

# EVALUATION OF THE MULTI-SITE REPRODUCIBILITY OF THE NxTAG® RESPIRATORY PATHOGEN PANEL

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## Background and Objective

The NxTAG® Respiratory Pathogen Panel assay 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. (See Figure 1 for the assay workflow.) The organism types and subtypes detected by the assay are listed in Table 1.

Figure 1. Overall Assay Workflow of the NxTAG Respiratory Pathogen Panel



Table 1. Targets Detected by the NxTAG Respiratory Pathogen Panel

Viral Targets		
Influenza A	Coronavirus 229E	Adenovirus
Influenza A H1	Coronavirus OC43	Parainfluenza 1
Influenza A H3	Coronavirus NL63	Parainfluenza 2
Influenza B	Coronavirus HKU1	Parainfluenza 3
Respiratory Syncytial Virus A	Human Metapneumovirus	Parainfluenza 4
Respiratory Syncytial Virus B	Rhinovirus/Enterovirus	Human Bocavirus
Bacterial Targets		
<i>Chlamydomphila pneumoniae</i>	<i>Mycoplasma pneumoniae</i>	

The objective of the study was to assess the total variability of the NxTAG® Respiratory Pathogen Panel assay across study sites, testing days, and operators/instruments.

## Materials and Methods

The sample set for the reproducibility assessment was composed of 17 unique un-extracted contrived samples (Table 2): 1 negative sample (Repro-1), 8 moderate positive multi-analyte samples (3xLoD, samples 2 - 9), and 8 low positive multi-analyte samples (1xLoD, samples 10 - 17). All pathogen targets were contrived in Universal Transport Medium (UTM) from high-titre pathogen stocks from commercial suppliers except for targets that were not commercially available, i.e., Coronavirus HKU1 and Human Bocavirus. Clinical specimens with known sequence identities were used in these cases. Sample extractions were performed using the NuCiSENS® easyMAG® (bioMérieux) automated system.

Reproducibility was evaluated across 3 sites with two operators at each site. Each assay run consisted of the 17 samples tested in triplicate, and the run was performed on 5 non-consecutive days, for a total of 30 runs (3 sites x 2 operators x 5 days).

Table 2. Composition of Reproducibility Sample Set and Titers

Sample ID	Target	Strain ID	Titre of MP (TCID <sub>50</sub> /mL)	Sample ID	Titre of LP (TCID <sub>50</sub> /mL)
Repro-1	Negative	N/A	N/A	N/A	N/A
Repro-2	Influenza A – H1 subtype	A/Brisbane/59/07 H1	9.24E+00	Repro-10	1.08E+00
	Respiratory Syncytial Virus A	A2	6.44E+00		2.15E+00
	Rhinovirus	1A	1.55E+00		5.18E-01
Repro-3	Influenza A-H3 subtype	A/Wisconsin/67/05	7.49E-01	Repro-11	2.50E-01
	Adenovirus C	A/Wisconsin/67/05 species C, type 01	9.76E+00		3.25E+00
	Influenza A- 2009 H1N1 subtype	A/Swain/03/2009	1.66E+00		5.53E-01
Repro-4	Parainfluenza 1	C35	8.46E+01	Repro-12	2.82E-01
	<i>Chlamydomphila pneumoniae</i>	TWAR strain TW-183	1.93E-01		6.43E-02
	Influenza A-H3 subtype	A/Wisconsin/67/05	2.81E-01		9.36E-02
Repro-5	Respiratory Syncytial Virus B	18537	4.07E+00	Repro-13	1.36E+00
	Human Bocavirus*	Type 1	1.17E+03		3.91E+02
	Parainfluenza 2	C 243	4.83E+01		1.61E+01
Repro-6	Coronavirus OC43	OC43 (Betacoronavirus 1)	8.15E-01	Repro-14	7.15E-02
	<i>Legionella pneumophila</i> †	Philadelphia	9.37E+02		3.12E+02
	Influenza B	B/Florida/04/2006	1.74E+00		5.81E-01
Repro-7	Parainfluenza 4A	Type 4A	7.63E+00	Repro-15	2.54E+00
	<i>Mycoplasma pneumoniae</i> ‡	M129	4.25E+02		1.42E+02
	Coronavirus NL63	NL63	1.01E-02		3.37E-03
Repro-8	Coronavirus HKU1* †	USA/HKU1-12/2009-2010	4.71E+04	Repro-16	1.57E-04
	Human Metapneumovirus	JA10-2003	4.15E-01		1.38E-01
	Parainfluenza 2	Greer	1.61E+00		1.38E-01
Repro-9	Parainfluenza 4B	CH 19503	1.83E+00	Repro-17	6.09E-01
	Coronavirus 229E	OC229E	3.22E-02		1.07E-02

MP: Moderate Positive (3xLoD); LP: Low Positive (LoD). \* Titer in Copies/mL; † Titer in CFU/mL; ‡ Titer in CCU/mL

## Results

A total of 90 data points (30 runs x 3 replicates) were generated for each of the 17 samples. Call agreement for targets detected at Moderate Positive dilution level (3xLoD), at Low Positive dilution level (1xLoD) and Negative samples are summarized in Tables 3, Table 4 and Table 5, respectively. An analysis of variance was also performed to assess four sources of variance: site, operator, day, and error (analysis not shown).

Table 3. Targets Detected at Moderate Positive Dilution Level (3xLoD)

Analyte	Site-1	Site-2	Site-3	All Sites
	Total	Total	Total	
Influenza A (H1s)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Influenza A (H1p)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Influenza A (H3)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Influenza A H1A	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	89/90 (98.9%)
Influenza A H1B	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Influenza B	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Respiratory Syncytial Virus A	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Respiratory Syncytial Virus B	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Coronavirus 229E	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Coronavirus OC43	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Coronavirus NL63	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Coronavirus HKU1	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	89/90 (98.9%)
Human Metapneumovirus	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Rhinovirus/Enterovirus	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Adenovirus	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Parainfluenza 1	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Parainfluenza 2	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Parainfluenza 3	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Parainfluenza 4A	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Parainfluenza 4B	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Human Bocavirus	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
<i>Chlamydomphila pneumoniae</i>	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
<i>Mycoplasma pneumoniae</i>	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)

Table 4. Targets Detected at Low Positive Dilution Level (1xLoD)

Analyte	Site -1			Site -2			Site -3			All Sites
	OP-A	OP-B	Total	OP-A	OP-B	Total	OP-A	OP-B	Total	
Influenza A (H1s)	15	15	30/30 (100%)	15	15	30/30 (100%)	15	15	30/30 (100%)	90/90 (100%)
Influenza A (H1p)	15	15	30/30 (100%)	15	14	29/30 (96.7%)	15	15	30/30 (100%)	89/90 (98.9%)
Influenza A (H3)	15	15	30/30 (100%)	15	15	30/30 (100%)	15	15	30/30 (100%)	90/90 (100%)
Influenza A H1A	15	15	30/30 (100%)	15	15	30/30 (100%)	15	14	29/30 (96.7%)	89/90 (98.9%)
Influenza A H1B	15	15	30/30 (100%)	15	14	29/30 (96.7%)	15	15	30/30 (100%)	89/90 (98.9%)
Influenza A H3	15	15	30/30 (100%)	15	14	29/30 (96.7%)	15	15	30/30 (100%)	89/90 (98.9%)
Influenza B	14	15	29/30 (96.7%)	15	15	30/30 (100%)	15	15	30/30 (100%)	89/90 (98.9%)
Respiratory Syncytial Virus A	15	15	30/30 (100%)	15	15	30/30 (100%)	15	15	30/30 (100%)	90/90 (100%)
Respiratory Syncytial Virus B	15	15	30/30 (100%)	15	15	30/30 (100%)	15	15	30/30 (100%)	90/90 (100%)
Coronavirus 229E	15	15	30/30 (100%)	15	15	30/30 (100%)	15	14	29/30 (96.7%)	89/90 (98.9%)
Coronavirus OC43	15	15	30/30 (100%)	15	15	30/30 (100%)	15	13	28/30 (93.3%)	88/90 (97.8%)
Coronavirus NL63	15	15	30/30 (100%)	15	15	30/30 (100%)	15	15	30/30 (100%)	90/90 (100%)
Coronavirus HKU1	15	15	30/30 (100%)	15	15	30/30 (100%)	12	15	27/30 (90.0%)	87/90 (96.7%)
Human Metapneumovirus	14	14	28/30 (93.3%)	15	15	30/30 (100%)	15	15	30/30 (100%)	88/90 (97.8%)
Rhinovirus/Enterovirus	15	15	30/30 (100%)	15	15	30/30 (100%)	15	15	30/30 (100%)	90/90 (100%)
Adenovirus	15	15	30/30 (100%)	15	15	30/30 (100%)	15	15	30/30 (100%)	90/90 (100%)
Parainfluenza 1	15	15	30/30 (100%)	15	13	28/30 (93.3%)	14	13	27/30 (90.0%)	85/90 (94.4%)
Parainfluenza 2	14	14	28/30 (93.3%)	15	15	30/30 (100%)	15	14	29/30 (96.7%)	87/90 (96.7%)
Parainfluenza 3	15	15	30/30 (100%)	15	15	30/30 (100%)	15	14	29/30 (96.7%)	89/90 (98.9%)
Parainfluenza 4A	13	15	28/30 (93.3%)	14	15	29/30 (96.7%)	15	15	30/30 (100%)	87/90 (96.7%)
Parainfluenza 4B	15	15	30/30 (100%)	14	15	29/30 (96.7%)	15	15	30/30 (100%)	89/90 (98.9%)
Human Bocavirus	14	15	29/30 (96.7%)	15	15	30/30 (100%)	15	15	30/30 (100%)	89/90 (98.9%)
<i>Chlamydomphila pneumoniae</i>	15	15	30/30 (100%)	15	15	30/30 (100%)	15	15	30/30 (100%)	90/90 (100%)
<i>Mycoplasma pneumoniae</i>	14	15	29/30 (96.7%)	15	15	30/30 (100%)	15	15	30/30 (100%)	89/90 (98.9%)

Table 5. Targets Detected as NEGATIVE in Negative Samples

Analyte	Site-1	Site-2	Site-3	All Sites
	Total	Total	Total	
Influenza A (H1s)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Influenza A (H1p)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Influenza A (H3)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Influenza A H1A	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Influenza A H1B	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Influenza A H3	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Influenza B	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Respiratory Syncytial Virus A	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Respiratory Syncytial Virus B	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Coronavirus 229E	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Coronavirus OC43	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Coronavirus NL63	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Coronavirus HKU1	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	89/90 (98.9%)
Human Metapneumovirus	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Rhinovirus/Enterovirus	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Adenovirus	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Parainfluenza 1	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Parainfluenza 2	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Parainfluenza 3	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Parainfluenza 4A	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Parainfluenza 4B	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
Human Bocavirus	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
<i>Chlamydomphila pneumoniae</i>	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
<i>Mycoplasma pneumoniae</i>	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)

## Conclusion

The NxTAG® Respiratory Pathogen Panel assay is reproducible across study sites, testing days, and operators/instruments.

The largest contributor to variability was predominantly "error", which referred to variance not uniquely assignable to one of the other three sources or represented a combination of 2 to 3 of the assignable sources. The variability contribution from the "operator" was minimal.