detected[b]
Type 1	M129	30 $TCID_{50}$/mL	1x
	M129-B7 ATCC 29342	300 CCU/mL[a]	10x
	PI 1428 ATCC 29085	3,000 CCU/mL	100x
Type 2	FH strain [NCTC 10119] ATCC 15531	300 CFU/mL[c]	n/a
	[Mac] ATCC 15492	300 CCU/mL	10x
Not Determined	[M52] ATCC 15293	300 CCU/mL	10x
	[Bru] ATCC 15377	30,000 CCU/mL	1,000x
	Mutant 22 ATCC 39505	30 CCU/mL	1x
	UMTB-10G ATCC 49894	300 CCU/mL	10x

[a] CCU = Color Changing Unit. Both $TCID_{50}$ and CCU were determined according to the Reed-Muench method. Quantification in CCU/mL was considered equivalent to quantification in $TCID_{50}$/mL.

[b] Following inclusivity testing, all *M. pneumoniae* isolates previously quantified in CCU/mL were re-evaluated by real-time PCR against a standard curve of the LoD strain (M129, $TCID_{50}$/mL). Based on relative molecular quantification, all isolates were detected at levels <1x – 10x LoD, rather than 1-1,000x LoD determined by CCU/mL. ATCC 49894, ATCC 15293, ATCC 29342, and ATCC 39505 were detected at or below 1x LoD, while ATCC 15492, ATCC 29085, and ATCC 15377 were detected between 1-10x LoD.

[c] This was the only *M. pneumoniae* isolate in the panel able to form colonies on plates and was quantified in CFU/mL.

Analytical Specificity (Cross-Reactivity and Exclusivity)

The potential for cross-reactivity between assays contained in the FilmArray RP was evaluated by testing simulated NPS samples containing high concentrations of respiratory panel organisms (tens to thousands-fold higher than LoD). Exclusivity testing was performed with the modified FilmArray RP, except where indicated. No cross-reactivity was observed with the strains and concentrations listed in Table 41.

NOTE: The Coronavirus OC43 assay may cross-react with certain strains of Coronavirus HKU1 when present in the sample at high concentrations.

Table 41. Results of Testing for Cross-Reactivity with FilmArray RP Organisms

Virus or Bacterium	Type / Strain	Test Concentration	Multiple of LoD Tested
Adenovirus	AdVC1	$1.00x10^5$ $TCID_{50}$/mL	1,000x
Coronavirus	229E ATCC VR-740	$5.67x10^3$ $TCID_{50}$/mL	1,418x
	HKU1[a] Clinical specimen 42/08	$1.34x10^8$ copies/mL	70x
	NL63 NR-470	$5.67x10^3$ $TCID_{50}$/mL	1,134x

Virus or Bacterium	Type / Strain	Test Concentration	Multiple of LoD Tested
	OC43 ATCC VR-759	$7.30x10^4$ $TCID_{50}$/mL	122x
Human Metapneumovirus	Type A1 hMPV-16 IA10-2003 A1	$8.17x10^3$ $TCID_{50}$/mL	4,085x
Human Rhinovirus / Enterovirus	Echovirus 6	$3.40x10^6$ $TCID_{50}$/mL	113x
	Rhinovirus A1	$5.67x10^3$ $TCID_{50}$/mL	5,670x
Influenza A H1N1	A/Brisbane/59/07	$1.00x10^5$ $TCID_{50}$/mL	500x
	A/New Caledonia/20/99[b]	$1.00x10^5$ $TCID_{50}$/mL	500x
	A/PR/8/34[b]	$1.00x10^6$ $TCID_{50}$/mL	5,000x
	A1/FM/1/47[b]	$4.70x10^3$ $TCID_{50}$/mL	24x
	A/NWS/33[b]	$4.70x10^3$ $TCID_{50}$/mL	24x
	A1/Denver/1/57[b]	$4.70x10^3$ $TCID_{50}$/mL	24x
	A/Solomon Islands/3/2006 [b]	$1.39x10^4$ $TCID_{50}$/mL	70x
	A/Weiss/43[b]	$4.70x10^3$ $TCID_{50}$/mL	24x
	A/Mal/302/54[b]	$1.39x10^4$ $TCID_{50}$/mL	70x
Influenza A H1-2009	A/SwineNY/03/2009	$8.40x10^4$ $TCID_{50}$/mL	840x
Influenza A H3N2	A/Wisconsin/67/2005	$8.17x10^3$ $TCID_{50}$/mL	1634x
	A/Victoria/3/75[b]	$4.70x10^3$ $TCID_{50}$/mL	940x
	A/Port Chalmers/1/73[b]	$5.67x10^3$ $TCID_{50}$/mL	1,134x
	A/Aichi/2/68[b]	$1.00x10^5$ $TCID_{50}$/mL	20,000x
	A/Hong Kong/8/68[b]	$1.00x10^5$ $TCID_{50}$/mL	20,000x
	A/Alice[b]	$4.70x10^3$ $TCID_{50}$/mL	940x
	A/MRC 2[b]	$8.17x10^3$ $TCID_{50}$/mL	1,634x
	A/Brisbane/10/07[b]	$8.17x10^3$ $TCID_{50}$/mL	1,634x
Influenza B	B/FL/04/06	$1.67x10^4$ $TCID_{50}$/mL	278x
	B/Lee/40[b]	$8.17x10^3$ $TCID_{50}$/mL	136x
	B/Taiwan/2/62[b]	$5.03x10^4$ $TCID_{50}$/mL	838x
	B/GL/1739/54[b]	$8.17x10^3$ $TCID_{50}$/mL	136x
	B/Maryland/1/59[b]	$8.17x10^3$ $TCID_{50}$/mL	136x
	B/Florida/07/04[b]	$1.00x10^5$ $TCID_{50}$/mL	1,667x
	B/Malaysia/2506/04[b]	$5.67x10^3$ $TCID_{50}$/mL	95x
	B/Allen/45[b]	$1.00x10^5$ $TCID_{50}$/mL	1,667x
	B/HongKong/5/72[b]	$8.17x10^3$ $TCID_{50}$/mL	136x
	B/Brigit[b]	$3.50x10^4$ $TCID_{50}$/mL	583x
Parainfluenza Virus	Type 1	$1.39x10^4$ $TCID_{50}$/mL	28x

Virus or Bacterium	Type / Strain	Test Concentration	Multiple of LoD Tested
	Type 2	$1.6x10^4$ $TCID_{50}$/mL	1,670x
	Type 3	$1.00x10^5$ $TCID_{50}$/mL	10,000x
	Type 4A	$5.67x10^3$ $TCID_{50}$/mL[c]	1.13x[c]
Respiratory Syncytial Virus	A	$1.39x10^4$ $TCID_{50}$/mL	6,950x
	B	$2.14x10^4$ $TCID_{50}$/mL	10,700x
Bordetella pertussis	E431[b]	$1.00x10^6$ CFU/mL	250x
	A639		
	ATCC 8467[b]		
	ATCC 9797[b]		
	ATCC 51445[b]		
	ATCC BAA-589[b]		
	ATCC 9340[b]		
	ATCC 10380[b]		
	ATCC BAA-1335[b]		
Chlamydophila pneumoniae	TW183	$2.42x10^5$ copies/mL	81x
Mycoplasma pneumoniae	M129	$1.88x10^5$ $TCID_{50}$/mL	6,267x
	ATCC 15531[b]	$4.27x10^5$ CFU/mL	n/a
	ATCC 15293[b]	$1.00x10^6$ CCU/mL	33,333x
	ATCC 15377[b]		
	ATCC 15492[b]		
	ATCC 29085[b]		
	ATCC 29342[b]		
	ATCC 39505[b]		
	ATCC 49894[b]		

[a] Cross-reactivity was not observed between this isolate of Coronavirus HKU1 and the Coronavirus OC43 assay at the concentration tested. However, in the clinical data set, 2 specimens showed cross-reactivity between the OC43 assay and the HKU1 virus contained in the specimen.

[b] Exclusivity testing was performed with the original FilmArray RP.

[c] Parainfluenza Virus 4A could not be tested at higher concentrations due to the relatively low titer of the stock viral culture.

The potential for the FilmArray RP to cross-react with non-FilmArray RP organisms was evaluated by testing an exclusivity panel consisting of 26 bacteria, 6 viruses, and 1 yeast. These organisms were selected based on their relatedness to FilmArray RP organisms, clinical relevance (cause respiratory symptoms or represent nasopharyngeal flora), or high prevalence within the population (e.g. Herpes Simplex Virus). Negative sample matrix was spiked with bacteria or fungi at a concentration of 10^6 CFU/mL and viruses at a concentration between 10^4 - 10^5 $TCID_{50}$/mL, or the highest concentration possible. The FilmArray RP did not cross-react with the exclusivity panel organisms listed in Table 42.

Table 42. Non-FilmArray RP Exclusivity Panel – No cross-reactivity was observed with the organisms listed

Virus	**Strain / Isolate**
Bocavirus	Clinical Specimen
Cytomegalovirus (CMV)	AD-169 (VR-538)
Epstein-Barr Virus (EBV)	B95-8
Herpes Simplex Virus	Type 1
Measles Virus	Edmonston
Mumps	Zeptometrix # 0810079CF
Yeast	**Strain / Isolate**
Candida albicans	Zeptometrix #0801504
Bacterium	**Strain / Isolate**
Bordetella bronchiseptica	clinical isolate
Bordetella holmesii	F061
Bordetella parapertussis	A747
Chlamydia trachomatis	D-UW3
Corynebacterium diptheriae	ATCC14779
Escherichia coli	O157:H7
Haemophilus influenzae	MinnA
Lactobacillus acidophilus	Type strain
Lactobacillus plantarum	17-5
Legionella longbeacheae	Long Beach 4
Legionella micdadei	Tatlock
Legionella pneumophilia	Philadelphia
Moraxella catarrhalis	Ne 11 (type strain)
Mycobacterium tuberculosis	H37Ra-1
Mycoplasma hominis	ATCC 23114
Mycoplasma genitalium	ATCC 33530
Neisseria elongate	type strain
Neisseria gonorrhoeae	ATCC 700825
Neisseria meningitidis	M1027 (type strain)
Pseudomonas aeruginosa	Zeptometrix #0801519
Staphylococcus aureus	COL
Staphylococcus epidermidis	RP62A
Streptococcus pneumoniae	type 59
Streptococcus pyogenes	Zeptometrix #0801512
Streptococcus salivarius	ATCC 13419
Ureaplasma urealyticum	ATCC 27618

Supplemental Analytical Exclusivity Testing for Influenza Strains of Avian Origin:

Additional analytical exclusivity testing was carried out (using the original FilmArray RP) with either live isolates or purified genomic RNA of avian host influenza A strains with the following results:

Table 43. Results of Exclusivity Testing of Virus or Nucleic Acid from Cultures of Avian Isolates of Influenza A

Host	Subtype	Isolate / Strain	Test Concentration[a]	FilmArray Result
Avian	H2N2	A/Japan/305/57	3.3 ng RNA	Influenza A (no subtype detected)
		Kilbourne F38 A/Korea/426/68 (HA, NA) x A/Puerto Rico/8/34	6.3 ng RNA	
	H5N1	A/Vietnam/1203/2004 R-H5	N/A[b]	
	H5N2	A/duck/Pennsylvania/10218/84	2.5 ng RNA	
	H5N3	Kilbourne F181 A/duck/Singapore/645/97	247 ng RNA	
	H7N2	A/NewYork/107/2003	N/A[b]	
	H7N3	A/Mallard/Netherlands/12/2000	N/A[b]	
	H10N7	A/chicken/Germany/N/49	68 ng RNA	

[a] Purified and quantified RNA from Avian Influenza cultures was obtained from BEI Resources
[b] Stock virus HA titer from CDC = 128. Twenty microliters of virus stock tested.

Supplemental SARS Coronavirus Exclusivity Information:

In silico analysis of SARS virus sequence (GenBank ID NC_004718.3) against primers for each of the 4 FilmArray RP Coronavirus assays was conducted. When the non-SARS Coronavirus assay primers were aligned with the corresponding target sequence from SARS virus, each primer contained at least 6 mismatches. Based on alignments, no reactivity was predicted between SARS virus and the 4 FilmArray RP Coronavirus assays.

Cross-Contamination and Carryover

To evaluate cross-contamination or carryover, high positive samples (Table 41) were loaded and tested back-to-back with other high positive samples containing different organisms. No false positive results were observed, indicating that the system design and recommended sample handling practices are effective in preventing carryover.

Reproducibility

A multicenter reproducibility study was performed to determine between-site and overall reproducibility of the FilmArray RP system. Reproducibility testing occurred at three test sites utilizing the original FilmArray RP and a panel of twelve simulated NPS specimens spiked with various combinations of organisms at three different test levels: Medium Positive (3X LoD), Low Positive (1X LoD), and High Negative (LoD/10). The High Negative test level was predicted to generate positive results in approximately 20-80% of the samples tested. On each testing day, two operators at each site tested two aliquots of specimens on two different FilmArray instruments (six specimens per operator per instrument per day). Every specimen was tested four times a day on five days at the three testing sites, for a total of at least 60 tests per organism per concentration. A total of 26 lots of reagents and 20 FilmArray instruments were utilized in the reproducibility study. Positive, Equivocal, Negative results and Tm data for each organism are summarized in the tables below:

Table 44. Summary of Positive Agreement, Negative Agreement, and Tm Results from Reproducibility Testing of Single Assay Organisms

Adenovirus AdVC1		# Positive	# Negative	% Agreement with Expected Result [a]	95% CI	Mean Tm	%CV Tm	Observed Tm Range
Medium Positive (3X LoD)[c] 900 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	83.74	0.24	83.33 - 84.26
	Site B	20/20	0/20	100%	83.2% – 100%	84.23	0.21	83.95 - 84.60
	Site C	20/20	0/20	100%	83.2% – 100%	83.76	0.28	83.03 - 84.13
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**83.93**	**0.36**	**83.03 - 84.60**
Low Positive (1X LoD)[c] 300 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	83.42	0.28	83.01 - 83.98
	Site B	20/20	0/20	100%	83.2% – 100%	83.90	0.26	83.54 - 84.30
	Site C	20/20	0/20	100%	83.2% – 100%	83.62	0.38	83.05 - 84.64
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**83.64**	**0.39**	**83.01 - 84.64**
High Negative[b] (LoD/10)[c] 30 $TCID_{50}$/mL	Site A	18/20	2/20	90.0%	68.3% – 98.8%	83.33	0.26	83.02 - 83.76
	Site B	16/20	4/20	80.0%	56.3% – 94.3%	83.71	0.30	82.93 - 84.29
	Site C	10/20	10/20	50.0%	27.2% – 72.8%	83.26	0.31	82.61 - 83.86
	All Sites	**44/60**	**16/60**	**73.3%**	**60.3% – 83.9%**	**83.43**	**0.38**	**82.61 - 84.29**
Negative	Site A	0/180	180/180	100%	98.0% - 100%			
	Site B	0/180	180/180	100%	98.0% - 100%			
	Site C	0/180	180/180	100%	98.0% - 100%			
	All Sites	**0/540**	**540/540**	**100%**	**99.3% – 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.
[c] Reproducibility testing was performed prior to the modification of the panel to include a second Adenovirus assay. Test concentrations and multiples of LoD reflect the Adenovirus LoD prior to panel modification (300 $TCID_{50}$/mL).

Coronavirus 229E ATCC VR-740		# Positive	# Negative	% Agreement with Expected Result [a]	95% CI	Mean Tm	%CV Tm	Observed Tm Range
Medium Positive (3X LoD) 12 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	81.20	0.20	80.81 - 81.47
	Site B	20/20	0/20	100%	83.2% – 100%	81.80	0.32	81.35 - 82.18
	Site C	20/20	0/20	100%	83.2% – 100%	81.35	0.34	81.03 - 82.13
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**81.37**	**0.40**	**80.81 - 82.18**
Low Positive (1X LoD) 4 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	81.19	0.21	80.83 - 81.66
	Site B	20/20	0/20	100%	83.2% – 100%	81.62	0.25	81.12 - 82.08
	Site C	20/20	0/20	100%	83.2% – 100%	81.09	0.32	80.30 - 81.56
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**81.31**	**0.38**	**80.30 - 82.08**
High Negative[b]	Site A	14/20	6/20	70%	45.7% - 88.1%	81.06	0.22	80.72 - 81.43

Coronavirus 229E ATCC VR-740		**# Positive**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**	**Mean Tm**	**%CV Tm**	**Observed Tm Range**
(LoD/10) 0.4 $TCID_{50}$/mL	Site B	7/20	13/20	35%	15.4% - 59.2%	81.54	0.24	81.15 - 82.08
	Site C	11/20	9/20	55%	31.5% - 76.9%	81.17	0.24	80.70 - 81.68
	All Sites	**32/60**	**28/60**	**53.3%**	**40.0% - 66.3%**	**81.25**	**0.34**	**80.70 - 82.08**
Negative	Site A	0/180	180/180	100%	98.0% - 100%			
	Site B	0/180	180/180	100%	98.0% - 100%			
	Site C	0/180	180/180	100%	98.0% - 100%			
	All Sites	**0/540**	**540/540**	**100%**	**99.3% – 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Coronavirus HKU1 Type B Clinical Specimen 6123		**# Positive**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**	**Mean Tm**	**%CV Tm**	**Observed Tm Range**
Medium Positive (3X LoD) 5.7 x 10^6 RNA copies/mL	Site A	20/20	0/20	100%	83.2% – 100%	75.61	0.23	75.37 - 76.02
	Site B	20/20	0/20	100%	83.2% – 100%	76.02	0.26	75.58 - 76.33
	Site C	20/20	0/20	100%	83.2% – 100%	75.49	0.32	74.96 - 75.90
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**75.69**	**0.41**	**74.96 - 76.33**
Low Positive (1X LoD) 1.9 x 10^6 RNA copies/mL	Site A	20/20	0/20	100%	83.2% – 100%	75.47	0.22	74.96 - 75.79
	Site B	20/20	0/20	100%	83.2% – 100%	75.89	0.20	75.59 - 76.12
	Site C	20/20	0/20	100%	83.2% – 100%	75.35	0.30	74.83 - 75.81
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**75.55**	**0.40**	**74.83 - 76.12**
High Negative[b] (LoD/10) 1.9 x 10^5 RNA copies/mL	Site A	15/20	5/20	75.0%	50.9% - 91.3%	75.51	0.28	75.06 - 75.90
	Site B	17/20	3/20	85.0%	62.1% - 96.8%	75.85	0.22	75.26 - 76.23
	Site C	19/20	1/20	95.0%	75.1% – 99.9%	75.33	0.22	74.98 - 75.59
	All Sites	**51/60**	**9/60**	**85.0%**	**73.4% – 92.9%**	**75.55**	**0.38**	**74.98 - 76.23**
Negative	Site A	0/180	180/180	100%	98.0% - 100%			
	Site B	0/180	180/180	100%	98.0% - 100%			
	Site C	0/180	180/180	100%	98.0% - 100%			
	All Sites	**0/540**	**540/540**	**100%**	**99.3% – 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Coronavirus NL63 BEI Resources NR-470		**# Positive**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**	**Mean Tm**	**%CV Tm**	**Observed Tm Range**
Medium Positive (3X LoD) 15 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	80.21	0.31	79.64 - 80.90
	Site B	20/20	0/20	100%	83.2% – 100%	80.55	0.33	79.99 - 81.03
	Site C	20/20	0/20	100%	83.2% – 100%	80.04	0.25	79.67 - 80.62
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**80.29**	**0.40**	**79.64 - 81.03**
Low Positive (1X LoD) 5 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	80.08	0.25	79.57 - 80.42
	Site B	20/20	0/20	100%	83.2% – 100%	80.40	0.24	79.98 - 80.89
	Site C	20/20	0/20	100%	83.2% – 100%	79.88	0.27	79.36 - 80.40
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**80.12**	**0.38**	**79.14 - 80.89**
High Negative[b] (LoD/10) 0.5 $TCID_{50}$/mL	Site A	13/20	7/20	65.0%	40.8% - 84.6%	80.08	0.34	79.21 - 80.82
	Site B	14/20	6/20	70.0%	45.7% - 88.1%	80.36	0.30	79.98 - 80.91
	Site C	10/20	10/20	50.0%	27.2% - 72.8%	79.91	0.26	79.24 - 80.30
	All Sites	**37/60**	**23/60**	**61.7%**	**48.2% - 73.9%**	**80.11**	**0.39**	**79.21 - 80.91**
Negative	Site A	0/180	180/180	100%	98.0% - 100%			
	Site B	0/180	180/180	100%	98.0% - 100%			
	Site C	0/180	180/180	100%	98.0% - 100%			
	All Sites	**0/540**	**540/540**	**100%**	**99.3% – 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Coronavirus OC43 ATCC VR-759		**# Positive**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**	**Mean Tm**	**%CV Tm**	**Observed Tm Range**
Medium Positive (3X LoD) 1,800 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	80.98	0.23	80.53 - 81.36
	Site B	20/20	0/20	100%	83.2% – 100%	81.39	0.25	81.04 - 81.82
	Site C	20/20	0/20	100%	83.2% – 100%	81.20	0.26	80.60 - 81.55
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**81.17**	**0.32**	**80.53 - 81.82**
Low Positive (1X LoD) 600 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	80.82	0.26	80.29 - 81.25
	Site B	20/20	0/20	100%	83.2% – 100%	81.31	0.24	80.95 - 81.85
	Site C	20/20	0/20	100%	83.2% – 100%	81.07	0.37	80.40 - 81.58
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**81.07**	**0.38**	**80.29 - 81.85**
High Negative[b] (LoD/10) 60 $TCID_{50}$/mL	Site A	18/20	2/20	90.0%	68.3% - 98.8%	80.86	0.24	80.49 - 81.25
	Site B	14/20	6/20	70.0%	45.7% - 88.1%	81.29	0.28	80.92 - 81.81
	Site C	16/20	4/20	80.0%	56.3% - 94.3%	81.04	0.32	80.11 - 81.45

Coronavirus OC43 ATCC VR-759		**# Positive**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**	**Mean Tm**	**%CV Tm**	**Observed Tm Range**
	All Sites	**48/60**	**12/60**	**80.0%**	**67.7% - 89.2%**	**81.07**	**0.36**	**80.11 - 81.81**
Negative	Site A	0/180	180/180	100%	98.0% - 100%			
	Site B	0/180	180/180	100%	98.0% - 100%			
	Site C	1/180	179/180	99.4%	96.9% - 100%			
	All Sites	**1/540**	**539/540**	**99.8%**	**99.0% - 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Human Metapneumovirus hMPV-16 (Type A1)		**# Positive**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**	**Mean Tm**	**%CV Tm**	**Observed Tm Range**
Medium Positive (3X LoD) 6 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	77.61	0.23	77.06 - 77.90
	Site B	20/20	0/20	100%	83.2% – 100%	78.07	0.28	77.67 - 78.61
	Site C	19/20	1/20	95.0%	75.1% - 99.9%	77.73	0.21	77.45 - 78.11
	All Sites	**59/60**	**1/60**	**98.3%**	**91.1% - 100%**	**77.82**	**0.36**	**77.06 - 78.61**
Low Positive (1X LoD) 2 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	77.39	0.21	77.04 - 77.79
	Site B	20/20	0/20	100%	83.2% – 100%	77.88	0.22	77.37 - 78.11
	Site C	20/20	0/20	100%	83.2% – 100%	77.60	0.24	77.16 - 77.99
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**77.62**	**0.35**	**77.04 - 78.11**
High Negative[b] (LoD/10) 0.2 $TCID_{50}$/mL	Site A	17/20	2/20	85.0%	62.1% - 96.8%	77.34	0.20	77.05 - 77.59
	Site B	19/20	6/20	95.0%	75.1% - 99.9%	77.76	0.22	77.06 - 78.11
	Site C	12/20	4/20	60.0%	36.1% - 80.9%	77.37	0.29	76.74 - 77.79
	All Sites	**48/60**	**12/60**	**80.0%**	**67.7% - 89.2%**	**77.50**	**0.35**	**76.74 - 78.11**
Negative	Site A	0/180	180/180	100%	98.0% - 100%			
	Site B	0/180	180/180	100%	98.0% - 100%			
	Site C	0/180	180/180	100%	98.0% - 100%			
	All Sites	**0/540**	**540/540**	**100%**	**99.3% – 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Influenza B B/FL/04/06		**# Positive**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**	**Mean Tm**	**%CV Tm**	**Observed Tm Range**
Medium Positive (3X LoD) 180 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	80.47	0.26	79.88 - 80.93
	Site B	20/20	0/20	100%	83.2% – 100%	80.88	0.31	80.30 - 81.30
	Site C	20/20	0/20	100%	83.2% – 100%	80.36	0.32	79.78 - 80.80
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**80.56**	**0.40**	**79.78 - 81.30**
Low Positive (1X LoD) 60 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	80.44	0.27	80.00 - 80.92
	Site B	20/20	0/20	100%	83.2% – 100%	80.79	0.29	80.40 - 81.33
	Site C	20/20	0/20	100%	83.2% – 100%	80.34	0.22	79.77 - 80.81
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**80.51**	**0.37**	**79.77 - 81.33**
High Negative[b] (LoD/10) 6 $TCID_{50}$/mL	Site A	12/20	8/20	60.0%	36.1% - 80.9%	80.42	0.32	79.84 - 80.90
	Site B	10/20	10/20	50.0%	27.2% - 72.8%	80.78	0.25	80.40 - 81.17
	Site C	8/20	12/20	40.0%	19.1% - 64.0%	80.30	0.21	79.79 - 80.69
	All Sites	**30/60**	**30/60**	**50.0%**	**36.8% - 63.2%**	**80.50**	**0.36**	**79.79 - 81.17**
Negative	Site A	0/180	180/180	100%	98.0% - 100%			
	Site B	0/180	180/180	100%	98.0% - 100%			
	Site C	0/180	180/180	100%	98.0% - 100%			
	All Sites	**0/540**	**540/540**	**100%**	**99.3% – 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Parainfluenza Virus 1 Zeptometrix # 0810014CFN		**# Positive**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**	**Mean Tm**	**%CV Tm**	**Observed Tm Range**
Medium Positive (3X LoD) 1,500 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	78.86	0.26	78.42 - 79.25
	Site B	20/20	0/20	100%	83.2% – 100%	79.32	0.28	78.83 - 79.78
	Site C	19/20	1/20	95.0%	75.1% - 99.9%	78.50	0.28	78.02 - 78.87
	All Sites	**59/60**	**1/60**	**98.3%**	**91.1% - 100%**	**78.91**	**0.50**	**78.02 - 79.78**
Low Positive (1X LoD) 500 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	78.60	0.31	77.99 - 79.05
	Site B	20/20	0/20	100%	83.2% – 100%	78.93	0.26	78.31 - 79.36
	Site C	20/20	0/20	100%	83.2% – 100%	78.50	0.38	77.90 - 79.16
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**78.67**	**0.40**	**77.90 - 79.36**
High Negative[b] (LoD/10) 50 $TCID_{50}$/mL	Site A	15/20	5/20	75.0%	50.1% - 91.3%	78.54	0.25	78.10 - 78.94
	Site B	15/20	5/20	75.0%	50.1% - 91.3%	78.94	0.25	78.52 - 79.36
	Site C	13/20	7/20	65.0%	41.0% - 84.6%	78.41	0.35	77.87 - 79.02

Parainfluenza Virus 1 Zeptometrix # 0810014CFN		**# Positive**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**	**Mean Tm**	**%CV Tm**	**Observed Tm Range**
	All Sites	**43/60**	**17/60**	**71.7%**	**58.6% - 82.6%**	**78.61**	**0.42**	**77.79 - 79.36**
Negative	Site A	0/180	180/180	100%	98.0% - 100%			
	Site B	0/180	180/180	100%	98.0% - 100%			
	Site C	0/180	180/180	100%	98.0% - 100%			
	All Sites	**0/540**	**540/540**	**100%**	**99.3% – 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Parainfluenza Virus 2 Zeptometrix #0810015CF		**# Positive**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**	**Mean Tm**	**%CV Tm**	**Observed Tm Range**
Medium Positive (3X LoD) 30 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	83.63	0.36	83.01 - 84.39
	Site B	20/20	0/20	100%	83.2% – 100%	84.06	0.40	83.44 - 84.79
	Site C	20/20	0/20	100%	83.2% – 100%	83.88	0.32	83.13 - 84.28
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**83.85**	**0.42**	**83.01 - 84.79**
Low Positive (1X LoD) 10 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	83.56	0.28	82.94 - 84.08
	Site B	20/20	0/20	100%	83.2% – 100%	84.00	0.31	83.52 - 84.63
	Site C	20/20	0/20	100%	83.2% – 100%	83.79	0.32	82.92 - 84.25
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**83.78**	**0.37**	**82.92 - 84.63**
High Negative[b] (LoD/10) 1 $TCID_{50}$/mL	Site A	12/20	8/20	60.0%	36.1% - 80.9%	83.43	0.34	82.71 - 83.96
	Site B	12/20	8/20	60.0%	36.1% - 80.9%	83.91	0.31	83.43 - 84.56
	Site C	11/20	9/20	55.0%	31.5% - 76.9%	83.71	0.36	82.91 - 84.30
	All Sites	**35/60**	**25/60**	**58.3%**	**44.9% - 70.9%**	**83.69**	**0.41**	**82.71 - 84.56**
Negative	Site A	0/180	180/180	100%	98.0% - 100%			
	Site B	0/180	180/180	100%	98.0% - 100%			
	Site C	0/180	180/180	100%	98.0% - 100%			
	All Sites	**0/540**	**540/540**	**100%**	**99.3% – 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Parainfluenza Virus 3 Zeptometrix #0810016CF		**# Positive**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**	**Mean Tm**	**%CV Tm**	**Observed Tm Range**
Medium Positive (3X LoD) 30 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	81.17	0.36	80.71 - 81.86
	Site B	20/20	0/20	100%	83.2% – 100%	81.48	0.35	81.03 - 81.89
	Site C	20/20	0/20	100%	83.2% – 100%	80.94	0.28	80.63 - 81.37
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**81.22**	**0.41**	**80.63 - 81.89**
Low Positive (1X LoD) 10 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	80.97	0.36	80.36 - 81.52
	Site B	20/20	0/20	100%	83.2% – 100%	81.35	0.26	80.93 - 81.79
	Site C	17/20	3/20	85.0%	62.1% - 96.8%	80.86	0.28	80.10 - 81.21
	All Sites	**57/60**	**3/60**	**95.0%**	**86.1% - 99.0%**	**81.08**	**0.40**	**80.10 - 81.79**
High Negative[b] (LoD/10) 1 $TCID_{50}$/mL	Site A	10/20	10/20	50.0%	27.2% - 72.8%	80.99	0.26	80.30 - 81.34
	Site B	7/20	13/20	35.0%	15.4% - 59.2%	81.29	0.28	80.61 - 81.77
	Site C	5/20	15/20	25.0%	8.7% - 49.1%	80.84	0.24	80.41 - 81.24
	All Sites	**22/60**	**38/60**	**36.7%**	**24.6% - 50.1%**	**81.05**	**0.34**	**80.30 - 81.77**
Negative	Site A	0/180	180/180	100%	98.0% - 100%			
	Site B	0/180	180/180	100%	98.0% - 100%			
	Site C	0/180	180/180	100%	98.0% - 100%			
	All Sites	**0/540**	**540/540**	**100%**	**99.3% – 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Parainfluenza Virus 4a Zeptometrix #0810060CF		**# Positive**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**	**Mean Tm**	**%CV Tm**	**Observed Tm Range**
Medium Positive (3X LoD) 15,000 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	77.70	0.30	77.36 - 78.10
	Site B	20/20	0/20	100%	83.2% – 100%	78.09	0.56	77.48 - 78.74
	Site C	20/20	0/20	100%	83.2% – 100%	77.73	0.40	77.05 - 78.21
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	77.82	0.47	77.05 - 78.74
Low Positive (1X LoD) 5,000 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	77.11	0.28	76.64 - 77.68
	Site B	20/20	0/20	100%	83.2% – 100%	77.65	0.41	76.73 - 78.71
	Site C	20/20	0/20	100%	83.2% – 100%	77.23	0.38	76.81 - 78.20
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	77.33	0.46	76.64 - 78.71
High Negative[b] (LoD/10)	Site A	4/20	16/20	20.0%	5.7% - 43.7%	77.07	0.26	76.63 - 77.58
	Site B	5/20	15/20	25.0%	8.7% - 49.1%	77.59	0.27	77.05 - 78.00

Parainfluenza Virus 4a Zeptometrix #0810060CF		# Positive	# Negative	% Agreement with Expected Result [a]	95% CI	Mean Tm	%CV Tm	Observed Tm Range
500 $TCID_{50}$/mL	Site C	11/20	9/20	55.0%	31.5% - 76.9%	77.24	0.30	76.62 - 77.84
	All Sites	**20/60**	**40/60**	**33.3%**	**21.7% - 46.7%**	77.29	0.40	76.62 - 78.00
Negative	Site A	0/180	180/180	100%	98.0% - 100%			
	Site B	0/180	180/180	100%	98.0% - 100%			
	Site C	0/180	180/180	100%	98.0% - 100%			
	All Sites	**0/540**	**540/540**	**100%**	**99.3% – 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Respiratory Syncytial Virus Type A		# Positive	# Negative	% Agreement with Expected Result [a]	95% CI	Mean Tm	%CV Tm	Observed Tm Range
Medium Positive (3X LoD) 6 $TCID_{50}$/mL	Site A	60/60	0/60	100%	94.0% - 100%	80.44	0.35	79.46 - 80.83
	Site B	60/60	0/60	100%	94.0% - 100%	80.86	0.25	80.41 - 81.56
	Site C	60/60	0/60	100%	94.0% - 100%	80.39	0.33	80.08 - 80.91
	All Sites	**180/180**	**0/180**	**100%**	**97.8% - 100%**	**80.58**	**0.40**	**79.46 - 81.56**
Low Positive (1X LoD) 2 $TCID_{50}$/mL	Site A	40/40	0/40	100%	91.2% - 100%	79.82	0.50	78.93 - 80.62
	Site B	40/40	0/40	100%	91.2% - 100%	80.40	0.46	79.47 - 81.03
	Site C	40/40	0/40	100%	91.2% - 100%	80.13	0.47	79.13 - 80.79
	All Sites	**120/120**	**0/120**	**100%**	**97.0% - 100%**	**80.10**	**0.57**	**78.93 - 81.03**
High Negative[b] (LoD/10) 0.2 $TCID_{50}$/mL	Site A	18/20	2/20	90.0%	68.3% - 98.8%	79.63	0.50	78.72 - 80.72
	Site B	17/20	3/20	85.0%	62.1% - 96.8%	80.12	0.50	79.26 - 80.89
	Site C	11/20	9/20	55.0%	31.5% - 76.9%	79.97	0.57	78.83 - 80.84
	All Sites	**46/60**	**14/60**	**76.7%**	**64.0% - 86.6%**	**79.90**	**0.58**	**78.72 - 80.89**
Negative	Site A	0/120	120/120	100%	97.0% - 100%			
	Site B	0/120	120/120	100%	97.0% - 100%			
	Site C	0/120	120/120	100%	97.0% - 100%			
	All Sites	**0/360**	**360/360**	**100%**	**99.0% - 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Bordetella pertussis A639		# Positive	# Negative	% Agreement with Expected Result [a]	95% CI	Mean Tm	%CV Tm	Observed Tm Range
Medium Positive (3X LoD) 12,000 CFU/mL	Site A	20/20	0/20	100%	83.2% – 100%	88.36	0.36	87.46 - 89.05
	Site B	20/20	0/20	100%	83.2% – 100%	88.81	0.29	88.26 - 89.20
	Site C	20/20	0/20	100%	83.2% – 100%	88.34	0.18	87.97 - 88.58
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**88.50**	**0.38**	**87.46 - 89.20**
Low Positive (1X LoD) 4,000 CFU/mL	Site A	20/20	0/20	100%	83.2% – 100%	88.39	0.28	87.73 - 88.96
	Site B	20/20	0/20	100%	83.2% – 100%	88.64	0.31	88.07 - 89.20
	Site C	20/20	0/20	100%	83.2% – 100%	88.22	0.28	87.74 - 88.67
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**88.42**	**0.34**	**87.73 - 89.20**
High Negative[b] (LoD/10) 400 CFU/mL	Site A	16/20	4/20	80.0%	56.3% - 94.3%	88.41	0.34	87.86 - 89.36
	Site B	12/20	8/20	60.0%	36.1% - 80.9%	88.70	0.32	88.33 - 89.29
	Site C	12/20	8/20	60.0%	36.1% - 80.9%	88.26	0.28	87.52 - 88.71
	All Sites	**40/60**	**20/60**	**66.7%**	**53.3% - 78.3%**	**88.46**	**0.37**	**87.52 - 89.36**
Negative	Site A	0/180	180/180	100%	98.0% - 100%			
	Site B	0/180	180/180	100%	98.0% - 100%			
	Site C	0/180	180/180	100%	98.0% - 100%			
	All Sites	**0/540**	**540/540**	**100%**	**99.3% – 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Chlamydophila pneumoniae TW183		# Positive	# Negative	% Agreement with Expected Result [a]	95% CI	Mean Tm	%CV Tm	Observed Tm Range
Medium Positive (3X LoD) 9,000 copies/mL	Site A	20/20	0/20	100%	83.2% – 100%	79.65	0.29	78.94 - 79.99
	Site B	20/20	0/20	100%	83.2% – 100%	80.27	0.30	79.88 - 80.82
	Site C	20/20	0/20	100%	83.2% – 100%	79.73	0.23	79.42 - 80.09
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**79.92**	**0.45**	**78.94 - 80.82**
Low Positive (1X LoD) 3,000 copies/mL	Site A	20/20	0/20	100%	83.2% – 100%	79.63	0.27	79.03 - 80.09
	Site B	19/20	1/20	95.0%	75.1% - 99.9%	80.15	0.28	79.68 - 80.62
	Site C	20/20	0/20	100%	83.2% – 100%	79.61	0.30	78.84 - 80.09
	All Sites	**59/60**	**1/60**	**98.3%**	**91.1% - 100%**	**79.79**	**0.42**	**78.84 - 80.62**
High Negative[b] (LoD/10) 300 copies/mL	Site A	11/20	9/20	55.0%	31.5% - 76.9%	79.55	0.25	79.25 - 80.08
	Site B	14/20	6/20	70.0%	45.7% - 88.1%	80.02	0.29	79.66 - 80.72
	Site C	10/20	10/20	50.0%	27.2% - 72.8%	79.61	0.29	79.16 - 80.21

Chlamydophila pneumoniae TW183		# Positive	# Negative	% Agreement with Expected Result [a]	95% CI	Mean Tm	%CV Tm	Observed Tm Range
	All Sites	**35/60**	**25/60**	**58.3%**	**44.9% - 70.9%**	**79.72**	**0.38**	**79.16 - 80.72**
Negative	Site A	0/180	180/180	100%	98.0% - 100%			
	Site B	0/180	180/180	100%	98.0% - 100%			
	Site C	0/180	180/180	100%	98.0% - 100%			
	All Sites	**0/540**	**540/540**	**100%**	**99.3% – 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Mycoplasma pneumoniae Type 1 - M129		# Positive	# Negative	% Agreement with Expected Result [a]	95% CI	Mean Tm	%CV Tm	Observed Tm Range
Medium Positive (3X LoD) 90 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%	77.42	0.29	77.04 - 77.71
	Site B	20/20	0/20	100%	83.2% – 100%	77.94	0.33	77.59 - 78.30
	Site C	20/20	0/20	100%	83.2% – 100%	77.83	0.28	77.44 - 78.12
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**77.75**	**0.38**	**77.04 - 78.30**
Low Positive (1X LoD) 30 $TCID_{50}$/mL	Site A	19/20	1/20	95.0%	75.1% - 99.9%	77.55	0.30	77.05 - 78.00
	Site B	17/20	3/20	85.0%	62.1% - 96.8%	77.96	0.33	77.36 - 78.37
	Site C	20/20	0/20	100%	83.2% – 100%	77.68	0.39	76.65 - 78.10
	All Sites	**56/60**	**4/60**	**93.3%**	**83.8% - 98.2%**	**77.73**	**0.40**	**76.65 - 78.37**
High Negative[b] (LoD/10) 3 $TCID_{50}$/mL	Site A	5/20	15/20	25.0%	8.7% - 49.1%	77.58	0.31	76.72 - 78.01
	Site B	4/20	16/20	20.0%	5.7% - 43.7%	78.01	0.27	77.67 - 78.45
	Site C	11/20	9/20	55.0%	31.5% - 76.9%	77.76	0.34	77.04 - 78.09
	All Sites	**20/60**	**40/60**	**33.3%**	**21.7% - 46.7%**	**77.78**	**0.38**	**76.72 - 78.45**
Negative	Site A	0/180	180/180	100%	98.0% - 100%			
	Site B	0/180	180/180	100%	98.0% - 100%			
	Site C	0/180	180/180	100%	98.0% - 100%			
	All Sites	**0/540**	**540/540**	**100%**	**99.3% – 100%**			

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Table 45. Summary of Positive Agreement, Negative Agreement, and Tm Results from Reproducibility Testing of Multi-Assay Organisms

Reproducibility Agreement Summary for Enterovirus (Human Rhinovirus/Enterovirus)

Enterovirus Echovirus 6 (Species B)		**# Positive**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**
Medium Positive (3X LoD) 90,000 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%
	Site B	20/20	0/20	100%	83.2% – 100%
	Site C	20/20	0/20	100%	83.2% – 100%
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**
Low Positive (1X LoD) 30,000 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%
	Site B	20/20	0/20	100%	83.2% – 100%
	Site C	20/20	0/20	100%	83.2% – 100%
	All Sites	**60/60**	**60/60**	**100%**	**94.0% - 100%**
High Negative[b] (LoD/10) 3,000 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%
	Site B	20/20	0/20	100%	83.2% – 100%
	Site C	20/20	0/20	100%	83.2% – 100%
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**
Negative	Site A	0/60	60/60	100%	94.0% - 100%
	Site B	0/60	60/60	100%	94.0% - 100%
	Site C	0/60	60/60	100%	94.0% - 100%
	All Sites	**0/180**	**180/180**	**100%**	**97.8% - 100%**

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Reproducibility Tm Summary (by assay) for Enterovirus

Assay	**Enterovirus** Echovirus 6 (Species B)		**Mean Tm**	**% CV Tm**	**Observed Tm Range**
Entero 1	Medium Positive 3x LoD 90,000 $TCID_{50}$/mL	Site A	87.12	0.24	86.79 - 87.73
		Site B	87.51	0.30	86.99 - 88.05
		Site C	86.98	0.33	86.37 - 87.64
		All Sites	**87.18**	**0.39**	**86.37 - 88.05**
	Low Positive 1x LoD 30,000 $TCID_{50}$/mL	Site A	87.00	0.33	86.17 - 87.64
		Site B	87.36	0.29	86.80 - 87.85
		Site C	86.81	0.35	86.15 - 87.41
		All Sites	**87.05**	**0.42**	**86.15 - 87.85**
	High Negative 0.1x LoD 3,000 $TCID_{50}$/mL	Site A	86.89	0.29	86.06 - 87.40
		Site B	87.34	0.31	86.67 - 87.86
		Site C	86.67	0.29	85.97 - 87.30
		All Sites	**86.96**	**0.44**	**85.97 - 87.86**

Assay	Enterovirus Echovirus 6 (Species B)		Mean Tm	% CV Tm	Observed Tm Range
Entero 2	Medium Positive 3x LoD 90,000 $TCID_{50}$/mL	Site A	87.09	0.28	86.68 - 87.73
		Site B	87.47	0.30	86.82 - 88.00
		Site C	86.93	0.36	86.16 - 87.64
		All Sites	**87.14**	**0.41**	**86.16 - 88.00**
	Low Positive 1x LoD 30,000 $TCID_{50}$/mL	Site A	86.98	0.30	86.28 - 87.53
		Site B	87.34	0.28	86.89 - 87.82
		Site C	86.77	0.35	86.05 - 87.52
		All Sites	**87.02**	**0.41**	**86.05 - 87.82**
	High Negative 0.1x LoD 3,000 $TCID_{50}$/mL	Site A	86.86	0.29	86.17 - 87.54
		Site B	87.26	0.35	86.59 - 87.94
		Site C	86.65	0.27	86.27 - 87.20
		All Sites	**86.92**	**0.42**	**86.17 - 87.94**
HRV4	Medium Positive 3x LoD 90,000 $TCID_{50}$/mL	Site A	85.70	0.31	85.21 - 86.18
		Site B	86.19	0.30	85.35 - 86.77
		Site C	85.59	0.34	84.91 - 86.19
		All Sites	**85.81**	**0.44**	**84.91 - 86.77**
	Low Positive 1x LoD 30,000 $TCID_{50}$/mL	Site A	85.45	0.26	84.81 - 86.06
		Site B	85.87	0.24	85.44 - 86.36
		Site C	85.32	0.40	84.69 - 86.26
		All Sites	**85.54**	**0.40**	**84.69 - 86.36**
	High Negative 0.1x LoD 3,000 $TCID_{50}$/mL	Site A	85.37	0.26	84.80 - 86.04
		Site B	85.82	0.23	85.43 - 86.23
		Site C	85.22	0.22	84.82 - 85.58
		All Sites	**85.46**	**0.39**	**84.80 - 86.23**

Reproducibility Agreement Summary for Rhinovirus (Human Rhinovirus/Enterovirus)

Human Rhinovirus A1		**# Positive**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**
Medium Positive (3X LoD) 3 $TCID_{50}$/mL	Site A	60/60	0/60	100%	94.0% - 100%
	Site B	60/60	0/60	100%	94.0% - 100%
	Site C	60/60	0/60	100%	94.0% - 100%
	All Sites	**180/180**	**0/180**	**100%**	**97.8% - 100%**
Low Positive (1X LoD) 1 $TCID_{50}$/mL	Site A	20/20	0/20	100%	83.2% – 100%
	Site B	20/20	0/20	100%	83.2% – 100%
	Site C	20/20	0/20	100%	83.2% – 100%
	All Sites	**60/60**	**0/60**	**100%**	**94.0% - 100%**
High Negative[b] (LoD/10) 0.1 $TCID_{50}$/mL	Site A	40/40	0/40	100%	91.2% - 100%
	Site B	40/40	0/40	100%	91.2% - 100%
	Site C	32/40	8/40	80.0%	64.4% - 91.0%
	All Sites	**112/120**	**8/120**	**93.3%**	**87.3% - 97.1%**
Negative	Site A	0/60	60/60	100%	94.0% - 100%
	Site B	0/60	60/60	100%	94.0% - 100%
	Site C	0/60	60/60	100%	94.0% - 100%
	All Sites	**0/180**	**180/180**	**100%**	**97.8% - 100%**

[a] Expected results for the “Medium Positive”, the “Low Positive”, and the “High Negative” panel members are positive. Expected result for the “Negative” panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Reproducibility Tm Summary (by assay) for Rhinovirus

Assay	**Human Rhinovirus** A1		**Mean Tm**	**% CV Tm**	**Observed Tm Range**
HRV1	Medium Positive 3x LoD 3 $TCID_{50}$/mL	Site A	83.79	0.44	83.25 - 85.09
		Site B	84.06	0.31	83.43 - 84.56
		Site C	83.68	0.23	83.22 - 84.09
		All Sites	**83.84**	**0.38**	**83.22 - 85.09**
	Low Positive 1x LoD 1 $TCID_{50}$/mL	Site A	83.71	0.29	83.07 - 84.35
		Site B	84.07	0.34	83.44 - 84.66
		Site C	83.93	0.34	83.24 - 84.71
		All Sites	**83.90**	**0.37**	**83.07 - 84.71**
	High Negative 0.1x LoD 0.1 $TCID_{50}$/mL	Site A	83.56	0.28	83.02 - 84.26
		Site B	83.91	0.34	83.22 - 84.52
		Site C	83.76	0.31	83.04 - 84.48
		All Sites	**83.74**	**0.36**	**83.02 - 84.52**
HRV2	Medium Positive 3x LoD	Site A	83.33	0.48	82.55 - 84.67
		Site B	83.65	0.29	83.11 - 84.17
		Site C	83.30	0.28	82.70 - 83.77

Assay	Human Rhinovirus A1		Mean Tm	% CV Tm	Observed Tm Range
	3 $TCID_{50}$/mL	**All Sites**	**83.42**	**0.41**	**82.55 - 84.67**
	Low Positive 1x LoD 1 $TCID_{50}$/mL	Site A	83.28	0.31	82.57 - 83.86
		Site B	83.66	0.36	83.02 - 84.37
		Site C	83.52	0.39	82.82 - 84.29
		All Sites	**83.48**	**0.40**	**82.57 - 84.37**
	High Negative 0.1x LoD 0.1 $TCID_{50}$/mL	Site A	83.17	0.34	82.42 - 83.88
		Site B	83.58	0.36	82.80 - 84.31
		Site C	83.37	0.33	82.51 - 83.92
		All Sites	**83.37**	**0.40**	**82.42 - 84.31**
HRV3	Medium Positive 3x LoD 3 $TCID_{50}$/mL	Site A	82.73	0.53	81.99 - 83.94
		Site B	83.25	0.36	82.49 - 83.88
		Site C	82.89	0.43	82.10 - 83.88
		All Sites	**82.96**	**0.51**	**81.99 - 83.94**
	Low Positive 1x LoD 1 $TCID_{50}$/mL	Site A	82.67	0.49	81.76 - 83.65
		Site B	83.24	0.44	82.40 - 84.08
		Site C	83.07	0.41	82.13 - 83.76
		All Sites	**82.98**	**0.54**	**81.76 - 84.08**
	High Negative 0.1x LoD 0.1 $TCID_{50}$/mL	Site A	82.68	0.50	81.78 - 83.67
		Site B	83.21	0.46	82.18 - 84.37
		Site C	82.97	0.42	81.85 - 83.64
		All Sites	**82.96**	**0.52**	**81.78 - 84.37**
HRV4	Medium Positive 3x LoD 3 $TCID_{50}$/mL	Site A	83.81	0.38	83.28 - 84.98
		Site B	84.14	0.34	83.43 - 84.83
		Site C	83.80	0.25	83.44 - 84.20
		All Sites	**83.90**	**0.37**	**83.28 - 84.98**
	Low Positive 1x LoD 1 $TCID_{50}$/mL	Site A	83.79	0.31	83.18 - 84.45
		Site B	84.08	0.31	83.55 - 84.61
		Site C	84.04	0.38	83.35 - 84.93
		All Sites	**83.94**	**0.37**	**83.18 - 84.93**
	High Negative 0.1x LoD 0.1 $TCID_{50}$/mL	Site A	83.58	0.26	83.11 - 84.05
		Site B	83.88	0.31	83.34 - 84.49
		Site C	83.86	0.27	83.24 - 84.58
		All Sites	**83.76**	**0.33**	**83.11 - 84.58**

Reproducibility Agreement Summary for Influenza A H1

Influenza A H1N1 A/Brisbane/59/07		**# Positive**	**# Equivocal**	**# Negative**	**% Agreement with Expected Result [a]**	**95% CI**
Medium Positive (3X LoD) 600 $TCID_{50}$/mL	Site A	20/20	0/20	0/20	100%	83.2% – 100%
	Site B	20/20	0/20	0/20	100%	83.2% – 100%
	Site C	20/20	0/20	0/20	100%	83.2% – 100%
	All Sites	**60/60**	**0/60**	**0/60**	**100%**	**94.0% - 100%**
Low Positive (1X LoD) 200 $TCID_{50}$/mL	Site A	20/20	0/20	0/20	100%	83.2% – 100%
	Site B	19/20	1/20	0/20	95.0%	75.1% - 99.9%
	Site C	17/20	2/20	1/20	85.0%	62.1% – 96.8%
	All Sites	**56/60**	**3/60**	**1/60**	**93%**	**83.8% – 98.2%**
High Negative (LoD/10)[b] 20 $TCID_{50}$/mL	Site A	20/20	0/20	0/20	100%	83.2% – 100%
	Site B	17/20	2/20	1/20	85.0%	62.1% – 96.8%
	Site C	14/20	5/20	1/20	70.0%	45.7% - 88.1%
	All Sites	**51/60**	**7/60**	**2/60**	**85.0%**	**73.4% - 92.9%**
Negative	Site A	0/180	0/180	180/180	100%	98.0% - 100%
	Site B	0/180	0/180	180/180	100%	98.0% - 100%
	Site C	0/180	0/180	180/180	100%	98.0% - 100%
	All Sites	**0/540**	**0/540**	**540/540**	**100%**	**99.3% – 100%**

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Reproducibility Tm Summary (by assay) for Influenza A H1

Assay	**Influenza A H1N1** A/Brisbane/59/07		**Mean Tm**	**% CV Tm**	**Observed Tm Range**
FluA-pan1	Moderate Positive 3x LoD 600 $TCID_{50}$/mL	Site A	84.67	0.24	84.27 - 85.12
		Site B	85.10	0.24	84.78 - 85.56
		Site C	84.64	0.34	83.76 - 85.23
		All Sites	**84.80**	**0.37**	**83.76 - 85.56**
	Low Positive 1x LoD 200 $TCID_{50}$/mL	Site A	84.57	0.27	84.17 - 85.31
		Site B	85.01	0.28	84.59 - 85.75
		Site C	84.68	0.26	84.16 - 85.18
		All Sites	**84.75**	**0.34**	**84.16 - 85.75**
	High Negative 0.1x LoD 20 $TCID_{50}$/mL	Site A	84.27	0.26	83.85 - 84.81
		Site B	84.75	0.23	84.29 - 85.32
		Site C	84.46	0.35	83.89 - 85.48
		All Sites	**84.48**	**0.37**	**83.85 - 85.48**
FluA-pan2	Moderate Positive 3x LoD 600 $TCID_{50}$/mL	Site A	80.42	0.25	79.78 - 80.63
		Site B	80.85	0.27	80.39 - 81.26
		Site C	80.31	0.28	79.89 - 80.72
		All Sites	**80.52**	**0.39**	**79.78 - 81.26**

Assay	Influenza A H1N1 A/Brisbane/59/07		Mean Tm	% CV Tm	Observed Tm Range
	Low Positive 1x LoD 200 $TCID_{50}$/mL	Site A	80.36	0.23	79.99 - 80.73
		Site B	80.80	0.21	80.42 - 81.15
		Site C	80.52	0.22	80.19 - 80.89
		All Sites	**80.57**	**0.32**	**79.99 - 81.15**
	High Negative 0.1x LoD 20 $TCID_{50}$/mL	Site A	79.91	0.37	79.15 - 80.41
		Site B	80.49	0.30	79.67 - 80.83
		Site C	80.10	0.35	79.56 - 80.73
		All Sites	**80.17**	**0.45**	**79.15 - 80.83**
FluA-H1-pan	Moderate Positive 3x LoD 600 $TCID_{50}$/mL	Site A	78.79	0.25	78.31 - 79.25
		Site B	79.20	0.31	78.30 - 79.57
		Site C	78.76	0.39	77.79 - 79.25
		All Sites	**78.91**	**0.42**	**77.67 - 79.57**
	Low Positive 1x LoD 200 $TCID_{50}$/mL	Site A	78.77	0.25	78.42 - 79.34
		Site B	79.20	0.27	78.72 - 79.67
		Site C	78.80	0.25	78.30 - 79.26
		All Sites	**78.93**	**0.36**	**78.30 - 79.67**
	High Negative 0.1x LoD 20 $TCID_{50}$/mL	Site A	77.65	0.33	77.15 - 78.21
		Site B	78.18	0.45	77.47 - 79.26
		Site C	77.93	0.49	77.43 - 79.04
		All Sites	**77.92**	**0.51**	**77.15 - 79.26**

Reproducibility Agreement Summary for Influenza A H1-2009

Influenza A H1-2009 A/Swine NY/03/2009		# Positive	# Equivocal	# Negative	% Agreement with Expected Result [a]	95% CI
Medium Positive (3X LoD) 300 $TCID_{50}$/mL	Site A	20/20	0/20	0/20	100%	83.2% – 100%
	Site B	20/20	0/20	0/20	100%	83.2% – 100%
	Site C	20/20	0/20	0/20	100%	83.2% – 100%
	All Sites	**60/60**	**0/60**	**0/60**	**100%**	**94.0% - 100%**
Low Positive (1X LoD) 100 $TCID_{50}$/mL	Site A	20/20	0/20	0/20	100%	83.2% – 100%
	Site B	20/20	0/20	0/20	100%	83.2% – 100%
	Site C	20/20	0/20	0/20	100%	83.2% – 100%
	All Sites	**60/60**	**0/60**	**0/60**	**100%**	**94.0% - 100%**
High Negative[b] (LoD/10) 10 $TCID_{50}$/mL	Site A	20/20	0/20	0/20	100%	83.2% – 100%
	Site B	19/20	1/20	0/20	95.0%	75.1% - 99.9%
	Site C	20/20	0/20	0/20	100%	83.2% – 100%
	All Sites	**59/60**	**1/60**	**0/60**	**98.3%**	**91.1% – 100%**
Negative	Site A	0/180	0/180	180/180	100%	98.0% - 100%
	Site B	0/180	0/180	180/180	100%	98.0% - 100%
	Site C	0/180	0/180	180/180	100%	98.0% - 100%

Influenza A H1-2009 A/Swine NY/03/2009		# Positive	# Equivocal	# Negative	% Agreement with Expected Result [a]	95% CI
	All Sites	**0/540**	**0/540**	**540/540**	**100%**	**99.3% – 100%**

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.
[b] High negative samples are targeted to be positive 20-80% of the time.

Reproducibility Tm Summary (by assay) for Influenza A H1-2009

Assay	Influenza A H1-2009 A/Swine NY/03/2009		Mean Tm	% CV Tm	Observed Tm Range
FluA-pan1	Moderate Positive 3x LoD 300 $TCID_{50}$/mL	Site A	84.67	0.24	84.27 - 85.12
		Site B	85.10	0.24	84.78 - 85.56
		Site C	84.64	0.34	83.76 - 85.23
		All Sites	**84.80**	**0.37**	**83.76 - 85.56**
	Low Positive 1x LoD 100 $TCID_{50}$/mL	Site A	84.57	0.27	84.17 - 85.31
		Site B	85.01	0.28	84.59 - 85.75
		Site C	84.68	0.26	84.16 - 85.18
		All Sites	**84.75**	**0.34**	**84.16 - 85.75**
	High Negative 0.1x LoD 10 $TCID_{50}$/mL	Site A	84.27	0.26	83.85 - 84.81
		Site B	84.75	0.23	84.29 - 85.32
		Site C	84.46	0.35	83.89 - 85.48
		All Sites	**84.48**	**0.37**	**83.85 - 85.48**
FluA-pan2	Moderate Positive 3x LoD 300 $TCID_{50}$/mL	Site A	80.62	0.20	80.31 - 80.83
		Site B	81.01	0.17	80.70 - 81.36
		Site C	80.69	0.23	80.19 - 81.02
		All Sites	**80.81**	**0.32**	**80.19 - 81.36**
	Low Positive 1x LoD 100 $TCID_{50}$/mL	Site A	80.39	0.29	79.87 - 80.73
		Site B	80.91	0.27	80.20 - 81.24
		Site C	80.30	0.17	80.05 - 80.61
		All Sites	**80.62**	**0.44**	**79.87 - 81.24**
	High Negative 0.1x LoD 10 $TCID_{50}$/mL	Site A	80.43	0.27	80.08 - 81.03
		Site B	80.72	0.27	80.11 - 81.14
		Site C	80.41	0.34	79.86 - 80.82
		All Sites	**80.54**	**0.34**	**79.86 - 81.14**
FluA-H1-pan	Moderate Positive 3x LoD 300 $TCID_{50}$/mL	Site A	78.87	0.40	78.20 - 79.56
		Site B	79.44	0.35	78.94 - 79.99
		Site C	78.62	0.43	77.92 - 79.44
		All Sites	**78.97**	**0.58**	**77.92 - 79.99**
	Low Positive 1x LoD 100 $TCID_{50}$/mL	Site A	78.37	0.30	77.89 - 79.24
		Site B	78.90	0.37	78.21 - 79.76
		Site C	78.16	0.25	77.83 - 78.69
		All Sites	**78.47**	**0.51**	**77.78 - 79.76**
	High Negative 0.1x LoD	Site A	78.34	0.37	77.90 - 79.37
		Site B	78.83	0.37	77.99 - 79.68

Assay	Influenza A H1-2009 A/Swine NY/03/2009		Mean Tm	% CV Tm	Observed Tm Range
FluA-H1-2009	10 $TCID_{50}$/mL	Site C	78.08	0.27	77.68 - 78.51
		All Sites	**78.40**	**0.53**	**77.68 - 79.68**
	Moderate Positive 3x LoD 300 $TCID_{50}$/mL	Site A	78.73	0.24	78.31 - 79.14
		Site B	79.20	0.24	78.84 - 79.67
		Site C	78.54	0.30	77.89 - 78.92
		All Sites	**78.81**	**0.44**	**77.89 - 79.67**
	Low Positive 1x LoD 100 $TCID_{50}$/mL	Site A	78.64	0.26	78.10 - 79.14
		Site B	79.03	0.25	78.53 - 79.48
		Site C	78.49	0.24	78.12 - 78.82
		All Sites	**78.71**	**0.39**	**78.10 - 79.48**
	High Negative 0.1x LoD 10 $TCID_{50}$/mL	Site A	78.60	0.28	77.90 - 79.04
		Site B	79.00	0.23	78.52 - 79.36
		Site C	78.52	0.28	78.10 - 78.93
		All Sites	**78.70**	**0.38**	**77.90 - 79.36**

Reproducibility Agreement Summary for Influenza A H3

Influenza A H3N2 A/Wisconsin/67/2005		# Positive	# Equivocal	# Negative	% Agreement with Expected Result [a]	95% CI
Medium Positive (3X LoD) 15 $TCID_{50}$/mL	Site A	20/20	0/20	0/20	100%	83.2% – 100%
	Site B	20/20	0/20	0/20	100%	83.2% – 100%
	Site C	20/20	0/20	0/20	100%	83.2% – 100%
	All Sites	**60/60**	**0/60**	**0/60**	**100%**	**94.0% - 100%**
Low Positive (1X LoD) 5 $TCID_{50}$/mL	Site A	20/20	0/20	0/20	100%	83.2% – 100%
	Site B	20/20	0/20	0/20	100%	83.2% – 100%
	Site C	20/20	0/20	0/20	100%	83.2% – 100%
	All Sites	**60/60**	**0/60**	**0/60**	**100%**	**94.0% - 100%**
High Negative[b] (LoD/10) 0.5 $TCID_{50}$/mL	Site A	3/20	11/20	6/20	15.0%	3.2% - 37.9%
	Site B	4/20	12/20	4/20	20.0%	5.7% - 43.7%
	Site C	3/20	8/20	9/20	15.0%	3.2% - 37.9%
	All Sites	**10/60**	**31/60**	**19/60**	**16.7%**	**8.3% - 28.5%**
Negative	Site A	0/180	0/180	180/180	100%	98.0% - 100%
	Site B	0/180	0/180	180/180	100%	98.0% - 100%
	Site C	0/180	0/180	180/180	100%	98.0% - 100%
	All Sites	**0/540**	**0/540**	**540/540**	**100%**	**99.3% – 100%**

[a] Expected results for the "Medium Positive", the "Low Positive", and the "High Negative" panel members are positive. Expected result for the "Negative" panel member is negative.

[b] High negative samples are targeted to be positive 20-80% of the time.

Reproducibility Tm Summary (by assay) for Influenza A H3

Assay	Influenza A H3N2 A/Wisconsin/67/2005		Mean Tm	% CV Tm	Observed Tm Range
FluA-pan1	Moderate Positive 3x LoD 15 $TCID_{50}$/mL	Site A	85.33	0.26	84.79 - 85.73
		Site B	85.48	0.42	84.91 - 86.27
		Site C	85.06	0.38	84.50 - 85.40
		All Sites	**85.36**	**0.38**	**84.50 - 86.27**
	Low Positive 1x LoD 5 $TCID_{50}$/mL	Site A	84.95	0.41	84.19 - 86.03
		Site B	85.31	0.31	84.79 - 86.02
		Site C	84.83	0.26	84.37 - 85.25
		All Sites	**85.03**	**0.41**	**84.19 - 86.03**
	High Negative 0.1x LoD 0.5 $TCID_{50}$/mL	Site A	84.91	0.39	84.22 - 85.60
		Site B	85.24	0.30	84.78 - 85.77
		Site C	84.86	0.27	84.48 - 85.33
		All Sites	**85.01**	**0.37**	**84.22 - 85.77**
FluA-pan2	Moderate Positive 3x LoD 15 $TCID_{50}$/mL	Site A	79.75	0.25	79.51 - 79.99
		Site B	79.94	0.30	79.40 - 80.37
		Site C	79.60	0.24	79.14 - 79.86
		All Sites	**79.81**	**0.33**	**79.14 - 80.37**
	Low Positive 1x LoD 5 $TCID_{50}$/mL	Site A	79.42	0.31	79.00 - 80.30
		Site B	79.69	0.35	79.14 - 80.29
		Site C	79.25	0.20	78.82 - 79.59
		All Sites	**79.45**	**0.37**	**78.82 - 80.30**
	High Negative 0.1x LoD 0.5 $TCID_{50}$/mL	Site A	79.22	0.35	78.64 - 79.75
		Site B	79.59	0.29	79.05 - 79.99
		Site C	79.24	0.20	78.84 - 79.54
		All Sites	**79.35**	**0.36**	**78.64 - 79.99**
FluA-H3	Moderate Positive 3x LoD 15 $TCID_{50}$/mL	Site A	82.58	0.35	81.87 - 83.01
		Site B	82.89	0.19	82.60 - 83.11
		Site C	82.44	0.33	81.98 - 82.81
		All Sites	**82.62**	**0.39**	**81.87 - 83.11**
	Low Positive 1x LoD 5 $TCID_{50}$/mL	Site A	82.32	0.29	81.88 - 82.69
		Site B	82.69	0.34	82.16 - 83.32
		Site C	82.21	0.25	81.76 - 82.73
		All Sites	**82.39**	**0.41**	**81.67 - 83.32**
	High Negative 0.1x LoD 0.5 $TCID_{50}$/mL	Site A	82.28	0.36	81.71 - 82.91
		Site B	82.61	0.29	82.17 - 83.05
		Site C	82.20	0.23	81.87 - 82.57
		All Sites	**82.37**	**0.36**	**81.71 - 83.05**

Repeatability

The repeatability of the FilmArray RP results was evaluated with the original FilmArray RP by repeated testing the same 12 specimens tested in the Reproducibility study while minimizing as many sources of variability as possible. The in-house Repeatability testing was performed at Site C over the course of 12 testing days. On each day, all 12 specimens were tested 4 times by 2 operators on 2 FilmArray instruments.

Table 46. Summary of Positive Agreement Results for Repeatability Testing

Organism Result	Moderate Positive (3x LoD)		Low Positive (1x LoD)		High Negative (0.1x LoD)	
	# Positive / Total	% Positive Results	# Positive / Total	% Positive Results	# Positive / Total	% Positive Results
Adenovirus[a]	48/48	100%	48/48	100%	34/48	70.8%
Coronavirus 229E	48/48	100%	48/48	100%	19/48	39.6%
Coronavirus HKU1	48/48	100%	48/48	100%	44/48	91.7%
Coronavirus NL63	48/48	100%	48/48	100%	27/48	56.3%
Coronavirus OC43	48/48	100%	47/48	97.9%	33/48	68.8%
Human Metapneumovirus	47/48	97.9%	48/48	100%	32/48	66.7%
Enterovirus	48/48	100%	48/48	100%	48/48	100%
Human Rhinovirus	144/144	100%	47/48	97.9%	83/96	86.5%
Influenza A H1	48/48	100%	43/48[b]	89.6%[b]	39/48[c]	81.3%[c]
Influenza A H1-2009	48/48	100%	48/48	100%	47/48[d]	97.9%[d]
Influenza A H3	48/48	100%	48/48	100%	7/48[e]	14.6%[e]
Influenza B	48/48	100%	48/48	100%	25/48	52.1%
Parainfluenza Virus 1	47/48	97.9%	48/48	100%	32/48	66.7%
Parainfluenza Virus 2	48/48	100%	47/48	97.9%	27/48	56.3%
Parainfluenza Virus 3	48/48	100%	41/48	85.4%	14/48	29.2%
Parainfluenza Virus 4	48/48	100%	48/48	100%	26/48	54.2%
Respiratory Syncytial Virus	144/144	100%	96/96	100%	32/48	66.7%
Bordetella pertussis	48/48	100%	48/48	100%	27/48	56.3%
Chlamydophila pneumoniae	48/48	100%	46/48	95.8%	18/48	37.5%
Mycoplasma pneumoniae	48/48	100%	47/48	97.9%	19/48	39.6%
% positive (all organisms) per test level	**1102/1104 99.8%**		**942/960 98.1%**		**599/960 62.4%**	

[a] Repeatability testing was performed prior to the modification of the panel to include a second Adenovirus assay. Test concentrations and multiples of LoD reflect the Adenovirus LoD prior to panel modification (300 $TCID_{50}$/mL).
[b] The five (5) non-positive results for Influenza A H1 at LoD include: (1) Negative, (1) Influenza A H1 Equivocal and (3) Influenza A Equivocal results.
[c] The nine (9) non-positive results for Influenza A H1 at 0.1 x LoD include: (1) Negative, (3) Influenza A (no subtype detected), (1) Influenza A H1 Equivocal, and (4) Influenza A Equivocal results.
[d] One (1) Influenza A H1-2009 Equivocal result at the 0.1 x LoD test level.
[e] The 42 non-positive results for Influenza A H3 at 0.1 x LoD include: (18) Negative, (2) Influenza A (no subtype detected) (15) Influenza A H3 Equivocal, and (7) Influenza A Equivocal results.

Interference

Substances that could be present in NPS samples or introduced during sample handling were evaluated for their potential to interfere with assay performance. Four different mixes containing FilmArray RP organisms were spiked into a simulated NPS (sNPS) sample matrix (human epithelial cells in VTM) at 5x their respective LoDs. The 5x LoD organism concentration was chosen to be near the LoD but also to provide consistent results for sample-to-sample comparison. Each FilmArray RP sample was tested with the original FilmArray RP in the presence of each potentially interfering substance listed in Table 47. None of the substances tested were found to compete or interfere with the control or organism assays in the FilmArray RP.

Table 47. List of Potentially Interfering Substances Evaluated (No Interference or Inhibition Observed)

Endogenous Substances	**Competing / Interfering Microorganisms**	
Human Blood (with Na Citrate) (1% v/v)	Respiratory Syncytial Virus A	2.8×10^4 $TCID_{50}$/mL
Mucin (bovine submaxillary gland) (1% v/v)	Human Rhinovirus	1.1×10^4 $TCID_{50}$/mL
Human Genomic DNA: 0.2 ng/μL	Influenza A H1-2009	1.0×10^5 $TCID_{50}$/mL
2 ng/μL	*Staphylococcus aureus*	1.0×10^6 CFU/mL
20 ng/μL	*Neisseria meningitidis*	1.0×10^6 CFU/mL
	Corynebacterium diptheriae	1.0×10^6 CFU/mL
Exogenous Substances		
Saline Nasal Spray with Preservatives (1% v/v)	Analgesic ointment (1% w/v)	
Nasal Decongestant Spray (Oxymetazoline HCl) (1%v/v)	Petroleum Jelly (1% w/v)	
Tobramycin (0.6 mg/mL)	Smokeless Tobacco (1% w/v)	
Mupirocin (2% w/v)	FluMist® Nasal Influenza Vaccine (2009-2010)	
Technique Specific Substances		
Laboratory Reagents:	**Viral Transport Media:**	**Swabs:**
Bleach (1%, 2%, 5% v/v)	Remel M4	Copan 168C (rayon / twisted aluminum shaft)
Disinfecting wipes	Remel M4-RT	Copan FloQ (flocked nylon / plastic shaft)
Ethanol (7% v/v)	Remel M5	Copan 175KS01 (polyester / aluminum shaft)
DNAzap (1% v/v)	Remel M6	Millipore 519CS01M (flocked nylon / plastic shaft)
RNaseOut (1% v/v)	Copan UTM	

Evaluation of the FilmArray RP was not performed using clinical NPS specimens obtained from individuals who had recently received the FluMist® nasal influenza vaccine (MedImmune, Gaithersburg, MD). However, analytical testing was performed (using the original FilmArray RP) with simulated samples containing various concentrations of the 2009-2010 formulation of the vaccine material. The FilmArray RP assays react with the Influenza A H1, Influenza A H3 and Influenza B viral material contained in the vaccine (Table 48). No cross-reactivity was observed with other, non-influenza FilmArray RP assays.

Table 48. Evaluation of FluMist® Nasal Vaccine as a Potentially Interfering Substance

FluMist 2009-2010 (% v/v)	Adenovirus	Coronavirus 229E	Coronavirus HKU	Coronavirus NL63	Coronavirus OC43	Human Metapneumovirus	Human Rhinovirus / Enterovirus	Influenza A			Influenza B	Parainfluenza Virus 1	Parainfluenza Virus 2	Parainfluenza Virus 3	Parainfluenza Virus 4	Respiratory Syncytial Virus	*Bordetella pertussis*	*Chlamydophila pneumoniae*	*Mycoplasma pneumoniae*
								H1	H1-2009	H3									
10%	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-
1%	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-
0.1%	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-
0.01%	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-
0.001%	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-
0.0001%	-	-	-	-	-	-	-	-	-	Equiv [a]	+	-	-	-	-	-	-	-	-
0.00001%	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
0.000001%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] Influenza A H3 Equivocal result

Analysis of Potential Interference from Medications

The clinical dataset from the original FilmArray RP was analyzed for evidence of the effects of potentially interfering medications (prescription and over-the-counter; OTC) administered to or taken by the study population. If clinically significant interference from medications had occurred, it would be expected that the organism detection rate in the medicated population would be lower than that in the non-medicated population. This effect was not observed in the clinical dataset. One or more organisms was detected by the reference/comparator methods in 118 of 257 (45.9%) non-medicated individuals. The FilmArray RP correctly identified all organisms in 112 (94.9%) of these individuals. One or more organisms were detected by the reference/comparator methods in 342 of 596 (57.4%) medicated individuals. The FilmArray RP correctly identified all organisms in 331 (96.8%) of these individuals. Table 49 lists the medications administered to or taken by the enrolled study population (self-reported and/or recorded from patient charts).

Table 49. List of Medications Administered/Taken During the FilmArray RP Clinical Evaluation

Acetaminophen	Clindamycin	Lansoprazole	Phenobarbital
Albuterol	Co-trimoxazole	Levalbuterol	Phenylephrine
Alendronate	Cough drops/syrups (various OTC)	Levothyroxine	Pilocarpine
Alprazolam	Cyproheptadine	Lisinopril	Pravachol (Pravastatin)
Amoxicillin	Deferasirox	Loperamide	Prednisolone
Amoxicillin Clavulanate	Dextromethorphan	Loratadine	Prednisone
Azithromycin	Dextromethorphan hydrobromide	Lorazepam	Propranolol
Baclofen	Diphenhydramine	Metoclopramide	Pseudoephedrine
Benzonatate	Doxylamine succinate	Metolazone	Ranitidine
Betamethasone	Electrolyte solutions (rehydration salts)	Metoprolol	Saline Nasal Drops/Sprays (various OTC)
Brimonidine	Enoxaparin	Midazolam	Salmeterol
Budesonide	Eszopiclone	Montelukast	Simethicone

Budesonide/Formoterol Fumarate Dihydrate	Fentanyl	Naproxen	Sirolimus
Bupropion hydrochloride	Fluticasone	Nizatidine	Spironolactone
Carvedilol	Furosemide	Omeprazole	Temazepam
Cefdinir	Gabapentin	Oseltamivir	Tiotropium
Cefepime	Gemfibrozil	Oxybutynin	Ursodiol
Ceftriaxone	Guaifenesin	Pancrelipase	Valacyclovir
Cephrazole	Hydrocodone	Pantoprazole	Vancomycin
Cetirizine	Ibuprofen	Penicillin	Vitamins/Multivitamins/ Minerals (various OTC)
Chlorpheniramine maleate	Inderal (Propranolol)	Pentamidine	Voriconazole
Ciprofloxacin	Isosorbide		

PERFORMANCE CHARACTERISTICS ON THE FILMARRAY 2.0

Clinical and non-clinical studies were carried out to establish that the performance characteristics of the FilmArray Respiratory Panel (RP) are equivalent on the FilmArray and FilmArray 2.0 systems.

Clinical Comparison

The clinical comparison study was performed using specimens previously obtained during the FilmArray RP prospective clinical evaluation supplemented with other archived specimens collected from external medical facilities and reference laboratories to increase the number of specimens being tested for low prevalence analytes. A total of 102 specimens were selected such that each analyte was represented 3-5 times. Each specimen was thawed and tested using the FilmArray and FilmArray 2.0 systems. Overall positive percent agreement (PPA) between systems was 96.8% with the lower bound of the two-sided 95% confidence interval (95% CI) at 92.0%. Overall negative percent agreement (NPA) was 99.9% with the lower bound of the two-sided 95% CI at 99.7%.

Table 50. Analyte Results from FilmArray System Clinical Comparison Study

Analyte	FilmArray 2.0 / FilmArray					
	PPA	%	95% CI	NPA	%	95% CI
Viruses						
Adenovirus	5/5	100%	47.8-100%	97/97	100%	96.3-100%
Coronavirus 229E	5/5	100%	47.8-100%	97/97	100%	96.3-100%
Coronavirus HKU1	6/6	100%	54.1-100%	95/96	99%	94.3-100%
Coronavirus NL63	6/6	100%	54.1-100%	96/96	100%	96.2-100%
Coronavirus OC43	4/5	80%	28.4-99.5%	97/97	100%	96.3-100%
Human Metapneumovirus	5/5	100%	47.8-100%	97/97	100%	96.3-100%
Human Rhinovirus/Enterovirus	8/10	80%	44.4-97.5%	92/92	100%	96.1-100%
Influenza A	16/16	100%	79.4-100%	86/86	100%	95.8-100%
Influenza A H1	3/3	100%	29.2-100%	99/99	100%	96.3-100%
Influenza A H1-2009	6/6	100%	54.1-100%	96/96	100%	96.2-100%
Influenza A H3	7/7	100%	59-100%	95/95	100%	96.2-100%
Influenza B	5/5	100%	47.8-100%	97/97	100%	96.3-100%
Parainfluenza Virus 1	7/7	100%	59-100%	95/95	100%	96.2-100%

Analyte	FilmArray 2.0 / FilmArray					
	PPA	%	95% CI	NPA	%	95% CI
Parainfluenza Virus 2	6/6	100%	54.1-100%	96/96	100%	96.2-100%
Parainfluenza Virus 3	6/6	100%	54.1-100%	96/96	100%	96.2-100%
Parainfluenza Virus 4	6/6	100%	54.1-100%	96/96	100%	96.2-100%
Respiratory Syncytial Virus	8/8	100%	63.1-100%	94/94	100%	96.2-100%
Bacteria						
Bordetella pertussis	4/4	100%	39.8-100%	98/98	100%	96.3-100%
Chlamydophila pneumoniae	3/3	100%	29.2-100%	99/99	100%	96.3-100%
Mycoplasma pneumoniae	5/6	83.3%	35.9-99.6%	96/96	100%	96.2-100%
Overall agreement	***121/125***	***96.8%***	***92.0-99.1%***	***1914/1915***	***99.9%***	***99.7-100%***

System performance for testing of 102 specimens on each platform was calculated. For the FilmArray, a total of 108 runs were attempted, 104 of which were completed (96.3%; 104/108). There were two run failures each for software (1.9%) and instrument (1.9%) errors. No control failures were observed. Two specimens were retested due to Influenza A 'equivocal' results. For the FilmArray 2.0, a total of 102 runs were attempted, all of which completed (100%; 102/102). No control failures were observed.

Reproducibility

A multicenter reproducibility study was performed to determine between-site and overall reproducibility of the FilmArray RP on multi-instrument FilmArray 2.0 systems. Reproducibility testing occurred at three test sites using contrived NPS samples, each spiked with various combinations of four different RP analytes representing the types of organisms detected by the panel (bacteria, DNA viruses and RNA viruses). Each analyte was evaluated at three different concentrations (Negative, Low Positive and Moderate Positive). Negative results for each assay were obtained from samples that were not spiked with a corresponding organism (analyte not in the sample).

The data include 90 replicates per analyte and incorporate a range of potential variation introduced by 7 different operators, 3 different pouch lots, and 10 different FilmArray 2.0 instruments configured on 3 different multi-instrument systems. Similar to the reproducibility of the FilmArray RP on the FilmArray (Tables 44-45 above), percent (%) agreement with the expected Detected, Not Detected or N/A result was 95.6% or better and the standard deviation in Tm was 0.5˚C or less for all assays.

Table 51. Summary of Reproducibility Results on the FilmArray 2.0

Analyte Tested	Concentration Tested	Expected Test Result	% Agreement with Expected Result			
			Site/System			Total (95% Confidence Interval)
			A	B	C	
Bordetella pertussis **Strain A639** Zeptometrix 0801459	**Moderate Positive** 3× LoD $1.2x10^4$ CFU/mL	Detected	29/29[a] 100%	30/30 100%	30/30 100%	89/89[a] 100% (95.9-100%)
	Low Positive 1× LoD $4x10^3$ CFU/mL	Detected	29/30 96.7%	30/30 100%	27/30 90.0%	86/90 95.6% (89.0-98.8%)

Analyte Tested	Concentration Tested	Expected Test Result	% Agreement with Expected Result			
			Site/System			Total
			A	B	C	(95% Confidence Interval)
	Negative	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0-100%)
Adenovirus Species C Serotype 1 Zeptometrix 0810050CF	**Moderate Positive** 3× LoD $3.0x10^2$ $TCID_{50}$/mL	Detected	30/30 100%	30/30 100%	28/30 93.3%	88/90 97.8% (92.2%-99.7%)
	Low Positive 1× LoD $1.0x10^2$ $TCID_{50}$/mL	Detected	28/29[a] 96.60%	30/30 100%	28/30 93.3%	86/89[a] 96.6% (90.5%-99.3%)
	Negative	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0-100%)
Influenza A H1N1-2009 A/SwineNY/03/2009 Zeptometrix 0810109CFN	**Moderate Positive** 3× LoD $3.0x10^2$ $TCID_{50}$/mL	Detected	29/29[a] 100%	30/30 100%	30/30 100%	89/89[a] 100% (95.9-100%)
	Low Positive 1× LoD $1.0x10^2$ $TCID_{50}$/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0-100%)
	Negative	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0-100%)
Respiratory Syncytial Virus Type A Zeptometrix 0810040ACF	**Moderate Positive** 3× LoD 6.0 $TCID_{50}$/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90% 100% (96.0-100%)
	Low Positive 1× LoD 2.0 $TCID_{50}$/mL	Detected	29/29[a] 100%	30/30 100%	30/30 100%	89/89[a] 100% (95.9-100%)
	Negative	Not Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0-100%)

[a] Due to multiple failures in a day, a valid result could not be obtained for one of the replicates, reducing the total number of replicates for Site/System A from 30 to 29 and for All Sites/Systems from 90 to 89.

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For additional information regarding our products and applications, please contact BioFire Diagnostics Customer Support Department, local sales representative or distributor.

Safety Data Sheet (SDS/MSDS)

FilmArray™ Reagent Kit

(According to regulation (EC) 1907/2006.)
Date SDS established : 05.2014
Revision number : 04 // ASAY-PRT-0643-04

1. IDENTIFICATION OF THE SUBSTANCE / MIXTURE AND OF THE COMPANY / UNDERTAKING

1.1 PRODUCT IDENTIFIER

Product Name:	FilmArray™ Reagent Kit
Catalog #:	RFIT-ASY-0002, RFIT-ASY-0007 (RUO) or RFIT-ASY-0114 (IVD), RFIT-ASY-0008 (RUO) or RFIT-ASY-0116 (IVD), RFIT-ASY-0094, RFIT-ASY-0104, RFIT-ASY-0105 (IVD) OR RFIT-ASY-0115 (RUO), RFIT-ASY-0107, RFIT-ASY-0109, RFIT-ASY-0118
Kit Components:	FilmArray™ Respiratory Panel Pouch, BioThreat Pouch, BCID Pouch, Hydration Solution, Sample Buffer, GI Panel Pouch, ME Panel Pouch

1.2 RELEVANT IDENTIFIED USES OF THE SUBSTANCE OR MIXTURE AND USES ADVICES AGAINST

In vitro diagnostic use and for research use.

1.3 DETAILS OF THE SUPPLIER OF THE SAFETY DATA SHEET

Address:	BioFire Diagnostics, LLC, 390 Wakara Way, Salt Lake City, Utah 84108, USA
Telephone Number:	1-801-736-6354
E-mail Address:	support@BioFireDX.com

1.4 EMERGENCY TELEPHONE NUMBER

Call your local emergency center.

2. HAZARDOUS IDENTIFICATION

2.1 CLASSIFICATION OF THE MIXTURE

Sample Buffer:	Acute toxicity (Category 4)
	Serious Eye damage (Category 1)
	Skin irritation (Category 2)

The other kit components are not classified as dangerous mixtures according to regulation 1272/2008.

2.2 LABEL ELEMENTS

Labeling according Regulation (EC) No 1272/2008 [CLP]

Pictogram/Signal Word:	 Danger

Hazardous Statements	
H302	Harmful if swallowed.
H318	Causes serious eye damage.
H315	Causes skin irritation.

Precautionary Statements	
P280	Wear protective gloves/eye protection/face protection.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P301 + P312	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.

Supplemental Hazard Statements

None

2.3 OTHER HAZARDS

None

3. COMPOSITION/INFORMATION ON INGREDIENTS

Component Name/ Hazardous Ingredient	EC nr.	Cas#	Classification acc. 1272/2008	Concentration
Sample Buffer: Guanidinium Chloride	200-0 02-3	50-01-1	Acute toxicity (Cat. 4) Eye irritation (Cat. 2) Skin irritation (Cat. 2)	50-60% w/w
Sample Buffer: Triton X-100	/	9002-93-1	Acute toxicity, Oral (Cat. 4) Serious eye damage (Cat. 1) Chronic aquatic toxicity (Cat. 2)	10-20% w/w

4. FIRST AID MEASURES

4.1 DESCRIPTION OF FIRST AID MEASURES

If Inhaled:	Remove to fresh air. Seek medical attention.
In Case of Skin Contact:	Immediately flush skin with plenty of water for at least 15 minutes. Cover irritated skin with an emollient. Remove contaminated clothing. Seek medical attention.
In Case of Eye Contact:	Check for and remove any contact lenses and immediately flush eyes with copious amounts of water. Seek medical attention.
If Swallowed:	Immediately seek medical attention. If swallowed, induce vomiting as directed to do so by medical personnel. Loosen tight clothing.

4.2 MOST IMPORTANT SYMPTOMS AND EFFECTS, BOTH ACUTE AND DELAYED

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

4.3 INDICATION OF ANY IMMEDIATE MEDICAL ATTENTION AND SPECIAL TREATMENT NEEDED

No data available

5. FIREFIGHTING MEASURES

5.1 EXTINGUISHING MEDIA

Suitable Extinguishing Media:	Use foam, carbon dioxide, water spray or dry chemical powder. Foam and water spray may cause frothing, but are still effective.
Unsuitable Extinguishing Media:	None

5.2 SPECIAL HAZARDS ARISING FROM THE SUBSTANCE OR MIXTURE

Carbon oxides, nitrogen oxides (NOx), Hydrogen chloride gas

5.3 ADVICE FOR FIREFIGHTERS

Wear self-contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

6.1 PERSONAL PRECAUTIONS, PROTECTIVE EQUIPMENT, EMERGENCY PROCEDURES

Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation.

6.2 ENVIRONMENTAL PRECAUTIONS

Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

6.3 METHODS AND MATERIAL FOR CONTAINMENT AND CLEANING UP

Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable, closed containers for disposal.

6.4 REFERENCE TO OTHER SECTIONS

For disposal, see section 13.

7. HANDLING AND STORAGE

7.1 PRECAUTIONS FOR SAFE HANDLING

Avoid contact with skin and eyes. Avoid formation of dust and aerosols.

7.2 CONDITIONS FOR SAFE STORAGE, INCLUDING ANY INCOMPATIBILITIES

Store at room temperature. Keep container closed and away from direct sunlight.

7.3 SPECIFIC END USE(S)

No data available

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 CONTROL PARAMETERS

Components with workplace control parameters: no data available.

8.2 EXPOSURE CONTROLS

Respiratory Protection:	Exhaust ventilation or other engineering controls.
Hand Protection:	Compatible chemical resistant gloves
Eye Protection:	Chemical safety goggles or face shield
Body Protection:	Lab coat
Other Information:	Change contaminated clothing. Wash hands after working with substances.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 INFORMATION ON BASIC PHYSICAL AND CHEMICAL PROPERTIES

Physical State/Form:	Colorless Liquid
Solubility in Water:	Soluble

9.2 OTHER INFORMATION

None

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10. STABILITY AND REACTIVITY

10.1 REACTIVITY

No data available

10.2 CHEMICAL STABILITY

No data available

10.3 HAZARDOUS REACTIONS

No data available

10.4 CONDITIONS TO AVOID

No data available

10.5 INCOMPATIBLE MATERIALS

Oxidizing agents

10.6 HAZARDOUS DECOMPOSITION PRODUCTS

COx and some metallic oxides

11. TOXICOLOGICAL INFORMATION

11.1 INFORMATION ON TOXICOLOGICAL EFFECTS

	Guanidinium Chloride	Triton X-100
Acute Toxicity:	LD50 Oral - rat - 475 mg/kg Remarks: Behavioral: Altered sleep time (including change in righting reflex). Behavioral: Excitement. Diarrhea LD50 Oral - mouse - 571 mg/kg Remarks: Behavioral: Altered sleep time (including change in righting reflex). Behavioral: Muscle contraction or spasticity. Behavioral: Irritability. LD50 Oral - rat – 1,120 mg/kg LC50 Inhalation - rat - 4 h - 5,3 mg/l	LD50 Oral - rat - male - 500 mg/kg LD50 Dermal - rabbit – 8,000 mg/kg
Skin Corrosion/Irritation:	Skin - rabbit - Skin irritation	No data available
Serious Eye Damage/ Eye Irritation:	Eyes - rabbit - Irritating to eyes	Eyes - rabbit - Severe eye irritation
Respiratory or Skin Sensitization:	Buehler Test - guinea pig - Did not cause sensitization on laboratory animals.	No data available
Germ Cell Mutagenicity:	Not mutagenic in Ames Test.	No data available
Carcinogenicity:	IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.	
Reproductive toxicity, Specific target organ toxicity - single exposure, Specific target organ toxicity - repeated exposure, Aspiration hazard: No data available		
Potential Health Effects:	Inhalation: May be harmful if inhaled. May cause respiratory tract irritation. Ingestion: Harmful if swallowed. Skin: May be harmful if absorbed through skin. May cause skin irritation. Eyes: Causes eye burns.	
Signs and Symptoms of Exposure:	To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.	
Additional Information:	RTECS: MF4300000	RTECS: MD0907700

12. ECOLOGICAL INFORMATION

12.1 TOXICITY

Guanidinium Chloride:	Toxicity to fish LC50 - Leuciscus idus (Golden orfe) – 1,759 mg/l
Triton X-100:	Toxicity to fish LC50 - Pimephales promelas (fathead minnow) - 8,9 mg/l - 96,0 h Toxicity to daphnia and other aquatic invertebrates. EC50 - Daphnia - 26 mg/l - 48 h

12.2 PERSISTENCE AND DEGRADABILITY

Guanidinium Chloride:	Result: - Not readily biodegradable.
Triton X-100:	Biodegradability Biotic/Aerobic Biochemical oxygen demand - Exposure time 28 d Result: 36 % - Not readily biodegradable. Method: Closed Bottle test

12.3 BIOACCUMULATIVE POTENTIAL

No data available

12.4 MOBILITY IN SOIL

No data available

12.5 RESULTS OF PBT AND VPVB ASSESSMENT

No data available

12.6 OTHER ADVERSE EFFECTS

Triton X-100:	Toxic to aquatic life with long lasting effects. Chemical Oxygen Demand (COD) 2,19 mg/g

13. DISPOSAL CONSIDERATIONS

13.1 WASTE TREATMENT METHODS

Recommendation:
Chemicals must be disposed of in compliance with the respective national regulations.

Uncleaned packaging:
Recommendation:
Disposal must be made according to official regulations.
Packagings that may not be cleansed are to be disposed of in the same manner as the product.
Recommended cleansing agents:
Water, if necessary together with cleansing agents.

14. TRANSPORT INFORMATION

No restrictions apply due to the low volumes.

15. REGULATORY INFORMATION

This safety datasheet complies with the requirements of Regulation (EC) No. 1907/2006.

15.1 SAFETY, HEALTH AND ENVIRONMENTAL REGULATIONS/LEGISLATION SPECIFIC FOR THE SUBSTANCE OR MIXTURE.

U.S. Federal Regulations:

Triton X-100:	TSCA 8(a) PAIR; TSCA 8(b) inventory; TSCA 8(d) H and S data reporting (1996). SARA 311/312 MSDS distribution – chemical inventory – hazard Identification: Immediate (Acute) and Delayed (Chronic) Health Hazard. SARA 302/304/311/312 hazardous chemicals
Guanidine:	SARA 311/312:; Acute: Yes; Chronic: No

15.2 CHEMICAL SAFETY ASSESSMENT

No data available

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16. OTHER INFORMATION

Classification and procedure used to derive the classification of the sample buffer: Calculation method.

Not for food, drug, household, agricultural or cosmetic use. The above information is correct to the best of our knowledge. The user should make independent decisions regarding completeness of the information based on all sources available. BioFire Diagnostics shall not be held liable for any damage resulting from handling or from contact with the above product.

It remains the user's own responsibility to make sure that the information is appropriate and complete for his specific use of this product. The user is also responsible for observing any laws and applicable guidelines.

Changes to previous version of the SDS: Revision date: 05.01.2014
Revised for compliance with Regulation (EC) 1907/2006 (REACH)

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