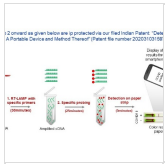

Sep 08, 2020

COVIRAP testing protocol

Suman Chakraborty[1], Arindam Mondal[2], Aditya Bandopadhyay[3], Sujay Kumar Biswas[4], Saptarshi Banerjee[5], Nandita Kedia[5], Subhamoy Chatterjee[6], Aratrika de[5], Indranath Banerjee[7]

[1]Professor, Department of Mechanical Engineering, IIT Kharagpur, India-721302;
[2]Asst. Professor, Department of Biosciences, IIT Kharagpur, India -721302;
[3]Asst. Professor, Department of Mechanical Engineering, IIT Kharagpur, India-721302;
[4]PhD, School of Medical Science and Technology, IIT Kharagpur, India -721302;
[5]PhD, Department of Biosciences, IIT Kharagpur, India -721302;
[6]PhD, Electronics and Electrical Communication Engg., IIT Kharagpur, India -721302;
[7]Doctor, BC Roy Technology Hospital, IIT Kharagpur, India-721302

1 Works for me dx.doi.org/10.17504/protocols.io.bkzgkx3w

Sujay Kumar Biswas

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.

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PROTOCOL CITATION

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KEYWORDS

COVIRAP

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GUIDELINES

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

MATERIALS

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

EQUIPMENT

NAME	CATALOG #	VENDOR
Micropipette	T10	

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 STARTING

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).

Micropipette
Dispenser
Tarsons **T10**

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

Micropipette
Dispenser
Tarsons **T10**

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

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Micropipette
Dispenser
Tarsons T10

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.

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