Assessment of a High Throughput Next Generation Multiplex Respiratory Pathogen Panel

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Introduction

Luminex next generation NxTAG® Respiratory Pathogen Panel (NxTAG RPP) pilot assay for research use is a high throughput assay designed to simultaneously detect and discriminate nucleic acids from 19 viruses and 3 atypical bacteria extracted from respiratory samples (Table 1). NxTAG RPP is a ready to use system with a throughput capability of up to 96 reactions per run. An aliquot of extracted nucleic acid is directly added to pre-plated lyophilized reagents. Multiplexed RT-PCR and bead hybridization is carried out as one single cycling program in a closed PCR vessel. Data is acquired on the Luminex MAGPIX® in <4 hours for a batch of 96 samples and the results are analyzed with the RPP assay-specific Software Accessory Package using SYNCT software (Figure 1). No post-PCR sample handling is required. This study evaluates the pilot assay performance with nasopharyngeal swabs collected from symptomatic subjects.

Table 1: Targets Probed by NxTAG® RPP Assay

Targets	
Influenza A	Coronavirus 229E
Influenza A – H1	Coronavirus NL63
Influenza A - 2009 H1N1	Coronavirus OC43
Influenza A H3	Coronavirus HKU1
Influenza B	Human Metapneumovirus
RSV A	Rhinovirus/Enterovirus
RSV B	Adenovirus
Parainfluenza 1	Human Bocavirus
Parainfluenza 2	*Chlamydophila pneumoniae*
Parainfluenza 3	*Mycoplasma pneumoniae*
Parainfluenza 4	*Legionella pneumophila*

Material and Methods

The NxTAG Respiratory Pathogen Panel (For Research Use Only) was evaluated with remnant de-identified nasopharyngeal swab samples collected during the 2014/2015 flu season. Samples were tested with BioFire ® FilmArray® RP assay which was the primary comparator method for the common targets between the two assays. For human Bocavirus and *Legionella pneumophila* (not probed by the FilmArray assay), in house real-time PCR assay and culture were used as primary comparator methods. Bi-directional sequencing was used for discrepancy analysis.

Material and Methods

Nucleic acid was extracted from 200µl specimen spiked in with 10µl MS2 using easyMAG extraction system with 110µl elution volume. Thirty five microliters of extracted nucleic acid were added to pre-plated NxTAG RPP assay plate as shown in Figure 1 below.

Figure 1: Workflow of the NxTAG RPP Assay

Step 1	Add 1 to 96 samples to pre-plated test wells.
Step 2	Multiplex PCR and hybridization.
Step 3	Read on MAGPIX®.

Results

• Overall positive and negative agreement between NxTAG RPP and FilmArray RP for the common 16 targets were 98.3% and 98.6% respectively (Table 2). 11 targets showed 100% positive agreement with the rest of the targets gave positive agreement > 94% except for coronavirus OC43 and HKU1 due to limited sample size. The negative agreement for most targets was > 98% except for hMPV (94.8%) and Rhino (94.6%).

• After sequencing discrepancy analysis, all targets achieved 100% positive agreement except for FluB and OC43. Sequencing confirmed additional calls by NxTAG RPP for PIV3, PIV4, NL63, Rhino and Adeno targets.

• For human Bocavirus (not reported by FilmArray RP), in-house real-time PCR was used as comparator method. NxTAG RPP showed 96.8% positive agreement (30/31).

• Three H7 samples (positive by in-house real-time PCR) were detected as FluA positive (unsubtypeable) by NxTAG RPP.

• There were two culture positive *Legionella* samples; RPP detected one as positive.

Results

Table 2: Positive and Negative Agreement between NxTAG RPP and FilmArray RP

Targets	Positive Agreement		Negative Agreement	
	TP/TP + FN	%	TN/TN + FP	%
2009H1N1	17/18 (17/17)	94.4 (100.0)	281/281 (282/282)	100.0
H3	35/35 (37/37)	100.0	263/265 (263/263)	99.2 (100.0)
Influenza B	20/21	95.2	279/279	100.0
RSV	40/40 (41/41)	100.0	254/260 (254/259)	97.7 (98.1)
PIV1	7/7	100.0	293/293	100.0
PIV2	4/4	100.0	294/296	99.3
PIV3	8/8 (12/12)	100.0	281/288 (281/284)	97.6 (98.9)
PIV4	6/6 (9/9)	100.0	289/294 (289/291)	98.3 (99.3)
229E	4/4	100.0	292/296	98.6
NL63	8/8 (9/9)	100.0	291/292 (291/291)	99.7 (100.0)
OC43	2/3	66.7	295/296	99.7
HKU1	2/3 (2/2)	66.7 (100.0)	297/297 (298/298)	100.0
hMPV	10/10	100.0	275/290	94.8
Rhino	75/76 (80/80)	98.7 (100.0)	212/224 (213/220)	94.6 (96.8)
Adeno	45/45 (46/46)	100.0	246/255 (246/254)	96.5 (96.9)
Mpneu	8/8	100.0	291/291	100.0
TOTAL	**291/296**	**98.3**	**4433/4497**	**98.6**

Note: number in bracket is based on bi-directional sequencing discrepancy analysis.

Conclusions

Results from this study indicate that the Luminex NxTAG Respiratory Pathogen Panel can detect multiple respiratory pathogens present in nasopharyngeal swab samples with a simple workflow, minimal hands-on time and a high throughput capacity of 96 samples per batch. For a 96 sample batch, it would take < 4 hours post extraction for NxTAG RPP assay, but as estimated from the FilmArray protocol would be ~ 96 hours with one FilmArray instrument or 4 hours with 24 FilmArray instruments.