



CoVan RT-LAMP protocol V.3

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

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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	GAAATTTGGATCTTTGTATCC	2 µM

Forward Internal Primer (FIP)	Color-N-FIP	TGCGGCCAATGTTTGTAAATCAGCCAAGGAAATTTGGGGAC	16 μ M
Backward Internal Primer (BIP)	Color-N-BIP	CGCATTGGCATGGAAGTCACTTTGATGGCACCTGTGTAG	16 μ M
Forward Loop Primer (LF)	Color-N-LF	TTCCTTGTCTGATTAGTTC	8 μ M
Backward Loop Primer (LB)	Color-N-LB	ACCTTCGGGAACGTGGTT	8 μ M

* 10X RT-LAMP Primer Stock

SAFETY WARNINGS

Because this protocol uses potentially infectious human samples, be sure to wipe down tubes and workstations frequently with 10% bleach to ensure safe handling of samples and a safe work environment. Instances where this is recommended are noted in the protocol.

Saliva collection area 2m

- 1 Prepare saliva collection tube by placing a p1000 pipet tip in a 1.5 ml tube
- 2 Obtain ~300 μ l 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 65°C 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 98°C 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 tubes with 10% bleach

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



Infection control: Wipe down the tubes with 10% bleach

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	
dH ₂ O	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



Infection control: Wipe down the tubes with 10% bleach

- 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. (More information on how to prepare a positive control stock [here](#).) 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 65°C 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. If any of the controls don't work, repeat the entire run.

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: positive, but retest –

- Re-run sample 4x using the same PBS/saliva mix
- Optional PCR confirmation of results