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Opentrons COVID-19 testing (RT-qPCR path, Station C, 24+3+1 sample special) V.2

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[1]Opentrons Labworks

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

Coronavirus Method Development Community | Opentrons COVID-19 Testing

Max Marrone

1 Clean the OT-2.

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

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.

Citation: Max Marrone (04/09/2020). Opentrons COVID-19 testing (RT-qPCR path, Station C, 24+3+1 sample special). https://dx.doi.org/10.17504/protocols.io.beqajdse

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 **6 °C** .

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3

Prepare the **reagent tube rack.**

In an **Opentrons 24 tube rack,** place the following reagents from the BP Genomics 2019-nCoV Detection Assay kit, prepared according to the kit's instructions.

- **Well A1: 1000 µl Reaction Mix**
- **Well B1: 500 µl Endogenous Control Mix**
- **Well B3: 50 µl nuclease-free water**
- **Well D3: 50 µl standard curve dilution 4** (Positive Control Template diluted to 200 copies per µL, per BP Genomics' instructions)

	Col. 1	Col. 2	Col. 3	Col. 4	Col. 5	Col. 6	Col. 7	Col. 8
Row A	Reaction Mix							
Row B	Endogenous Control Mix		Nuclease-free water					
Row C								
Row D			Standard curve dilution 4					

Robot loading

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4 Load the labware into the robot.

In **Slot 4,** an empty, sterile **qPCR plate** atop an **Opentrons 96 well aluminum block** atop the **Opentrons Temperature Module:**

Temperature Module with 96 well aluminum block. qPCR plate not shown.

In **Slot 5,** the **reagent tube rack,** as prepared earlier.

In **Slot 3,** A full, sterile **Opentrons 20 µL filter tip rack:**

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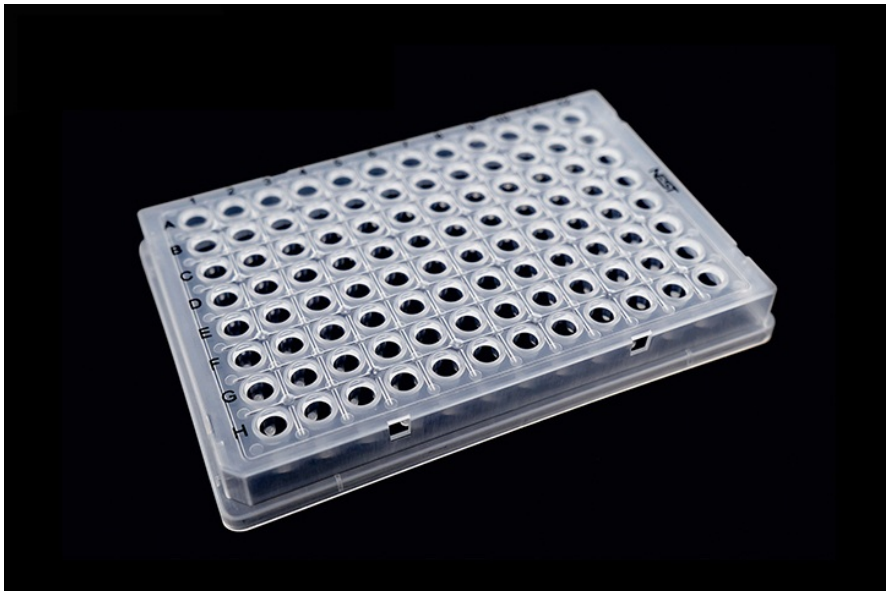
In **Slot 1,** the **elution plate.**

The elution plate (a **NEST 96 Well Plate 100 µL PCR Full Skirt**): should have:

- 27 samples from Station B starting at the top-left corner.
- Water filling the remaining wells, up to 6 column**s.**
- An extra sample in **well H12** (bottom-right).



	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	W	W						

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B	2	10	18	26	W	W						
C	3	11	19	27	W	W						
D	4	12	20	W	W	W						
E	5	13	21	W	W	W						
F	6	14	22	W	W	W						
G	7	15	23	W	W	W						
H	8	16	24	W	W	W						Manual samp.

5 Run **StationC-24-plus3-plus1-2020-04-09.py** on the robot.

Operating an OT-2 for COVID-19 testing
by Max Marrone

PREVIEW RUN

5.1 Open the Opentrons App.

5.2 Ensure you are connected to the robot. In the **Robots** tab, you can try flipping the robot's lights on and off to test the connection.

5.3 Go to the **Run** tab.

Double-check the name at the top to make sure the correct protocol is uploaded.

5.4 Click **Start run**. The OT-2 will home its motors and then begin the protocol.

Do not click **Start run** more than once. If you do, a known bug will make the OT-2 run the protocol back-to-back.

If something goes wrong and you need to abort the protocol:

1. Shut down the OT-2 with the power switch on its back left side.
2. Turn the OT-2 back on. Wait a couple of minutes for the pipettes to rise.
3. Manually remove any tips attached to the pipettes. (This ensures that the pipettes will not aspirate liquid into themselves when they home.)
4. Reconnect to the OT-2 in the Opentrons App. Click the Home button to move the gantry out of the way so you can access the labware on the deck.

6 Wait for the run to finish.

7 Add any additional manually-prepared samples, including their master mix, to empty wells of your choosing on the qPCR plate.

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