Sections Action Sequence

**Section A**

1) Set the temperature module at 4°C

2) Put 96 deepwell plate into slot 1

3) Put 20 µl filter tips rack into slot 7

4) Put 300 µl filter tips rack into slot 8

5) Load 96 patient samples into 1.5 ml Eppendorf tubes (depends on sample collections, are they already delivered in these?)

\* Ideally samples are already in the tubes which are put into the opentron rack

4 racks x 24 tubes (slots 2, 3, 5, 6)

6) Load the positive control into 2ml Eppendorf and put it into temperature module (slot 4)

7) Calibrate opentron (there are instructions on computer)

8) Run the opentron protocol A

9) After 30 min take out the extraction plate from slot 1 and load it to the opentron B

10) Repeat steps 2-9

**Section B**

1. Move results plate from Opentrons A to Opentrons B.
2. Run the RNA Extraction Program using Opentrons B.

**Section C**

1. Thaw 8x 4X Master Mix and primer/probe mix
2. Mix by inversion, briefly centrifuge and return to cold block
3. Prepare 8x195 uL sample reservoirs in OT2
4. Prepare 500 uL nuclease-free reservoir in OT2
5. Prepare 500 uL nCoVPC resevoir in OT2
6. Move results plate from Opentrons B to Opentrons C.
7. Run Assay Prep Program using Opentrons C.
8. Move prep assay plate into the qPCR thermocycler.
9. Run nCov-19 qPCR program on the qPCR thermocycler.
10. Obtain results & enter them.
11. Dispose of samples.
12. Repeat

NB: Preparing Section C stock 4X Master Mix & Primer/Probe mix

Using aseptic technique, suspend dried reagents in 1.5 mL nuclease-free water and allow to rehydrate for 15 min at room temperature in the dark. Mix gently and aliquot primers/probe in 300 μL volumes into 5 pre-labeled tubes. Store a single aliquot of primers/probe at 2-8oC in the dark. Do not refreeze (stable for up to 4 months). Store remaining aliquots at ≤-20oC in a non-frost-free freezer.