Opentrons COVID-19 testing (24 samples) V.3

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[1]Opentrons Labworks

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Opentrons COVID-19 Testing

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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

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.beesjbee

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 20 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 If needed, 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.)

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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 D6:** 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-left) 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.**

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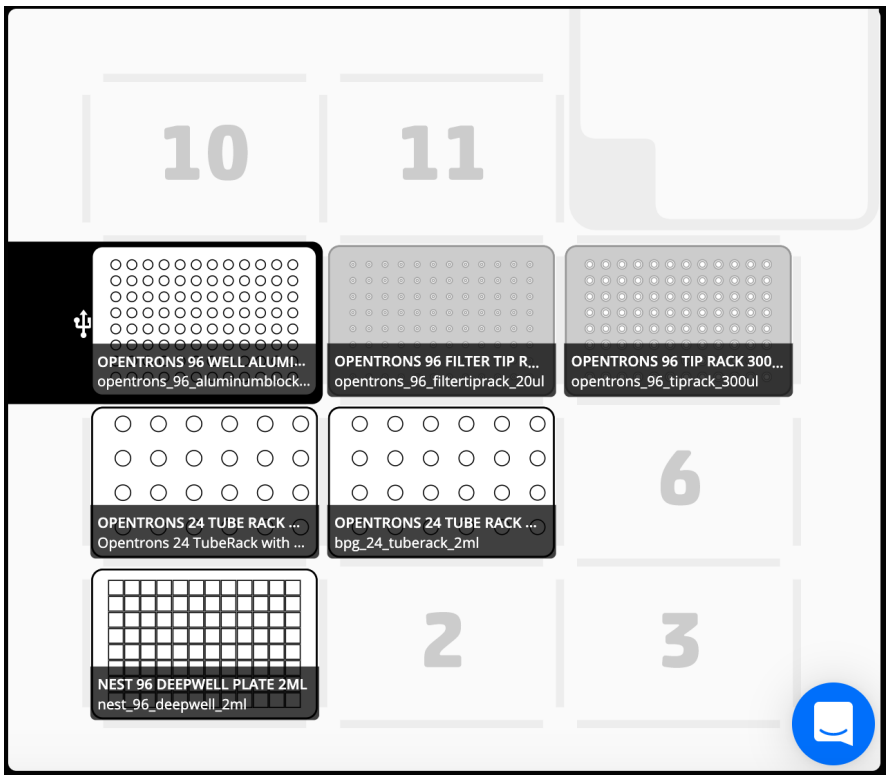
10 Double-check all the labware to make sure it looks correct.

- 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.

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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.** You will move it to Station B later.

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.

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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.**

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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

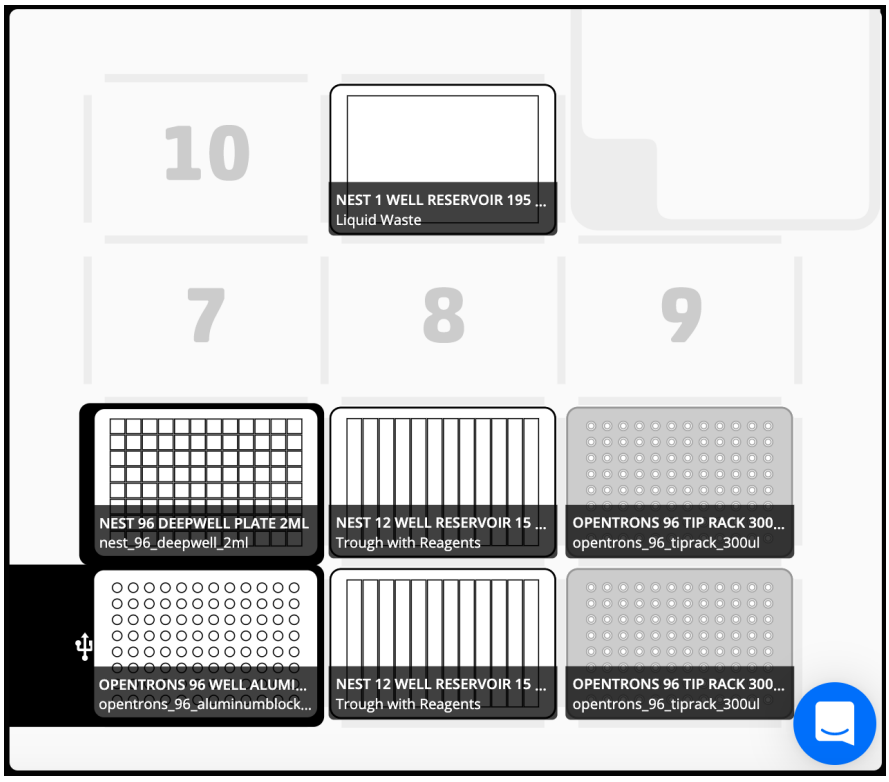
21 Place the **NEST 96 deep well plate** that was output from Station A onto the Magnetic Module in **slot 4**.

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.

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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.

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