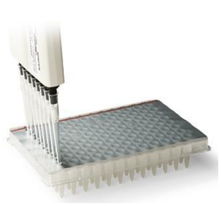
Performance Assessment of the Luminex® NxTAG™ Respiratory Pathogen Panel

Sarah Gonsalves, Margot Boszko, Sabina Fernandes, Wing Lau, Yuen-Yee Wong, Scott Morrison, Hongwei Zhang
Luminex Molecular Diagnostics, Toronto, Canada

Background and Objective

Respiratory tract infection presents a significant burden to health care system worldwide. Respiratory viral infections are causes of morbidity and mortality in young children, elderly and immunocompromised adults. Fast and accurate detection of respiratory infectious agent helps physicians provide better patient care and management. Multiplex molecular detection of respiratory viral nucleic acid has become part of the routine diagnostic algorithm in clinical laboratories since the introduction the first syndromic panel of Luminex xTAG ® Respiratory Virus Panel (RVP) which received CE marking in 2008. High throughput capacity of xTAG RVP has demonstrated its utility during nosocomial respiratory outbreaks and pandemic influenza season[1-3].

The next generation respiratory (NxTAG) Respiratory Pathogen Panel currently in development is a ready-to-use close-tube system with simplified workflow and 96-well high throughput capacity (Figure 1). NxTAG Respiratory Pathogen Panel can simultaneously detect and distinguish nucleic acids from 19 viruses and three atypical bacteria from nasopharyngeal swabs in Universal Transport Medium (UTM). Table 1 lists the 19 respiratory viruses and three atypical bacteria that are probed in NxTAG Respiratory Pathogen Panel.



Step 1

Add 1-96 extracted samples to pre-plated test wells

Step 2

Integrated multiplex PCR and bead hybridization

Step 3

Read on MAGPIX®

Figure 1. Overall Assay Workflow of NxTAG Respiratory Pathogen Panel

The objective of this study was to evaluate the performance of the NxTAG Respiratory Pathogen Panel pilot assay in nasopharyngeal swabs collected from symptomatic subjects. Positive and negative agreement between NxTAG Respiratory Pathogen Panel and xTAG RVP was determined for the 17 analytes covered by both assays. For two viruses covered by NxTAG Respiratory Pathogen Panel but not xTAG RVP, positive and negative agreement between NxTAG Respiratory Pathogen Panel and xTAG RVP FAST v2 was determined. Bi-directional sequencing was used to confirm positive calls made by the NxTAG Respiratory Pathogen Panel for the three atypical bacteria as well as to resolve discrepant calls between NxTAG Respiratory Pathogen Panel and xTAG RVP FAST v2.

Table 1. List of 19 Respiratory Viruses and three Atypical Bacteria Detected By NxTAG Respiratory Pathogen Panel

Viral Targets		
Influenza A	Parainfluenza 1	Coronavirus HKU1
Influenza A H1	Parainfluenza 2	Coronavirus NL63
Influenza A H3	Parainfluenza 3	Coronavirus 229E
Influenza A 2009 H1N1	Parainfluenza 4	Coronavirus OC43
Influenza B	Human Metapneumovirus	Human Bocavirus
Respiratory Syncytial Virus A	Rhinovirus/Enterovirus	
Respiratory Syncytial Virus B	Adenovirus	
Bacterial Targets		
Chlamydophila pneumoniae	Legionella pneumophila	Mycoplasma pneumoniae

Results

Table 2. Positive and Negative Agreement between NxTAG Respiratory Pathogen Panel and xTAG RVP for 17 Common Analytes

Target	Positive Agreement		Negative Agreement		Post bi-directional sequencing *	
	%	TP/ (TP+FN)	%	TN/ (TN+FP)	%	TN/ (TN+FP)
FluA	100.0%	(40/40)	99.8%	(404/405)	99.8%	(404/405)
H1s	N/A		99.8%	(444/445)	99.8%	(444/445)
H3	100.0%	(26/26)	98.8%	(414/419)	**99.3%**	(414/417)
FluB	100.0%	(43/43)	99.0%	(398/402)	**99.5%**	(398/400)
RSVA	100.0%	(30/30)	97.3%	(404/415)	**99.5%**	(404/406)
RSVB	100.0%	(23/23)	99.1%	(418/422)	**99.5%**	(418/420)
PIV1	100.0%	(9/9)	99.1%	(432/436)	**100.0%**	(432/432)
PIV2	100.0%	(18/18)	100.0%	(427/427)	100.0%	(427/427)
PIV3	100.0%	(23/23)	97.9%	(413/422)	**98.3%**	(413/420)
PIV4	100.0%	(8/8)	97.3%	(425/437)	97.3%	(425/437)
229E	100.0%	(4/4)	100.0%	(441/441)	100.0%	(441/441)
NL63	100.0%	(4/4)	99.3%	(438/441)	**99.8%**	(438/439)
OC43	N/A		99.1%	(441/445)	99.1%	(441/441)
HKU1	100.0%	(8/8)	99.5%	(435/437)	**99.8%**	(435/436)
hMPV	100.0%	(34/34)	97.8%	(402/411)	97.8%	(402/409)
Rhino/Entero	94.3%	(66/70)	98.1%	(368/375)	**99.5%**	(368/370)
Adeno	100.0%	(40/40)	91.9%	(372/405)	**94.4%**	(372/394)

* Bold highlights analytes showing improved negative agreement after bi-directional sequencing analysis

Table 3. Positive and Negative Agreement between NxTAG Respiratory Pathogen Panel and xTAG RVP FAST v2 for 2009H1N1 and Bocavirus

Target	Positive Agreement		Negative Agreement	
	%	TP/ (TP+FN)	%	TN/ (TN+FP)
2009 H1N1	100.0%	(11/11)	100%	(434/434)
Boca	100.0%	(7/7)	99.1%	(427/431)

Table 4. Positive Agreement between NxTAG Respiratory Pathogen Panel and Bi-directional sequencing for three Atypical Bacteria

Target	Positive Agreement		Negative Agreement	
	%	TP/ (TP+FN)	%	TN/ (TN+FP)
Mpneu	100.0%	(2/2)	99.5%	(441/443)
Cpneu	NA		100.0%	(445/445)
Lpneu	NA		100.0%	(445/445)

For Research Use Only. Not for use in Diagnostic Procedures.

Conclusions

- 100% positive agreement was achieved between NxTAG Respiratory Pathogen Panel and xTAG RVP for 14 targets and 94% for rhino/entero (no positive detected for Coronavirus OC43 and Influenza A seasonal H1 by xTAG RVP) (Table 2)
- > 98% negative agreement was achieved between NxTAG Respiratory Pathogen Panel and xTAG RVP for 12 common targets, between 97% and 98% for four common targets, and 91.9% for Adenovirus
- After bi-directional sequencing discrepancy analysis on specimens that gave positive calls for FluB, RSVA, RSVB, PIV1, NL63, OC43, HKU1, and Rhino/Entero with the NxTAG Respiratory Pathogen Panel but not with xTAG RVP, sequencing confirmed the majority of positive calls made by NxTAG Respiratory Pathogen Panel, improving the negative agreement as highlighted in bold in Table 2
- 100% positive and negative agreement for 2009H1N1, and 100% positive agreement and 99.1% negative agreement for Bocavirus was achieved between NxTAG Respiratory Pathogen Panel and xTAG RVP FAST v2
- Bi-directional sequencing confirmed two *M. pneumoniae* positive calls made by the NxTAG Respiratory Pathogen Panel

In Summary:

- NxTAG Respiratory Pathogen Panel pilot assay provides a scalable, closed-tube format for the detection of 22 clinically-relevant respiratory pathogens with a simple workflow
- NxTAG Respiratory Pathogen Panel showed comparable performance to xTAG RVP IVD assay
- This study indicates that NxTAG Respiratory Pathogen Panel may have improved detection for certain analytes
- NxTAG Respiratory Pathogen Panel demonstrated capability of detecting *M. pneumoniae,* although with limited data set.
- Further clinical studies with NxTAG Respiratory Pathogen Panel are underway

Material and Methods

Material

A total of 445 samples, de-identified remnant nasopharyngeal swabs in UTM, were used for the study.

Nucleic Acid Extraction

Nucleic acid from 200 µl of raw sample spiked with 10 µl of MS2 bacteriophage was extracted using the NucliSENS® easyMAG® extractor with Generic protocol 2.0.1. Extracted nucleic acid was stored at -80° C until testing.

NxTAG Respiratory Pathogen Panel

Thirty-five (35ul) microliters of extracted nucleic acid were added directly to NxTAG Respiratory Pathogen Panel pre-plated lyophilized reagents. Multiplexed RT-PCR and bead hybridizations were performed in closed PCR tubes under a single cycling program. The sealed plates required no post-PCR handling and were placed directly on the MAGPIX® instrument for data acquisition (NxTAG Respiratory Pathogen Panel workflow is shown in figure 1). NxTAG Respiratory Pathogen Panel analyte call algorithm is based on Multi-dimensional detection (MDD), a value generated from mean fluoresence intensity (MFI) signal acquired from each analyte in each sample. Thresholds applied to make calls are preliminary and subject to change.

Comparator Method

The xTAG RVP was used as the primary comparator for 17 viral analytes that are common to NxTAG RPP and xTAG RVP. xTAG RVP FAST v2 was used as the primary comparator for influenza A subtype 2009H1N1 and human Bocavirus. The same nucleic acid extracts were used for xTAG RVP and xTAG RVP FAST v2 testing which was carried according to their respective package inserts. Bi-directional sequencing was used to confirm positive calls for the three atypical bacteria made by NxTAG Respiratory Pathogen Panel and discrepant calls between NxTAG RPP and xTAG RVP or NxTAG RPP and xTAG RVP FAST v2.

References: [1] Buller RS. Molecular detection of respiratory viruses. *Clin Lab Med*. 2013;33(3):439-60. [2]Yan Y, Zhang S, Tang YW. Molecular assays for the detection and characterization of respiratory viruses. *Semin Respir Crit Care Med.* Aug 2011;32(4):512-26. [3]Ginocchio CC, Zhang F, Manji R, et al. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. *J Clin Virol.* Jul 2009;45(3):191-5.