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CoVan RT-LAMP protocol V.1

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1 *Works for me* This protocol is published without a DOI.

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PROTOCOL CITATION

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MATERIALS

NAME	CATALOG #	VENDOR
1X PBS (Phosphate-buffered saline)		
1.5 mL Eppendorf tubes		
WarmStart Colorimetric LAMP 2X Master Mix (DNA and RNA) - 100 rxns	M1800S	New England Biolabs
PCR tubes		
Microcentrifuge		
Digital Heating Shaking Bath, Block 96 well	88880125	Thermo Fisher
1.5 mL reaction tubes	72.690.001	Sarstedt
P1000 pipette tips		
10X stock of RT-LAMP primers (Color Genomics N-gene)*		
1e6 copies/ml UV-inactivated SARS-CoV-2 positive control		
Cooler with freeze-packs		

MATERIALS TEXT

Primer	Abbrev.	Primer sequence	10x concentration
Forward Outer Primer (F3)	Color-N-F3	AACACAAGCTTTCGGCAG	2 µM
Backward Outer Primer (B3)	Color-N-B3	GAAATTTGGATCTTTGTCATCC	2 µM

Forward Internal Primer (FIP)	Color-N-FIP	TGCGGCCAATGTTTGTAATCAGCCAAGGAAATTTTGGGGAC	16 µM
Backward Internal Primer (BIP)	Color-N-BIP	CGCATTGGCATGGAAGTCACTTTGATGGCACCTGTGTAG	16 µM
Forward Loop Primer (LF)	Color-N-LF	TTCCTTGTCTGATTAGTTC	8 µM
Backward Loop Primer (LB)	Color-N-LB	ACCTTCGGGAACGTGGTT	8 µM

* 10X RT-LAMP Primer Stock

Saliva collection area

1 Prepare saliva collection tube by placing a p1000 pipet tip in a 1.5 ml tube

2 Obtain ~100 µl of saliva – have the individual spit into a tube using a P1000 pipet tip to funnel saliva into the tube

Infection control: Have individual throw the p1000 pipet tip away in a biohazard bag, close the cap of the 1.5ml tube, and wipe down the outside of the tube with 10% bleach

3 Place the saliva sample in a cooler with ice packs and transport the saliva to the sample preparation table

Sample preparation area

4

Infection control: Wipe down the outside of the tube again with 10% bleach.

Heat saliva sample at 65ºC for 30 minutes, while shaking gently at 300 rpm, to inactivate virus

This is a good time to start preparing the RT-LAMP master mix (step 9)

5 Heat and shake sample at 98ºC for 3 minutes to liberate RNA and help inactivate some of the enzymes in saliva

6 Spin down tubes in microcentrifuge for 2 minutes to pellet cell debris in the saliva

Infection control: Wipe down the microcentrifuge with 10% bleach and 70% ethanol after spin; wipe down tubes with 10% bleach

7 Add 50 µl of saliva to a 1.5 ml Sarstedt tube with 50 µl of 1x PBS to buffer against variability in pH and make the sample easier to handle

Infection control: Wipe down the PCR tube with 10% bleach

8 Transport the saliva + PBS tubes to the RT-LAMP testing area

RT-LAMP area

9 Prepare RT-LAMP master mix:

Reagent	Master mix (1 reaction)	Your reaction volumes:
WarmStart 2X MM	10 µl	
LAMP primer mix (10X)	2 µl	
dH2O	5 µl	
Sample	3 µl	
Total Volume	20 µl	

10 Aliquot 17 µl of RT-LAMP master mix into a new set of PCR strip tubes – two for each sample, plus two each for positive and negative controls

11 Add 3 µl of saliva/PBS mix to 17 µl RT-LAMP master mix in duplicate – two tests per individual

12 For a positive control, add 3 µl of a 1e6 copies/ml UV-inactivated SARS-CoV-2 stock to two 17 µl RT-LAMP master mix tubes. For a negative control, add 3 µl of water to two 17 µl RT-LAMP master mix tubes.

13 Heat and shake sample + master mix at 65ºC for 30 minutes

14 Observe RT-LAMP reaction result for each individual. All four controls (two positive and two negative) must be correct in order to consider results valid:

Both sample tubes are PINK: negative result
Both sample tubes are YELLOW: positive result
One sample tube is PINK and one sample tube is YELLOW: retest sample using PCR