ID01

A Comparison of the FilmArray, xTAG and Viral Culture for the Detection of Respiratory Viruses

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Introduction

Multiplex reverse transcriptase respiratory viral PCR has been shown to be more sensitive than standard respiratory virus culture, direct fluorescent antigen and direct ELISA antigen detection methods (1,2). Viral culture is labor intensive, detects some viruses poorly (e.g. Rhinovirus, Coronavirus), and requires 3 – 5 days to detect most agents. As a result, the positive results are generally not available in an early clinical decision making time frame. Direct fluorescent antibody (DFA) and chromatographic immunoassays are rapid enough to support real time clinical decisions, but DFA is highly labor intensive and chromatographic immunoassays are relatively insensitive. The FilmArray multiplex respiratory viral panel uses a pouch system that contains all reagents for the identification of 18 respiratory viruses and 3 bacterial respiratory pathogens within 1 hour after inoculation of a patient sample, potentially obviating both labor and turnaround time issues. We compared the performance of the FilmArray with the FDA approved Luminex xTAG multiplex panel and traditional viral culture for 200 retrospective clinical respiratory virus culture samples.

Methods

Patient Samples
Patient specimens sent to the Shands at the University of Florida Hospital Clinical Virology laboratory between October, 2008 and May, 2010 were frozen at - 70℃ after standard viral culture was performed. There were 139 upper respiratory samples (NP swabs, N=101; throat cultures, N=25; miscellaneous, N=14) and 59 lower respiratory tract specimens (BAL, N=45; bronchial brushings, N=2; endotracheal aspirates, N=11, and one autopsy lung).

Viral Culture and Antigen Detection
One hundred eighty specimens were cultured using standard tube cultures and shell vials containing human diploid fibroblasts, Monkey Kidney cells, and A 549 cells. Shell vials were stained on days 3 and 5 using the Light Diagnostics (Temecula, CA) 7 way fluorescent antibody screen, and further identified with specific antisera if positive. Five samples were tested by direct antigen testing only (3 Influenza A and 2 RSV). Fifteen samples were tested by multiplex PCR only.

Multiplex Respiratory Virus PCR
The FilmArray detects the following agents: Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype H1N1 swine-origin variant, Influenza B, Respiratory Syncytial Virus, human Metapneumovirus, Coronavirus NL63, Coronavirus OC43, Coronavirus 229E, Coronavirus HKU1, Adenovirus, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Bocavirus, Rhinovirus/Enterovirus, *Bordetella pertussis*, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*. The xTAG detects Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus, human Metapneumovirus, Adenovirus, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3,and Rhinovirus. Both assays were performed according to the manufacturer's instructions, following training by the respective companies. Nucleic acid extraction was done with a Qiagen kit using a 0.4 ml sample. The remaining extracted nucleic acid and aliquots of the original frozen specimen were stored at - 70℃ for further testing.

Resolution of Discordant Results
Samples that tested positive in any 2 of the 3 methods were considered positive for that agent. If a virus was found in either of the multiplex PCR systems but not in the other, the sample was tested by an independent PCR method based on the literature (sequences available on request). If the independent PCR was negative, further PCR testing was performed using the proprietary sequences from Idaho Technology, Inc followed by sequencing the PCR product. Complete agreement was calculated between the two multiplex PCR methods and required the results to be exactly the same, i.e. if two or more viruses were found in one method, the same viruses had to be identified in the other. Essential agreement was defined as having at least one virus the same between both methods if the results were a mixture of viruses. Viruses that were "equivocal" by xTAG (i.e. MFI 150 – 299) were considered positive for statistical purposes, since these results were reported to physicians. Viruses not included in the xTAG e.g. Coronavirus, Bocavirus that were identified by the FilmArray were not considered discordant between the two methods.

Statistics
The Kappa statistic was calculated from:
http://people.dbmi.columbia.edu/homepages/chuangj/kappa/calculator.htm
Sensitivity and specificity were calculated using the confirmed results as the gold standard at:
http://www.chestx-ray.com/statistics/twobytwo.html

Results

Table 1

Sensitivity and Specificity of the FilmArray and xTAG vs PCR Confirmed Results

	FA + xTAG+	FA + xTAG-	FA- xTAG+	FA- xTAG-	Sensitvity		Specificity		PPV		NPV	
					FA	xTAG	FA	xTAG	FA	xTAG	FA	xTAG
Influenza A	32	0	1	167	97	100	100	100	100	100	99.4	100
Influenza B	7	0	0	193	100	100	100	100	100	100	100	100
RSV	37	8	0	155	100	82.2	100	100	100	100	100	95.1
Parainfluenza	15	1	0	184	100	93.8	100	100	100	100	100	99.5
Rhino/Entero	39	4	2	155	95.6	91.1	100	100	100	100	98.7	97.5
Adenovirus	9	0	1	190	90	100	100	100	100	100	99.5	100
Metapneumo	6	1	0	193	100	85.7	100	100	100	100	100	99.5

Table 2

Viruses Detected by FilmArray, xTAG and Standard Culture/Antigen

	Culture/Antigen* N = 185	FilmArray** N = 200	xTAG*** N = 200
Influenza A	32	32	33
Influenza B	7	7	7
RSV	36	45	37
Rhino/Entero	6	43	41
Parainfluenza	14	16	15
Adenovirus	11	10	10
Metapneumovirus		7	6
Unable to Identify	1		
Negative	81	62	68
Total # Viruses	107	160	149

*Culture N=180; Influenza N=3, RSV N=2
** $p < 0.00001$ Chi2 Culture vs Filmarray
*** $p < 0.00001$ Chi2 Culture vs xTAG

Table 3

Total Viruses Detected*

	FilmArray	
	Positive	Negative
xTAG		
Positive	145	4
Negative	15	57

*Excludes Coronavirus and Bocavirus

$p = 0.0192$, Binomial probability http://www.quantitativeskills.com/sisa/distributions/binohlp.htm

$p = 0.049$, Liddell's correction, McNemar Test

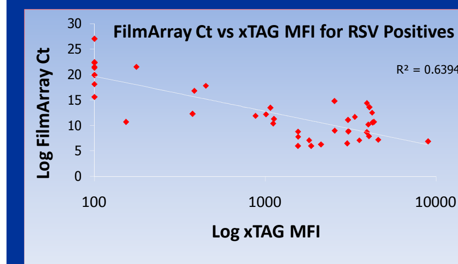
Table 4

Resolution of Discordant Results

xTAG	FilmArray	Resolution
Negative	RSV	RSV
Negative	RSV	RSV
Negative	RSV/Rhino	RSV/Rhino
Negative	HMP**	HMP
Negative	Rhino	Rhino
Negative	RSV	RSV
Rhino	Negative	Rhino
Flu A	Negative	Flu A
Adeno	Adeno/Rhino/RSV	Adeno/Rhino/RSV
Para 2	Para 2/RSV	Para 2/RSV
Para 2	Para 2/RSV	Para 2/RSV
Flu A/Rhino	Flu A	Flu A/Rhino
Flu A	Flu A/RSV	Flu A/RSV
Para 1/Adeno***	Para 1	Para 1/Adeno***
RSV	RSV/Rhino	RSV/Rhino
RSV	RSV/Para 2	RSV/Para 2
Flu B/RSV/Rhino	Flu B/RSV/Rhino/Adeno***	Flu B/RSV/Rhino/Adeno***

* Independent PCR and Sequencing
** HMP = Human Metapneumovirus
*** Discordant Adenovirus not yet confirmed by molecular testing

Figure 1



The Figure shows the relationship between the FilmArray cycle threshold (Ct) and xTAG Mean Fluorescence Intensity (MFI).

Conclusions

1. Both the FilmArray and the xTAG significantly detected significantly more viruses than standard culture and rapid methods, mostly Rhinovirus/Enterovirus, but also RSV in the case of the FilmArray (See Table 2).
2. The FilmArray appeared to detect more total viruses and more RSVs than the xTAG (Table 3); in 6 instances (4 RSV, 2 Rhinovirus) the xTAG was negative, while in the other 8 instances, the xTAG was positive for at least 1 of the 2 or 3 viruses found in the FilmArray. Pabbaruju et al. (3) also noted a greater number of positive RSV results using an in house PCR than found by the xTAG (92 vs 78, in 9 of which RSV was a single positive result).
3. Most of the discordant RSVs had low titers in the FilmArray, suggesting sensitivity is more likely to explain the results than sequence differences but this question is not resolved (See Figure 1).
4. The FilmArray is far easier to use than the xTAG (literally 3 – 5 minutes hands-on time vs 2 -3 hours); and provides results in 1 hour vs 5 ½ - 6 hours. However, as a single unit test, multiple instruments or instruments with batch capacity will need to be developed.

References and Acknowledgement

1. Mahony J., et al. Development of a Respiratory Virus Panel Test for Detection of Twenty Human Respiratory Viruses by Use of Multiplex PCR and a Fluid Microbead-Based Assay. J. Clin. Micro. , Sept. 2007, p. 2965–2970.
2. Schindera C., et al. Immunofluorescence versus xTAG multiplex PCR for the detection of respiratory picornavirus infections in children. J. Clin. Virol. 48 (2010) 223–225
3. Pabbaraju K., et al., Comparison of the Luminex xTAG Respiratory Viral Panel with In-House Nucleic Acid Amplification Tests for Diagnosis of Respiratory Virus Infections. J. Clin. Micro. Sept. 2008, p. 3056–3062.

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