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Comparison of Luminex NxTAG Respiratory Pathogen Panel and xTAG Respiratory Viral Panel FAST Version 2 for the Detection of Respiratory Viruses

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Owing to advancements in molecular diagnostics, recent years have seen an increasing number of laboratories adopting respiratory viral panels to detect respiratory pathogens. In December 2015, the NxTAG respiratory pathogen panel (NxTAG RPP) was approved by the United States Food and Drug Administration. We compared the clinical performance of this new assay with that of the xTAG respiratory viral panel (xTAG RVP) FAST v2 using 142 clinical samples and 12 external quality assessment samples. Discordant results were resolved by using a laboratory-developed respiratory viral panel. The NxTAG RPP achieved 100% concordant negative results and 86.6% concordant positive results. It detected one coronavirus 229E and eight influenza A/H3N2 viruses that were missed by the xTAG RVP FAST v2. On the other hand, the NxTAG RPP missed one enterovirus/rhinovirus and one metapneumovirus that were detected by FAST v2. Both panels correctly identified all the pathogens in the 12 external quality assessment samples. Overall, the NxTAG RPP demonstrated good diagnostic performance. Of note, it was better able to subtype the influenza A/H3N2 viruses compared with the xTAG RVP FAST v2.

Key Words: Respiratory tract infections, Respiratory viral panel, Evaluation, Molecular diagnostics

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Respiratory tract infection is a leading cause of death worldwide [1]. Laboratory testing is required to identify the underlying etiologic agent of respiratory infections, as they commonly present with similar signs and symptoms [2]. The xTAG respiratory viral panel (xTAG RVP) FAST v2 is a multiplexed molecular assay for respiratory viral infections manufactured by Luminex Corp. (Austin, TX, USA) that has been routinely used in our clinical laboratory to detect respiratory viruses.

In December 2015, Luminex introduced the NxTAG respiratory pathogen panel (NxTAG RPP), following approval from United States Food and Drug Administration. Both the NxTAG RPP and xTAG RVP FAST v2 have the same number of viral targets, including influenza A virus (A/H3N2, A/H1N1, and A/H1N1/2009 strains), influenza B virus, parainfluenza virus types 1 to 4 (PIV 1-4), enterovirus/rhinovirus, coronaviruses (OC43, NL63, 229E, and HKU1), respiratory syncytial virus (RSV) A and B, metapneumovirus, adenovirus, and bocavirus. The NxTAG RPP has two additional atypical bacterial targets, namely *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*. Recent studies have compared the performance of the new NxTAG RPP with that of other respiratory panels such as the BioFire FilmArray RVP [3, 4], RespiFinder-22 [5], Anyplex II RV16 [6], and xTAG RVP FAST v2 [7]. Overall, these reports demonstrated that the NxTAG RPP is at least comparable to, if not better than, some of the comparators. Here, we assessed the clinical performance of the NxTAG RPP versus the xTAG RVP FAST v2 in detecting respiratory

viruses.

This study was approved by the local institutional ethics board (National Healthcare Group Domain-Specific Review Board A, reference: 2016/00044) and was performed between May and December 2015. Here, 142 de-identified clinical respiratory samples submitted to the Molecular Diagnosis Centre of the Singapore National University Hospital were included (see Table 1 for the list of viral pathogens included). Additionally, 12 external quality assessment (EQA) samples from the College of American Pathologists (CAP) infectious disease respiratory panel, received in year 2015, were tested (Table 2). Total nucleic acid was extracted with the Qiagen EZ1 Virus Mini Kit v2.0 on the BioRobot EZ1 extractor (Qiagen, Hilden, Germany).

All samples were initially tested with the xTAG RVP FAST v2 as part of our routine clinical service. In brief, the extracted nucleic acid (10 µL) was used for target amplification by multiplex

Table 2. Summary of the 12 College of American Pathologists 2015 external quality assessment samples used in the study

Sample	Intended result
2015 IDR-A-01	Influenza A/H3N2 (Brisbane/10/2007), PIV1
2015 IDR-A-02	Influenza B (Florida/02/06), RSV B
2015 IDR-A-03	Influenza B (Florida/04/06), Metapneumovirus B2
2015 IDR-A-04	Coxsackie A9, Adenovirus type 14
2015 IDR-A-05	Rhinovirus Type 1A, Metapneumovirus B2
2015 IDR-A-06	Coronavirus 229E
2015 IDR-C-13	Influenza A/H3N2 (Brisbane/10/2007), Adenovirus type 21
2015 IDR-C-14	PIV2, Adenovirus type 3
2015 IDR-C-15	RSV A, Rhinovirus 1A
2015 IDR-C-16	Influenza A/H1N1 (California/07/2009), Metapneumovirus B2
2015 IDR-C-17	Influenza B (Florida/04/06), Enterovirus type 71
2015 IDR-C-18	Bocavirus (Lambda recombinant)

Abbreviations: PIV, parainfluenza virus; RSV, respiratory syncytial virus.

Table 1. Summary of the performance of the NxTAG respiratory pathogen panel (NxTAG RPP) and the xTAG respiratory viral panel (xTAG RVP) FAST v2 for the detection of viral pathogens in 142 clinical samples

Viral targets	Number of samples with the following result				Assay performance with the true-positive result*			
	Assays	Number of samples				Sensitivity (95% CI)		Specificity (95% CI)	
	NxTAG RPP	+	-	+	-				
	xTAG RVP FAST v2	+	-	-	+	NxTAG RPP	xTAG RVP FAST v2	NxTAG RPP	xTAG RVP FAST v2
	LDT	NA	NA	+	+				
Influenza A		12	130	0	0	1 (0.7–1)	1 (0.7–1)	1 (0.96–1)	1 (0.96–1)
Influenza A/H3N2		3	131	8	0	1 (0.7–1)	**0.27** (0.1–0.6)	1 (0.96–1)	1 (0.96–1)
Influenza A/H1N1/2009		1	141	0	0	1 (0.1–1)	1 (0.1–1)	1 (0.97–1)	1 (0.97–1)
Influenza B		2	140	0	0	1 (0.2–1)	1 (0.2–1)	1 (0.96–1)	1 (0.96–1)
Parainfluenza virus type 1		1	141	0	0	1 (0.1–1)	1 (0.1–1)	1 (0.97–1)	1 (0.97–1)
Parainfluenza virus type 2		1	141	0	0	1 (0.1–1)	1 (0.1–1)	1 (0.97–1)	1 (0.97–1)
Parainfluenza virus type 3		7	135	0	0	1 (0.6–1)	1 (0.6–1)	1 (0.96–1)	1 (0.96–1)
Parainfluenza virus type 4		2	140	0	0	1 (0.2–1)	1 (0.2–1)	1 (0.97–1)	1 (0.97–1)
Enterovirus/rhinovirus		39	102	0	1	**0.98** (0.9–1)	1 (0.9–1)	1 (0.95–1)	1 (0.95–1)
Coronavirus OC43		2	140	0	0	1 (0.2–1)	1 (0.2–1)	1 (0.97–1)	1 (0.97–1)
Coronavirus NL63		2	140	0	0	1 (0.2–1)	1 (0.2–1)	1 (0.97–1)	1 (0.97–1)
Coronavirus 229E		1	140	1	0	1 (0.2–1)	**0.50** (0–0.97)	1 (0.97–1)	1 (0.97–1)
Coronavirus HKU1		1	141	0	0	1 (0.1–1)	1 (0.1–1)	1 (0.97–1)	1 (0.97–1)
Respiratory syncytial virus		9	133	0	0	1 (0.63–1)	1 (0.63–1)	1 (0.97–1)	1 (0.97–1)
Metapneumovirus		7	134	0	1	**0.88** (0.5–1)	1 (0.6–1)	1 (0.97–1)	1 (0.97–1)
Adenovirus		2	140	0	0	1 (0.2–1)	1 (0.2–1)	1 (0.97–1)	1 (0.97–1)
Bocavirus		1	141	0	0	1 (0.1–1)	1 (0.1–1)	1 (0.97–1)	1 (0.97–1)

*When NxTAG RPP and xTAG RVP FAST v2 results were discordant, a laboratory-developed respiratory viral panel was applied to the sample. A true-positive result was defined as one agreed by any two of the three assays.
Abbreviations: CI, confidence interval; NA, not applicable; NxTAG RPP, NxTAG respiratory pathogen panel; xTAG RVP FAST v2, xTAG respiratory viral panel FAST v2; LDT, laboratory-developed test.

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reverse transcription PCR (RT-PCR). The PCR product (2 μL) was hybridized to a bead mix; next, reporter dye was added in a new reaction vessel, which was sealed and incubated. The amplification and hybridization/incubation were performed on the Applied Biosystems Veriti thermal cycler (Thermo Fisher Scientific, Wohlen, Switzerland), as per the manufacturer's recommendations. Signal acquisition was performed on the MAGPIX instrument (Luminex Corp). After testing, the extracted nucleic acids were immediately frozen at –80°C until further testing.

Residual frozen archival samples were retrieved and tested with the NxTAG RPP, a closed-tube nucleic acid assay containing premixed lyophilized reagents for target amplification, PCR product hybridization/incubation, and detection. All procedures were carried out according to the manufacturer's instructions. The extracted nucleic acid (35 μL) was added to resuspend the preplated lyophilized bead reagents in the vessel. Multiplex RT-PCR, bead hybridization, and reporter dye incubation were performed on the Veriti thermal cycler, as per the manufacturer's recommendations. Finally, the vessel was placed onto the MAGPIX instrument for signal acquisition.

When discordant results were found between the two assays for a particular sample, a third method—a laboratory-developed, clinically validated RVP—was used for confirmation. The laboratory-developed RVP methodology is described in Supplemental file S1. In this scenario, the result concurrent between any two of the three methods was considered true. The concordance rate and Cohen's kappa coefficient of the two Luminex assays were determined by using GraphPad QuickCalcs (GraphPad, La Jolla, CA, USA).

Of the 142 clinical samples tested, 131 had concordant results, 60 and 71 of which were negative and positive, respectively. The 11 discordant samples containing metapneumovirus, enterovirus/rhinovirus, coronavirus 229E, and eight influenza A/H3N2 viruses tested positive in the laboratory-developed RVP assay and thus, were considered true positives (Table 1). The overall concordance rate between the two Luminex assays was 92.3% (131/142) with a Cohen's kappa coefficient of 0.85 (95% confidence interval [CI] 0.757–0.932), indicating a substantial degree of agreement. Of the discordant samples, the xTAG RVP FAST v2 missed eight influenza A/H3N2 viruses and one coronavirus 229E, while the NxTAG RPP missed one enterovirus/rhinovirus and one metapneumovirus. On the basis of the CAP results, both NxTAG RPP and xTAG RVP FAST v2 correctly identified all the pathogens in the samples tested.

The NxTAG RPP detected the presence of *M. pneumoniae* in one of the samples included in this study. This finding was confirmed by using a commercial real-time PCR assay for *Mycoplasma*, the Venor GeM qEP *Mycoplasma* detection kit (Minerva Biolabs GmbH, Berlin, Germany). Subsequent sensitivity testing using the lyophilized 10 colony-forming units (CFU) Sensitivity Standards (Minerva Biolabs GmbH) showed that NxTAG assay was capable of detecting *M. pneumoniae* strain down to 10 CFUs/PCR.

We assessed the clinical performance of the new NxTAG RPP against that of the xTAG RVP FAST v2 using a representative panel of viral pathogens and negatives. Notably, the seasonal influenza A/H1N1 virus was not detected in our local population, and was not included in this study. This strain appears to have been completely replaced by the pandemic influenza A/H1N1/2009 virus since 2009/2010 [8]. Overall, both assays showed comparable sensitivity and specificity for all viral targets, except for the influenza A/H3N2 virus. Notably, the xTAG RVP FAST v2 showed poor performance in influenza A/H3N2 subtyping, which may be due to primer mismatches. To the best of our knowledge, only one study has compared the performance of the NxTAG RPP with that of the xTAG RVP FAST v2; however, missed detection of influenza A/H3N2 was not reported [7]. It is unclear whether the missed detection by the xTAG RVP FAST v2 was related to the variant H3N2 virus reported by the Centers for Disease Control and Prevention (Atlanta) recently [9, 10]. Nonetheless, the inability to simultaneously detect and subtype these H3N2 viruses is a major hindrance for clinical laboratories to return test results within established turn-around-time. The influenza A/H3N2 virus is a clinically significant respiratory pathogen. Therefore, the ability to rapidly provide subtype information is important during an outbreak or in epidemiologic investigations. By contrast, the influenza A/H3N2 primers in the NxTAG RPP have been updated to detect these untypable strains. Our study suggests that existing xTAG RVP FAST v2 users should switch to the NxTAG RPP, which has better sensitivity for influenza A/H3N2, without a significant drop in sensitivity for the other respiratory viral targets.

Enterovirus/rhinovirus infections comprised 27.5% (39/142) of our study population. However, the inability of both Luminex assays to distinguish enterovirus from rhinovirus infections in patients lowers their overall clinical utility. This distinction is clinically important, particularly for septic workups in neonates and other vulnerable/immunocompromised patients, as enteroviruses can disseminate to cause systemic infection and involve multiple organs, whereas rhinoviruses generally do not [11]. The RVP assay can detect multiple viral targets simultaneously. Our study revealed nine cases of co-infections. Most of the co-infections

A

Target	Call	Signals (MFI)	
		Raw	Background
Run Control	ABS	51.0	51.0
Internal Control	PRES	3311.0	47.0
Influenza A	NEG		
├ Matrix		50.0	51.0
├ 2009 H1N1		44.0	50.0
├ H1		48.0	48.0
└ H3		48.0	50.0
Influenza B	NEG	126.5	50.5
RSV	NEG		
├ Probe-1		50.0	41.5
└ Probe-2		44.0	49.0
Corona 229E	NEG	50.0	51.0
Corona HKU1	POS	925.0	44.5
Corona NL63	NEG	49.0	50.0
Corona OC43	NEG	49.0	48.0
Para 1	NEG	47.0	46.5
Para 2	NEG	51.0	54.0
Para 3	NEG	45.0	46.5
Para 4	NEG	52.0	52.0
Enterovirus/Rhinovirus	NEG	45.0	51.0
Metapneumovirus	NEG	54.0	48.0
Adenovirus	NEG	49.5	46.0
Bocavirus	NEG	41.0	42.0

B

Target	Call	Signals (MFI)	
		Raw	Background
Run Control	ABS	56.0	51.0
Internal Control	PRES	2730.5	47.0
Influenza A	2009 H1N1,H1 D		
├ Matrix		6061.0	51.0
├ 2009 H1N1		7238.0	50.0
├ H1		799.5	48.0
└ H3		52.5	50.0
Influenza B	NEG	63.0	50.5
RSV	NEG		
├ Probe-1		50.0	41.5
└ Probe-2		47.0	49.0
Corona 229E	NEG	52.0	51.0
Corona HKU1	NEG	49.0	44.5
Corona NL63	NEG	63.0	50.0
Corona OC43	NEG	57.0	48.0
Para 1	NEG	56.0	46.5
Para 2	NEG	147.0	54.0
Para 3	NEG	50.0	46.5
Para 4	NEG	55.0	52.0
Enterovirus/Rhinovirus	POS	5327.0	51.0
Metapneumovirus	NEG	49.0	48.0
Adenovirus	NEG	46.5	46.0
Bocavirus	NEG	45.0	42.0

Target	Call	Signals (MFI)	
		Raw	Background
Run Control	ABS	50.5	77.0
Internal Control	PRES	1378.0	73.0
Influenza A	NEG		
├ Matrix		50.0	74.5
├ 2009 H1N1		42.0	63.5
├ H1		45.5	75.0
└ H3		44.0	69.0
Influenza B	NEG	52.0	75.0
RSV	NEG		
├ Probe-1		40.0	66.0
└ Probe-2		45.5	64.0
Corona 229E	NEG	46.0	75.0
Corona HKU1	NEG	45.5	73.0
Corona NL63	NEG	41.0	74.0
Corona OC43	NEG	46.0	66.0
Para 1	NEG	48.0	73.0
Para 2	NEG	46.0	80.5
Para 3	NEG	43.5	66.0
Para 4	NEG	52.0	78.0
Enterovirus/Rhinovirus	NEG	42.0	71.0
Metapneumovirus	NEG	43.0	65.0
Adenovirus	NEG	44.0	66.0
Bocavirus	NEG	43.0	72.0

Target	Call	Signals (MFI)	
		Raw	Background
Run Control	ABS	55.5	77.0
Internal Control	PRES	1393.0	73.0
Influenza A	2009 H1N1 D		
├ Matrix		3667.0	74.5
├ 2009 H1N1		4568.0	63.5
├ H1		47.0	75.0
└ H3		47.0	69.0
Influenza B	NEG	51.0	75.0
RSV	NEG		
├ Probe-1		48.0	66.0
└ Probe-2		47.0	64.0
Corona 229E	NEG	54.0	75.0
Corona HKU1	NEG	49.0	73.0
Corona NL63	NEG	57.0	74.0
Corona OC43	NEG	58.0	66.0
Para 1	NEG	46.0	73.0
Para 2	NEG	54.5	80.5
Para 3	NEG	52.0	66.0
Para 4	NEG	49.5	78.0
Enterovirus/Rhinovirus	POS	3018.0	71.0
Metapneumovirus	NEG	52.0	65.0
Adenovirus	NEG	41.5	66.0
Bocavirus	NEG	41.0	72.0

Fig. 1. High background noise observed with the Luminex bead hybridization technology in a run. (A) Sample A initially tested positive for coronavirus HKU1 with the xTAG respiratory viral panel (RVP) FAST v2 (top left). Of note, the internal control signal intensity was higher than that in previous runs. After repeating the bead hybridization step, sample A was negative for all viral targets (false-positive) and the internal control signal intensity was within the expected range (bottom left). (B) Sample B initially tested positive for seasonal influenza A/H1N1 virus, influenza A/H1N1/2009 virus, and enterovirus/rhinovirus (top right). Again, the internal control signal intensity was higher than that in previous runs. After repeating the bead hybridization step, seasonal influenza A/H1N1 virus signal was found to be negative (false-positive), and the internal control signal intensity was within the expected range (bottom right). Subsequent investigation revealed that the high background is likely due to operator variations.

Abbreviations: Corona, coronavirus; RSV, respiratory syncytial virus; Para, parainfluenza virus; MFI, median fluorescence intensity.

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involved enterovirus/rhinovirus (78%), consistent with results of previous studies [12, 13], and 43% of these cases involved enterovirus/rhinovirus and RSV.

A limitation of the current study is that the numbers per target were relatively low for influenza B, parainfluenza virus types 1, 2, and 4, coronaviruses, adenovirus, and bocavirus, and may not be sufficient to reflect the true diagnostic capability of the two assays. Such low detection rates of these viruses have been observed elsewhere [12, 13]. Additionally, we were unable to evaluate the performance of the bacterial panel in the NxTAG RPP, as the additional bacterial targets were not detectable by the xTAG RVP FAST v2 or the laboratory-developed RVP. Furthermore, we detected only a single case of *M. pneumoniae* with the NxTAG RPP.

Experimentally, the xTAG RVP FAST v2 assay had a turnaround time of 5 hr for 48 samples. However, the need to manipulate post-amplification products presents an inherent risk for laboratory contamination. Moreover, the need to remove the seal from the vessel during the detection presents another potential source of sample cross-contamination, leading to false-positives. Besides cross-contamination, high background noise (Fig. 1) is another source of false-positives, which is commonly associated with the Luminex bead-based suspension array technology due to suboptimal hybridization conditions involving temperature divergences or operator variations. In contrast, the NxTAG RPP is a closed-tube, one-step system, which abolishes the need for post-amplification product manipulation and removal of the seal. The hands-on time is significantly reduced with the simplified workflow, alleviating process variations and giving a turnaround time of <4 hr for 48 samples. Overall, the streamlined workflow minimizes cross-contamination and background noise. However, initially, where the extracted nucleic acid is used to resuspend the preplated lyophilized bead reagents, the repeat pipetting can cause possible cross-over contamination. Finally, the NxTAG RPP can process between 1 and 96 samples per run, without wasting additional consumables or reagents. This flexible throughput can cater to the needs of laboratories with different and/or variable volume demands.

In conclusion, the two Luminex assays performed comparably for most pathogens, with the NxTAG RPP having the advantages of being able to detect atypical bacteria and having better diagnostic sensitivity for certain viruses.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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