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# FQ-LAMP Assay for Detection of CoV2 in Clinical Nasal Swabs

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COVID-19 is a public health challenge requiring rapid testing for detecting infections and transmission. Nucleic acid amplification tests (NAAT) targeting SARS-CoV-2 (CoV2) are used to detect CoV2 in clinical samples.Real-time reverse transcription-quantitative PCR (RT-qPCR) is the standard NAAT for CoV2, although Reverse Transcription Loop-mediated isothermal amplification (RT-LAMP) is used in diagnostics. We show a sequence-specific RT-LAMP-based NAAT assay that is finished within 30 min using minimally processed clinical nasal swab samples and describe a fluorescent-quenched RT-LAMP assay (FQ-LAMP) using labeled primers and a quencher oligo. This assay can achieve rapid (30 min) and sensitive (1000 PFU/ml) fluorescent detection of CoV2 (WA1/2020), B.1.1.7 (Alpha), and variants of concern Delta (B.1.617.2) and Omicron (B.1.1.529) in nasal samples.

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FQ-LAMP, RT-LAMP, fluorescence, SARS-CoV-2, diagnostics, variant, VOC

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1. WarmStart LAMP Kit (DNA & RNA) New England Biolabs (E1700)

2. Oligo Primers and detection from IDT

| | |
|---|---|
| COVID-F3 | TGGCTACTACCGAAGAGCT |
| COVID-B3 | TGCAGCATTGTTAGCAGGAT |
| COVID-LoopF | GCCATTTTACTTTCTAGAGTCAGGT |
| COVID-FLB | **[6FAM]**ACTGAGGGAGCCTTGAATAC |
| COVID-FIP (F1c) | GACGAATTCGTGGTGGTGA |
| COVID-FIP (F2) | TCTGGCCCAGTTCCTAGGTAGT |
| COVID-BIP (B1c) | CGGGTGCCAATGTGATCT |
| COVID-BIP (B2) | AGACGGCATCATATGGGTTGCA |
| COVID-QLB | GCTCCCTCAGT**[IBHQ]** |

Primers 5' - 3' from IDT

3. Molecular grade deionized water
4. Proteinase K Molecular Grade New England Biolabs (P8107S)
5. Guanidine-HCL Sigma
6. EDTA Sigma
7. Triton X-100 detergent Sigma
8. Tris-HCL pH 7.5 Sigma

## FQ-LAMP Sample Preparation

1 mix 20ul of clinical swab material with 20 ul of SPS buffer containing Proteinase K.

2 Incubate at 37C for 15 minutes, then 95C for 5 minutes

3 Sample is ready to use in FQ-LAMP at 2ul / 25ul reaction

## Prepare FQ-LAMP Master Mix

4 Determine the number of 25ul FQ-LAMP reactions needed and add 10%



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5 Mix the following in clean tube per 25ul FQ-LAMP reaction

5.1 2X WarmStart LAMP reagent 12.5ul
10X Detection Oligo/Primer Mix 2.5ul
deionized water 8ul

6 Dispense 23ul of FQ-LAMP master mix to each required wells of a 96 well PCR plate

7 Add 2ul of prepared sample in SPS buffer per assay

8 Centrifuge the plate to settle all contents and seal the plate with appropriate cover material.

## Run FQ-LAMP assay on real time PCR machine

9 Set up the real time machine to measure fluorescence in the FAM channel.

10 Set up the real time machine amplification program:
HOLD 65C / 30 minutes
HOLD 25C / 30 seconds
measure fluorescence

11 Record results



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