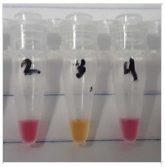

Sep 08, 2020

One Hour Covid Test Protocol

Sarah Ware[1], Ellen D Jorgensen[2], Chris Monaco[3]

[1][Bioblaze]; [2]Aanika Biosciences; [3]Centers for Disease Control

1 Works for me dx.doi.org/10.17504/protocols.io.bktakwie

XPRIZE Rapid Covid Testing Open Science

Ellen Jorgensen
Aanika Biosciences

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 easilty scaled up on a pipetting robot such as an Opentrons OT2 because the protocol requires no cengtrifugation.

It leverages a commercially-avaialble 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.

DOI

dx.doi.org/10.17504/protocols.io.bktakwie

PROTOCOL CITATION

Sarah Ware, Ellen D Jorgensen, Chris Monaco 2020. One Hour Covid Test Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bktakwie

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

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GUIDELINES

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MATERIALS

NAME	CATALOG #	VENDOR
primers		
Water, nuclease-free	R0581	Thermo Fisher
Sodium Hydroxide 1N	S2770-100ml	Sigma Aldrich
Guanidine hydrochloride	G3272-1KG	Sigma Aldrich
Sodium hydroxide	306576	Sigma-aldrich
prepIT.Q2A kit Solution AG	PT-Q2A-384 AG	DNA Genotek
prepIT.Q2A kit Solution ST	PT-Q2A-384 ST	DNA Genotek
OR-100 OraCollectRNA kit	OR-100	DNA Genotek
WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA)	M1800L	New England Biolabs

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Sodium Hydroxide 1N	S2770-100ml	Sigma Aldrich
OR-100 OraCollectRNA kit	OR-100	DNA Genotek
prepIT.Q2A kit Solution ST	PT-Q2A-384 ST	DNA Genotek
prepIT.Q2A kit Solution AG	PT-Q2A-384 AG	DNA Genotek
Guanidine hydrochloride	G3272-1KG	Sigma Aldrich
Water, nuclease-free	R0581	Thermo Fisher
WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA)	M1800L	New England Biolabs

MATERIALS TEXT

Note that our kit will contain:

- PCR tubes preloaded with reagents including LAMP Master Mix and primers recognizing a human control RNA and primers recognizing the SARS-CoV-2 virus. One tube of each is used per assay.
- A tube preloaded with 270uL nuclease-free water
- prepIT.Q2A Reagent Solution AG
- prepIT.Q2A Reagent Solution ST
- DNA Genotek OR-100 OraCollect RNA collection swab kit

There is no need to buy OR-100 swab kit, primers, prepIT Solutions, WarmStart Colorimetric Master Mix, nuclease-free water, guanidine HCl or sodium hydroxide outside of the kit unless you plan to make the kit yourself. We consider this an option for labs that have the know-how since this is not a commercial protocol and is open for use by anyone. Labs wanting to make their own kits can follow the Optional Step 1. in our protocol.

Note also that the primer sets can be changed as we learn more about how the virus evolves. The main innovation in this kit is the use of available components in a new way to make the test easy, fast cheap and doable with minimal equipment.

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EQUIPMENT

NAME	CATALOG #	VENDOR
SCILOGEX Pipette Controllers	740300029999	Lab Depot
Serological pipette, 10mL	1071-0810	USA Scientific
PLASTIC PH TEST STRIPS, UNIVERSAL APPLICATION (PH 0.0-14.0, 0.5 PH INTERVALS), 100 STRIPS	pH 0-14	LabRat Supplies
80 place 1.5 mL microfuge tube rack	2380-1008	USA Scientific
Set of micropipettes with rack: 100-1000 µl, 20-200 µl, 2-20 µl, and 0.5-10 µl	QP-1001-07	miniPCR
Precision Lid for Anova Precision Cooker	6371115	Best Buy
Sous Vide - Precision Cooker Nano	6238306	Best Buy
10uL Filter tips	1121-2710	USA Scientific
200uL filter tips	1120-8710	USA Scientific
Compact polypropylene PCR tube rack	2396-5048	USA Scientific
20uL filter tips	1123-1710	USA Scientific
TipOne Pipette tips in racks, yellow 200uL	1111-0816	USA Scientific
0.2 ML PCR PULL-APART 8-TUBE STRIPS, ATTACHED INDIVIDUAL DOME CAPS	1402-2900	USA Scientific
Adventurer™ Analytical Balances	30100600	Fisher Scientific
1.5mL microcentrifuge tube, sterile	1615-5510	
Cellstar 15 mL polypropylene conical screw cap centrifuge tube	5618-8271	USA Scientific
80-place tube rack	2380-1008	USA Scientific
TipOne Pipette tips in racks, natural 10uL	1111-3810	USA Scientific
4-way tube rack	2304-8044	USA Scientific
Disposable polystyrene weighing dishes	Z154873	Millipore Sigma
Floating Test Tube Rack	1201U03	Thomas Scientific
TipOne Pipette tips in racks, blue graduated 1000uL	1111-2831	USA Scientific

SAFETY WARNINGS

Use lab coat, gloves and safety glasse when working in the lab.

DISCLAIMER:

BEFORE STARTING

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

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OPTIONAL pre-preparation of Master Mix 1h

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

Citation: Sarah Ware, Ellen D Jorgensen, Chris Monaco (09/08/2020). One Hour Covid Test Protocol. https://dx.doi.org/10.17504/protocols.io.bktakwie

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**

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TipOne Pipette tips in racks, natural 10uL

Pipette tips in racks, natural, 10uL

TipOne **1111-3810**

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**

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Cellstar 15 mL polypropylene conical screw cap centrifuge tube
Sterile conical screw cap centrifuge tube, 15 mL

GREINER BIO-ONE 5618-8271

Guanidine hydrochloride
by Sigma Aldrich
Catalog #: G3272-1KG
CAS Number: 50-01-1

WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA)
by New England Biolabs
Catalog #: M1800L
RRID: n/a

Sodium Hydroxide 1N
by Sigma Aldrich
Catalog #: S2770-100ml

Water, nuclease-free
by Thermo Fisher
Catalog #: R0581

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.

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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 ACCAGGAACTAATCAGACAAG
N2-B3 GACTTGATCTTTGAAATTTGGATCT
N2-FIP TTCCGAAGAACGCTGAAGCGGAACTGATTACAAACATTGGCC
N2-BIP CGCATTGGCATGGAAGTCACAATTTGATGGCACCTGTGTA
N2-LF GGGGGCAAATTGTGCAATTTG
N2-LB CTTCGGGAACGTGGTTGACC

For E1 Primer Mix:

E1-F3 TGAGTACGAACTTATGTACTCAT
E1-B3 TTCAGATTTTTAACACGAGAGT
E1-FIP ACCACGAAAGCAAGAAAAAGAAGTTCGTTTCGGAAGAGACAG
E1-BIP TTGCTAGTTACACTAGCCATCCTTAGGTTTTACAAGACTCACGT
E1-LB GCGCTTCGATTGTGTGCGT
E1-LF CGCTATTAACTATTAACG

For actinR Primer Mix:

ACTB-F3 AGTACCCCATCGAGCACG
ACTB-B3 AGCCTGGATAGCAACGTACA
ACTB-FIP GAGCCACACGCAGCTCATTGTATCACCAACTGGGACGACA
ACTB-BIP CTGAACCCCAAGGCCAACCGGCTGGGGTGTTGAAGGTC
ACTB-LF TGTGGTGCCAGATTTTCTCCA
ACTB-LB CGAGAAGATGACCCAGATCATGT

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 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.

Store frozen at **-20 °C** until ready to use them.

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

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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.

SCILOGEX Pipette Controllers
Pipette Controller 10mL
SCILOGEX 740300029999

Serological pipette, 10mL
10ml serological pipette, sterile, individually wrapped
USA Scientific 1071-0810

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PLASTIC PH TEST STRIPS, UNIVERSAL APPLICATION (PH 0.0-14.0, 0.5 PH INTERVALS), 100 STRIPS
pH test strips, 0-14

LabRat pH 0-14

Sodium Hydroxide 1N
by Sigma Aldrich
Catalog #: S2770-100ml

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** 1
- **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.

Test Protocol: Sample Collection

2 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 apporval 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 sfe and effective.

Additionally, we chose swabs rather than liquid saliva collection because in talking with nursing homes many of their

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residents cannot produce much saliva. Furthermore physicians told us that it is necessary to test patients who are on ventilators and unconcious and in these cases saliva collection is impossible but swabbing is doable.

OR-100 OraCollectRNA kit
by DNA Genotek
Catalog #: OR-100
RRID: n/a

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

3 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 colorimetic 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 becuase 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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prepIT.Q2A kit Solution ST
by DNA Genotek
Catalog #: PT-Q2A-384 ST
RRID: n/a

and

prepIT.Q2A kit Solution AG
by DNA Genotek
Catalog #: PT-Q2A-384 AG
RRID: none

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.

Precision Lid for Anova Precision Cooker
lid for pot
Anova 6371115

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

Sous Vide - Precision Cooker Nano
Waterbath heater
Anova 6238306

Other stuff you will need will be:

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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

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**

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

3.1 Turn on sous vide heater and set to **68 °C** . 1m

Place a 1.5mL microfuge tube in the tube rack. Using the P-200 pipettor and filter tips, transfer 100uL 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 µl** prepIT.Q2A Solution AG 1m

3.3 Using the P-20 pipettor and tips, add **20 µl** prepIT.Q2A Solution ST 1m

3.4 Pipette up and down 12 times to mix. 1m

3.5 Incubate tube **Room temperature** for **00:15:00** 15m

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 µl** 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 µl** 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 °C** sous vide waterbath and incubate for **00:30:00**

Assay Readout

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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 ther 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 positve 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.

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