

Rapid, reliable, and cheap point-of-care bulk testing for SARS-CoV-2 by combining hybridization capture with improved colorimetric LAMP (Cap-iLAMP)

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Method Article

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Abstract

Here we present a step-by-step protocol for Cap-iLAMP (capture and improved loop-mediated isothermal amplification) which combines a hybridization capture-based RNA extraction of gargle lavage samples with an improved colorimetric RT-LAMP assay and smartphone-based color scoring. Cap-iLAMP is compatible with point-of-care testing and enables the detection of SARS-CoV-2 positive samples in less than one hour. The sensitivity is 97% and the specificity is 99%. In contrast to direct addition of the sample to improved LAMP (iLAMP), Cap-iLAMP prevents false positives and allows single positive samples to be detected in pools of 25 negative samples.

Introduction

Reagents

Chemicals

Reagent Manufacturer Catalog #

WarmStart® Colorimetric LAMP 2X Master Mix New England Biolabs M1800L

ATP (100mM) Thermo Fisher Scientific R0441

Protector RNase Inhibitor (40U/μl) Roche 3335399001

Thermostable Inorganic Pyrophosphatase (2U/μl) New England Biolabs M0296L

Tte UvrD Helicase (20ng/ μl) New England Biolabs M1202S

SYTO-9 (5mM) Thermo Fisher Scientific S34854

SYBR green I (10000x) Thermo Fisher Scientific S7563

Dynabeads™ MyOne™ Streptavidin C1 Thermo Fisher Scientific 65001

Tris-HCL (1M, pH 7.5) Jena Bioscience BU-125L

LiCl Alfa Aesar 10515.3

Lithium dodecyl sulfatate VWR Chemicals 782-25G

Dithiothreitol Molekula Group 19733320

EDTA (0.5M, pH 8.0) VWR Chemicals E522-100ML

Tween-20 Thermo Fisher Scientific 85113

Sodium hydroxyde (1M) Sigma Aldrich S2770

Twist synthetic SARS-CoV-2 RNA control 2 Twist Bioscience 102024

Oligonucleotides (Sigma Aldrich, desalted)

ID Sequence (5' -> 3')

CV1 (F3) TCCAGATGAGGATGAAGAAGA

CV2 (B3) AGTCTGAACAACCTGGTGTAAG

CV3 (FIP) AGAGCAGCAGAAGTGGCACAGGTGATTGTGAAGAAGAAGAG

CV4 (BIP) TCAACCTGAAGAAGAGCAAGAACCTGATTGTCCTCACTGCC

CV5 (LF) CTCATATTGAGTTGATGGCTCA

CV6 (LB) ACAAACTGTTGGTCAACAAGAC

CV15 (F3) TGGCTACTACCGAAGAGCT

CV16 (B3) TGCAGCATTGTTAGCAGGAT

CV17 (FIP) TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG

CV18 (BIP) AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT

CV19 (LF) GGACTGAGATCTTTTATTTCACCGT

CV20 (LB) ACTGAGGGAGCCTTGAATACA

CV2_btn (capture oligonucleotide)

/BtnTg/TTAAATAACCACTAAAACCTATTCACTTCAATAGTCTGAACAACCTGGTGTAAG

CV16_btn (capture oligonucleotide)

/BtnTg/AATGTTGTTTCTTGAGGAAGTTGTAGCACGATTGCAGCATTGTTAGCAGGAT

Recipe Low Salt buffer

Reagent Amount Final concentration

Tris HCl (1M, pH 7.5) 4ml 20mM

LiCl 1.694g 200mM

EDTA (0.5M, pH 8.0) 0.4ml 1mM

Water to 200ml

Recipe Elution buffer

Reagent Amount Final concentration

Tris HCl (1M, pH 7.5) 4ml 20mM

EDTA (0.5M, pH 8.0) 0.4ml 1mM

Water to 200ml

Recipe Wash buffer

Reagent Amount Final concentration

Tris HCl (1M, pH 7.5) 4ml 20mM

LiCl 4.235g 500mM

EDTA (0.5M pH 8.0) 0.4ml 1mM

Water to 200ml

Recipe Lysis Binding Buffer (2x)

Reagent Amount Final concentration

Tris HCl (1M, pH 7.5) 40ml 200mM

LiCl 8.47g 500mM

EDTA (0.5M pH 8.0) 0.8ml 2mM

LiDS 2g 1% (w/V)

DTT 0.3085g 10mM

Water to 200ml

Recipe alkaline melt solution

Reagent Amount Final concentration

Tween 20 1 μ l 0.1% (v/v)

NaOH (1M) 125 μ l 125mM

Water to 1000 μ M

Recipe primer mix (10x)

Primer V/ μ l Final concentration (10x)

F3 (100 μ M) 8 2 μ M

B3 (100 μ M) 8 2 μ M

FIP (100 μ M) 64 16 μ M

BIP (100 μ M) 64 16 μ M

LF (100 μ M) 16 4 μ M

LB (100 μ M) 16 4 μ M

Water to 400 μ l

Recipe LAMP master mix

Reagent V/ μ l Final concentration

WarmStart LAMP MM (2x) 15 1x

ATP (100mM) 0.3 1mM

RNase In (40U/ μ l, Roche) 0.3 0.4U/ μ l

Thermostable PPase (2 U/ μ l) 0.75 0.05U/ μ l

Primer mix (10x) 3 1x Water 0.2 4

Tte UvrD helicase (20ng/μl) 0.15 1ng/μl

SYTO-9 (100μM) 0.3 1μM

Equipment

Thermo block (e.g. Thermomixer compact (Eppendorf))

Magnetic rack for 1.5ml tubes

Smartphone

Procedure

Bead preparation for capture

1. Mix an aliquot of 2ml Lysis Binding Buffer (LysBB, 2x concentrated) with 2ml of water to obtain 1x concentrated LysBB for bead washing
2. Transfer 20μl Dynabeads™ MyOne™ Streptavidin C1 per reaction to 1.5ml tubes (beads for up to 15 reactions can be prepared at once in a 1.5ml tube), place in a magnetic rack and discard the supernatant
3. Resuspend beads in 500μl 1x LysBB, place in a magnetic rack and discard supernatant, repeat this step once
4. Resuspend the washed beads in 100μl 1x LysBB per reaction and add of 2μl of CV2_btn (100 μM) and 2μl CV16_btn (100 μM) per reaction, rotate tubes for 10min at room temperature for binding of the oligonucleotides to the beads
5. Place tube on a magnetic rack, discard the supernatant, resuspend in 40μl alkaline melt solution per reaction
6. Incubate the suspension for 5min at room temperature, then place tube in a magnetic rack, discarded the supernatant
7. Resuspend beads in 40μl 1x LysBB per reaction, place in a magnetic rack and discard the supernatant
8. Resuspend the washed beads in 100μl 2x LysBB per reaction, distribute 100μl of suspension to single 1.5ml tubes if you prepared multiple reactions at once
9. Add 400μl 2x LysBB to each tube to obtain a volume of 500μl

Capture reaction

1. Add 500µl of sample (e.g. gargle lavage) or water (negative control) or 499.5µl of water and 0.5µl synthetic SARS-CoV-2 RNA control 2 (positive control) to 500µl bead suspension and mix by pipetting
2. Incubate at 55°C for 10min
3. Place the suspension on a magnetic rack, discard the supernatant
4. Resuspend the beads in 200µl wash buffer, place the suspension on a magnetic rack, discard the supernatant
5. Repeat this wash step once
6. Resuspend the beads in 200µl low salt buffer, place the suspension on a magnetic rack, discard the supernatant
7. Resuspend the beads in 25µl elution buffer, incubate at 60°C for 2min
8. Place the reaction on a magnetic rack, transfer the supernatant to a fresh tube, discard the beads

LAMP assay

1. To 20µl of LAMP master mix, add 10µl of eluate from the captures, mix by pipetting, add 0.5µl of SYBR green I to the cap of the tube without getting in contact with the reaction volume
2. Incubate the reactions for 40min at 65°C
3. To stop the reaction and record the outcome of the assay, shake the tube to mix the reaction volume with the SYBR green I in the cap
4. Photos can be taken with any available smartphone and hue values can be extracted from the images with freely available color picker apps. We used the 'Palette Cam' app (Alexander Mathers, App Store) to score red, green, and blue (RGB) values and extracted the hue. This can be easily done using a web tool (e.g. <https://www.rapidtables.com/convert/color/rgb-to-hsv.html>) or in Microsoft Excel ($=\text{ROUNDDOWN}(\text{IF}(180/\text{PI}()*\text{ATAN2}(2*R-G-B,\text{SQRT}(3)*(G-B))<0,180/\text{PI}()*\text{ATAN2}(2*R-G-B,\text{SQRT}(3)*(G-B))+360,180/\text{PI}()*\text{ATAN2}(2*R-G-B,\text{SQRT}(3)*(G-B))),0)$). Hue can also be scored directly when using e.g. 'Color Grab (color detection)' app for android or 'Aurora' app for iOS.