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Short Communication

Validation of the NeuMoDx™ SARS-CoV-2 assay with COPAN eNAT® and E&O Viral PCR Sample Solution collection media types in comparison with other validated SARS-CoV-2 RNA assays

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ABSTRACT

Objectives: This study aimed to confirm NeuMoDx™ SARS-CoV-2 assay (NeuMoDx assay) functionality using off-label collection media, determine assay performance versus other SARS-CoV-2 RNA assays, and assess any cross-reactivity with other respiratory viruses (human coronavirus NL63, influenza, and respiratory syncytial virus).

Methods: Nasopharyngeal swab samples in off-label collection media and external quality assessment (EQA) samples were dual-tested, first using either the RealStar® SARS-CoV-2 reverse transcriptase polymerase chain reaction assay or the QIAstat-Dx® Respiratory SARS-CoV-2 Panel and then using the NeuMoDx assay. Samples found to be positive for respiratory viruses and negative for SARS-CoV-2 were then tested using the NeuMoDx assay to assess cross-reactivity.

Results: Overall, 274 samples (244 patient and 30 EQA samples) were dual-tested; 154 were SARS-CoV-2 positive and 120 were negative. No false-positive or false-negative results were identified, regardless of collection medium used. The NeuMoDx assay sensitivity was 100% (95% confidence interval [CI] 97.63-100.00) and the specificity was 100% (95% CI 96.97-100.00). The assay did not exhibit any cross-reactivity with other respiratory viruses.

Conclusion: The NeuMoDx assay demonstrated high sensitivity and specificity on a platform well-suited for fully automated SARS-CoV-2 testing.

Introduction

Reverse transcriptase polymerase chain reaction (RT-PCR) assays for the detection of SARS-CoV-2 RNA are the gold standard for diagnosis because of their high sensitivity and specificity (Park et al., 2020). Assay validation during the pandemic was challenging because of the need for rapid implementation of novel tests (Vandenberg et al., 2021).

Although certain collection media are recommended in commercial assays (altona Diagnostics, 2021; NeuMoDx Molecular, 2021), their use depends on supplier availability. Because of supply issues during the pandemic, assays should offer functionality with various media, and laboratories must adopt a flexible approach (Locher et al., 2021).

The NeuMoDx™ SARS-CoV-2 assay (NeuMoDx assay) implemented on the NeuMoDx 96 Molecular System is a rapid, automated, random-access, real-time RT-PCR test for SARS-CoV-2 RNA detection. This study aimed to assess NeuMoDx (1) assay functionality using off-label collection media; (2) assay sensitivity and specificity versus other validated SARS-CoV-2 RNA assays; and (3) assay cross-reactivity.

Methods

Assessment of off-label collection media compatibility with the NeuMoDx assay

To confirm functionality of the NeuMoDx assay using off-label collection media (eNat® [COPAN Diagnostics, Murrieta, CA, USA] and Viral PCR Sample Solution [VPSS; E&O Laboratories, Bonny-

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bridge, UK]), patient nasopharyngeal swab (NPS) samples previously determined to be positive for SARS-CoV-2 RNA were retested. Samples were from individuals in acute and community settings who required confirmation of SARS-CoV-2 infection status in January 2021-June 2021.

Sample positivity was initially determined using existing *in vitro* diagnostic RT-PCR assays. These included either the RealStar® SARS-CoV-2 RT-PCR assay (altona Diagnostics GmbH, Hamburg, Germany) on the QIAsymphony and Rotor-Gene Q platforms (QIAGEN, Manchester, UK), hereafter called RealStar/QS/RGQ or the QIAstat-Dx® Respiratory SARS-CoV-2 Panel (QIAGEN) on the QIAstat-Dx Analyzer (QIAGEN), which allows for detection of 21 additional respiratory pathogens. Samples were stored at −80°C before testing using the NeuMoDx assay (QIAGEN) according to the manufacturer's instructions (NeuMoDx Molecular, 2021). These samples were included in the pool of samples used to determine assay sensitivity and specificity.

Assessment of NeuMoDx assay sensitivity and specificity

Additional patient NPS samples and external quality assessment (EQA) samples were dual-tested using first the RealStar/QS/RGQ and then the NeuMoDx assay (Supplementary Methods).

Assessment of cross-reactivity

Several samples were known to be positive for human coronavirus NL63 (HCoV-NL63), influenza, or respiratory syncytial virus (RSV) (Supplementary Methods) and negative for SARS-CoV-2. These samples were tested using the NeuMoDx assay to determine cross-reactivity.

Statistical analysis

Clopper-Pearson confidence intervals (CIs) were determined using an online calculator (MedCalc Software Ltd., 2022).

Results

Overall, 274 samples (244 patient and 30 EQA) were dual-tested; 154 were SARS-CoV-2 positive and 120 were negative.

Retrospective evaluation in off-label collection media

Patient NPS samples, previously determined to be positive using RealStar/QS/RGQ or QIAstat-Dx Respiratory SARS-CoV-2 Panel, were retested using the NeuMoDx assay. A total of 28 samples in eNat (Tables S1a and S1b) and 32 samples in VPSS (Tables S2a and S2b) were confirmed to be positive in the NeuMoDx assay.

Prospective evaluation of the NeuMoDx assay

In addition, 64 patient NPS samples in eNat, 120 patient NPS samples in VPSS, and 30 EQA samples, all of unknown positivity status, were dual-tested using RealStar/QS/RGQ and the NeuMoDx assay with the operator blinded to results whenever possible (Tables S3a–S3c). Thus, a pool of 274 samples overall was used to calculate sensitivity and specificity. The NeuMoDx assay sensitivity and specificity were both 100% (Table 1).

Cross-reactivity

During routine testing, three patient and three EQA samples that were positive for HCoV-NL63 RNA were tested using the NeuMoDx assay (Table S4). Six additional EQA samples tested using RealStar/QS/RGQ and the NeuMoDx assay were SARS-CoV-2-negative but positive for other respiratory viruses (influenza or RSV; Table S3c). These results show that the NeuMoDx assay did not exhibit cross-reactivity with other detected respiratory viruses.

Table 1
Sensitivity and specificity of the NeuMoDx SARS-CoV-2 assay.

	Concordance, n/N	%	95% CI, %
Sensitivity	274/274	100.00	97.63-100.00
Specificity	274/274	100.00	96.97-100.00

Sensitivity = true positives / (true positives + false negatives) × 100.
Specificity = true negatives / (false positives + true negatives) × 100.
Confidence intervals were determined using the Clopper-Pearson method.
CI, confidence interval.

Discussion

Here we demonstrate that the NeuMoDx assay has a performance similar to that of other validated SARS-CoV-2 RNA assays. Although eNat and VPSS collection media are not manufacturer-recommended for use with the NeuMoDx assay, we validated their compatibility. The assay's functionality with off-label media will allow laboratories to adopt a flexible approach.

There are several operational advantages of the NeuMoDx assay. Run time is <2 hours, compared with <5 hours for RealStar/QS/RGQ. Unlike RealStar/QS/RGQ, NeuMoDx assay techniques do not require detailed operational training or low-volume liquid handling, enabling the assay to be performed by staff who are not registered with the Health and Care Professions Council.

Regarding study limitations, some samples were subject to freeze-thaw and potential degradation of viral material. Assessment of cross-reactivity is necessary to ensure an assay meets Food and Drug Administration requirements (Motley et al., 2020); however, because of lack of circulating viruses during the pandemic (Olsen et al., 2021), prospective assessment of cross-reactivity was limited by low sample numbers. However, the detection of SARS-CoV-2 RNA without exception demonstrates that the NeuMoDx assay performs comparably to other validated assays.

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Author contributions

DB contributed to practical study and data collation. AM contributed to training and supervision. LL contributed to departmental management and resources management. MM contributed to experimental design, quality management, and quality assurance. All authors were involved in drafting, critical revision, and final approval of the manuscript.

Declaration of Competing Interest

The authors have no competing interests to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.07.024.

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