Mar 31, 2020

Opentrons COVID-19 testing (24 samples) V.2

Max Marrone[1]

[1]Opentrons Labworks

In Development dx.doi.org/10.17504/protocols.io.bd8ui9ww

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

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
- Internal extraction control RNA

Citation: Max Marrone (03/31/2020). Opentrons COVID-19 testing (24 samples). https://dx.doi.org/10.17504/protocols.io.bd8ui9ww

- 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

BEFORE STARTING

When placing labware on the OT-2's deck:

1. Make sure the labware is properly inserted. You should feel a click, and the labware should sit flat.
2. Make sure the labware is inserted the right way around, with well A1 at the top left.

Station A: Initial OT-2 setup

1 Clean the Station A OT-2.

Cleaning an OT-2 COVID-19 Diagnostic Station
by Max Marrone
PREVIEW RUN

Citation: Max Marrone (03/31/2020). Opentrons COVID-19 testing (24 samples). https://dx.doi.org/10.17504/protocols.io.bd8ui9ww

1.1 Wipe these parts of the OT-2 down with a 1:10 dilution of bleach:
1. The clear polycarbonate windows.
2. The black pipette stems. (Avoid the rest of the pipettes, including the ejectors.)
3. The aluminum deck.
4. The removable black trash bin.

1.2 Wait **00:00:30** , then quickly rinse the bleach off with distilled water.

The aluminum on the OT-2 will be discolored if the bleach sits for too long. In the long term, it may also cause more serious corrosion.

1.3 Wipe these parts of the OT-2 down with RNaseZap or RNase AWAY.

The same parts that you wiped down with bleach:
1. The clear polycarbonate windows.
2. The black pipette stems. (Avoid the rest of the pipettes, including the ejectors.)
3. The aluminum deck.
4. The removable black trash bin.

Plus these additional parts:
1. The bottoms of the pipette ejectors.
2. Any Temperature Modules or Magnetic Modules that the OT-2 has on its deck.
3. Any 96 well aluminum blocks that are going to be used on the OT-2.

1.4 Rinse the RNaseZap or RNase AWAY off with distilled water.

1.5 Wipe the OT-2 dry, or let the water evaporate.

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

3 Place the following labware on the OT-2's deck:

- **Slot 1:** An empty, sterile NEST 96 deep well plate.
- **Slot 7:** Temperature Module with Opentrons 96 aluminum block.
- **Slot 8:** A full, sterile rack of Opentrons 10 µL filter tips.
- **Slot 9:** A full, sterile rack of Opentrons 200 µL filter tips.

Station A: Reagent preparation

4 Prepare the **sample collection tube rack.**

4.1 Give the collection tubes a flick to make sure all the liquid has settled to the bottom.

(Some liquid might be trapped against the swab scraper built into the tube.)

Citation: Max Marrone (03/31/2020). Opentrons COVID-19 testing (24 samples). https://dx.doi.org/10.17504/protocols.io.bd8ui9ww

4.2 Uncap the collection tubes and place them in an Opentrons 24 tube rack.

Sample 1 goes in position A1, sample 2 goes in position B1, and so on. Top to bottom, then left to right.

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Row A	#1	#5	#9	#13	#17	#21
Row B	#2	#6	#10	#14	#18	#22
Row C	#3	#7	#11	#15	#19	#23
Row D	#4	#8	#12	#16	#20	#24

5 Prepare the **reagent tube rack.**

In an Opentrons 24 2 mL tube rack, place:

- **Positions C1, C2, C3, D1, D2, D3:** a sterile 1.5 mL tube containing **1.3 ml lysis buffer** , each.
- **Position D2:** a sterile 1.5 mL tube containing **580 µl proteinase K** .

Remember to fold the tubes' caps back and secure them in their slots in the tube rack.

6 Ensure the Temperature Module has reached **4 °C** .

7 Place a single generic 200 µL PCR tube containing **110 µl internal extraction control RNA** in **well H1** (the bottom-right) of the Opentrons 96 aluminum block.

Station A: Final OT-2 setup

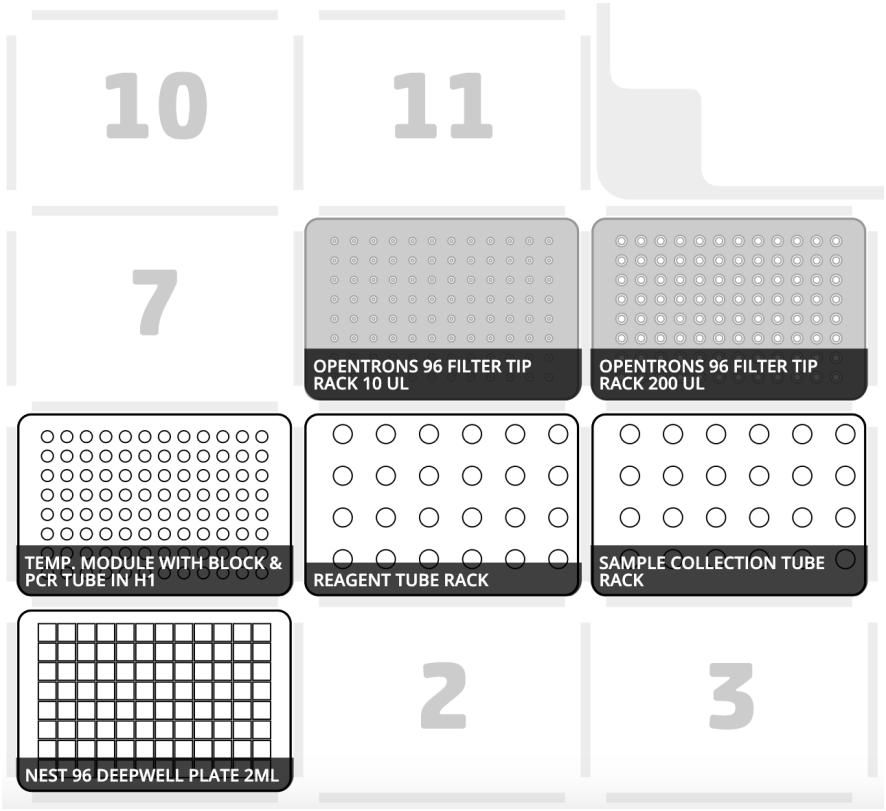
8 Place the **reagent tube rack** in **slot 4.**

9 Place the **sample collection tube rack** in **slot 5.**

Citation: Max Marrone (03/31/2020). Opentrons COVID-19 testing (24 samples). https://dx.doi.org/10.17504/protocols.io.bd8ui9ww

10 Double-check all the labware to make sure it looks correct.

- Check that labware are inserted the right way around (well A1 at the top-left).
- Check that labware are properly clicked into the deck slots.
- Check that the tubes are seated flat in their tube racks.



Station A: Running the OT-2

11 Run the Station A protocol on the OT-2.

11.1 In the **Run** tab, click **Start run**. The OT-2 will home its motors and then begin the protocol.

Do not click **Start run** more than once. A known bug will make the OT-2 run the protocol back-to-back.

If you need to cancel the protocol for any reason, use the power switch to turn off the OT-2. When it turns back on, the pipettes will rise. If the pipettes had tips attached, you will need to manually remove them before starting again.

Citation: Max Marrone (03/31/2020). Opentrons COVID-19 testing (24 samples). https://dx.doi.org/10.17504/protocols.io.bd8ui9ww

11.2 Wait for the run to finish.

You can use this time to start preparing Station B.

Station A: Finishing up

12 Collect the **NEST 96 deep well plate** from **slot 1.** Move it to Station B.

13 Throw out the partially used tip racks and empty reagent tubes. Remove the used sample collection tubes.

Station B: Initial OT-2 setup

14 Clean the Station B OT-2.

Cleaning an OT-2 COVID-19 Diagnostic Station
by Max Marrone

PREVIEW RUN

14.1 Wipe these parts of the OT-2 down with a 1:10 dilution of bleach:
1. The clear polycarbonate windows.
2. The black pipette stems. (Avoid the rest of the pipettes, including the ejectors.)
3. The aluminum deck.
4. The removable black trash bin.

14.2 Wait **00:00:30** , then quickly rinse the bleach off with distilled water.

The aluminum on the OT-2 will be discolored if the bleach sits for too long. In the long term, it may also cause more serious corrosion.

14.3 Wipe these parts of the OT-2 down with RNaseZap or RNase AWAY.

The same parts that you wiped down with bleach:
1. The clear polycarbonate windows.
2. The black pipette stems. (Avoid the rest of the pipettes, including the ejectors.)
3. The aluminum deck.
4. The removable black trash bin.

Plus these additional parts:
1. The bottoms of the pipette ejectors.
2. Any Temperature Modules or Magnetic Modules that the OT-2 has on its deck.
3. Any 96 well aluminum blocks that are going to be used on the OT-2.

Citation: Max Marrone (03/31/2020). Opentrons COVID-19 testing (24 samples). https://dx.doi.org/10.17504/protocols.io.bd8ui9ww

14.4 Rinse the RNaseZap or RNase AWAY off with distilled water.

14.5 Wipe the OT-2 dry, or let the water evaporate.

15 Place the following labware on the OT-2's deck:

- **Slot 1:** A Temperature Module with an Opentrons 96 well aluminum block and an empty, sterile NEST 100 µL PCR plate.
- **Slot 3:** A full, sterile rack of Opentrons 200 µL filter tips.
- **Slot 4:** A Magnetic Module with nothing on it.
- **Slot 6:** A full, sterile rack of Opentrons 200 µL filter tips.
- **Slot 11:** An empty NEST 1-well reservoir, for organic liquid waste.

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

Station B: Reagent preparation

17 Prepare 3x **4.2 ml IPA + magnetic beads** :

17.1 In a sterile 15 mL Falcon tube, add **4 ml isopropyl alcohol** .

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

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

17.4 Repeat for a total of **3 Falcon tubes** of **IPA + magnetic beads.** **go to step #17**

18 Wait for the Temperature Module to reach **4 °C** .

19 Prepare **reagent trough 1.**

In a sterile NEST 12 well reservoir, add:

- **6 ml 70% ethanol** to each of the **wells 1—9** (**54 ml 70% ethanol** total).
- **3 ml nuclease-free water** to **well 11**.

20 Prepare **reagent trough 2.**

In a separate, sterile NEST 12 well reservoir:

20.1 Add **6 ml Wash Buffer 1** to each of the **wells 10–12** (**18 ml Wash Buffer 1** total).

20.2 Vortex the tubes of **IPA + magnetic beads.**

Citation: Max Marrone (03/31/2020). Opentrons COVID-19 testing (24 samples). https://dx.doi.org/10.17504/protocols.io.bd8ui9ww

20.3 For each of the **wells 1–3**, pipette an entire tube of **4.2 ml IPA + magnetic beads** into the well.

This step is time-sensitive. As soon as you pipette the beads into well 1, they will start to settle. Continue quickly through the next steps.

Station B: Final OT-2 setup

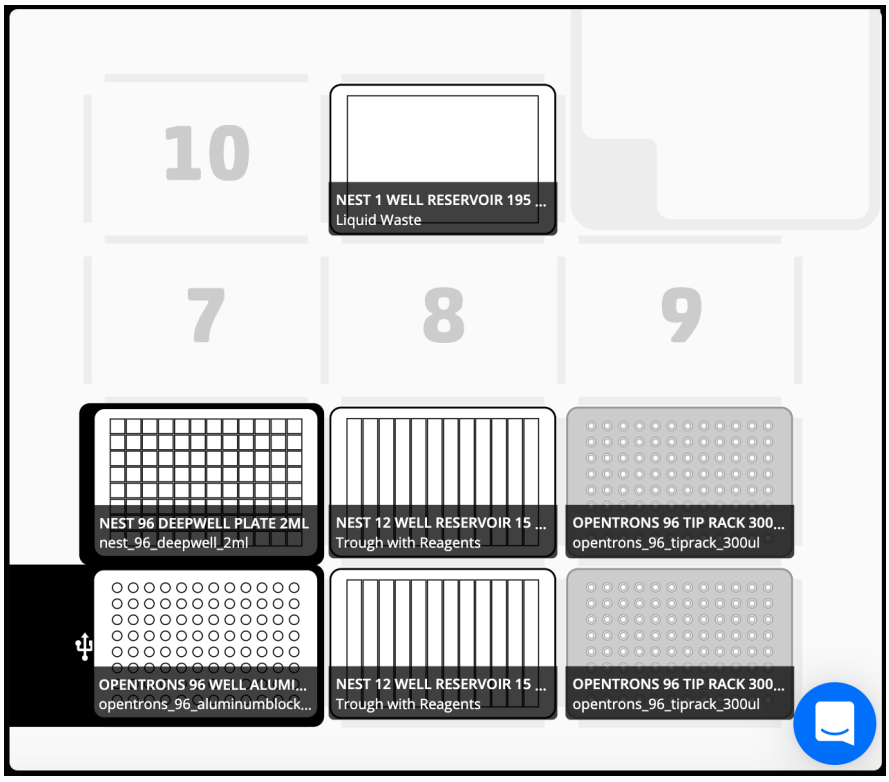
21 Place the **NEST 96 deep well plate** that was output from Station A onto the Magnetic Module in **slot 4**.

22 Place **reagent reservoir 1** in **slot 2.**

23 Place **reagent reservoir 2** in **slot 5.**

24 Double-check all the labware to make sure it looks correct.

- Check that labware are inserted the right way around (well A1 at the top-left for the plates, well 1 at the left for the troughs).
- Check that labware are properly clicked into the deck slots.



Station B: Running the robot

25 Run the Station B protocol on the OT-2.

Citation: Max Marrone (03/31/2020). Opentrons COVID-19 testing (24 samples). https://dx.doi.org/10.17504/protocols.io.bd8ui9ww

25.1 In the **Run** tab, click **Start run**. The OT-2 will home its motors and then begin the protocol.

Do not click **Start run** more than once. A known bug will make the OT-2 run the protocol back-to-back.

If you need to cancel the protocol for any reason, use the power switch to turn off the OT-2. When it turns back on, the pipettes will rise. If the pipettes had tips attached, you will need to manually remove them before starting again.

25.2 Wait for the run to finish.

26 The output is the **NEST 100 µL PCR plate** sitting on the aluminum block atop the Temperature Module in **slot 1.**

When you're ready to move on to station C, collect the PCR plate.

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

27 Prepare reaction mixtures:

27.1 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
Nuclease-free water	3	26.4

27.2 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
Nuclease-free water	4	35.2

Citation: Max Marrone (03/31/2020). Opentrons COVID-19 testing (24 samples). https://dx.doi.org/10.17504/protocols.io.bd8ui9ww

27.3 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
Nuclease-free water	4	35.2

28 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

29 Prepare the Internal Control RNA working solution:

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

30 Prepare the Negative Control working solution:

Add **20 µl nuclease free water** to a 1.5 mL Eppendorf tube.

31 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

32 Clean the OT-2.

1. Wipe down the OT-2 with a 10% dilution of bleach. Let dry.
2. Wipe down the OT-2 with RNaseZap or RNase AWAY.

See also: Cleaning your OT-2

Citation: Max Marrone (03/31/2020). Opentrons COVID-19 testing (24 samples). https://dx.doi.org/10.17504/protocols.io.bd8ui9ww

33 Load the labware into the robot:

33.1 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	Negative Control		
B						
C	Internal Control RNA				PCD 8	PCD 7
D	PCD 6	PCD 5	PCD 4	PCD 3	PCD 2	PCD 1

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

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

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

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

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

36 Seal the qPCR plate.

37 Move the qPCR plate to the qPCR machine for analysis.

38

Citation: Max Marrone (03/31/2020). Opentrons COVID-19 testing (24 samples). https://dx.doi.org/10.17504/protocols.io.bd8ui9ww