Clinical Evaluation of the FilmArray® Respiratory Panel

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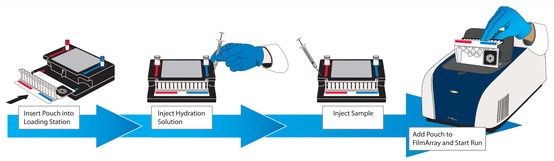
INTRODUCTION

Respiratory infections are responsible for significant morbidity and mortality especially in children, the elderly, and immunocompromised individuals. A wide variety of viral, bacterial, and fungal pathogens have been associated with respiratory tract infection, many causing similar symptoms. Antigen and culture-based diagnostic methods used to identify a subset of these pathogens including influenza A, influenza B, respiratory syncytial virus (RSV), adenovirus, and parainfluenza viruses 1, 2, and 3. For many other respiratory pathogens such as coronaviruses and parainfluenza virus 4, antigen and culture-based methods are not available or lack adequate sensitivity to be diagnostically useful. Nucleic acid amplification tests (PCR) are also currently used in many laboratories (primarily singleplex or limited multiplex assays). PCR offers increased sensitivity and/or specificity over antigen and culture-based methods; however, these methods can be very labor and time intensive. No pathogens at all are detected in many respiratory specimens due to the restricted range of testing, the limitations of current testing methods, and the fact that many agents of respiratory infection remain to be identified.

The **FilmArray Respiratory Panel (RP)** is a simple-to-use, rapid, multiplexed PCR in vitro diagnostic system comprised of an instrument and reagent pouch that simultaneously detects any of 21 respiratory pathogens in a single nasopharyngeal swab specimen (Figure 1). The pouch contains all of the freeze-dried reagents (primers, buffers, enzymes, LC Green Plus dye, etc.) required for nucleic acid extraction, nested RT-PCR, and high resolution melting. The instrument interacts with the pouch to disrupt the sample via bead beating, purify nucleic acids via magnetic bead extraction, perform a highly-multiplexed first-stage RT-PCR reaction, perform individual second-stage PCR reactions, and perform high-resolution melting analysis. Based upon the melting profile of the PCR products, the FilmArray software algorithms automatically determine whether or not the analytes are detected in the specimen. Please see poster by Amiott et al. "Analytical Evaluation of the FilmArray Respiratory Panel" for a more in-depth description of the system and analytical performance.

Figure 1. FilmArray RP System Components.

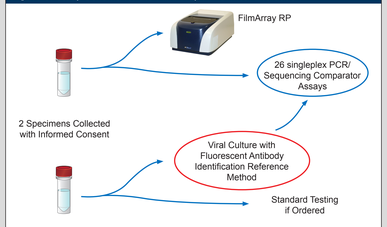
Figure 2. Preparation of a FilmArray RP Test



To perform a FilmArray RP test (hands-on time approx. 2 minutes):

1. Add Hydration Solution to the pouch to rehydrate the freeze-dried reagents.
2. Mix direct unprocessed sample with Sample Buffer.
3. Load the sample/buffer mix into the pouch.
4. Insert the prepared pouch into the FilmArray instrument and start the run. Results are available in approximately 1 hour.

Figure 3. Study Schema for the FilmArray RP Clinical Evaluation



PURPOSE

The aims of this study were to establish the clinical performance of the FilmArray RP system for each analyte on the panel and to investigate rates of infection and co-infection in subjects with signs and/or symptoms of respiratory infection.

STUDY DESIGN

Subjects presenting with signs and/or symptoms of respiratory infection were invited to participate in the study. After acquiring informed consent/assent, two respiratory specimens were collected from each subject. One specimen (Nasopharyngeal Swab; NPS) was used for FilmArray testing and PCR/sequencing comparator assay testing. The second specimen (NPS or other respiratory specimen type) was used for viral culture reference testing and any other testing ordered by the health care providers. An aliquot of each viral culture was also used for PCR/sequencing comparator assay testing (Figure 3). The respective reference/comparator methods used to assess each analyte are presented in Table 2. Operators performing one method (either the FilmArray testing or the reference/comparator testing) were blinded to the results from the other method.

Table 1. FilmArray RP Clinical Study Enrollment

Enrollment Sites	Enrolled Populations	Enrollment Dates	Number of Subjects
Medical University of South Carolina (MUSC); Charleston, SC	Adult Emergency Department	12/2009 - 5/2010	275
Detroit Medical Center (DMC; including Karmanos Cancer Institute Bone Marrow Transplant Clinic); Detroit, MI	Pediatric Emergency Department; Immunocompromised Adults	1/2010 - 5/2010; 9/2010 - 12/2010	513
Children's Medical Center of Dallas (CMC); Dallas, TX	Pediatric Emergency Department and Urgent Care	1/2010 - 5/2010; 9/2010 - 1/2011	329

The study was performed at 3 geographically distinct U.S. sites (Table 1). Subjects were enrolled during the 2009/2010 respiratory season (853 subjects at 3 sites) and the 2010/2011 respiratory season (264 subjects at 2 sites).

A total of 433 archived pre-selected positive and negative specimens were also tested to supplement the prospective data. All specimens were previously determined to be positive or negative by the source site (specimens acquired from five U.S. hospital/reference laboratories) and screened using PCR/sequencing assays for limited sets of the target analytes. Operators performing the PCR/sequencing testing or the FilmArray RP testing were blinded to the previous test results.

Table 2. Comparator/Reference Methods Used to Assess FilmArray RP Performance

Organism/Virus	Reference/ Comparator Method(s)
Adenovirus Influenza A Influenza B Parainfluenza virus 1 Parainfluenza virus 2 Parainfluenza virus 3 Respiratory Syncytial Virus	Culture followed by Fluorescent Antibody ID
Parainfluenza virus 4 FluA/H1 subtyping FluA/H3 subtyping FluA/H1-2009 subtyping	1 PCR test on culture with bi-directional sequence confirmation
Human Rhinovirus Enterovirus Bocavirus Coronavirus 229E Coronavirus NL63 Coronavirus HKU1 Coronavirus OC43 Human Metapneumovirus Bordetella pertussis Chlamydophila pneumoniae Mycoplasma pneumoniae	2 PCR tests on direct specimen with bi-directional sequence confirmation

RESULTS

Specimens from a total of 1117 subjects were analyzed. The age range of the subjects was <1 year to 86 years of age. Greater than 60% of the subjects were young children (≤ 5 years of age) (Figure 4.). The highest rate of FilmArray RP analyte detection was observed in young children; 78% of their specimens were positive for one or more analytes compared to 32-40% of specimens in the other age groups (Figure 5.)

Figure 5. Observed Positivity Rates by Age Group

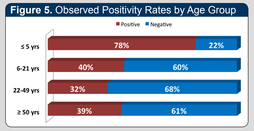
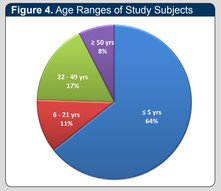


Figure 4. Age Ranges of Study Subjects



The prevalence of specific analytes also differed between age groups (Table 3.) Most analytes had higher prevalence in young children, with the exceptions of several coronaviruses and influenza A/2009 H1. Rhinoviruses were the most prevalent viruses detected in all four age groups.

Table 3. Prevalence Within/Between Age Groups

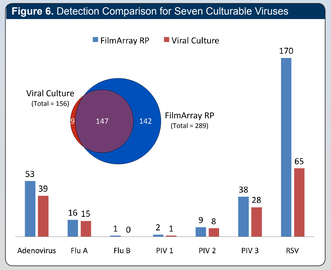
Prevalence Within/Between Age Groups
(December 2009 - May 2010 and September 2011 - January 2011)

Analyte	≤ 5 years		6-21 years		22-49 years		≥ 50 years	
	# Pos	Prevalence (n=722)	# Pos	Prevalence (n=124)	# Pos	Prevalence (n=190)	# Pos	Prevalence (n=84)
Adenovirus	47	6.5%	2	1.6%	3	1.6%	1	1.2%
Influenza A	5	0.7%	2	1.6%	7	3.7%	2	2.4%
Flu A/H1	0	0%	0	0%	0	0%	0	0%
Flu A/H3	4	0.6%	1	0.8%	0	0%	0	0%
Flu A/2009 H1	1	0.1%	1	0.8%	7	3.7%	2	2.4%
Influenza B	1	0.1%	0	0%	0	0%	0	0%
PIV1	1	0.1%	1	0.8%	0	0%	0	0%
PIV2	9	1.2%	0	0%	0	0%	0	0%
PIV3	36	5.0%	1	0.8%	0	0%	1	1.2%
PIV4	9	1.2%	1	0.8%	0	0%	0	0%
RSV	157	21.7%	4	3.2%	4	2.1%	5	6.0%
Bocavirus	34	4.7%	0	0%	0	0%	1	1.2%
CoV 229E	6	0.8%	2	1.6%	5	2.6%	1	1.2%
CoV HKU1	12	1.7%	1	0.8%	8	4.2%	4	4.8%
CoV NL63	18	2.5%	2	1.6%	2	1.1%	2	2.4%
CoV OC43	13	1.8%	2	1.6%	2	1.1%	2	2.4%
hMPV	80	11.1%	4	3.2%	10	5.3%	4	4.8%
Rhino/Entero	279	38.6%	31	25.0%	29	15.3%	11	13.1%
B. pertussis	5	0.7%	1	0.8%	0	0%	1	1.2%
C. pneumoniae	1	0.1%	0	0%	0	0%	0	0%
M. pneumoniae	2	0.3%	0	0%	2	1.1%	0	0%

FilmArray RP had an 85% greater detection rate than the viral culture reference method for the seven cultured viruses (adenovirus, influenza A, influenza B, PIV1, PIV2, PIV3, and RSV) (Figure 6). The greatest difference was for RSV; FilmArray RP detected 162% more RSV than viral culture.

Prospective study performance calculations for positive percent agreement and negative percent agreement were first determined by comparing FilmArray RP results with comparator/reference test results, where the comparator/reference results were taken as "truth". Following this initial assessment, analyses were performed for subsets of the discrepant results using alternate PCR/sequencing assays to determine which method (FilmArray RP or comparator/reference) was likely correct in each instance. Both calculations are presented below in Tables 4a, 5a, and 6a. Retrospective study performance from the testing of known positive and negative archived specimens is presented in the accompanying tables 4b, 5b, and 5c.

Figure 6. Detection Comparison for Seven Culturable Viruses



The FilmArray RP demonstrated very high positive and negative percent agreement with the comparator/reference methods for nearly all analytes (85 - 100% prospective, 90 - 100% retrospective). One exception was bocavirus where positive percent agreement between FilmArray RP and the singleplex comparator PCR assays was approximately 67%. Investigation into the lower performance of this assay is ongoing.

Table 4a. Prospective Clinical Study Performance (FilmArray RP vs Viral Culture Reference Method)

Analyte	Total FilmArray Positives	Positive Percent Agreement (PPA)		Negative Percent Agreement (NPA)	
		FilmArray Agreement with Viral Culture	Agreement + PCR Discrepancy Resolution	FilmArray Agreement with Viral Culture	Agreement + PCR Discrepancy Resolution
Adenovirus	53	84.6% (33/39)	88.5% (46/52)	98.1% (1061/1081)	99.3% (1061/1068)
FluA	16	93.8% (15/16)	94.7% (18/19)	99.8% (1099/1101)	100% (1099/1099)
FluB	1	n/a (0/0)		99.9% (1116/1117)	
PIV1	2	100% (1/1)	100% (2/2)	99.9% (1115/1116)	100% (1115/1115)
PIV2	9	87.4% (7/8)	100% (9/9)	99.9% (1107/1109)	100% (1108/1108)
PIV3	38	96.4% (27/28)	97.4% (37/38)	99.0% (1078/1089)	99.9% (1078/1079)
RSV	170	100% (65/65)	100% (148/148)	90.0% (947/1052)	97.7% (947/969)

Table 4b. Supplemental Archived Specimens

Analyte	PPA	NPA
	FilmArray Agreement with Known Positives	FilmArray Agreement with Known Negatives
Adenovirus	100% (27/27)	100% (28/28)
FluA	100% (120/120)	100% (357/357)
FluB	100% (54/54)	100% (129/129)
PIV1	97.1% (34/35)	100% (94/94)
PIV2	100% (28/28)	100% (101/101)
PIV3	100% (36/36)	100% (93/93)
RSV		

As very little influenza was circulating during the 2009/2010 respiratory season, performance of the FilmArray RP for influenza viruses was established primarily using pre-selected archived specimens collected during previous respiratory seasons.

Table 5a. Prospective Clinical Study Performance (FilmArray RP vs PCR/Sequencing from Viral Culture Comparator Method)

Analyte	Total FilmArray Positives	Positive Percent Agreement (PPA)		Negative Percent Agreement (NPA)	
		FilmArray Agreement with PCR from Viral Culture	Agreement + PCR Discrepancy Resolution	FilmArray Agreement with PCR from Viral Culture	Agreement + PCR Discrepancy Resolution
FluAH1	0	n/a (0/0)		100% (1117/1117)	
FluA2009 H1	11	88.9% (8/9)	91.7% (11/12)	99.7% (1105/1108)	100% (1105/1105)
FluAH3	5	100% (6/6)		100% (1111/1111)	
PIV4	10	100% (9/9)	100% (10/10)	99.9% (1107/1108)	100% (1107/1107)

Table 5b. Supplemental Archived Specimens

Analyte	PPA	NPA
	FilmArray Agreement with Known Positives	FilmArray Agreement with Known Negatives
FluAH1	100% (32/32)	100% (127/127)
FluA2009 H1	100% (34/34)	100% (125/125)
FluAH3	100% (54/54)	100% (105/105)
PIV4	100% (11/11)	100% (6/6)

Table 6a. Prospective Clinical Study Performance (FilmArray RP vs PCR from Direct Specimen Comparator Method)

Analyte	Total FilmArray Positives	Positive Percent Agreement (PPA)		Negative Percent Agreement (NPA)	
		FilmArray Agreement with PCR from Direct Specimen	Agreement + PCR Discrepancy Resolution	FilmArray Agreement with PCR from Direct Specimen	Agreement + PCR Discrepancy Resolution
Bocavirus	35	66.7% (28/42)	68.9% (31/45)	99.4% (1068/1075)	99.6% (1068/1072)
CoV229E	14	100% (12/12)	100% (13/13)	99.8% (1103/1105)	99.9% (1104/1105)
CoVHKU1	25	95.8% (23/24)	96.2% (25/26)	99.8% (827/829)	100% (827/827)
CoVNL63	24	95.8% (23/24)		100% (829/829)	
CoVOC43	19	100% (14/14)	100% (16/16)	99.6% (1098/1103)	99.7% (1098/1101)
hMPV	98	94.6% (88/93)		99.2% (754/760)	
Rhino/Entero	350	92.7% (190/205)		94.6% (613/648)	
B.pertussis	7	100% (6/6)		99.9% (1110/1111)	
C.pneumoniae	1	100% (1/1)		100% (1116/1116)	
M.pneumoniae	4	100% (4/4)		100% (1113/1113)	

Table 6b. Supplemental Archived Specimens

Analyte	PPA	NPA
	FilmArray Agreement with Known Positives	FilmArray Agreement with Known Negatives
Bocavirus	100% (7/7)	98.3% (56/57)
CoV229E		
CoVHKU1		
CoVNL63		
CoVOC43		
hMPV		
Rhino/Entero	95.7% (22/23 EVs)	100% (90/90)
B.pertussis	100% (17/17)	100% (41/41)
C.pneumoniae		
M.pneumoniae	90% (36/40)	100% (24/24)

Figure 8. Prevalence of Individual Analytes in Co-infections

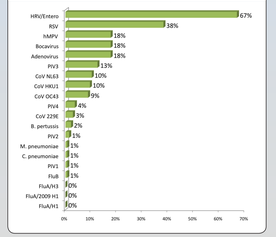
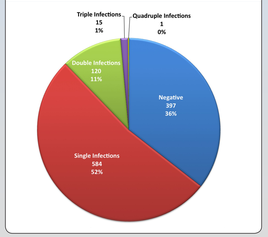


Figure 7. Proportions of Positive/Negative Specimens by FilmArray RP (n=1117)



Sixty-four percent (64%) of all specimens were positive for at least one FilmArray RP analyte. Of the 720 positive specimens, 19% (136/720) had greater than one analyte (i.e. co-infection) (Figure 7).

The most common viruses involved in co-infections were rhinovirus/enterovirus (67% of co-infections) and RSV (38% of co-infections) (Figure 8). Additionally, the single most common co-infection profile was rhinovirus/enterovirus with RSV (Table 7). These viruses were the most prevalent in the study. Also prevalent in co-infections were human metapneumoviruses, adenoviruses, bocaviruses, and coronaviruses.

Table 7. Most Common Co-infections (Detected in >1 Subject)

Number of Co-infections	Analyte 1	Analyte 2	Analyte 3
27	Rhino/Entero	RSV	
10	Adenovirus	Rhino/Entero	
9	Rhino/Entero	PIV 3	
8	Bocavirus	Rhino/Entero	
7	hMPV	Rhino/Entero	
4	Adenovirus	Bocavirus	Rhino/Entero
4	CoV NL63	Rhino/Entero	
4	CoV OC43	Rhino/Entero	
3	Bocavirus	RSV	
3	CoV HKU1	hMPV	
3	CoV HKU1	Rhino/Entero	
3	CoV HKU1	RSV	
3	CoV NL63	hMPV	
3	CoV NL63	RSV	
3	CoV OC43	RSV	
3	hMPV	PIV 3	
3	Rhino/Entero	PIV 4	
2	Adenovirus	Rhino/Entero	PIV 3
2	Bocavirus	hMPV	RSV
2	Bocavirus	PIV 3	
2	CoV HKU1	CoV OC43	
2	hMPV	RSV	

Table 8. Analysis of Potential Medication Effects on FilmArray RP Performance

Study Population (Medication Status)	Total # of Subjects	Positive by Comparator/ Reference Method(s)	Correctly Identified by FilmArray RP
Medicated	597	335/597 (56%)	319/335 (95.2%)
Non-medicated	256	125/256 (49%)	118/125 (94.4%)

An analysis of FilmArray RP performance in medicated versus non-medicated subjects demonstrated that the test system is insensitive to over-the-counter and prescribed topical and systemic medications (Table 8). The FilmArray correctly identified all analytes in 95.2% of medicated individuals and 94.4% of non-medicated individuals (as compared to the comparator/reference method results).

Idaho Technology, Inc. has received FDA clearance for greater than 2/3 of the analytes detected by FilmArray RP and is currently pursuing clearance of the remaining 6 analytes (Table 9).

Table 9. FDA Clearance of FilmArray RP

FDA Cleared Analytes (K103175, K110764)	Analytes Not Yet FDA Cleared
Adenovirus Influenza A/H1 Influenza A/2009 H1 Influenza A/H3 Influenza B Parainfluenza Virus 1 Parainfluenza Virus 2 Parainfluenza Virus 3 Parainfluenza Virus 4 Respiratory Syncytial Virus Coronavirus HKU1 Coronavirus NL63 Human Metapneumovirus Human Rhinovirus/Enterovirus	Bocavirus Coronavirus 229E Coronavirus OC43 *Bordetella pertussis* *Chlamydophila pneumoniae* *Mycoplasma pneumoniae*

CONCLUSIONS

- FilmArray RP is a rapid, robust, highly specific and highly sensitive in vitro diagnostic device for the simultaneous detection of multiple respiratory viruses and bacteria from nasopharyngeal swab specimens.
- FilmArray RP improves respiratory diagnostic testing by increasing the number of pathogens that can be assayed from a single specimen and by decreasing the resources required to perform extensively multiplexed nucleic acid testing.
- FilmArray RP may also be a useful epidemiological research tool for investigations into the importance of mixed respiratory infections and emerging respiratory viruses.

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