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

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10 **Highlights:**

11 • A publicly available SARS-CoV-2 RT-PCR assay was adapted and evaluated on the open mode
12 of the NeuMoDx 96 system (Qiagen)
13 • The assay showed comparable analytical and clinical performance to the reference assay
14 • Fast turn-around times (80 minutes) and random-access workflow of the system makes the
15 assay well suited for urgent clinical samples.

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1 Abstract

Background: The ongoing SARS-CoV-2 pandemic presents a unique challenge for diagnostic laboratories around the world. Automation of workflows in molecular diagnostics are 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 NeuMoDx-sarbeco-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 Sarbeco-LDT and the reference assay. Positive and negative agreement were 100% and 99.2% respectively. Invalid-rate was 6.3%. **Conclusion:** The NeuMoDx-sarbeco-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 minutes, the device is very well suited for providing fast-tracked SARS-CoV-2 diagnostics for urgent clinical samples in the hospital setting.

2 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

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50 common respiratory pathogens such as e.g. Influenza (4) and polymerase chain reaction (PCR) assays
51 are necessary to confirm or rule out the novel virus (5). A variety of suitable assays were made available
52 early on during the outbreak, notably by Corman et al. (6) and the CDC, which were swiftly adopted by
53 many labs in Europe and around the world. However, their overall testing capacity remained limited
54 (7). We and others have previously demonstrated how automation in molecular diagnostics enables
55 easy scaling of testing capacity by substantially cutting back hands-on time for PCR-assays (8, 9).
56 For the assay presented in this study, we used a fully automated random-access platform for molecular
57 diagnostics, handling everything from extraction, amplification, signal detection to reporting of results
58 (10). For RNA targets, the time-to-result is approximately 80 minutes, given optimal conditions. The
59 availability of an open mode allows for the rapid implementation of lab developed tests (LDT). The aim
60 of this study was to adapt and evaluate a previously published SARS-CoV-2 PCR assay (by Corman et
61 al. (6)) for the NeuModx 96/288 system.

62 3 Sarbeco-LDT assay setup

63 Primers (fwd: 5´-ACAGGTACGTTAATAGTTAATAGCmGT-3´, rev 5´-ATATTGCAGCAGTACGCACAmCA-3´)
64 and probe (5´-Fam-ACACTAGCC/ZEN/ATCCTTACTGCGCTTCG-Iowa Black FQ-3') used for Sarbeco-LDT
65 were custom made and purchased from IDT DNA Technologies (Coralville, USA). Both primers were
66 modified with 2'-O-methyl bases in their penultimate base to prevent formation of primer dimers (mG
67 or mC). A double-quenched probe was used in order to reduce background fluorescence.
68 In accordance with instructions issued by the manufacturer, a 6x Primer/Probe mix was prepared and
69 5µL of the mix were loaded into the LDT-Strip well by well for each reaction (e.g. 400nM primers, 75
70 nM probe per reaction). For a complete run protocol see the test-summary displayed in table 1.
71
72

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NeuMoDx software setup:

Ct Calling Algorithm: Second Derivative

Result Type: Qualitative

Speciment Type: TransportMedium

Specimen Aspirate Volume (µL): 400

Lysis: 600 sec. (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 sec, 50°C)

PCR Stage: InActivation (Hold, 240 sec, 95°C)

PCR Stage: Cycle (Cycle, 50 Cycles)

Step Denature: 6 sec, at 95°C, No Detect

Step Anneal: 19 sec, at 60°C, Detect

73

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

75

76 Clinical specimens used for this study were oropharyngeal and nasopharyngeal swabs (E-Swab
77 collection kits, Copan, Italy). Prior to analysis, 1ml Roche cobas PCR medium (≤ 40% guanidine
78 hydrochloride in Tris-HCL buffer) was added to the sample in order to inactivate potential pathogens
79 within and facilitate further handling. Samples were then briefly vortexed before being loaded into the
80 instrument.

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81
4 Assessment of analytical performance

82 For analytical evaluation, in-vitro transcribed RNA (IVT-RNA) of the viral E-gene was generated as
83 described previously (6) using the following primers: 5´-
84 TACTAATACGACTCACTATAGATACAGGTACGTTAATAGTTAATAGCGT-3´ and 5´-
85 ttttttttgtatacATATTGCAGCAGTACGCACACA-3´. IVT-RNA was adjusted for copy-numbers to a
86 predefined RNA standard obtained from "European virus archive" (EVA), (https://www.european-
87 virus-archive.com).
88 A total of 8 replicates of 4 different concentrations (400, 100, 40 and 10 copies/ml) and negative
89 control were used to determine LoD by probit-analysis (MedCalc, MedCalc Software Ltd). Limit of
90 detection was determined as 95.55 cp/ml at 95% probability of detection (CI 63.56 cp/ml – 241.46
91 cp/ml). (Figure 1)
92 Inter-run and intra-run variability were evaluated using spiked swab samples containing IVT-RNA at
93 approximately 5x and 10x LoD, running 5 repeats each on two different days. Median Ct values were
94 27.045 (+/- 0.695 ct) and 27.640 (+/- 1.14 ct) for 10x LoD and 5x LoD respectively.
95 In order to rule out potential cross-reactivity with other organisms present in respiratory swabs, a set
96 of predetermined clinical samples containing a variety of respiratory pathogens and external quality
97 assessment panel samples were selected and subjected to the Sarbeco-LDT. There were no false
98 positive results, see table 2.
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100

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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	

102

103 **Table 2:** Clinical samples and external quality control samples (provided by INSTAND e.V., Düsseldorf,

104 Germany) were tested for potential cross-reactivity with the Sarbeco-LDT.

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105 # 5 Comparing clinical performance

106 Clinical performance of the assay was analyzed by comparing the Sarbeco-LDT to the reference method
107 within the lab, the cobas6800-based “SARS-CoV-2 UCT” assay (11). A total of 176 clinical samples
108 (collected during the time between 17/03/20 and 30/03/20) were prepared according to the above-
109 mentioned protocol, split into aliquots and tested in parallel on both systems. Samples that did not
110 yield valid results on the NeuMoDx system are reported as “Invalid”. The inhibition rate was 6.3%
111 (11/176 samples, all of which were tested negative in the reference assay). Positive agreement was
112 100% (35/35, amplification curves see figure 2) and negative agreement was 99.2% (129/130). A single
113 discrepant sample occurred, returning positive on the NeuMoDx system (late ct, close to LoD) and
114 negative on the cobas6800. Root cause investigation revealed that this patient had previously been
115 diagnosed with COVID-19 elsewhere.

116 # 6 Discussion

117 When comparing the current SARS-CoV-2 outbreak to the SARS pandemic in 2003/04, it is immediately
118 apparent how much faster emerging pathogens can be identified and characterized in the modern day
119 (2, 12). TaqMan based RT-PCR-assays for the novel virus were available online mere days after the
120 initial sequence of SARS-CoV-2 had been published (6). However, while these assays can be
121 implemented relatively swiftly by local diagnostic laboratories, their reliance on manual PCR setups
122 sets narrow limits to overall capacity. A study by Reusken et al. reported readiness to test for the novel
123 Coronavirus by the end of January 2020 in almost all countries of the European union, but with a
124 capacity of 250 tests per week or less for the vast majority of them (7). Similar issues were reported
125 early on in China, where testing could not be performed for all suspected cases due to limitations in
126 capacity (13).
127 In a recent study we demonstrated that a previously published TaqMan based SARS-CoV-2 RT-PCR
128 assay, endorsed by ECDC and WHO, can be adapted to run on an automated batch-based high-

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129 throughput system, the cobas6800 (11). Utilizing this assay, more than 10,000 samples were tested for
130 SARS-CoV-2 during the month of March 2020 while maintaining all other routine diagnostics in our
131 laboratory, proving the potency of rapid automation to cope with massive surges in demand. However,
132 taking into consideration sample registration, pretreatment, preparation of batches, and generating
133 reports, it usually takes more than 5 hours before results can be made available to clinicians (14).
134 Consequently, alternative workflows are required to enable fast-tracking of high-priority samples.
135 The NeuMoDx 96 system is a fully automated RT-PCR platform, performing extraction, amplification
136 and signal detection without requiring any human interaction. it provides random-access capabilities
137 and turn-around times of 80 minutes for RNA targets. In this study we have adapted the SARS-CoV-2
138 RT-PCR assay by Corman et al. (6) for use on the NeuMoDx 96 automated system. Analytical and clinical
139 performance was comparable to the cobas6800-based reference assay (11), showing an LoD of
140 approximately 100 copies/ml and positive and negative agreement of 100% and 99.2% respectively.
141 The relatively high inhibition rate of 6.3% suggests that sample preparation procedures can be
142 optimized further. The NeuMoDx-based Sarbeco-LDT represents a valuable complement to routine
143 SARS-CoV-2 testing by offering the ability to run individual high-priority samples at any time and
144 reporting results within two hours if necessary.

145 7 Conclusion

146 In this study we have adapted a publicly available SARS-CoV-2 screening assay for use on the open
147 channel of the NeuMoDx 96/288 system (Qiagen). The assay demonstrates comparable analytical and
148 clinical performance to established LDTs currently in use for SARS-CoV-2 diagnostics. Due to its
149 random-access capabilities and short turn-around times (80 minutes), the system is well suited for
150 automating medium-throughput routine SARS-CoV-2 testing, or as an addition to high-throughput
151 systems to allow fast-tracking for highly urgent clinical samples.

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152 # 8 Competing interest

153 All authors declare no conflict of interest.

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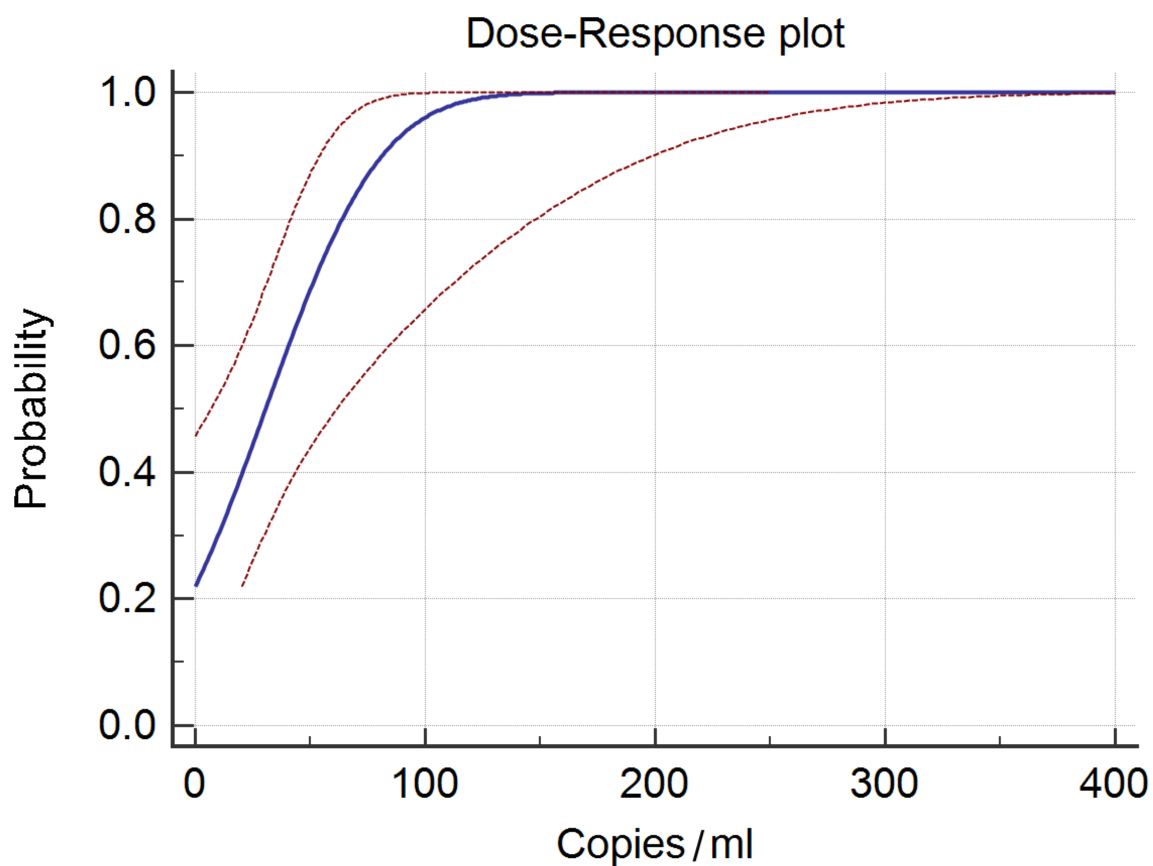

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193 **Figure 1:** Probit-curve of LoD assessment (Blue curve, Probability of detection; Red curves, 95%

194 confidence interval)

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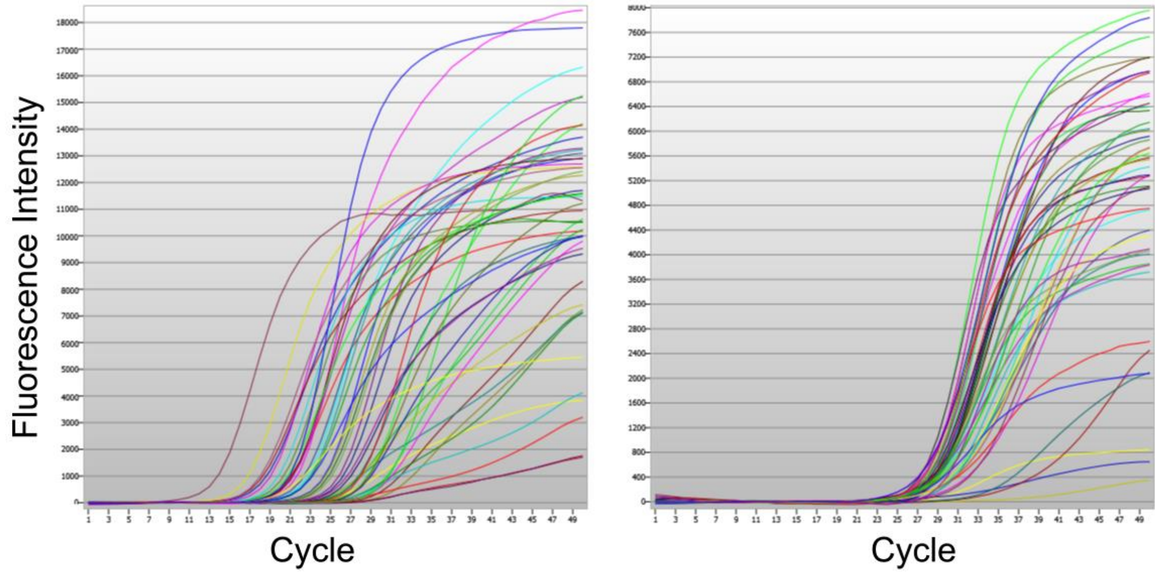

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197 **Figure 2**: Amplification curves of all positive clinical samples as displayed by the NeuMoDx Software.

198 Left: FAM-probe (E-gene), normalized; Right: VIC/JOE-probe (IC), normalized.

199