A lateral flow-based at-home test for detection of SARS-CoV-2 V.2

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ABSTRACT

This protocol was a finalist in the XPRIZE Global Rapid Covid Testing competition

We have developed a highly sensitive and specific LAMP(loop-mediated amplification)-based assay for the detection of SARS-CoV-2 that can potentially be used for at-home and point-of-care (POC) testing. The assay includes only three simple steps: (1) heat inactivate the sample mixed with lysis buffer and add LAMP reagent mix, (2) incubate the reaction on a heating device, and (3) read the result by inserting a lateral flow strip into the tube. Performance is comparable to gold-standard RT-PCR testing, as the assay is based on nucleic acid detection rather than antigen detection which is used by the majority of lateral flow strip-based assays. There is minimal pre-processing involved and no bulky instrumentation needed, and non-lab professionals can follow the instructions and understand the results easily. The assay targets the SARS-CoV-2 nucleoprotein (N) gene and is capable of detecting as low as 4.0 copies per reaction (0.5 copies/µL) of SARS-CoV-2 RNA, demonstrating no cross-reactivity with a panel of 20 other respiratory pathogens. Furthermore, the test can be run on multiple sample types, including nasopharyngeal and/or oropharyngeal swab and saliva samples. Overall, this assay provides a rapid (sample-to-answer turnaround time of ~40 min), accurate, and affordable solution to the challenge of at-home diagnostics for SARS-CoV-2.

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GUIDELINES

This protocol is used for laboratory validation.

MATERIALS TEXT

ITEM	SUPPLIER	CATALOG#	STORAGE
DEPC-treated nuclease-free water	Thermo-Fisher	750023	Room Temp
QuickExtract™(QE) Solution	Lucigen	QE09050	-20˚C
Dithiothreitol (DTT), 1M	Thermo-Fisher	P2325	-20˚C
LAMP Reagent Mix*	UCSF	-	-20˚C
Lateral flow strip/dipstick	TwistDx	MILENIA01	2-8˚C
PCR tubes, 0.2 mL	Thermo-Fisher	AB0620	Room Temp
Pipette and barrier tips	Rainin or equivalent	-	Room Temp
Vortex/Mixer	Vortex Genie or equivalent	-	Room Temp
Mini Centrifuges	Thermo-Fisher or equivalent	-	Room Temp
Dry heating bath	VWR or equivalent	-	Room Temp

*includes SARS-CoV-2 nucleoprotein (N) gene primer mix and LAMP mastermix prepared at UCSF using reagents sourced from IDT Technologies and New England Biolabs (NEB)

NOTE: Reagents should not be used beyond their expiration dates.

SPECIMEN REQUIREMENTS

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1. Nasopharyngeal (NP) or nasopharyngeal/oropharyngeal (NP/OP) swab samples are collected with flocked swabs in universal transport media (UTM) or viral transport media (VTM).
    a. Specimens may be stored at 4˚C for up to 72 hours post-collection.
    b. Specimens should be stored at -80˚C if not processed within 72 hours of collection.
    c. Specimens should not undergo >3 freeze-thaw cycles.

2. Saliva samples are collected in sterile containers without any media. A minimum of 1 mL saliva is collected by pooling saliva in the mouth and gently expelling it into the collection tube (e.g. 50 mL falcon tube). Thirty (30) minutes prior to saliva collection, avoid brushing teeth, using mouthwash, smoking, or eating or drinking anything except water.
    a. Saliva may be kept at room temperature for 3-5 days. For longer storage, store the samples at -80˚C.
    b. Prior to processing, add 10µL of 1M dithiothreitol (DTT) per 1 mL of saliva to minimize viscosity.

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2

QUALITY CONTROL

3

1. Positive control (PC) – pooled positive samples aliquoted into single-use tubes with 80µL/tube and stored in -80˚C until needed (prepared at UCSF).
a. Multiple freeze/thaw cycles should be avoided. Maintain on ice when thawed.
2. Negative template control (NTC) – nuclease-free water aliquoted into single-use tubes with 80µL/tube
a. Stored at room temperature. In-use vials are stored at 2-8°C.

PROCEDURE

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1. Preparation of pre-amplification workspace prior to use:
a. Turn on the ultraviolet (UV) light of the biosafety cabinet (BSC) for 15 minutes.
b. Wipe all pipets and surfaces of the BSC with 10% bleach followed by 70% ethanol.
2. Take the LAMP Reagent Mix and QE Solution out of the -20°C freezer. Place in a rack at room temperature and allow to thaw completely. Place on ice as soon as the reagents are thawed. Vortex and briefly spin down to remove bubbles.
Note: Thawed reagents can be used within 24 hours. Maintain on ice or refrigerate when thawed!
3. Remove the patient samples and positive controls from the -80°C freezer and allow to thaw. Place on ice as soon as they are thawed. If the sample is freshly collected, keep refrigerated or on ice until ready to test. Thoroughly mix the samples and controls by vortexing.
4. Preheat a dry bath or a PCR thermal cycler at 95˚C for sample inactivation.
5. In a clean PCR tube, add 20ul QE solution to 80µL of patient sample or control (or a 1:4 ratio) . Mix well by pipetting up and down 10 times and do a quick spin.
6. Heat the tube from step 5 using a dry bath (or a thermal cycler) at 95˚C for 3 minutes (Note: longer incubation may degrade sample RNA). Cool on ice immediately.
7. In a new PCR tube or 96-well plate, add 42µL of the LAMP Reagent Mix from step 2 into each tube or well.Then add 8µL of the test or control sample from step 6. Mix well by pipetting up and down 10 times. Cap the tubes or seal the plate and briefly spin down to remove bubbles. The assay can be scaled according to the number of samples.
8. Incubate the PCR tubes or plate from step 7 at 65°C on a heating block or PCR thermal cycler for 40 minutes, and cool on ice.
9. Take the lateral flow dipstick out of the refrigerator and bring to room temperature before use. Protect dipsticks from humidity and close the container when not in use. Take the required number of dipsticks out of the container and mark them. Use new gloves when removing dipstick(s) from the container. Touch and label only the foil-covered areas.
10. After a 40-min incubation, bring the tubes or plate from step 8 to a post-amplification workspace/hood. Open the tubes or adhesive seal carefully to prevent splashing or spilling of the amplified product.
NOTE: It is important to separate pre-amplification and post-amplification workspaces/rooms to prevent cross-contamination. One-way directional workflow is critical to minimize carrying amplicons to less contaminated and amplicon-free (pre-amp room) zones. Operators should don and doff separate disposable lab coats when entering and exiting the pre-amp or post-amp rooms.

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11. Insert the lateral flow strip vertically with the arrow pointing up into each tube or well, making sure that the sample application spot is submerged in the assay solution. Wait 3 minutes for the bands to appear.

12. Record and interpret the results immediately using the following criteria:

LATERAL FLOW STRIP RESULT	INTERPRETATION
Bands present in Control (top) and Test (bottom) lines	Positive
Band present in Test (bottom) line only	Positive
Band present in Control (top) line only	Negative
No bands in Control (top) and Test (bottom) lines	Invalid*

*If the result is invalid, the test needs to be repeated.

CONTROL EVALUATION

5 1. NTC should be negative and display no band on the Test line.
a. If a false positive occurs, contamination may have occurred. The run is invalid. Repeat the assay after thorough cleaning of workspaces and using a new batch of reagents.
2. PC reaction should produce a positive result and bands should be present on the Test and/or Control lines.
a. If the PC does not exhibit positivity, the run is invalid. A failure in PC may be due to the following: PC sample may be degraded, the wrong reagent was used, the assay was run incorrectly, or the lateral flow strip is defective. The assay needs to be repeated. If a repeat run also tests invalid, the result is reported as “Invalid, collect another sample for testing”.

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