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Comparison of Two Multiplex Respiratory Pathogen PCR Panels: The FilmArray™ 20-Pathogen Panel Versus Luminex xTAG® 12-Pathogen Panel.

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ABSTRACT

Background: FilmArray™ RP is a user friendly multiplex-PCR system for comprehensive detection of 20 different bacterial and viral respiratory pathogens in clinical specimens within one hour from receipt. Pathogens detected are influenza A (Flu A), influenza B (Flu B), Respiratory syncytial virus (RSV), Adenovirus (AdV), Parainfluenza viruses (PIV 1, PIV1, PIV3, PIV4), human metapneumovirus (hMPV) Rhinovirus (RhV), Coronaviruses (NL63, 229E, HKU1, OC43), human BoCa virus (BV), *Mycoplasma pneumoniae*(Mpne), *Chlamydiophila pneumonia* and *Bordetella pertussis*.

Objective: To evaluate performance of FilmArray™ RP using respiratory specimens from children that were previously tested by Luminex xTAG multiplex PCR (xTAG-RVP).

Method: Frozen aliquots of xTAG RVP-tested specimens were used for the comparison study. About 250 ul of respiratory specimen was added to lysis buffer and immediately inoculated in to hydrated FilmArray™ RP pouch and loaded on the instrument. Turn-around-time (TAT) from sample in to result out is about one hour. Additionally seven pertussis specimens (5 positive and 2 negative) previously characterized by laboratory developed realtime PCR was also used for evaluation.

xTAG ® -RVP and FilmArray™ -RP results from 92 respiratory specimens

Results	FluA	FluB	RSV	AdV	hMPV	PIV	RhV/EV	CoV
xTAG-RVP	10	11	11	10	12	28	24	NR*
FilmArray ™ RP	10	11	15	11	13	29	33	8

* Not reportable in FDA approved xTAG version.

FilmArray™ RP detected all pathogens reported by xTAG RVP in 87/92 (95% concordance) specimens. In the 92 specimens tested, xTAG detected a total of 106 viruses while FilmArray™ RP detected 132 pathogens; 14 of these pathogens are either not reportable (NL63 = 4, HKU1 = 1, 229E=1, PIV4=1) or not on xTAG panel (BV =6, Mpne=1). 4/5 pertussis-positive specimens and 2/2 pertussis-negative specimens were accurately detected by FilmArray™ RP (86% concordance).

Conclusion: FilmArray™ RP had a higher detection rate for pathogen when compared to xTAG RVP. The increased detection rate observed in FilmArray™ RP may be attributed to the use of highly sensitive nested PCR chemistry and addition of new pathogens to the panel. Limitation of the current FilmArray™ RP is low-throughput of one sample per hour on a single instrument; however hands-on-time of less than five minutes and rapid TAT of about 1 hour makes this assay user friendly and rapid compared to the >1hr set-up time and 8 hr TAT for xTAG RVP. A cluster of 4 FilmArray™ RP instruments will potentially allow processing of 28, 56, or 84 specimens in one, two or three laboratory shifts respectively making it a suitable option for medium to large size laboratories. The FilmArray™ RP assay is easy to setup and provides rapid and highly sensitive detection of respiratory pathogen. The improved diagnostic yield may result in decreased antibiotic usage, reduced diagnostic testing and reduced hospital stay.

INTRODUCTION

Acute viral infections are the most common pathogens associated with respiratory infections and rapid antigen detection tests are currently available only for few pathogens such as influenza and RSV. Rapid detection of respiratory infections is important for the proper management of patients, appropriate use of antibiotics, avoidance of unnecessary diagnostic evaluations, infection control decisions and overall cost savings to the health care system.

Highly sensitive and specific nucleic acid amplification tests (NAAT) aid in the accurate detection of respiratory pathogens. Recently Luminex xTAG RVP introduced a FDA cleared multiplex respiratory viral panel for detection and identification of 12 different viruses and their subtypes. The assay uses target specific amplification of different viral sequences and microfluidic-based hybridization to fluorescently labeled beads and detection by Luminex xTAG ® instrument. Idaho technology has developed a new FilmArray™ platform to detect and identify 20 different respiratory pathogens and their subtypes. The assay uses nested multiplex PCR chemistry and identification by high resolution melt curve analysis.

The aim of the current study was to evaluate the performance of FilmArray™ RP using respiratory specimens previously characterized by Luminex xTAG ® RVP testing. The two multiplex systems were compared with respect to turn-around-time to result, detection rate for pathogens and user friendliness.

MATERIALS AND METHODS

Study Design: Frozen aliquots (-70C) of respiratory specimens (n=92) from children were used in this study. Luminex xTAG® multiplex PCR (xTAG® RVP) assay was previously used to test these freshly collected specimens for routine clinical care following manufacturers instruction.

FilmArray™ Testing:

A 200ul frozen aliquot of the specimen was allowed to rapidly thaw and mixed with FilmArray™ lysis buffer. A new FilmArray™ pouch was hyrdated using the hydration medium followed by the inoculation of specimen lysate. The FilmArray™ pouch is filled with freeze dried reagents for nucleic acid extraction and subsequent two-stage nested PCR reaction. The negative pressure in the pouch is titrated to suction appropriate amount of hydration medium and specimen lysate. The FilmArray™ pouch has a fitment containing all needed freeze dried reagents (figure 1). The FilmArray™ instrument depresses plungers (B) in the fitment to move reagents through channels and in to blisters in the pouch (C-H). PCR primers are dried in to the wells of the array and each primer set amplifies a unique product of the first stage multiplex PCR. A fluorescent double stranded DNA binding dye LC Green plus, developed by ITI is used to detect amplification. A CCD camera collects fluorescent signals and the software generates PCR amplification curves and the product is identified by high resolution melt profiling.

Discrepant Analysis: A result was considered discrepant if one of the multiplex PCR system detected an analyte that was missed by another system

RESULTS

Figure 1 : FilmArray™ RV Pouch and Instrument

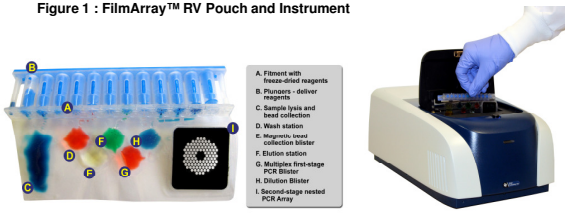

Table 1: Respiratory Specimen Testing (n=92) Luminex xTAG® RVP Vs FilmArray™ RP

Respiratory Pathogen	Luminex xTAG®	FilmArray™ RP
Influenza A	10	10
Influenza B	11	11
Respiratory syncytial virus	11	15
Human Metapneumovirus	12	13
Parainfluenza 1	10	10
Parainfluenza 2	7	5
Parainfluenza 3	11	13
Parainfluenza 4*	Not reportable	1
Adenovirus	10	11
Rhinovirus/Enterovirus	24	30
Bocavirus	Not on Panel	6
Corona Virus NL63*	Not reportable	4
Corona Virus 229E*	Not reportable	1
Corona Virus OC43*	Not reportable	0
Corona Virus HKU1*	Not reportable	1
Chlamydiophila pneumoniae	Not on Panel	0
Mycoplasma penumoniae	Not on Panel	1
Total	106	132

*** not reportable in the U.S. IVD Test (FDA Cleared)**

Table 2: Pertussis specimen testing (n=7) FilmArray™ RP Vs Lightcycler realtime Pertussis PCR

Respiratory Pathogen	LightCycler *IS*481 PCR	FilmArray™ RP
Pertussis-Positive	5	4#
Pertussis-Negative	2	3*

•one pertussis negative specimen was positive for CoV-HKU1 and CoV-OC43
•# RhV was also found in 2 pertussis positive and one pertussis false-negative specimen

Table 3: Discrepant analysis

Luminex xTAG RVP	FilmArray RP (Original Result)	FilmArray RP (Repeat Result)*
Fresh Specimen	One Freeze Thaw cycle	Two Freeze Thaw cycles
HRV, PIV2	HRV	HRV(14), PIV4(30)
HRV	HRV, Mpne, PIV4	HRV(18), PIV4(25), Mpne(18)
AdV	AdV, RSV	AdV(24), RSV(27)
HRV	NL63, HRV	NL63(14), HRV(19)
HRV	Boca, HRV (Cp19)	Boca(5)
MPV	NL63, MPV	NL63(22), MPV(15)
RSVB	AdV (Cp27), RSV	RSV(15)
PIV2	Negative	Negative
RSVB	229E, RSV	229E(13), RSV(17)
MPV	Boca (Cp27), MPV	MPV(16)
FluA H1	FluA, HRV (Cp27)	FluA-H1(12)
MPV	MPV, HRV (Cp20)	MPV(16)
MPV	Negative	MPV(27)
FluA H1,HRV	MPV, FluA	MPV(22), FluA-H1(20)
MPV	MPV, RSV (Cp27)	MPV(19)
MPV	NL63 (Cp24), MPV	hMPV(15)
FluB	Boca, NL63 (Cp25), HRV (Cp27), FluB	Boca(22), FLuB(12)
AdV	AdV, HRV	AdV(15), HRV(24)
MPV	HKU1 (Cp27), MPV	MPV(25)
PIV3	PIV3, RSV (Cp23)	PIV3(8)
AdV	AdV (Cp30), Boca, HRV (Cp28)	Boca(15)
AdV	AdV, Boca, HRV (Cp27), PIV3	AdV(22), Boca(19), PIV3(26)
PIV1	Boca (Cp27), HRV (Cp27), PIV1	PIV1(11)
HRV	Failed	HRV (20)
PIV2	HRV, PIV2	HRV(27), PIV2(20)
27 Viruses	50 Pathogens	37 Pathogens

Discrepant analysis was performed by repeat testing of frozen FilmArray lysates by another investigator (KN) in a blinded fashion. Viruses noted in red font failed to repeat positive on second attempt Cp = crossing point.

CONCLUSIONS

•Film Array™ RP detected all pathogens reported by xTAG RVP in 87/92 (95% concordance) specimens.

•FilmArray™ RP failed to detect 5 viruses previously detected by xTAG (PIV2 = 2, MPV=1, HRV = 2), but on repeat analysis on FilmArray™ RP one HRV and one MPV was detected.

•FilmArray™ RP detected 26 additional pathogens that xTAG RVP missed. 14 of these pathogens are either not reportable (NL63 = 4, HKU1 = 1, 229E=1, PIV4=1) or not on xTAG panel (BV =6, Mpne=1)

•4/5 pertussis-positive specimens and 2/2 pertussis-negative specimens were accurately detected by FilmArray™ RP (86% concordance).

•FilmArray™ RP is a highly sensitive multiplex PCR system to detect 20 different respiratory pathogens. The FilmArray™ RP assay is a user-friendly and rapid assay; it requires less than 5 minutes to set-up and results are available within 1 hour. A cluster of 4 FilmArray™ instruments may allow processing of 28 to 84 specimens in one to three laboratory shifts making it a suitable option for medium to large sized laboratories.

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