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Step 2: RNA extraction and RT-qPCR

In 1 collection

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DISCLAIMER

J.V. is co-founder and CSO of InActiv Blue; S.M. is an employee of Tecan Trading AG; L.M.P.D., M.M.C.B., R.J.T.M.R., L.B.J.V. and J.H.B.B. are employees of Stichting PAMM Veldhoven; A.vW. is an employee of Sanquin;, E.D. is a founder and employee of Bodegro; and F.J.D. is CEO and co-founder HiFIBiO France.

ABSTRACT

STRIP is a start-to-end streamlined and automated procedure for COVID-19 testing, centering on a single Tecan Fluent liquid-handling robot that can process over 14,000 samples per day.

Key features of the customized Tecan Fluent robot are (1) on-board 1D and 2D barcode scanners, (2) an automated tube decapper, (3) two robotic arms for simultaneous processing of different procedural steps, (4) a newly-designed spin vessel to keep magnetic beads in solution and immediately transferable to 384-well plates, (5) a built-in magnetic deck and a 384-channel pipetting head for rapid RNA isolation, (6) a heating device for fast drying of RNA prior to elution, (7) a built-in plate sealer and (8) a plate storage system to allow processing of multiple sample plates in a single run (See Materials).

Here we describe the RNA extraction and RT-qPCR protocol as currently applied in STRIP.

PROTOCOL CITATION

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MATERIALS TEXT

- the negative amplification control (NAC): a Micronic tubefilled up with 0.4 ml inactive blue.
- the negative extraction (NEC) control : a Miconic tube filled up with 0.4 ml inactive blue mixed with PDV.
- positive extraction control (PEC): a micronic tube filled up with 0.4 ml inactive blue mixed with PDV and inactivated SARS-CoV-2.
- 70% EtOH: Mix 7 parts of 100% Ethanol (200-proof) and 3 parts MilliQ water.
- Elution H2O: Nuclease-Free Water, ThermoFisher, #AM9932.
- RNA extraction plate: 150ul 384-well deep well plates, Greiner masterblock 384 deep well plate, #781270
- EtOH plate: 150ul 384-well deep well plates, Greiner masterblock 384 deep well plate, #781270.
- Waste plate: 300ul 384-well deep well plate, Sigma-Aldrich, #BR701355.
- 384-well qPCR plates: microAmp optical 384 reaction plate with barcode, ThermoFisher, #4309849.
- 125 µl tips : 125 ul disposable tip for MCA384, with filter Catalogus # 30138092.
- 50ul tips: 50 ul disposable tip for MCA384, with filter Catalogus #, 30138089.
- Plate seal: Clear-Seal-Weld, Kbiosystems Ltd, #9095-10102-078LR.

prepare the beads as described in:

Bead preparation protocol
by Peter Krijger
PREVIEW RUN

prepare the RT-qPCR mix as described in:

Multiplex SARS-CoV-2 RT-qPCR protocol
by Peter Krijger
PREVIEW RUN

Tecan Fluent configuration and protocol:

STRIP1_System configuration.pdf
STRIP-1_protocol_Fluent.pdf

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Preparation of the Fluent

1 Startup the Tecan Fluent.

2 Take out 10ml aliquots beads/isopropanol mixture from **4 °C** storage, vortex and resuspend in 2-Propanol as described in step 11-14 of the bead preparation protocol.

Bead preparation protocol
by Peter Krijger
PREVIEW RUN

Transfer the diluted beads into the spinvessel. Start the spinvessel, this will keep the beads in solution. Beads can be used for 5 days at RT.

2.1 Mix the beads by vortexing for **00:01:00** . 1m

2.2 Put the beads on a rollerbank for **00:30:00** . 30m

2.3 Aliquot 10 ml beads in 50ml tubes. 1m

2.4 Put the 50 ml tube on the magnet. 1m

2.5 When the solution is clear carefully remove the supernatant. 5m

2.6 Add 20 ml 2-Propanol. 1m

2.7 Mix by vortexing. 1m

2.8 Repeat step 4-7. 15m

2.9 Repeat step 4-5.

2.10 Resuspend beads in 10 ml 2-Propanol. Beads resuspended in 2-Propanol can be stored at 4°C for at least 5 days.

2.11 When ready to be used mix by vortexing and transfer the 10 ml beads to 150 ml 2-Propanol.

2.12 Add 20 ml 2-Propanol to the 50 ml tube to resuspend the remaining beads and transfer the diluted beads.

2.13 Repeat step 11.

2.14 Transfer the diluted beads (Total volume 200 ml) into the spinvessel. Start the spinvessel, this will keep the beads in solution. Beads can be used for 5 days at RT.

3 Refill Nuclease-Free Water in **80 °C** H2O bath and turn on H2O bath.

4 Refill 70% EtOH in the hydrospeed tank (Note: in future designs the hydrospeed will be replaced with a multidrop).

5 Place the RNA extraction plates, EtOH plates, plates, 384-well qPCR plates, 125 µl disposable tips, and 50 µl disposable tips in appropriate positions of the stacker.

6 Check if a sufficient amount of seal is present in the plate sealer.

7 Check if waste bins need to be emptied.

8 Prepare qPCR mix in a 50 ml tube wrapped in aluminum foil and place in cooling unit. Note: for each 384-well plate a new 50 ml tube with qPCR mix is prepared. 700 reactions of the RT-qPCR mix is prepared per 384-well plate due to the dead volume of the 50ml tube and tubing.

RNA extraction and qPCR setup 1m

9 Combine in 1 micronic tube rack:
- 1 micronic tube with the positive extraction control (position A1)
- 1 micronic tube carrying the negative extraction control (position B1)
- 1 micronic tube carrying the negative amplification control (position C1)
- 93 micronic tubes carrying swab specimens

10 Briefly spin the micronic tube racks in an under bench centrifuge (**00:01:00** , **500 x g**) and place in the Tecan Fluent carousel with the barcode facing the user. 1m

11 Start the RNA extraction and qPCR setup script to initiate the automated workflow:

11.1 The 2D barcodes of the collection tubes are scanned using a Ziath Mirage Barcode Scanner.

11.2 A 150 µl RNA extraction plate is placed at the deck.

11.3 Micronic tubes are de-capped per tube-rack (96 samples) in the Brooks IntelliXCap 96 Capping/Decapping Unit.

11.4 125 µl pipet tips are attached to the MCA96 head. 30 µl bead solution is aspirated, then 60 µl sample is aspirated, and the entire 90 µl is dispensed in the RNA extraction plate and mixed 5x.

11.5 The Micronic tubes are recapped and returned to the Tecan carousel.

11.6 Steps 11.1-11.5 are repeated for 3 other 96-tube racks, to completely fill the RNA extraction plate.

11.7 Incubate for **00:02:00** . 2m

11.8 The RNA extraction plate is placed on the magnetic deck and incubated for **00:03:00** . 3m

11.9 The 300 µl waste collection plate is placed at the deck.

11.10 The 150 µl EtOH plate is filled up with 150ul 70% ethanol using the Hydrospeed and placed at the deck.

11.11 The MCA 96 head is replaced for the MCA 384 head and 384x 125 µl tips are attached.

11.12 The supernatant present in the RNA extraction plate is aspirated using the 125 µl tips and transferred to the waste plate.

11.13 The RNA extraction plate is placed at the deck.

11.14 Using the same pipet tips, 30 µl 70% EtOH is aspirated from the EtOH plate, dispensed into the RNA extraction plate, and mixed 3 times.

11.15 The RNA extraction plate is placed on the magnetic deck and incubated for **00:00:30** . 30s

11.16 Using the same pipet tips, the Etoh is aspirated from the extraction plate and transferred to the waste plate.

11.17 Step 11.13- 11.16 is repeated 3x (4x total).

11.18 The RNA extraction plate is placed at the Bioshake and heated at **80 °C** for **00:02:00** . 2m

11.19 The waste plate is sealed and transferred to the waste bin.

11.20 The RNA extraction plate is placed at the deck.

11.21 The lid from the **80 °C** H2O bad is removed and using 50 µl 384 pipet tips attached to the MCA 384 head 25 µl H2O is aspirated, dispensed in the extraction plate, and mixed 21x (**00:02:00** total incubation) to elute the RNA. 2m

11.22 In parallel, 7.5 µl of the multiplex RT-qPCR master mix is added to a barcoded 384-well qPCR plate.

11.23 7.5 µl of RNA extracted from the samples (out of 25 µl total RNA) is transferred from the extraction plate to the qPCR plate using the tips to pipet the H2O.

11.24 The qPCR plate is sealed.

11.25 The barcode of the sealed qPCR plate is scanned and the plate is stored in the robot, ready for RT-qPCR analysis.

11.26 The EtOH plate is transferred to the waste bin.

12 Briefly spin the qPCR plate (**500 x g, 00:01:00**) and transfer immediately to a Quantstudio 7 thermocycler. 1m

13 Run the RT-qPCR program.

A	B	C
	Time (s)	Temp (°C)
1x	300	55
1x	20	95
40x	3	95
	30	58

RT-qPCR program Quantstudio 7 thermocycler

14 The processing of the RT-qPCR output is automated using custom-designed LabTrain software by Bodegro. Positive samples are manually curated by trained professionals. Negative samples that show inhibition of PDV amplification (Cq > 33) are repeated. After manually curation, the results (positive or negative for SARS-CoV-2/COVID-19) are sent digitally to CoronIT (software designed for the Dutch Municipal Health service / GGD). A clinical GGD doctor informs and advises the person who has undergone the covid-19 test about the PCR result.