VaNGuard assay protocol for SARS-CoV-2 detection

Kean Hean Ooi
Mengying Mandy Liu
Jie Wen Douglas Tay
Seok Yee Teo
Pornchai Kaewsapsak
Shengyang Jin
Chun Kiat Lee
Jingwen Hou
Sebastian Maurer-Stroh
Weisi Lin
Benedict Yan
Gabriel Yan
Yong-Gui Gao
Meng How Tan (mh.tan@ntu.edu.sg)
https://orcid.org/0000-0003-3627-5586

Method Article

Keywords: CRISPR, COVID-19, diagnostics

Posted Date: May 26th, 2021

DOI: https://doi.org/10.21203/rs.3.pex-1373/v1

Abstract

This protocol presents the Variant Nucleotide Guard (VaNGuard) assay, which is robust towards viral mutations and can be performed on purified RNA or directly on nasopharyngeal (NP) swab samples. The procedure typically comprises three parts, namely sample preparation, RT-LAMP reaction, and Cas12a-based detection via fluorescence or lateral flow assay. Sample preparation from NP swabs involves Proteinase K digestion followed by heat inactivation. Purified RNA or digested NP swab samples are then added as templates into RT-LAMP reactions and incubated at 65ºC for 22 minutes. Next, enAsCas12a and ssDNA-probes are added and the reactions are incubated at 60ºC for another 5 minutes. End-point fluorescence can be detected by a plate reader or a real-time PCR machine. Alternatively, a lateral flow strip can be inserted into each reaction tube for equipment-free read-out. The VaNGuard assay is a rapid and convenient point-of-care test for SARS-CoV-2 and is applicable to resource poor settings.

Introduction

Reagents

· CRISPR RNA S2(+4DNA) rUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArUArCrUrCrCrUrGGrUrGrArUrUrCrUrUrCrUTC (Integrated DNA Technologies)

· CRISPR RNA S6(+4DNA) rUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArUArArArCrCrUrAGrUrGrArUrGrUrUrArArUAC (Integrated DNA Technologies)

· FAM-Biotin ssDNA probe: /56-FAM/TTATTATT/3Bio/ (Integrated DNA Technologies)

· Cy5-Quencher ssDNA probe: /5Cy5/TTATTATT/3IABKRQ/ (Integrated DNA Technologies)

· WarmStart® LAMP Kit (DNA & RNA) (NEB cat. no. E1700L)

· Tango Buffer (10X) (Thermo Scientific, cat. no. BY5)

· Q5® High-Fidelity DNA Polymerase (NEB, cat. no. M0491L)

· Proteinase K, Molecular Biology Grade (NEB, cat. no. P8107S)

· CoV2-S LAMP Primer F3: TTAATTTAGTGCGTGATCTCC (Integrated DNA Technologies)

· CoV2-S LAMP Primer B3: AGCATCTGTAATGGTTCCAT (Integrated DNA Technologies)

· CoV2-S LAMP Primer FIP(PM): TGTAAAGCAAGTAAAGTTTGAAACCCTCAGGGTTTTTCGGCT (Integrated DNA Technologies)

· CoV2-S LAMP Primer FIP(tPM-1): TGTAAAGCAAGTAAAGTTTGAAACCCTCAGGGTTTTTCGGC (Integrated DNA Technologies)

· CoV2-S LAMP Primer BIP(PM): TGGACAGCTGGTGCTAATAGAAAAGTCCTAGGTTGAAG (Integrated DNA Technologies)

· CoV2-S LAMP Primer BIP(tPM-1): TGGACAGCTGGTGCTAATAGAAAAGTCCTAGGTTGAA (Integrated DNA Technologies)

· CoV2-S LAMP Primer LB: GCAGCTTATTATGTGGGTTAT (Integrated DNA Technologies)

· CoV2-S LAMP Primer LF: GGCAAATCTACCAATGGTTCTAA (Integrated DNA Technologies)

· ACTB LAMP Primer F3: GGCATCCACGAAACTACCTT (Integrated DNA Technologies)

· ACTB LAMP Primer B3: GCCGATCCACACGGAGTAC (Integrated DNA Technologies)

· ACTB LAMP Primer FIP: TGCCGCCAGACAGCACTGTGTGAAGTGTGACGTGGACATC (Integrated DNA Technologies)

· ACTB LAMP Primer BIP: TTGCCGACAGGATGCAGAAGGGCGCTCAGGAGGAGCAAT (Integrated DNA Technologies)

· ACTB LAMP Primer LF: GGCGTACAGGTCTTTGCG (Integrated DNA Technologies)

· ACTB LAMP Primer LB: CCTGGCACCCAGCACAAT (Integrated DNA Technologies)

· Milenia® HybriDetect (Milenia Biotec, cat. no. MGHD 1)

Equipment

· CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories)

· SpectraMax® M5 Microplate Reader (Molecular Devices)

· SimpliAmp Thermal Cycler (Thermo Scientific)

Procedure

NP swab sample preparation.

1. 8.3µL of heat-inactivated NP swab samples was added with 1µL of Proteinase K
2. Vortexed for 1 minutes.
3. Heat inactivate Proteinase K at 95ºC for 5minutes.

4. 2µL of the treated sample is then used for RT-LAMP amplification.

RT-LAMP reaction (with ACTB internal control)

1. Prepare 10X SARS-CoV2 S primer mix with 22µL of water, 2µL for F3(100µM), 4µL for B3(100µM), 8µL for FIP(PM)(100µM), 8µL for BIP(PM)(100µM), 8µL for FIP(tPM-1)(100µM), 8µL for BIP(tPM-1)(100µM), 4µL for LF(100µM) and 4µL for LB(100µM), 16µL for swarm F1c(100µM) and 16µL for swarm B1c

2. Prepare 10X ACTB primer mix with 56µL of water, 2µL for F3(100µM), 2µL for B3(100µM), 16µL for FIP(100µM), 16µL for BIP(100µM), 4µL for LF(100µM) and 4µL for LB(100µM).

3. Dilute 3µL of Q5 Polymerase at 2U/µL (NEB) with 99µL of water to 0.06U/µL.

4. Setup RT-LAMP reaction in a 0.2mL PCR strip tube with 2.5µL of 10X SARS-CoV2 S primer mix, 1.25µL of 10X ACTB primer mix, 2.5µL of 0.4M Guanidine HCl, 2.5µL of Q5 Polymerase (0.06U/µL), 1.75µL of water, 0.5µL of LAMP dye and 12.5µL of WarmStart LAMP mastermix.

5. Pipette 2µL of Proteinase K-treated NP swab samples into the assembled reaction in Step 4.

6. Incubate final reaction at 65ºC for 22mins. Real time fluorescence can be measure via FAM channel (Ex:450/90, Em:515/30).

quasiOnePot Cas12a reaction (with ACTB internal control)

1. Pre-complex Cas12a RNP by mixing 0.75µL of S2(+4DNA)(5µM), 0.75µL of S6(+4DNA)(5µM) and 7.5µL of enAsCas12a(100ng/µL)

2. Incubate RNP at 37ºC for at least 15minutes.

3. Prepare RNP-probe mix with 32µL of water, 7.5µL of 10X Tango Buffer, 1.5µL of Cy5-Quencher reporter(10µM) and 9µL of complexed RNP.

4. Pipette 50µL of Cas12a-probe mix directly into RT-LAMP products.

5. Incubate reaction mix from Step 4 at 60ºC for 5 minutes. Fluorescence detection can be done at Ex: 640/Em: 670.

RT-LAMP reaction (Lateral Flow reaction)

1. Prepare 10X SARS-CoV2 S primer mix with 22µL of water, 2µL for F3(100µM), 4µL for B3(100µM), 8µL for FIP(PM)(100µM), 8µL for BIP(PM)(100µM), 8µL for FIP(tPM-1)(100µM), 8µL for BIP(tPM-1)(100µM), 4µL for LF(100µM) and 4µL for LB(100µM), 16µL for swarm F1c(100µM) and 16µL for swarm B1c

2. Dilute 3µL of Q5 Polymerase at 2U/µL (NEB) with 99µL of water to 0.06U/µL.

3. Setup RT-LAMP reaction in a 0.2mL PCR strip tube with 2.5µL of 10X SARS-CoV2 S primer mix, 2.5µL of 0.4M Guanidine HCl, 2.5µL of Q5 Polymerase (0.06U/µL), 3µL of water and 12.5µL of WarmStart LAMP mastermix.

4. Pipette 2µL of Proteinase K-treated NP swab samples into the assembled reaction in Step 4.

5. Incubate final reaction at 65ºC for 22mins.

quasiOnePot Cas12a reaction (Lateral flow readout)

1. Pre-complex Cas12a RNP by mixing 0.75µL of S2(+4DNA)(5µM), 0.75µL of S6(+4DNA)(5µM) and 7.5µL of enAsCas12a (100ng/µL).

2. Incubate RNP at 37ºC for 30minutes.

3. Prepare Cas12a-probe mix with 20µL of water, 7.5µL of 10X Tango Buffer, 13.5µL of FAM-Biotin reporter(500nM) and 9µL of complexed RNP.

4. Pipette 50µL of RNP-probe mix from Step 3 directly into RT-LAMP products.

5. Incubate reaction mix from Step 4 at 60ºC for 5 minutes.

6. Pipette 75µL of HybriDetect assay buffer (Milenia Biotec) into the reaction mix from Step 5.

7. Insert dipstick into reaction mix in an upright position.

8. Allow reaction to flow up the flow strip for 2 minutes before inspection.