COLUMBIA | FERTILITY

Step-by-step guide to Rapid, Point-of-Care SARS-CoV-2 Detection in Clinical Samples using a Colorimetric RT-LAMP reaction.

Method Overview: This protocol is intended for detection of SARS-CoV-2 directly from clinical samples (nasopharyngeal swab placed into viral transport media) in 30 minutes. The only reaction equipment required is a heat block. No isolation or purification of RNA is required. The method utilizes reverse transcription, loop-mediated isothermal amplification (LAMP) using 6 custom designed primers (3 primer sets) to amplify viral RNA and colorimetric indicator to give a visual display of the result.

To perform this test, a master mix is made that consists of the 6 primers, lysis buffer, amplification reagents and the color indicator solution. The master mix is placed into a microcentrifuge tube. The clinical sample (e.g. VTM) is placed into the microcentrifuge tube containing the master mix, mixed, and then ½ of the solution in placed into a second microcentrifuge tube. Both tubes are then placed into a 63.0˚C dry bath for 30 min. After 30 min, the sample is removed, placed on ice for 1 min and visualized. A red color indicates a negative result, a yellow color indicates a positive result.

Below is a detailed step-by step guide. It is divided into three parts. The first part is a list of the reagents and supplies that you will need. The second part is the instructions for preparing the reaction buffer. The third part is the protocol for conducting the assay.

Reagents and Supplies

1. LAMP primers (CUFC-FIP, CUFC-BIP, CUFC-LF, CUFC-LB, CUFC-F3, CUFC-B3)
1. Nuclease-free water (Ambion, AM9937).
2. 100mM dUTP,
3. 0.5µL UDG, and
4. 5mM SYTO 9 (Invitrogen, S34854)
5. 1250µL WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA) (NEB, M1800L).
6. TE buffer pH 8.0
7. Tween-20
8. 1% Thermolabile Proteinase K (NEB, P8111S),
9. 2% ezDNase (Invitrogen, 11766051),
10. 0.3ng/µL human genomic DNA
11. 1.5mL LoBind microcentrifuge tube (Eppendorf, 022431021)
12. Ice and a container to hold the ice.
13. Sterile disposal transfer pipette (Fisherbrand, 13-711-20)
14. 63.0˚C dry bath (Fisherbrand, 14-955-219)

Preparation:

1. A 25-fold primer mix of LAMP primers (CUFC-FIP, CUFC-BIP, CUFC-LF, CUFC-LB, CUFC-F3, CUFC-B3) was prepared by assembling 40µM CUFC-FIP and CUFC-BIP,

10µM CUFC-LF and CUFC-LB, and 5µM CUFC-F3 and CUFC-B3 primers in nuclease-free water (Ambion, AM9937).

2. 2X colorimetric RT-LAMP master mix were prepared by adding 3.5µL 100mM dUTP, 0.5µL UDG, and 0.25µL 5mM SYTO 9 (Invitrogen, S34854) in 1250µL WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA) (NEB, M1800L).

3. The reaction mix is prepared by mixing 125µL 2X colorimetric RT-LAMP master mix, 10µL 25-fold LAMP primer mix, 95µL nuclease-free water for one 250µL reaction, and scaled up according to the actual number of samples.

4. Lysis buffer consist of 0.1-fold TE buffer pH 8.0 with 0.1% tween-20, 1% Thermolabile Proteinase K (NEB, P8111S), 2% ezDNase (Invitrogen, 11766051), 0.3ng/µL human genomic DNA from a normal male. For one reaction, 460µL reaction mix and 20µL lysis buffer was preloaded in a clean 1.5mL LoBind microcentrifuge tube (Eppendorf, 022431021) and kept on ice until use.

Performing the assay:

1. Add 20µL clinical sample directly into a 1.5mL LoBind microcentrifuge tube (Eppendorf, 022431021) containing the reaction mix (460µL) and lysis buffer (20µL)

   1b: Alternative method: Add 10µL clinical sample directly into a 1.5mL LoBind microcentrifuge tube (Eppendorf, 022431021) containing the reaction mix (230µL) and lysis buffer (10µL). Repeat for a second tube. Proceed directly to Step 4.

2. Mix using a sterile disposal transfer pipette (Fisherbrand, 13-711-20)
3. Aliquot 250uL of the 500 uL solution into a new clean 1.5mL LoBind microcentrifuge tube.
4. Place both tubes with ~250µL each into a 63.0˚C dry bath (Fisherbrand, 14-955-219)
5. Incubate tubes for 30min in a 63.0˚C dry bath.
6. Place tubes on ice for 1 min to pause the reaction.
7. Record colorimetric results.
   - ❖ Red=Negative
   - ❖ Yellow=Positive

Table 1. Sequence information of LAMP primers for SARS-Cov-2 detection

Primer Name	Sequence
CUFC1-F3	TGGATACAACTAGCTACAGAGAAG
CUFC1-B3	AGCCAAAGACCGTTAAGTGTA

CUFC1-FIP	GTGGTGGTTGGTAAAGAACATCAGACTTGTTGTCATCTCGCAAAGG
CUFC1-BIP	CCTCTATCACCTCAGCTGTTTTGCTGTACCATACAACCCTCAACTT
CUFC1-LF	ACCTGAGTTACTGAAGTCATTGAGA
CUFC1-LB	TGGTTTTAGAAAAATGGCATTCCC