Version 1

Opentrons COVID-19 testing V.1

Max Marrone[1]

[1]Opentrons Labworks

In Development dx.doi.org/10.17504/protocols.io.bd5wi87e

Opentrons COVID-19 Testing

Max Marrone

ABSTRACT

Opentrons and the Open Medicine Institute are developing an automated high-throughput COVID-19 testing protocol to submit to the FDA for an Emergency Use Authorization as a diagnostic.

The standard assay for this type of infectious disease testing is quantitative PCR (qPCR), and in this case reverse transcriptase qPCR since COVID-19 is an RNA virus. After patient samples are collected in public health facilities, doctors offices, and hospitals, they are sent to the lab for processing, which happens in four steps:

1. Sample Intake
2. RNA Extraction
3. qPCR Setup
4. RT-qPCR Assay

Opentrons OT-2 robots carry out most of this work. However, human operators are needed for some key tasks, like:

- Moving samples between stations
- Preparing certain reagents
- Running the qPCR machine
- Logging data

GUIDELINES

Robot calibration: To calibrate labware height on the OT-2, we recommend placing something thin and rigid atop the labware and jogging the tip down until it just barely touches, so you don't have to try to eyeball the top plane. We used NYC Metrocards, which are 0.010 inches thick.

Labware: This protocol was designed and tested with the exact labware mentioned in the .py files. If you use other labware, the OT-2 may not properly position the pipette tip within wells.

MATERIALS

NAME	CATALOG #	VENDOR
Ethyl alcohol, Pure 200 proof, for molecular biology	E7023	Sigma Aldrich
Molecular grade H20	W4502	Sigma Aldrich
2-propanol	I9516	Sigma Aldrich
NEST 1 Well Reservoir 195 mL	360103	Opentrons
NEST 12 Well Reservoir 15 mL	360102	Opentrons
NEST 96 Well Plate 100 µL PCR Full Skirt	402501	Opentrons
Eppendorf Safe-Lock Tubes 1.5 mL PCR clean colorless 500 tubes	022363212	Eppendorf

MATERIALS TEXT

Materials

Station A: Sample Intake Reagents

- Collection tubes

Citation: Max Marrone ( ). Opentrons COVID-19 testing. https://dx.doi.org/10.17504/protocols.io.bd5wi87e

- Internal extraction control RNA
  - From BP Genomics' pureBASE COVID19 Detection Assay kit
  - Resuspended according to BP's instructions
- Lysis buffer
  - From BP Genomics' pureBASE RNA for Buccal, Saliva & Tissue kit
- Proteinase K
  - From BP Genomics' pureBASE RNA for Buccal, Saliva & Tissue kit

Station B: RNA Extraction Reagents
- BP Genomics wash buffer 1
  - From BP Genomics' pureBASE RNA for Buccal, Saliva & Tissue kit
- BP Genomics magnetic beads
  - From BP Genomics' pureBASE RNA for Buccal, Saliva & Tissue kit
- 100% microbiology grade isopropanol
- Freshly-prepared 70% ethanol (microbiology grade)
- Nuclease free water

Station B: RNA Extraction Labware
- NEST 1-well reservoir (for liquid waste)
- Sterile NEST 12-well reservoir (for reagents)
- Sterile NEST 100 µL PCR plate

Station C: qPCR Prep Reagents
- BP Genomics Test Kit
  - pureBASE 2X RT-qPCR Master Mix
  - 2019-nCoV primer/probe mix
  - Internal extraction control primer/probe mix
  - Endogenous human control primer/probe mix
  - 2019-nCoV positive control RNA
- RNase/DNase free water

Station C: qPCR Prep Equipment
- Sterile, RNase/DNase free 1.5 mL eppendorf tubes
- Pipettes with sterile, RNase free filter tips
- Vortexer
- Cold block or ice bucket

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

BEFORE STARTING

Download the OT-2 protocol and labware files from GitHub.

Station A: Sample Intake

1 Clean the robot and labware.

2 Set up the OT-2's deck:
- **Slot 7:** Temperature Module with Opentrons 96 aluminum block
- **Slot 8:** A full, sterile rack of Opentrons 20 µL filter tips
- **Slot 9:** A full, sterile rack of Opentrons 200 µL filter tips

3 Start pre-cooling the Temperature Module to **4 °C** .

Citation: Max Marrone ( ). Opentrons COVID-19 testing. https://dx.doi.org/10.17504/protocols.io.bd5wi87e

4 Centrifuge the collection tubes at **14000 rpm 00:05:00** .

5 Place the tubes in an Opentrons 24 tube rack (the sample tube rack). A1 is sample 1, B1 is sample 2, etc.

6 Set up the reagents in an Opentrons 24 tube rack:

- **Position C1:** a sterile 1.5 mL thin-walled PCR tube containing **1.3 ml lysis buffer**
- **Position D1:** a sterile 1.5 mL thin-walled PCR tube containing **1.3 ml lysis buffer**
- **Position D2:** a sterile 1.5 mL thin-walled PCR tube containing **200 µl proteinase K**

7 Uncap the sample tubes in their rack on the deck.

8 Place a single generic 200 µL PCR tube containing **35 µl internal extraction control RNA** in **well H1** of the aluminum block.

9 Run the Station A protocol on the OT-2. Wait for the run to finish.

10 The NEST 96 deep well plate in slot 1 contains the samples ready for Station B. Collect it.

11 Throw out the partially used tip racks and the sample collection tubes.

Station B: RNA Extraction

12 Clean the robot.

13 On the OT-2's deck, set up the labware that isn't time-sensitive.

- **Slot 1:** a Temperature Module with:
- an Opentrons 96 well aluminum block and empty NEST 100 µL PCR plate, sterile
- **Slot 4:** An Opentrons Magnetic Module, with the magnets disengaged
- **Slot 6:** A full, sterile rack of Opentrons 200 µL filter tips
- **Slot 11:** an empty NEST 1-well reservoir, for organic liquid waste

14 Optionally, start pre-cooling the Temperature Module to **4 °C** . This will save waiting for it to cool down when the run starts on the OT-2.

Citation: Max Marrone ( ). Opentrons COVID-19 testing. https://dx.doi.org/10.17504/protocols.io.bd5wi87e

15 Prepare **4.2 ml IPA + magnetic beads** :

15.1 In a sterile 15 mL Falcon tube, add **4 ml IPA** .

15.2 Vortex BP Genomics magnetic beads in their container for 20 seconds at high speed.

15.3 Add **200 µl vortexed magnetic beads** to the Falcon tube.

16 Prepare a sterile NEST 12 well reservoir:

- **Well 6:** Pipette **6 ml BP Genomics wash buffer 1** (prepared according to the manufacturer).
- **Wells 8, 9, and 10:** Pipette **6 ml freshly-prepared 70% ethanol** into each well.
- **Well 12:** Pipette **3 ml nuclease-free water** .
- **Well 1:** Vortex the Falcon tube of **4.2 ml IPA + magnetic beads** once more, for 20 seconds at high speed. Then, pipette the entire solution to well 1 of the NEST 12 well reservoir. The solution should look uniform (no beads settled).

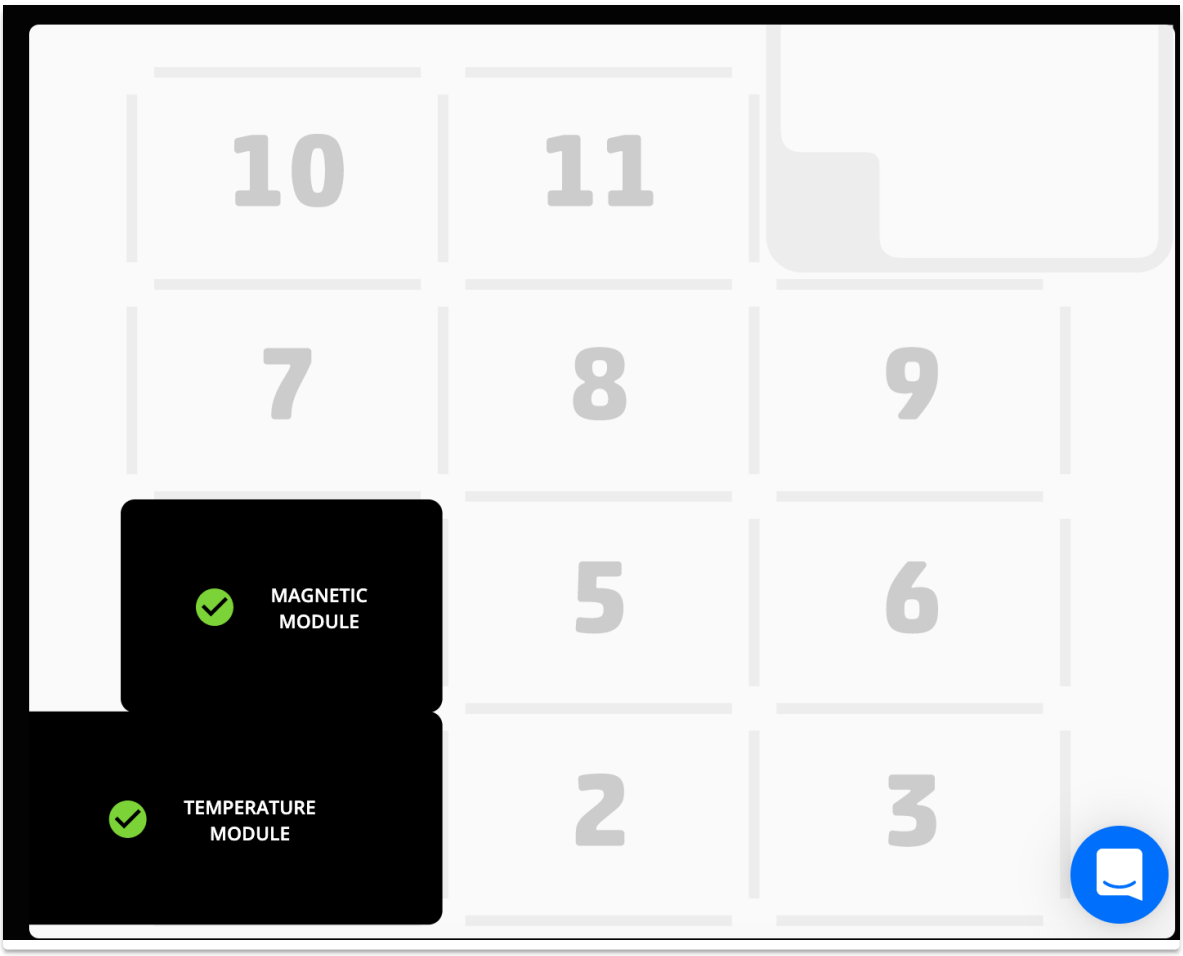
This step is time-sensitive. As soon as you pipette the beads into well 1, they will start to settle slowly. You should continue quickly to start the run on the OT-2.

Place the NEST 12 well reservoir on slot 2 of the robot.

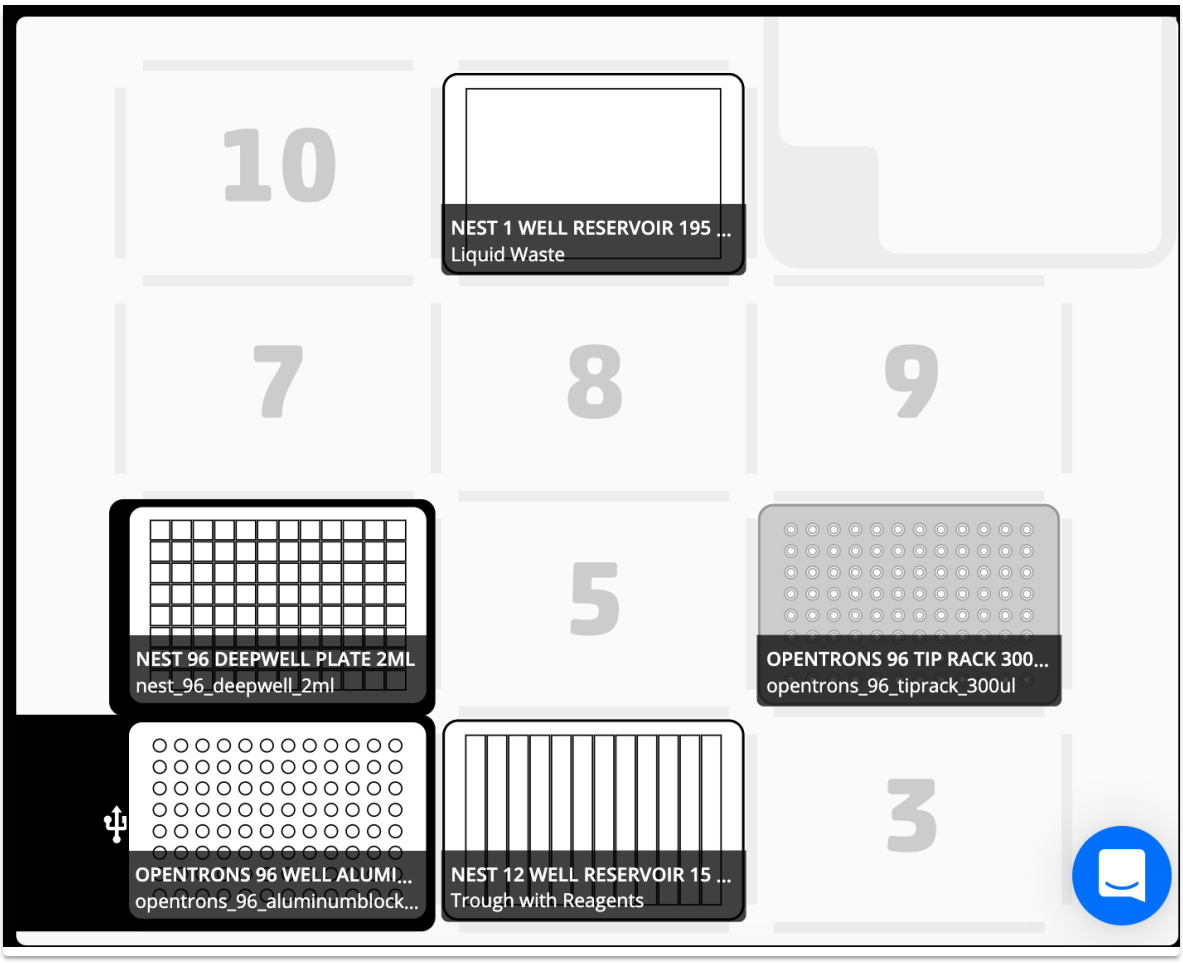
17 Place the NEST 96 deep well plate that was output from Station A onto the Magnetic Module in slot 4. You don't need to lock it down with the Magnetic Module bracket.

18 The OT-2's deck should now look like this:

Citation: Max Marrone ( ). Opentrons COVID-19 testing. https://dx.doi.org/10.17504/protocols.io.bd5wi87e



Citation: Max Marrone ( ). Opentrons COVID-19 testing. https://dx.doi.org/10.17504/protocols.io.bd5wi87e



19 Run the Station B protocol on the OT-2. Wait for the run to finish.

20 The output is the 96 PCR plate atop the Temperature Module.

When you're ready to move on to station C, collect the cool aluminum block with the PCR plate on top.

The condensation on the aluminum block might make it stick to the Temperature Module a little. Be careful when removing it.

We've left the output on Station B for up to an hour before moving it to Station C.

Station C: qPCR Prep Reagent Preparation

21 Prepare reaction mixtures:

Citation: Max Marrone ( ). Opentrons COVID-19 testing. https://dx.doi.org/10.17504/protocols.io.bd5wi87e

21.1 In a 1.5 mL Eppendorf tube, prepare the Reaction Mix **On ice** :

Name	Volume per sample (µL)	Total for 8 samples, plus 10% margin (µL)
pureBASE 2X RT-qPCR Master Mix	10	88
2019-nCoV primer/probe mix (BROWN)	1	8.8
Internal extraction control primer/probe mix	1	8.8
RNase/DNase free water	3	26.4

21.2 In a 1.5 mL Eppendorf tube, prepare the Endogenous Control Reaction Mix **On ice** :

Name	Volume per reaction (uL)	Total
pureBASE 2X RT-qPCR Master Mix	10	88
Endogenous control primer/probe mix	1	8.8
RNase/DNase free water	4	35.2

21.3 In a 1.5 mL Eppendorf tube, prepare the Standard Curve Reaction Mix **On ice** :

Name	Volume per reaction (uL)	Total
pureBASE 2X RT-qPCR Master Mix	10	88
2019-nCoV primer/probe mix	1	8.8
RNase/DNase free water	4	35.2

22 Prepare the Standard Curve Dilution Series:

Make a 1:10 dilution series of the Positive Control Template (RED) **On ice** across 7 dilutions.

Name	Dilution	Copy Number (#/uL)	Reaction Copy Number
PCD 1	0	2 x 10^5	1000000
PCD 2	1	2 x 10^4	100000
PCD 3	2	2 x 10^3	10000
PCD 4	3	2 x 10^2	1000
PCD 5	4	2 x 10^1	100
PCD 6	5	2 x 10^0	10
PCD 7	6	2 x 10^-1	1
PCD 8	7	2 x 10^-2	0.1

23 Prepare the Internal Control RNA working solution:

Add **4 µl internal extraction control RNA** to **26 µl DNase/RNase free water** in a 1.5 mL Eppendorf tube **On ice** for a total volume of 30 uL, and briefly vortex.

24 Prepare the Negative Control working solution:

Add **20 µl DNase/RNase free water** to a 1.5 mL Eppendorf tube.

Citation: Max Marrone ( ). Opentrons COVID-19 testing. https://dx.doi.org/10.17504/protocols.io.bd5wi87e

25 If you're ready to load the robot immediately, continue to the next step. If not, freeze the reaction mixtures at **-20 °C** until needed.

Station C: qPCR Prep Robot Loading

26 Clean the inside of the robot with RNaseZap:

26.1 Apply RNaseZap liberally to a paper towel and wipe all work surfaces inside the robot.

26.2 Rinse with water.

26.3 Wipe dry.

27 Load the labware into the robot:

27.1 Load the reaction mixtures and standards into the tube rack:

Row	1	2	3	4	5	6
A	Endogenous Control Mix	Sample Reaction Mix	Standard Curve Mix	RNase/DNase free water		
B						
C	Internal Control RNA				PCD 8	PCD 7
D	PCD 6	PCD 5	PCD 4	PCD 3	PCD 2	PCD 1

27.2 Load two racks of sterile p20 filter tips into positions 2 and 3.

27.3 Place a new, sterile, half-skirt 96 well PCR plate on the cold block, on the temperature deck at position 4.

27.4 Load the sample elution plate from **Station B**.

28 Run the Station C protocol on the robot. Wait for the run to finish.

Citation: Max Marrone ( ). Opentrons COVID-19 testing. https://dx.doi.org/10.17504/protocols.io.bd5wi87e

29 Unload the qPCR plate from the Temperature Module on slot 4 by removing both the plate and the aluminum block.

30 Seal the qPCR plate.

31 Move the qPCR plate to **Station D** for analysis.

Citation: Max Marrone ( ). Opentrons COVID-19 testing. https://dx.doi.org/10.17504/protocols.io.bd5wi87e