Supplementary Method 2: RT-LAMP RNA Loading and Running RT-PCR Machine

Equipment / Consumables

- Pipettes: Rainin 5-50µl LTS multichannel; Rainin L-200 single pipette; Rainin L-10 single pipette

- Pipette tips: 1 full, unopened box of Rainin filtered tips per RNA plate plus an additional box; 1 box Rainin filtered tips

- Clean beaker for waste

- MicroAmp™ Optical Adhesive Film

- MicroAmp™ adhesive film applicator (seal roller)

- PCR chiller plate

- Benchtop centrifuge

- Barcode Scanner and laptop computer

- Vortex

- Quantstudio Real time PCR System

Reagents

- ABI MicroAmp™ Fast Optical PCR plate containing RT-PCR Master Mix for detecting SARS-CoV-2

- Extracted viral RNA samples in a 96 or 384-well barcoded plate

- Positive control

Procedure

Reagents preparation:

1 Prepare a PCR Hood by loading pipettes, tips, waste beaker, MicroAmp™ adhesive film and seal roller and switch on UV.

2 Retrieve RNA plate from storage on ice

3 Spin RNA plate briefly (1000rpm, 1min)

4 Open the hood. In the hood, unseal the barcoded plate containing the PCR master mix.

5 In the hood, unseal the barcoded plate containing the RNA template.

6 CHECK THAT THE RNA and RT-LAMP MASTERMIX PLATES ARE IN THE SAME ORIENTATION, with A1 at upper left of plate. With well A1 at the top left the Barcode

should be on long side of plate facing the operator

7   Using an L-200 single pipette with a filtered tip (green box), remove and discard contents of well H12 in RNA plate

8   Using a 5-50 µL LTS multichannel with filtered tips from a brand-new green box, transfer 4.5 µL of RNA from RNA plate row by row into corresponding wells of the PCR plate, using new tips for each well.

9   Pipette setting: From Main menu, select Multi-Disp setting with Aliquot volume 4.5 µL, 1 aliquot, 1/1 Asp/Dsp Speed

10 Discard blowout.

11 When complete, reseal RNA plate.

12 Using a L-10 single pipette with a filtered tip from red box, transfer 4.5 µL of positive control into well H12 for 96 well or P24 for 384 well plate.

13 Seal the PCR plate carefully

14 Label the RNA plate on seal with "RNA plate" and date it

15 Return Positive Control tube to box in -20°C freezer, and place RNA and PCR plate on ice

16 Remove plates from hood, on ice. Discard used tips and replace waste beaker with new unused one, spray working surface with DNAzap (both solutions in equal amount) and wipe down, then close the hood, and turn on UV lamp. The hood will beep when ready to be used again.
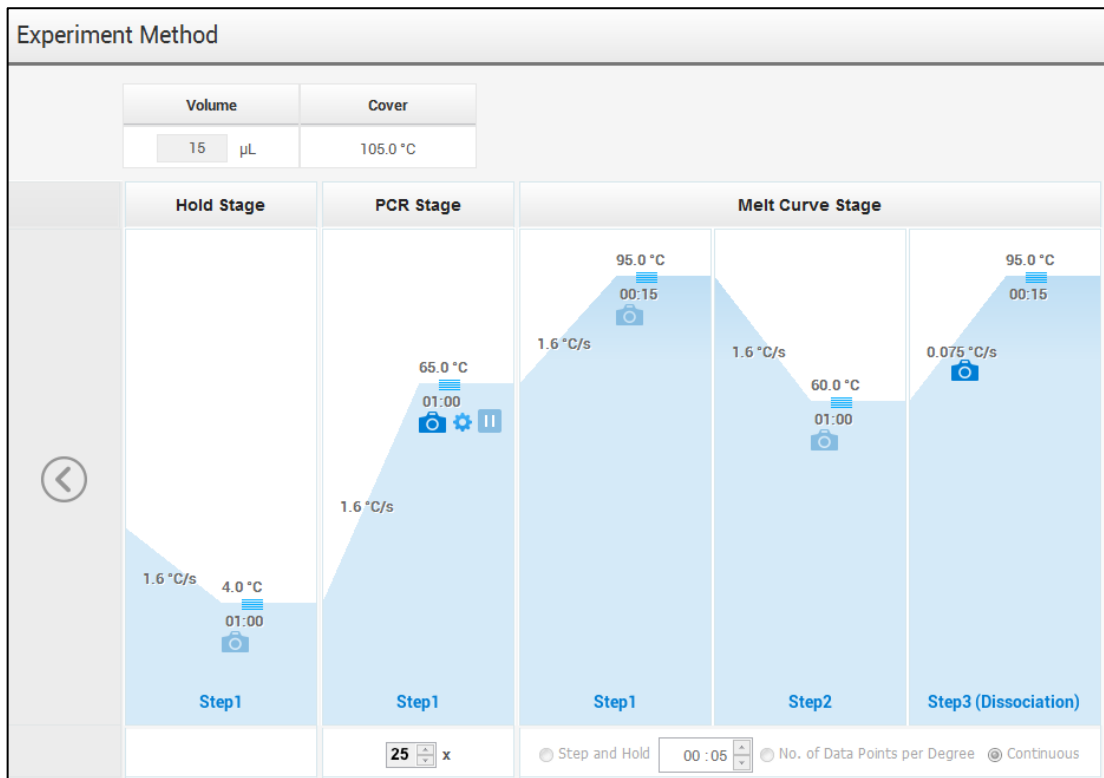
RT-LAMP Set up on RT-PCR Machine

1   Take PCR Plate to PCR lab. Ensure that the PCR plate is correctly assigned to the PCR stage of the process in a LIMS system. If using Clarity LIMS to confirm receipt of plate, click in empty field box in app, then scan the barcode with a handheld barcode scanner to enter PCR plate name. Hit **SUBMIT**.

2   Check seal integrity on PCR plate and spin briefly in underbench centrifuge (1000rpm, 1min)

3   Open the sample drawer of an available QuantStudio 3 machine (96-well plate) or QuantStudio 5 machine (384-well plate) and place PCR Plate in the machine.
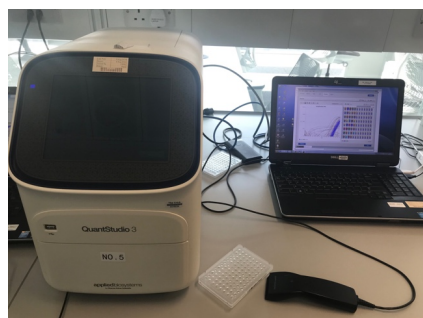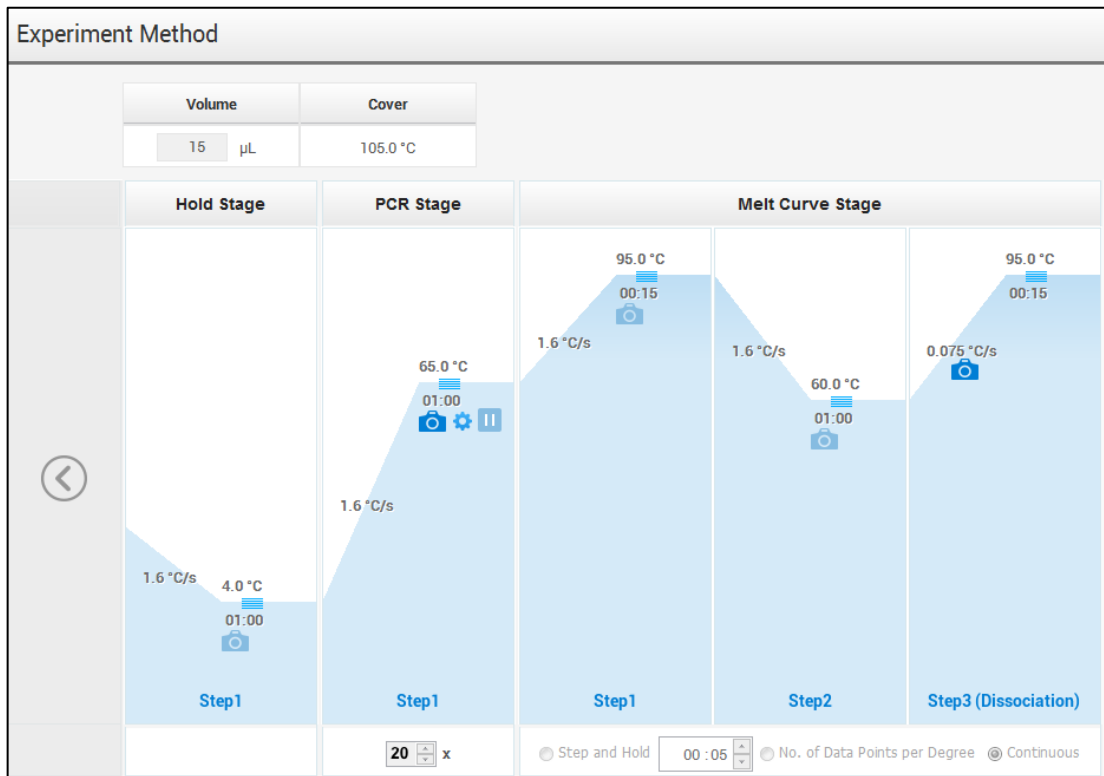
4   Confirm that the barcode is visible and facing the front of the machine, and A1 is on top-left side

5   On the PCR machine-associated laptop, ensure that the correct annotation for the specific PCR plate is downloaded from a LIMS system. If using Clarity LIMS, click in empty field box in app and scan the barcode on the PCR plate with handheld barcode scanner to enter PCR plate name. Hit **SUBMIT.**

6   Download of text file will happen automatically. Save to 'Plate Layout' folder on Desktop with the Barcode as filename.

7   **At this point, a second operator must confirm plate orientation.**

8   Open QuantStudio software.

9   Click on the button to "Open Existing Experiment" and find the **RT-LAMP Covid-19 Master Template.edt** if running N gene RT-LAMP assay. Select **RT-LAMP Internal Control Master Template.edt** if running 18S rRNA RT-LAMP assay on Desktop.

10   When prompted, click on 'Edit' and enter designated password.

11   Click in field box for **Barcode** and use handheld scanner to scan plate barcode again to populate the field and replace default.

12   Click in field box for **Name** and scan barcode again to replace default name.

13   Click in field box for **User** and enter your Crick user name.

14   Under 'File' menu click on 'Import Plate Setup' and browse to identify the text file with same barcode on Desktop\Plate Layout. Hit '**Apply**'.

15   You should be prompted that current info will be lost and replaced. Hit '**yes**'.

16   Confirm the following settings by clicking on 'Method' on menu near top of window:

N GENE RT-LAMP METHOD

Experiment Method

Volume	Cover
15 µL	105.0 °C

Hold Stage	PCR Stage	Melt Curve Stage

Hold Stage — Step1
1.6 °C/s   4.0 °C   01:00

PCR Stage — Step1
65.0 °C   01:00
1.6 °C/s
25 x

Melt Curve Stage
Step1: 95.0 °C   00:15   1.6 °C/s
Step2: 60.0 °C   01:00   1.6 °C/s
Step3 (Dissociation): 95.0 °C   00:15   0.075 °C/s

○ Step and Hold   00:05   ○ No. of Data Points per Degree   ◉ Continuous

18S rRNA RT-LAMP METHOD

Experiment Method

Volume	Cover
15 µL	105.0 °C

Hold Stage	PCR Stage	Melt Curve Stage

Hold Stage — Step1
1.6 °C/s   4.0 °C   01:00

PCR Stage — Step1
65.0 °C   01:00
1.6 °C/s
20 x

Melt Curve Stage
Step1: 95.0 °C   00:15   1.6 °C/s
Step2: 60.0 °C   01:00   1.6 °C/s
Step3 (Dissociation): 95.0 °C   00:15   0.075 °C/s

○ Step and Hold   00:05   ○ No. of Data Points per Degree   ◉ Continuous

17 Confirm following settings by clicking on 'Plate'

(Advanced Setup Tab) Target1: SYBR, Quencher: None
(Quick Setup Tab) Passive Reference Dye: None

18 In 'Plate' window in Quick Setup Tab that plate is populated with sample barcodes.

19 Click on 'Run' window, and hit 'Start Run' button

20 When prompted, save barcode.eds file in 'Covid19 Results' folder on Desktop.

21 **FINAL CHECKPOINT (stop the run if the following is not true):** The PCR run should last ~45 minutes for N gene RT-LAMP assay and ~40 minutes for 18S rRNA RT-LAMP assay.

22 After the amplification is complete, remove PCR plates from the thermal cycler and discard plate for autoclave and decontamination in line with laboratory procedures.