EVALUATION OF LUMINEX® NxTAG™ RESPIRATORY PATHOGEN PANEL (RPP) ON NASOPHARYNGEAL SWABS

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INTRODUCTION

The Luminex NxTAG™ Respiratory Pathogen Panel (RPP) is a qualitative nucleic acid multiplex test that provides simultaneous detection and identification of 19 viruses and 3 atypical bacteria associated with respiratory tract infections. NxTAG RPP is a ready to use system requiring very little hands-on time and is performed in a closed PCR vessel, reducing the chances of contamination. Nucleic acid is simply added directly to pre-plated lyophilized reagents for RT-PCR and bead hybridization. Results are read on the MAGPIX® instrument. The objective of this study is to evaluate the performance of the prototype NxTAG RPP assay currently in development for nasopharyngeal swabs.

MATERIALS AND METHODS

Specimens: Anonymized remnants of 345 nasopharyngeal specimens submitted to the Regional Virology Laboratory at St. Joseph's Healthcare (Hamilton, Canada) between January to March 2013, were used in this study.

Nucleic Acid Extraction: 200 µl of nasopharyngeal swab spiked with 10 µl of MS2 bacteriophage was extracted for total nucleic acid in an elution volume of 110 µl using the easyMAG® extractor (bioMérieux, St. Laurent, Canada).

NxTAG RPP Testing: The prototype NxTAG RPP assay, a qualitative multiplex test that provides simultaneous detection and identification of multiple viral and atypical bacterial pathogens causing respiratory infections (Table 1), was performed as per manufacturer's instructions (Figure 1).

Table 1: Pathogens Detected in the NxTAG RPP Assay

	Pathogens
Virus	Influenza A Influenza A subtypes (H1, 2009 H1N1, H3) Influenza B Respiratory Syncytial Virus A and B Parainfluenza 1 to 4 Coronavirus 229E Coronavirus NL63 Coronavirus OC43 Coronavirus HKU-1 Human Metapneumovirus Rhinovirus/Enterovirus Adenovirus Bocavirus
Atypical Bacteria	*Chlamydophila pneumoniae* *Legionella pneumophila* *Mycoplasma pneumoniae*

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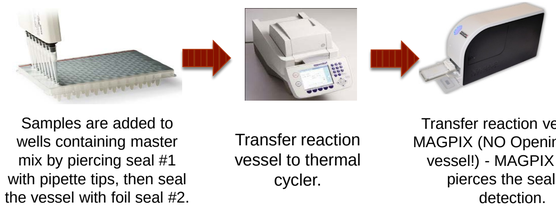
MATERIALS AND METHOD

Figure 1: Workflow of the NxTAG RPP Assay



Samples are added to wells containing master mix by piercing seal #1 with pipette tips, then seal the vessel with foil seal #2.

Transfer reaction vessel to thermal cycler.

Transfer reaction vessel to MAGPIX (NO Opening of the vessel!) - MAGPIX probe pierces the seal for detection.

Reference Testing: All specimens were tested for Adenovirus, Parainfluenza 1 to 3, Influenza A, Influenza B, Respiratory Syncytial Virus and Human Metapneumovirus by a well-characterized RT-PCR test. Specimens that yielded a positive call in the NxTAG RPP for a pathogen not assessed by the RT-PCR, were confirmed by bi-directional sequencing and/or xTAG® Respiratory Viral Panel (RVP).

RESULTS

Table 2: General Demographic Details for the Specimen Set (N=345)

Sex	Number of Subjects
Male	179
Female	166
Age (years)	
0-5	105
6-17	19
≥18	221

Table 3: NxTAG RPP Positivity Rates for Various Pathogens

Pathogen	Positivity Rate
Influenza A	33/345 (9.6%)
Influenza A 2009 H1N1	10/345 (2.9%)
Influenza A H3	22/345 (6.4%)
Influenza B	6/345 (1.7%)
RSV A	34/345 (9.9%)
RSV B	33/345 (9.6%)
Parainfluenza 2	1/345 (0.3%)
Parainfluenza 3	7/345 (2.0%)
Parainfluenza 4	1/345 (0.3%)
Coronavirus NL63	4/345 (1.2%)
Coronavirus OC43	4/345 (1.2%)
Metapnuemovirus	28/345 (8.1%)
Rhinovirus	26/345 (7.5%)
Adenovirus	2/345 (0.6%)

- Overall positive agreement between the NxTAG RPP and the RT-PCR for the common viruses was 99.3% (138/139) (Table 4). Overall negative agreement in this comparison was 99.8% (2960/2966).
- There were 29 specimens positive in the NxTAG RPP assay for viruses not assessed in the RT-PCR. The positive and negative agreement between the NxTAG RPP and bi-directional sequencing or xTAG® RVP for these additional viruses was 96.7% and 99.7%, respectively (Table 5).

RESULTS

Table 4: Positive and Negative Agreement between NxTAG RPP and RT-PCR

Pathogen	Positive Agreement		Negative Agreement	
	TP/TP + FN	%	TN/TN + FP	%
Adenovirus	2/2	100.0	343/343	100.0
Influenza A	31/31	100.0	312/314	99.4
Influenza B	6/6	100.0	339/339	100.0
Metapneumovirus	27/28	96.4	316/317	99.7
Parainfluenza 1	0/0	NA	345/345	100.0
Parainfluenza 2	0/0	NA	344/345	99.7
Parainfluenza 3	6/6	100.0	338/339	99.7
RSV A	33/33	100.0	311/312	99.7
RSV B	33/33	100.0	312/312	100.0
TOTAL	138/139	99.3	2960/2966	99.8

Table 5: Positive and Negative Agreement between NxTAG RPP and Bi-Directional Sequencing or xTAG RVP

Pathogen	Positive Agreement		Negative Agreement	
	TP/TP + FN	%	TN/TN + FP	%
Parainfluenza 4	0/1	0	343/344	99.7
Coronavirus 229E	0/0	NA	345/345	100.0
Coronavirus HKU-1	0/0	NA	345/345	100.0
Coronavirus NL63	4/4	100.0	341/341	100.0
Coronavirus OC43	2/2	100.0	341/343	99.4
Rhinovirus	23/23	100.0	319/322	99.1
TOTAL	29/30	96.7	2034/2040	99.7

CONCLUSIONS

- In a study of 345 nasopharyngeal swabs specimens, the prototype NxTAG RPP assay showed a combined positive and negative agreement to RT-PCR, bi-directional sequencing or xTAG RVP of 98.8% (167/169) and 99.8% (4994/5006), respectively.
- Multiplexed RT-PCR and bead hybridization of the NxTAG RPP assay occurs in a closed PCR vessel. There is no post PCR transfer steps, reducing the possibility of contamination. The assay requires very little hands-on time and enables batch processing of up to 192 samples within 8 hours.
- Further studies are required to evaluate the performance of the NxTAG RPP for the lower prevalent viruses and the atypical bacterial pathogens.