



Robust and High-Throughput SARS-CoV-2 Viral RNA Detection, Research, and Sequencing Using RNAdvance Viral and the OT-2 Platform

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Introduction

The current global pandemic (SARS-CoV-2) has highlighted a critical need to support high-throughput RNA detection and genetic sequencing for clinical research purposes. The ability to extract and purify sequencing grade viral RNA from samples has been hindered by non-optimized protocols. Here we demonstrate an automated SARS-CoV-2 RNA extraction workflow using a Beckman Coulter Life Sciences RNAdvance Viral kit (C63510) on an Opentrons OT-2 robot; an open-source robotic liquid handling platform. We present analytical data showing the SARS-CoV-2 limit of detection (LoD) quantified by qRT-PCR and sequence alignment data that establishes proof of principle for automated sequencing sample and library preparation for possible genetic identification of mutated strains.

Overview of the RNAdvance Viral kit and OT-2 platform

The RNAdvance Viral kit efficiently extracts viral RNA from samples by utilizing SPRI paramagnetic bead-based technology. We employed this reagent chemistry on the automated Opentrons platform to extract viral RNA from synthetic nasal matrix (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) samples spiked with different concentrations of heat-inactivated SARS-CoV-2 (ATCC® VR-1986HK™). Each concentration included 20 test samples, 2 positive controls, and 2 negative controls.

Workflow Throughput

The demonstrated OT-2 deck layout for an automated RNAdvance Viral extraction workflow is shown in **Figure 1**. The workflow started from the addition of Bind (BBB) to the sample lysate and only requires 10 tips/sample with flexibility to run batch sizes of 8 up to 96 samples. Reported workflow durations include hands-on time, sample lysing time, and run-time for the samples after optimization (**Figure 2**). The 96 samples workflow takes a total of 3.5 hours using the RNAdvance Viral kit and OT-2 platform and can be accessed here: <https://protocols.opentrons.com/protocol/bc-rnadvance-viral>. Users have the flexibility to alter method variables to achieve the desired results for batch size, sample input, and elution volume.

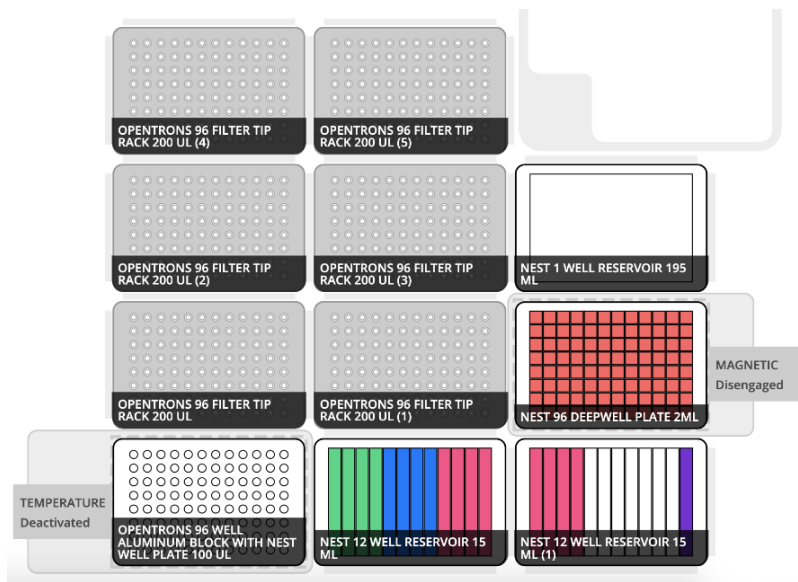


Figure 1. Opentrons OT-2 deck layout equipped with modules, labware, and RNAdvance Viral Reagents. With 10 tips/sample, this workflow is fully automated on the OT-2 starting with the addition of Bind (BBB). Deck layout includes up to 10 tip racks, 1 x NEST 1 well reservoir, 2 x NEST 12 well reservoirs, 1 x 96 well aluminum block, 1 x NEST 0.1mL PCR plate and 1 x NEST 2mL Deep well plate for labware. Modules include a GEN 2 Magnetic Module and a GEN 2 Temperature module with a GEN 2 P300 Multichannel pipette. All NEST consumables and modules are supplied by Opentrons.

A

Batch size	Run time	Lysing time	Hands-on time	Total workflow time
8 samples	45 minutes	20 minutes	10 minutes	1 hour, 15 minutes
24 samples	1 hour, 2 minutes	20 minutes	20 minutes	1 hour, 40 minutes
48 samples	1 hour, 20 minutes	20 minutes	30 minutes	2 hour, 10 minutes
96 samples	2 hours, 38 minutes	20 minutes	40 minutes	3 hour, 38 minutes

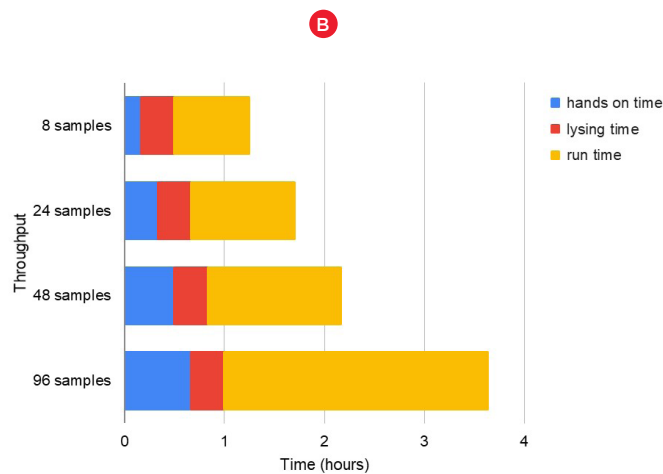


Figure 2. Throughput Utilizing the RNAdvance Viral kit on the OT-2 platform. Workflow completion times include 3 sections: hands on time, lysing time, and run time. Hands on time includes preparing the samples and the OT-2. Run time refers to the time it takes for the OT-2 to complete the protocol from start to finish. **A)** The run time ranges from 45 minutes for 8 samples to 2 hours and 38 minutes for 96 samples. **B)** Graphical representation of the timing.

RNAdvance Viral Kit Limit of Detection

The limit of detection (LoD) was used to determine the automated RNAdvance Viral extraction efficiency on the OT-2 platform and was considered the lowest concentration of viral copy number that can be detected in $\geq 95\%$ of repeat measurements. Samples were probed with the 2019-nCoV CDC N1 and RP Primers and Probes (2019-nCoV CDC EUA kit, Integrated DNA Technologies (IDT)), the master mix and enzyme (Luna® Universal Probe One-Step RT-qPCR Kit, New England BioLabs) on the PCRmax ECO48 Real-Time PCR System. Viral RNA (copies/ μL) were detected in SARS-CoV-2 samples (n=20), positive controls (n=2) but not negative controls (n=2). As summarized in **Table 1**, all the contrived positive samples across the analytical range were determined to be positive, with 1 copy/ μL being the lowest concentration of viral RNA detected by PCR. The PCR parameters were as follows.

Target level (copies/ μL)	No. of Positives / Total No of replicates	Mean Ct
50	20/20	32
10	20/20	34
5	20/20	35
1	20/20	37

Table 1. Viral sensitivity depicted through the SARS-CoV-2 target N1 limit of detection using the OT-2 platform and RNAdvance Viral kit. 1 copy/ μL was the lowest concentration to detect $\geq 95\%$ of the samples and is defined as the limit of detection (LoD).

To investigate the automation performance of RNAdvance Viral on the OT-2, we spiked in synthetic nasal matrix with 850 copies/ μL concentrations of heat-inactivated SARS-CoV-2 and extracted RNA. The specifications, including mean Ct value, standard deviation, CV (%), and detection rate, were determined for N1 and RP (**Figure 3A**). Amplification curves for the N1 target were quantitated in experimental samples (n=20), positive (n=2) and negative (n=2) controls (**Figure 3B**) versus quantitation of RP targets in samples, including controls (**Figure 3C**).

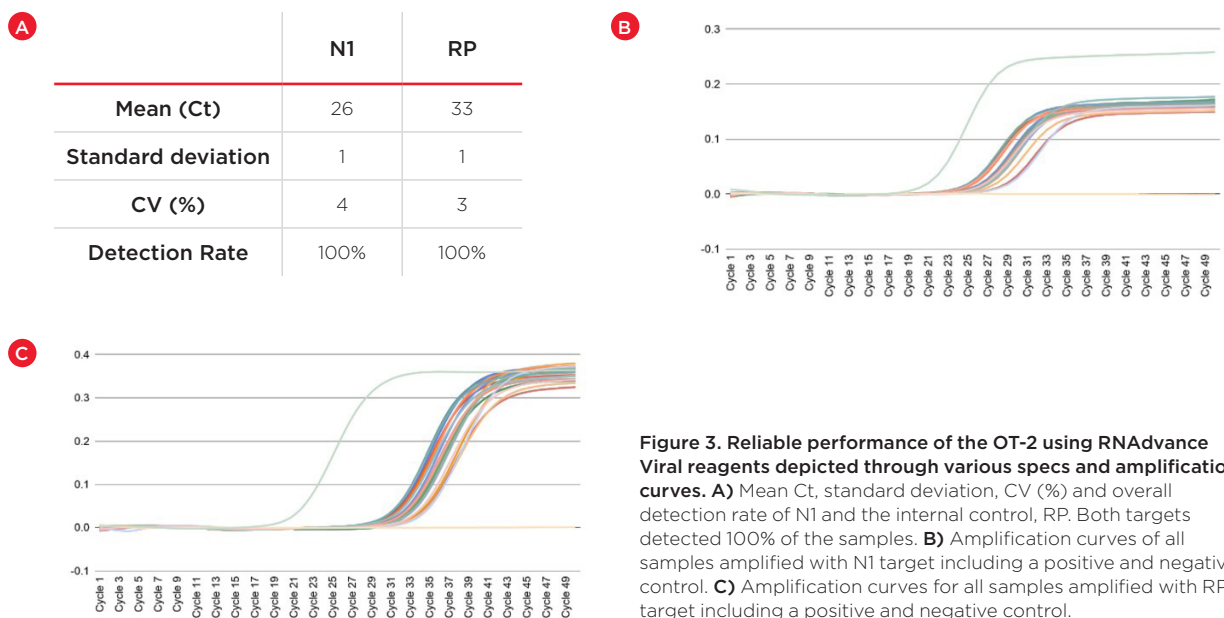


Figure 3. Reliable performance of the OT-2 using RNAdvance Viral reagents depicted through various specs and amplification curves. A) Mean Ct, standard deviation, CV (%) and overall detection rate of N1 and the internal control, RP. Both targets detected 100% of the samples. **B)** Amplification curves of all samples amplified with N1 target including a positive and negative control. **C)** Amplification curves for all samples amplified with RP target including a positive and negative control.

DNA Sequencing and Data Analysis

To further understand the sequence performance, the N1 amplified samples were generated using the Swift 2S Turbo DNA library prep kit and Swift Unique Dual Indexing Primer Plate. The comprehensive data and sequence alignment from the 850 copies/ μL samples can be found in **Figure 4**. We observed a sample mean coverage of 455x and mean reads aligned to reference genome, SARS-CoV-2 isolate Wuhan-Hu-1 (NC_045512.2) at 91%. The read length matches the N1 amplified region, which is about 72 bp. The expected insert size according to the Swift 2s Turbo DNA Library Prep manufacturer is ~200bp.

Sequencing was performed using the Illumina MiSeq and screening data was uploaded to Illumina BaseSpace. Coverage was determined using Bowtie2 in Galaxy and visualized using Integrative Genome Viewer (IGV). NC_045512.2, SAR2-COV-2, isolated Wuhan-Hu-1, was the NCBI reference genome used for sequencing. Alignment, read length, insert size, and GC content was identified using QualiMap-BAMQC in Galaxy.

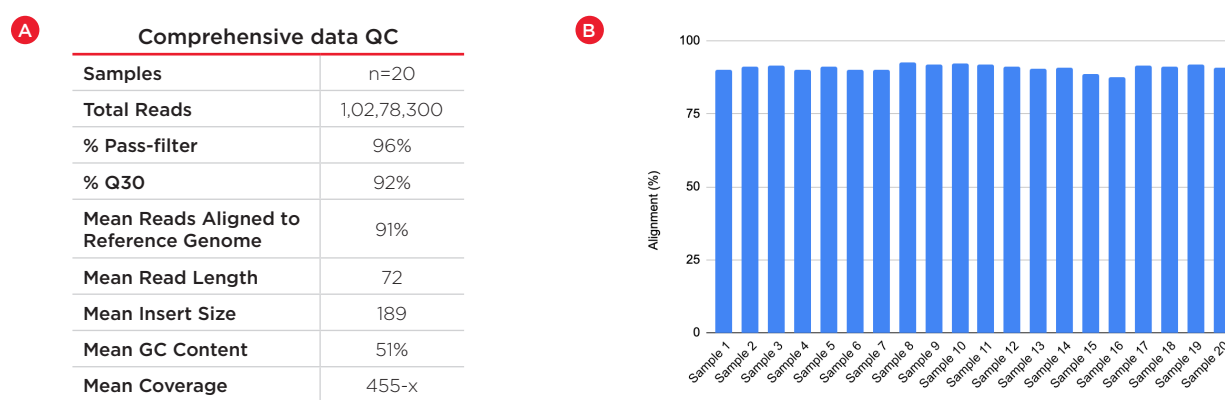


Figure 4. Sequencing comprehensive data and coverage visualization from samples extracted on the OT-2. A) Comprehensive data QC table depicting several data points including mean coverage at 455-x and mean reads aligned to reference genome, SARS-CoV-2 isolate Wuhan-Hu-1 (NC_045512.2) at 91%. The expected insert size according to the Swift 2s Turbo DNA Library Prep manufacturer is ~200bp. **B)** Percent aligned to reference genome for each sample.

Conclusions

We present here a robust, highly affordable automated extraction solution utilizing the Beckman Coulter Life Sciences RNAdvance Viral kit and Opentrons OT-2. The workflow was optimized for viral RNA PCR detection with analytical performance measured at LoD of 1 copy/ μL for SARS-CoV-2. We also show the protocol is suitable for NGS with high mean sample coverage at 455x and mean reads aligned to SARS-CoV-2 reference genome at 91%. This evaluation enables the use of RNAdvance Viral reagents and the Opentrons OT-2 in a streamlined workflow to obtain sequenceable input samples for clinical researchers studying the SARS-CoV-2 virus.

RNAdvance Viral is for molecular biology research use only. Not for use in diagnostic procedures.

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