Biotin labelled DNA-probe based detection of nucleic acid in reverse transcription-LAMP amplified oropharyngeal viral swab samples via a lateral flow assay

Saloni Agarwal [1], Mojdeh Hamidizadeh [1], Frank F. Bier* [1,2]
*frank.bier@uni-potsdam.de

1. Institute for Biochemistry and Biology, Chair of Molecular Bioanalysis and Bioelectronics, University of Potsdam, Karl-Liebknecht-Strasse 24/25, 14476 Golm, Potsdam, Germany
2. Institute for Molecular Diagnostics and Bioanalysis-IMDB gGmbH, Am Mühlenberg 10,14476 Golm, Potsdam, Germany

Supplementary Material

S.1 LAMP primers:

LAMP primers and PCR primers for N-gene 466 bp amplification was designed using Primer Explorer.

Primers	Sequences (5' -> 3')	Length (nt)
F3	CCCAAAATCAGCGAAATGCA	20
B3	AGCCAATTTGGTCATCTGGA	20
FIP	TGTTTTGATCGCGCCCCACTGATTACGTTTGGTGGACCCTC	41
BIP	TGCGTCTTGGTTCACCGCTCATTGGAACGCCTTGTCCTC	39
FITC-LF	**FITC—**TCCATTCTGGTTACTGCCAGTTGAA	25
LB	ACTCAACATGGCAAGGAAGACCTTA	25
Fwd_PCR	GACCCCAAAATCAGCGAAAT	20
Rev_PCR	TGTAGCACGATTGCAGCATTG	20

Table S1: LAMP/RT-LAMP and PCR primer details.

S.2 RT-LAMP for SARS-CoV-2 negative and positive samples. Positive swab samples with a CT value range: 18.55 to 31.8 were used. RT-LAMP was performed for 20 min and the results were visualised both on 1.5% agarose gel and LFA.

S.No.	CT-value
j	31.8
k	30.11
l	28.55
m	27.7
n	26.57
o	25.4
p	23.09
q	21.68
r	20.54
s	18.55

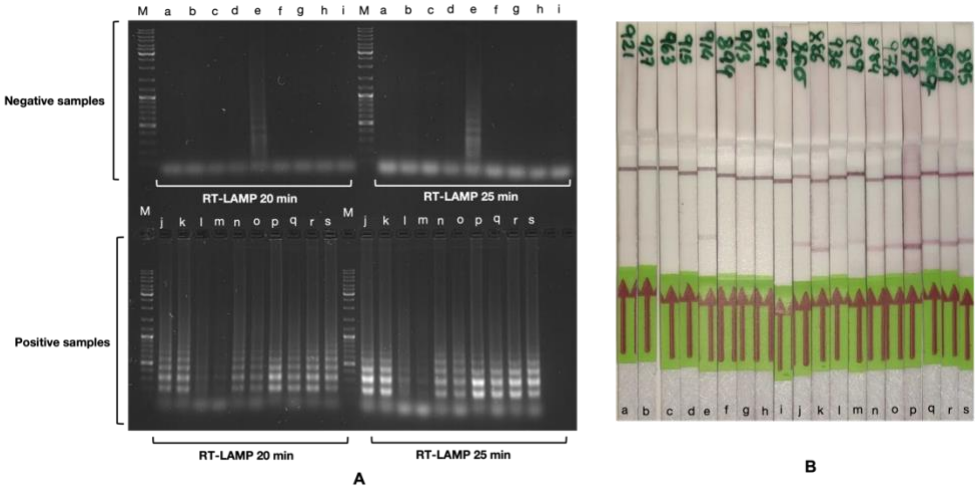

Figure S1: The table shows the CT-values of the 10 positive swab samples used. The optimisation of RT-LAMP-LFA annealing time for 20 and 25 min with 10 negative and 10 positive SARS-CoV-2 swab samples was performed. **A)** The gel shows 10 negative samples (upper half) subjected to RT-LAMP for 20 min and 25 min annealing time. One of the negative samples (e) show amplification which could be a false negative obtained from qRT-PCR. 10 Positive samples (lower half) were also subjected to RT-LAMP for 20 min and 25 min annealing time. A characteristic concatemeric pattern was observed for RT-LAMP 20 min annealing time. The RT-LAMP reaction shows brighter bands for 25 min, but 20 min was chosen as the optimal time for swab sample amplification. **B)** The result for RT-LAMP was readout via LFA using Biotin-DNA probes (see section 4.5). The LFA results were coherent with the results observed on the gel.