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Short communication

Clinical evaluation of a SARS-CoV-2 RT-PCR assay on a fully automated system for rapid on-demand testing in the hospital setting

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ABSTRACT

Background: The ongoing SARS-CoV-2 pandemic presents a unique challenge for diagnostic laboratories around the world. Automation of workflows in molecular diagnostics is instrumental for coping with the large number of tests ordered by clinicians, as well as providing fast-tracked rapid testing for highly urgent cases. In this study we evaluated a SARS-CoV-2 LDT for the NeuMoDx 96 system, a fully automated device performing extraction and real-time PCR.

Methods: A publicly available SARS-CoV-2 RT-PCR assay was adapted for the automated system. Analytical performance was evaluated using in-vitro transcribed RNA and clinical performance was compared to the cobas 6800-based reference assay within the lab.

Results: The Envelope (E) Gene-LDT displayed good analytical performance with an LoD of 95.55 cp/mL and no false positives during evaluation of cross-reactivity. A total of 176 patient samples were tested with both the E-Gene-LDT and the reference assay. Positive and negative agreement were 100 % and 99.2 % respectively. Invalid-rate was 6.3 %.

Conclusion: The E-Gene-LDT showed analytical and clinical performance comparable to the cobas6800-based reference assay. Due to its random-access workflow concept and rapid time-to-result of about 80 min, the system is very well suited for providing fast-tracked SARS-CoV-2 diagnostics for urgent clinical samples in the hospital setting.

1. Introduction

In early January 2020, SARS-CoV-2 was first identified as the likely causative agent of a cluster of cases of viral pneumonia in the city of Wuhan, China [1]. The novel virus is situated in the 'sarbecovirus' subgenus along with its genetically distinct relative, the original SARS-coronavirus [2]. SARS-CoV-2 saw rapid spread worldwide eventually prompting the WHO to declare a 'global health emergency' by the end of January [3].

Outbreak scenarios present a unique challenge for diagnostic laboratories. Particularly in the case of respiratory viruses such as SARS-CoV-2, clinical symptoms can be largely indistinguishable from other common respiratory pathogens such as e.g. Influenza [4] and polymerase chain reaction (PCR) assays are necessary to confirm or rule out the novel virus [5]. A variety of suitable assays were made available early on during the outbreak, notably by Corman et al. [6] and the CDC, which were swiftly adopted by many labs in Europe and around the world. However, their overall testing capacity remained limited [7]. We and others have previously demonstrated how automation in molecular diagnostics enables easy scaling of testing capacity by substantially cutting back hands-on time for PCR-assays [8,9].

For the assay presented in this study, we used a fully automated random-access platform for molecular diagnostics, handling everything from extraction, amplification, signal detection to reporting of results [10]. For RNA targets, the time-to-result is approximately 80 min, given optimal conditions. The availability of an open mode allows for the rapid implementation of lab developed tests (LDT). The aim of this study was to adapt and evaluate a previously published SARS-CoV-2

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Table 1
NeuMoDx-Software run-protocol summary displaying settings and PCR protocol.

NeuMoDx software setup:
Ct Calling Algorithm: Second Derivative
Result Type: Qualitative
Speciment Type: TransportMedium
Specimen Aspirate Volume (µL): 400
Lysis: 600 s. (Lysis Buffer 4)
Target: SPC2, Speciment Type: TransportMedium
(Internal Control)
Reporter: Yellow (530/555)
Peak Minimum Cycle: 25
Peak Maximum Cycle: 40
Minimum End Point Fluorescence: 1000
Minimum Peak Height: 10
Target: FAM (Sarbeco-E), Speciment Type: TransportMedium
Reporter: Green (470/510)
Peak Minimum Cycle: 25
Peak Maximum Cycle: 40
Minimum End Point Fluorescence: 1000
Minimum Peak Height: 10
PCR Stage: RT (Hold, 900 s, 50 °C)
PCR Stage: InActivation (Hold, 240 s, 95 °C)
PCR Stage: Cycle (Cycle, 50 Cycles)
Step Denature: 6 s, at 95 °C, No Detect
Step Anneal: 19 s, at 60 °C, Detect

PCR assay (by Corman et al. (6)) for the NeuModx 96 system.

2. Materials and methods

2.1. Envelope (E) Gene-LDT assay setup (NeuMoDx 96 system)

Primers (fwd: 5´-ACAGGTACGTTAATAGTTAATAGCmGT-3´, rev 5´-ATATTGCAGCAGTACGCACAmCA-3´) and probe (5´-Fam-ACACTA GCC/ZEN/ATCCTTACTGCGCTTCG-Iowa Black FQ-3′) used for the Envelope (E) Gene-LDT were custom made and purchased from IDT DNA Technologies (Coralville, USA). Both primers were modified with 2′-O-methyl bases in their penultimate base to prevent formation of primer dimers (mG or mC). A double-quenched probe was used in order to reduce background fluorescence.

In accordance with instructions issued by the manufacturer, a 6x Primer/Probe mix was prepared and 5 µL of the mix were loaded into the LDT-Strip well by well for each reaction (e.g. 400 nM primers, 75 nM probe per reaction). For a complete run protocol see the test-summary displayed in Table 1. Reagents and materials for extraction and PCR were from NeuMoDx inc. (Ann Arbor, USA; distributed by QIAGEN) and used according to instructions by the manufacturer.

2.2. Assessment of Limit of detection and intra-/inter-run variability

For analytical evaluation, in-vitro transcribed RNA (IVT-RNA) of the viral E-gene was generated as described previously [6] using the following primers: 5´- TACTAATACGACTCACTATAGATACAGGTACGTT AATAGTTAATAGCGT-3´ and 5´-ttttttttgtatacATATTGCAGCAGTACGC ACACA-3´. IVT-RNA was adjusted for copy-numbers to a predefined RNA standard obtained from “European virus archive” (EVA), (https://www.european-virus-archive.com).

A total of 4 different concentrations (400, 100, 40 and 10 copies/mL) and negative control, 8 replicates each, were used to determine Limit of detection (LoD) by probit-analysis (MedCalc, MedCalc Software Ltd). Inter-run and intra-run variability were evaluated using spiked swab samples containing IVT-RNA at approximately 5× and 10× LoD, running 5 repeats each on two different days.

2.3. Evaluation of cross-reactivity

In order to rule out potential cross-reactivity with other organisms present in respiratory swabs, a set of predetermined clinical samples containing a variety of respiratory pathogens and external quality assessment panel samples (INSTAND e.V., Germany) were selected and subjected to the E-Gene-LDT.

2.4. Comparing of clinical performance

Clinical specimens used for this study were oropharyngeal and nasopharyngeal swabs (E-Swab collection kits, Copan, Italy). Prior to analysis, 1 mL Roche cobas PCR medium (≤ 40 % guanidine hydrochloride in Tris-HCL buffer) was added to the sample for pre-lysis and inactivation. Samples were then briefly vortexed before being loaded into the instrument. A total of 176 clinical were prepared, split into aliquots and tested in parallel on both systems. Samples that did not yield valid results on the NeuMoDx system are reported as “Invalid”.

3. Results

3.1. Assessment of analytical performance

Limit of detection was determined as 95.55 cp/mL at 95 % probability of detection (CI 63.56 cp/mL – 241.46 cp/mL). Intra-/inter-run variability yielded median Ct values of 27.045 (+/- 0.695 ct) and 27.640 (+/- 1.14 ct) for 10× LoD and 5× LoD respectively. This is in line with previously published data for comparable assays [11].

There were no false positive results in cross-reactivity experiments indicating solid specificity, see Table 2.

3.2. Comparing clinical performance

Clinical performance of the assay was analyzed by comparing the E-Gene-LDT to the reference method within the lab, the cobas6800-based “SARS-CoV-2 UCT” assay [11]. Inhibition rate was 6.3 % (11/176 samples, all of which were tested negative in the reference assay). Positive agreement was 100 % (35/35) and negative agreement was 99.2 % (129/130), see Table 3. Median ct of clinical samples was 25.15 (IQR 20.87–27.04) for the NeuMoDx E-Gene-LDT and 28.16 (IQR

Table 2
Clinical samples and external quality control samples (provided by INSTAND e.V., Düsseldorf, Germany) were tested for potential cross-reactivity with the E-Gene-LDT.

Positive clinical samples	Number	Result
hCoV 229E	2	Negative
hCoV HKU1	2	Negative
Influenza A	3	Negative
Influenza A H1N1	2	Negative
Influenza B	2	Negative
RSV	3	Negative
Rhino-/Enterovirus	2	Negative
Human Metapneumovirus	2	Negative
Parainfluenzavirus 3	1	Negative
Adenovirus	1	Negative
Boca-virus	2	Negative
Mycoplasma pneumoniae	1	Negative
Chlamydophila pneumoniae	1	Negative
Pneumocystis jirovecii	1	Negative
External quality assessment panels (INSTAND)		
MERS Coronavirus	2	Negative
hCoV NL63	1	Negative
hCoV 229E	1	Negative
hCoV OC43	1	Negative
Parainfluenzavirus 2	1	Negative
Parainfluenzavirus 3	1	Negative
Total number tested:	32	

Table 3
Results of clinical samples were compared between the novel E-Gene-LDT and the routine assay (SARS-CoV UCT on the cobas6800 system).

		SARS-CoV UCT cobas6800	
		Positive	Negative
E-Gene-LDT NeuMoDx 96	**Positive**	35	1
	Negative	0	129
	Invalid	0	11
		Total number:	176

24.36–31.66) for the cobas6800 SARS-CoV-2 UCT. A single discrepant sample occurred, returning positive on the NeuMoDx system (ct 28.73, close to LoD) and negative on the cobas6800. Root cause investigation revealed that this patient had previously been diagnosed with COVID-19 elsewhere.

4. Discussion

Reliance on manual PCR setups is one of the fundamental limitations in molecular diagnostics when it comes to scalability and speed during outbreak scenarios such as the current SARS-CoV-2 situation. A study by Reusken et al. reported readiness to test for the novel Coronavirus by the end of January 2020 in almost all countries of the European union, but with a capacity of 250 tests per week or less for the vast majority of them [7]. Similar issues were reported early on in China, where testing could not be performed for all suspected cases due to limitations in capacity [12].

In a recent study we demonstrated that a previously published TaqMan based SARS-CoV-2 RT-PCR assay, endorsed by ECDC and WHO, can be adapted to run on an automated batch-based high-throughput system, the cobas6800 [11]. Incidentally, Roche recently released their own SARS-CoV-2 assay for this system under “Emergency Use Authorization” by the FDA [13]. However, taking into consideration sample registration, pretreatment, preparation of batches, and generating reports, it usually takes more than 5 h before results can be made available to clinicians [14]. Consequently, alternative workflows are required to enable fast-tracking of high-priority samples.

The NeuMoDx 96 system is a fully automated RT-PCR platform, performing extraction, amplification and signal detection without requiring any human interaction. it provides random-access capabilities, turn-around times of 80 min for RNA targets and a throughput of 144 samples/8 h [15]. In this study we have adapted the SARS-CoV-2 RT-PCR assay by Corman et al. [6] for use on the NeuMoDx 96 automated system. Analytical and clinical performance was comparable to the cobas6800-based reference assay [11], showing an LoD of approximately 100 copies/mL and positive and negative agreement of 100 % and 99.2 % respectively. The relatively high inhibition rate of 6.3 % suggests that sample preparation procedures can be further optimized.

During the preparation of this manuscript, several commercial assays were released offering rapid random-access testing (< 80 min), including Xpert Xpress (Cepheid), QIAstat-Dx (QIAGEN) and ID NOW COVID-19 (Abbott) [16,17], however clinical evaluation of these assays is not yet available in peer-reviewed literature.

5. Conclusion

In this study we have adapted a publicly available SARS-CoV-2 screening assay for use on the open mode of the NeuMoDx 96 system. The assay demonstrates comparable analytical and clinical performance to established LDTs currently in use for SARS-CoV-2 diagnostics. Due to its random-access capabilities and short turn-around times (80 min), the system is well suited for automating medium-throughput routine SARS-CoV-2 testing, or as an addition to high-throughput systems to allow fast-tracking for highly urgent clinical samples.

Authors contribution

ML, SP, MA, NF, AS, SK, and UM conceptualized and supervised the study. DN performed the experiments. DN, ML and SP wrote the initial draft of the manuscript. All authors agreed to the publication of the final manuscript.

Declaration of Competing Interest

All authors declare no conflict of interest.

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