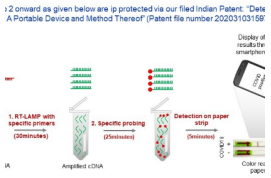
Sep 08, 2020

COVIRAP testing protocol



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XPRIZE Rapid Covid Tes...

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Protocol status: Working
We are using this protocol for COVID 19 testing with extracted viral RNA samples in a simple portable device.

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Keywords: COVIRAP, subsequent colorimetric detection of the covid19 infection, covid19 infection, reverse transcription, protocol for isothermal amplification, mediated reverse transcription, subsequent colorimetric detection, cdna products in the first step, based probe, detection technique, based detection technique, cdna product, isothermal amplification, rapid dissemination of the result,

Abstract

We have developed an RT-LAMP based protocol for isothermal amplification and subsequent colorimetric detection of the COVID19 infection. This protocol essentially relies upon the RT-LAMP mediated reverse transcription and subsequent amplification into large copies of cDNA products in the first step of the protocol. Subsequently, we have fused a second step where we label the cDNA with a fluorophore based probe to increase the sensitivity and specificity of the results. This step also makes the overall process to be compatible with the paper strip based detection technique where the product from the second step gets captured by the molecule impregnated in the paper strip. This leads to the development of the control and test line which could be detected with a smart phone based app for the prediction and rapid dissemination of the result.

Guidelines

The protocol can be performed by any semi-trained person. There are total nine steps to follow for performing the test.

Materials

MATERIALS

- WarmStart LAMP Kit (DNA and RNA) - 500 rxns **New England Biolabs** **Catalog #**E1700L
- Primers Probes **Catalog #**NA
- 0.2ml PCR tube **Catalog #**510051
- Paper strip **Catalog #**MGHD 1
- Filter tips **Catalog #**BT20/AXYZTF200LRS

Troubleshooting

Safety warnings

Do not touch the heating block while the machine is running.

Use safety PPEs before handling the samples.

As the device is using extracted RNA for nucleic acid based COVID 19 detection, no biosafety level-II safety protocol is required for testing with the device.

Before start

Go through the protocol before starting the test.

DNA/RNA amplification

1 Aliquot 10 µL RT-LAMP master mix into PCR tubes considering the total number of reactions. For one RNA sample, three reactions need to be carried out; one corresponding to RNaseP (internal control) and two corresponding to viral gene targets (N gene and ORF 1b).

Equipment	
Micropipette	NAME
Dispenser	TYPE
Tarsons	BRAND
T10	SKU

2 Add specific primers (mixture of all the RT LAMP primers) corresponding to RNaseP, N gene and ORF 1b.

Equipment	
Micropipette	NAME
Dispenser	TYPE
Tarsons	BRAND
T10	SKU

3 Add 1 µL to 9 µL of RNA extracted from patient swab sample.

Equipment	
Micropipette	NAME
Dispenser	TYPE
Tarsons	BRAND
T10	SKU

4 Switch on the device. Put the microchamber on the isothermal heating platform of the portable device with the help of microchamber holder

5 The target temperature for isothermal heating of the RNA mix is pre-set at 65 °C . This isothermal heating will continue for next 00:30:00 minutes. System will increase the temperature to 85 °C after 30 minutes and at 85 °C will continue for 00:05:00 minutes

6 *Add specific probes into the microchamber and continue the isothermal heating at specified temperature for a specific time. Then cool down the chamber to room temperature at 25 °C

*This step is IP protected by this team.

Colorimetric Detection

7 Apply the amplified reaction mixtures to the paper-strip and allow the lateral flow to occur. This will lead to the development of control and test lines on the strip.

8 Put the smartphone in its designated place. Wait for 00:05:00 to 00:10:00 minutes. and then start the smartphone app.

9 The result will be dispalyed onto the smartphone screen after 1-2 minutes.