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Short communication

Increasing diagnostic possibilities using the geneLEAD VIII platform for detection of SARS-CoV-2

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ABSTRACT

At the time SARS-CoV-2 was identified as the cause of coronavirus disease 2019 (COVID-19) no in vitro diagnostic (IVD) tests were available since it was a new virus. Very shortly after the release of the genomic sequence of SARS-CoV-2, laboratory-developed tests (LDTs) were developed, made available and implemented in several laboratories in the Netherlands and globally. In this study, the performance of an E-gene Sarbeco specific real-time reverse-transcriptase PCR (RT-PCR) was verified on the open modus of the geneLEAD VIII sample-to-answer platform. The results obtained from 134 clinical samples, of which 63 had been tested positive, showed almost complete concordance compared to the same PCR on the routine diagnostic systems and that was validated according to the national reference standard. The only discordant sample tested positive using the routine diagnostic workflow with a cycle threshold (C_T) value of 37.7, while the sample tested negative using the geneLEAD VIII workflow. In addition, good performance was achieved in analyzing a blinded SARS-CoV-2 external quality assurance (EQA) panel. Implementation of the geneLEAD VIII platform as routine diagnostic tool resulted in testing 871 clinical samples with 115 positive results. In conclusion, the geneLEAD VIII SARS-CoV-2 workflow presented in this study showed excellent diagnostic performance and with a rapid turn-around time of approximately two hours it proved a valuable option for STAT SARS-CoV-2 testing in the absence of (rapid, CE-IVD) point-of-care testing platforms.

Molecular diagnostics is the gold standard for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detection. Rapid identification of infected patients directs appropriate patient isolation, treatment in acute care and ability to return to work for medical staff members (Loeffelhoz and Tang, 2020; Keeley et al., 2020). However, during the early phase of the coronavirus disease 2019 (COVID-19) pandemic, in vitro diagnostic (IVD) tests were not available as it takes time to develop, manufacture, and distribute new commercially available molecular-based assays. And once these assays became available, the global demand for SARS-CoV-2 diagnostics led to a shortage of reagents and consumables for both high-throughput PCR-based workflows and rapid (point-of-care) testing platforms (Butler-Wu et al., 2020; Lima et al., 2020). For these reasons, the development of laboratory-developed tests (LDTs) plays a critical role in efforts to increase diagnostic possibilities in times of crisis, such as the COVID-19 pandemic.

In this study, the application of the widely used E-gene Sarbeco specific real-time reverse-transcriptase PCR (RT-PCR) as described by Corman et al. (2020) has been evaluated on the open modus of the geneLEAD VIII (Diagenode Diagnostics). This sample-to-answer platform can handle up to eight samples in a single run with a turnaround time of 1.5–2 hours, depending on the amplification protocol. Open modus is an intrinsic functionality of the platform that enables the implementation of LDTs in the sample-to-answer workflow. The performance of SARS-CoV-2 detection via the geneLEAD VIII workflow was compared to the routine diagnostic workflow at the Leiden University Medical Center (LUMC), using 134 clinical samples that were previously submitted for clinical diagnosis of SARS-CoV-2 infection and eight samples that were part of an external quality assurance (EQA) panel.

Prior to nucleic acid (NA) extraction through both workflows, equine arteritis virus (EAV) was added to all samples that served as an internal extraction and amplification control (IC) (Scheltinga et al., 2005). Also, sputum samples and bronchoalveolar lavage fluids were pretreated by diluting these sample types 1:5 in phosphate-buffered saline and

Table 1
Primers and probes used for SARS-CoV-2 detection. The EAV primers and probe were combined with the E-gene primers and probe, resulting in an internally controlled Sarbeco specific RT-PCR assay.

Target	Oligonucleotide	Sequence (5′ -> 3′)	Refs.
E-gene	Forward primer	ACAGGTACGTTAATAGTTAATAGCGT	(Corman et al., 2020)
	Reverse primer	ATATTGCAGCAGTACGCACACA	
	Probe	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ-1	
EAV	Forward primer	CATCTCTTGCTTTGCTCCTTAG	(Scheltinga et al., 2005)
	Reverse primer	AGCCGCACCTTCACATTG	
	Probe	CY5-CGCTGTCAGAACAACATTATTGCCCAC-BHQ-2	

Table 2
Performance of the geneLEAD VIII workflow as compared to the routine diagnostic workflow.

Initial result	Concordance geneLEAD VIII workflow	Median CT value difference
Positive (C$_T$ <30)	13/13 (100 %)	0.9 (± 0.9)
Positive (C$_T$ 30−35)	45/45 (100 %)	0.9 (± 1.0)
Positive (C$_T$ >35)	4/5 (80 %)	3.3 (± 1.7)
Negative	71/71 (100 %)	

Table 3
Comparison of results obtained with the Dutch SARS-CoV-2 EQA panel.

ID	Virus	Routine diagnostic workflow	geneLEAD VIII workflow
LEQA1_CoV20−1	SARS-CoV-2 (D1)	30.4	29.5
LEQA1_CoV20−2	Human coronavirus NL63	–	–
LEQA1_CoV20−3	Human coronavirus 229E	–	–
LEQA1_CoV20−4	SARS-CoV-2 (D4)	–	–
LEQA1_CoV20−5	SARS-CoV-2 (D3)	36.3	35.1
LEQA1_CoV20−6	Human coronavirus OC43	–	–
LEQA1_CoV20−7	SARS-CoV-2 (D3)	36.3	34.7
LEQA1_CoV20−8	Influenzavirus A (H3N2)	–	–
LEQA1_CoV20−9	None	–	–
LEQA1_CoV20−10	SARS-CoV-1	30.1*	29.7*

D1, D3 and D4 indicate that D3 is a 1:100 dilution of D1 and D4 is a 1:10 dilution of D3; values shown represent C$_T$ values; '-', RT-PCR negative result.

* Sarbeco specific RT-PCR.

homogenizing the dilutions by bead-beating. No pretreatment was performed for the other types of samples. The geneLEAD VIII workflow consisted of an on-board NA extraction followed by RT-PCR. In short, NA was extracted from 200 μL of sample using MagDEA® Dx cartridges (Diagenode Diagnostics) and eluted in 100 μL buffer. Next, the internally controlled RT-PCR assay targeting the E-gene was performed with a 25 μL reaction mixture consisting of 6.25 μL TaqMan Fast Virus 1-Step mastermix [4x] (ThermoFisher), 0.4 μM of each primer, 0.2 μM of each probe (Table 1), and 10 μL of the NA extracts. The RT-PCR run time could be reduced to 80 min and consisted of a reverse transcription step at 50 °C for 5 min, followed by 95 °C for 10 s and then 45 cycles of 95 °C for 3 s, 55 °C for 10 s, and a final extension at 60 °C for 30 s. RT-PCR amplification signals were automatically interpreted by the geneLEAD VIII software. For the routine diagnostic workflow, NA was extracted from 200 μL of sample and eluted in 100 μL buffer using a MagNa Pure 96 instrument (Roche Diagnostics). Ten microliters extract was added to the same Sarbeco specific RT-PCR assay targeting the E-gene and EAV detection as described for the geneLEAD VIII workflow, but performed on a CFX96 PCR instrument (Bio-Rad): 50 °C for 5 min, followed by 95 °C for 20 s and then 45 cycles of 95 °C for 3 s, 55 °C for 10 s, and 60 °C for 30 s.

Table 4
SARS-CoV-2 detection using the geneLEAD VIII SARS-CoV-2 workflow.

Sample type	Result	geneLEAD VIII workflow
Throat/nasopharyngeal swab (n = 746)	Positive*	87
	Negative	659
Sputum (n = 115)	Positive*	27
	Negative	88
Bronchoalveolar lavage fluid (n = 9)	Positive	1
	Negative	8
Conjunctivital swab (n = 1)	Positive	0
	Negative	1

* The viral load of three SARS-CoV-2 positive throat/nasopharyngeal swabs and three SARS-CoV-2 positive sputum samples was too high for an automatic C$_T$ calculation by the geneLEAD VIII software and required visual evaluation of the amplification signal for a final result.

One hundred thirty-four (134) samples initially tested using the routine diagnostic workflow showed 99.3 % agreement between the geneLEAD VIII workflow and the routine diagnostic workflow (Table 2). There was only one discrepant result, which can be explained by a low SARS-CoV-2 viral load in this sample, as it initially tested positive with a cycle threshold (C$_T$) value of 37.7. The C$_T$ values obtained using the geneLEAD VIII workflow were comparable to the C$_T$ values obtained using the routine diagnostic workflow, with a median C$_T$ value difference of 0.9 (± 1.1). However, the viral load of one SARS-CoV-2 positive sample was too high for an automatic C$_T$ value calculation by the geneLEAD VIII software. This resulted in an error and required visual evaluation of the amplification signal for a final result. Importantly, the addition of the EAV-based IC to this previously described E-gene Sarbeco specific RT-PCR protocol did not affect test performance, but improved test quality by allowing direct assessment of the efficiency of extraction and the absence of inhibitory substances.

The sensitivity and specificity of the geneLEAD VIII workflow was investigated using an EQA panel consisting of 10 samples that were distributed blinded by the National Institute for Public Health and the Environment (RIVM). As shown in Table 3, all but one of the EQA samples were correctly detected. The discrepant sample (i.e. LEQA1_CoV20−4) contained only 128 SARS-CoV-2 E-gene digital copies per milliliter (dc/mL), according to the RIVM. One sample containing SARS-CoV-1 tested positive using both the routine diagnostic workflow and the geneLEAD VIII workflow, as these workflows contain a Sarbeco specific oligo set. In addition, serial dilutions of a quantified SARS-CoV-2 standard (Qnostics) showed that both workflows were able to detect 50 SARS-CoV-2 dc/mL (data not shown). These results are in agreement with comprehensive validation studies using the same oligo sets (Corman et al., 2020; Nalla et al., 2020).

After verification, the geneLEAD VIII SARS-CoV-2 workflow was added to the diagnostic portfolio at the LUMC. As of March 2020, 871 clinical samples were tested and resulted in 115 positive results (Table 4). These samples represented a variety of sample types and no PCR inhibition effects were observed during the analysis. The only errors represented the inability to automatically calculate the C$_T$ value of positives with low C$_T$ values (<15) by the geneLEAD VIII software in 6/115 positive samples.

The clinical management of the SARS-CoV-2 pandemic (i.e. triage of

patients) is critically dependent on rapid molecular workflows (Fournier et al., 2020; Wolters et al., 2020). The geneLEAD VIII SARS-CoV-2 workflow was available shortly after the start of the pandemic and can be considered as relatively fast. Results can be provided within two hours with limited hands-on time. This workflow is an intermediate between the rapid, cartridge-based platforms and the medium/high throughput, random access and/or batchwise testing platforms that came available in late spring and summer 2020 (Wolters et al., 2020; Opota et al., 2020). However, a drawback of the geneLEAD VIII software is the inability to calculate C_T values for samples containing high template concentrations, which requires visual inspection of PCR results. Therefore, automated reporting to the hospital information system was not possible. Nevertheless, given the robust performance and the short time-to-result, the geneLEAD VIII SARS-CoV-2 workflow has been implemented in our laboratory with over 1,000 samples tested. A second geneLEAD VIII enabled a STAT-like service every hour for a maximum of eight samples.

Future validations of geneLEAD VIII non-SARS-CoV-2 workflows can be expected that will expand sample-to-answer capabilities in diagnostic laboratories. In particular, the open modus of this platform facilitates the continuation of LDTs to detect pathogens for which no commercial test exist yet. Altogether the geneLEAD VIII platform increases the diagnostic capacity for (rapid) SARS-CoV-2 testing and can be a valuable addition for microbiological laboratories to prepare for rapid detection of emerging infectious diseases.

Author statement

Conceived the study: SB, EW and EC. Wrote the manuscript: SB and BM. Performed the laboratory experiments: MB and CB. Supervised data collection and laboratory work: SB, MB, CB, EW and EC. Critical reviewed the manuscript: EW and EC. All authors read and approved the final manuscript.

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Declaration of Competing Interest

The authors report no declarations of interest.

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