Affordable COVID-19 testing automation with the Opentrons OT-2

Authors
Jethary Rader, Kinnari Watson

ABSTRACT

Using the Opentron's OT-2 and platform, we demonstrate a highly sensitive, affordable, and hands-off pipeline for PCR-based SARS-CoV-2 testing.

Key Findings

- The pipeline can detect trace amounts of the virus down to 0.5 copies/µL with a ≥ 96% accuracy across all targets, demonstrating the sensitivity of the OT-2 that is comparable to other automation systems.
- A coronavirus cross-reactivity panel showcases the assay specificity for SARS-CoV-2 detection, in contrast to SARS, MERS, and human coronaviruses 229E, NL63, and OC43.
- Viral RNA extraction of 96 samples was completed on the OT-2 in under 3 hours, with an average threshold cycle (Ct) of 36, average standard deviation of 1.0, and average coefficient of variation (CV) of 2.8%.

INTRODUCTION

COVID-19, a disease caused by SARS-CoV-2, was first declared a global pandemic by the World Health Organization on March 11, 2020 (1). Since then, there has been an enormous global emphasis on wide-scale, high-throughput testing. While numerous preventative measures like social distancing and mask mandates have been enforced in many countries around the world, large-scale testing has been proven to be a powerful option in minimizing the spread of the disease and threat to global economies and public health. Countries such as South Korea (2) and Singapore (3) have scaled up extraordinary testing efforts and have become models for containing the disease. With a reliable vaccine still expected to be months away (4) and numerous testing choices exhibiting high rates of false negatives (5), increasing the amount of trustworthy testing options is critical.

In this study, we illustrate a simple and reproducible strategy for SARS-CoV-2 RT-PCR based detection using the Opentrons OT-2, modules, and labware. This automation-friendly workflow is highly sensitive, incredibly affordable, and can be an immeasurable help in increasing testing capacities globally.

MATERIALS AND METHODS

Synthetic positive samples were created by adding SARS-CoV-2 RNA control 2 (Twist Biosciences) and Synthetic Nasal Matrix (SNM) (6) to each sample containing 200 µL of lysis buffer and 35 µL of proteinase K (PK). SNM was created similarly to what is outlined in Panpradist, Nuttada, et al (6). (SNM: 110 mM NaCl, 1% w/v mucin from porcine stomach type II (Sigma M2378-100G) and 10 µg/mL w/v human genomic DNA (Coriell NA12878) at 90% v/v of TE/SNM. Each sample was incubated at room temperature for 15 minutes for lysing.

RNA extractions were performed using Promega Maxwell® HT Viral TNA Kit on the OT-2, which occurred immediately after lysing. After beads and isopropanol were added to the samples, a pause on the OT-2 occurred to mix the plate for 10 minutes on an off-deck vortexer. Once mixing was complete, the OT-2 run resumed. Each sample was eluted in 65 µL. The OT-2 used a GEN2 P300 8-channel pipette on the left mount. Up to ten 200 µL filter tipracks were used per run, including a GEN2 Magnetic Module and a GEN2 Temperature Module (Figure 1). Total run time including hands-on time and lysing, varied between 1 hours and 3.5 hours (Figure 2).

Center of Disease Control and Prevention (CDC) primers and probes:

- 2019-nCoV_N1-F (GACCCCAAAATCAGCGAAAT)
- 2019-nCoV_N1-R (TCTGGTTACTGCCAGTTGAATCTG)
- 2019-nCoV_N1- P (FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1)
- 2019-nCoV_N2-F (TTACAAACATTGGCCGCAAA)

- 2019- nCoV_N2-R (GCGCGACATTCCGAAGAA)
- 2019-nCoV_N2-P (FAMACAATTTGCCCCCAGCGCTTCAG-BHQ1)
- RP-F (AGATTTGGACCTGCGAGCG)
- RP-R (GAGCGGCTGTCTCCACAAGT)
- RP-P (FAM-TTCTGACCTGAAGGCTCTGCGCG-BHQ1)

They were ordered from Integrated DNA Technologies (IDT, 2019-nCoV CDC EUA kit). N1, N2 and RNase P came premixed at the recommended concentrations by the CDC. 2019-nCoV_N_Positive Control (GenBank: NC_045512.2) and Hs_RPP30 Positive Control were both from IDT and were used for a SARS-CoV-2 positive control and Human RNA positive control respectively. Either the One Step PrimeScript™ III RT-PCR Kit (Takara Bio Inc.) was used at concentrations recommended by Takara or the Luna® Universal Probe One-Step RT-qPCR Kit (NEB) was used at the recommended concentrations as tested by Lista, Maria Jose, et al (7). The following program was performed for RT-PCR: Reverse transcription was performed for 10 minutes at 55°C. Initial denaturation was for 1 minute at 95°C followed by 50 cycles of denaturation for 10 seconds at 95°C and annealing for 30 seconds at 60°C.

Charité, Berlin/World Health Organization (WHO) primers and probes:

- E_Sarbeco_F1 (ACAGGTACGTTAATAGTTAATAGCGT)
- E_Sarbeco_R2 (ATATTGCAGCAGTACGCACACA)
- E_Sarbeco_P1 (FAM-ACACTAGCCATCCTTACTGCGCTTCGBBQ)
- RdRP_SARSr-F2 (GTGARATGGTCATGTGTGGCGG)

FIGURE 1

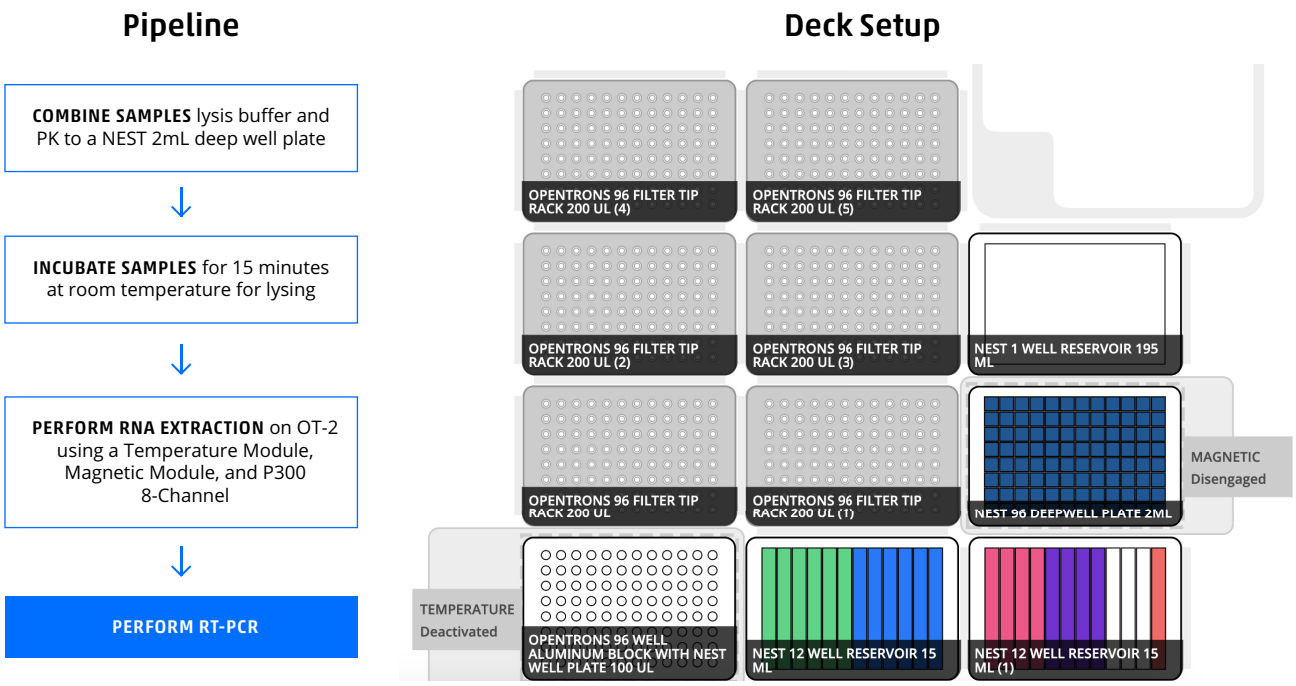

Figure 1: Easily adopted SARS-CoV-2 PCR-based detection pipeline A. *The pipeline starts with synthetic samples. After reformatting the samples into a NEST 96 Deepwell Plate 2mL, the samples are kept at room temperature for 15 minutes for lysing, followed by RNA extraction performed on the OT-2 using an Opentrons GEN2 Magnetic Module and GEN2 Temperature Module. Finally, RT-PCR is performed on a RT-qPCR machine.* **B.** *This layout on the OT-2 includes the Opentrons GEN2 P300 8-channel pipette, GEN2 Temperature Module, and GEN2 Magnetic Module. The labware requirements include up to ten 200µL filtered tipracks, one NEST 1 Well Reservoir 195 mL, one NEST 2mL deep well plate, up to two NEST 12 Well Reservoir 15 mL, and one NEST 96 Well Plate 100 µL PCR Full Skirt. Overall, the total labware per sample costs $14.35.*

FIGURE 2

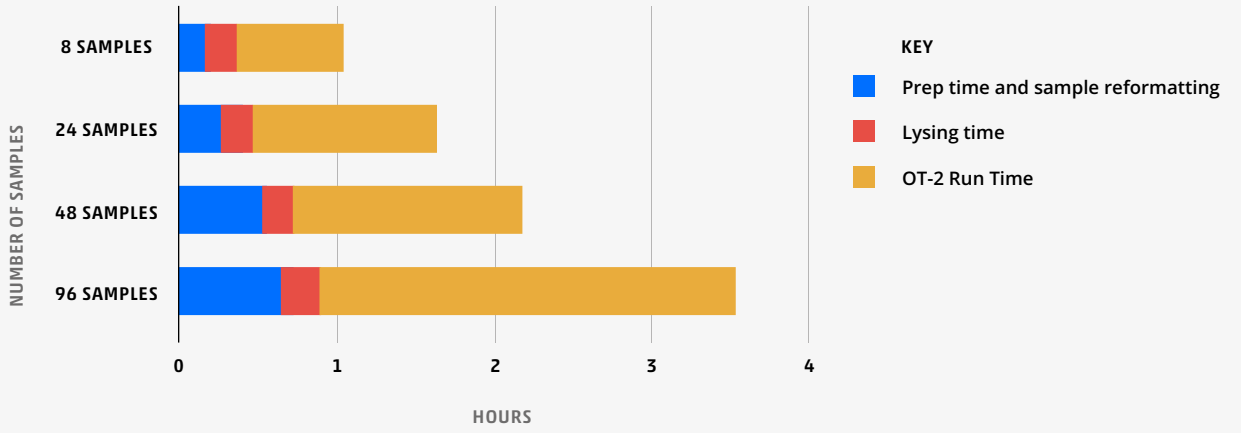

Figure 2: Quickly complete COVID testing on the Opentrons OT-2. *The prep time and sample reformatting was between 10 minutes to 40 minutes, depending on the throughput. The lysing time was always 15 minutes. The run time, described as the amount of time the OT-2 took to perform the run, varied between 37 minutes for 8 samples to 2 hours and 38 minutes for 96 samples. Overall, 96 samples can be prepped and extracted in about 3.5 hours.*

FIGURE 3A

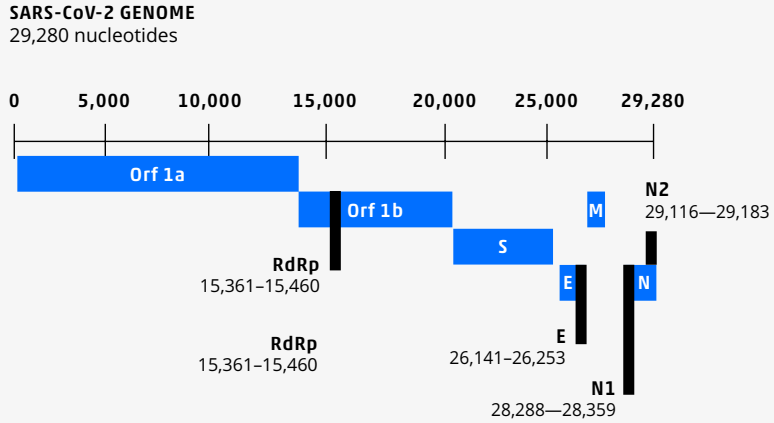

Figure 3: Successful cross-reactivity panel demonstrating high quality OT-2 extractions.

A. *Related amplicon target positions in the SARS-CoV-2 genome. The genome consists of 29,280 nucleotides and genome positions are according to GenBank NC_004718. CDC's targets N1 and N2 (11) and WHO's targets RdRp and E (12) are labeled.*

FIGURE 3B

			POSITIVE				
VIRUS	STRAIN	GENOME	N1	N2	E	RDRP (DISCRIMINATORY)	RDRP (CONFIRMATORY)
SARS		NC_004718.3	0/2	0/2	2/2	0/2	2/2
MERS		2 JX869059.2	0/2	0/2	0/2	0/2	0/2
Human coronavirus	229E	NC_002645.1	0/2	0/2	0/2	0/2	0/2
Human coronavirus	NL63	NC_005831.2	0/2	0/2	0/2	0/2	0/2
Human coronavirus	OC43	NC_006213.1	0/2	0/2	0/2	0/2	0/2

Figure 3: Successful cross-reactivity panel demonstrating high quality OT-2 extractions. B. *Cross-reactivity coronavirus panel testing 5 coronaviruses and 5 SARS-CoV-2 targets from the CDC and WHO. Each target did not amplify except for E and RdRp (exclusive) amplified SARS.*

- RdRP_SARSr_R1 (CARATGTTAAASACACTATTAGCATA)
- RDRP_SARSr_P2 (FAM-CAGGTGGAACCTCATCAGGAGATGCBBQ)
- RdRP_SARSr-P1 (FAM-CCAGGTGGWACRTCATCMGGTGATGCBBQ)

Primers from IDT were employed in this pipeine. Each primer/probe came premixed at the concentrations recommended by the WHO. The Luna® Universal Probe One-Step RT-PCR Kit (NEB) was used in concentrations and volumes outlined by Corman, Victor, et al (8). The following program was performed for RT-PCR: Reverse transcription was performed for 10 minutes at 55°C. Initial denaturation was for 1 minute at 95°C followed by 50 cycles of denaturation for 10 seconds at 95°C and annealing for 30 seconds at 60°C.

RESULTS

The sensitivity of the Opentrons platform is demonstrated through a Limit of Detection (LoD) of 0.5 copies/µL. 5 concentrations ranging from 50 copies/µL to 0.5 copies/µL of SARS-CoV-2 RNA control (Twist Biosciences) were tested with 20 samples per concentration. The LoD for both N1 and N2 targets is ~0.5 copies/µL, where 95% of the samples successfully amplified, with a mean Ct of 30 and 36 respectively (Table 1). These results are in scope of IDT (9) and Promega (10) specs. This pipeline eliminates the chances of false negatives and shows comparable performance to compete with alternative automation systems.

Related coronaviruses that can be present in clinical specimens were evaluated for the effectiveness of different primers/probes through a cross-reactivity coronavirus panel. 5 synthetic coronaviruses were tested to amplify with the CDC N1 and N2 targets, and WHO E and RdRp targets (Figure 3A). First, 2.5 x 10^6 copies/µL for each coronavirus was tested to evaluate amplification for each target. Next, there were 5 samples run on the OT-2. Every sample total concentration was 5,000 copies/µL of one of the five coronaviruses. The cross-reactivity coronavirus panel for 2.5 x 10^6 copies/µL and 5,000 copies/µL showed no amplification for each target except for WHO targets E gene and RdRp (exclusive), which showed positive detection for SARS. E gene and RdRp (exclusive) are targets designed to amplify SARS-associated coronaviruses (Figure 3B). This illustrates that the OT-2 can eliminate the chance for cross-contamination, and that positive samples can be attributed to SARS-CoV-2 detection, with great confidence.

To further reinforce the sensitivity and efficiency, a ninety-

TABLE 1

TARGET LEVEL	SARS-COV-2 (N1) POSITIVE N	SARS-COV-2 (N1) POSITIVE MEAN CT	SARS-COV-2 (N1) DETECTION RATE	SARS-COV-2 (N1) POSITIVE N	SARS-COV-2 (N1) POSITIVE MEAN CT	SARS-COV-2 (N2) DETECTION RATE
50 copies/µL	20/20	28	100%	20/20	35	100%
10 copies/µL	20/20	29	100%	20/20	36	100%
5 copies/µL	20/20	28	100%	20/20	37	100%
1 copies/µL	20/20	30	100%	20/20	35	100%
.5 copies/µL	19/20	30	95%	19/20	36	95%

Table 1: Sensitive pipeline illustrated through a low LoD for N1 and N2 SARS-CoV-2 targets. *The LoD is defined by the lowest concentration that can be reliably detected ≥95% of the time. The lowest concentration with ≥95% success was 0.5 copies/µL, therefore 0.5 copies/µL is the LoD.*

TABLE 2

TARGET	AVERAGE CT	STANDARD DEVIATION	CV (%)	ACCURACY (%)
N1	37	1.1	3.1	96
N2	37	0.7	1.9	96
RNase P	34	1.2	1.2	100
Overall average	**36**	**1.0**	**2.8**	**97**

Table 2: OT-2 extraction results from a full PCR plate of samples exhibiting a high level of success. *The overall results across the 3 targets had a Ct of 36, standard deviation of 1.0, CV of 2.8% and accuracy of ≥ 96%. These results are in range with the FDA's EUA criteria.*

six sample run was performed. The starting concentrations were 50 copies/µL of SARS-CoV-2 RNA control (Twist Biosciences) and 50 pg/µL of gDNA from the SNM per sample. Results showed an overall Ct of 36, standard deviation of 1.0, and CV of 2.8%. At least 96% accuracy was achieved in all targets, where amplification in the RNase P target was observed for all samples, demonstrating 100% accuracy (Table 2, Figure 4). This detection level and sensitivity correlate with the FDA guidelines for Emergency Use Authorization (10) and demonstrate that the Opentrons platforms for COVID-19 PCR-based testing can lead to success in an affordable and easily deployable platform.

CONCLUSION

With COVID-19 testing urgently needed, the OT-2 can provide a simple and reliable pipeline. While it isn't as fast as competitor automation systems, the OT-2 system is highly affordable and can produce exceptional quality results.

- 0.5 copies/µL LoD and successful cross-reactivity coronavirus panel showcasing sensitivity and high quality of the OT-2, comparable to other automation systems and in range of FDA EUA guidelines.
- Ninety-six samples can be processed with at least 96% accuracy in under 4 hours, using the Opentrons platform.

FIGURES 4A-4D

FIGURE 4A

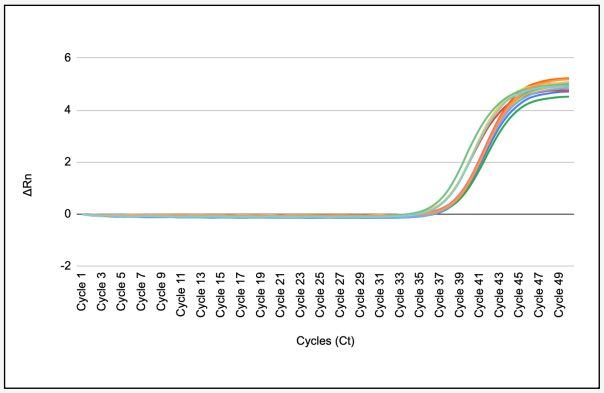

FIGURE 4B

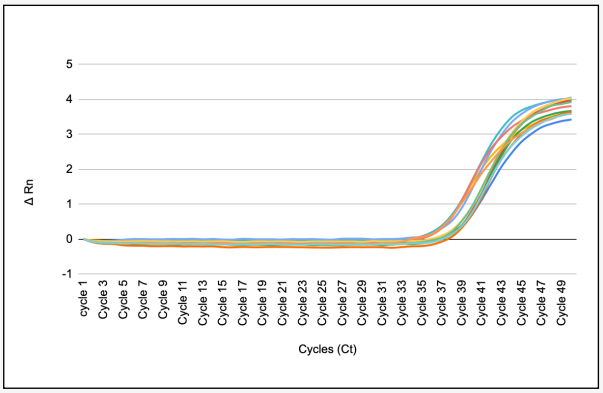

FIGURE 4C

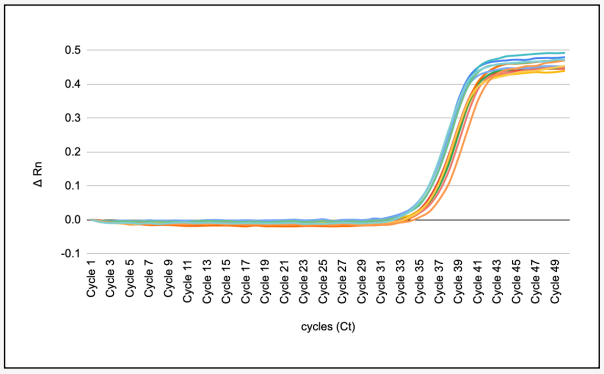

FIGURE 4D

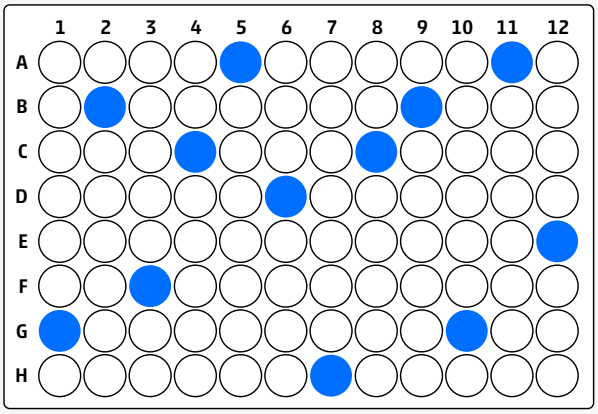

Figure 4: Sensitive and reliable COVID control assay. *Amplification curves from the ΔRn values from RT-PCR for 12 of the 96 samples.* **A.** *amplification curves for N1.* **B.** *amplification curves for N2.* **C.** *amplification curves for RNase P.* **D.** *illustration of which samples in the 96 well plate are graphed in the amplification curves.*

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