Detection of Influenza A H1 Subtype, Parainfluenza Viruses, Coronaviruses, Adenovirus, Enterovirus D68, *Chlamydophila Pneumoniae* and *Mycoplasma Pneumoniae* with Luminex NxTAG® Respiratory Pathogen Panel (For *In Vitro* Diagnostic Use) in Clinical and Contrived Specimens

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Introduction

Respiratory tract infections (RTIs) are the most common, and potentially most severe, type of infections in adults and children and a major cause of morbidity and mortality both in developed and developing countries. Most RTIs occur during the fall and winter months and include viruses such as influenza, Respiratory syncytial virus (RSV), rhinoviruses and human metapneumovirus (hMPV). Enteroviruses (EV), parainfluenza viruses (PIV), coronaviruses (CoV), and adenoviruses (AdV) also account for many RTIs but are most active in spring, summer and early autumn or occur throughout the year. Bacteria including *Chlamydophila pneumoniae (C. pneumoniae)* and *Mycoplasma pneumoniae (M. pneumoniae)* are also important causative agents of RTIs but are less common.[1] The ability of Luminex NxTAG® Respiratory Pathogen Panel to detect H1 subtype of influenza A, PIV viruses, CoV viruses, AdV, enterovirus D68 (EV D68), *C. pneumoniae* and *M. pneumoniae* in archived clinical specimens and contrived samples prepared at clinically relevant titers was assessed in this evaluation.

Material and Methods

Clinical Specimen Collection

A total of 326 archived, anonymized, nasopharyngeal swab specimens were collected at multiple North American clinical laboratories from pediatric and adult subjects who tested positive for Influenza A H1 subtype (N=35), Adenovirus (N=30[2]), PIV-1 (N=38), PIV-2 (N=33), PIV-3 (N=34[2]), PIV-4 (N=42), CoV 229E (N=17), CoV OC43 (N=16), CoV NL63 (N=15), CoV HKU1 (N=49), EV D68 (N=14), *C. pneumoniae* (N=2) or *M. pneumoniae* (N=4). All archived specimens were previously characterised at the collection sites using either FDA-cleared or homebrew RT-PCR assays. The presence of the expected pathogen in each of the clinical specimens was further confirmed by xTAG® Respiratory Viral Panel (Luminex Corp.) or well-characterized nucleic acid amplification tests (NAATs) followed by bi-directional sequencing using analytically validated primers that targeted genomic regions distinct from those of the NxTAG® Respiratory Pathogen Panel.

Contrived Specimen Preparation

Contrived samples were prepared by spiking varying concentrations of *C. pneumoniae* or *M. pneumoniae* into negative clinical specimens. For each bacteria, six (6) characterised strains were used to prepare 50 contrived specimens at clinically relevant titers as reported in the published scientific literature. Titers selected for *C. pneumoniae* and *M. pneumoniae* specimens ranged from 1 x 10^2 to 2 x 10^6 copies/mL and from 1 x 10^2 to 1x10^8 copies/mL respectively (See Table 2).

NxTAG® Respiratory Pathogen Panel Testing

In order to minimize bias, all archived and contrived specimens were tested by NxTAG® Respiratory Pathogen Panel assay along with negative clinical specimens in a randomized, blinded fashion at 4 testing sites. Specimens were tested by NxTAG® Respiratory Pathogen Panel following their extraction using the NucliSENS® easyMAG® method (BioMérieux, Inc.). Extracted nucleic acid was stored at -80^{0}C prior to testing with NxTAG® Respiratory Pathogen Panel. Assay runs and re-runs were carried out according to manufacturer's instructions and the results acquired on MAGPIX® instruments at each testing sites.

[1] *www.cdc.gov*

[2] Three (3) specimens were confirmed mixed-infections for both Adenovirus and PIV-3

Results

Table 1: Viral and Bacterial Detection Rate of NxTAG® Respiratory Pathogen Panel in the Archived Clinical Specimens

Pathogen	TP / (TP+FN)	Percent Agreement	95% CI
Adenovirus	30/30	100.0%	88.6% - 100.0%
Influenza A H1	35/35	100.0%	90.1% - 100.0%
Parainfluenza 1	38/38	100.0%	90.8% - 100.0%
Parainfluenza 2	33/33	100.0%	89.6% - 100.0%
Parainfluenza 3	34/34	100.0%	89.8% - 100.0%
Parainfluenza 4	41/42	97.6%	87.7% - 99.6%
Coronavirus 229E	17/17	100.0%	81.6% - 100.0%
Coronavirus OC43	16/16	100.0%	80.6% - 100.0%
Coronavirus NL63	15/15	100.0%	79.6% - 100.0%
Coronavirus HKU1	44/49	89.8%	78.2% - 95.6%
Enterovirus D68	14/14	100.0%	78.5% - 100.0%
C. pneumoniae	2/2	100.0%	34.2% - 100.0%
M. pneumoniae	4/4	100.0%	51.0% - 100.0%

Table 2: Summary Information of Contrived Specimens

Analyte	Strain Information	Source	Spiking Level (copies/mL)	Number of Samples
C. pneumoniae	AR39	ATCC 53592	10^2	5
C. pneumoniae	TWAR strain 2023	ATCC VR-1356	10^3	15
C. pneumoniae	J-21	ATCC VR-1435	10^3	15
C. pneumoniae	AO3	ATCC VR-1452	10^4	5
C. pneumoniae	CM-1	ATCC VR-1360	10^5	5
C. pneumoniae	TW-183	ATCC VR-2282	10^6	5
M. pneumoniae	UTMB-10P	ATCC 49894	10^2	5
M. pneumoniae	[Mac] (Type 2)	ATCC 15492	10^3	15
M. pneumoniae	M129-B7 (Type 1)	ATCC 29342	10^3	15
M. pneumoniae	PI 1428 (Type 1)	ATCC 29085	10^4	5
M. pneumoniae	[Bru]	ATCC 15377	10^6	5
M. pneumoniae	FH strain of Eaton Agent [NCTC 10119]; (Type 2)	ATCC 15531-TTR	10^8	5

Table 3: Summary of the Results Generated on Contrived Specimens

Pathogen	TP / (TP+FN)	Percent Agreement	95% CI
C. pneumoniae	50/50	100.0%	92.9% - 100.0%
M. pneumoniae	50/50	100.0%	92.9% - 100.0%

Discussion

- NxTAG® Respiratory Pathogen Panel accurately detected 323 out of 329 (98.2%; 95% CI, 96.1% - 99.2%) viral and bacterial pathogens present in the 326 archived clinical specimens tested.
- NxTAG® Respiratory Pathogen Panel was also able to detect all clinically relevant concentrations of *C. pneumoniae* and *M. pneumoniae* that were spiked into contrived specimens (100%; 50/50; 95% CI, 92.9% - 100%).
- An additional 89 positive results were reported in archived clinical specimens (i.e. mixed infections). These represented analytes probed by the NxTAG® Respiratory Pathogen Panel which were not initially pre-selected at the banking sites. The majority of these additional positives were Rhinovirus (N=32) whilst the remaining positives represented other viruses and bacteria included in the panel.
- The presence of the additional pathogens detected in clinical specimens was confirmed by bi-directional sequencing in 56 of these mixed-infections (63%).
- The ability of NxTAG® Respiratory Pathogen Panel to detect Influenza A (matrix), Influenza A H1, Influenza A H3, influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Coronaviruses 229E, OC43, NL63, HKU1, Human Metapneumovirus, Rhinovirus/Enterovirus, Adenovirus, Parainfluenza viruses 1, 2, 3, 4, Human Bocavirus *C. pneumoniae* and *M. pneumoniae* was also assessed in prospectively collected clinical specimens (see Poster #223).

Conclusion

Luminex NxTAG® Respiratory Pathogen Panel shows excellent detection rates for H1 subtype of influenza A, Parainfluenza viruses, Coronaviruses, Adenovirus, EV D68, *C. pneumoniae* and *M. pneumoniae*. By detecting 20 respiratory pathogens in a single closed-tube multiplex reaction, this assay is another valuable tool for diagnostic and public health laboratories for RTI etiology determination .

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