Performance of a Modified FilmArray® Respiratory Panel for Improved Detection of Respiratory Adenovirus Serotypes

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INTRODUCTION/BACKGROUND

Rapid and reliable detection of respiratory pathogens is a critical step in the diagnosis and treatment of respiratory infections. The FilmArray Respiratory Panel (RP) is a user-friendly, highly multiplexed in vitro diagnostic test for the detection of twenty different agents of upper respiratory tract infection from a nasopharyngeal swab (NPS) specimen in about an hour.

One of the 17 viral targets of the FilmArray RP test is Adenovirus. *Adenoviridae* is a genetically diverse family of viruses. Human adenoviruses (AdV) are classified into 7 species (A-G) and further distinguished into over 50 distinct serotypes. Adenovirus species B, C, and E are associated with respiratory illness, while infection with other species (A, D, F and G) typically leads to conjunctivitis or gastroenteritis. The Adenovirus assay in the FilmArray RP will detect many, but not all Adenovirus species and serotypes. The original FDA-cleared FilmArray RP was labeled with a limitation on the detection of AdV2 (C2) and AdV6 (C6) serotypes and the sensitivity for detection of several other respiratory AdV serotypes was reported to be lower than some published or commercial molecular adenovirus assays.

The FilmArray RP has recently been modified to improve detection of all respiratory adenovirus serotypes from NPS specimens. The performance of the modified panel was validated with analytical and clinical studies. Analytical studies were performed to characterize the sensitivity (limit of detection), reactivity (inclusivity) and specificity (cross-reactivity and performance in the presence of co-infecting organisms) for adenovirus in the modified panel. In addition, a clinical method comparison study was performed in which 222 archived clinical NPS specimens and 44 contrived specimens were tested with both the modified and unmodified FilmArray RP. Performance was compared for detection of AdV and all analytes on the panel. These studies validate that detection of all respiratory AdV serotypes, including C2 and C6, is improved with the modified FilmArray RP; while the performance of all other panel assays is unchanged compared to the original version of the test.

METHODS

Analytical studies were performed by spiking quantified viruses and/or bacteria into a simulated NPS sample matrix (human epithelial cells in Remel M4 Viral Transport medium) followed by testing with the original and/or modified FilmArray RP test.

The clinical comparison study was performed by testing 222 archived NPS specimens and 44 contrived NPS specimens with both original and modified FilmArray RP versions. Archived specimens were acquired from multiple source laboratories across the U.S. and Scotland and chosen based upon previous positive test results (from various tests including culture, DFA, lab developed PCR tests, or original FilmArray RP) to cover all analytes in the panel. Any specimen positive for Adenovirus by original or modified FilmArray RP was subject to bi-directional sequencing (hexon gene target) to determine the serotype of the virus. To supplement the archived specimens, contrived specimens were prepared by spiking *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, or Adenovirus serotypes C2 and C6 into archived NPS specimens. Operators performing the FilmArray RP testing were blinded to previous test results and contrived spiking scheme.

Modified FilmArray RP Can Detect Adenovirus and other Analytes in the Presence of Potentially Competing FilmArray RP Viruses (Co-Infection)

Low Concentration Virus (LoD)	High Concentration Virus (~105 $TCID_{50}$/mL)	Modified RP Results
AdV1	HRV	Adenovirus Human Rhinovirus/Enterovirus
HRV	AdV1	Adenovirus Human Rhinovirus/Enterovirus
AdV5	hMPV	Adenovirus Human Metapneumovirus
hMPV	AdV5	Adenovirus Human Metapneumovirus
AdV6	RSV	Adenovirus Respiratory Syncytial Virus
RSV	AdV6	Adenovirus Respiratory Syncytial Virus
AdV7	AdV4	Adenovirus
AdV4	AdV7	Adenovirus
AdV2	AdV21	Adenovirus
AdV21	AdV2	Adenovirus

Contrived samples were prepared, each containing one FilmArray RP analyte at LoD concentration and another analyte at a high, potentially competitive, concentration (~10^3 – 10^5 $TCID_{50}$/mL). Samples were tested with the modified FilmArray RP and all spiked analytes were detected.

Analytical Sensitivity (Limit of Detection): Adenovirus Detection is Improved in Modified FilmArray RP as Compared to Original FilmArray RP

Adenovirus Serotype	Concentration Detected by Original RP ($TCID_{50}$/mL)	Modified RP Limit of Detection[a] ($TCID_{50}$/mL)	Fold Improvement in Detection with Modified RP
AdV1 (C1)	300	100	3x
AdV2 (C2)	30,000	100	300x
AdV6 (C6)	3,000,000	100	30,000x
AdV4 (E4)	300	100	3x

[a] LoD confirmed by detection of Adenovirus in 20/20 (100%) of spiked samples at the indicated concentration

Contrived specimens were created by spiking respiratory serotypes of adenovirus (AdV1, AdV2, AdV6 and AdV4) into simulated NPS specimens at a concentration of 100 $TCID_{50}$/mL. Twenty replicate specimens were tested to confirm 100 $TCID_{50}$/mL as the Adenovirus Limit of Detection (LoD) for the modified FilmArray RP (Adenovirus detected in at least 19/20 replicates). LoD of the modified panel was compared to the concentration of each serotype previously detected by the original FilmArray RP to determine the fold improvement in detection.

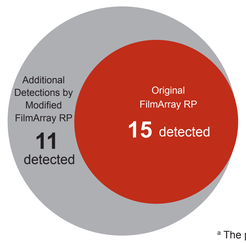
Modified FilmArray RP has Improved Adenovirus C2 and C6 Detection as Compared to Original FilmArray RP

Spike Level	# of Specimens Spiked	Detected by Original FilmArray RP	Detected by Modified FilmArray RP
Adenovirus C6			
12 $TCID_{50}$/mL (0.12 × LoD)	1	0/1 (0%)	1/1 (100%)
120 $TCID_{50}$/mL (1.2 × LoD)	2	0/2 (0%)	2/2 (100%)
1200 $TCID_{50}$/mL (12 × LoD)	5	0/5 (0%)	5/5 (100%)
12000 $TCID_{50}$/mL (12 0× LoD)	2	0/2 (0%)	2/2 (100%)
Total Adenovirus C6	**10**	**0/10 (0%)**	**10/10 (100%)**
Adenovirus C2			
12 $TCID_{50}$/mL (0.12 × LoD)	1	0/1 (0%)	1/1 (100%)
120 $TCID_{50}$/mL (1.2 × LoD)	2	0/2 (0%)	2/2 (100%)
1200 $TCID_{50}$/mL (12 × LoD)	5	0/5 (0%)	5/5 (100%)
12000 $TCID_{50}$/mL (120 × LoD)	2	0/2 (0%)	2/2 (100%)
Total Adenovirus C2	**10**	**0/10 (0%)**	**10/10 (100%)**

Contrived specimens were created by spiking Adenovirus C2 or C6 into archived NPS specimens at concentrations ranging from 12 to 12,000 $TCID_{50}$/mL (0.12 – 120 × LoD). Modified FilmArray RP detected Adenovirus in all 20 specimens; original FilmArray RP failed to detect Adenovirus in all of the contrived specimens.

Clinical Sensitivity: Modified FilmArray RP Detects Adenovirus in More Specimens than Original FilmArray RP Testing of 222 Archived Clinical NPS Specimens

Modified FilmArray RP = 26 Detected[a]



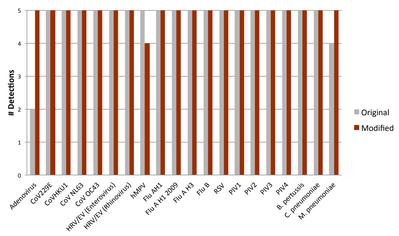
Original FilmArray RP detected Adenovirus in 15 of 222 archived NPS specimens. Modified FilmArray RP detected Adenovirus in the same 15 specimens and also detected Adenovirus in 11 additional specimens (26 total Adenovirus detections by modified FilmArray RP; 26/15 = 173% relative detection in this specimen set). Bi-directional sequencing was able to determine the serotype of Adenovirus in 24 specimens. Modified FilmArray RP demonstrated improved detection of C2, C5, C6, and E4 serotypes in archived clinical NPS specimens.

Adenovirus Subtype	Modified FilmArray RP # Detected	Original FilmArray RP Detections
AdV1 (C1)	8	100% (8/8)
AdV2 (C2)	4	50% (2/4)
AdV5 (C5)	2	50% (1/2)
AdV6 (C6)	6	17% (1/6)
AdV3 (B3)	1	100% (1/1)
AdV4 (C4)	3	67% (2/3)
AdV4 serotype unknown	2	0% (0/2)
Total	**26** (26/15 = 173% of Original FilmArray RP)	**15** (15/26 = 58% of Modified FilmArray RP)

[a] The presence of adenovirus in all 26 specimens was confirmed by bi-directional sequence analysis.

Non-Adenovirus Analyte Performance is Equivalent in Original and Modified FilmArray RP

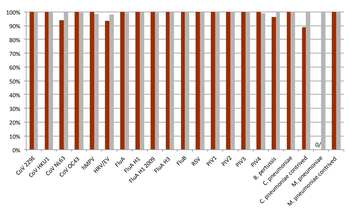
Detection of Non-Adenovirus Analytes is Equivalent between the Original and Modified FilmArray RP at LoD Levels



Five replicates of contrived samples spiked with each FilmArray RP analyte at its respective LoD concentration were tested with both the original and modified FilmArray RP and results were compared. Detection of each non-adenovirus FilmArray RP analyte at LoD concentration was as expected (at least 4/5 detected) and equivalent between the original and modified versions of the panel.

Detection of all non-Adenovirus analytes by modified FilmArray RP was compared to detection by original FilmArray RP when testing 222 archived specimens and 44 contrived specimens. Positive percent agreement (PPA) was 100% for the majority of analytes. A single discrepancy was observed for CoV NL63 (15/16; 93.8%), *B. pertussis* (25/26; 96.2%), and *C. pneumoniae* (8/9; 88.9% in contrived specimens). Four discrepancies were observed for Rhinovirus/Enterovirus (57/61; 93.4%). Negative Percent Agreement (NPA) was 98% or higher for all analytes.

Detection of Non-Adenovirus Analytes in Clinical NPS Specimens is Equivalent in Original and Modified FilmArray RP



Analyte	RSV	PIV1	PIV2	PIV3	PIV4	*B. pert*	*Cpne*	*Cpne* Contrived	*Mpne*	*Mpne* Contrived
Positive Agreement	100% (21/21)	100% (11/11)	100% (8/8)	100% (18/18)	100% (6/6)	96.2% (25/26)	100% (1/1)	88.9% (8/9)	n/a (0/0)	100% (14/14)
Negative Agreement	100% (201/201)	100% (211/211)	100% (214/214)	100% (204/204)	99.1% (214/216)	100% (196/196)	100% (221/221)	100% (35/35)	100% (222/222)	100% (30/30)

Analyte	CoV 229E	CoV HKU1	CoV NL63	CoV OC43	hMPV	HRV/EV	FluA	FluA H1	FluA H1 2009	FluA H3	FluB
Positive Agreement	100% (6/6)	100% (8/8)	93.8% (15/16)	100% (13/13)	100% (10/10)	93.4% (57/61)	100% (36/36)	100% (9/9)	100% (15/15)	100% (13/13)	100% (10/10)
Negative Agreement	100% (216/216)	100% (214/214)	100% (206/206)	99.5% (208/209)	98.6% (209/212)	98.1% (158/161)	99.5% (184/185)	100% (213/213)	99.5% (205/206)	100% (209/209)	100% (212/212)

Analytical Reactivity (Inclusivity): Modified FilmArray RP Detects More Adenovirus Serotypes at Lower Concentrations as Compared to Original FilmArray RP

Modified FilmArray RP is More Inclusive for Adenovirus Respiratory Serotypes (Species B, C and E) Compared to Original FilmArray RP

Species	Serotype	Isolate	Test Level $TCID_{50}$/mL	x LoD	Original RP	Modified RP
B	AdV3	Zepto #0810062CF	100	1x	D	D
	AdV7a	Zepto #0810021CF	100	1x	D	D
	AdV7d2	Iowa/2001	100	1x	D	D
	AdV7h	Iowa/1999	100	1x	D	D
	AdV11	Wisconsin/2005	100	1x	ND	D
	AdV14	Missouri/2005	100	1x	ND	D
	AdV16	ATCC VR-17	100	1x	D	D
	AdV21	Missouri/2005	100	1x	ND	D
	AdV34	UIRF-Texas/2005	100	1x	ND	D
	AdV35	ATCC VR-718	100	1x	ND	D
	AdV50	ATCC VR-1602	100	1x	ND	D
C	AdV1	Zepto#0810050CF	100	1x	D	D
	AdV2	New York/2004	100	1x	ND	D
		ATCC VR-846	100	1x	ND	D
	AdV5	Zepto#0810020CF	100	1x	ND	D
	AdV6	Colorado/2005	100	1x	ND	D
		ATCC VR-6	100	1x	ND	D
E	AdV4a	South Carolina/2004	100	1x	D	D
	AdV4p3	New Jersey/2005	100	1x	D	D

Detection of Non-Respiratory Adenovirus Serotypes (Species A, D and F) Is Variable with Both the Original and Modified FilmArray RP

Species	Serotype	Isolate	Test Level $TCID_{50}$/mL	x LoD	Original RP	Modified RP
A	AdV12	ATCC VR-863	1,000	10x	ND	ND
	AdV18	ATCC VR-19	1,000	10x	ND	ND
	AdV31	Zepto#0810073CF	1,000	10x	D	D
D	AdV8	Zepto#0810069CF	100	1x	ND	D
	AdV20	Zepto#0810115CF	100	1x	D	D
	AdV37	Zepto#0810119CF	100	1x	D	D
F	AdV40	Zepto#0810084CF	1,000	10x	ND	ND
	AdV41	Indiana/2004	100	1x	D	D

Representative isolates of 17 different AdV respiratory serotypes (species B,C, and E) and 8 different non-respiratory serotypes (species A, D and F) were spiked into simulated NPS sample matrix at a concentration of 100 $TCID_{50}$/mL and tested with both the original and modified FilmArray RP. All respiratory serotypes were detected (D) at this concentration (1 × LoD) by the modified panel, while several were not detected (ND) by the original panel. Non-respiratory serotypes that were not detected by either the modified or original panel were re-tested at a 10-fold higher concentration. If an isolate was not detected by either panel at 10 × LoD, no additional testing was performed.

SUMMARY

The modified FilmArray RP exhibited a 3-fold increase in analytical sensitivity (LoD) compared to the original panel and was shown to detect 17 different respiratory adenovirus serotypes within species B, C and E (including AdVC2 and AdVC6) at a concentration of 100 $TCID_{50}$/mL. No cross-reactivity with other respiratory pathogens was observed (data not shown) and the panel is able to accurately identify multiple pathogens from a single sample. In the clinical study, the modified FilmArray RP detected Adenovirus in 73% more archived specimens than the unmodified test (26 vs. 15 specimens respectively). Improved detection was observed for Adenovirus serotypes AdVC2, AdVC5, AdVC6, and AdVE4. The archived specimen testing data also demonstrate equivalent performance between the two test versions for all other analytes with Positive Percent Agreement of 93.4 – 100% and Negative Percent Agreement of 97.9 – 100%.

These data validate that the modified FilmArray RP, which recently received FDA clearance, has increased sensitivity for several Adenovirus serotypes and is capable of detecting more Adenoviruses in clinical specimens than the original version of the test.

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