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EVALUATION OF THE LIMIT OF DETECTION AND ANALYTICAL REACTIVITY OF THE NxTAG® RESPIRATORY PATHOGEN PANEL

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Background and Objective

Acute respiratory infections are the most common human illness and the primary cause of morbidity and mortality from infectious diseases worldwide. Most susceptible are the youngest and oldest populations from low and middle income nations. In the USA respiratory tract infections impose a huge economic burden estimated in 2003 at 17 billion a year from direct costs such as healthcare expenses and 22.5 billion a year from indirect costs such as loss of workplace productivity[1]. Because many viral and bacterial respiratory pathogens present with similar clinical manifestations, rapid identification of the causative pathogen is both challenging and critical for effective patient management.

The Luminex® NxTAG® Respiratory Pathogen Panel is a qualitative (*in vitro diagnostic*) test used 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 a respiratory tract infection. 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 organism types and subtypes detected by the NxTAG® Respiratory Pathogen Panel 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*.

The objective of this work was to measure the limit of detection (LoD) for each target probed by the NxTAG® Respiratory Pathogen Panel and to evaluate the ability of the assay to detect multiple strains, genotypes, serotypes, or subtypes of each targeted pathogen.

Methods

Study Design

LoD testing of the NxTAG® Respiratory Pathogen Panel targets was carried out in two parts. In Part-1, single analyte simulated samples were tested at 10 serial dilution levels (each in triplicate) at concentrations resulting in signal saturation at the highest concentration to background signal levels at the lowest. Each successive dilution level was related by a 4-fold dilution factor. The lowest dilution level positive for 3/3 replicates was selected for LoD confirmation by testing 20 replicates in Part-2 of the study. Following LoD confirmation, each analyte was tested at the confirmed LoD titer in simulated multi-analyte mixes where 2 – 4 analytes were included in one contrived sample. Following LoD determination of each NxTAG® Respiratory Pathogen Panel target, the analytical reactivity of the assay was investigated. Two hundred and two (202) reactivity strains were prepared and tested with three replicates at three times the measured LoD. Strains that were not detected with 3/3 positive calls at 3xLoD, were retested at a higher concentration until positive results were obtained for all 3 replicates or the maximum concentration possible for that strain stock was reached.

Sample Preparation

Simulated samples were generated using high-titer pathogen stocks from commercial suppliers which were spiked into Universal Transport Medium (UTM). Due to the lack of available commercial strains for Coronavirus HKU1 and Human Bocavirus, well characterized clinical samples were used. Nucleic acid purification of the simulated samples was performed on a NucliSENS® easyMAG® (bioMerieux®) as per the manufacturer's instructions. A negative extraction control (UTM only) was included in every nucleic acid extraction run.

NxTAG Respiratory Pathogen Panel

The extracted nucleic acid from the simulated samples were analyzed with the NxTAG® Respiratory Pathogen Panel following the instructions for use. 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.

Results

Limit of Detection (LoD) Analysis

The LoD titer for each of the NxTAG® Respiratory Pathogen Panel targets for which ≥19/20 (≥95%) of the simulated sample replicates generated a positive call by the assay is shown in Table 1 for both single and multi-analyte samples. The composition of multi- analyte samples are shown in Table 2.

Table 1: Confirmed LoD for Targets Detected by the NxTAG Respiratory Pathogen Panel

Target	Strain	LoD Titer	LoD Confirmation	LoD in Multi-Analyte Samples
Influenza A Matrix	A/Brisbane/59/07	3.08E+00 $TCID_{50}$/mL	20/20 POS	20/20 POS
	A/SwineNY/03/2009	5.53E - 01 $TCID_{50}$/mL	20/20 POS	20/20 POS
	A/Wisconsin/67/05	2.50E - 01 $TCID_{50}$/mL	20/20 POS	20/20 POS
Influenza A H1 Subtype	A/Brisbane/59/07	3.08E+00 $TCID_{50}$/mL	20/20 POS	19/20 POS
	A/SwineNY/03/2009	5.53E - 01 $TCID_{50}$/mL	20/20 POS	19/20 POS
Influenza A H3 Subtype	A/Wisconsin/67/05	9.36E - 02 $TCID_{50}$/mL	20/20 POS	20/20 POS
Influenza B	B/Florida/04/2006	5.81E - 01 $TCID_{50}$/mL	20/20 POS	20/20 POS
Respiratory Syncytial Virus A	A2	2.15E+00 $TCID_{50}$/mL	20/20 POS	20/20 POS
Respiratory Syncytial Virus B	18537	1.36E+00 $TCID_{50}$/mL	20/20 POS	20/20 POS
Coronavirus 229E	229E	1.07E - 02 $TCID_{50}$/mL	20/20 POS	20/20 POS
Coronavirus OC43	Betacoronavirus 1	7.15E - 02 $TCID_{50}$/mL	19/20 POS	20/20 POS
Coronavirus NL63	NL63	3.37E - 03 $TCID_{50}$/mL	20/20 POS	20/20 POS
Coronavirus HKU1	Clinical Specimen	1.57E+04 Copies/mL	20/20 POS	20/20 POS
Human Metapneumovirus	Human Metapneumovirus	1.38 - 01 $TCID_{50}$/mL	20/20 POS	19/20 POS
Rhinovirus/Enterovirus	Rhinovirus type 1A	5.18E-01 $TCID_{50}$/mL	20/20 POS	20/20 POS
	Enterovirus D68	3.34E+00 $TCID_{50}$/mL	20/20 POS	N/A
Adenovirus	C, type 1	3.25E+00 $TCID_{50}$/mL	20/20 POS	20/20 POS
	B, type 14	1.52E - 01 $TCID_{50}$/mL	20/20 POS	N/A
	E, type 4	6.91E - 02 $TCID_{50}$/mL	20/20 POS	N/A
Parainfluenza 1	C35	2.82E+01 $TCID_{50}$/mL	20/20 POS	20/20 POS
Parainfluenza 2	Greer	5.36E - 01 $TCID_{50}$/mL	19/20 POS	20/20 POS
Parainfluenza 3	C 243	1.61E+01 $TCID_{50}$/mL	20/20 POS	20/20 POS
Parainfluenza 4A	Type 4A	2.54E+00 $TCID_{50}$/mL	20/20 POS	20/20 POS
Parainfluenza 4B	CH19503	6.09E - 01 $TCID_{50}$/mL	20/20 POS	20/20 POS
Human Bocavirus	Type 1	3.91E+02 Copies/mL	20/20 POS	20/20 POS
Chlamydophila pneumoniae	*Chlamydophila pneumoniae*	6.43E - 02 $TCID_{50}$/mL	20/20 POS	20/20 POS
Mycoplasma pneumoniae	*Mycoplasma pneumoniae*	1.42E+02 CCU/mL	20/20 POS	20/20 POS

Table 2: Composition of Multi-Analyte Samples

Sample ID	Analyte-1	Analyte-2	Analyte-3
MA-1	Influenza A H1	Rhinovirus	Respiratory Syncytial Virus A
MA-2	Influenza A H3 (for matrix)	Adenovirus C	N/A
MA-3	Influenza A 2009 H1N1	Parainfluenza 1	*Chlamydophila pneumoniae*
MA-4	Influenza A H3 (for subtype)	Respiratory Syncytial Virus B	Human Bocavirus
MA-5	Parainfluenza 3	Coronavirus OC43	N/A
MA-6	Influenza B	Parainfluenza 4A	*Mycoplasma pneumoniae*
MA-7	Coronavirus NL63	Human Metapneumovirus	Coronavirus HKU1
MA-8	Parainfluenza 4B	Parainfluenza 2	Coronavirus 229E

Evaluation of Analytical Reactivity

A total of 202 reactivity strains were tested to evaluate the ability of NxTAG® Respiratory Pathogen Panel to detect multiple strains, genotypes, serotypes, subtypes, or isolates probed by the assay. The titer at which the strains were detected are shown in Table 3 with the number of strains detected at a particular concentration range shown in brackets.

Table 3: Summary of the NxTAG® Respiratory Pathogen Panel Analytical Reactivity

NxTAG® Respiratory Pathogen Panel TARGET	# of Strains, Isolates, Subtypes or Genotypes Detected		Concentration of Detected Analytes	
			Detectability Range	Unit
Influenza A Matrix	75	41 H1 subtype	(20) 5.53E-01 - 3.80E+06	$TCID_{50}$/mL
			(20) 9.24E+00 - 1.00E+04	$CEID_{50}$/mL
			(1) 1.66E+00	PFU/mL
		24 H3 subtype	(6) 2.50E-01 - 3.00E+00	$TCID_{50}$/mL
			(18) 3.00E+00 - 9.59E+02	$CEID_{50}$/mL
		6 H5 subtype	1.51E+02	Copies/mL
		2 H7 subtype	1.51E+02	Copies/mL
		2 H9 subtype	1.00E+02	$CEID_{50}$/mL
Influenza B	24		(11) 5.81E-01 - 1.39E+01	$TCID_{50}$/mL
			(12) 1.74E+00 -1.00E+04	$CEID_{50}$/mL
			(1) 3.63E+00	Copies/mL
Respiratory Syncytial Virus A	3		2.15E+00 - 1.65E+03	$TCID_{50}$/mL
Respiratory Syncytial Virus B	5		1.36E+00 - 6.51E+01	$TCID_{50}$/mL
Coronavirus 229E	2		1.07E-02 - 5.15E-01	$TCID_{50}$/mL
Coronavirus OC43	2		7.15E-02 - 2.15E-01	$TCID_{50}$/mL
Coronavirus NL63	2		3.37E-03 - 1.01E-02	$TCID_{50}$/mL
Coronavirus HKU1	3 (additional 57 clinical samples)		(3) 1.57E+04 -9.45E+04	Copies/mL
Human Metapneumovirus	10		1.38E-01 - 2.65E+01	$TCID_{50}$/mL
Rhinovirus/Enterovirus	12 Rhinovirus		(11) 5.18E-01 - 9.95E+01	$TCID_{50}$/mL
			(1) 3.43E+02	Copies/mL
	15 Enterovirus		3.34E+00 - 1.00E+04	$TCID_{50}$/mL
Adenovirus	18		6.91E-02 - 4.00E+04	$TCID_{50}$/mL
Parainfluenza 1	2		2.82E+01 -8.46E+01	$TCID_{50}$/mL
Parainfluenza 2	2		5.36E-01 - 1.03E+02	$TCID_{50}$/mL
Parainfluenza 3	4		1.61E+01 - 4.83E+01	$TCID_{50}$/mL
Parainfluenza 4A	2		2.54E+00 -7.63E+00	$TCID_{50}$/mL
Parainfluenza 4B	3		6.09E-01 - 4.68E+02	$TCID_{50}$/mL
Human Bocavirus	27 clinical samples, all Human Bocavirus type 1		N/A	N/A
Chlamydophila pneumoniae	8		6.43E-02 – 1.24E+01	Copies/mL
Mycoplasma pneumoniae	10		7.04E+02 - 2.11E+03	Copies/mL

Discussion

- Laboratory testing was supplemented with *in silico* data where prediction rules were used to predict reactivity of NxTAG Respiratory Pathogen Panel to additional Influenza A, Adenovirus strains and Human Poliovirus strains (data not shown).
- Bidirectional sequencing analysis was performed for 27 clinical samples positive for Human Bocavirus by the NxTAG® Respiratory Pathogen Panel and confirmed all 27 samples to be Human Boca virus 1. Sequencing analysis was also performed for 57 clinical samples positive for Coronavirus HKUI and the results suggest that the NxTAG® Respiratory Pathogen Panel can detect HKU1 genotypes A and B.

Conclusion

This study established the limit of detection for all 20 NxTAG® Respiratory Pathogen Panel targets. The limit of detection of each target in single analyte samples was equivalent to the limit of detection for each target in multi-analyte mixes. This study also demonstrated the inclusivity of 202 strains representing multiple strains, genotypes, serotypes and subtypes of targets probed by the assay.

[1] Fendrick et al. (2003), Arch Intern Med. 163(4):487-494.