## Supplementary Method 1: RT-LAMP Mastermix Preparation

### *Equipment / Consumables*

- Rainin L-100 manual single pipette
- Rainin L-1000 manual single pipette
- Rainin 5-50 µL LTS multichannel pipette
- Rainin 10-100 µL or 20-200 µL LTS electronic single channel pipette
- Rainin filtered tips: green box, blue large box, and grey/yellow box
- Eppendorf Tube rack
- 25 mL individually wrapped reservoirs
- PCR plate (MicroAmp™ Fast Optical 96-well reaction plate with barcode, cat no 4346906)
- PCR plate (MicroAmp™ Optical 96-well reaction plate with barcode, cat no 4309849)
- AlumaSeal II Film (cat no Z721530)
- DNA Zap solutions ( cat no AM9890)
- Seal roller
- PCR chiller plate
- Vortex

### *Reagents*

**WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA), NEB Cat M1800**

*1.25 mL aliquots (~166 reactions)*
*Store at -20ºC until use. After thawing, store at -20ºC or keep at 4ºC if using within 2 days. After thawing, solution must be vortexed well to dissolve any precipitate in solution before use. Avoid multiple freeze/thaw cycles.*

**SYTO 9 Green Fluorescent Nucleic Acid Stain, ThermoFisher Cat S34854**

*Stock solution supplied in 100 µL aliquots of 5 mM solution in DMSO*
*Working stock of 5 µM (10X) is made by diluting 1:1000 in nuclease free water*
*Store at -20ºC until use. Once thawed, store at -20ºC or keep at 4ºC if using within 2 days. After thawing, solution must be vortexed well. Store stocks with dessicant material in 50 mL conical. Avoid multiple freeze/thaw cycles.*

**Primers for SARS-CoV-2 Detection (N Gene)**

| | |
|---|---|
| **N-F3** | TGGCTACTACCGAAGAGCT |
| **N-B3** | TGCAGCATTGTTAGCAGGAT |
| **N-FIP** | TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG |

| | |
|---|---|
| **N-BIP** | AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT |
| **N-LF** | GGACTGAGATCTTTCATTTTACCGT |
| **N-LB** | ACTGAGGGAGCCTTGAATACA |

**Primers for Internal Control Detection (18S rRNA)**

| | |
|---|---|
| **18S-F3** | GTTCAAAGCAGGCCCGAG |
| **18S-B3** | CCTCCGACTTTCGTTCTTGA |
| **18S-FIP** | TGGCCTCAGTTCCGAAAACCAACCTGGATACCGCAGCTAGG |
| **18S-BIP** | GGCATTCGTATTGCGCCGCTGGCAAATGCTTTCGCTCTG |
| **18S-LF** | AGAACCGCGGTCCTATTCCATTATT |
| **18S-LB** | ATTCCTTGGACCGGCGCAAG |

**Primer Preparation (order from Sigma, DST purity)**

- *Prepare N gene and 18S mixes separately in nuclease free tubes and water for 10X concentrated mixes*
- *Store at -20ºC until use. Once thawed, store at -20ºC or keep at 4ºC if using within 2 days. After thawing, solution must be vortexed well. Avoid multiple freeze/thaw cycles.*

| | | For 700 µL of 10X Mix (~450 reactions) |
|---|---|---|
| F3/B3 primers | (10X = 2 µM) | 14 µL of each |
| FIP/BIP primers | (10X = 16 µM) | 112 µL of each |
| LF/LB primers | (10X = 4 µM) | 28 µL of each |
| | | 392 µL of nuclease free water |

***Manual Preparation of PCR master mix in 96/384 well MicroAmp™ plates***

NOTE: Reagents are stored at -20ºC. Take out all the necessary reagents (WarmStart Colorimetric LAMP 2X Mix, Primer Mix (10X) and SYTO 9 (10X) and thaw them thoroughly at ambient temperature. Immediately once thawed, vortex well and centrifuge briefly. The enzyme mix should be kept on ice at all times. RT-PCR mastermix is aliquoted into PCR plates in designated hoods in the HTS tissue culture room. All personnel must have full PPE when entering the tissue culture room, which includes clean lab coat, fresh nitrile gloves, and full-face guard. Additionally, the individual pipetting in the hood must wear disposable forearm sleeves.

1 When email indicating 'RNA plate is ready' is received, take required number of reagent aliquots from the -20°C freezer for number of samples (+10 extra) and maintain on ice immediately once thawed at RT.

2 Vortex well to remove any precipitate and quick spin down all tubes.

3 Clean hood and everything that enters hood with DNA ZAP by spraying Solution 1 followed by Solution 2 and then wiping dry with clean paper towels.

4 Using L-100 pipette with filtered tip from green tip box or L-1000 pipette with filtered tip from blue tip box, dispense required amount of each reagent for number of sample reactions (plus 10 extra) into a nuclease free tube. Mix well once assembled by vortexing and spin down.

**1X Reaction**
7.5 µL WarmStart Colorimetric LAMP 2X Mix
1.5 µL N Gene Primer Mix (10X) **OR** Internal Control 18S rRNA Primer Mix (10X)
1.5 µL SYTO 9 Dye (10X)
10.5 µL per well (+ 4.5 µL RNA for 15 µL total reaction volume)

5 Dispense RT-LAMP Master mix into plates as follows:

- For half plate, leave PCR mix in tube and use an electronic single channel pipette fitted with a filter tip to draw up 100 µL or 200 µL and repeatedly dispense 10.5 µL into individual wells of the PCR plate.

- For full plate or more: Using an L-1000 pipette with filtered tip from Blue tip box, pipette all the Master Mix from individual tubes into a 25 mL individually wrapped reservoir. Dispense 10.5 µL of Master mix into all wells of a barcoded optical PCR plate, using an LTS 5-50 µL multichannel pipette.

**Pipette settings: From Main menu, select Multi-Disp setting with Aliquot volume 10.5 µl, 1/1 Asp/Dsp Speed**

6 Eject any remaining PCR master mix left in tips back into reservoir.

7 Seal the plates with AlumaSeal. Check each well is sealed.

8 Transfer remaining RT-LAMP master mix back into prepared tube, label with date, and put in box in -20°C.

9 Clean up hood by spraying with both DNA ZAP solution 1 followed by DNA ZAP solution 2 and wiping with paper towel.

10 Briefly **spin** the plate at 1000rpm, 1min, and store on ice together with the thawed positive control tube.

11 On designated computer, enter the date, the name of the operator, the PCR plate barcodes, RT-LAMP lot numbers, and RNA plate barcode from automated email into the spreadsheet.

12 Carry the prepared RT-PCR plates and positive control tube to 5$^{th}$ floor and leave in designated fridge outside ASF lab.

13 4.5 µL of RNA is loaded into the RT-LAMP reaction mix with the following wells designated for control wells:

96 well plate format:
Negative Control 1 (Well F12, L6 inactivation buffer)
Negative Control 2 (Well G12, no template control)
Positive Control (Well H12)

384 well plate format:
Negative Control 1 (Well N24, L6 inactivation buffer)
Negative Control 2 (Well O24, no template control)
Positive Control (Well P24)