ABSTRACT
This is a fast (one hour), very simple LAMP-based Covid-19 diagnostic test using readily-available components that is appropriate for point-of-care use and also can be easily scaled up on a pipetting robot such as an Opentrons OT2 because the protocol requires no centrifugation.

It leverages a commercially-available swab kit to collect a sample from the mouth of the patient in a safe manner. Viruses are inactivated by the buffer in the swab kit so risk of exposure is minimal. The sample can be safely handled once the swab is placed back in the collection tube. The collection kit has an EUA from FDA.

A commercially-available reagent kit called prepIT.Q2A is used to prepare an aliquot of the sample for LAMP analysis. The two kit reagents are added to 100uL of the sample, a precipitate forms and falls to the tube bottom with no centrifugation necessary, and the RNA is pipetted off the top of the liquid in the tube.

The RNA solution is diluted 1:10 with water and analyzed by adding 1uL to a preloaded PCR tube with primers and the NEB WarmStart Colorimetric LAMP reagent. Incubation at 68C for 30min produces a color change visible to the eye for a simple yes/no readout.

The whole process takes an hour from start to finish.

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Covid-19, diagnostic, fast, isothermal amplification, LAMP

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MATERIALS TEXT

MATERIALS

- **primers** Contributed by users
- **Water, nuclease-free** Thermo
- Aldrich Catalog #0581 Step 1
- **Sodium Hydroxide 1N** Sigma
- Aldrich Catalog #S2770-100ml Step 1
- **Guanidine hydrochloride** Sigma
- Aldrich Catalog #G3272-1KG Step 1
- **Sodium hydroxide** Sigma
- Aldrich Catalog #306576
- **prepIT.Q2A kit Solution AG** DNA
- Genotek Catalog #PT-Q2A-384 AG Step 3
- **prepIT.Q2A kit Solution ST** DNA
- Genotek Catalog #PT-Q2A-384 ST Step 3
- **OR-100 OraCollectRNA kit** DNA
- Genotek Catalog #OR-100 Step 2
- **WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA)** New England Biolabs Catalog #M1800L Step 1

STEP MATERIALS

- **Sodium Hydroxide 1N** Sigma
- Aldrich Catalog #S2770-100ml Step 2
- **OR-100 OraCollectRNA kit** DNA
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- **prepIT.Q2A kit Solution ST** DNA
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- **prepIT.Q2A kit Solution AG** DNA
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SAFETY WARNINGS
Use lab coat, gloves and safety glasses when working in the lab.

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BEFORE STARTING
Use PPE appropriate to your lab and the population you are swabbing.

OPTIONAL pre-preparation of Master Mix

1

LAMP Master Mix preparation (OPTIONAL, supplied premixed in our kit)
NOTE: Kits will have LAMP Master Mix already prepared with guanidine and primers in it in PCR tubes. We are leveraging the work of scientists at New England Biolabs and include the preparation because of our shared commitment to open science. This is an open source protocol and anyone can buy the materials and make it themselves.

Please wear proper lab gloves and safety glasses when preparing this. The primers, water and NEB WarmStart Colormetric Master Mix do not contain any harsh chemicals but take appropriate care in making the 1M guanidine HCl, especially if you need to adjust the pH with sodium hydroxide.

Here is what you need—these things are common labware and can be purchased from a variety of vendors without affecting the quality of the Master Mix, so equivalents can be used:

4-way tube rack
Tube rack capable of holding 15mL tubes
USA Scientific 2304-8044

0.2 ML PCR PULL-APART 8-TUBE STRIPS, ATTACHED INDIVIDUAL DOME CAPS
0.2mL PCR tube strips
TempAssure 1402-2900

Floating Test Tube Rack
PCR tube floating foam rack
Jaece 1201U03

Disposable polystyrene weighing dishes
Weigh boat 40mm x 40 mm
Sigma Z154873
Set of micropipettes with rack: 100-1000 µl, 20-200 µl, 2-20 µl, and 0.5-10 µl
Pipettor set
Pipetman QP-1001-07
Can use equivalent Pipettors

80-place tube rack
1.5mL microfuge tube rack
USA Scientific 2380-1008
Rack that holds 1.5mL microfuge tubes

TipOne Pipette tips in racks, yellow 200uL
200uL Pipette tips in racks
TipOne 1111-0816

TipOne Pipette tips in racks, natural 10uL
Pipette tips in racks, natural, 10uL
TipOne 1111-3810
Guanidine hydrochloride Sigma
Aldrich Catalog #G3272-1KG

WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA) New England
Biolabs Catalog #M1800L

TipOne Pipette tips in racks, blue graduated 1000uL
Pipette tips in racks, 1000uL
TipOne 1111-2831

1.5mL microcentrifuge tube, sterile
1.5mL sterile microfuge tube
Seal-Rite 1615-5510

Adventurer™ Analytical Balances
Analytical balance
Ohaus 30100600

Cellstar 15 mL polypropylene conical screw cap centrifuge tube
Sterile conical screw cap centrifuge tube, 15 mL
GREINER BIO-ONE 5618-8271
1.1 **Purchase LAMP primer sets from IDT or other oligo synthesis company.**

You will need three sets of primers. Two for detecting Covid-19 and one as a control to show that you have successfully extracted RNA from human samples. The primers can be changed to reflect the latest consensus of scientific opinion on the best primers to use. In this iteration we will use actinR for control primers and the N2/E1 set of primers developed by New England Biolabs.

**LAMP primer sets have six primers each, and they are designated BIP, FIP, F3, B3, LoopF (LF) and LoopB (LB).**

The primer sequences (read 5prime to 3prime) are as follows:

For N2 Primer mix:

- N2-F3: ACCAGGAACATATCGACAG
- N2-B3: GACCTGACTTGTGAAATTTGGATCT
- N2-FIP: TTCCGAAGAACGCTGAAGCGGAACTGATTACAAACATTGGCC
- N2-BIP: CGCATTGGCATGGAAGTCACAATTTGATGGCACCTGTGTA
- N2-LF: GGGGCAAATTGTGCAATTTG
- N2-LB: CTTCGGGAACGTTGGTGACC

For E1 Primer Mix:

- E1-F3: TGAGTACGAACTTATGTACTCAT
- E1-B3: TTCAGATTTTTAACACGAGAGT
- E1-FIP: ACCACGAAAGCAAGAAAAAGAAGTTCGTTTCGGAAGAGACAG
- E1-BIP: TTGCTAGTTACACTAGCCATCCTTAGTATAAGACTCACGT
- E1-LB: GCGCTTCGATTGTGTGCGT
- E1-LF: CGCTATTAACTATTACAG

For actinR Primer Mix:

- ACTB-F3: AGTACCCCCATCGAGACACC
- ACTB-B3: AGCCTGAGTAGCAAGCTAC
- ACTB-FIP: GAGCCACCAAGCAAGCTAACGAGCTACGT
- ACTB-BIP: CTGACCACCAAGGCGTAGGTGGTGAGGCTAC
- ACTB-LF: TGGGTGCCAGATTTCTCCA
- ACTB-LB: CGAGAGATGACCCAGATCATGT

1.2 **Resuspend Primers.**

When primers arrive they will be in plastic screw-cap vials in a dry form. Set the vials in the tube rack and for each vial use the P-200 or P-1000 pipettor and appropriate tips add nuclease-free water to achieve a final primer concentration of \[100 \text{ Micromolar (µM)}\]. A rule of thumb is to add ten times the number of microliters as there are nanomoles of primer in the vial. For example, if the vial label says it contains 20.3 nanomoles of primer DNA, add 203 microliters of water.
1.3 Make 10x Primer Mix.

You will need one 1.5mL microfuge tube for each of the 3 sets of primers. Make separate 10x mixes for each by placing the tubes in the tube rack and using the P-10, P-20 and P-200 pipettors and appropriate tips to Make separate 10X Primer Mixes for each primer set.

To make **100 µl 10x Primer Mix** you must add

- 16 µl BIP
- 16 µl FIP
- 2 µl F3
- 2 µl B3
- 8 µl LoopF
- 8 µl LoopB
- 48 µl nuclease free water

Note that the amount can be scaled up by multiplying all component volumes by the same value (e.g. ten times each component will give you one milliliter final volume of 10x Primer Mix). Store frozen at **-20 °C** until ready to use.

1.4 Prepare 1M Guanidine HCl

To make **1 Molarity (M) Guanidine HCl**, use the weigh boat and analytical balance to weigh out **955 mg Guanidine HCl**. Place in a 15mL conical tube in rack. Using the 10mL serological pipette and pipette controller (NEVER pipette by mouth) add **10 mL nuclease free water** to the tube, cap and invert several times to dissolve the guanidine. Adjust pH to around 7 using sodium hydroxide and test strips if needed.
1.5 Prepare and Aliquot the LAMP Master Mix

Place a 1.5mL tube in the 1.5mL tube rack and using the pipettors add the following for 1mL Master Mix with Covid Primers:

- 500 µl NEB 2x Colorimetric LAMP Master Mix
- 100 µl N2 Primer Mix
- 100 µl E1 primer mix
- 100 µl 1M Guanidine HCl
- 50 µl nuclease free water

Place PCR tubes in rack. Using the p-20 pipettor and tips, dispense the Master Mix into 0.2mL PCR tubes in the amount of 19 µl per tube.

Store frozen at -20 °C until use.
Using the DNA Genotek OR-100 (aka ORE-100) swab kit for RNA collection

In developing the simplest kit, we decided to take advantage of the fact that DNA Genotek has already gotten an EUA approval for these kits, and they have already tested and confirmed that viruses are inactivated and the RNA preserved for analysis when the swab is submerged in the kit buffer. Thus they are safe and effective.

Additionally, we chose swabs rather than liquid saliva collection because in talking with nursing homes many of their residents cannot produce much saliva. Furthermore physicians told us that it is necessary to test patients who are on ventilators and unconscious and in these cases saliva collection is impossible but swabbing is doable.

OR-100 OraCollectRNA kit DNA
Genotek Catalog # OR-100

2.1 Open the individually-wrapped swab kit, taking care not to touch the swab itself. Note that when swabbing someone other than oneself, appropriate PPE such as gloves, face shields, medical coveralls and N95 masks may be necessary for safety.

2.2 Swab towards the back of the throat for about ten seconds. Note that the swab remains attached to the top of the tube during this time.

2.3 Carefully unscrew the cap and reverse it, sealing the swab inside. Do not touch the swab during this procedure. Make sure the cap is securely tightened.

2.4 Shake the tube vigorously for five seconds.

2.5 Store at Room temperature until ready to assay.

Test Protocol: Sample Assay 19m

Assaying the sample

In developing this assay we took into account multiple considerations including speed, ease-of-use and minimizing expensive lab equipment. We tried literally a dozen different methods that addressed how to go from a human sample into the pH-dependent colorimetric LAMP assay that can be read with no special equipment. In the end it was a conversation between us and our DNA Genotek rep that sparked the idea of trying a reagent that they sell. It is sold and normally used for DNA extraction and nobody had ever tried it for viral RNA. We obtained samples of it and it worked beautifully. The best part is it is not only fast but requires no centrifugation. This opens up great possibilities for scale-up because the whole procedure can be done on a lab robot with no need to remove tubes and spin them.

You will need:

Our kit, which will include:

2 PCR tubes prefilled with LAMP MAster Mix + guanidine and primers, one with Covid primers N2/E1 and one with actinR control primers
Tube containing 270 µl nuclease-free molecular biology grade water

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A way to heat the tubes to 68 °C for 00:30:00
This could be anything that achieves this- a heat block, a water bath etc. We used a cheap sous vide cooker set we got (sold on Amazon, Target and BestBuy). Buy the heater and lid separate and set them on top of any thing of appropriate size that holds water and can take 68C temperatures.

on top of an ordinary kitchen pot, total cost less than $150.

Other stuff you will need will be:

Set of micropipettes with rack: 100-1000 µl, 20-200 µl, 2-20 µl, and 0.5-10 µl
Can use equivalent Pipettors

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10uL Filter tips
Pipette tips in racks, natural, 10uL
TipOne  1121-2710

20uL filter tips
Pipette tips in racks, 20uL
TipOne  1123-1710

200uL filter tips
Pipette tips in racks, 200uL
TipOne  1120-8710

80 place 1.5 mL microfuge tube rack
Tube rack
BestRack  2380-1008

Compact polypropylene PCR tube rack
PCR tube rack
USA Scientific  2396-5048

3.1  Turn on sous vide heater and set to \( 68 \, ^{\circ}C \).  1m

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Place a 1.5mL microfuge tube in the tube rack. Using the P-200 pipettor and filter tips, transfer 100µL of the liquid from the OR-100 kit used for the sample into the microfuge tube.

3.2 Using the P-20 pipettor and tips, add \(10 \mu\text{l}\) prepIT.Q2A Solution AG

3.3 Using the P-20 pipettor and tips, add \(20 \mu\text{l}\) prepIT.Q2A Solution ST

3.4 Pipette up and down 12 times to mix.

3.5 Incubate tube \(\text{Room temperature}\) for \(00:15:00\). A precipitate will form and settle to the tube bottom, leaving a clear aqueous layer on top.

3.6 Using the P-200 pipettor and tips, transfer \(30 \mu\text{l}\) from the top of the aqueous phase to the tube with 270 µl nuclease-free water supplied in the kit. This is your working dilution.

3.7 Thaw the two tubes from the kit containing LAMP Master Mix, one with N2/E1 primers and one with the control actinR primers. Using the P10 pipettor and tips, add \(1 \mu\text{l}\) of the working dilution from step 3.6 to each PCR tube.

3.8 Place the PCR tubes in a floating rack in the \(68 \, ^\circ\text{C}\) sous vide waterbath and incubate for \(00:30:00\).

**Assay Readout**

4 The tubes can be read by eye. Remove the tubes from the waterbath and observe the color. Yellow indicates that the target sequence is present. If the actinR control tube is not yellow, discard the test result. It means that there was no human RNA collected and there was probably something wrong with the sample collection or preparation.

A true negative result is the N2/E1 PCR tube remaining pink while the actinR control turns yellow. A true positive result is when both tubes turn yellow.

This procedure can be automated by the use of a 96-well plate and a plate reader in place of individual PCR tubes. In that case it may be necessary to provide the kit with Master Mix made using NEB WarmStart®Colorimetric LAMP 2X Master Mix with UDG to prevent cross-contamination in plates.