ANALYTICAL SPECIFICITY EVALUATION OF THE NxTAG® RESPIRATORY PATHOGEN PANEL ASSAY

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Background and Objective

NxTAG® Respiratory Pathogen Panel is a qualitative *in vitro* diagnostic test intended for use on the Luminex® MAGPIX® instrument for the simultaneous detection and identification of nucleic acids from multiple respiratory viruses and bacteria, extracted from nasopharyngeal swabs collected from individuals with clinical signs and symptoms of respiratory tract infection. The organism types and subtypes detected by the test are Influenza A, Influenza A H1, Influenza A H3, Influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Coronavirus 229E, Coronavirus OC43, Coronavirus NL63, Coronavirus HKU1, Human Metapneumovirus, Rhinovirus/Enterovirus, Adenovirus, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Human Bocavirus, *Chlamydophila pneumoniae, and Mycoplasma pneumoniae*. NxTAG Respiratory Pathogen Panel uses the proprietary Luminex xMAP Technology and NxTAG platform in a closed-tube system that incorporates all reagents required for reverse transcription, PCR and bead hybridization of a sample following nucleic acid extraction.

The objective of the study was to assess the analytical specificity (cross-reactivity) of NxTAG Respiratory Pathogen Panel with respect to potential cross-reactivity with pathogens that cause respiratory infections that are not probed by the assay or pathogens that may be found in respiratory specimens. As well, potential cross-reactivity with pathogens that are a part of the assay was assessed.

Materials and Methods

Material

Simulated specimens were prepared by spiking cultured organisms into Universal Transport Medium (UTM). Viral and bacterial targets were prepared at 1 x 10^5 $TCID_{50}$/mL or 1 x 10^6 CFU/mL, respectively, or the highest concentration possible was used based on the organism stock concentration.

Nucleic Acid Extraction

Nucleic acid from 200µL of simulated specimen or stock organism (depending on the organism) was spiked with 10µL of MS2 bacteriophage, and each specimen was extracted in 3 replicates, using the bioMérieux® NucliSENS® easyMAG® extractor with Generic protocol 2.0.1. Extracted nucleic acid was stored at -80° C until testing. At least one negative extraction control (NEC) was included in every nucleic acid extraction run.

NxTAG Respiratory Pathogen Panel

Thirty-five microliters (35µL) of extracted nucleic acid were added directly to NxTAG Respiratory Pathogen Panel pre-plated lyophilized reagents. Each run included a negative control (NTC), which was DNase/RNase-free water. NECs, where possible, were also included as a negative control in the assay runs. Positive controls representing analytes probed by the assay were included with each run in a rotating manner so that each analyte was covered at least once. 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.

In addition to experimental testing, *in silico* analysis was performed to predict cross-reactivity of certain strains that were difficult to obtain.

Results

Table 1: Pathogens not probed by NxTAG Respiratory Pathogen Panel Tested for Cross-reactivity

Organisms			
Acholeplasma laidlawii (PG8 [NCTC 10116, PG8; A])	*Haemophilus influenza* (strain Minn A)	*Mycobacterium avium* (Serotype 2)	*Pseudomonas aeruginosa* (strain Boston 41501)
Acinetobacter baumanii (strain 307-0294)	Herpesvirus (Simplex Type 1) (strain Macintyre)	*Mycobacterium intracellulare* (strain 3600 [TMC 1406])	*Pseudomonas pertucinogena*
Bordetella bronchiseptica	Herpesvirus 3 (VZV) (strain Ellen)	*Mycobacterium tuberculosis* (strain H37Rv)	*Pseudomonas pseudoalcaligenes* (Stanier U-188 [FERM-P 2922])
Bordetella holmesii (strain F061)	*Klebsiella pneumoniae*	*Mycoplasma arginini* (G230 [NCTC 10129])	*Serratia proteamaculans* (subsp. quinovora Grimont et al; strain 4364 [CIP 8195])
Bordetella parapertussis (strain A747)	*Lactobacillus acidophilus* (strain Scav [IFO 13951, M. Rogosa 210X, NCIB 8690, P.A. Hansen L 917])	*Mycoplasma fermentans* (PG18 [G, NCTC 10117])	*Serratia proteamaculans* (subsp. proteamaculans (Paine and Stansfield) Grimont et al, strain NCPPB 245 [D. Dye ZL1, ICPB XP176, NCTC 394])
Bordetella pertussis (strain A639)	*Lactobacillus casei* (strain 03)	*Mycoplasma gallisepticum* [NCTC 10115, PG 31, X95]	*Staphylococcus aureus subsp. aureus* (protein A producer) (strain NCTC 8530)
Burkholderia cepacia (strain Z066)	*Lactobacillus plantarum* (strain 17-5 [BUCSAV 217, BUCSAV 449, Glaxo 664, ICPB 2080, NCDO 82, NCIB 6376, NCIB 8014, NCIB 8030])	*Mycoplasma genitalium* (Tully et al., [UMTB-10G])	*Staphylococcus aureus* (MSSA, delta mecA)
Candida albicans (strain 3147)	*Lactobacillus reuteri* (strain type F275)	*Mycoplasma hominis* [LBD-4]	*Staphylococcus epidermidis* (MRSE, RP62A)
Candida glabrata (strain Z007)	*Legionella anisa* (Gorman et al., strain WA-316-C3 [NCTC 11974])	*Mycoplasma hyorhinis* (BTS-7 [ATCC 23234, D.G. ff. Edward PG 42, NCTC 10130])	*Staphylococcus epidermidis* (strain MSSE, HER 1292)
Chlamydia trachomatis (strain IC-Cal-3)	*Legionella birminghamensis* (strain 1407-AL-H)	*Mycoplasma orale* (CH 19299 [NCTC 10112])	*Staphylococcus epidermidis* (strain PCI 1200)
Corynebacterium diphtheriae	*Legionella cincinnatiensis* (strain 72-OH-0)	*Mycoplasma pneumoniae* (strain M129)	*Staphylococcus haemolyticus* (strain Z067)
Corynebacterium genitalium (strain 392-1)	*Legionella feeleii* (Herwaldt et al., strain WO-44C [NCTC 12022])	*Mycoplasma salivarium* ([H110, NCTC 10113, PG 20])	*Streptococcus dysgalactiae*
Corynebacterium glutamicum (Type strain 534 [NCIB 10025])	*Legionella hackeliae* (strain Lansing 2 [NCTC 11979])	*Mycoplasma synoviae* (WVU 1853 [NCTC 10124])	*Streptococcus mitis*
Cytomegalovirus (strain AD-169)	*Legionella hackeliae* (strain 798-PA-H [NCTC 11980])	Mumps virus	*Streptococcus pneumoniae* (strain Z022, 19F)
Epstein-Barr virus (strain B95-8)	*Legionella lansingensis* (strain 1677-MI-H)	*Neisseria elongata* (strain Z071)	*Streptococcus pyogenes* (strain M-3 [DLS 88002, Weller])
Escherichia coli (strain Crooks)	*Legionella longbeachae* (strain Long Beach 4 [NCTC 11477])	*Neisseria gonorrhoeae* (strain Z017)	*Streptococcus salivarius* (strain 275 [NCTC 8618])
Escherichia coli ((Migula) Castellani and Chalmers; serotype O17:K52:H18; strain UMN 026)	*Legionella micdadei* (strain Tatlock)	*Neisseria meningitides* (Serotype A)	*Tatlockia micdadei* (strain TATLOCK [CIP 103882, NCTC 11371])
Fluoribacter bozemanae (Brenner et al.) Garrity et al., strain WIGA)	*Legionella pneumophila* (strain Philadelphia)	*Neisseria sicca* (strain Z043)	*Thermanaerovibrio acidaminovorans* (Guangsheng et al.; Baena et al, strain Su883 [DSM 6589])
Fluoribacter dumoffii (strain NY 23)	Measles virus (Rubeola) (strain Edmonston)	*Porphyromonas gingivalis* (strain 2561)	*Thielavia terrestris* ((Apinis) Malloch et Cain, teleomorph, NRRL 8126)
Fluoribacter gormanii (strain LS-13 [ALLO3])	*Moraxella catarrhalis* (strain Ne11)	*Proteus vulgaris*	*Ureaplasma urealyticum* (T-strain 960)

Table 2: Pathogens Tested for Within Panel Cross-reactivity of the NxTAG Respiratory Pathogen Panel

Organisms		
Adenovirus (Type 01 Species C)	Human parainfluenza virus (Type 1)	Influenza A H1N1 (A/Brisbane/59/07)
Bocavirus (clinical specimen)	Human parainfluenza virus (Type 2)	Influenza A H1N1 (strain A1/Mal/302/54)
Chlamydophila pneumoniae (strain CM-1)	Human parainfluenza virus (Type 3)	Influenza A H1N1 (strain A/New Caledonia/20/99)
Human coronavirus 229E	Human parainfluenza virus (Type 4A)	Influenza A H1N1 (strain A/NWS/33)
Human coronavirus HKU1 (recombinant, in Sendai virus)	Human respiratory syncytial virus (Type A)	Influenza A H1N1 (strain A/Singapore/63/04)
Human coronavirus NL63	Human respiratory syncytial virus (CH93-18(18), Type B)	Influenza A H1N1 (strain A/Solomon Islands/3/2006)
Human coronavirus OC43 (Betacoronavirus 1)	Human rhinovirus (strain 1A)	Influenza A H3N2 (strain A/Victoria/3/75)
Human metapneumovirus (strain IA10-2003)	Influenza A H1N1 pandemic 2009 (A/SwineNY/03/2009)	Influenza B virus (B/Florida/04/2006)

Table 3: Simulated Cross-Reactivity of NxTAG Respiratory Pathogen Panel with Avian, Human and Swine Influenza strains

Host	Subtype Tested	Number of Strains Tested	Host	Subtype Tested	Number of Strains Tested
Avian	H2N2	3	Avian	H10N7	1
	H3N1	1		H11N9	1
	H3N2	1	Human	H1N1	1
	H3N5	1		H1N1 2009	1
	H3N6	1		H1N2	1
	H3N7	1		H2N2	1
	H3N8	1		H3N2	1
	H4N6	1		H5N1	4
	H5N1	15		H7N2	1
	H5N2	11		H7N3	1
	H5N3	1		H7N7	1
	H6N1	1		H9N2	1
	H6N2	1	Swine	H1N1	1
	H7N2	8		H1N2	2
	H7N7	7		H3N2	3
	H9N2	1		H5N1	1

Discussion

- One hundred and seven pathogens were tested for cross-reactivity, of which 80 are not probed by the NxTAG Respiratory Pathogen Panel; the remaining 27 are probed by the assay (Tables 1 and 2).
- None of these pathogenic agents cross-reacted with the targets probed by the assay, with the exception of three strains of non-pandemic Influenza A H1 (A/Brisbane/59/07, A/Solomon Islands/3/2006 and A/Singapore/63/04) cross-reacting with Coronavirus 229E, when the titer of these Influenza A H1 strains was above 1 x 10^4 TCID50/mL.
- Based on both laboratory testing and *in silico* prediction analysis (data not shown), high titers of these 3 non-pandemic Influenza A H1 may result in a false positive call for Coronavirus 229E.
- Based on *in silico* analysis, there is potential that the presence of Coronavirus 229E may cause a false positive Influenza H1 call and the presence of Parainfluenza 2 may cause a false positive Influenza H3 call, although no false positive calls were observed with these two targets in this study.
- Laboratory testing was supplemented with *in silico* data where prediction rules were used to predict cross-reactivity of NxTAG Respiratory Pathogen Panel to specific Influenza A strains (Table 3).
- With the exception of an H5N1 swine strain (A/swine/East Java/UT6010/2007(H5N1), the strains listed are predicated to react to the Influenza A primers but show no cross-reactivity with the other analyte primers in the NxTAG Respiratory Pathogen Panel assay.

Conclusion

The NxTAG Respiratory Pathogen Panel does not cross-react with tested pathogens that cause respiratory infections that are not probed by the assay, or pathogens that are found in respiratory specimens. No cross-reactivity with pathogens that are a part of the assay was seen with the exception of three strains of non-pandemic Influenza A H1 (A/Brisbane/59/07, A/Solomon Islands/3/2006 and A/Singapore/63/04) cross-reacted with Coronavirus 229E at concentrations above 1 x 10^4 $TCID_{50}$/mL.

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