Evaluation of the Luminex® NxTAG™ Respiratory Pathogen Panel

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Introduction

Introduction. Both random-access and batched testing platforms are needed based on routine and unexpected clinical microbiology practice needs. The Luminex next generation (NxTAG™) Respiratory Pathogen Panel in development incorporates multiplex RT-PCR and suspension bead array to qualitatively detect and identify nineteen (19) viruses and three (3) atypical bacteria simultaneously (Table 1). The Luminex next generation respiratory pathogen panel is a ready to use system that allows a scalable batch testing from 1 to 96 reactions with minimal hands on time. The NxTAG Respiratory Pathogen Panel comes in a closed-tube format of sealed 96-well plate with pre-plated lyophilized reagents. After sample addition, multiplexed RT-PCR and bead hybridization occurs in the closed PCR vessel under a single cycling program, eliminating the need for post-PCR transfer steps. Post-cycling the sealed plate is directly placed onto the MAGPIX® for data acquisition.

The objective of this study was to evaluate the performance of the NxTAG Respiratory Pathogen Panel pilot assay in nasopharyngeal swab (NPS) specimens collected from patients with symptoms of respiratory tract infections.

Table 1. NxTAG Respiratory Pathogen Panel

Viral Pathogens (N=19)	Bacterial Pathogens (N=3)
Influenza A (H1, 2009H1, H3), B	***Chlamydophila pneumoniae***
Respiratory Syncytial virus A, B	***Mycoplasma pneumoniae***
Parainfluenza 1, 2, 3, and 4	***Legionella pneumophila***
Human metapneumovirus	
Adenovirus	
Coronavirus (HKU1, NL63, 229E, OC43)	
Human rhinovirus/Enterovirus	
Bocavirus	

Materials & Methods

Materials: Evaluation was carried out with a total of 221 remnant NPS in viral transport medium (VTM) received at MSKCC for FilmArray® Respiratory Panel (RP) testing during the 2013-2014 influenza season.

FilmArray RP testing: All samples were tested as per RP assay package insert.

NxTAG Respiratory Pathogen Panel assay: Total nucleic acids were extracted from 200 µl of raw sample spiked with 10 µl of MS2 bacteriophage using the easyMAG® extractor following Generic protocol 2.0.1 and eluted in 110 µl of elution buffer. Extracts (35 µl) were added directly to NxTAG pre-plated well by simply piercing through the seal with pipette tip. The wells are then sealed by applying a foil seal provided with the reagents. Multiplexed RT-PCR and bead hybridization was performed by using a single cycling program in a closed format. Upon completion of the cycling program, the sealed plate is placed directly on the MAGPIX for data acquisition. There is no opening PCR vessels required, minimizing amplicon contamination. The workflow of NxTAG is shown in Figure 1. A calculated Multi Dimension Detection (MDD) signal based cut-off was used for NxTAG RPP analyte calls using preliminary thresholds.

Discrepancy Analysis: RT-PCR followed by bi-directional sequencing was used for discrepant results between FilmArray RP assay and the NxTAG Respiratory Pathogen Panel assay.

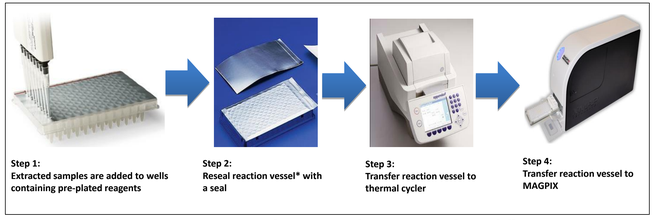

Figure 1. NxTAG Respiratory Pathogen Panel Workflow

Results & Conclusions

Table 2. NxTAG Respiratory Pathogen Panel Agreement with FilmArray RP assay (n=221)

Target	TP	FN	%	TP/ (TP + FN)	TN	FP	%	TN / (TN + FP)
FluA	44	2	95.7%	(44/46)	172	1	99.4%	(172/173)
FluB	12	1	92.3%	(12/13)	205	2	99.0%	(205/207)
RSVA	5	0	100.0%	(5/5)	215	1	99.5%	(215/216)
RSVB	5	0	100.0%	(5/5)	216	0	100.0%	(216/216)
PIV1	8	1	88.9%	(8/9)	212	0	100.0%	(212/212)
PIV2	6	0	100.0%	(6/6)	214	1	99.5%	(214/215)
PIV3	12	0	100.0%	(12/12)	204	4	98.1%	(204/208)
PIV4	10	2	83.3%	(10/12)	198	11	94.7%	(198/209)
229E	6	0	100.0%	(6/6)	215	0	100.0%	(215/215)
NL63	6	0	100.0%	(6/6)	214	1	99.5%	(214/215)
OC43	5	0	100.0%	(5/5)	216	0	100.0%	(216/216)
HKU1	4	0	100.0%	(4/4)	217	0	100.0%	(217/217)
hMPV	8	2	80.0%	(8/10)	201	10	95.3%	(201/211)
RhV/EnV	14	3	82.4%	(14/17)	197	7	96.6%	(197/204)
AdV	14	2	87.5%	(14/16)	193	12	94.1%	(193/205)
Boca	0	0	N/A		219	2	99.1%	(219/221)
Cpneu	4	1	80.0%	(4/5)	216	0	100.0%	(216/216)
Lpneu	0	0	N/A		220	4	98.2%	(220/224)
Mpneu	4	2	66.7%	(4/6)	214	1	99.5%	(214/215)

Table 3. NxTAG Respiratory Pathogen Panel Positive Agreement after discrepancy analysis

Target	TP	FN	%	TP / (TP + FN)	TN	FP	%	TN / (TN + FP)
FluA	45	1	97.8%	(45/46)	173	0	100.0%	(173/173)
FluB	12	0	100.0%	(12/12)	206	2	99.0%	(206/208)
RSVA	5	0	100.0%	(5/5)	215	1	99.5%	(215/216)
RSVB	5	0	100.0%	(5/5)	216	0	100.0%	(216/216)
PIV1	8	0	100.0%	(8/8)	213	0	100.0%	(213/213)
PIV2	6	0	100.0%	(6/6)	214	1	99.5%	(214/215)
PIV3	14	0	100.0%	(14/14)	204	2	99.0%	(204/206)
PIV4	10	2	83.3%	(10/12)	198	11	94.7%	(198/209)
229E	6	0	100.0%	(6/6)	215	0	100.0%	(215/215)
NL63	6	0	100.0%	(6/6)	214	1	99.5%	(214/215)
OC43	5	0	100.0%	(5/5)	216	0	100.0%	(216/216)
HKU1	4	0	100.0%	(4/4)	217	0	100.0%	(217/217)
hMPV	10	0	100.0%	(10/10)	203	8	96.2%	(203/211)
RhV/EnV	21	1	95.5%	(21/22)	199	0	100.0%	(199/199)
AdV	15	1	93.8%	(15/16)	194	11	94.6%	(194/205)
Boca	0	0	N/A		219	2	99.1%	(219/221)
Cpneu	4	1	80.0%	(4/5)	216	0	100.0%	(216/216)
Lpneu	4	0	100.0%	(4/4)	216	0	100.0%	(216/216)
Mpneu	4	0	100.0%	(4/4)	216	1	99.5%	(216/217)

Table 4. NxTAG Respiratory Pathogen Panel made more FluA subtyping calls than FilmArray RP

FluA subtype	Luminex NxTAG RPP	FilmArray RP	McNemar's P value
H1 (N = 1*)	0	0	N.D.
H1-2009 (N = 20)	20 (100%)	15 (75%)	0.074
H3 (N = 24)	24 (100%)	12 (50%)	0.0015
Total (N=45)	44 (98%)	27 (60%)	0.00010

* Sequencing indicated a single FluA specimen with an H1S-like sequence

1. The NxTAG Respiratory Pathogen Panel performs comparably to the FilmArray RP assay for simultaneous detection and identification of panels of microbial pathogens in NPS: overall positive agreement of 92% and negative agreement of 98%.
2. After discrepancy analysis, the positive agreement for NxTAG was 100% for 15 analytes, 97.8% for FluA (45/46), 95% for RhV (21/22), 94% for AdV (15/16), 83% for PIV-4 (10/12) and 80% for *C. pneumoniae* (4/5), the negative agreement for NxTAG RPP ranged from 95-100%.
3. The NxTAG Respiratory Pathogen Panel identified more FluA subtypes (-2009H1 and -H3 subtypes).
4. The NxTAG Respiratory Pathogen Panel detected four *L. pneumophila* which were confirmed by subsequent culture.
5. The NxTAG Respiratory Pathogen Panel allows for scalable testing of 1 to 96 specimens at a time with a total test-to-result time of less than 4 hours.
6. The NxTAG Respiratory Pathogen Panel assay provides an appropriate platform when a high throughput testing is needed during burdensome influenza seasons and pandemics.

NxTAG Respiratory Pathogen Panel is currently in development and for Research Use Only. Not for Diagnostics Use.