To: Daniel Young[dyoung@theranos.com]; Arnold Gelb[agelb@theranos.com]; Nicholas Menchel[nmenchel@theranos.com]
Cc: Sani Hadziahmetovic[shadziahmetovic@theranos.com]; Chinmay Pangarkar[cpangarkar@theranos.com]
From: Andrea Cuppoletti
Sent: Mon 10/31/2011 11:01:05 PM
Importance: Normal
Subject: RE: LDT "Validation" under CLIA
Received: Mon 10/31/2011 11:01:06 PM
510(K)_Filmarray.pdf

Arnie,

Attached is the FDA approval document for the Idaho Technologies panel. We used it as a guideline for our internal validation of the synthetic targets.

Andrea

From: Daniel Young
Sent: Friday, October 28, 2011 8:15 AM
To: Arnold Gelb; Nicholas Menchel
Cc: Sani Hadziahmetovic; Chinmay Pangarkar; Andrea Cuppoletti
Subject: RE: LDT "Validation" under CLIA

Attached are two FDA guidances relevant for nucleic acid amplification tests and panels. Andrea, I believe I sent these to you previously – these should serve as a useful template for us for establishing our protocol.

1. "Establishing Performance Characteristics of In Vitro Diagnostic Devices for Detection or Detection and Differentiation of Influenza Viruses"

2. "Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay"

-Daniel

From: Arnold Gelb
Sent: Friday, October 28, 2011 12:00 AM
To: Nicholas Menchel
Cc: Daniel Young; Sani Hadziahmetovic; Chinmay Pangarkar; Andrea Cuppoletti
Subject: FW: LDT "Validation" under CLIA

It would be extremely helpful to get this type of information from Andrea and Chinmay. Thanks.

From: Surekha Gangakhedkar
Sent: Thursday, October 27, 2011 1:56 PM
To: Arnold Gelb
Cc: Sani Hadziahmetovic; Nicholas Menchel
Subject: RE: LDT "Validation" under CLIA

Hi Arne,

As discussed here is the copy of a validation protocol used previously for a project to revise to CLIA guidelines.

Thanks,

Surekha

From: Arnold Gelb
Sent: Wednesday, October 26, 2011 2:38 PM
To: Surekha Gangakhedkar
Subject: LDT "Validation" under CLIA

Hi Surekha,

Here is the general approach for "validating" an LDT under CLIA:

- Decide on final version of test procedure to be used for method validation studies
- Develop a method validation protocol including the following:
  - Determine applicable test performance specifications to be established
  - Identify which reagents, specimens, etc. will require stability studies to establish storage and expiration specifications
  - Determine if validation needs to address the issue of interfering substances in the patient sample; if yes, develop study protocol to assess the impact of these interfering substances on test accuracy
  - Establish patient specimen acceptability criteria
  - Develop a study protocol to generate data for the purposes of establishing test performance specifications; clinical trials may be required if clinical significance of test results will be reported.
  - Develop appropriate maintenance and control methods to properly control all aspects of the test system
  - If applicable develop a protocol to establish reference ranges (i.e. normal values)
  - Develop a "proficiency testing" protocol to independently assess test accuracy after approval to test and report patient results (can be done post-validation)
  - Establish a review and approval process for method validation studies
  - Determine if test system uses one or more Analyte Specific Reagents (ASR's); if yes, final report must include FDA-mandated qualifier
  - Establish format for final reporting of test results
- Conduct studies to generate performance specification data
- Analyze study data and determine performance specifications
- Document review and approval of test validation study
- Ensure that the test procedure and effective start date for the new procedure are approved by laboratory director
- Document training of all appropriate laboratory personnel
- Retain study source data, analyses, and approval documentation for the life of the test plus 2 years

Once Daniel has provided a prioritized list of tests, I will review it with you to determine which ones are sufficiently developed/have above data to start pursuing a validation. Thanks.

Arne

K103175

11 510(k) Summary

FEB 17 2011

510(k) Summary
Idaho Technology Inc. FilmArray RP Test System

Introduction: According to the requirements of 21 CFR 807.92, the following information provides sufficient detail to understand the basis for a determination of substantial equivalence.

Submitted by:

Idaho Technology Inc
390 Wakara Way
Salt Lake City, UT 84108

Telephone: 801-736-6354
Facsimile: 801-588-0507

Contact: Beth Lingenfelter, ext. 407

Date Submitted: February 2, 2011

Device Name and Classification:

Trade Name: FilmArray RP System

Regulation Number: 21 CFR 866.3980

Classification Name: Respiratory Viral Panel Multiplex Nucleic Acid Assay

Predicate Device:

K063765, K081483, K091677 - Luminex® xTAG™ Respiratory Viral Panel (RVP).

Intended Use:

The FilmArray Respiratory Panel (RP) is a multiplexed nucleic acid test intended for use with the FilmArray instrument for the simultaneous qualitative detection and identification of multiple respiratory viral nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections. The following virus types and subtypes are identified using the FilmArray RP: Adenovirus, Coronavirus HKU1, Coronavirus NL63, Human Metapneumovirus, Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype 2009 H1, Influenza B, Parainfluenza virus 3, Rhinovirus/Enterovirus, and Respiratory Syncytial Virus. The detection and identification of specific viral 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. Negative results do not preclude respiratory infection and should not be used as the sole basis for diagnosis, treatment or other management decisions. Positive results do not rule out bacterial infection or co-infection with other organisms. The agent detected may not be the definite cause of disease. The use of additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) and clinical presentation must be taken into considertation in order to obtain the final diagnosis of respiratory infection.

Due to seasonal prevalence, performance characteristics for Influenza A/H1, Influenza A/H3, Influenza A/2009 H1, and Influenza B were established primarily with retrospective 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 detects Adenovirus species C serotype 6 with reduced sensitivity. It is recommended that specimens found to be negative for Adenovirus after examination using FilmArray RP be confirmed by an alternate method (e.g. FDA-cleared molecular test or cell culture).

Performance characteristics for influenza A were established when influenza A/2009 H1N1, A/H1, and A/H3 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary. 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.

Device Description:

The FilmArray RP System is multiplex nucleic acid test system composed of the FilmArray instrument, the FilmArray software (preinstalled on a laptop computer) and the FilmArray RP pouch. The FilmArray RP pouch contains freeze-dried reagents to perform nucleic acid purification, reverse transcription, and nested, multiplex PCR with DNA melt analysis. The Respiratory Panel (RP) pouch identifies 12 common and emerging viral respiratory pathogens (see Table 1).

Table 1. Viruses Detected by the FilmArray Respiratory Panel

Viral Respiratory Pathogens
Influenza A
H1 subtype
H3 subtype
2009 H1 subtype
Influenza B
Adenovirus
Coronavirus HKU1
Coronavirus NL63
Human Metapneumovirus
Parainfluenza Virus 3
Respiratory Syncytial Virus
Rhinovirus and Enterovirus

A test is initiated by loading Hydration Solution and an unprocessed patient nasopharyngeal swab (NPS) specimen (i.e. specimen mixed with Sample Buffer) into the FilmArray RP pouch. The pouch contains all of the reagents required for specimen testing and analysis in a freeze-dried format; the addition of Hydration Solution and Sample/Buffer Mix rehydrates the reagents. After the pouch is prepared, the FilmArray software guides the user though the steps of placing the pouch into the instrument, scanning the pouch barcode, entering the sample identification and initiating the run.

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

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

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

Substantial Equivalence:

The Luminex® xTAG™ RVP is a PCR-based system for detecting the presence of viral nucleic acid in nasopharyngeal swabs collected from individuals with signs and symptoms of respiratory illness. The similarities and differences between the xTAG RVP and the FilmArray RP are outlined below.

Table 2. Similarities between the xTAG RVP and the FilmArray RP

Element	FilmArray Respiratory Panel Test System	Luminex® xTAG™ RVP
Organisms Detected	Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus, human Metapneumovirus, Adenovirus, Parainfluenza 3, and Rhinovirus/Enterovirus	Same See below for differences
Analyte	RNA/DNA	Same
Technological Principles	multiplex nucleic acid	Same See below for differences
Specimen Types	Nasopharyngeal swabs	Same

Table 3. Differences between the xTAG RVP and the FilmArray RP

Element	FilmArray Respiratory Panel Test System	Luminex® xTAG™ RVP
Organisms Detected	Can distinguish Influenza A subtype 2009 H1 from Influenza A subtype H1. Also detects Coronavirus NL63 and Coronavirus HKU1.	Can distinguish Respiratory Syncytial Virus Type A from Respiratory Syncytial Virus Type B. Detects Parainfluenza virus 1 and Parainfluenza virus 2.
Technological Principles	Nested multiplex RT-PCR followed by high resolution melting analysis to confirm identity of amplified product.	Multiplex RT-PCR and multiplex TSPE followed by Fluorescence-activated sorting of labeled beads coupled to streptavidin-conjugated biotinylated products
Instrumentation	FilmArray Instrument	PCR Thermocycler Luminex® 100 IS or 200 system
Time to result	Less than 1 hour	Approximately 8 hours
Test Interpretation	Automated test interpretation and report generation. User cannot access raw data.	Semi-automated test interpretation. User must review all "no call" results to determine cause and retesting strategy.
Sample Preparation Method	Sample Processing is automated in the FilmArray instrument.	Up front sample processing is required to extract nucleic acid.

Element	FilmArray Respiratory Panel Test System	Luminex® xTAG™ RVP
Reagent Storage	Reagents are stored at room temperature.	Reagents stored at 4°C and -20°C.
Controls	Two controls are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis.	Internal control added to each sample. External control processed with each batch of samples.
User Complexity	Moderate/Low	High

Summary of Performance Data

Clinical Performance

The clinical performance of the FilmArray RP system was established during a prospective study at 3 U.S. clinical sites over a 6 month time period (December 2009 through May 2010). Subjects with signs and/or symptoms of respiratory infection were invited to participate. Upon obtaining informed consent, NPS samples were collected for FilmArray and comparator testing; a second respiratory sample was collected from each subject for viral culture reference testing. A total of 857 subjects were initially enrolled in the study and four were withdrawn. Table 4, provides a summary of demographic information for the 853 subjects that participated in the prospective study.

Table 4. Demographic Summary for FilmArray RP Prospective Study

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

Each NPS specimen 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 5.

Table 5. Reference/Comparator Methods Used to Assess FilmArray RP Performance

Organism/Virus	Reference/Comparator Method(s)
Adenovirus	Viral culture followed by DFA identification[1]
Influenza A	
Influenza B	
Parainfluenza virus 3	
Respiratory Syncytial Virus	
FluA/H1 subtyping	1 PCR test of influenza A positive viral culture with bi-directional sequence confirmation[2]
FluA/H3 subtyping	
FluA/2009 H1subtyping	
Human Rhinovirus	2 PCR tests of patient specimen with bi-directional sequence confirmation[3]
Enterovirus	
Coronavirus NL63	
Coronavirus HKU1	
Human Metapneumovirus	

[1] Performance of the FilmArray RP system detecting Adenovirus, Flu A, Flu B, PIV3, or RSV, respectively, was compared to viral culture followed by fluorescent antibody identification. "True" Adenovirus, Influenza A, Influenza B, Parainfluenza virus 3, or RSV positives, respectively, were considered as any sample that tested positive for Adenovirus, Influenza A, Influenza B, Parainfluenza virus 3, or RSV, respectively, by viral culture followed by DFA testing. "True" Adenovirus, Influenza A, Influenza B, Parainfluenza virus 3, or RSV negatives, respectively, were considered as any sample that tested negative for Adenovirus, Influenza A, Influenza B, Parainfluenza virus 3, or RSV, respectively, by viral culture followed by DFA testing.

[2] Performance of the FilmArray RP system detecting Influenza A/H1, A/H3, or A/2009 H1, 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" Influenza A/H1, A/H3, or A/2009 H1 positives, respectively, were considered as any sample that tested positive for Influenza A by viral culture, and had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched Influenza A/H1, A/H3, or A/2009 H1 sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), respectively, with acceptable E-values. "True" Influenza A/H1, A/H3, or A/2009 H1 negatives, respectively, were considered as any sample that tested negative for Influenza A by viral culture, or any sample that tested positive for Influenza A virus by viral culture, but tested negative by the respective Influenza A subtype specific PCR assay.

[3] Performance of the FilmArray RP system detecting Human Rhinovirus, Enterovirus, Coronavirus NL63, Coronavirus HKU1, or Human Metapneumovirus, 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" Human Rhinovirus, Enterovirus, Coronavirus NL63, Coronavirus HKU1, or Human Metapneumovirus positives, respectively, were considered as any sample that had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched Human Rhinovirus, Enterovirus, Coronavirus NL63, Coronavirus HKU1, or Human Metapneumovirus sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), respectively, with acceptable E-values. "True" Human Rhinovirus, Enterovirus, Coronavirus NL63, Coronavirus HKU1, or Human Metapneumovirus negatives, respectively, were considered as any sample that tested negative by both of the comparator PCR assays.

A total of 853 specimens were evaluated in this study. 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 analyte and false negative (FN) indicates that the FilmArray result was negative while the comparator result was positive. Specificity 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 results was negative. The exact binomial two-sided 95% confidence interval was calculated. The results are summarized in Table 6.

Table 6. Clinical Sensitivity and Specificity for the FilmArray RP Prospective Clinical Study

	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 [d]	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/2009 H1	8/9	88.9%	51.8 - 99.7%	841/844 [d]	99.6%	99.0 - 99.9%
Influenza B	0/0	n/a	n/a	853/853	100%	99.6 - 100%
Parainfluenza Virus 3	23/24 [e]	95.8%	78.9 – 99.9%	819/829 [f]	98.8%	97.8 - 99.4%
Respiratory Syncytial Virus	52/52	100%	93.2 - 100%	714/801 [g]	89.1%	86.8 - 91.2%
	PPA		**95% CI**	**NPA**		**95% CI**
Coronavirus HKU1	23/24	95.8%	78.9 - 99.9%	827/829 [c]	99.8%	99.1 - 100%
Coronavirus NL63	23/24	95.8%	78.9 - 99.9%	829/829	100%	99.6 - 100%
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%

[a] The FilmArray RP system 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] CoV-HKU1 viruses were identified in 2/2 false positive specimens using bi-directional sequence analysis with an alternate assay.

[d] Influenza A viruses (2009 H1 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.

[e] The FilmArray RP system detected PIV3 in the single false negative specimen when retested.

[f] PIV3 viruses were identified in 10/10 false positive specimens using bi-directional sequence analysis.

[g] RSV viruses were identified in 83/87 false positive specimens using bi-directional sequence analysis.

The FilmArray RP system detected a total of 81 mixed infections in the prospective clinical evaluation. This represents 16.1% of the total positive specimens (81/502). Seventy-six (76/81; 93.8%) were double infections, and 5 (5/81; 6.2%) were triple infections. The total number of test results comprising these co-infections was 167. The single most common co-infection (21/81; 25.9%) was Human Rhinovirus/Enterovirus with Respiratory Syncytial Virus. These viruses were the most prevalent in the tested population. Out of the 81 co-infections, 47 contained one or more analytes that had not been detected with the reference/comparator methods, i.e. discrepant co-infection.

Table 7. Mixed Infections Detected by FilmArray RP and Prevalence of Individual Analytes in Mixed Infections

Organism Combinations	Number of Positive Samples	Percentage of Total Samples Tested	Organism	Number of Mixed Infections	Prevalence in Mixed Infections
HRV/Entero + RSV	21	2.46%	Adenovirus	16	19.75%
Adenovirus + HRV/Entero	9	1.06%	CoVHKU1	10	12.35%
HRV/Entero + PIV3	8	0.94%	CoVNL63	13	16.05%
hMPV + HRV/Entero	7	0.82%	hMPV	22	27.16%
hMPV + RSV	4	0.47%	HRV/Entero	56	69.14%
CoVNL63 + HRV/entero	4	0.47%	FluA/H1	0	0.00%
CoVHKU1 + hMPV	3	0.35%	FluA/H1-2009	0	0.00%
CoVHKU1 + HRV/Entero	3	0.35%	FluA/H3	0	0.00%
CoVHKU1 + RSV	3	0.35%	FluB	0	0.00%
CoVNL63 + hMPV	3	0.35%	PIV3	14	17.28%
CoVNL63 + RSV	3	0.35%	RSV	36	44.44%
hMPV + PIV3	3	0.35%			
Adenovirus + HRV/Entero + PIV3	2	0.23%			
Adenovirus + RSV	2	0.23%			
Adenovirus + CoVNL63	1	0.12%			
Adenovirus + hMPV	1	0.12%			
Adenovirus + PIV3	1	0.12%			
CoVNL63 + HRV/Entero + RSV	1	0.12%			
CoVHKU1 + HRV/Entero + RSV	1	0.12%			
CoVNL63 + hMPV + RSV	1	0.12%			
Total Mixed Infections	**81**	**9.50%**			

Table 8 provides the prevalence and age distribution of FilmArray RP detected analytes in the clinical study. The most prevalent analytes were Human Rhinovirus/Enterovirus, Respiratory Syncytial Virus, and Human Metapneumovirus; these three analytes comprised 78% of all positive results. Positive results were obtained across all age groups tested; however, the majority was detected in children 5 years or younger.

Table 8. Prevalence and Age Distribution of Analytes in the Clinical Study

Analyte	Total (Prevalence)	≤ 5 years	6-21 years	22-49 years	≥ 50 years
Adenovirus	38 (4.5%)	32	2	3	1
Coronavirus HKU1	25 (2.9%)	12	1	8	4
Coronavirus NL63	23 (2.7%)	17	2	2	2
Human Metapneumovirus	94 (11%)	76	4	10	4
Human Rhinovirus/Enterovirus	225 (26.4%)	161	24	29	11
Influenza A (all subtypes)	11 (1.3%)	1	1	7	2
Influenza A/H1	0 (0%)	0	0	0	0
Influenza A/2009 H1	11 (1.3%)	1	1	7	2
Influenza A/H3	0 (0%)	0	0	0	0
Influenza B	0 (0%)	0	0	0	0
Parainfluenza Virus 3	33 (3.9%)	31	1	0	1
Respiratory Syncytial Virus	139 (16.3%)	127	3	4	5

Several analytes, such as influenza viruses were either not encountered in the clinical study or had a low prevalence. To supplement the results of the prospective clinical study, an evaluation of preselected archived samples was performed.

Testing of Preselected Archived Specimens

In addition to the prospective clinical study, archived clinical NPS specimens were also tested using the FilmArray RP. The specimens were selected because they had previously tested positive for one of the following organisms: Adenovirus, Enterovirus, Influenzas A/H1, 2009 H1N1, and H3, Influenza B, and Parainfluenza Virus 3, or had been negative by previous testing methods. Prior to testing with the FilmArray RP, the presence or absence of the analyte of interest was confirmed in each specimen using analyte specific PCR and bi-directional sequencing. Of 400 specimens, 349 were confirmed to contain the analyte of interest (or lack thereof for negative specimens). The specimens were organized into "test panels" and randomized such that the users testing the samples with the FilmArray RP were blinded as to the expected test result. Each panel contained specimens known to be positive and negative for the specific analyte being evaluated allowing 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 9 and the results of the FilmArray testing are presented in Table 10.

Table 9. Demographic Summary of FilmArray RP Archived Specimen Study

Total Specimens		349
Sex	Female (%)	82 (23.5%)
	Male (%)	79 (22.6%)
	Unknown	188(53.9%)
Age	Avg	14.1
	Median	4.0
	Min	0.5
	Max	83.0
Age Range	≤5	89 (25.5%)
	6-21	35 (10.0%)
	22-49	23 (6.6%)
	≥50	14 (4.0%)
	Unknown[a]	188(53.9%)

[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.

Table 10. FilmArray Archived Specimen Performance Data Summary

	Positive Percent Agreement (PPA)			Negative Percent Agreement (NPA)		
	TP/TP +FN	Percent	95% CI	TN/TN+FP	Percent	95% CI
Adenovirus	27/27	100.0%	87.2 - 100%	28/28	100.0%	87.7 - 100%
Enterovirus	22/23	95.7%	78.0 - 99.9%	90/90	100.0%	96.0 - 100%
FluA/H1	32/32	100.0%	89.1 - 100%	127/127	100.0%	97.1 - 100%
FluA/H1-2009	34/34	100.0%	89.7 - 100%	125/125	100.0%	97.1 - 100%
FluA/H3	54/54	100.0%	93.4 - 100%	105/105	100.0%	96.5 - 100%
Influenza B	30/30	100.0%	88.4 - 100%	129/129	100.0%	97.2 - 100%
PIV3	36/36	100.0%	90.3 - 100%	93/93	100.0%	96.1 - 100%

Selected Analytic Studies

Limit of Detection

The analytical sensitivity or Limit of Detection (LoD) for each FilmArray RP analyte (except for Coronavirus HKU1) was determined by testing limiting dilutions of live, quantified viruses. The LoD for Coronavirus HKU1 was determined by testing limiting dilutions of a clinical specimen containing a high viral load of Coronavirus HKU1. LoD is defined as the lowest concentration at which the analyte is consistently detected (detection in ≥95% of samples tested). Simulated NPS sample matrix (cultured human cells in VTM) was spiked with one or more analytes and at least 20 replicates were tested at the LoD concentration. The LoD for each FilmArray RP analyte is listed in Table 11.

Table 11. LoD for Analytes Detected by FilmArray RP

Organism	Strain Identification	Limit of Detection
Adenovirus	Serotype 1 (Species C)	300 $TCID_{50}$/mL
Coronavirus HKU1[a]	Clinical Specimen (Type B)	1.9 x 10^6 RNA copies/mL
Coronavirus NL63	NR-470	5 $TCID_{50}$/mL
Human Metapneumovirus	Type A1 (hMPV-16, IA10-2003)	2 $TCID_{50}$/mL
Enterovirus	Echovirus 6	30,000 $TCID_{50}$/mL
Human Rhinovirus	A1	1 $TCID_{50}$/mL
Influenza A/H1	A/Brisbane/59/07	200 $TCID_{50}$/mL
	A/New Caledonia/20/99	2,000 $TCID_{50}$/mL
Influenza A/2009 H1	A/SwineNY/03/2009	100 $TCID_{50}$/mL
Influenza A/H3	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	60 $TCID_{50}$/mL
Parainfluenza Virus 3	Type 3	10 $TCID_{50}$/mL
Respiratory Syncytial Virus	Type A	2 $TCID_{50}$/mL

[a] Coronavirus HKU1 was quantified by a non-FilmArray real-time PCR assay against a standard curve of synthetic Coronavirus HKU1 RNA transcript to obtain quantification of the viral nucleic acid in the clinical specimen (RNA copies/mL).

NOTE: Most analytes were re-grown and quantified in $TCID_{50}$ (50% Tissue Culture Infectious Dose). 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 system assays was evaluated with an inclusivity panel consisting of 99 strains or isolates that represent the genetic, temporal, and geographic diversity of the FilmArray RP analytes. The tested organisms include: 17 Adenovirus, 4 Coronavirus (3 HKU1 and 1 NL63), 10 human Metapneumovirus, 12 Enterovirus, 14 Rhinovirus, 22 Influenza A (including 10 Influenza A/H1, 3 Influenza A/2009 H1 and 9 Influenza A/H3), 11 Influenza B, 3 Parainfluenza virus 3 and 6 Respiratory Syncytial Virus. Each organism was initially tested in a simulated NPS sample matrix at or near the system LoD. Higher concentrations were tested if the analyte was not detected at LoD.

Each of the 99 strains tested in this study were reactive with the FilmArray RP system. Reactivity of the FilmArray RP system with Adenovirus C serotype 6 was 10,000-fold less than the system LoD due to sequence variation in the region targeted by the FilmArray RP Adenovirus assay.

Additional clinical data analyses, and *in silico* analyses were also carried out to supplement the testing of the inclusivity panel.

Results from inclusivity testing are presented below. The concentration and multiple of LoD at which each strain was detected by the FilmArray RP system is indicated.

Table 12. Results of Inclusivity Testing for Adenovirus

Species	Serotype (Isolate)	Concentration	Multiple of LoD
A	**31**	300 $TCID_{50}$/mL	1x
B	**3**	300 $TCID_{50}$/mL	1x
	7a	300 $TCID_{50}$/mL	1x
	7d2 (Iowa/2001)	300 $TCID_{50}$/mL	1x
	7h (Iowa/1999)	300 $TCID_{50}$/mL	1x
	11 (Wisconsin/2005)	3,000 $TCID_{50}$/mL	10x
	14 (Missouri/2005)	300 $TCID_{50}$/mL	1x
	21 (Missouri/2005)	300 $TCID_{50}$/mL	1x
	34 (Texas/2005)	300 $TCID_{50}$/mL	1x
C	**1**	300 $TCID_{50}$/mL	1x
	2 (New York/2004)	30,000 $TCID_{50}$/mL	100x
	5	3,000 $TCID_{50}$/mL	10x
	6 (Colorado/2005)*	3,000,000 $TCID_{50}$/mL	10,000x
D	**8**	3,000 $TCID_{50}$/mL	10x
E	**4a** (S Carolina/2004)	300 $TCID_{50}$/mL	1x
	4p3 (New Jersey/2005)	300 $TCID_{50}$/mL	1x

Species	Serotype (Isolate)	Concentration	Multiple of LoD
F	**41** (Indiana/2004)	300 $TCID_{50}$/mL	1x

*Due to sequence variation, the FilmArray RP Adenovirus assay reacts with adenovirus serotype 6 (species C) less efficiently than with other adenovirus serotypes.

Supplemental Adenovirus Reactivity Information
(Clinical Data and *in silico* analyses):

A subset of prospective and retrospective archived clinical specimens that were FilmArray 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 polymerase gene identified adenovirus species B (serotypes 3, 3+11p, 7, 16 and 21), species C (serotypes 1, 2, and 5), and species E (serotype 4) in these clinical specimens. Species B, C and E are the most common Adenovirus species associated with respiratory illness. Species A, D, and F (often associated with conjunctivitis and gastroenteritis) were not identified in clinical specimens.

In addition to laboratory testing, bioinformatics resources were also used to predict reactivity of additional Adenovirus species and serotypes with the FilmArray RP System. Simulated reactivity was based on the number and location of mismatches between the target sequence and the assay primer(s). Table 13 lists the adenovirus types that were not tested by the FilmArray system either in analytical or clinical testing. Based on the bioinformatics analysis, the FilmArray RP system is predicted to react with all of the indicated Adenovirus species and serotypes.

Table 13. Simulated FilmArray RP Reactivity with Untested Adenovirus Serotypes

Virus	Species	Serotype	GenBank ID	Simulated FilmArray Adenovirus Result
Human Adenovirus	A	12	AB330093	Positive
		18	DQ149610	Positive
	B	16	AB330097	Positive
		35	AB052912	Positive
		50	DQ149643	Positive
	D	9	AB330090	Positive
		10	DQ149615	Positive
		13	DQ149616	Positive
		15	DQ149617	Positive
		17	AB330098	Positive
		19	DQ149618	Positive
		20	DQ149619	Positive
		22	DQ149620	Positive
		23	DQ149621	Positive
		24	DQ149622	Positive

Table 13. Simulated FilmArray RP Reactivity with Untested Adenovirus Serotypes

Virus	Species	Serotype	GenBank ID	Simulated FilmArray Adenovirus Result
		25	DQ149623	Positive
		26	DQ149624	Positive
		27	DQ149625	Positive
		28	DQ149626	Positive
		29	DQ149627	Positive
		30	DQ149628	Positive
		32	DQ149629	Positive
		33	DQ149630	Positive
		36	DQ149631	Positive
		37	DQ149632	Positive
		38	DQ149633	Positive
		39	DQ149634	Positive
		42	DQ149635	Positive
		43	DQ149636	Positive
		44	DQ149637	Positive
		45	DQ149638	Positive
		46	DQ149639	Positive
		47	DQ149640	Positive
		48	AB330129	Positive
		49	DQ149641	Positive
		51	DQ149642	Positive
		53	FJ169625	Positive
	F	40	AB330121	Positive
	G	52	DQ923122	Positive

Table 14. Results of Inclusivity Testing for Coronaviruses

Type	Strain / Isolate	Concentration [ab]	Multiple of LoD
HKU1	Clinical Sample #1120	2.08×10^6 RNA copies/mL	1.1x
	Clinical Sample # 6123	1.41×10^4 $TCID_{50}$/mL ~1.9×10^6 RNA copies/mL	1x
	Clinical Sample #6213 (Type B)[c]	1.9×10^6 RNA copies/mL	1x
NL63	BEI Resources NR-470	50 $TCID_{50}$/mL	1x

[a] Virus contained in Clinical Sample #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] Phylogenetic information for Coronavirus HKU1 Clinical Sample #6213 was obtained from bi-directional sequence analysis of the nucleocapsid (N) gene.

Table 15. Results of Inclusivity Testing for Human Metapneumovirus

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

Table 16. Results of Inclusivity Testing for Enterovirus and Rhinovirus

Species	Strain	Concentration [a]	Multiple of LoD
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	9400 $TCID_{50}$/mL [b]	<1x
Enterovirus B	Coxsackievirus A9	9400 $TCID_{50}$/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	9400 $TCID_{50}$/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
	A34 (137-3) ATCC VR-507	1 $TCID_{50}$/mL	1x
	A57 (Ch47) ATCC VR-1600	100 $TCID_{50}$/mL	100x

Species	Strain	Concentration [a]	Multiple of LoD
	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 is 30,000 $TCID_{50}$/mL. The LoD for Rhinovirus is 1 $TCID_{50}$/mL.

[b] Strains were tested below the 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 (5' UTR) 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 (PV1, PV2, and PV3). 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 GenBank sequences indicates the FilmArray RP assay will react with Poliovirus types 1, 2 and 3, giving a Human Rhinovirus/Enterovirus result.

Table 17. 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

Table 18. Results of Inclusivity Testing for Influenza A

Type	Strain	Concentration	Multiple of LoD
Influenza A (H1N1)	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 (H1N1-2009)	Swine NY/01/2009	100 $TCID_{50}$/mL	1x
	Swine NY/02/2009	100 $TCID_{50}$/mL	1x
	Swine NY/03/2009	100 $TCID_{50}$/mL	1x
Influenza A (H3N2)	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

Type	Strain	Concentration	Multiple of LoD
	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
	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 and *in silico* analyses):

Results of testing viral isolates or nucleic acid from viral culture indicate that the FilmArray RP pan-influenza and subtyping assays react with virus of swine and avian origin as expected (Table 19).

Table 19. 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)	14.8 ng RNA [b]	Influenza A H1

[a] No reactivity was observed with the 2009 H1 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. The strains listed in Table 20 are predicted to react with the FilmArray RP pan-influenza A and H1, H1-2009 or H3 subtyping assays as indicated.

Table 20. Simulated Reactivity of FilmArray Influenza A Assays with Human, Swine, and Avian Influenza Strains

Host	Subtype	Strain	GenBankID	Simulated FilmArray RP Reactivity
Human	H1N1	A/California/UR06-0393/2007(H1N1)	CY026540	Influenza A H1
			CY026543	
			CY026539	
	H1N1-2009	A/Aalborg/INS133/2009(H1N1)	CY063606	Influenza A H1-2009
			CY063610	
			CY063607	
	H1N2	A/New York/297/2003(H1N2)	CY002668	Influenza A H1
			CY002665	
			CY002664	
	H2N2	A/Albany/20/1957(H2N2)	CY022013	Influenza A (no subtype detected)
			CY022014	
			CY022017	
	H3N2	A/Boston/38/2008(H3N2)	CY044581	Influenza A H3
			CY044584	
			CY044580	
	H5N1	A/Cambodia/R0405050/2007(H5N1)	HQ200572	Influenza A (no subtype detected)
			HQ200573	
			FJ225472	
		A/Hong Kong/486/97(H5N1)	AF084281	
			AF255368	
			AF115289	
	H7N2	A/New York/107/2003(H7N2)	EU587368	Influenza A (no subtype detected)
			EU587373	
			EU587374	
	H7N3	A/Canada/rv504/2004(H7N3)	CY015006	Influenza A (no subtype detected)
			CY015007	
			CY015010	
	H7N7	A/Netherlands/219/03(H7N7)	AY340089	Influenza A (no subtype detected)
			AY342422	
			AY338459	
	H9N2	A/Hong Kong/1073/99(H9N2)	AJ404626	Influenza A (no subtype detected)
			AJ278647	
			AJ278649	
Swine	H1N1	A/swine/Wisconsin/1/1971(H1N1)	CY022414	Influenza A H1
			CY022417	
			CY022413	
	H1N2	A/swine/Hong Kong/NS857/2001(H1N2)	GQ229348	Influenza A H1
			GQ229350	
			GQ229347	
		A/swine/Sweden/1021/2009(H1N2)	GQ495135	Influenza A (no subtype detected)
			GQ495136	
			GQ495132	
	H5N1	A/swine/East Java/UT6010/2007(H5N1)	HM440124	Influenza A (no subtype detected)
			HM440111	
			HM440123	
Avian	H2N2	A/chicken/New York/13828-3/1995(H2N2)	CY014822	Influenza A (no subtype detected)
			CY014825	
			CY014821	
		A/Japan/305/1957(H2N2)	CY045804	Influenza A (no subtype detected)
			CY014977	
			CY014980	

Table 20. Simulated Reactivity of FilmArray Influenza A Assays with Human, Swine, and Avian Influenza Strains

Host	Subtype	Strain	GenBankID	Simulated FilmArray RP Reactivity
		A/Korea/426/1968(H2N2)	CY031595	Influenza A (no subtype detected)
			CY031596	
			CY031599	
	H3N1	A/blue-winged teal/ALB/452/1983(H3N1)	CY004635	Influenza A H3
			CY004638	
			CY005940	
	H3N2	A/American black duck/North Carolina/675-075/2004(H3N2)	GU051135	Influenza A (no subtype detected)
			GU051136	
			GU051137	
	H3N5	A/mallard/Netherlands/2/1999(H3N5)	CY060261	Influenza A H3
			CY060264	
			CY060265	
	H3N6	A/American black duck/New Brunswick/25182/2007(H3N6)	CY047696	Influenza A (no subtype detected)
			CY047697	
			CY047700	
	H3N7	A/northern shoveler/California/HKWF1367/2007(H3N7)	CY033372	Influenza A H3
			CY033375	
			CY033376	
	H3N8	A/American black duck/Washington/699/1978(H3N8)	GU052299	Influenza A H3
			GU052302	
			GU052300	
	H4N6	A/blue-winged teal/Minnesota/Sg-00043/2007(H4N6)	CY063977	Influenza A (no subtype detected)
			CY063978	
			CY063981	
	H5N1	A/rook/Rostov-on-Don/26/2007(H5N1)	EU814503	Influenza A (no subtype detected)
			EU814504	
			EU814507	
		A/turkey/VA/505477-18/2007(H5N1)	GU186509	Influenza A (no subtype detected)
			GU186510	
			GU186513	
		A/chicken/Bangladesh/1151-10/2010(H5N1)	HQ156765	Influenza A (no subtype detected)
			HQ156766	
			HQ156764	
	H5N2	A/duck/Pennsylvania/10218/1984(H5N2)	AB295603	Influenza A (no subtype detected)
			AB286120	
			AB286652	
	H5N3	A/duck/Singapore/F119/3/1997(H5N3)	GU052802	Influenza A (no subtype detected)
			GU052803	
			GU052805	
	H6N1	A/duck/PA/486/1969(H6N1)	EU743286	Influenza A (no subtype detected)
			EU743287	
			EU743289	
	H6N2	A/mallard/Czech Republic/15902-17K/2009(H6N2)	HQ244430	Influenza A (no subtype detected)
			HQ244433	
			HQ244434	
	H7N7	A/mallard/Korea/GH171/2007(H7N7)	FJ750872	Influenza A (no subtype detected)
			FJ959087	
			FJ959090	
	H9N2	A/turkey/Wisconsin/1/1966(H9N2)	CY014663	Influenza A

Table 20. Simulated Reactivity of FilmArray Influenza A Assays with Human, Swine, and Avian Influenza Strains

Host	Subtype	Strain	GenBankID	Simulated FilmArray RP Reactivity
			CY014664	(no subtype detected)
			CY014667	
	H10N7	A/chicken/Germany/N/1949(H10N7)	GQ176136	Influenza A (no subtype detected)
			GQ176135	
			GQ176132	
	H11N9	A/duck/Memphis/546/1974(H11N9)	CY014691	Influenza A (no subtype detected)
			GQ257441	
			CY014687	

Table 21. Results of Inclusivity Testing for Influenza B

Strain	Concentration	Multiple of LoD
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.

Table 22. Results of Inclusivity Testing for Parainfluenza Virus 3

Type	Strain or Source	Concentration	Multiple of LoD
3	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

Table 23. Results of Inclusivity Testing for Respiratory Syncytial Virus

Type	Strain or Source	Concentration	Multiple of LoD
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

Analytical Specificity (Cross-reactivity and Exclusivity)

The potential for cross-reactivity between assays contained in the FilmArray RP system was evaluated by testing simulated NPS samples containing high concentrations of respiratory panel viruses (tens to thousands-fold higher than LoD). No cross-reactivity was observed at the concentrations listed in **Error! Reference source not found.**24.

Table 24. Results of Testing for Cross-Reactivity with FilmArray RP Analytes

Virus or Bacterium	Type / Strain	Test Concentration	Multiple of LoD
Adenovirus	**Serotype 1** (Species C)	$1.00x10^5$ $TCID_{50}$/mL	333 x
Coronavirus	**HKU1 – Type B** Clinical specimen	$2.78x10^9$ copies/mL	1,463 x
	NL63 NR-470	$5.67x10^3$ TCID50/mL	1,134 x
Human Metapneumovirus	**Type A1 - hMPV-16** IA10-2003 A1	$8.17x10^3$ $TCID_{50}$/mL	4,085 x
Human Rhinovirus / Enterovirus	**Echovirus 6**	$3.40x10^6$ $TCID_{50}$/mL	113 x
	Rhinovirus A1	$5.67x10^3$ TCID50/mL	5,670 x
Influenza A H1N1	**A/Brisbane/59/07**	$1.00x10^5$ $TCID_{50}$/mL	500 x
	A/New Caledonia/20/99	$1.00x10^5$ $TCID_{50}$/mL	500 x
	A/PR/8/34	$1.00x10^6$ $TCID_{50}$/mL	5,000 x
	A1/FM/1/47	$4.70x10^3$ $TCID_{50}$/mL	24 x
	A/NWS/33	$4.70x10^3$ $TCID_{50}$/mL	24 x
	A1/Denver/1/57	$4.70x10^3$ $TCID_{50}$/mL	24 x
	A/Solomon Islands/3/2006	$1.39x10^4$ $TCID_{50}$/mL	70 x

Virus or Bacterium	Type / Strain	Test Concentration	Multiple of LoD
	A/Weiss/43	$4.70x10^3$ $TCID_{50}$/mL	24 x
	A/Mal/302/54	$1.39x10^4$ $TCID_{50}$/mL	70 x
Influenza A H1N1-2009	A/SwineNY/03/2009	$4.00x10^5$ $TCID_{50}$/mL	4,000 x
Influenza A H3N2	A/Wisconsin/67/2005	$8.17x10^3$ $TCID_{50}$/mL	1634 x
	A/Victoria/3/75	$4.70x10^3$ $TCID_{50}$/mL	940 x
	A/Port Chalmers/1/73	$5.67x10^3$ $TCID_{50}$/mL	1,134 x
	A/Aichi/2/68	$1.00x10^5$ $TCID_{50}$/mL	20,000 x
	A/Hong Kong/8/68	$1.00x10^5$ $TCID_{50}$/mL	20,000 x
	A/Alice	$4.70x10^3$ $TCID_{50}$/mL	940 x
	A/MRC 2	$8.17x10^3$ $TCID_{50}$/mL	1,634 x
	A/Brisbane/10/07	$8.17x10^3$ $TCID_{50}$/mL	1,634 x
Influenza B	B/FL/04/06	$1.67x10^4$ $TCID_{50}$/mL	278 x
	B/Lee/40	$8.17x10^3$ $TCID_{50}$/mL	136 x
	B/Taiwan/2/62	$5.03x10^4$ $TCID_{50}$/mL	838 x
	B/GL/1739/54	$8.17x10^3$ $TCID_{50}$/mL	136 x
	B/Maryland/1/59	$8.17x10^3$ $TCID_{50}$/mL	136 x
	B/Florida/07/04	$1.00x10^5$ $TCID_{50}$/mL	1,667 x
	B/Malaysia/2506/04	$5.67x10^3$ $TCID_{50}$/mL	95 x
	B/Allen/45	$1.00x10^5$ $TCID_{50}$/mL	1,667 x
	B/HongKong/5/72	$8.17x10^3$ $TCID_{50}$/mL	136 x
	B/Brigit	$3.50x10^4$ $TCID_{50}$/mL	583 x
Parainfluenza Virus	Type 3	$1.00x10^5$ $TCID_{50}$/mL	10,000 x
Respiratory Syncytial Virus	A	$1.39x10^4$ $TCID_{50}$/mL	6,950 x
	B	$2.14x10^4$ $TCID_{50}$/mL	10,700 x

The potential for the FilmArray RP system to cross-react with non-FilmArray RP organisms was evaluated by testing an exclusivity panel consisting of 45 bacteria, 13 viruses, and 1 fungus. These organisms were selected based on their relatedness to FilmArray RP analytes, 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 system did not cross-react with the exclusivity panel organisms listed in Table 25. One measles virus strain was found to contain Adenovirus, which was detected by the FilmArray RP.

Table 25. Non-FilmArray RP Exclusivity Panel

Bacteria	Strain / Isolate
Bordetella bronchiseptica	clinical isolate
Bordetella holmesii	F061
Bordetella parapertussis	A747
Bordetella pertussis	E431
Bordetella pertussis	A639
Bordetella pertussis	ATCC 8467
Bordetella pertussis	ATCC 9797
Bordetella pertussis	ATCC 51445
Bordetella pertussis	ATCC BAA-589
Bordetella pertussis	ATCC 9340
Bordetella pertussis	ATCC 10380
Bordetella pertussis	ATCC BAA-1335
Chlamydia trachomatis	D-UW3
Chlamydophila pneumoniae	TW183
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
Mycoplasma pneumoniae	M129
Mycoplasma pneumoniae	ATCC 15531
Mycoplasma pneumoniae	ATCC 15293
Mycoplasma pneumoniae	ATCC 15377
Mycoplasma pneumoniae	ATCC 15492
Mycoplasma pneumoniae	ATCC 29085
Mycoplasma pneumoniae	ATCC 29342
Mycoplasma pneumoniae	ATCC 39505
Mycoplasma pneumoniae	ATCC 49894

Viruses	Strain / Isolate
Bocavirus	Type 1 - Clinical specimen
Coronavirus 229E	ATCC VR-740
Coronavirus SARS	Zeptometrix –Nucleic Acid
Coronavirus OC43	ATCC VR-759
Cytomegalovirus (CMV)	AD-169 (VR-538)
Epstein-Barr Virus (EBV)	B95-8
Herpes Simplex Virus	Type 1
Parainfluenza Virus 1	Zeptometrix # 0810014CF
Parainfluenza Virus 2	Zeptometrix # 0810015CF
Parainfluenza Virus 4	Zeptometrix #0810060CF
Measles Virus	Edmonston
Measles Virus	Zeptometrix # 0810025CF[a]
Mumps	Zeptometrix # 0810079CF
Fungi	**Strain / Isolate**
Candida albicans	Zeptometrix #0801504

Neisseria elongata	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

[a]This viral stock produced one false positive Adenovirus result. The false positive was confirmed to be caused by Adenovirus contamination of the viral stock and was not due to cross-reactivity between the Adenovirus assay and Measles virus.

Supplemental Analytical Exclusivity Testing for Influenza Strains of Avian Origin:

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

Table 26. Results of Exclusivity Testing of Virus or Nucleic Acid from Culture of Avian 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 titre from CDC = 128. Twenty microliters of virus stock tested.

Precision (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 a panel of twelve simulated NPS specimens spiked with various combinations of live respiratory pathogens (analytes) at three different test levels (high negative (LoD/10), low positive (1X LoD), and medium positive (3X LoD)). 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 60 tests per analyte per concentration. A total of 26 lots of reagents and 20 FilmArray instruments were utilized in the reproducibility study. Summary results for each analyte are summarized below.

Results for each analyte are summarized below:

Summary of Positive Agreement, Negative Agreement, and Tm Results from Reproducibility Testing of Single Assay Analytes

Adenovirus Species C Serotype 1		# Positive	# Negative	% Agreement with Expected Result[a]	95% CI	Mean Tm	%CV Tm	Observed Tm Range
Medium Positive (3X LoD) 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) 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) 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	100.0%	98.0% - 100%			
	Site B	0/180	180/180	100.0%	98.0% - 100%			
	Site C	0/180	180/180	100.0%	98.0% - 100%			
	All Sites	**0/540**	**540/540**	**100.0%**	**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)	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
5.7×10^6 RNA copies/mL	**All Sites**	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**75.69**	**0.41**	**74.96 - 76.33**
Low Positive (1X LoD)	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
1.9×10^6 RNA copies/mL	**All Sites**	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**75.55**	**0.40**	**74.83 - 76.12**
High Negative[b] (LoD/10)	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
1.9×10^5 RNA copies/mL	**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)	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
15 $TCID_{50}$/mL	**All Sites**	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**80.29**	**0.40**	**79.64 - 81.03**
Low Positive (1X LoD)	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
5 $TCID_{50}$/mL	**All Sites**	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**80.12**	**0.38**	**79.14 - 80.89**
High Negative[b] (LoD/10)	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
0.5 $TCID_{50}$/mL	**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.

Human Metapneumovirus hMPV-16 (A1)		# Positive	# Negative	% Agreement with Expected Result [a]	95% CI	Mean Tm	%CV Tm	Observed Tm Range
Medium Positive (3X LoD)	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
6 $TCID_{50}$/mL	**All Sites**	**59/60**	**1/60**	**98.3%**	**91.1% - 100%**	**77.82**	**0.36**	**77.06 - 78.61**
Low Positive (1X LoD)	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
2 $TCID_{50}$/mL	**All Sites**	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**77.62**	**0.35**	**77.04 - 78.11**
High Negative[b] (LoD/10)	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
0.2 $TCID_{50}$/mL	**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)	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
180 $TCID_{50}$/mL	**All Sites**	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**80.56**	**0.40**	**79.78 - 81.30**
Low Positive (1X LoD)	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
60 $TCID_{50}$/mL	**All Sites**	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**80.51**	**0.37**	**79.77 - 81.33**
High Negative[b] (LoD/10)	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
6 $TCID_{50}$/mL	**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 3 Zeptometrix #0810016CF		# Positive	# Negative	% Agreement with Expected Result [a]	95% CI	Mean Tm	%CV Tm	Observed Tm Range
Medium Positive (3X LoD)	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
30 $TCID_{50}$/mL	**All Sites**	**60/60**	**0/60**	**100%**	**94.0% - 100%**	**81.22**	**0.41**	**80.63 - 81.89**
Low Positive (1X LoD)	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
10 $TCID_{50}$/mL	**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)	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
1 $TCID_{50}$/mL	**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.

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)	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
6 $TCID_{50}$/mL	**All Sites**	**180/180**	**0/180**	**100%**	**97.8% - 100%**	**80.58**	**0.40**	**79.46 - 81.56**
Low Positive (1X LoD)	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
2 $TCID_{50}$/mL	**All Sites**	**120/120**	**0/120**	**100%**	**97.0% - 100%**	**80.10**	**0.57**	**78.93 - 81.03**
High Negative[b] (LoD/10)	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
0.2 $TCID_{50}$/mL	**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.

Summary of Positive Agreement, Negative Agreement, and Tm Results from Reproducibility Testing of Multi-Assay Analytes

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)	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%
90,000 $TCID_{50}$/mL	**All Sites**	**60/60**	**0/60**	**100%**	**94.0% - 100%**
Low Positive (1X LoD)	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%
30,000 $TCID_{50}$/mL	**All Sites**	**60/60**	**60/60**	**100%**	**94.0% - 100%**
High Negative [b] (LoD/10)	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%
3,000 $TCID_{50}$/mL	**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	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
	90,000 $TCID_{50}$/mL	**All Sites**	**87.18**	**0.39**	**86.37 - 88.05**
	Low Positive 1x LoD	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
	30,000 $TCID_{50}$/mL	**All Sites**	**87.05**	**0.42**	**86.15 - 87.85**
	High Negative 0.1x LoD	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
	3,000 $TCID_{50}$/mL	**All Sites**	**86.96**	**0.44**	**85.97 - 87.86**
Entero 2	Medium Positive 3x LoD	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
	90,000 $TCID_{50}$/mL	**All Sites**	**87.14**	**0.41**	**86.16 - 88.00**
	Low Positive 1x LoD	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
	30,000 $TCID_{50}$/mL	**All Sites**	**87.02**	**0.41**	**86.05 - 87.82**

Assay	Enterovirus Echovirus 6 (Species B)		Mean Tm	% CV Tm	Observed Tm Range
HRV4	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**
	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	Site A	83.79	0.44	83.25 - 85.09
	3x LoD	Site B	84.06	0.31	83.43 - 84.56
		Site C	83.68	0.23	83.22 - 84.09
	3 $TCID_{50}$/mL	**All Sites**	**83.84**	**0.38**	**83.22 - 85.09**
	Low Positive	Site A	83.71	0.29	83.07 - 84.35
	1x LoD	Site B	84.07	0.34	83.44 - 84.66
		Site C	83.93	0.34	83.24 - 84.71
	1 $TCID_{50}$/mL	**All Sites**	**83.90**	**0.37**	**83.07 - 84.71**
	High Negative	Site A	83.56	0.28	83.02 - 84.26
	0.1x LoD	Site B	83.91	0.34	83.22 - 84.52
		Site C	83.76	0.31	83.04 - 84.48
	0.1 $TCID_{50}$/mL	**All Sites**	**83.74**	**0.36**	**83.02 - 84.52**
HRV2	Medium Positive	Site A	83.33	0.48	82.55 - 84.67
	3x LoD	Site B	83.65	0.29	83.11 - 84.17
		Site C	83.30	0.28	82.70 - 83.77
	3 $TCID_{50}$/mL	**All Sites**	**83.42**	**0.41**	**82.55 - 84.67**
	Low Positive	Site A	83.28	0.31	82.57 - 83.86
	1x LoD	Site B	83.66	0.36	83.02 - 84.37
		Site C	83.52	0.39	82.82 - 84.29
	1 $TCID_{50}$/mL	**All Sites**	**83.48**	**0.40**	**82.57 - 84.37**
	High Negative	Site A	83.17	0.34	82.42 - 83.88
	0.1x LoD	Site B	83.58	0.36	82.80 - 84.31
		Site C	83.37	0.33	82.51 - 83.92
	0.1 $TCID_{50}$/mL	**All Sites**	**83.37**	**0.40**	**82.42 - 84.31**
HRV3	Medium Positive	Site A	82.73	0.53	81.99 - 83.94
	3x LoD	Site B	83.25	0.36	82.49 - 83.88
		Site C	82.89	0.43	82.10 - 83.88
	3 $TCID_{50}$/mL	**All Sites**	**82.96**	**0.51**	**81.99 - 83.94**
	Low Positive	Site A	82.67	0.49	81.76 - 83.65
	1x LoD	Site B	83.24	0.44	82.40 - 84.08
		Site C	83.07	0.41	82.13 - 83.76
	1 $TCID_{50}$/mL	**All Sites**	**82.98**	**0.54**	**81.76 - 84.08**
	High Negative	Site A	82.68	0.50	81.78 - 83.67
	0.1x LoD	Site B	83.21	0.46	82.18 - 84.37
		Site C	82.97	0.42	81.85 - 83.64
	0.1 $TCID_{50}$/mL	**All Sites**	**82.96**	**0.52**	**81.78 - 84.37**
HRV4	Medium Positive	Site A	83.81	0.38	83.28 - 84.98
	3x LoD	Site B	84.14	0.34	83.43 - 84.83
		Site C	83.80	0.25	83.44 - 84.20
	3 $TCID_{50}$/mL	**All Sites**	**83.90**	**0.37**	**83.28 - 84.98**
	Low Positive	Site A	83.79	0.31	83.18 - 84.45
	1x LoD	Site B	84.08	0.31	83.55 - 84.61
		Site C	84.04	0.38	83.35 - 84.93
	1 $TCID_{50}$/mL	**All Sites**	**83.94**	**0.37**	**83.18 - 84.93**
	High Negative	Site A	83.58	0.26	83.11 - 84.05
	0.1x LoD	Site B	83.88	0.31	83.34 - 84.49
		Site C	83.86	0.27	83.24 - 84.58
	0.1 $TCID_{50}$/mL	**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 [b] (LoD/10) 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**
	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**

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	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/2009 H1

Influenza A 2009 H1N1 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%
	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/2009 H1

Assay	Influenza A 2009 H1N1 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**

Assay	Influenza A 2009 H1N1 A/Swine NY/03/2009		Mean Tm	% CV Tm	Observed Tm Range
FluA H1-pan	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**
	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 10 $TCID_{50}$/mL	Site A	78.34	0.37	77.90 - 79.37
		Site B	78.83	0.37	77.99 - 79.68
		Site C	78.08	0.27	77.68 - 78.51
		All Sites	**78.40**	**0.53**	**77.68 - 79.68**
FluA H1-2009	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	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
	15 $TCID_{50}$/mL	**All Sites**	**85.36**	**0.38**	**84.50 - 86.27**
	Low Positive 1x LoD	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
	5 $TCID_{50}$/mL	**All Sites**	**85.03**	**0.41**	**84.19 - 86.03**
	High Negative 0.1x LoD	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
	0.5 $TCID_{50}$/mL	**All Sites**	**85.01**	**0.37**	**84.22 - 85.77**
FluA pan2	Moderate Positive 3x LoD	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
	15 $TCID_{50}$/mL	**All Sites**	**79.81**	**0.33**	**79.14 - 80.37**
	Low Positive 1x LoD	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
	5 $TCID_{50}$/mL	**All Sites**	**79.45**	**0.37**	**78.82 - 80.30**
	High Negative 0.1x LoD	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
	0.5 $TCID_{50}$/mL	**All Sites**	**79.35**	**0.36**	**78.64 - 79.99**
FluA H3	Moderate Positive 3x LoD	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
	15 $TCID_{50}$/mL	**All Sites**	**82.62**	**0.39**	**81.87 - 83.11**
	Low Positive 1x LoD	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
	5 $TCID_{50}$/mL	**All Sites**	**82.39**	**0.41**	**81.67 - 83.32**
	High Negative 0.1x LoD	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
	0.5 $TCID_{50}$/mL	**All Sites**	**82.37**	**0.36**	**81.71 - 83.05**

Precision (Repeatability)

The repeatability of the FilmArray RP System results was evaluated by repeated testing of the same 12 specimens tested in the reproducibility study while minimizing as many sources of variability as possible. The in-house repeatabilty testing was performed at Site C over the course of 12 testing days for a total of 48 test results per specimen. On each day, all 12 specimens were tested 4 times by two operators on two FilmArray instruments.

Table 27. Summary of Positive Agreement Results for Repeatability Testing

Spiked Organism	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	48/48	100.0%	48/48	100.0%	34/48	70.8%
Coronavirus HKU1	48/48	100.0%	48/48	100.0%	44/48	91.7%
Coronavirus NL63	48/48	100.0%	48/48	100.0%	27/48	56.3%
Human Metapneumovirus	47/48	97.9%	48/48	100.0%	32/48	66.7%
Enterovirus	48/48	100.0%	48/48	100.0%	48/48	100%
Human Rhinovirus	144/144	100.0%	47/48	97.9%	83/96	86.5%
Influenza A/H1	48/48	100.0%	43/48[a]	89.6%[a]	39/48[b]	81.3%[b]
Influenza A/2009 H1N1	48/48	100.0%	48/48	100.0%	47/48[c]	97.9%[c]
Influenza A/H3	48/48	100.0%	48/48	100.0%	7/48[d]	14.6%[d]
Influenza B	48/48	100.0%	48/48	100.0%	25/48	52.1%
Parainfluenza Virus 3	48/48	100.0%	41/48	85.4%	14/48	29.2%
Respiratory Syncytial Virus	144/144	100.0%	96/96	100.0%	32/48	66.7%
% positive (all analytes) per test level	**767/768 99.9%**		**611/624 97.9%**		**432/624 69.2%**	

[a] 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.
[b] 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.
[c] One (1) equivocal Influenza A/H1-2009 result at the 0.1 x LoD test level.
[d] 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 organism mixes containing FilmArray RP analytes were spiked into a simulated NPS (sNPS) sample matrix (human epithelial cells in VTM) at 5 x their respective LoDs. The 5x LoD organism concentration was chosen to be near the analyte LoD but also to provide consistent results for sample-to-sample comparison. Each FilmArray RP analyte was tested in the presence of each potentially interfering substance listed in Table . None of the substances tested were found to compete or interfere with the control or analyte assays in the FilmArray RP.

Table 28. List of Potentially Interfering Substances Evaluated

Endogenous Substances	**Competitive / 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 2009 H1N1	1.0×10^5 $TCID_{50}$/mL
2 ng/µL	*Staphylococcus aureus*	1.0×10^6 CFU/mL
20 ng/µL	*Neisseria meningitides*	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)		
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 system was not performed using clinical NPS specimens obtained from individuals who had recently received the FluMist® nasal influenza vaccine (MedImmune). However, analytical testing was performed 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 29). No cross-reactivity was observed with other, non-influenza FilmArray RP assays.

Table 29. Evaluation of FluMist® Nasal Vaccine as a Potentially Interfering Substance

FluMist® 2009-2010 (% v/v)	Adenovirus	Coronavirus HKU1	Coronavirus NL63	Human Metapneumovirus	Human Rhinovirus / Enterovirus	Influenza A			Influenza B	Parainfluenza virus 3	Respiratory Syncytial Virus
						H1	H1-2009	H3			
10%	-	-	-	-	-	+	-	+	+	-	-
1%	-	-	-	-	-	+	-	+	+	-	-
0.1%	-	-	-	-	-	+	-	+	+	-	-
0.01%	-	-	-	-	-	+	-	+	+	-	-
0.001%	-	-	-	-	-	+	-	+	+	-	-
0.0001%	-	-	-	-	-	-	-	Equiv[a]	+	-	-
0.00001%	-	-	-	-	-	-	-	-	+	-	-
0.000001%	-	-	-	-	-	-	-	-	-	-	-

[a] Equivocal Influenza A/H3 result

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

Beth Lingenfelter, M.S.
Director, Regulated Products
Idaho Technology Inc.
390 Wakara Way
Salt Lake City, UT 84108

FEB 17 2011

Re: k103175

Trade/Device Name:	FilmArray Respiratory Panel (RP) System
Regulation Number:	21 CFR §866.3980
Regulation Name:	Respiratory Viral Panel Multiplex Nucleic Acid Assay
Regulatory Class:	Class II
Product Codes:	OCC, OEM, OOU, OEP, OTG, NXD, OOI
Dated:	February 2, 2011
Received:	February 3, 2011

Dear Ms. Lingenfelter:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please go to http://www.fda.gov/AboutFDA/CentersOffices/CDRH/CDRHOffices/ucm115809.htm for the Center for Devices and Radiological Health's (CDRH's) Office of Compliance. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number: k103175
Device Name: FilmArray Respiratory Panel (RP) System

Indications for Use:

The FilmArray Respiratory Panel (RP) is a multiplexed nucleic acid test intended for use with the FilmArray instrument for the simultaneous qualitative detection and identification of multiple respiratory viral nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections. The following virus types and subtypes are identified using the FilmArray RP: Adenovirus, Coronavirus HKU1, Coronavirus NL63, Human Metapneumovirus, Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype 2009 H1, Influenza B, Parainfluenza virus 3, Rhinovirus/Enterovirus, and Respiratory Syncytial Virus. The detection and identification of specific viral 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. Negative results do not preclude respiratory infection and should not be used as the sole basis for diagnosis, treatment or other management decisions. Positive results do not rule out bacterial infection or co-infection with other organisms. The agent detected may not be the definite cause of disease. The use of additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) and clinical presentation must be taken into consideration in order to obtain the final diagnosis of respiratory infection.

Due to seasonal prevalence, performance characteristics for Influenza A/H1, Influenza A/H3, Influenza A/2009 H1, and Influenza B were established primarily with retrospective 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 detects Adenovirus species C serotype 6 with reduced sensitivity. It is recommended that specimens found to be negative for Adenovirus after examination using FilmArray RP be confirmed by an alternate method (e.g. FDA cleared molecular test or cell culture).

Performance characteristics for influenza A were established when influenza A/2009 H1N1, A/H1, and A/H3 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary. 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.

Prescription Use ___X___ (Part 21 CFR 801 Subpart D) AND/OR Over-The-Counter Use _____ (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-
CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD)

Division Sign-Off
Office of In Vitro Diagnostic Device Evaluation and Safety
510(k) k 103175