Performance assessment of the Luminex NxTAG Respiratory Pathogen Panel in clinical testing at the National University Hospital, Singapore

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Background

Respiratory tract infections (RTIs) are a significant cause of morbidity and mortality worldwide. The xTAG respiratory viral panel (RVP; Luminex Corporation, Austin, TX, USA) assay, commonly known as the xTAG Classic assay, was the first multiplexed molecular assay that was approved by the United States Food and Drug Administration (FDA) for comprehensive respiratory pathogen detection. This xTAG Classic assay was later streamlined to reduce the turnaround time and simplify the workflow, and was renamed the xTAG RVP FAST v1 (xTAG v1). Despite the efforts to improve the efficiency of the original assay, studies have reported a significant loss in the overall diagnostic sensitivity for the xTAG v1, which is a poor trade-off for clinical laboratories.

Subsequently, the xTAG v1 was further modified and introduced as the xTAG RVP FAST v2 (xTAG v2). In December 2015, the NxTAG respiratory pathogen panel (NxTAG), the latest respiratory panel by Luminex, received approval from the FDA. Both the xTAG v2 and the NxTAG assays have the same number of viral targets, including influenza A virus, influenza A/H3N2 virus, seasonal influenza A/H1N1 virus, influenza A/H1N1/2009 virus, influenza B virus, parainfluenza virus types 1 to 4, enterovirus/rhinovirus, coronaviruses (OC43, NL63, 229E, and HKU1), respiratory syncytial virus A and B, metapneumovirus, adenovirus, and bocavirus. The NxTAG has three additional atypical bacterial targets, namely *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *Legionella pneumophila* (Figure 1).

The use of the xTAG Classic and the xTAG v1 for the detection of respiratory viruses had been extensively evaluated in previous reports. Here, we compared the performance of the xTAG v2 and the NxTAG assays in a routine clinical laboratory setting.

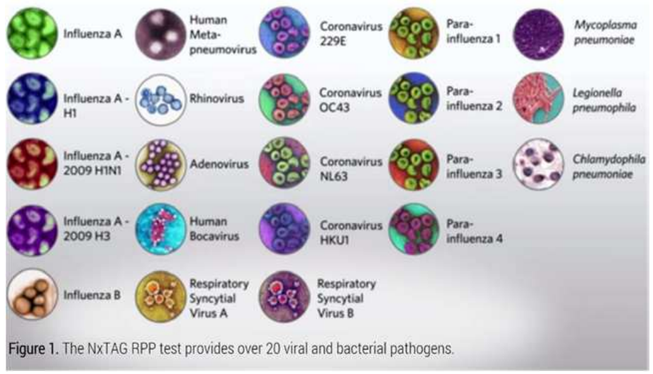

Figure 1. The NxTAG RPP test provides over 20 viral and bacterial pathogens.

Material/Methods

Eighty-one upper and lower respiratory samples submitted to the Singapore National University Hospital between May to November 2015, were used in this study. The 81 clinical samples were freshly collected and initially tested with a modified version of the xTAG v2 assay on the Applied Biosystems Veriti thermal cycler (Thermo Fisher Scientific, Wohlen, Switzerland), as part of our routine clinical service. The original number of PCR cycles of the xTAG v2 assay recommended by the manufacturer was 36. This was increased to 39 cycles during our in-house validation to improve the analytical sensitivity. This modified version of the assay has been clinically validated and put in use for routine clinical testing at our laboratory since early May 2015. After testing, the original samples and extracted nucleic acid were immediately frozen at -80ºC until further testing with the NxTAG.

Table 1. Number of observed agreements: 87 (91.58% of the observations).

Kappa is 0.806 and the strength of agreement is considered to be very good.

		xTAG v2		
		Detected	Not Detected	Total
NxTAG	Detected	61	7	68
	Not Detected	1	26	27
	Total	62	33	95

Archived residual clinical samples were retrieved and tested with the NxTAG, which is a closed-tube nucleic acid assay that contains pre-mixed lyophilised reagents for target amplification and detection. All procedures were performed according to the instructions of the manufacturer. The testing algorithm for this comparative study is illustrated in Figure 2. We were unable to compare the performance of the xTAG v2 and NxTAG assays in detecting bacterial targets, as the former assay only detects viral targets.

Results

Performance comparison between NxTAG and xTAG v2 was shown in Table 1. xTAG v2 was unable to type for six influenza A-positive samples. Five were subsequently determined to be H3 by the laboratory-developed RVP and NxTAG. The remaining untypable sample was detected as H3 by NxTAG. xTAG v2 detected an additional metapneumovirus positive that was missed by the NxTAG. This was further confirmed by the laboratory-developed RVP. An additional *Mycoplasma pneumoniae*-positive sample was detected by NxTAG which was confirmed by real-time PCR (Venor® GeM qEP Mycoplasma detection kit; Minerva Biolabs GmbH, Berlin, Germany).

Conclusion

NxTAG showed comparable performance to xTAG v2 with marked improvement in H3 detection. The xTAG v2 showed poor performance in its ability to subtype the influenza A/H3N2 virus when compared to the NxTAG.

This may be due to the presence of primer mismatches against the A/H3N2 virus in the xTAG v2. It is unclear whether the missed detection by the xTAG v2 was related to the variant H3N2 virus that has been reported by the Centers for Disease Control and Prevention (Atlanta) recently.

Limited data is available to demonstrate the NxTAG capability in detecting the bacterial targets. Only a single case of *M. pneumoniae* was detected in this study. Subsequent sensitivity test showed that NxTAG can detect 10 CFU of *M. pneumoniae* extracted from lyophilised 10CFU™ Sensitivity Standards (Minerva Biolabs GmbH).

In conclusion, we have found that the two Luminex respiratory pathogen panel assays perform comparably for most pathogens, with the NxTAG having the advantages of being able to detect atypical bacteria and having better diagnostic sensitivity for certain viruses.

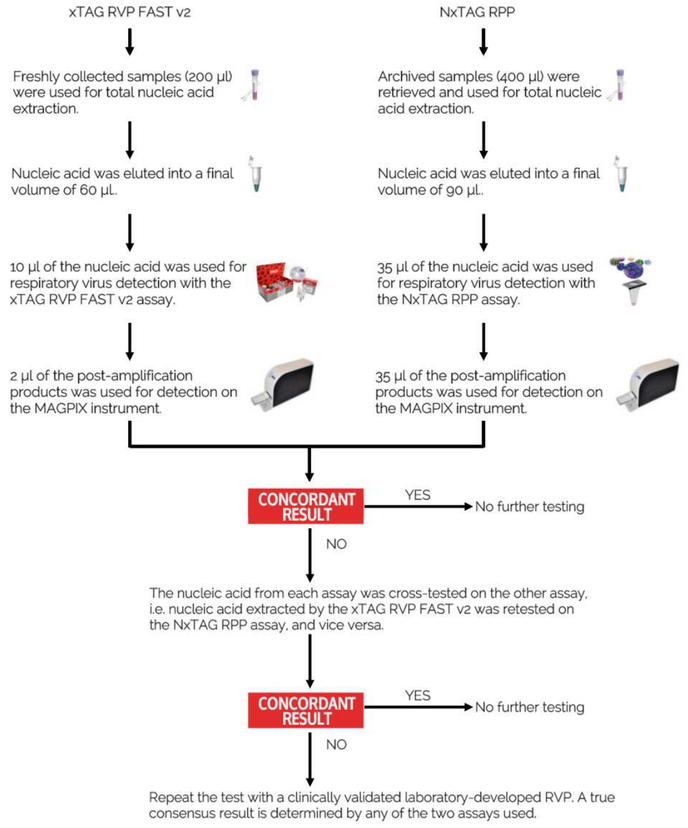

Figure 2. An overview of the comparison study. When discordance occurs between the xTAG v2 and NxTAG assays, the nucleic acid from each assay will be cross-tested on the other assay. If the result remains discordant, a laboratory-developed respiratory viral panel will be used to determine the true consensus result.