Real-time detection of SARS-CoV-2 in clinical samples via ultrafast ligation-dependent RNA transcription amplification

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Supplementary Information

Table S1. Sequences of nucleic acids used in this work

Name		Sequence (5'-3')
Promoter probe		[a]AACACTATTAGCATAAGCAGTTGTGCCCTATAGTGAGTCGTATTAGGAAGG
Reporter probe		[b]TCGACCTAGCATATCCATGATATCTGTTAGTTTTTTTCCTGAAAGACCGCCGGACAAGTGACAGCTTGACAAATGTTAAA
Reporter probe (no terminator)		CCGCCGGACAAGTGACAGCTTGACAAATGTTAAA
T7 promoter	20-nt	TAATACGACTCACTATAGGG
	22-nt	CCTAATACGACTCACTATAGGG
	24-nt	TTCCTAATACGACTCACTATAGGG
	26-nt	CCTTCCTAATACGACTCACTATAGGG
Molecular beacon	MB1	FAM- CCGCCGGACAAGGAGGCTGGAGTCAACCGGCGG -DABCYL
	MB2	FAM-AGTATACCTGCAGTGACTCTTAAGATCCAGGTATACT-DABCYL
	MB3	FAM-ACCTTCTATAGCAAACACCATTGTCACACTCCACCTCAGCCTATAGAAGGT-DABCYL
	MB4	FAM-ACTATAGGTATACAACCTACTACCTCACCCTATAGT-DABCYL
qRT-PCR primer set ([c]LR898678.2)	F	GTGATGTAGAAAACCCTCACC
	R	AGCAAGAACAAGTGAGGC
qRT-LAMP primer set ([c]LR898678.2)	F3	TGCTTCAGTCAGCTGATG
	B3	TTAAATTGTCATCTTCGTCCTT
	FIP	TCAGTACTAGTGCCTGTGCCCACAATCGTTTTTAAACGGGT
	BIP	TCGTATACAGGGCTTTTGACATCTATCTTGGAAGCGACAACAA
	LF	CTGCACTTACACCGCAA
	LB	GTAGCTGGTTTTGCTAAATTCC
RNA fragment with SARS-CoV-2 *orf1ab* gene specific sequence ([c]OQ117226.1)		AUGCCACAACUGCUUAUGCUAAUAGUGUUUUUAACAUUUGUCAAGCUGUCACGGCC
RNA fragment with SARS-CoV *orf1ab* gene specific sequence ([c]MN312831.1)		[d]AUGC*U*ACAACUGCUUAUGCUAAUAGUGU*C*UUUAACAUUUGUCAAGCUGU*U*AC*A*GCC

[a] Sequences marked in red and blue were the sequence complementary to the part of the target RNA and T7 promoter binding site, respectively.

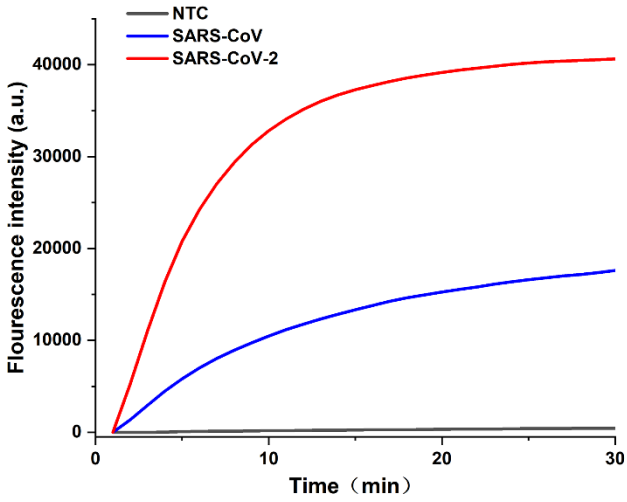
[b] Sequences marked in orange, green, and red were the VSV terminator, the sequence consistent with the loop part of the MB, and the sequence complementary to the part of the target RNA, respectively.

[c] GenBank accession number.

[d] The portion in italic type represented the nucleotides variation compared to the RNA fragment with SARS-CoV-2 *orf1ab* gene specific sequence.

Procedure of qRT-PCR and qRT-LAMP

The reaction system of qRT-PCR with a total volume of 20 μL consists of 2 μL sample, 0.4 μL dNTPs (10 mM each), 0.8 μL forward and backward primer (10 μM each), 0.3 μL *Taq* DNA polymerase (5 U/μL), 1.3 μL 10× *Taq* buffer, 0.5 μL EvaGreen Dye, 0.2 μL HiScript II Reverse Transcriptase (200 U/μL), 1.3 μL 5× HiScript II Buffer, 0.2 μL Murine RNase inhibitor (40 U/μL), 0.08 $MgSO_4$ solution (100 mM) and 12.12 μL DEPC-treated water. The reaction procedure included reverse transcription at 55℃ for 5 min, denaturation at 95℃ for 3 min, and 40 cycles consisting of 95℃ for 10 s, and 58℃ for 15 s for amplification. qRT-LAMP was performed by adding 2.5 μL sample, 4 μL dNTPs (10 mM each), 1 μL F3 and B3 (10 μM each), 0.8 μL FIP and BIP (100 μM each), 0.4 μL LF and LB (100 μM each), 0.5 μL *Bst* 2.0 WarmStart DNA polymerase (8000 U/mL), 2.5 μL 10× ThermoPol reaction buffer, 0.5 μL $MgSO_4$ solution (100 mM), 0.625 μL EvaGreen Dye, 0.3 μL HiScript II Reverse Transcriptase (200 U/μL) and 11.875 μL DEPC-treated water into a total volume of 25 μL at 65℃ for 45 min. Both qRT-PCR and qRT-LAMP assays were carried out by a CFX Connect™ Real-Time PCR System (Bio-Rad, CA, USA). The fluorescence signal of the qRT-PCR was read after each thermal cycling, while that of the qRT-LAMP was monitored at 1-min intervals.



SARS-CoV-2 AUGCCACAACUGCUUAUGCUAAUAGUGU*UU*UUAACAUUUGUCAAGCUGUCACGGCC
SARS-CoV AUGCUACAACUGCUUAUGCUAAUAGUGU*C*UUUAACAUUUGUCAAGCUGUUACAGCC

Fig.S1. Capability of SCAN to distinguish RNA with similar sequence to the target. The concentration of the RNA fragments was 1 μM. The portion in italic type represented the ligation site of the two probes (between the two nucleotides), while the variation nucleotides of the RNA fragment with SARS-CoV *orf1ab* gene specific sequence were marked in red. NTC represented no template control.