Validation of the IdahoTechnology Inc. FilmArray® Respiratory Panel for Clinical Use at Primary Children's Medical Center, Salt Lake City, UT

Marc Roger Couturier, PhD[1,2], Trenda Barney[3], Garrison Alger[3], Weston Hymas[2], Jeffery Stevenson, PhD[2], David Hillyard, MD[1,2], and Judy Daly, PhD[1,3]

[1]Department of Pathology, University of Utah, Salt Lake City, UT [2]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT [3]Primary Children's Medical Center, Salt Lake City, UT

Abstract

The FilmArray®, from Idaho Technology Inc., (Salt Lake City, UT) is a user-friendly multiplex PCR designed to integrate readily into routine clinical microbiology laboratories. The assay requires minimal molecular biology expertise and 2 minutes of sample preparation time. The FilmArray® utilizes a self-contained extraction and nested PCR assay that is capable of detecting 21 respiratory pathogens. No end-user analysis is required, as the software determines the test results based on the positive reaction and quality control reaction performance. We validated 12 of the 21 analytes for the film FilmArray® using nasopharyngeal sources submitted in M4 transport media (Adenovirus, *Bordetella pertussis*, human Metapneumovirus, Influenza A [H1N1 seasonal, H3N2, & 2009 H1N1], Influenza B, Parainfluenza [1, 2, & 3], Respiratory Syncytial Virus, & Rhinovirus). Positive and negative samples (n= 28-33 positive & 28-38 negative per analyte) were pooled in groups of 5 for accuracy determination. Samples identified by prior testing as positive for specific respiratory pathogens were used for the majority of true-positive samples with the exception of *B. pertussis* which was performed largely with five isolates of cultivated bacteria spiked in thirty different M4 media samples. DFA was used as the standard reference method for all analytes. Discrepant analysis was resolved by positive culture combined with fluorescent antibody stain or PCR assays (ARUP Laboratories). The accuracy of all analytes was overall in concordance with the established DFA results with the exception of Adenovirus which was 89.9% accurate. Additionally, several DFA-positive samples were deemed negative by singleplex PCR and FilmArray®. Overall the accuracy of FilmArray® was greater than DFA in this study, making the FilmArray® an attractive alternative to laboratories looking to replace multiple DFA assays in favor of a less subjective and less labor-intensive assay. This assay is also an attractive alternative for laboratories with minimal molecular capabilities that cannot readily establish laboratory developed singleplex PCR assays for respiratory pathogens.

Background

Respiratory infections are a leading cause of acute hospital admission and represent serious complications in patients with chronic immunomodulatory conditions. Pediatric and elderly patients are particularly at risk for these infections in an immunecompetent population. Several of the causative agents of respiratory illnesses cannot readily be cultured *in vitro* in the clinical microbiology laboratory, and others have slow growth rates, leading to delay in diagnosis using classical culture techniques. Historically, culture and detection by direct fluorescence antibody (DFA) staining have been the hallmark for diagnostics of respiratory infections. Culture for many organisms is notoriously insensitive due to fastidious growth, and DFAs are insensitive due to low level infections that fall below the practical limit of detection for this technology as well as being subject to technical error and subjective/inconsistent result interpretation.

The Respiratory FilmArray® platform from Idaho Technology Incorporated allows simultaneous detection of respiratory pathogens using nested and probe-based real-time PCR, as well as chemiluminescent reporting. The respiratory pouch can evaluate over 20 targets, however this validation study investigated the performance of 12 analytes (Adenovirus [Adeno], Respiratory Syncytial Virus [RSV], human Metapneumo virus [hMPV], Influenza A H1N1 seasonal [FluA H1N1], Influenza A seasonal H3N2 [FluAH3N2], Influenza A H1N1 2009 pandemic [FluA H1N1 2009], Influenza B [FluB], Rhinovirus [Rhino], Parainfluenza 1 [Para1], Parainfluenza 2 [Para2], Parainfluenza 3 [Para3], and *Bordetella pertussis* [Bpert].

Methods

Test Specimens

Nasopharyngeal specimens in M4 transport media (swab and nasal suction) previously tested by direct fluorescent antibody at Primary Children's Medical Center (Salt Lake City, UT) or Luminex® Respiratory Virus Panel (Rhinovirus only, Intermountain Central Laboratory, Murray, UT) were analyzed for this validation study. In order to obtain 30 positive samples for each analyte, cultivated organisms (FluA H3N2 [n=9], FluA H1N1 [n=23], Para2 [n=9], FluB [n=11], Adeno [n=9]) and Bpert (n=29) were used to simulate positive samples. The majority of samples were previously frozen for 1-3 years as primary patient specimens, however fresh specimens were included in the study when identified by DFA on the day of testing (n=4). Each machine is able to run one pouch at a time and samples were pooled in groups of 5 analytes per run. Pooling specimens allowed for a reasonable timeframe for the study as well as allowed for cost control.

FilmArray® Run

325 µL of pooled sample was injected into the FilmArray® pouch for analysis. The extraction, amplification, detection, and analysis was completely automated within the machine. No end-user interpretation was involved in the result determination. All results were reported through the software interface if all quality control reactions were detected for the run. Any QC failures resulted in a rerun of the sample.

Discrepant Analysis

All discrepant samples in this study were analyzed by culture and fluorescent antibody stain and/or PCR. For FilmArray false negative results, culture positive results were considered for discrepent analysis and negative culture was tested by PCR. PCR assays were performed at Associated Regional and University Pathologists (ARUP) Laboratories. PCR for FluA, FluB, and RSV were performed in a single assay. The remaining analytes were performed as singleplex PCR reactions using laboratory developed assays. Resolution was achieved when culture/FA and/or PCR results correlated to either the initial DFA result or the FilmArray® result.

Analytical Sensitivity Determination

Clinical samples were chosen at random from each cohort of analytes and quantified by ARUP laboratories. Serial 1:5 fold dilutions were then prepared in M4 media and pooled in groups of 4 analytes. The limit of detection was determined and the theoretical concentration (copies/mL) was reconfirmed by ARUP to ensure accuracy of dilutions. The limit of detection was tested for two anlalytes (Adeno and Bpert) without pooling in order to account for any loss of sensitivity due to pooling.

Reproducibility

Each analyte was tested four times on a single day in three concentrations defined as "medium-positive" (3X LoD), "low-positive" (1xLoD), and "high-negative" LoD/10. The samples were also ran four times over five days. The Analytical sensitivity studies also provide intra-run reproducibility as each sample was run three times in a single day at the established LoD.

Results

- **The calculated accuracy, sensitivity, & specificity values for each analyte was greater than 95% for each analyte except Adeno (Table 1)**
- **The FilmArray® did not cross-react with organisms that can be routinely encountered in nasopharyngeal specimens including: *Candida albicans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae* (type B), *Staphylococcus aureus* (methicillin resistant), *Moraxella catarrhalis*, *Aspergillus* spp., and *Bordetella bronchiseptica***
- **Limit of detection values were comparable to (≤1 log difference) ARUP PCR assays for FluB, hMPV, Para2, RSV, and Para3 (Table 2.)**

Table 1. Initial sample runs without resolution of discrepant samples (n=30 positive and negative) and with discrepant resolution. Accuracy, sensitivity, and specificity values are included.

	Initial Run Results					Resolved Run Results				
		TRUE	FALSE							
FluA H3N2	Positive	30	0	Accuracy	100.00%	No discrepant samples				
	Negative	30	0	Sensitivity	100.00%					
				Specificity	100.00%					
		TRUE	FALSE							
FluA H1N1 Seasonal	Positive	31	0	Accuracy	100.00%	No discrepant samples				
	Negative	30	0	Sensitivity	100.00%					
				Specificity	100.00%					
		TRUE	FALSE				TRUE	FALSE		
Parainfluenza 1	Positive	27	1	Accuracy	93.33%	Positive	30	0	Accuracy	100.00%
	Negative	29	3	Sensitivity	90.00%	Negative	30	0	Sensitivity	100.00%
				Specificity	96.67%				Specificity	100.00%
		TRUE	FALSE				TRUE	FALSE		
Parainfluenza 2	Positive	31	0	Accuracy	84.72%	Positive	31	0	Accuracy	100.00%
	Negative	30	11	Sensitivity	73.81%	Negative	37	0	Sensitivity	100.00%
				Specificity	100.00%				Specificity	100.00%
		TRUE	FALSE				TRUE	FALSE		
Parainfluenza 3	Positive	29	3	Accuracy	93.33%	Positive	31	1	Accuracy	98.33%
	Negative	27	1	Sensitivity	96.67%	Negative	28	0	Sensitivity	100.00%
				Specificity	90.00%				Specificity	96.55%
		TRUE	FALSE				TRUE	FALSE		
RSV	Positive	30	1	Accuracy	98.33%	Positive	30	1	Accuracy	98.33%
	Negative	29	0	Sensitivity	100.00%	Negative	29	0	Sensitivity	100.00%
				Specificity	96.67%				Specificity	96.67%
		TRUE	FALSE				TRUE	FALSE		
Influenza B	Positive	30	0	Accuracy	84.51%	Positive	30	0	Accuracy	100.00%
	Negative	30	11	Sensitivity	73.17%	Negative	38	0	Sensitivity	100.00%
				Specificity	100.00%				Specificity	100.00%
		TRUE	FALSE				TRUE	FALSE		
2009 Influenza A H1N1	Positive	28	0	Accuracy	96.67%	Positive	28	0	Accuracy	98.31%
	Negative	30	2	Sensitivity	93.33%	Negative	30	1	Sensitivity	96.55%
				Specificity	100.00%				Specificity	100.00%
		TRUE	FALSE				TRUE	FALSE		
Adeno	Positive	32	1	Accuracy	88.41%	Positive	33	0	Accuracy	89.86%
	Negative	29	7	Sensitivity	82.05%	Negative	29	7	Sensitivity	82.50%
				Specificity	96.67%				Specificity	100.00%
		TRUE	FALSE							
hMPV	Positive	30	0	Accuracy	100.00%	No discrepant samples				
	Negative	30	0	Sensitivity	100.00%					
				Specificity	100.00%					
		TRUE	FALSE							
B. pertussis	Positive	30	0	Accuracy	100.00%	No discrepant samples				
	Negative	30	0	Sensitivity	100.00%					
				Specificity	100.00%					
		TRUE	FALSE							
Rhinovirus	Positive	30	0	Accuracy	100.00%	No discrepant samples				
	Negative	10	0	Sensitivity	100.00%					
				Specificity	100.00%					

Table 2. Analytical sensitivity determination for the FilmArray® using ARUP PCR assays for quantitative determination. The difference in LoD is reported in relation to the FilmArray® values.

Organism	FilmArray® LoD (copies/mL)	FilmArray® LoD (Log copies/mL)	ARUP LoD (copies/mL)	ARUP LoD (Log copies/mL)	Log difference
Adeno	600000	5.78	1200	3.08	2.70
B. pertussis	500000	5.70	200	2.30	3.40
2009H1N1	2150000	6.33	43000	4.63	1.70
H3N2	275000	5.44	1100	3.04	2.40
Seasonal H1	24750	4.39	99	2.00	2.40
FluB	77500	4.89	31000	4.49	0.40
hMPV	375000	5.57	150000	5.18	0.40
Para1	5750	3.76	230	2.36	1.40
Para2	145000	5.16	29000	4.46	0.70
Para3	17400	4.24	8700	3.94	0.30
RSV	242.5	2.38	9700	3.99	-1.60

Results (cont.)

- **Each Influenza A analyte reported as "Equivocal" when diluted 1.0 log below the established limit of detection in Table 2**
- **All Medium- and Low-positive samples were detected for each analyte for both intra- and inter-run reproducibility testing**
- **The LoD for Adeno and Bpert were repeated in the absence of pooling and the values were unchanged**
- **A maximum of 7 analytes were detected in one run (5 expected and two unexpected positives)**

Discussion

- **Of the 12 analytes tested in this validation study only Adeno was determined to have unacceptable performance (less than 95% Accuracy/Specificity/Sensitivity vs DFA).**
- **Ongoing validation of Adeno will continue in order to identify those serotypes that are unable to be reproducibly detected.**
- **The LoD for all 12 analytes were variable, as would be expected with a multiplexed PCR reaction. The absolute LoD values are not directly comparable since the targets for each analyte vary between the FilmArray® and ARUP assays, however the values do reflect a relative difference in analytical sensitivity.**
- **Pooling samples did not lower the sensitivity of the assay, which provides confidence for our laboratory in the ability to detect simultaneous infections. This allows laboratories to conduct an in depth validation while limiting expenses.**
- **The FilmArray was also able to detect positives previously misidentified or undetected by DFA.**
- **Implementation of the FilmArray in our laboratory will effectively supplant 4 assays: DFA for *B. pertussis*, DFA for respiratory viruses, Luminex® RVP for Rhinovirus, and Influenza A subtyping PCR. Since two of these tests are referred to local laboratories, the turn around time will greatly improve for respiratory pathogen reporting.**

Primary Children's Medical Center

Contact Information

Marc Roger Couturier, Ph.D.
Medical Microbiology Fellow
University of Utah
Department of Pathology
ARUP Laboratories
500 Chipeta Way
Salt Lake City, UT 84108
marc.couturier@aruplab.com

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