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Article

Accessible Platform for High-Throughput COVID-19 Molecular Diagnostics and Genome Sequencing Using a Repurposed 3D Printer for RNA Extraction

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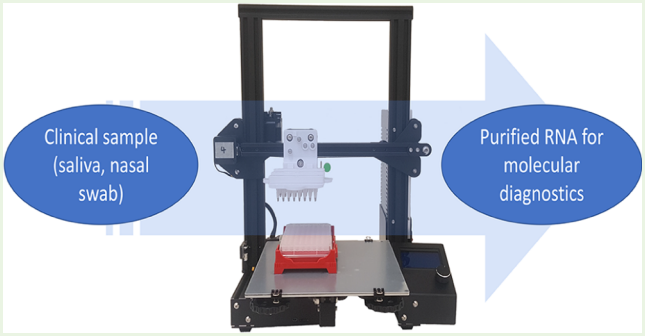
ABSTRACT: The COVID-19 pandemic has exposed the dependence of diagnostic laboratories on a handful of large corporations with market monopolies on the worldwide supply of reagents, consumables, and hardware for molecular diagnostics. Global shortages of key consumables for RT-qPCR detection of SARS-CoV-2 RNA have impaired the ability to run essential, routine diagnostic services. Here, we describe a workflow for rapid detection of SARS-CoV-2 RNA in upper respiratory samples including nasal swabs and saliva, utilizing low-cost equipment and readily accessible reagents. Using repurposed *Creality3D Ender-3* three-dimensional (3D) printers, we built a semiautomated paramagnetic bead RNA extraction platform. The hardware for the system was built for $300 USD, and the material cost per reaction was $1 USD. Named the *Ender VX500*, instrument performance when paired with RT-qPCR for SARS-CoV-2 detection in nasal and saliva specimens was two virus copies per microliter. There was a high-performance agreement (assessed using 458 COVID-19 nasal swab specimens) with the Aptima SARS-CoV-2 assay run on the Hologic Panther, a commercial automated RNA extraction and detection platform. Inter- and intrainstrument precision was excellent (coefficients of variation (CoV) of 1.10 and 0.66−1.32%, respectively) across four instruments. The platform is scalable with throughput ranging from 23 specimens on a single instrument run by one user in 50 min to 364 specimens on four instruments run by four users in 190 min. Step-by-step instructions and protocols for building and running the *Ender VX500* have been made available without restriction.



KEYWORDS: *RNA extraction and purification, 3D printer, COVID-19, SARS-CoV-2, RT-PCR, molecular diagnostics, genome sequencing*

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the etiological agent of the coronavirus disease 2019 (COVID-19) pandemic, has emerged as a global health concern with governments worldwide taking drastic infection control measures. Prompt identification of individuals shedding SARS-CoV-2 to prevent further transmission is integral to an effective public health response, a measure predicated upon the widespread availability of rapid diagnostics for SARS-CoV-2.[1] Viral RNA extraction from a nasopharyngeal swab in universal transport medium (UTM) followed by reverse transcription quantitative polymerase chain reaction (RT-qPCR) is the current gold standard method for detecting SARS-CoV-2, as it offers exceptional sensitivity and specificity.[2]

Despite its proven diagnostic utility, access to RT-qPCR is largely restricted to well-resourced, central laboratories, partly attributable to dependence on expensive, automated, high-throughput RNA extraction platforms and their dedicated kits. Such nucleic acid purification platforms are bound by servicing contracts to maintain accredited validation and often use proprietary reagents. Furthermore, the dominance of a handful of corporations providing the robotic platforms for RNA extraction has, at times, led to a fragile supply chain of consumables and reagents.[2−4] This combination of market oligopoly and very high global demand has kept the price of materials for RNA extraction kits high and resulted in

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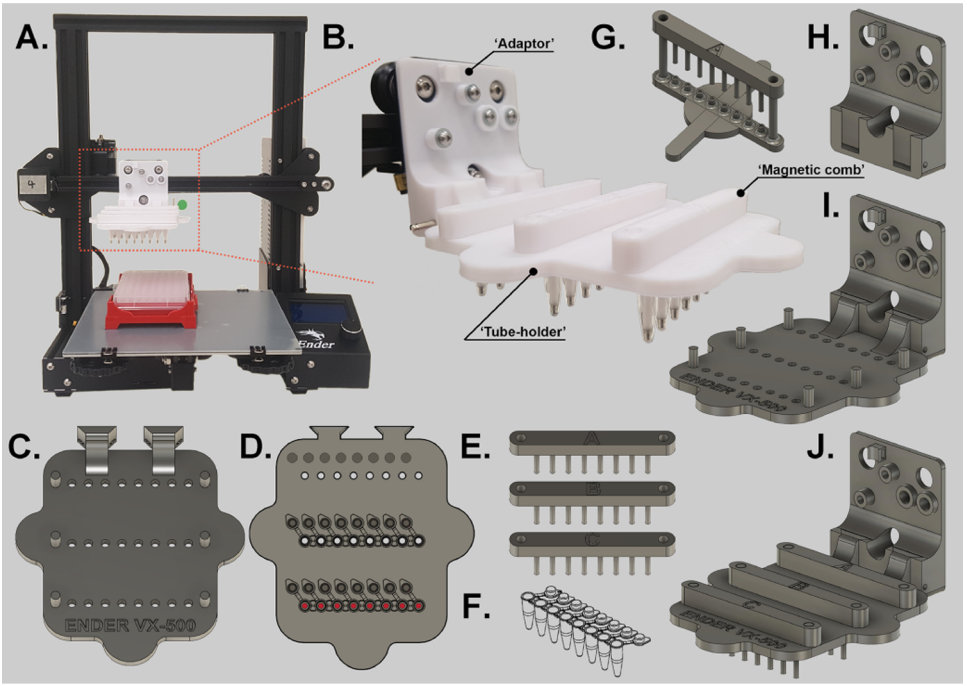

Figure 1. Overview of *Ender VX500* and components. (A) *Ender VX500*, fully assembled for extraction with a 96-well extraction plate loaded on the plate-holding rack (red). (B) Details of the interlocking 3D-printed *Ender VX500* attachments (adaptor, tube-holder, and magnetic combs) mounted on the printer extruder mounting mechanism. (C) Top view of the tube-holder. Note the double-trapezoidal lock system fitted with a set of extrusions that accept an Allan wrench locking pin to secure the attachment firmly into place. (D) Bottom view of the tube-holder. Note the three rows of eight holes that can hold the caps of the rigid 8-tube strip. The other three rows of eight holes pass entirely through the surface of the tube-holder and allow the magnetic combs (colored red) to be inserted into the 8-tube strip. (E) A set of three magnetic combs. Notice the two extrusions on the magnetic combs that tightly fit a set of rods on the tube-holder. (F) Technical drawing of the rigid 8-tube 0.2 mL polymerase chain reaction (PCR) strip. (G) Accessory magnet loader that can reproducibly fix rear-earth magnets onto the tips of the magnetic combs. (H–I) Detailed overview of how the adaptor, tube-holder, and magnetic combs interlock together.

unreliable and unpredictable availability for many diagnostic laboratories, and in some cases, no access, particularly in lower-income countries.

Since RNA extraction from clinical samples is critical to ensure high sensitivity and specificity for the detection of SARS-CoV-2 by molecular methods, we sought to evaluate a noncommercial, innovative approach to nucleic acid testing in the face of the current supply chain issues.[2,5,6] Building on a body of recent research that has shown three-dimensional (3D) printers can be repurposed to automate nucleic acid extractions, particularly the work of Chan et al.;[7] here, we "hijack" a 3D printer's robotics to emulate the movements required for a plate-based RNA extraction protocol.

In this study, a custom paramagnetic bead-based RNA extraction protocol is automated on a repurposed printer, which we named *Ender VX500*. Utilizing *Ender*-3 to 3D-print the components required to convert it into a functional nucleic acid extraction platform, we subsequently demonstrate our *Ender VX500* extraction protocol to yield RNA that is comparable to a commercial automated extraction system and compatible with downstream molecular methods including RT-qPCR and amplicon-based genome sequencing. Our designs for the 3D-printed parts are open source and have been deposited in the public domain. It is hoped that this work will facilitate the deployment of similar cost-effective, yet efficient systems to assist diagnostic capacity in the fight against COVID-19.

MATERIALS AND METHODS

Physical Modifications to the *Creality3D Ender-3* and Additional Parts Required for RNA Extraction. The 3D printers used in this study were purchased through Amazon from various authorized resellers of *Creality3D* (Sainsmart and Comgrow). The 3D modeling and CAD design of printer modifications was done with Autodesk Fusion 360, and all models were deposited in the Thingiverse repository (thing:4591346). The main modification to the *Ender*-3 was the replacement of the plastic-extruding heated nozzle and its surrounding fan shroud assembly with three custom-designed, interlocking attachments we named "adaptor," "tube-holder," and "magnetic comb," which were 3D-printed in PLA filament (Figure 1). The adaptor was assembled directly on the extruder's mounting mechanism using the same four bolts that attached the heated nozzle and its fan shroud (Figure 1). The adaptor is equipped with a double-trapezoidal lock system, which allows different attachments to be mounted reproducibly (Figure 1). The tube-holder was mounted onto the adaptor using this toolless locking mechanism (Figure 1). The two pieces were firmly locked into position by inserting an Allan wrench—supplied in the *Ender*-3 assembly toolkit—through a set of overlapping holes provided in both parts. The bottom of the tube-holder was designed with three rows of eight holes that fit and tightly hold the caps of a specific rigid 8-tube 0.2 mL PCR strip (SSIbio, ref 3247-00) (Figure 1). A set of three "magnetic combs" was inserted through the tube-holder directly into the bottom of these 8-tube strips (Figure 1). Each inserted magnetic comb was locked into position by a set of two rods on the tube-holder that tightly fitted a compatible set of holes on the combs (Figure 1). The tips of the magnetic combs were equipped with cylindrical neodymium rear-earth magnets (Amazing Magnetics, ref R125-094). We designed an accessory "Magnet loader" that assists with accurately fixing the magnets onto the tips of the magnetic combs using acrylic

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glue (Figure 1). During automated extraction, the magnets are shielded from direct contact with the RNA binding paramagnetic beads by the certified RNase-free 8-tube strips (Figure 1).

The spacing between the rows of 8-tube strips attached to the tube-holder was designed to match the layout of the 96-well plate format. As a result, the *Ender VX500* can process microtiter plates pre-loaded with both samples and the reagents required for paramagnetic bead-based RNA extraction. We specifically chose 96-well plates with V-shaped wells (Greiner, ref 651201) to limit the required elution volume. The 96-well plates were reproducibly loaded into the *Ender VX500* by mounting on a 96-well-plate-holding rack (Interpath, ref 524009), which was glued to the building platform of *Ender-3* (Figure 1).

To limit environmental contamination, the *Ender VX500* was housed in a custom-built acrylic enclosure, equipped with a hinged door. We also chose to include the following optional 3D-printed accessories: a fan shroud on the main board enclosure to redirect the airflow away from the RNA extraction platform (thing:2935204); four supporting feet (thing:3020865) printed using a flexible filament (NinjaTek, ref 3DNF0117510) that dampen machine vibrations to reduce noise; four cable clips that fit tightly to the printer's aluminum frame to keep cables tidy (thing:2960375).

Creating an RNA Extraction Workflow Using G-Code. 3D printers use a human-readable programming language called G-Code that is made up of a series of commands, which have an assigned movement or action. To repurpose the *Ender-3* for RNA extraction, we wrote a G-Code script that controls the motion and speed of the extruder mount relative to the building platform. The script directs the magnetic combs on the interlocking attachments to move from column to column on a 96-well plate and shake, transporting the RNA−paramagnetic bead complexes through the wash steps and into the elution buffer.

Preparation of RNA Extraction Reagents. Solid-phase reversible immobilization (SPRI) on carboxylated paramagnetic beads (Sera-Mag Magnetic SpeedBeads, GE Healthcare) were prepared for RNA binding as described.[8] Prior to use, each batch of SPRI beads was assessed for satisfactory performance as described.[8] Buffer for the extraction of RNA, called guanidinium thiocyanate (GTC) lysis buffer, was composed of 6 M guanidine thiocyanate (GTC), 150 mM Tris HCl (pH 8.0), 90 mM dithiothreitol, 30 mM ethylenediaminetetraacetic acid (pH 8.0), and 3% Triton-X 100. Both the activated SPRI beads and 6 M GTC lysis buffer were batch tested for satisfactory performance prior to use with the *Ender VX500*, using a defined concentration of SARS-CoV-2 RNA (approx. 5×10^2 $TCID_{50}$/mL). Batch acceptance criteria included percent coefficients of variation < 2% for at least triplicate RNA extractions as measured by E-gene RT-qPCR C_t values and no-template controls returning negative results, indicating an absence of RNA or DNA contamination (see RT-qPCR details below).

Setting Up the *Ender VX500* for RNA Extraction. To prepare the *Ender VX500* platform for use, three 8-tube strips were attached onto the tube-holder. We recommend handling the strips by the caps to prevent contamination of the tubes that will come into direct contact with the samples. The tube-holder was then seated into the adaptor's trapezoidal lock and fixed in place using the Allan wrench. Finally, three magnetic combs were inserted into the 8-tube strips. A maximum of 24 samples can be processed simultaneously during the instrument run.

A 96-well plate with V-shaped wells was prepared for extraction following a designated plate layout,[9] with 18.5 μL of GTC lysis buffer added to each well in columns 1, 5, and 9; 150 μL of 80% (v/v) ethanol (wash buffer) added to columns 2, 3, 6, 7, 10, and 11; and 80 μL of nuclease-free water (elution buffer) added to columns 4, 8, and 12. The plate was then transferred into a Class II Biological Safety Cabinet for addition of clinical samples. The following steps only apply to columns 1, 5, and 9 that already contain lysis buffer: 37 μL of sample was added to sequential wells and mixed by pipetting, then incubated at room temperature for 1 min. Each clinical sample was processed only once; 30 μL of SPRI bead mix was added and mixed by pipetting; 85 μL of 100% ethanol was added and mixed by pipetting, and the plate was incubated at room temperature for 5 min.

The prepared plate was then immediately mounted on the *Ender VX500* plate-holding rack, and the RNA extraction G-Code "print" protocol was initiated. Fully automated, the protocol runs for 20 min, requiring no user intervention. The *Ender VX500* first collects the bead−RNA complex from the lysate (5 min) in columns 1, 5, and 9. This is then followed by two wash steps in 80% (volume/volume) ethanol that each comprise a micro-shaking phase (1 min) and a bead−RNA complex collection phase (1.5 min), in columns 2, 3, 6, 7, 10, and 11. The beads are subsequently air-dried (5 min) and eluted in nuclease-free water with micro-shaking (1 min) in columns 4, 8, and 12. Finally, the beads are collected by the magnets and the magnetic combs raised out of the eluate (1 min). The machine sounds a beep to indicate the successful completion of the run. The plate is removed from the *Ender VX500* and transferred onto a 96-well magnetic rack to transfer the eluate to the downstream detection assays, free of any residual carryover beads. The 8-tube strips on the tube-holder are replaced with new strips at the end of each run. A detailed SOP of the *Ender VX500* RNA extraction protocol is available on *protocols.io*.[9] We have also prepared a step-by-step video guide to setting-up, running, and cleaning the Ender VX500 (https://youtu.be/aMUB5sEuJ2U).

Specimen Collection and Handling. Bilateral deep nasal oropharyngeal swabs were collected by qualified healthcare professionals from patients meeting the epidemiological and clinical criteria as specified by the Victorian Department of Health and Human Services at the time of swab collection between August 10 and 18, 2020. A range of commercial respiratory swabs and UTM were used by the collecting centers during this time (Copan 321C, Copan 346C, Copan 155C, Kang Jian KJ502-19D). Samples were collected in Melbourne, Victoria, Australia, and transported to the Microbiological Diagnostic Unit Public Health Laboratory, Doherty Institute, for further testing as per World Health Organization recommendations.[10] All swabs were processed in a class II biological safety cabinet according to standard diagnostic and microbiological practices.

SARS-CoV-2 E-Gene and Human RNaseP RT-qPCR. Two RT-qPCR assays were performed: a diagnostic assay targeting the SARS-CoV-2 viral envelope protein gene (E-gene)[11] and a *Homo sapiens* ribonuclease (RNase) P gene assay[12] used to assess *Ender VX500* precision. All oligonucleotide sequences are provided in Table 1 and were synthesized by Integrated DNA Technologies (IDT). For both assays, one-step RT-qPCR assays were performed with the Luna Universal Probe One-Step RT-qPCR Kit (NEB E3006). Each 20 μL reaction consisted of 10 μL of 2X Luna reaction mix, 400 nM forward and reverse primers, 200 nM probe, 2 μL of nuclease-free water, and 5 μL of purified RNA. Thermocycling conditions were as follows: 55 °C for 10 min; 95 °C for 1 min; then 45 cycles of 95 °C for 10 s and 58 °C for 30 s. Fluorescence was assessed after each extension step. All reactions were performed on a QuantStudio 1 Real-Time PCR System (Thermo Fisher). Run analysis was performed using

Table 1. Primer and Probe Sequences Used in This Study[a]

oligo	sequence (5′−3′)	refs
E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT	11
E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA	11
E_Sarbeco_P1	/FAM/-ACACTAGCCATCCTTACTGCGCTTCG-/BBQ/	11
RP-F	AGATTTGGACCTGCGAGCG	12
RP-R	GAGCGGCTGTCTCCACAAGT	12
RP-P	/5YAkYel/TTCTGACCT/ZEN/GAAGGCTCTGCGCG/3IABkFQ/	12

[a]Notes: /FAM/ = 6-carboxyfluorescein, /BBQ/ = BlackBerry Quencher, /5YAkYel/ = Yakima Yellow, /3IABkFQ/ = Iowa Black FQ, /ZEN/ = ZEN Internal Quencher.

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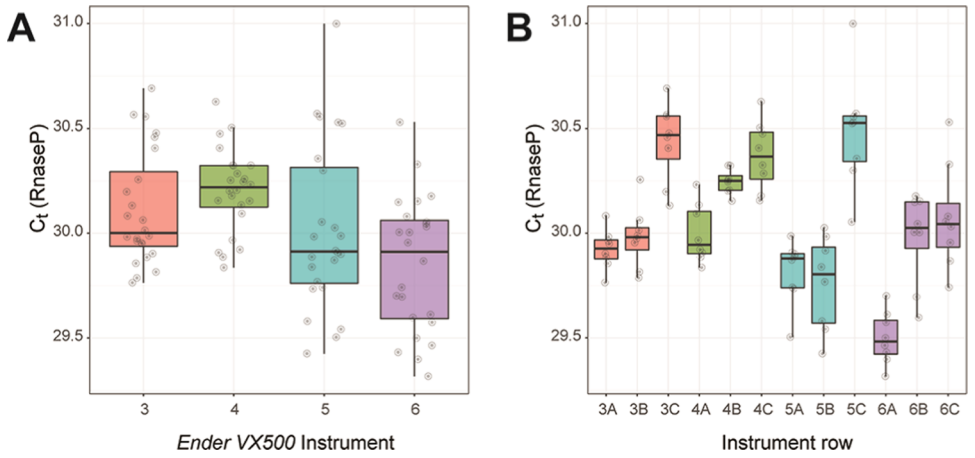

Figure 2. Assessment of *Ender VX500* intra- and interinstrument precision. 92 replicate RNA extractions of a single buccal swab were performed on four different *Ender VX500* instruments (named 3, 4, 5, and 6). The 92 RNA extracts were assayed with the human RNaseP RT-qPCR assay. Results are grouped per instrument (A) and per instrument row (B).

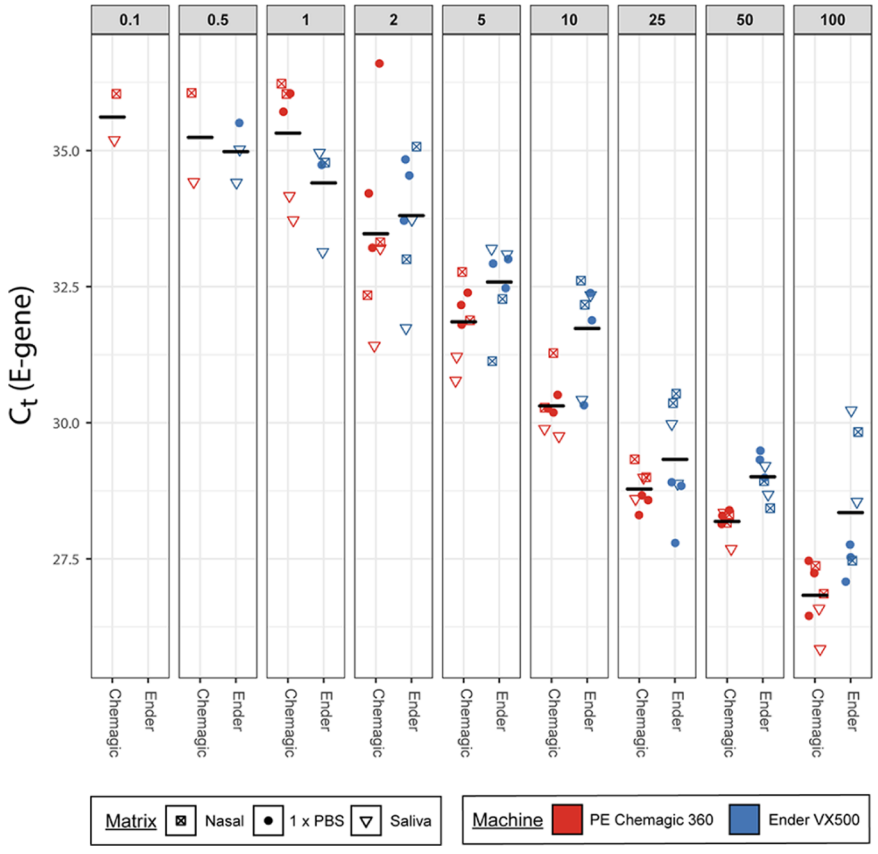

Figure 3. Analytical detection sensitivity. Comparison of the *Ender VX500* to the PE Chemagic 360 nucleic acid extraction platform, using dilutions of inactivated virus particles (Zeptometrix) in three different matrices. The *Y*-axis is C_t (E-gene) RT-qPCR, and the facets along the categorical *x*-axis represent a sample dilution series across a concentration range of 0.1−100 virus copies/μL. Black lines indicate platform-specific mean C_t (E-gene) RT-qPCR values at particular virus particle concentrations.

QuantStudio Design and Analysis Software v1.5.1 (Thermo Fisher). Copy number controls for RnaseP and E-gene for SARS-Cov-2 were purchased from IDT.

XPRIZE Proficiency Panel. While participating in the semifinal round of the XPRIZE "Rapid Covid Testing" competition,[13] we obtained a blinded SARS-CoV-2 proficiency panel, which allowed us to establish the analytical sensitivity of the *Ender VX500*. The panel consisted of a dilution series of inactivated SARS-CoV-2 particles (ZeptoMetrix, NATSARS(COV2)-ERC) spiked in a number of different sample matrix types including synthetic nasal and saliva matrices and phosphate-buffered saline (PBS).

RNA Extraction on the PerkinElmer Chemagic 360 Instrument. RNA was extracted using the Chemagic Viral DNA/RNA kit on a PerkinElmer (PE) Chemagic 360 instrument according to the manufacturer's instructions.

Hologic Panther Aptima SARS-CoV-2 Assay. SARS-CoV-2 testing was performed using the Aptima SARS-CoV-2 assay run on the Hologic Panther System (Hologic, Marlborough, MA) according to manufacturer's instructions.[14]

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Genome Sequencing and Analysis. SARS-CoV-2 RNA extracted by the PE Chemagic 360 or the *Ender VX500* was prepared and sequenced as previously described,[15] using a tiled amplicon PCR with ARTIC version 3 primers and Illumina sequencing. Reads were aligned to the SARS-CoV-2 reference genome (Wuhan-Hu-1; GenBank MN908947.3), and consensus sequences were generated.[15]

Statistical Analysis. Data analysis was managed using GraphPad Prism (v8.4.1). Performance agreement testing was performed using the Wilson/Brown hybrid method as deployed in GraphPad Prism. Graphs were made using the data visualization package ggplot2 for R v4.1.0.

Ethics Statement. This study was conducted in accordance with the National Health and Medical Research Council of Australia National Statement for Ethical Conduct in Human Research 2007 (Updated 2018). The study was exempt from requiring specific approvals, as it involved the use of existing collections of data or records that contained nonidentifiable data about human beings.[16]

RESULTS

Assessment of *Ender VX500* Analytical Precision. We first sought to assess *Ender VX500* precision, that is, the ability of the platform to reproducibly extract RNA from a defined specimen. To do this, we took a buccal swab from a single human volunteer and eluted the cellular material in 10 mL of PBS. This 10 mL sample was then used to prepare 92 replicate RNA extractions with four PBS extraction negative controls, run across the three rows of four different *Ender VX500* instruments. The resulting 92 RNA preparations and extraction controls were then assayed for human RNaseP mRNA by RT-qPCR to measure intra- and interinstrument precision. The average C_t obtained across the 92 samples for all instruments was 30.05 (range 29.32−31.00, coefficient of variation (CoV) 1.10%). Detailed results for individual instruments and each instrument row are provided (Table S1). The combined results indicate excellent intra- and interinstrument precision (Figure 2). The four extraction controls were RT-qPCR-negative, indicating an absence of cross-contamination between wells.

***Ender VX500* Analytical Sensitivity and Comparison with a Commercial RNA Extraction Platform.** We next assessed the analytical sensitivity of the *Ender VX500*. SARS-CoV-2 RNA was extracted from a set of 69 blinded proficiency samples, prepared for the COVID-19 Testing XPRIZE competition. These samples were a twofold dilution series of inactivated SARS-CoV-2 virus particles, prepared as two to three replicates in each of 1× PBS, synthetic nasal media, and synthetic saliva. A 37 μL volume of each sample was processed by the *Ender VX500*, and 150 μL volume was processed in parallel by the PE Chemagic 360 platform. The SARS-CoV-2 E-gene RT-qPCR assay was then used to assess the RNA yield from each extraction system and compare performance. Analytical detection sensitivity (defined as the lowest sample concentration where 7/7 replicates were detected) was 2 virus copies/μL for both the *Ender VX500* and PE Chemagic 360 (Figure 3, Table S2). Precision was acceptable for the *Ender VX500* with coefficients of variation (CoV) ranging from 1.2−4.4% across the dilution series. Similar, but trending lower CoV values were observed for the PE Chemagic 360 and ranged from 0.86 to 4.9% (Figure 3). There was no observable impact of sample matrix (PBS/nasal/saliva) on *Ender VX500* performance (Figure 3), showing the combination of our lysis/purification chemistry and instrument effectively removed potential PCR inhibitors.

***Ender VX500* Performance with COVID-19 Clinical Specimens.** Having demonstrated that our protocol could detect SARS-CoV-2 RNA at clinically relevant concentrations, we also validated *Ender VX500* RNA extraction with clinical samples. A panel of 75 SARS-CoV-2 positive and 383 SARS-CoV-2 negative nasal swab specimens as determined by the Aptima SARS-CoV-2 Assay (Hologic Panther System) were also processed using the *Ender VX500* and the extracted RNA tested by E-gene RT-qPCR (Table S3). The sensitivity and specificity for *Ender VX500* extracted RNA compared to the Hologic Aptima SARS-CoV-2 assay is shown in Table 2. Using E-gene RT-qPCR, the *Ender VX500* was demonstrated to have 96% positive predictive value and 100% negative predictive value. One of the three false negatives was explained by low viral load (weak positive on the Panther instrument) presumably at the limit of detection for the E-gene RT-qPCR (Table 2). The 75 positives samples were interspersed throughout the 384 negative samples on multiple *Ender VX500* runs. We did not observe any well-to-well cross-contamination and false positives.

Table 2. Performance Agreement between *Ender VX500*-Based RNA Extraction Coupled with E-Gene RT-qPCR and the Aptima SARS-CoV-2 Assay Run on the Hologic Panther System[a]

	Ender VX500 E-gene RT-qPCR+	*Ender VX500* E-gene RT-qPCR−
Hologic Aptima SARS-CoV-2+	72	3
Hologic Aptima SARS-CoV-2−	0	383

[a]*Ender VX500* RNA suitability for genome sequencing.

To assess the compatibility of the RNA prepared by the *Ender VX500* with SARS-CoV-2 genome sequencing, RNA from three positive clinical specimens prepared for the comparison with the Hologic Aptima SARS-CoV-2 assay (see above) was subjected to genome sequencing (ARTIC tiled amplicon protocol with Illumina sequencing; see the Materials and Methods section). More than 700 000 150 bp paired-end sequence reads were obtained for each sample (Table 3).

Table 3. *Ender VX500* Genome Sequencing Summary Statistics

specimen ID	C_t E-gene RT-qPCR	no. of reads mapped to reference	original accession no.
VIC7435	24.01	707 117	EPI_ISL_564407
VIC7433	20.55	857 967	EPI_ISL_564409
VIC7431	18.23	868 392	EPI_ISL_564407

The resultant consensus genome sequences were then compared to consensus sequences obtained from the same clinical specimens using RNA prepared by the PE Chemagic 360 instrument, from which high-quality, high-confidence genomes had previously been derived.[17] Genome sequences derived from *Ender VX500* RNA were distinct from each other by 5−7 SNPs but were identical to those sequences previously derived from the PE Chemagic 360 instrument, across a 29 782 bp contiguous length of the SARS-CoV-2 genome (Figure 4). These comparisons show that RNA prepared from the *Ender VX500* is suitable for SARS-CoV-2 amplicon-based genome sequencing.

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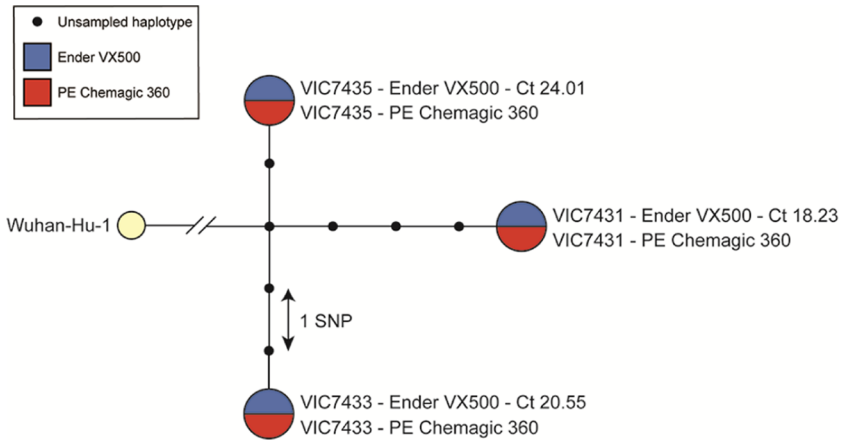

Figure 4. Whole genome comparisons for three SARS-CoV-2 positive clinical specimens. Shown is a haplotype network of SNP differences between three SARS-CoV-2 genomes, assembled using the ARTIC tiled amplicon protocol and Illumina sequencing. RNA was prepared from the three specimens using both the *Ender VX500* and the PE Chemagic 360 and the resulting consensus sequences were compared.

DISCUSSION

The COVID-19 global pandemic is an unprecedented event that has stretched the capacity of diagnostic services, necessitating the development of cost-effective, flexible, rapid, and scalable diagnostic solutions. The research presented here is an effort to enhance diagnostic capacity through an easily deployed, automated approach that yields scalable and robust nucleic acid purification chemistry at a low cost with readily available materials.

Automation of the RNA extraction workflow greatly enhances diagnostic capability, standardizes sample processing, and considerably minimizes handling and pipetting errors. Typical commercial automated nucleic acid purification instruments from key suppliers like Qiagen, Roche, and Abbot can uniformly process hundreds of samples per day with minimal hands-on time. Unfortunately, these instruments are costly ($20 000−$100 000 USD), are subject to consumable supply shortages, and most diagnostic centers do not have the number of instruments required to process the large influx of samples that are currently being collected. There is thus an urgent need for alternative nontraditional workflows that increase nucleic acid testing capacity while offering several contingency options in the face of consumable and reagent shortages. We therefore developed and validated a paramagnetic bead-based RNA extraction protocol that can be automated on a repurposed 3D printer, which we named the *Ender VX500*.

We used a paramagnetic bead-based RNA extraction due to the ready availability of the reagents, unaffected by global shortages; amenability for high-throughput processing; and no requirement for specialized plasticware such as silica-based spin columns.

We demonstrated that the *Ender VX500* platform can detect SARS-CoV-2 RNA at clinically relevant concentrations and showed inter- and intrainstrument precision was excellent. Magnetic bead-based RNA extraction on the *Ender VX500* followed by E-gene RT-qPCR was benchmarked against the Aptima SARS-CoV-2 assay implemented on the Hologic Panther automated RNA extraction and detection platform. Similar sensitivity and specificity were obtained for the protocol described here, suggesting that RNA extraction yields were comparable to the commercial platform. This also indicates that the extracted RNA quality was compatible with various downstream detection methods, a finding that is in alignment with other studies.[1]

A potential area for improvement we observed with the *Ender VX500* was the slight decrease in RNA extraction efficiency for samples in row C of the plate-holder compared to rows A and B. This conclusion was suggested by the tendency of C_t values for samples processed on row C to be around 0.5 cycles higher than the other two rows (Figure 2B). We explain this "row effect" by the tube-holder sitting at a very slight downward angle relative to the 96-well lysis plate such that the height of row C above the lysis plate is less than rows A and B. We speculate that this small height variation is sufficient to impact the efficiency of RNA elution in the final step by slightly changing the insertion depth of the magnetic combs in the plate wells.

Furthermore, the high-quality RNA yielded by our described method allows the flexibility of additional analysis such as whole genome sequencing, which is not an option with fully integrated extraction-to-RT-PCR commercial assays, such as the Panther Aptima SARS-CoV-2 assay, since such closed systems do not allow the diversion of extracted RNA for alternative downstream analysis.

Our presented diagnostic workflow can be rapidly assembled and is amenable to high-throughput scaling. The *Ender VX500* RNA extraction protocol took approximately 50 min to process 23 nasopharyngeal swabs on a single machine, with a single user processing samples/preparing extraction plates. When multiple *Ender VX500*s were run concurrently, RNA could be extracted from 364 swabs on four instruments in 190 min, with four people processing samples/preparing extraction plates. Similar to commercial extraction platforms, the registration and manual aliquoting of the clinical samples was the most time-consuming step of the protocol.

Due to physical restrictions determined by the placement of the magnet and 8-well strip tube within the 96-well plate, the minimum elution volume that could be used in the *Ender VX500* was 80 μL, which prevented effective concentration of the yielded RNA. Further refinement of the design could potentially optimize the RNA yield and sensitivity of downstream testing.

The *Ender VX500* itself was built for approximately $300 USD, which includes the cost of the interlocking attachments and the optional accessories that were 3D-printed on the

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instrument before converting it into an RNA extraction platform. The *Ender 3* 3D printer accounted for the majority of the cost. We estimated the combined consumable and reagent cost per reaction to be approximately $1.00 USD, which represents a fraction of an extraction performed on a commercial platform. However, as with any diagnostic platform used in human medicine, there are additional costs that need to be factored including batch testing and quality control of the lysis (GTC buffer) and purification reagents (activated SPRI beads) and periodic, routine quality assurance testing of the *Ender VX500* to ensure that the instrument is performing as expected. The specifics of these quality control and assurance activities will vary by country and jurisdiction and will need to comply with the requirements of the relevant pathology laboratory accreditation bodies and regulations.

When automating nucleic acid extraction for diagnostic testing, a major concern is presented by the potential of aerosols to cross-contaminate samples, which could result in false-positive diagnostic results. We demonstrated that no cross-contamination occurred between wells of the *Ender VX500* when extracting from strongly positive COVID-19 samples interspersed with negative controls in adjacent wells. Furthermore, to prevent external aerosol contamination, we housed all *Ender VX500* instruments in acrylic enclosures, each equipped with a hinged door.

Alleviating the cost barrier and supply chain bottlenecks to RNA extraction systems is important; however, similar impediments remain for the diagnostic instrumentation required to run the molecular detection assays. Consistent with our innovation reported here, there have been recent efforts to develop cost-effective diagnostic hardware alternatives, such as those that utilize loop-mediated isothermal amplification (LAMP), as this technology requires less sophisticated instrumentation than that of qPCR.[18,19] A parallel project in our research group has been the development of a low-cost and open-source device for high-performance fluorescence detection of LAMP reactions that can be manufactured locally from readily available parts (submitted).

As the COVID-19 pandemic continues and future pandemics are likely, devising innovative effective strategies for scalable and cost-effective nucleic acid extraction will be a continuing global challenge. It is hoped that the *Ender VX500* paramagnetic bead-based method outlined here will provide a frontline end-to-end diagnostic platform solution for low- to middle-income countries or developed nations in times of emergency.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsbiomaterials.1c00775.

> *Ender VX-500* intra- and interinstrument precision (Table S1); XPRIZE COVID-19 Testing proficiency sample results (Table S2); and analytical performance of the *Ender VX-500* using clinical specimens (Table S3) (PDF)

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K.V., T.H., B.P.H., and T.P.S. conceived the study. K.V., A.H.B., L.L., L.K.S., M.H., S.J.P., I.R.M., T.S., and J.Y.H.L. conducted experiments. K.V., A.H.B., and T.PS. wrote the manuscript. All authors read and approved the final draft of the manuscript.

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■ ABBREVIATIONS

CoV	coefficients of variation
COVID-19	coronavirus disease 2019
E-gene	viral envelope protein gene
GTC	guanidine thiocyanate
IDT	integrated DNA technologies
LAMP	loop-mediated isothermal amplification
PBS	phosphate-buffered saline
PE	Perkin Elmer
RNase	ribonuclease
RT-qPCR	reverse transcription quantitative polymerase chain reaction
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SPRI	solid-phase reversible immobilization
UTM	universal transport medium

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