

Development and Validation of a Portable Device for Lab-Free Versatile Nucleic Acid Extraction

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Abstract

Nucleic acid testing (NAT) has revolutionized diagnostics by providing precise, rapid, and scalable detection methods for diverse biological samples. These recent advancements satisfy the increasing demand for on-site diagnostics, yet sample preparation remains a significant bottleneck for achieving highly sensitive diagnostic assays. There is an unmet need for compatible, efficient, and lab-free sample preparation for point-of-care NAT. To address this, we developed a portable, lab-free, and battery-powered device for extracting nucleic acids. We explored using low centrifugal forces with existing commercial chemistry, demonstrating excellent performance. We designed and tested a battery powered device to enable lab-free extractions, and verified reagents stored out to 6 months, suggesting exceptional deployment capabilities. We evaluated our device, comparing our results against those from a benchtop centrifuge across three types of samples: HIV RNA in buffer, HIV RNA in plasma, and SARS-CoV-2 RNA in saliva. The portable device demonstrated excellent agreement with the benchtop centrifuge, indicating high reliability. By providing an effective on-site sample preparation solution, the widespread adoption of low

centrifugal extractions will improve the sensitivity and reliability of NAT and will positively impact other point-of-care technologies such as next generation sequencing (NGS), biomarker detection, and environmental monitoring.

Keywords

Sample preparation, lab-free extraction, nucleic acids, point-of-need, field deployable, HIV, SARS-CoV-2

Method Summary

This method utilizes a low-power, portable centrifuge to significantly improve the deployment of nucleic acid extractions. As a result, this method offers comparable extraction performance to benchtop devices while offering superior portability and ease-of-use. Minimizing centrifugal force allows for reliable nucleic acid extraction from a low-power device. Our approach is simple and uses low-cost electronics, presenting high potential for clinical preparation of RNA in field settings.

Article Highlights

- Sample preparation continues to be a major bottleneck for sensitive diagnostic assays.
- We developed a portable, lab-free, and battery-powered device for extracting nucleic acids.
- We demonstrated that a commercial extraction kit could be processed at low centrifugal forces, enabling point-of-care development.
- We designed a battery-powered, semi-automated centrifuge that rivaled a benchtop centrifuge in performance and efficiency.
- Reagents remained stable for 6 months and extractions were robust without carrier RNA.
- Our device offers an effective on-site sample preparation solution that enhances NAT sensitivity and reliability while maintaining compatibility with commercial chemistry.
- Consequently, our portable centrifuge is well-positioned to impact other point-of-care applications like NGS, biomarker detection, and environmental monitoring.

1. Introduction

Nucleic acid testing (NAT) offers rapid and precise detection of nucleic acids from various biological samples [1, 2]. The process of NAT begins with the isolation of DNA or RNA from a relevant biospecimen, such as blood, saliva, or tissue [3, 4]. The purified nucleic acids are then amplified, typically using Polymerase Chain Reaction (PCR) or Reverse Transcription-PCR (RT-PCR) [5] and detected by electrophoresis, fluorescence, or next-generation sequencing [2, 6]. This process is well established in laboratory settings and is the gold standard method for many applications. Nonetheless, there is a growing trend to make this technology readily accessible in non-laboratory settings to enable on-site, rapid, and robust diagnostics [2, 7]. The translation of NAT technology outside of the laboratory is particularly relevant for the detection of infectious diseases at the point-of-care (POC). The integration of microfluidics [8, 9], miniaturized electronics [10–13], and advancements in molecular assays are pivotal for this transition to point-of-care NAT (POC-NAT) [14–17].

One common format for POC-NAT devices is the combination of sample preparation and amplification assays in a single microfluidic cartridge [5, 18, 19]. This style of device is highly needed for applications that require sample-in-answer-out [20]. Several studies demonstrated automated devices using integrated magnetic beads [21], paper-based [22], and solid-phase matrices [20, 23–25]. However, this requires redesigning these cartridges for each new application, taking into consideration various sample types, volumes, and approaches [18]. The sensitivity of integrated sample preparation frequently falls short of traditional laboratory methods due to the need for streamlined, microfluidic, and low-power systems [15, 26]. It is well known that poor sample preparation can significantly diminish the sensitivity of downstream assays due to the presence of carry-over contaminants, inhibitory reagents, and insufficient targets [27]. Therefore, an alternative to integrated POCT is to separate the sample preparation and detection device, creating two general-purpose devices that can handle various sample types (i.e. plasma or saliva) and amplification assays (i.e. PCR or LAMP [Loop Mediated Isothermal Amplification]) [16, 28]. In this arrangement, systems aim to achieve higher accuracy and broader deployment by eliminating integration and single-use cartridges.

The two most common extraction technologies found in sample preparation devices are magnetic bead and solid phase based devices [8]. Magnetic bead-based extractions are commonly found in integrated systems due to their ease of automation, versatility, and lack of centrifuge

dependence. In our previous studies, we found that automated magnetic bead-based extraction systems exhibited reduced efficiency compared to solid phase extractions [29–33]. On the other hand, solid phase-based systems aim to translate laboratory grade extractions to the point-of-care. This format has been widely adopted into integrated systems using microfluidics [34–36], syringes [37, 38] or centrifuges [39, 40]. Portable devices such as these are particularly advantageous in situations where rapid, on-site extraction is needed [41–45]. However, these studies lack general-purpose deployment and specifically examine only one application scenario. Laboratory equipment is required for these devices to operate, and cold storage remains challenging to implement at the point-of-care. More importantly, the use of low centrifugal forces to process solid phase-based systems has been left unexplored. There is an unmet need for widely compatible, efficient, and lab-free sample preparation of nucleic acids for the point-of-care.

In this work, we developed a portable device for RNA extraction from a wide variety of samples. This lab-free device is compatible with existing commercial chemistries and materials while operating at lower centrifugal forces than conventional methods, making it versatile for various sample types. We found that the battery-powered device could generate sufficient centrifugal force for at least 30 complete extractions, however our design could be easily replicated with mini centrifuges and portable batteries. Over a 6-month storage period the portable device remained stable, therefore extending its deployment and storage time. The device was rigorously tested by comparing its extraction results against those from a conventional laboratory centrifuge. Across three types of samples (HIV RNA in buffer, HIV RNA in plasma, and SARS-CoV-2 RNA in saliva), the portable device demonstrated excellent agreement with the benchtop centrifuge, therefore indicating high reliability and sensitivity. By providing an effective on-site sample preparation solution that does not require carrier RNA or cold storage, the device’s versatility and usability could apply to many point-of-care applications. Our device widens the scope of point-of-care applications beyond nucleic acid amplification tests (NAAT) toward next generation sequencing (NGS), biomarker detection, protein analysis, and environmental monitoring.

2. Materials & Methods

2.1. Portable device design and fabrication

The portable device is composed of 3D printed parts (case and rotor) [Makerbot and PTC Creo], a DC motor (Autotoolhome via Amazon), Arduino Nano (Arduino.cc), and a 28.8 Wh Li-ion battery

(Daytona Industries) (**Figure 1a**). All electronic components, resistors, MOSFETs, switches, LEDs, and buttons, were purchased from Digikey. The device is 14 cm tall and 13 cm wide (diameter) and costs approximately \$118 (**Supplementary Figure S1a** and **Supplementary Table S2**). The extraction steps using the portable device are listed in **Table 1**. Reagents are pre-aliquoted and sealed using a commercially available vacuum sealer from FoodSaver® (**Supplementary Figure S1d**).

2.2. Extraction Protocol

Portable device. The sample extraction was achieved by using the Viral RNA Mini kit from Qiagen. All reagents and supplies for portable extraction are stored inside a vacuum pack (**Figure 1a** and **Supplementary Figure S1b**). Nucleic acid extraction follows four stages: DNA binding, Washing, Drying, and Elution (**Figure 1b**). The preloaded cartridge contains 500 µL AVL Buffer, 500 µL 95% Ethanol, 500 µL Wash Buffer I, 500 µL Wash Buffer II, 500 µL 95% Ethanol, and 80 µL water (**Table 1** and **Supplementary Figure S1c**)[46]. During all extraction stages the portable device runs at max speed (6000 rpm/ 1743 rcf.). Spiked samples were prepared by combining 100-140 µL of sample medium (TE buffer, plasma, or saliva) with lysis buffer and then immediately spiking the mixture with varied concentrations of RNA. The portable methods are demonstrated in **Supplementary Video V1** and can be found at DOI: [dx.doi.org/10.17504/protocols.io.kxygxyj4wl8j/v1](https://doi.org/10.17504/protocols.io.kxygxyj4wl8j/v1)

Benchtop device. Benchtop extractions were conducted according to the Viral RNA Mini kit from Qiagen. Extractions were performed with 100-140 µL sample, 500 µL AVL Buffer, 500 µL 95% Ethanol, 500 µL Wash Buffer I, 500 µL Wash Buffer II, 80 µL water (**Table 1**). The benchtop centrifuge was set to 1 min. at 6000 rcf. for all steps except Wash Buffer II where it ran for 3 min. at 20,000 rcf. Spiked samples were prepared by combining 100-140 µL of sample medium (TE buffer, plasma, or saliva) with lysis buffer and then immediately spiking the mixture with varied concentrations of RNA.

2.3. qRT-PCR Assay

The PCR HIV assay was previously validated by Palmer et al. and the SARS-CoV-2 assay was used from the recommended CDC sequences for the N1 region (See **Supplementary Table S1**). [47, 48]. For PCR analysis, 10 µL out of the 80 µL of elution was examined. Therefore, the total

PCR volume consisted of 25 μL : 6.25 μL of Fast Taq One-Step Master Mix (Applied Biosystems, Waltham, MA), 1.5 μL of Forward and Reverse primer, 0.63 μL of Probe, 10 μL of extracted RNA sample, and 5.13 μL of Nuclease-free water (New England Biolabs, Ipswich, MA). Analysis was conducted using a Bio-Rad C1000 Thermal Cycler (Hercules, CA). Thermal Cycling was set as 50 °C for 5 min, 95 °C for 3 s, 65 °C for 30s, and repeated 60 $\times$. Primers and probes were purchased from Integrated DNA Technologies (Coralville, IA). Positive samples were identified and tagged with a quantitative cycle (C_q) value when the background RFU reached a threshold defined as: $\mu + 3\sigma$. Gel electrophoresis was conducted on this assay to confirm the amplicon products (**Supplementary Figure S2**).

2.4. Data Analysis and Statistics

All data processing, analysis, and figure creation was completed using Python. Data is displayed as the mean of triplicates plus or minus three standard deviations, unless otherwise noted. Positive samples are classified using quantitative cycle (C_q) when RFU reaches a threshold of $\mu + 3\sigma$. Pearson's correlation coefficient (r) and least squares regression coefficients (R^2) were computed using the SciPy library.

3. Results and Discussion

3.1. Optimization of the portable device

Centrifugal force. To verify that sample preparation could be achieved at low centrifugal forces, we examined the extraction performance of a solid phased-based system using a benchtop centrifuge at various centrifugal forces. We varied the relative centrifugal force (RCF) from 20,000 down to 312.5 and processed three replicates at high concentration (10^5 cp/rxn). We found that samples processed with centrifugal forces lower than 1250 g were unable to be detected (**Figure 1c**). We suspect this is caused by the low centrifugal force which is unable to effectively pull fluid through the silica matrix, causing an inhibition in downstream elution. We noted there was a maximum 1.44 C_q difference between the samples processed with 20,000 g and 1250 g (**Figure 1f**). Therefore, the relationship between centrifugal force and extraction performance reveals why low-power, point-of-care systems struggle to process samples. The inherent tradeoff between complexity and sensitivity continues to bottleneck sample preparation for resource limited settings. Therefore, by understanding this limit, we can design ultra-portable devices to operate above the

cutoff. We found we were able to achieve a device capable of a maximum RCF of 1750 g with further details in the following sections.

Rotor Size. To enable semi-automated control, we designed a portable device and examined the effect of rotor size on extraction performance. First, we examined the variation of extraction performance using three rotor sizes, 1 cm, 1.25 cm, and 1.5 cm, to vary the relative centrifugal force of our device. Using **Equation (1)** for centrifugal force with a DC motor operating at 6000 rpm:

$$g \text{ Force (RCF)} = 1.118R\left(\frac{RPM}{1000}\right)^2 \quad (1)$$

We can approximate the maximum RCF of our three rotors to be 1542, 1642, and 1743, assuming the radius (R) is equal to the rotor size (1 cm, 1.25 cm, or 1.5 cm) plus the total length of a spin column multiplied by the sin (45°) (3.825, 4.075, and 4.325 cm, respectively). We saw minimal variation between maximum force (**Figure 1d**), and the portable device performed similarly to the benchtop centrifuge (Maximum ΔC_q of 0.45) (**Figure 1g**). These results suggest minimal variation using different centrifugal forces and demonstrate high performance extractions using low forces. We demonstrate that a portable device that achieves > 1250 g (1743 g) can achieve similar extraction performance to a benchtop centrifuge.

Processing Time. To improve processing time, we minimized the processing time of each extraction performance using our portable device. We began with the manufacturer's recommended time of eight minutes, and tested incremental decreases of time at four, two, and one minute stages (timing for each extraction step is shown in **Table 1**). We found that at high concentrations, there was little variation in extraction performance ($\Delta C_q = 0.71$) (**Figure 1e**) and the portable device using 1-min stages performed very similar to the benchtop device (**Figure 1h**). We examined the 1-min protocol further with diluted samples and found with reduced time we were unable to detect samples (**Supplementary Figure S3a-b**). To maintain sensitivity, the portable device used 8 min. (manufacturer's recommended protocol) with the 1.5 cm rotor (See **Table 1**).

3.2. Evaluation of endurance and stability

To validate the battery performance of the portable device, we recorded the voltage during repeated extractions (simulated 1 min spin cycles with 30s breaks). We observed a significant decrease in

battery voltage over time (**Figure 2a**), but the device remained operational for more than 6 hrs. (or 30-31 extractions) (See **Figure 2d**). We examined the extraction performance during six of those 31 extractions (#1, 2, 4, 8, 16, and 30) and found the extraction performance remained stable and independent (**Figure 2b**). The largest change in C_q value ($\Delta C_q = 0.53$) was observed between the baseline and 30th trial (**Figure 2e**). These results suggest that extraction performance is independent of battery voltage, thereby demonstrating the usefulness and reliability of our device to process samples in field locations.

To explore the potential side-effects of storage time on sample extractions, we examined identical samples using extraction reagents that were stored at room temperature for one, three, and six months. We found that all samples regardless of storage time showed similar amplification performance (**Figure 2c**) and demonstrated minimal variation (max $\Delta C_q = 0.25$) (**Figure 2f**). Our device does not use the manufacturer's recommended carrier RNA (Qiagen Viral RNA #52904) as it introduces the need for cold-chain storage. Therefore, by eliminating its use we are able to demonstrate a shelf-stable extraction protocol that is cold-chain independent. In Section 3.4, we compare our device's performance against the benchtop protocol demonstrating carrier RNA is not required. These results suggest our device and reagents have the potential to be used and stored away from lab settings, significantly improving the deployment of nucleic acid testing.

3.3. Examination of extraction performance

To examine the extraction performance and efficiency of our portable device we conducted several extractions using buffer samples. First, we extracted serially diluted samples of 10^5 down to 10 copies of HIV RNA in 100 μ L of buffer using the portable device. Using triplicates, the extracted samples showed amplification curves with the expected cycle delay between sample concentrations (**Figure 3a**). To summarize, we analyzed C_q value vs input copy concentration. In **Figure 3b**, we found a moderate linear trend ($R^2 = 0.811$) between the C_q value and log of the sample concentration, demonstrating the potential for quantitative measurements from our portable extraction methods.

To examine the extraction performance of our device, we calculated the ratio for a range of sample concentrations. We found that at high sample concentrations (10^5 copies per extraction [100 μ L buffer/plasma]), the ratio remains relatively high (0.84 ± 0.09); however, as we examined samples with smaller copy numbers, we found the extraction ratio gradually decreases to $0.50 \pm$

0.08 for 100 copies (**Figure 3c**). We expect this decrease in performance to be caused by the physical limitations of the spin column and the effects of sub-sampling on qRT-PCR testing. The silica material used in spin columns can irreversibly bind a portion of the DNA/RNA input. Samples demonstrate more tolerance to these errors when concentrations are high, and the irreversible bonds are a small percentage of the overall sample quantity. However, the error becomes more profound as the input sample decreases and the ratio of irreversible to reversible binding grows to a larger percent of the overall sample. We saw this using our device, samples with high copy numbers demonstrated high extraction ratios (84%) and as copy number decreased there was also a reduction in extraction ratio (as low as 50%). This phenomenon is most likely why the manufacturer recommends the use of carrier RNA, to decrease the likelihood that the sample DNA is irreversibly bound.

To evaluate our performance at clinically relevant concentrations we analyzed the probability of detecting our serially diluted samples (**Supplementary Figure S5**). Using logistic regression, we plotted probability vs input sample concentration (converted to clinical cp/mL) to visualize the hit rate curve. We found that our device was able to process and detect (with downstream PCR) samples as low as 750 cp/mL (0.75 cp/ μ L at 50 μ L). These results highlight our devices wide range of compatibility with clinical samples while using a small volume sample. These results also suggest increased efficiency and performance while demonstrating moderately linear relationships, therefore establishing our device as a suitable technology for point-of-care semi-quantitative analysis (**Supplementary Table S3**).

3.4. Comparison of portable vs laboratory methods

Contrived Samples. To benchmark the performance of the portable device against the benchtop device, we examined three different samples at clinically relevant concentrations: buffer, plasma, and saliva. First, we extracted serially diluted samples of 10^5 down to 25 copies of HIV RNA in 100 μ L of buffer using both the portable device and benchtop centrifuge (Curves shown in **Figure 4a**). We found that the portable device performed very similar to the benchtop method, showing a very strong correlation ($r = 0.961$) and strong linearity ($R^2 = 0.923$) (**Figure 4d**). Second, we extracted three concentrations from 10^5 to 10^3 cp/mL of HIV RNA spiked plasma (100 μ L) using both methods (**Figure 4b**). When plotted against each other, the largest deviation was seen in 1000 cp/mL samples, which showed a maximum delta C_q of 1.78 with x and y standard

deviations of $\sigma = 0.78$ and 0.30 , respectively. Overall, we found that the portable device performance matched very well to the benchtop method, showing a very strong correlation ($r = 0.982$) and very strong linearity ($R^2 = 0.964$) (**Figure 4e**). Last, we extracted three concentrations from 10^6 to 10^4 cp/mL of SARS-CoV-2 RNA spiked saliva ($200\ \mu\text{L}$) using both the portable device and benchtop centrifuge (**Figure 4c**). From saliva, 10^4 cp/mL samples showed a maximum delta C_q of 1.01 with x and y standard deviations of $\sigma = 0.42$ and 0.23 , respectively. Using our device, we found the portable device performed very similar to the benchtop, showing a very strong correlation ($r = 0.993$) and very strong linearity ($R^2 = 0.986$) (**Figure 4f**). Previous studies have validated the use of direct detection of SARS-CoV-2 from heat treated saliva. While this method is very simple and point-of-care friendly, it compromises sensitivity and limits the testing volume. With very strong agreement ($r > 0.96$) to the benchtop machine and minimal C_q variation (cycle variance < 3), our device demonstrates high performance extractions enabling our device to process and detect (using downstream PCR) low concentrated samples from patients outside of the acute infection window.

Clinical Samples. To validate our devices capabilities with real-world samples, we extracted and analyzed three clinical plasma samples. To process three archived clinical samples, we separated each sample into two $50\ \mu\text{L}$ aliquots and extracted them in parallel using our portable device or benchtop centrifuge. Ten microliters of the elution were tested using PCR, replicated three times showing similar performance between portable and benchtop (**Supplementary Figure S4a**). Plotting the C_q values against each other's shows a very strong agreement ($r = 0.994$) and very strong linearity ($R^2 = 0.989$) (**Supplementary Figure S4b**). These results suggest our portable device has similar performance to the benchtop centrifuge, therefore demonstrating our device's real-world ability.

4. Conclusion

Point-of-care nucleic acid testing is in demand for portable, efficient, and lab-free sample preparation devices. We developed a portable device that is compatible with RNA extractions from two clinical sample types and at low centrifugal forces. The portable device satisfies the minimum centrifugal forces required for successful extractions, therefore demonstrating its potential for field applications. Our device performed at least 30 extractions without significant performance loss using a simple design that could be replicated with mini centrifuges and battery packs.

Demonstrating minimal storage requirements, the portable device offers robust and reliable sample preparation without the need for laboratory equipment. When examining three types of samples (HIV RNA in buffer, HIV RNA in plasma, and SARS-CoV-2 RNA in saliva), the portable device demonstrated excellent agreement with the benchtop centrifuge, indicating reliability and sensitivity. This observation indicates the portable device provides an effective on-site sample preparation solution compatible with existing commercial chemistries. We will explore the development of a fully automated version of this device with plans to simplify the user experience, create a streamlined solution, and deploy it into the field for point of care testing. In the meantime, the device's strong performance demonstrates its potential for other nucleic acid targets, applications, and translation to preparation pipelines for next generation sequencing (NGS), biomarker detection, and environmental monitoring.

5. Future Perspectives

Lab-free and reliable extractions are crucial for highly sensitive nucleic acid tests operated away from traditional medical infrastructure. We created a low-power device for low copy number detection of HIV and SARS-CoV-2 by integrating commercial materials with automated electronics. We show that at low centrifugal forces and long storage times, QIAamp materials processed by our device produce highly reliable extractions for a wide variety of clinically relevant samples. Considering that this approach is simple, low-power, and portable, it presents a great tool for point of care NAT as well as other molecular analysis pipelines such as NGS and environmental monitoring. A highly reliable and integrated sample preparation platform is needed for the future of point of care molecular diagnostics. In the future, we plan to explore simplified and integrated versions of this method to provide a streamlined platform for sample preparation.

6. Author Contributions

Anthony J. Politza: conceptualization, software, investigation, writing – original draft. **Tianyi Liu:** conceptualization, resources, writing – review & editing. **Aneesh Kshirsagar:** conceptualization, resources, writing – review & editing. **Ming Dong:** methodology, supervision, visualization, writing – review & editing. **Md. Ahasan Ahamed:** visualization, writing – review & editing. **Weihua Guan:** conceptualization, supervision, writing – review & editing, project administration, and funding acquisition.

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8. Figures and Captions

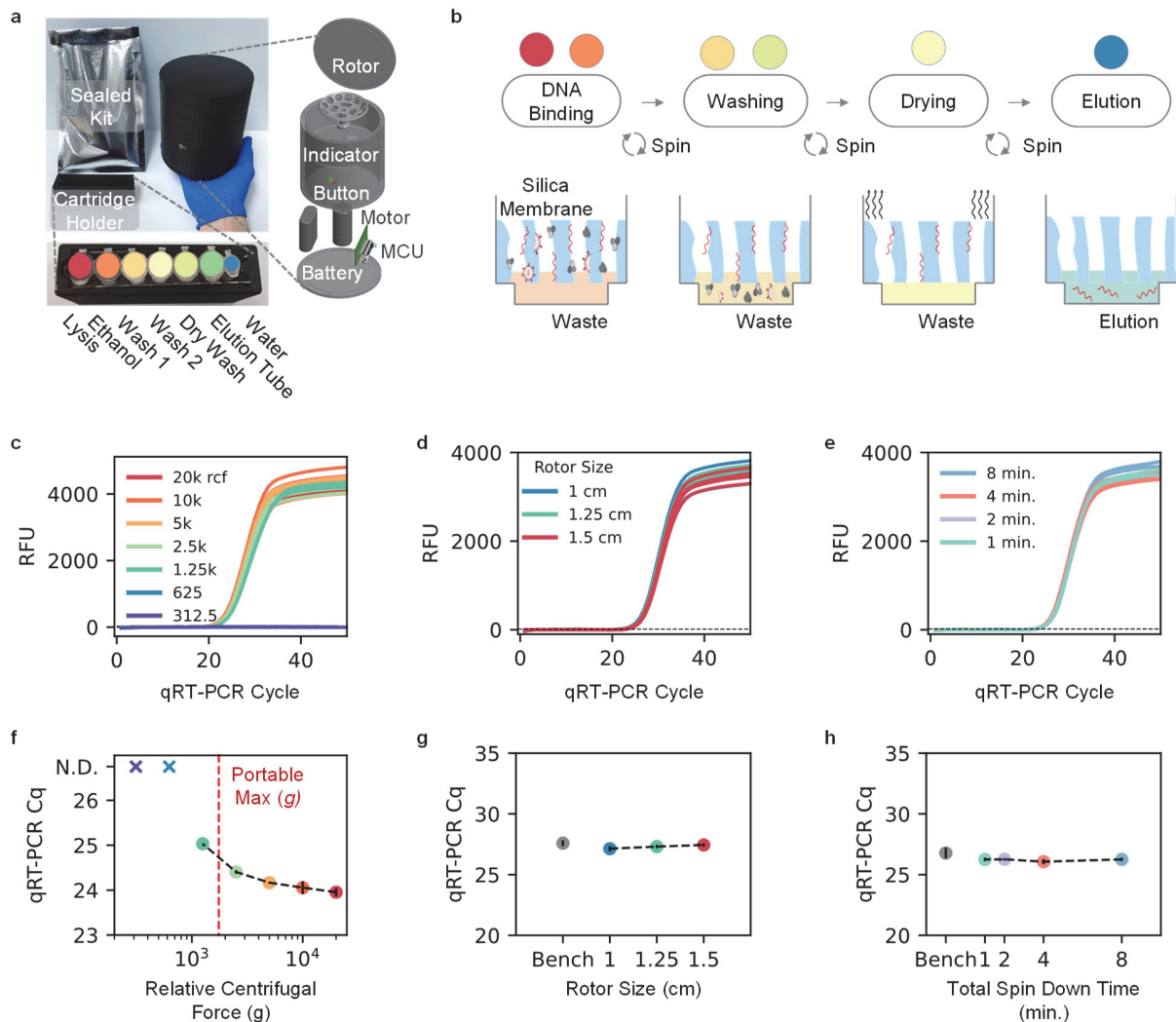


Figure 1. Workflow overview and device characterization. **a)** The necessary materials for our setup include the sealed reagent kit, cartridge holder, and portable device. Inside the sealed kit, a prepackaged cartridge contains a lysis buffer, ethanol, washing buffer 1, washing buffer 2, drying buffer, an extra elution tube, and water. **b)** Solid-phase extraction is conducted through four major steps: DNA binding, washing, drying and elution. All liquid reagents are forced through the spin column from top to bottom using centrifugal force from the portable device. **c)** Amplification curves from samples extracted with varying relative centrifugal force (RCF) from 20000 g down to 312.5 g. **d)** Amplification curves from samples extracted using the portable device with different rotor sizes (1, 1.25, 1.5 cm) [1550, 1650, and 1750 g]. **e)** Amplification curves from samples extracted with varying spin-down times from 8 to 1 minute. **f)** Summarized C_q values from varied RCF on the benchtop centrifuge. Samples below 1250 g were undetected. The portable device demonstrates a maximum RCF of 1750 g (red line). **g)** Summarized C_q for varied rotor size on the portable device compared to the benchtop device. The maximum observed difference in C_q was 0.45. **h)** Summarized C_q for varied spin-down times compared to the benchtop device. The maximum observed difference in C_q was 0.71.

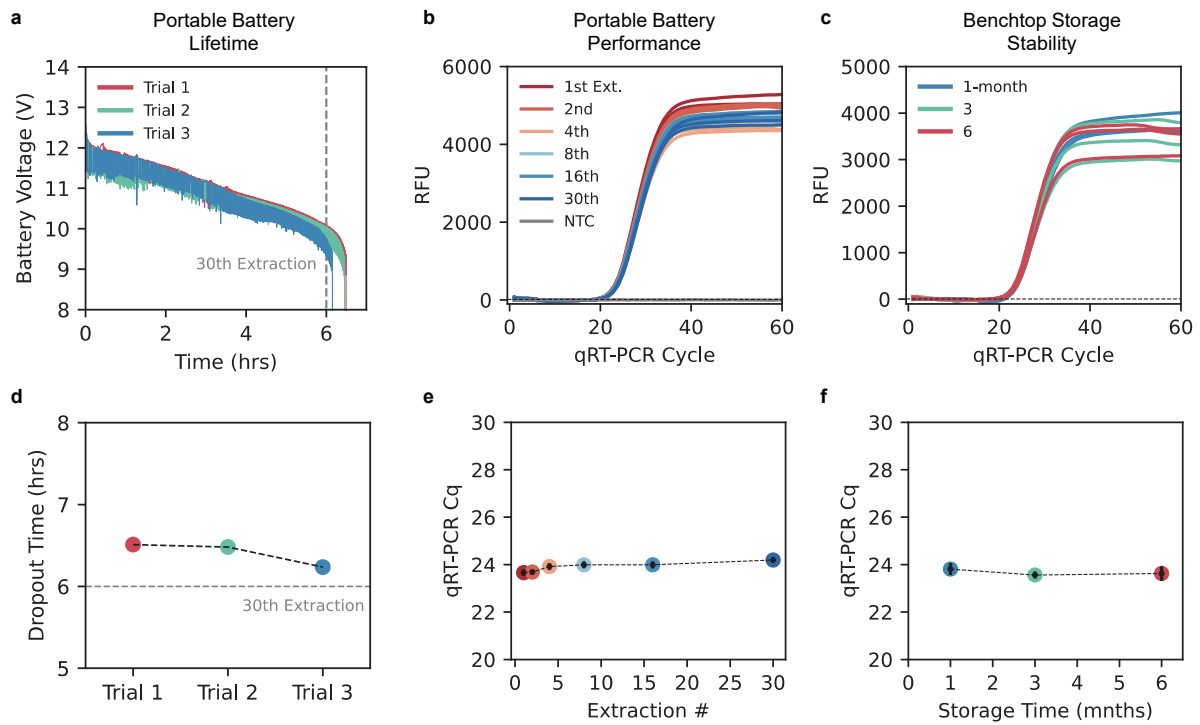


Figure 2. Deployment validation. **a)** Voltage response during the battery lifetime of the portable device. Three trials were tested with repeated extractions until the battery died. **b)** Amplification curves from extractions on the portable device (#1, 2, 4, 8, 16, 30). The maximum observed change in Cq was 0.53. **c)** Amplification curves from extractions using reagents stored at room temperature for one, three, and six months. **d)** Dropout times for each battery voltage endurance test. All three trials showed endurance passed six hours (the time for 30 extractions). **e)** Cq value versus extraction when operating on battery power. The maximum observed change in Cq was 0.53 from #1 to #30. **f)** Cq value versus storage time. The maximum observed change in Cq was 0.25 between all tests.

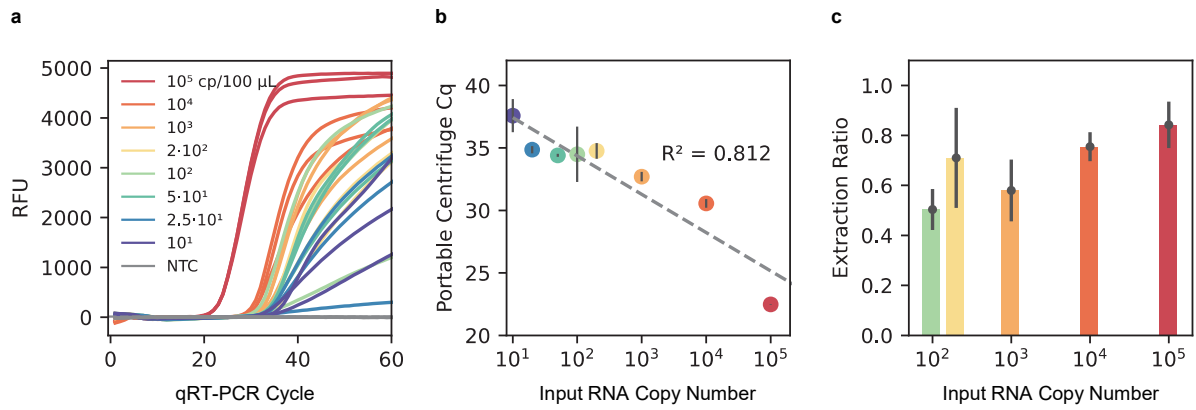


Figure 3. Portable device performance vs copy number. **a)** Amplification curves for extracted samples with 10^5 down to 10 copies of HIV RNA in 100 μL of buffer. **b)** Cq values versus known input concentration of RNA. A linear fit shows a strong negative correlation ($r = -0.901$) and moderate linearity ($R^2 = 0.811$) using triplicates. **c)** Extraction ratio versus starting input RNA copy number. Ratio was back calculated using triplicates of qRT-PCR standards.

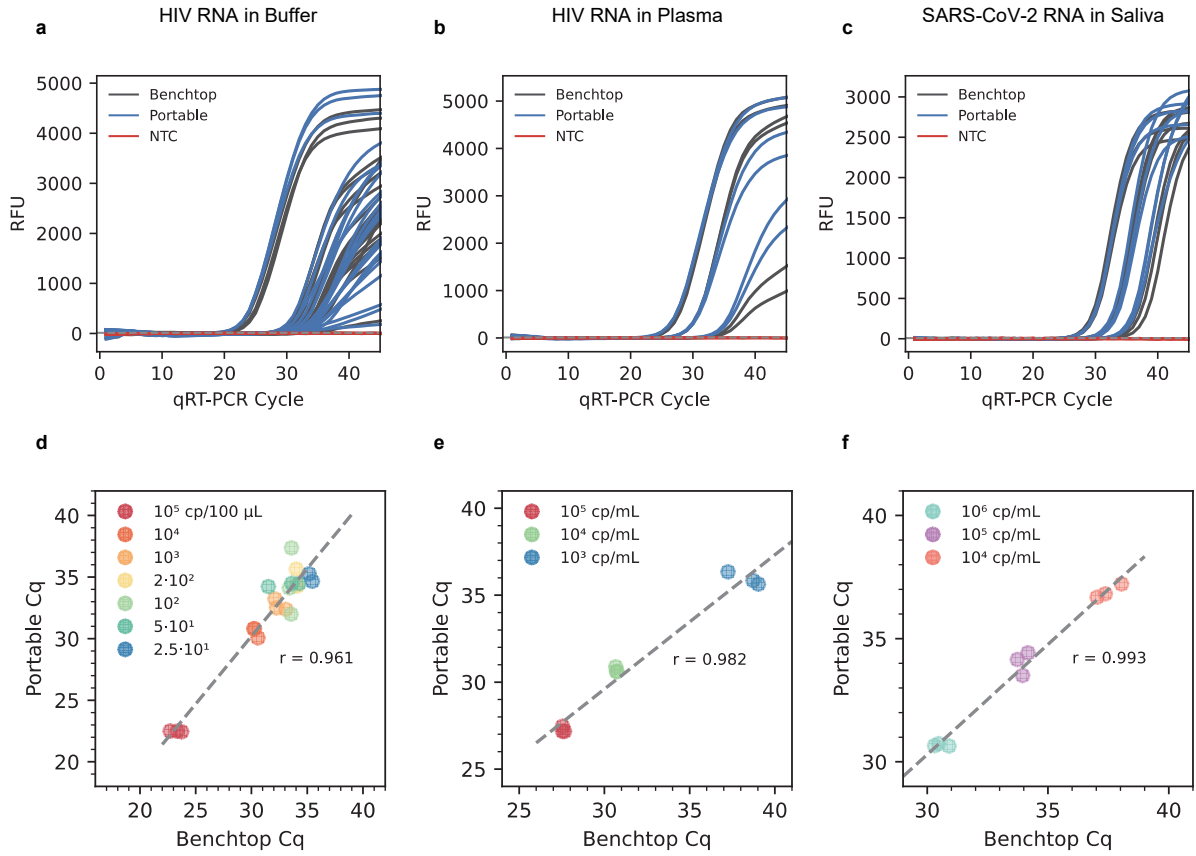


Figure 4. Cq benchmarking, portable vs benchtop. **a)** Amplification curves for all samples with 10^5 down to 25 copies of HIV RNA when extracted using the benchtop or portable device. **b)** Amplification curves for three plasma samples (HIV, 10^5 , 10^4 , and 10^3 cp/mL) when extracted using the benchtop or portable device. **c)** Amplification curves for three saliva samples (SARS-CoV-2, 10^6 , 10^5 , and 10^4 cp/mL) when extracted using the benchtop or portable device. **d)** Cq comparison between benchtop and portable devices when a 100 μ L buffer sample is used. Correlation shows a strong positive fit ($r = 0.961$) and strong linearity ($R^2 = 0.923$). **e)** Cq comparison between benchtop and portable devices when a 100 μ L plasma sample is used. Correlation shows a strong positive fit ($r = 0.982$) and strong linearity ($R^2 = 0.964$). 1000 cp/mL samples showed a maximum delta Cq of 1.78 with x and y standard deviations of $\sigma = 0.78$ and 0.30, respectively. **f)** Cq comparison between benchtop and portable devices when a 200 μ L saliva sample is used. Correlation shows a strong positive fit ($r = 0.993$) and strong linearity ($R^2 = 0.986$). 10^4 cp/mL samples showed a maximum delta Cq of 1.01 with x and y standard deviations of $\sigma = 0.42$ and 0.23, respectively. **Figures d-f** show individual data points to demonstrate two axis co-correlation and linearity.

9. Tables

Table 1. Centrifuge Protocols & Timings

	Stage Timing (s)						Total time (min)
	Lysate #1	Lysate #2	Wash 1	Wash 2	Dry Wash	Elution	
Benchtop (s)	60	60	60	180	-	60	7
Portable (s)							
8 min.	60	60	60	60	180	60	8
4 min.	30	30	30	30	90	30	4
2 min.	15	15	15	15	45	15	2
1 min.	7.5	7.5	7.5	7.5	22.5	7.5	1
Portable (s)							
Rotor Radius 1 cm.	60	60	60	60	180	60	8
Rotor Radius 1.25 cm	60	60	60	60	180	60	8
Rotor Radius 1.5 cm	60	60	60	60	180	60	8
Kit Volume (μL)	550	550	500	500	500	80	

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Special References (#):

Of interest *:

3. Excellent review of the field and expectations for future development.
5. Highlights the critical sensor types that interface with portable sample preparation.
6. An intriguing perspective to sample preparation technologies given the needs of SARS-CoV-2 testing.
27. Compiled literature review of the sample preparation technologies used prior to nucleic acid amplification testing and the effects on testing.

Considerable Interest **:

12. Has high potential for future impact using their sample preparation devices.
10. Showed a promising format of sample preparation using integrated magnetic beads.
45. An interesting format of microfluidic sample preparation that explores mineral oil pressure to process spin columns without centrifugation.