A Multi-Center Clinical Evaluation of the Luminex NxTAG® Respiratory Pathogen Panel (For *In Vitro* Diagnostic Use) in Patients with Signs and Symptoms of Respiratory Tract Infection

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Introduction

Luminex® NxTAG® Respiratory Pathogen Panel is a high throughput, closed-tube multiplex nucleic acid assay which simultaneously detects and identifies 20 of the most common viruses and bacteria implicated in respiratory tract infections in adults and children. Unlike most molecular-based closed tube systems, NxTAG® Respiratory Pathogen Panel enables clinical laboratories to manage variable sample demand by processing a single sample or up to 96 samples per run. The ability of NxTAG® Respiratory Pathogen Panel to detect respiratory pathogens from nasopharyngeal swab specimens (NPS) prospectively collected from pediatric and adult patients suspected of having respiratory tract infection was assessed in this investigative study.

Materials and Methods

Specimen Collection

A total of 2132 nasopharyngeal swab specimens were prospectively collected during the 2013/2014 and 2014/2015 flu seasons from pediatric and adult individuals presenting with signs and symptoms of respiratory tract infection at four clinical laboratories located in the US and Canada. Of these, 934 specimens were collected during the 2013/2014 flu season and the remaining 1198 specimens were enrolled during the 2014/2015 flu season.

Reference Method Testing

In order to generate un-biased estimates of clinical sensitivity (or positive percent agreement) and specificity (or negative percent agreement) for each pathogen probed by NxTAG® Respiratory Pathogen Panel , all specimens were assessed by comparator assays. The performance of NxTAG® Respiratory Pathogen Panel for detecting Influenza A (matrix gene), H3 subtype of Influenza A, Influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3 and Human Metapneumovirus was compared to xTAG® Respiratory Viral Panel (RVP) (Luminex Corp.). xTAG® RVP was performed according to instruction provided in the FDA-cleared product labeling and within sample stability claims. Comparator results for Adenovirus, Influenza A H1, Parainfluenza 4, Coronavirus 229E, Coronavirus OC43, Corona virusNL63, Coronavirus HKU1, Rhinovirus/Enterovirus, Human Bocavirus, *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* were calculated against a composite method consisting of well-characterized nucleic acid amplification tests (NAATs) followed by bi-directional sequencing using analytically validated primers that targeted genomic regions distinct from the NxTAG® Respiratory Pathogen Panel.

NxTAG® Respiratory Pathogen Panel Testing

NxTAG® Respiratory Pathogen Panel runs and re-runs were carried out at the clinical sites on all clinical specimens following their extraction from either the fresh state (fresh specimen) or thawed state (frozen specimens). Nucleic acid from 200µL of raw sample spiked with 10µL of MS2 bacteriophage was extracted using the bioMérieux® NucliSENS® easyMAG® extractor with Generic protocol 2.0.1. (BioMérieux, Inc.). Extracted nucleic acid was stored at -80°C until testing with NxTAG® Respiratory Pathogen Panel. Thirty-five microliters (35µL) 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 each plate well under a single cycling program. The sealed plates required no post-PCR handling and were placed directly on the MAGPIX® instrument for data acquisition. Raw signals generated by the MAGPIX® instrument were subsequently analyzed by the software component of the NxTAG® Respiratory Pathogen Panel assay to establish the presence or absence of each pathogen in each sample.

Results

Table 1: General Demographic Data for Prospective Data Set (n=2132)

SEX	# SUBJECTS	IMMUNE STATUS	# SUBJECTS
Male	1022 (47.9%)	Immuno-compromised	264 (12.4%)
Female	1110 (52.1%)	Immuno-competent	1316 (61.7%)
		Not Determined	552 (25.9%)
AGE (yrs)	**# SUBJECTS**	**PATIENT STATUS**	**# SUBJECTS**
0 – 1	453 (21.2%)	Outpatients	554 (26.0%)
>1 – 5	250 (11.7%)	Hospitalized	1060 (49.7%)
>5 – 21	353 (16.6%)	Emergency Department	518 (24.3%)
>21 – 65	584 (27.4%)		
>65	492 (23.1%)		

Table 2: Sensitivity and Specificity of the NxTAG® Respiratory Pathogen Panel Pathogen

Pathogen	Sensitivity (PPA)	95% CI	Specificity (NPA)	95% CI
Influenza A (matrix)	94.9% 259/273	91.5% - 97.2%	98.0% 1822/1859	97.3% - 98.6%
Influenza A H1	100% 21/21	83.9% - 100.0%	99.1% 2091/2111	98.5% - 99.4%
Influenza A H3	98.5% 203/206	95.8% - 99.7%	97.7% 1872/1917	96.9% - 98.3%
Influenza B	95.6% 87/91	89.1% - 98.8%	99.3% 2019/2033	98.8% - 99.6%
Respiratory Syncytial Virus A	100% 73/73	95.1% - 100.0%	99.3% 2037/2052	98.8% - 99.6%
Respiratory Syncytial Virus B	98.5% 131/133	94.7% - 99.8%	99.4% 1978/1990	98.9% - 99.7%
Coronavirus 229E	100% 21/21	83.9% - 100.0%	99.4% 2098/2111	98.9% - 99.7%
Coronavirus OC43	96.8 30/31	83.3% - 99.9%	99.6% 2092/2101	99.2% - 99.8%
Coronavirus NL63	95.4% 62/65	87.1% - 99.0%	99.4% 2053/2065	99.0% - 99.7%
Coronavirus HKU1	92.9% 13/14	66.1% - 99.8%	99.8% 2113/2118	99.4% - 99.9%
Human Metapneumovirus	93.8% 135/144	88.5% - 97.1%	99.1% 1958/1976	98.6% - 99.5%
Rhinovirus/ Enterovirus	95.3% 286/300	92.3% - 97.4%	96.3% 1764/1832	95.3% - 97.1%
Adenovirus	100% 20/20	83.2% - 100.0%	98.4% 2078/2112	97.8% - 98.9%
Parainfluenza 1	100% 5/5	47.8% - 100.0%	99.9% 2115/2116	99.7% - 100.0%
Parainfluenza 2	50% 1/2	1.3% - 98.7%	99.9% 2121/2122	99.7% - 100.0%
Parainfluenza 3	95.2% 20/21	76.2% - 99.9%	99.2% 2086/2103	98.7% - 99.5%
Parainfluenza 4	60% 3/5	14.7% - 94.7%	99.5% 2116/2127	99.1% - 99.7%
Human Bocavirus	96.4% 27/28	81.7% - 99.9%	98.9% 2081/2104	98.4% - 99.3%
Chlamydophila pneumoniae	0% 0/1	0.0% - 97.5%	100% 2131/2131	99.8% - 100.0%
Mycoplasma pneumoniae	77.8% 7/9	40.0% - 97.2%	99.9% 2121/2123	99.7% - 100.0%

Discussion

- Fifty-nine (59) specimens were identified as positive by the reference method but negative by NxTAG® Respiratory Pathogen Panel (i.e. False Negative). Of these, 35 specimens (59.3%) were confirmed as negative by either FDA-cleared RT-PCR assay routinely used at the clinical sites (FilmArray RP Panel or xTAG® RVP) or bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG® Respiratory Pathogen Panel Assay. These included 14 Influenza A, 2 H3 sub-type of Influenza A, 3 Influenza B, 6 hMPV, 1 Parainfluenza 2, 1 Parainfluenza 3 and 8 Rhinovirus/Enterovirus. Site testing results on discrepant specimens were not available for coronaviruses, Bocavirus , *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae.*
- Three hundred and fifty-six (356) specimens were identified as negative by the reference method but positive by NxTAG® Respiratory Pathogen Panel (i.e. False Positive). Of these, 96 specimens (27.0%) were confirmed as positive by either FDA-cleared RT-PCR assay routinely used at the clinical sites (FilmArray RP Panel or xTAG® RVP) or bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG® Respiratory Pathogen Panel Assay. These included 12 Influenza A, 11 H1 sub-type of Influenza A, 34 H3 sub-type of Influenza A, 4 Influenza B, 8 RSV-A, 3 RSV-B, 2 hMPV, 2 Parainfluenza 3, 18 Rhinovirus/Enterovirus and 2 Adenovirus. Site testing results on discrepant specimens were not available for coronaviruses, Bocavirus , *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae.*
- In this prospective evaluation, the prevalence of parainfluenza viruses, *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* was too low for accurate estimates of sensitivity (or PPA). Additional studies using archived and contrived specimens were performed (see Poster #247). The detection rate of NxTAG® Respiratory Pathogen Panel for these pathogens ranged from 97.6% to 100%.

Conclusion

Results generated from this investigative study indicate that NxTAG® Respiratory Pathogen Panel is a sensitive and specific multiplex panel that simultaneously detects the most important causative agents found in respiratory tract infections. By combining 20 tests in a single closed-tube multiplex reaction with higher sample throughput, this assay is a valuable tool to diagnostic and public health laboratories.

Acknowledgment

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