AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

VIROLOGY

Clinical Performance of the Luminex NxTAG CoV Extended Panel for SARS-CoV-2 Detection in Nasopharyngeal Specimens from COVID-19 Patients in Hong Kong

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ABSTRACT In December 2019, the coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was first reported in the Hubei province of China and later spread all over the world. There was an urgent need of a high-throughput molecular test for screening the COVID-19 patients in the community. The Luminex NxTAG CoV extended panel is a high-throughput FDA emergency use-authorized molecular diagnostic assay for SARS-CoV-2 detection. This system targets three genes (ORF1ab, N, and E genes) of SARS-CoV-2, the ORF1ab region of SARS-CoV, and the ORF5 region of MERS-CoV. In this study, we evaluated the diagnostic performance of this system with nasopharyngeal swab specimens of 214 suspected COVID-19 patients in Hong Kong. The results were compared with our routine COVID-19 reverse transcription-PCR (RT-PCR) protocol with a LightMix SarbecoV E-gene kit and an in-house RdRp/Hel RT-PCR assay. The NxTAG CoV extended panel demonstrated 97.8% sensitivity and 100% specificity to SARS-CoV-2 in nasopharyngeal specimens. On low-viral load specimens, the sensitivity of the NxTAG panel could still maintain at 85.71%. Strong agreement was observed between the NxTAG panel and the routine COVID-19 RT-PCR protocol (kappa value = 0.98). Overall, the E gene target of the NxTAG panel demonstrated the highest sensitivity among the three SARS-CoV-2 targets, while the N gene targets demonstrated the least. In conclusion, the NxTAG CoV extended panel is simple to use, and it has high diagnostic sensitivity and specificity to SARS-CoV-2 in nasopharyngeal specimens. We recommend this diagnostic system for high-throughput COVID-19 screening in the community.

KEYWORDS COVID-19, Luminex NxTAG, RT-PCR, SARS-CoV-2

In December 2019, the coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was first reported in the Hubei province of China and then spread throughout the country and later over the world (1–5). By 3 May 2020, over 3.35 million confirmed cases were reported to the World Health Organization (WHO) from 215 countries or territories, with nearly 238,000 confirmed deaths (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). In responding to this public health emergency, the U.S. Food and Drug Administration (FDA) authorized over 30 *in vitro* diagnostic commercial assays for SARS-CoV-2 detection under an emergency use authorization (EUA) (https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#covidinvitrodev).

The NxTAG CoV extended panel (Luminex Molecular Diagnostics, Toronto, Canada)

Citation Chen JH-K, Yip CC-Y, Chan JF-W, Poon RW-S, To KK-W, Chan K-H, Cheng VC-C, Yuen K-Y. 2020. Clinical performance of the Luminex NxTAG CoV extended panel for SARS-CoV-2 detection in nasopharyngeal specimens from COVID-19 patients in Hong Kong. J Clin Microbiol 58:e00936-20. https://doi.org/10.1128/JCM.00936-20.

Editor Alexander J. McAdam, Boston Children's Hospital

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Received 4 May 2020
Returned for modification 26 May 2020
Accepted 29 May 2020
Accepted manuscript posted online 1 June 2020
Published 23 July 2020

is a recently EUA-approved qualitative, multiplex, high-throughput molecular diagnostic test for the detection of SARS-CoV-2 in nasopharyngeal samples. The assay simultaneously targets the SARS-CoV-2 open reading frame 1ab (ORF1ab) region, the nucleoprotein (N) gene, and the envelope protein (E) gene (6). If any of the three targets show positive, that sample will be considered SARS-CoV-2 detected. The assay also targets the SARS-CoV ORF1ab region and the Middle East respiratory syndrome coronavirus (MERS-CoV) ORF5 region for SARS-CoV and MERS-CoV detection, respectively (7, 8). The assay is designed for differentiating all of the three most life-threatening coronaviruses, SARS-CoV, MERS-CoV, and SARS-CoV-2, in a single-tube reaction.

In this study, we evaluated the clinical performance of the new NxTAG CoV extended panel with nasopharyngeal specimens of COVID-19 patients in Hong Kong. The diagnostic performance of the NxTAG CoV extended panel was compared with our routine COVID-19 testing protocol using the LightMix SarbecoV E-gene assay (TIB-Molbiol, Berlin, Germany) as the screening assay and our in-house RT-PCR confirmatory assay targeting the RNA-dependent RNA polymerase/helicase (RdRp/Hel) gene of SARS-CoV-2 (9).

MATERIALS AND METHODS

Sample collection and ethical approval. A total of 214 archived nucleic acid extracts isolated from 214 nasopharyngeal swab (NPS) specimens of 91 confirmed COVID-19-positive and 123 COVID-19-negative patients staying in Queen Mary Hospital were included. Some of these specimens had been evaluated previously using the LightMix SarbecoV E-gene kit and the validated in-house COVID-19-RdRp/Hel RT-PCR assay (10). The 214 patients selected in this study had a median age of 51 years (interquartile range [IQR], 31 to 69), were admitted to Queen Mary Hospital through the Accident and Emergency Unit between 5 March and 26 April 2020, and developed COVID-19 clinical symptoms or had COVID-19 patient contact history or travel history.

Another four nasopharyngeal samples with different human coronaviruses (HCoV-HKU1, HCoV-NL63, HCoV-OC43, andHCoV-229E), one cell lysate of SARS-CoV, and two MERS-CoV external quality control samples from Quality Control for Molecular Diagnostics (QCMD) were also included in this evaluation for specificity analysis of the NxTAG CoV extended panel. Eight external quality assurance program (EQAP) specimens for COVID-19 of the QCMD 2020 Coronavirus Outbreak Preparedness EQA pilot panel were also included. This study was approved by the Institutional Review Board of The University of Hong Kong/Hospital Authority Hong Kong West Hospital Cluster (UW20-224).

Nucleic acid extraction. Total nucleic acid (TNA) extraction of the 214 clinical specimens was performed using the eMAG extraction system (bioMérieux, Marcy l'Étoile, France) according to the manufacturer's instructions and as previously described (11). In brief, 250 μl of NPS specimen was used for extraction, and the elution volume was set to 55 μl. The MS-2 internal control provided by the NxTAG CoV extended panel was spiked into each sample for monitoring the extraction and amplification steps. The TNA extracts were either used for routine COVID-19 RT-PCR protocol immediately and were then stored at −80°C for this evaluation. The same extracted product of each specimen was used for all the RT-PCRs.

Routine COVID-19 RT-PCR screening and confirmation protocol. For routine diagnosis of COVID-19 in Queen Mary Hospital from January 2020 until the time of writing, the LightMix SarbecoV E-gene assay (TIB Molbiol, Berlin, Germany) and the LightCycler multiplex RNA virus master kit (Roche Diagnostics, Mannheim, Germany) were used as the screening assays (10, 12). This RT-PCR aimed to target the E gene of SARS-CoV-2, but the assay cross-reacted with the E gene of SARS-CoV or bat SARS-like coronaviruses (Sarbecovirus) as mentioned in the manufacturer's instructions. Briefly, each 20-μl RT-PCR mixture contained 4 μl of 5× reverse transcriptase quantitative PCR (RT-qPCR) mix, 0.1 μl of 200× RT enzyme solution, 0.5 μl of LightMix primer and probe reagent mix, 5.4 μl of nuclease-free H_2O, and 10 μl of template. The thermal cycling condition was 5 min at 55°C for reverse transcription, 5 min at 95°C for denaturation, and 45 cycles of 5 s at 95°C, 15 s at 60°C, and 15s at 72°C. According to the manufacturer's instructions, samples with a cycle threshold (C_T) value of <39.0 will be regarded as SARS-CoV-2 or SARS-CoV or bat SARS-like coronaviruses (Sarbecovirus) detected.

Specimens showing positive in the LightMix SarbecoV E-gene RT-PCR assay will be further confirmed by another laboratory-developed SARS-CoV-2 RNA-dependent RNA polymerase/helicase (RdRp/Hel) real-time RT-PCR assay in the LightCycler 480 real-time PCR system (Roche, Basel, Switzerland) as previously described (9). The assay has been demonstrated to have an analytical sensitivity of 1.8 50% tissue culture infective dose ($TCID_{50}$/ml) and has high specificity to the SARS-CoV-2 RdRp/Hel gene region.

Luminex NxTAG CoV extended panel. A total of 35 μl of TNA of each sample stored at −80°C was added to the preplated NxTAG lyophilized bead reagents to resuspend the reaction reagents. RT-PCR was then performed according to the NxTAG CoV extended panel product insert instructions. The RT-PCR amplification was performed on an Eppendorf thermocycler (Eppendorf AG, Hamburg, Germany) with the following cycling parameters: one reverse transcription step at 42°C for 20 min; one template denaturation step at 95°C for 2 min and 30 s; 15 first amplification cycles at 95°C for 20 s, 65°C for 60 s, and 72°C for 10 s; 24 nested amplification cycles at 95°C for 20 s, 58°C for 60 s, and 72°C for 10 s; and

a hybridization step at 37°C for 45 min. The reaction plate was then transferred to the 37°C preheated MagPix heater plate of the MagPix instrument (Luminex Corporation, Austin, TX), and the signal acquisition was performed using the xPONENT and SYNCT software (Luminex Molecular Diagnostics, Toronto, Canada). Each running batch could handle up to 94 clinical specimens plus the positive and negative experiment controls. The total turnaround time was around 4 h.

Statistical analysis. McNemar's test was used to compare the performance of the assays. A *P* value of <0.05 was considered statistically significant. Computation was performed using MedCalc software version 14.12.0 (MedCalc Software, Ostend, Belgium). The area under the curve (AUC) calculated from the receiver operating characteristic (ROC) curve was compared between the two methods for performance evaluation.

RESULTS

Analytical performance of the NxTAG CoV extended panel. The specificity of the NxTAG CoV extended panel was tested by using different coronaviruses. All four samples containing human coronaviruses (HCoV-HKU1, HCoV-NL63, HCoV-OC43, and HCoV-229E) were reported negative by the assay. The two MERS-CoV samples were detected as MERS-CoV positive, and the SARS-CoV cell lysate samples was reported SARS-CoV positive. For the eight QCMD EQAP specimens, the results of the NxTAG panel and routine COVID-19 RT-PCR protocol were all matched with the expected results. Five out of eight specimens were identified as SARS-CoV-2 positive with viral load ranging from 2.30 $\log_{10}$ copies/ml (200 copies/ml) to 5.30 $\log_{10}$ copies/ml (200,000 copies/ml).

Routine COVID-19 RT-PCR protocol results. Among the 214 collected samples, the LightMix SarbecoV E-gene screening assay of the routine COVID-19 RT-PCR protocol showed positive for 91 samples with a mean C_T value of 26.44 (95% confidence interval [CI] C_T, 24.95 to 27.94). The other 123 samples all tested negative. The 91 screening positive samples were further confirmed by the RdRp/Hel confirmatory RT-PCR test, and 89 out of 91 (97.8%) were confirmed RT-PCR positive with mean C_T value of 27.17 (95% CI, C_T 25.63 to 28.72). The two RdRp/Hel RT-PCR negative samples were sent to the government reference laboratory for confirmation. The two samples were later reported as SARS-CoV-2 detected by their reference RT-PCR assay targeting the SARS-CoV-2 RdRp region. The C_T values in the LightMix screening assay for the two discrepant samples were 36.04 and 38.39.

Diagnostic performance of the NxTAG CoV extended panel. In the study, the extraction, amplification, and bead counting steps were shown to be successful with MS-2 internal control passed in the Luminex SYNCT software showing no inhibitor in the NPS specimens. Among the 91 COVID-19-positive samples, the NxTAG CoV extended panel detected 89 out of 91 samples. The assay demonstrated a diagnostic sensitivity of 97.80% (95% CI, 92.29% to 99.73%). The two false-negative samples were found to have LightMix E-gene C_T values of 37.66 and 38.39, and we believe the viral loads of the samples were at detection levels that were borderline for the NxTAG panel. For the other 123 COVID-19-negative samples, all of them (123/123; 100%) were reported negative by the NxTAG CoV extended panel assay, demonstrating a diagnostic specificity of 100% (95% CI, 97.1% to 100%). The positive predictive value was 100% (95% CI, 95.94% to 100%), and the negative predictive value was 98.40% (95% CI, 94.34% to 99.81%). The AUC of the NxTAG panel was 0.99 (95% CI, 0.96 to 1.00) (Table 1).

We further divided the 91 positive samples into two groups with a cutoff at LightMix C_T 35.00, which was roughly equal to 200 copies/ml according to the QCMD EQAP expected results. There were 77 samples found to have a LightMix C_T value of <35.00, while another 14 samples had a LightMix C_T value of ≥ 35. We observed that the diagnostic sensitivity for the sample group with C_T of <35.00 reached 100%, while the sensitivity of the sample group with C_T of ≥ 35.00 remained at 85.71% (Table 2).

Although the NxTAG panel simultaneously detected the ORF1ab, N, and E genes of SARS-CoV-2, we observed different diagnostic sensitivities for different gene targets. The overall sensitivity of individual gene targets was 91.21% (89/91) for the ORF1ab region, 85.71% (78/91) for the N gene, and 96.70% (88/91) for the E gene among the 91 COVID-19-positive samples (Table 2). When handling the 77 samples within the C_T

TABLE 1 Results of comparative evaluation of the NxTAG CoV extended panel and routine COVID-19 RT PCR protocol of 214 nasopharyngeal samples

Routine COVID-19 RT-PCR protocol result (LightMix E-gene + RdRp/Hel RT-PCR)	NxTAG CoV extended panel results (no.)			Kappa value (95% CI)	McNemar's test (*P*)
	Positive	Negative	Total		
Positive	89[a]	2[a]	91	0.98 (0.95–1.00)	0.500
Negative	0	123	123		
Total	89	125	214		

[a]There are in total 2 samples with LightMix E-gene PCR-positive and RdRp/Hel RT-PCR-negative results. The samples were further confirmed to be SARS-CoV-2-positive by the government reference laboratory.

value $<$ 35.00 sample group, the individual sensitivities of the NxTAG panel for ORF1ab, N gene, and E gene were 97.40%, 93.51%, and 100%, respectively. For the 14 samples of the C_T value $\geq$ 35 sample group, the individual sensitivities for ORF1ab, N gene, and E gene were 57.14%, 42.86%, and 78.57%, respectively (Table 2).

Agreement between the NxTAG panel and routine COVID-19 RT-PCR protocol. Comparing the NxTAG panel with the routine RT-PCR protocol, no statistically significant difference was observed using McNemar's test ($P = 0.5$). On the other hand, strong agreement between the two methods was observed (kappa = 0.98 [95% CI, 0.95 to 1.00]) (Table 1).

DISCUSSION

From January to April 2020, the number of requests for the routine COVID-19 RT-PCR protocol per day in Queen Mary Hospital, Hong Kong, ranged from 65 to 140. A high-throughput diagnostic system was necessary to accommodate the daily service need.

In this study, we evaluated the clinical performance of the NxTAG CoV extended panel, which is a new high-throughput FDA EUA diagnostic assay detecting not only SARS-CoV-2, but also MERS-CoV and SARS-CoV. Our results demonstrated that the assay differentiated SARS-CoV-2 well from the other two lethal coronaviruses simultaneously in the same reaction. However, due to the limited availability of SARS-CoV and MERS-CoV clinical specimens in Hong Kong, full evaluation of SARS-CoV and MERS-CoV detection of the NxTAG assay will need further analysis. The NxTAG assay included a single-tube nested RT-PCR amplification and provided ultrasensitive target detection and sealed tube amplification and detection minimized the risk of cross-contamination. From our study, we found that the three targets of the NxTAG panel were highly specific through testing. This NxTAG panel differentiated SARS-CoV-2 from the closely related SARS-CoV, MERS-CoV, and other human coronaviruses. The QCMD EQAP results also demonstrated that the NxTAG panel detected SARS-CoV-2-positive specimens with viral load reaching 2.30 $\log_{10}$ copies/ml (200 copies/ml).

For clinical specimens, we tested 214 NPS specimens with the NxTAG CoV extended panel and compared their results with our routine COVID-19 RT-PCR protocol, which was a combination use of the LightMix SarbecoV E-gene assay and the in-house RdRp/Hel RT-PCR assay. The NxTAG panel demonstrated good diagnostic performance with 97.80% sensitivity and 100% specificity for SARS-CoV-2 detection. Out of the 214 clinical samples, 212 of them showed concordant results between the two methods.

TABLE 2 Diagnostic sensitivity of the Luminex NxTAG CoV extended panel and the TIB Molbiol LightMix modular SarbecoV E-gene assay in the routine RT-PCR protocol

LightMix E gene C_T value	No. of samples	NxTAG CoV extended panel targets:			
		Overall result	ORF1ab	N	E
$<$35	77	77 (100%)	75 (97.40%)	72 (93.51%)	77 (100%)
$\geq$35	14	12 (85.71%)	8 (57.14%)	6 (42.86%)	11 (78.57%)
Overall	91	89 (97.80%)	83 (91.21%)	78 (85.71%)	88 (96.70%)

The two discordant samples were expected to have low viral load, and this may be the cause of the false-negative results in the NxTAG panel. Overall, the NxTAG panel demonstrated high agreement in performance with the routine COVID-19 RT-PCR protocol. which included the TIB-Molbiol LightMix SarbecoV E-gene kit, a commercially available WHO reference assay for screening and the in-house RdRp/Hel RT-PCR test for confirmation.

Our study demonstrated that the LightMix C_T value of 35.00 was roughly equal to 200 copies/ml viral load by using the QCMD EQAP samples. Therefore, we showed that all three gene targets (ORF1ab, N, and E) used in the NxTAG panel performed well when the viral loads of samples were higher than 200 copies/ml. When handling samples with viral loads lower than 200 copies/ml, we observed that the sensitivity of the gene targets in the NxTAG panel dropped significantly. The E gene target of the NxTAG panel was found to be the most sensitive gene target in the assay, while the N gene target was demonstrated to be the least sensitive target among the three targets in the assay for low-viral load COVID-19 samples. As the E gene of SARS-CoV-2 has been reported to have higher gene expression efficiency (13), this can explain the better performance of the E gene among the 3 gene targets in the NxTAG assay. The low sensitivity of the N gene target may be due to the incomplete optimization of the target amplification in the assay.

In addition to diagnostic performance, sample throughput and ease of use are also important factors to consider during this pandemic. Our routine COVID-19 RT-PCR protocol included a nucleic acid extraction step, a screening RT-PCR step with the commercial LightMix E-gene screening RT-PCR, and a confirmation RT-PCR with our in-house RdRp/Hel RT-PCR. The average processing time for a COVID-19-positive specimen would be around 4.5 to 5 h. Since the whole process required two times of master-mix preparation and template inoculation steps, it was not user-friendly to laboratory workers, especially when they were handling over 90 samples at a time. Multiplexing the screening E gene and confirmatory RdRp/Hel gene RT-PCR would be useful to shorten the processing time. However, it would require extra optimizations and evaluations. The Luminex MagPix system has been widely installed around the world, and the NxTAG assay is designed for high-throughput pathogen detection. The processing time for a complete NxTAG assay run is 4 h, and the manual handling procedures are simple (14). As the throughput of the system can be up to 96 samples per batch, it is suitable for routine screening of COVID-19-suspected cases in regional hospitals during the COVID-19 pandemic. However, the NxTAG assay used quite large amounts of TNA (35 μl) for each reaction, and repeat testing or further confirmation work may require extra nucleic acid extraction.

In conclusion, the NxTAG CoV extended panel demonstrated diagnostic performance comparable to the other WHO-recommended commercially available COVID-19 RT-PCR assays. The NxTAG system is simple to handle and supports high-throughput sample screening. This system will be a useful tool for large-scale screening of COVID-19 cases.

ACKNOWLEDGMENT

We acknowledge the kind generosity of Luminex for providing the NxTAG CoV extended panel kits used in this study. Luminex had no role in the design and conduct of the study, in the collection, analysis and interpretation of data, or in the preparation, review, or approval of the manuscript.

We declare no conflict of interest.

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