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

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**We use this protocol and it's working**

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## Abstract

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.

## Materials

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

2. Oligo Primers and detection from IDT

|  |                    |                                        |
|--|--------------------|----------------------------------------|
|  |                    |                                        |
|  | COVID-F3           | TGGCTACTACCGAAGA<br>GCT                |
|  | COVID-B3           | TGCAGCATTGTTAGCA<br>GGAT               |
|  | COVID-LoopF        | GCCATTTTACTTTCTA<br>GAGTCAGGT          |
|  | COVID-FLB          | <b>[6FAM]</b> ACTGAGGGAG<br>CCTTGAATAC |
|  | COVID-FIP<br>(F1c) | GACGAATTCGTGGTG<br>GTGA                |
|  | COVID-FIP<br>(F2)  | TCTGGCCCAGTTCCTA<br>GGTAGT             |
|  | COVID-BIP<br>(B1c) | CGGGTGCCAATGTGAT<br>CT                 |
|  | COVID-BIP<br>(B2)  | AGACGGCATCATATGG<br>GTTGCA             |
|  | COVID-QLB          | GCTCCCTCAGT <b>[IBHQ]</b>              |

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%
- 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