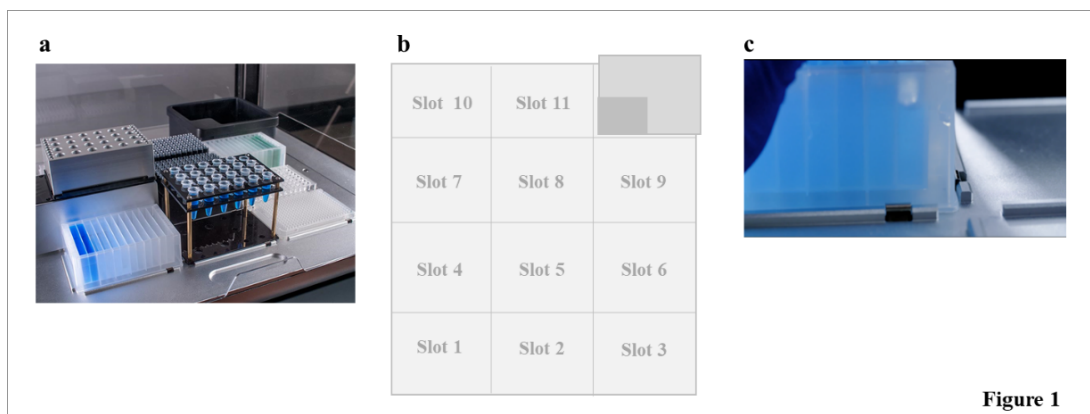


OPERATING OPENTRONS MACHINES BELONGING TO STATION A

ATTENTION

- Before starting, clean each OpenTrons machine belonging to Station A, following the instructions reported in the Standard Operating Procedure for Cleaning
- The deck of each OpenTrons machine has 11 different slots for the placement of the labware required for different processes (Figure 1a). A number is virtually assigned to each slot (Figure 1b). When placing any of the labware described below in the dedicate slot
 - check that the labware is properly inserted by pressing the corner into the metal springs (Figure 1c). You should feel a slight click and the labware should sit completely flat



- make sure that the labware is inserted in the right direction, *i.e.* the well numbered as A1 or as 1 is at the top left corner

- **This procedure applies to the OpenTrons Machines belonging to Station A, which are equipped with a p20 multi channel and a p300 single channel pipettes**

- **Materials required for operating each OpenTrons Machine belonging to Station A**

Item	Quantity
Temperature module	1
Rack for 200 μ L filter tips	3
200 μ L filter tips	288 (96*3)
Rack for 20 μ L filter tips	1
20 μ L filter tips	96
Sterile NEST 96 deep well plate	1
24 position tube rack	4
96 position aluminum block	1
200 μ L PCR strip tube	1
6 position tube rack	1
50 mL sterile Falcon tube	1
Pipette for measuring 1 mL	1
Tips for 1 mL	2
Graduated cylinder for measuring 20 mL	1
Ethanol for cleaning the external surface of tubes	n.a.
Heat plate / heat shaker for heating Lysates at 55-57 °C for 20 min	1
Aluminum seal	1

-

STEPS

1. **Start pre-cooling the temperature module to 4 °C. This is used to actively cool the internal extraction control RNA that will be added to each sample**
2. **Place**
 - a sterile rack full of 200 µL filter tips in **SLOT 11** of the deck of each OpenTrons machine belonging to Station A (**Figure 2**)



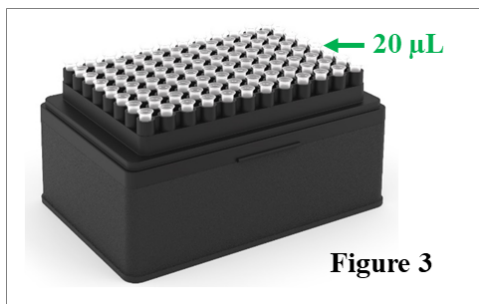
- a sterile rack full of 200 µL filter tips in **SLOT 9** of the deck of each OpenTrons machine belonging to Station A (**Figure 2**)



- a sterile rack full of 200 µL filter tips in **SLOT 8** of the deck of each OpenTrons machine belonging to Station A (**Figure 2**)



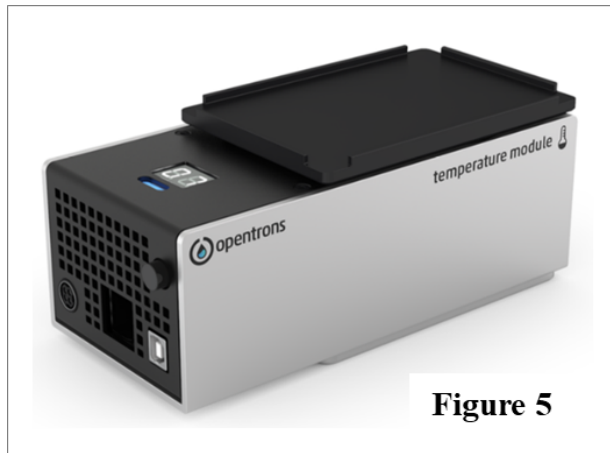
- a sterile rack full of 20 µL filter tips in **SLOT 10** of the deck of each OpenTrons machine belonging to Station A (**Figure 3**)



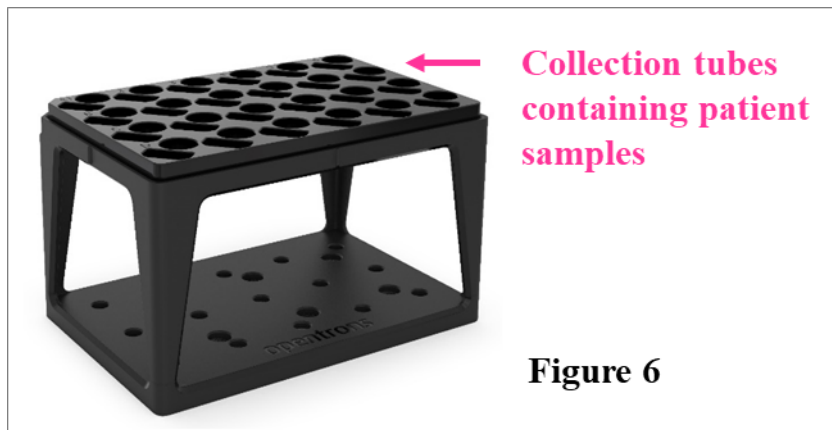
- an empty, sterile NEST 96 deep well plate in **SLOT 4** of the deck of each OpenTrons machine belonging to Station A (**Figure 4**)



- the temperature module in **SLOT 1** of the deck of each OpenTrons machine belonging to Station A (**Figure 5**)



3. **Prepare 4 different 24 position tube racks for patient samples (*i.e.* Rack 1, Rack 2, Rack 3, Rack 4) as follows. Each rack can hold 24 collection tubes**
- Important notes
 - clean with ethanol the external surface of collection tubes before opening
 - observe best practices for safely handling the collection tubes full of patient sample
 - if needed, to give the collection tubes a flick so that all the liquid should sit on the bottom
 - always place in position A1 of the Rack 1 a blank collection tube, *i.e.* a collection tube only containing the initial collection medium in which no swabs have been placed. These samples must result negative at the end of the testing process. This way we will have an extra control on the quality of the results from the very beginning of the process
 - always keep position A1 of the Rack 4 empty. This empty position will be used to add the positive control in the Station C
 - uncap the collection tubes containing the samples from the patients and insert them in the 24 tube rack (**Figure 6**)



- place the first collection tube in the A1 position, the second one in the B1 position and so on. In other words, the rack has to be filled top to bottom, then left to right. (**Figure 7, Table 1**)

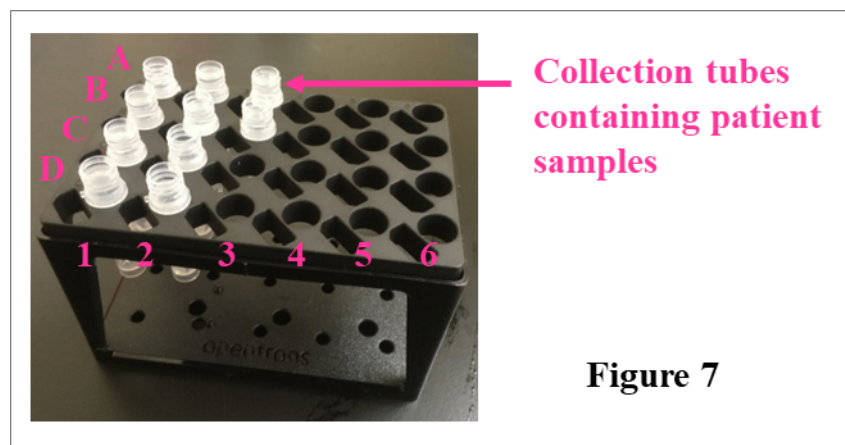


Table 1a: Outline of Rack 1

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

Table 1b: Outline of Rack 2 and Rack 3

		Column					
		1	2	3	4	5	6
Row	A	Sample 1	Sample 5	Sample 9	Sample 13	Sample 17	Sample 21

w	B	Sample 2	Sample 6	Sample 10	Sample 14	Sample 18	Sample 22
	C	Sample 3	Sample 7	Sample 11	Sample 15	Sample 19	Sample 23
	D	Sample 4	Sample 8	Sample 12	Sample 16	Sample 20	Sample 24

Table 1a: Outline of Rack 4

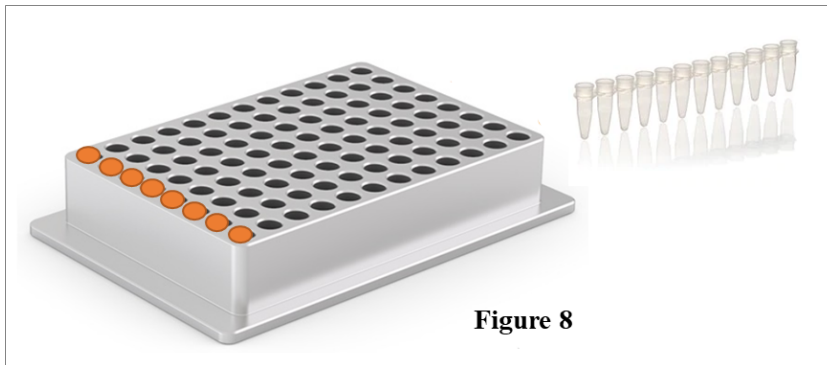
		Column					
		1	2	3	4	5	6
Row	A	EMPTY	Sample 4	Sample 8	Sample 12	Sample 16	Sample 20
	B	Sample 1	Sample 5	Sample 9	Sample 13	Sample 17	Sample 21
	C	Sample 2	Sample 6	Sample 10	Sample 14	Sample 18	Sample 22
	D	Sample 3	Sample 7	Sample 11	Sample 15	Sample 19	Sample 23

4. Place

- Rack 1 in **SLOT 2** of the deck of each OpenTrons machine belonging to Station A
- Rack 2 in **SLOT 3** of the deck of each OpenTrons machine belonging to Station A
- Rack 3 in **SLOT 5** of the deck of each OpenTrons machine belonging to Station A
- Rack 4 in **SLOT 6** of the deck of each OpenTrons machine belonging to Station A

5. Ensure the temperature module has reached 4 °C

6. Prepare the 96 position aluminum block for internal extraction control RNA (Figure 8)



- by placing in column A a 200 μ L PCR strip tube, each of the tube containing 125 μ L of internal extraction control RNA (1 mL in total)

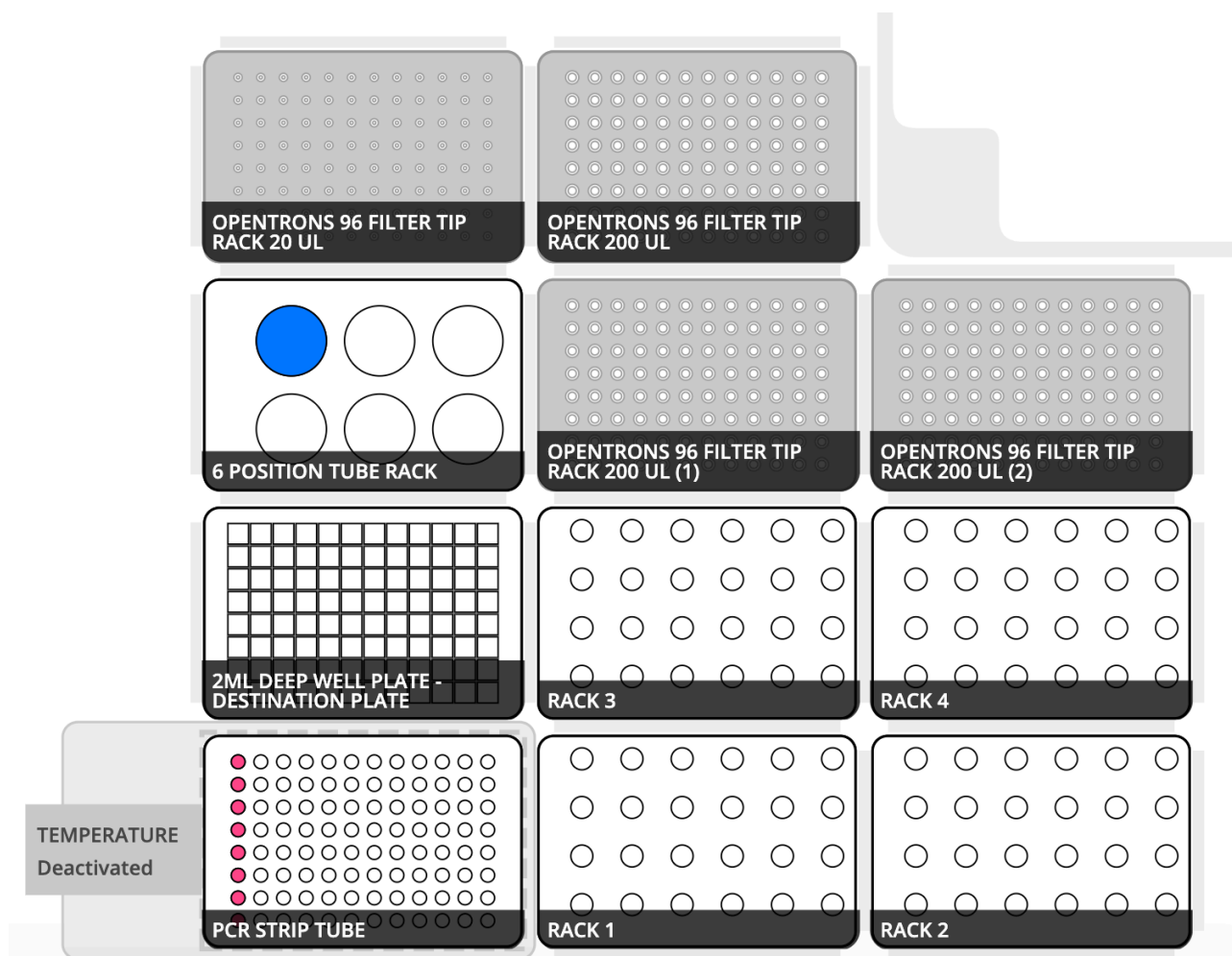
7. Place the 96 position aluminum block containing the 200 μ L PCR strip tube on top of the temperature module already located in SLOT 1 of each OpenTrons machine belonging to Station A

8. Prepare the 6 position tube rack for Lysis buffer and Proteinase K (Figure 9)



- by placing in A1 a 50 mL sterile Falcon tube containing
 - 20 mL of lysis buffer
 - 1.0 mL of proteinase K
- Important note
 - combine Lysis buffer and Proteinase K right before starting the run. Avoid preparing the mixture in advance as it can potentially deactivate the Proteinase K

9. Place the 6 position tube rack containing the Falcon tube with the lysis buffer in SLOT 7 of each OpenTrons machine belonging to Station A



10. **Double-check all the labware to make sure it looks correct. Follow the outline reported below to verify the right positioning of the labware (Figure 10). Notably, it has to be check that**
 - the labware is inserted the right way around (well A1 or 1 at the top-left)
 - the labware is properly clicked into each deck slots
 - the tubes are seated flat in their tube racks.

11. **Run the Station A protocol whose file name should be “`___.py`”, by clicking the button “Start run” in the “Run tab”**
 - do not click “Start run” more than once
 - if you need to cancel the protocol for any reason, use the power switch to turn off the machine. 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

12. **After the addition of the samples, lysis buffer, and proteinase K, the robot will pause**
 - remove the NEST 96 deep well plate from SLOT 4
 - seal the NEST 96 deep well plate with a plastic an aluminum seal
 - incubate the NEST 96 deep well plate using a heat deck or heat shaker
 - incubate at 55 - 57° C for 20 minutes

- once complete, remove the seal and put back the NEST 96 deep well plate in SLOT 4
- Hit “Resume” and the robot will resume until the end of the run

13. Wait for the run to finish

- in the meantime, start preparing what is required to run the OpenTrons machine belonging to Station B (see the Standard Operating Procedure for Station B)

14. Collect the NEST 96 deep well plate from SLOT 4, which is be the process output

14. Cleaning/Disposal of the labware

- throw out
 - the used filter tips
 - the 200 µL PCR strip tube
 - the collection tubes contacting the patient samples
 - the 50 mL sterile Falcon tube
- remove for cleaning/reuse
 - filter tips that were not used
 - the rack for 20 µL filter tips
 - the temperature module
 - the 3 racks for 1000 µL filter tips
 - the 4 diverse 24 position tube racks
 - the 96 position aluminum block
 - the 6 position tube rack
 - the graduate cylinder for measuring 20 mL
 - the heat plate/heat shaker

REFERENCES

- **Protocols provided by OpenTrons**
 - <https://docs.google.com/document/d/1wrQzhcGeOSTI12vW5RPaLsGE3ri-HIBiysv5bCClwwk8/edit>
- **Labware Library provided by OpenTrons**
 - <https://labware.opentrons.com/>

		Date	Operator (First and last name)	Signature
Draft 5 (May 7, 2020)	Check the protocol for compliance with what is reported on the OpenTrons website (Protocols and Labware Library provided by OpenTrons)			
	Check the protocol for compliance with the process			
	Acceptance			

NOTES

Any changes required in this draft will be reported below (indicating data, type of changes required by whom and why) and a new draft will be accordingly written.