

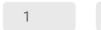


Apr 08, 2022

Les Jones¹, Hemant K. Naikare², Yung-Yi C. Mosley², and Ralph A. Tripp¹

¹Department of Infectious Disease, College of Veterinary Medicine, University of Georgia;

²Tifton Veterinary Diagnostic and Investigational Laboratory, University of Georgia



dx.doi.org/10.17504/protocols.io.14egn74bpv5d/v1

University of Georgia

Les Jones

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.

DOI

dx.doi.org/10.17504/protocols.io.14egn74bpv5d/v1

Les Jones, Hemant K. Naikare, Yung-Yi C. Mosley, and Ralph A. Tripp 2022. FQ-LAMP Assay for Detection of CoV2 in Clinical Nasal Swabs. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn74bpv5d/v1

B

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

protocol,

Apr 08, 2022

Apr 08, 2022



1

Citation: Les Jones, Hemant K. Naikare, Yung-Yi C. Mosley, and Ralph A. Tripp FQ-LAMP Assay for Detection of CoV2 in Clinical Nasal Swabs https://dx.doi.org/10.17504/protocols.io.14egn74bpv5d/v1

60503

- 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	GACGAATTCGTGGTGGTGA
(F1c)	
COVID-FIP	TCTGGCCCAGTTCCTAGGTAGT
(F2)	
COVID-BIP	CGGGTGCCAATGTGATCT
(B1c)	
COVID-BIP	AGACGGCATCATATGGGTTGCA
(B2)	
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%

protocols.io

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 Dispense 23ul of FQ-LAMP master mix to each required wells of a 96 well PCR plate Add 2ul of prepared sample in SPS buffer per assay Centrifuge the plate to settle all contents and seal the plate with appropriate cover material. Run FQ-LAMP assay on real time PCR machine 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