High performance automated nucleic acid extraction with the OT-2 platform

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INTRODUCTION

Modern molecular biology, driven by ever progressing technology, continues to deliver impressive and exciting new advancements — larger studies, more powerful therapeutics, and wholly new research questions. Meanwhile, a tried-and-true workhorse, nucleic acid extraction, continues to power these exciting developments with the same lengthy and tedious procedures from decades past. Recently, a modern solution has emerged — automation. With the consistency and speed of robotics, automated nucleic acid extraction should modernize the biology lab's old workhorse. But to deliver on this promise, such systems will need to meet or exceed the performance of highly skilled technicians, producing quality samples for downstream workflows.

The present study sought to test the performance of the Opentrons OT-2 Nucleic Acid Workstation as a tool to effectively automate magnetic bead-based nucleic acid extraction. Extraction was performed with several common templates used by researchers and clinicians: human saliva, human buccal swab, bacterial culture, and an RNA virus. Likewise, the industry's most popular brands and commonly used kits were used. Performance was measured in terms of yield, coefficient of variability (CV), and qPCR data and compared to manual procedures of highly skilled technicians.

The OT-2 performed well in all of these tests. The OT-2 reduced variation and improved reproducibility while delivering similar yield at lower costs and faster turnaround times. Importantly, these performance improvements and financial savings scale with throughput. Thus, this test showed that automation of nucleic acid extraction with the OT-2 can modernize the procedure and push extraction to meet the high-performance standards of cutting-edge molecular biology.

MATERIALS AND METHODS

Saliva: Fresh saliva was collected from anonymous donors and stored in a 15 mL conical tube until use.

Buccal swab: Using the Collection Swab, 20 mm break point (Zymo®), buccal swabs were collected from anonymous donors and stored in 1 mL Zymo DNA/RNA shield at room temperature until use.

Bacterial culture: One Shot® TOP10 Chemically Competent *E. coli* (Thermo Fisher Scientific®) was grown in lysogeny broth overnight at 37°C shaking at 225 rpm. Upon usage, 200 µL per sample was spun down in a centrifuge at 2000 rpm for 1.5 minutes. The supernatant was removed, and the pellet was resuspended in the equivalent volume of chilled phosphate-buffered saline. Additionally, for the Lactobacillus samples, approximately 1 x 10^7 copies/µL of *Lactobacillus plantarum* (ATCC® 8014MINIPACK™) was used per sample.

Virus: Due to the start of the COVID-19 global pandemic in March 2020 (1), synthetic SARS-CoV-2 RNA Control 2 (Twist Biosciences) was used at 10 copies/µL per sample for the viral RNA kits.

Internal Control: Synthetic nasal matrix (SNM) was used and created as stated in Panpradist, Nuttada, *et al* (2). (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. 50 pg/µL was used per viral sample.

Kits: DNA, RNA, and viral RNA extraction kits were selected from similar manufacturers **(Table 1)**.
A total of 13 kits were examined from 7 manufacturers in this study.

Kit	Manufacturer	Nucleic Acid	Identifier
Mag-Bind® Blood & Tissue DNA HDQ 96 Kit	Omega Bio-tek®	DNA	A
MagaZorb® DNA Mini-Prep Kit	Promega®	DNA	B
MagneSil® Total RNA mini-Isolation System	Promega	RNA	C
Mag-Bind Total RNA 96 Kit	Omega Bio-tek	RNA	D
Quick-DNA/RNA™ MagBead	Zymo Research	RNA	E
Direct-zol-96 MagBead RNA	Zymo Research	RNA	F
NucleoMag® Virus Viral DNA/RNA Isolation	Macherey-Nagel®	viral RNA	G
Maxwell® HT Viral TNA Kit	Promega	viral RNA	H
RNadvance viral XP	Beckman Coulter®	viral RNA	I
Quick-DNA/RNA Viral MagBead	Zymo Research	viral RNA	J
MGIEasy Nucleic Acid Extraction Kit	MGI®	viral RNA	K
Mag-Bind® Viral DNA/RNA 96 Kit	Omega Biotek	viral RNA	L
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	Thermo Fisher®	viral RNA	M

Table 1: Extraction kits investigated in this study. There are two DNA, four RNA and seven viral RNA kits automated on the OT-2 across seven popular reagent brands.

Extraction: Prior to automating, each DNA and RNA extraction kit and template was tested manually to get a baseline concentration. Each template was tested in 7 replicates with 1 negative control per kit.

Following manual extraction, automated extractions were performed using the Opentrons platform. For the DNA and RNA extraction kits, each sample was extracted in 7 replicates. For the viral RNA extraction kits, 22 samples with the same SARS-CoV-2 and SNM template were included. Each OT-2 run included 1 negative control of nuclease-free H_2O. The OT-2 deck layout included the GEN2 Magnetic Module, the p300 multichannel pipette, and the optional GEN2 Temperature Module, which is highly recommended for RNA work. Up to 14 x 300 µL tip racks are required depending on the kit, 1 x NEST 1 well reservoir, 1 x NEST 2 mL deep well plate, 2 x NEST 12 well reservoirs, 1 x PCR plate.

Primers:

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)

CDC primers and probes were ordered from Integrated DNA Technologies, aka IDT (2019-nCoV CDC EUA kit). N1, N2, and RNase P came premixed at the recommended concentrations by the CDC.

16s Primers:

- Forward (CCTATAAGACTGGGATAACTTCGGG)
- Reverse (CTTTGAGTTTCAACCTTGCGGTCG)

16s primers were created and ordered from IDT and were resuspended in IDTE 1x TE Solution pH: 8.0 (IDT) and diluted to a working concentration of 10 µM.

Quantification: DNA and RNA samples were quantified using the Qubit 4.0 Fluorometer™ (Thermo Fisher).

Additionally, all samples were quantified for qPCR on the PCRmax ECO48 Real time PCR system. The RNA samples were tested using the Luna® Universal Probe One-Step RT-qPCR Kit (NEB) at concentrations tested by Lista, Maria Jose, *et al* (3). The following program was performed: Reverse transcription was performed for 10 minutes at 55°C. Initial denaturation was performed 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. The DNA samples were tested using the Luna Universal qPCR Master Mix (NEB) at concentrations recommended by the manufacturer. The following program was used: initial denaturation was performed for 60 seconds at 95°C, subsequently denaturation and extension were performed for 15 and 30 seconds and 95°C and 60°C, respectively for 40 cycles.

RESULTS

The OT-2 achieves similar quantity but improved consistency of nucleic acid samples, as measured with qPCR and fluorometer analysis.
Human saliva and *L. plantarum* bacterial culture were processed using nine popular extraction kits and analyzed with qPCR using two common qPCR targets: RNase P for saliva samples and 16s for bacterial samples. *E. coli* bacterial culture was processed with six kits and analyzed with fluorometric analysis.

Yield was assessed with qPCR in terms of cycle threshold (Ct) and with the fluorometer in terms of ng/µL. Consistency was assessed in terms of CV for Ct values.

Ct values reflect the amount of replication cycles necessary for a sample to cross a threshold above background signal, and thus they convey an inverse measure of quantity. For these targets, in these kinds of samples, Ct values of 25–35 are considered reliable.

Across all extraction kits, automation with the OT-2 delivered comparable Ct values with lower CV and comparable yield **(Figure 1** and **Table 2)**. Both skilled technicians and the OT-2 returned mean Ct values of 25-35, while manual processing CV's were 1.41-4.8 and the OT-2's CV values were 1.2-2.6. Skilled technicians and the OT-2 delivered a comparable and consistent yield of 24-26 ng/µL. These results suggest that the OT-2 can deliver high yield while improving consistency and repeatability.

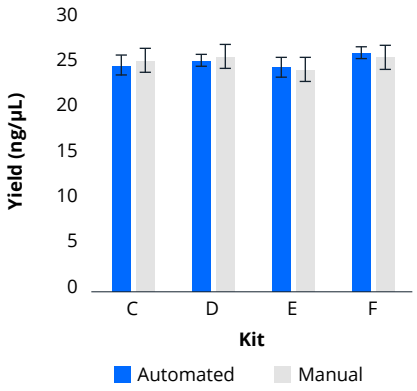

Figure 1: Automated extraction yields exhibits less variation and comparable yields to manual processing. Blue: Less variation from automated compared to manual for both DNA and RNA samples across six kits. **Gray:** Comparable RNA qubit yields (ng/uL) extracted from wild-type *E. coli* across four kits.

The OT-2 passes EUA standard qPCR performance with a wide array of extraction kits
In a performance test relevant to the COVID-19 pandemic, qPCR was conducted using synthetic SARS-CoV-2 samples and seven popular extraction kits. Human genomic DNA in SNM was used as an internal control. The CDC's SARS-CoV-2 N1 and N2 primers were used for quantification with the CDC's RP primer as a positive control.

qPCR performance was assessed in relation to the PCR test emergency use authorization (EUA) standards. To meet EUA acceptance criteria, a test must achieve 95% amplification, i.e., 95% of the samples must reach a detectable N1 and N2 signal in qPCR.

Performance was also assessed in terms of qPCR Ct values. For these targets, in these kinds of samples, Ct values of 30-40 are considered reliable.

The OT-2 produced EUA-standard-passing results with all but two kits **(Table 3)**. Two tested kits delivered only 91% pass results, as the kits' lysis buffers were viscous enough to impede extraction. However, across all tested kits, Ct values of 31-39 were achieved. These results suggest that the flexible OT-2 can meet clinical standards with a wide range of kits.

The OT-2 achieves high throughput and short turn-around-times with a wide array of extraction kits
To test throughput and speed, RNA and DNA extraction were performed with 13 popular kits, spanning a range of protocol length. To thoroughly estimate speed for various throughputs, batches of 8, 24, 48, and 96 samples were tested. Results were measured in terms of time taken from raw sample to purified nucleic acid.

Across these extraction kits and protocol lengths, the OT-2 achieved speedy results — taking 25 minutes for 8 samples on the shortest protocol and less than 5 hours for 96 samples on the longest protocol **(Figure 2)**. Restricted to 8 samples, the OT-2 took an hour or less on 9 of the 13 kits with an average time of 52 minutes across all kits. With 96 samples, the OT-2 took less than 2.5 hours with 8 of the 13 kits, with an average time of 2 hours 45 minutes across all kits. These results indicate that the OT-2 can couple its high performance with high throughput and drive significant improvements to extraction workflows.

		Manual		Automated	
Kit	**Template**	**Mean CV (%)**	**Mean (Ct)**	**Mean CV (%)**	**Mean (Ct)**
A	Human saliva	4.8	25.6	2.2	25.8
B	Human saliva	3.2	28.4	1.5	28.9
E	Human saliva	4.3	27	2	26.8
A	*L. plantarum*	3.15	34.2	2.6	34.1
B	*L. plantarum*	2.8	34.8	1.4	34.9
C	*L. plantarum*	1.43	30.9	1.2	31.4
D	*L. plantarum*	4.1	29.7	2	28.2
E	*L. plantarum*	3.5	29.8	2.6	30.7
F	*L. plantarum*	2	29.1	1.6	29.5

Table 2: Comparable yield and precision across various extraction kits using manual and automated processing. Mean coefficient of variation, CV, and cycle threshold, Ct, are shown on the left.

	N1		N2		RP		
Kit	**Average Ct**	**% passed**	**Average Ct**	**% passed**	**Average Ct**	**% passed**	**Overall % Passed**
G	36	100	38	91	33	100	97
H	33	100	37	95	36	100	98
I	35	95	39	100	36	95	96
J	34	100	36	100	34	100	100
K	34	100	33	100	31	100	100
L	35	100	37	91	33	100	96
M	35	100	38	95	35	95	96

Table 3: Viral QC specs using SARS-CoV-2 demonstrating wide versatility. 7 viral kits across 7 manufacturers were automated yielding >96% samples passed for every kit (n = 22).

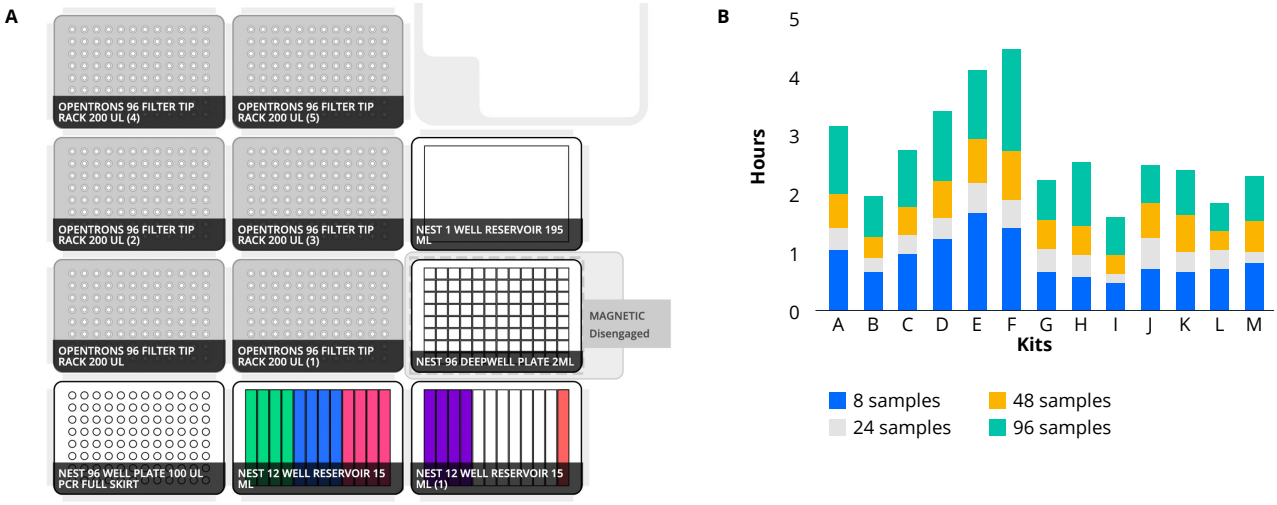

Figure 2: OT-2 Deck Layout and run times for up to 96 samples.
(A) Extraction OT-2 deck layout for 96-sample throughput with labware supplied by Opentrons.
(B) Run times for 8, 24, 48 and 96 for RNA and DNA extraction protocols.

DISCUSSION

The OT-2 Nucleic Acid Extraction Workstation delivered excellent-quality RNA and DNA samples from organic and synthetic raw templates. qPCR and fluorometric tests revealed that the automated system produced comparable yields as manual procedures performed by highly trained technicians but exceeded manual performance in reproducibility and precision. The OT-2 was shown to successfully meet EUA criterion-level performance as assessed with greater than 95% successful qPCR detection of signal. Timing tests showed that the OT-2 took less than an hour to process eight samples and less than three hours for 96 samples, averaged across kits with varying protocol lengths. In fact, with the fastest kits, the OT-2 took only 25 minutes for eight samples and only one and a half hours for 96 samples.

These tests were all performed with multiple samples of clinical and laboratory significance and across a wide range of extraction kits. The OT-2's flexibility accommodated the various protocols, reagents, and sample types to continually deliver quality results.

The cost to automate extraction

The OT-2's improvements to nucleic acid extraction can lead to a significant return on its initial investment. Automation with the OT-2 Nucleic Acid Extraction Workstation costs $11,340. That cost can be compared to approximately 380 hours of full-time-equivalent time (FTE)* in technician work. Assuming medium-sized throughput of 24 samples, manual extraction can take approximately 5 hours. The OT-2 processed 24 samples in less than 1.5 hours on average. At a relatively modest throughput of 500 samples per month, that amounts to approximately 70 hours of reduced FTE cost. At that rate, the OT-2 would pay for itself within 6 months, and these savings would increase dramatically with larger-scale throughput **(Table 4)**. *Assuming an hourly wage of $30.

SUMMARY

- The OT-2 can pay for itself, simply in terms of saved time, in less than 6 months for moderate-throughput laboratories.
- The OT-2 saved 3.5 hours of hands-on time for extraction runs of 24 samples.
- Higher throughput came with better precision, reduced variability, and improved reproducibility compared to manual performance.
- This high performance can facilitate better quality control and reduce variability in downstream data.
- Further, the elimination of lengthy hands-on procedures can reduce errors and the associated costs for rerunning procedures with expensive reagents.

Initial Investment				**$11,340**
	Manual	vs	Automated	Savings
Savings Per Run: 24 Sample Runs	5 Hours HOT / 24 samples		1.5 Hours HOT / 24 samples	3.5 hours saved / 24 samples
Throughput: 500 Runs Per Month	104.17 hours / month		31.25 hours / month	72.92 sours saved / month
FTE: wage of $30/hour	$3,125.10 FTE / month		$937.50 FTE / month	$2,187.60 FTE saved / month
Time To Recover Investment	**$11,340 / 2,187.60 = ~5 months**			

Table 4: Automated RNA Extraction with the OT-2 Recovers Investment in Approximately 6 Months. Savings calculated in terms of FTE hands-on time (HOT) saved. Calculations based on a relatively modest run size of 24 samples and moderate throughput of 500 samples per month. Full time equivalent considered as $30 hourly wage for a full-time technician.

REFERENCES

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2. Panpradist, Nuttada, *et al*. "Swab sample transfer for point-of-care diagnostics: characterization of swab types and manual agitation methods." *PloS one* 9.9 (2014): e105786.
3. Lista, Maria Jose, *et al*. "Resilient SARS-CoV-2 diagnostics workflows including viral heat inactivation." *medRxiv* (2020).