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Rapid simultaneous self-testing of HIV and HCV viral loads with integrated RNA extraction and multiplex RT-PCR in under 1 hour

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ABSTRACT

HIV and HCV Co-infection continues to be a significant public health problem globally especially within high-risk groups. Monitoring viral loads with precision helps direct treatment choices and measure treatment success while preventing resistance to drugs. Traditional laboratory-based testing faces limitations due to restricted accessibility and dependence on centralized facilities along with the complex process of quantifying both HIV and HCV viral loads which impedes worldwide control measures for these viruses. We developed a portable self-testing device that measures HIV and HCV viral loads from a 100 μL finger-prick blood sample at the same time. The system combines RNA extraction with rapid multiplex RT-PCR to provide semi-automated testing capabilities and generate results in under 1 h. The system extracts RNA at 80 % efficiency and employs a three-channel optical detection system that detects as low as 5 copies per reaction while delivering high sensitivity and accuracy. Validated studies found a robust connection with Bio-Rad benchtop systems (R^2 = 0.97–0.99) which verified that detection sensitivity and accuracy matched standard laboratory testing standards. The testing device enables parallel processing of four patients which results in enhanced efficiency and access to testing services. Individuals affected by HIV/HCV co-infection can use this self-testing solution to track their viral loads on their own to enable prompt treatment changes and lower transmission risks. The technology delivers an effective self-monitoring option for viral load management through its combination of precision, portability and an easy-to-use design which advances HIV and HCV treatment outcomes.

1. Introduction

Human Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV) represent a major global health concern that critically affects both disease management approaches and treatment plans (CDC, 2024a; 2024b, "People Coinfected with HIV and Viral Hepatitis | CDC," 2021). Precise viral load monitoring is essential for guiding treatment decisions and preventing drug resistance to ensure effective disease management (Deeks et al., 2021; Zhang et al., 2023). Precise viral load monitoring plays a critical role in starting treatment and assessing the success of particular therapeutic approaches in diverse patient groups (Dietz and Maasoumy, 2022; Reeves et al., 2023). The World Health Organization's 2030 goals of reaching 95 % HIV diagnosis/treatment coverage and cutting HCV incidence by 90 % face obstacles because of insufficient diagnostic tools for HIV and HCV viral load self-testing. The main barriers include the following: Traditional lab-based testing limits access due to reliance on centralized facilities and the complexity of dual viral load quantification (Cox et al., 2020; Djiyou et al., 2023). Furthermore, the difficulty of achieving public health targets increases due to the widespread occurrence of HIV and HCV co-infection among essential populations.

HIV and HCV co-infection occurs frequently because they share the same transmission pathways (Semá Baltazar et al., 2020). The occurrence of this co-infection remains widespread among high-risk groups, including people who inject drugs, which plays a major role in transmitting bloodborne infections (Chapin-Bardales et al., 2024; Kandathil et al., 2021). HIV and HCV transmission occurs through additional methods such as sexual contact with an infected person (Mukhatayeva

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et al., 2021). Co-infection not only complicates disease management but also leads to more severe health consequences: HIV attacks the immune system while HCV targets the liver which leads to faster disease progression (Tassachew et al., 2022; Yousefpouran et al., 2020). HIV infection accelerates the progression of HCV-related liver diseases which leads to higher chances of developing cirrhosis, liver failure and liver cancer (Jeyarajan and Chung, 2020). Therapeutic outcomes require simultaneous testing of HIV and HCV viral loads to make informed treatment decisions, achieve precise therapeutic approaches, assess treatment effectiveness and avert drug resistance (Abutaleb and Sherman, 2018).

Traditional laboratory-based testing needs samples to be transported to special facilities where trained staff perform the analysis (Drain et al., 2019). The lengthy procedure that forces patients to visit healthcare facilities serves as a major deterrent for many people seeking prompt testing. Self-testing methods for HIV and HCV viral load greatly enhance accessibility by enabling individuals to check their infection status from any location at any time. Point-of-care (POC) PCR-based testing devices now serve as vital tools because they deliver dependable results and enable direct health monitoring and disease management (Xun et al., 2021). Diagnostic tools for HIV and HCV viral load self-testing need to display high sensitivity and specificity while remaining cost-effective and user-friendly without the need for expert training (Bardon et al., 2020; "ITAP for HCV POC Diagnostics - POCTRN - GAITS," n.d.; W. Tang et al., 2022; Xu et al., 2020; Yang et al., 2022). These diagnostic tools must have compact portability along with the ability to deliver quick results to facilitate prompt clinical decisions and disease surveillance. Commercial self-testing solutions like the Biosynex Triplex HIV/HCV/HBsAg test exist but mainly focus on identifying infections without measuring viral loads (Kalla et al., 2018; Mukherjee et al., 2015). Multiple studies have investigated POC PCR technology for HIV and HCV viral load testing but it remains underutilized despite the necessity of precise viral load measurement in treatment tracking (Ahamed et al., 2025, 2024; Ahamed and Guan, 2024; Choi et al., 2018, 2016; Curtis et al., 2016; Damhorst et al., 2015; Li et al., 2024, 2024; Liu et al., 2011, 2023, 2022; Mauk et al., 2017; Nouri et al., 2023, 2021; Phillips et al., 2018; Politza et al., 2024, 2023; Safavieh et al., 2016; Z. Tang et al., 2022; Zhang et al., 2024). Whole blood processing difficulties, complete quantitative analysis attainment issues, and quality control system integration obstacles account for the slow uptake of these new technologies (Choi and Guan, 2022a, 2022b; Liao et al., 2016; Liu et al., 2022; Phillips et al., 2019; Z. Tang et al., 2022). In our previous work, we developed a portable platform capable of quantifying HIV-1 and HIV-2 viral loads from finger-prick blood using a sample-to-answer workflow. However, the RT-PCR reaction itself required approximately 70 min, which constrained its practicality for time-sensitive applications. Furthermore, the system was not equipped to detect additional pathogens such as HCV, which is of high clinical relevance in co-infected populations (Liu et al., 2025). There exists a crucial demand to create self-testing devices that allow simultaneous HIV and HCV viral load measurement which would enable patients and healthcare providers to perform precise self-tests independently of centralized lab facilities.

In this study, we developed a portable HIV/HCV self-testing device that uses only 100 μL of finger-prick blood to determine HIV and HCV viral loads at various disease stages while tackling the existing deficit of self-testing tools for viral load assessment. The system created for non-professional users combines RNA extraction and rapid multiplex RT-PCR to enable semi-automation of testing procedures which deliver results in under an hour and serve as a reliable tool for personalized treatment decisions and disease monitoring. Although our previous study required approximately 70 min for the RT-PCR reaction alone on a portable system (Liu et al., 2025), the present work significantly reduces this RT-PCR reaction time to 45 min while maintaining quantitative performance. This improvement was enabled by implementing an accelerated thermal cycling protocol with 1-s denaturation and extension steps, and by systematically optimizing reagent concentrations to ensure reliable amplification under rapid cycling conditions. The system delivers a self-testing solution that integrates RNA extraction and rapid multiplex RT-PCR to perform a complete sample analysis and viral load quantification in 1 h while maintaining 80 % RNA extraction efficiency and optimized portability design. The system enables concurrent analysis of four different patients using semi-automation to improve testing efficiency. The system achieves simultaneous measurement of HIV and HCV viral loads through a three-channel optical detection system that provides high-sensitivity and precise results with a limit of detection (LoD) of 5 copies/reaction which ensures robust and reliable performance for clinical decision-making and disease monitoring. Validation studies established a high degree of correlation with Bio-Rad benchtop systems (R^2 = 0.97–0.99) thereby proving that test sensitivity and accuracy match those of standard laboratory testing. The innovation allows HIV/HCV co-infected patients to self-manage their viral load monitoring and treatment adjustments precisely while enabling high-risk groups to identify infections sooner for prompt intervention and decreased progression and transmission risks. This technology establishes accurate self-testing of HIV and HCV viral loads as a practical option for disease management through its highly efficient and user-friendly solution that enables individuals to maintain control over their health without laboratory diagnostics.

2. Materials and methods

2.1. Materials and chemicals

Suppliers provided the materials used for the study. In particular, the list of all RNA extraction kits, and in particular, the QIAGEN QIAamp Viral RNA Mini Kit cat. # 52904, is available in Supplementary Table S1. The HIV Analyzer slides with a list and photographs of electronic and optical parts were obtained using DigiKey, which is mentioned in Supplementary Table S2. The RT-PCR primers and probes were purchased at IDT, while the reagents and chemicals used for the experiments were delivered by Sigma-Aldrich and Thermo Fisher. Assay validation was conducted by means of using the Bio-Rad CFX96 system. As for the components used for the analysis, the viral RNAs of HIV-1, HCV, and RNase P are represented by ATCC cat. #VR-3245SD, VR-3233SD, and 1006626, respectively. All materials were used as delivered and stored as it is recommended by the manufacturers.

2.2. Multiplex RT-PCR reaction

To detect the HIV-1, HCV, and RNase P, we implemented the dual-enzyme one-step RT-PCR protocol. We prepared a 20 μL reaction mixture that contained 5 μL of TaqMan® Fast Virus 1-Step Master Mix alongside 1 μL each of 1 μM forward and reverse primers and a 1 μM probe, added 1 μL of RNA templates and completed the volume with 11 μL of PCR-grade water. The developed assay incorporated the HIV-1, HCV, and RNase P RT-PCR primers. The Multiplex RT-PCR primers were applied in the developed assay. The RT-PCR procedure was performed by means of particular thermal conditions: the initial phase of reverse transcription was conducted at 50 °C for 5 min for the purpose of the conversion of HIV-1 RNA into cDNA, which was followed by the denaturation at 95 °C for 20 s, and then, there were 40 cycles of amplification. Each cycle of amplification included a 3-s denaturation at 95 °C and a 30-s annealing and extension at 60 °C. We used primer-probe sets previously validated in the literature for specificity and efficiency. BLAST alignment was performed to eliminate cross-reactivity, and primers were selected with closely matched melting temperatures for optimal multiplex amplification. The specific details of the primers and probes are presented in the Supplementary Tables S3-5. The reporters of artists in our experiment were ATTO425, HEX, and Cy5.

3. Results and discussion

3.1. Overall workflow

Self-testing capabilities for HIV and HCV viral load enable individuals to independently track infection status and manage their health. Standard viral load assessments help track disease progression and treatment success but current methods require lab-based testing which prevents self-testing. The absence of dependable self-testing systems results in delays for individuals trying to measure their viral load which creates treatment ambiguities and raises both disease progression and transmission risks. We have created a comprehensive self-testing system that enables rapid and straightforward HIV and HCV viral load measurement without needing laboratory or professional help.

As shown in Fig. 1a, the self-testing workflow consists of two key stages: RNA extraction and RNA detection. This system bypasses the external sample processing required by conventional point-of-care tests by incorporating a fully automated RNA extraction module that enables users to complete the testing process independently. The system uses a previously developed portable centrifuge module to perform RNA isolation through plasma separation and RNA extraction while completing the whole procedure within 10–15 min to minimize manual handling (J Politza et al., 2024; Liu et al., 2025; Politza et al., 2024). This study validated the feasibility and stability of the portable RNA extraction device using standard plasma samples. Future work will systematically evaluate the extraction efficiency and PCR performance in samples with varying blood cell counts (e.g., anemia, leukocytosis) and assess the effects of potential inhibitors such as hemoglobin and antibodies to ensure broad clinical applicability. RT-PCR amplification immediately utilizes the extracted RNA in a preloaded test tube where Cq values assess the viral load. This automated system allows users to reach final results from sample collection within 1 h thus proving to be an efficient and accessible self-testing option. The system achieves a 36 % reduction in total RT-PCR reaction time (45 min versus 70 min) by operating on our previously optimized portable PCR analyzer while preserving laboratory-level sensitivity (5 copies/reaction) (Liu et al., 2025). The enhanced performance comes from several optimizations that reduced reverse transcription time from 5 to 3 min and qPCR activation phase from 3 to 1 min while trimming DNA denaturation and extension times from 3 s each to 1 s each. The total per-test cost of the developed system was estimated to be $4.28, including reagents and consumables, with an approximate Bill of Materials (BOM) cost of $63.54 (Supplementary Tables S1–2). For comparison, GeneXpert HIV assays cost ~$16.12–42.34 per test depending on scale, and Abbott m2000 PCR tests cost ~$17–18 per test (Nyirenda-Nyang'wa et al., 2022). LAMP or RPA lateral flow assays cost ~$2–5 per test but typically do not provide viral load quantification (Hull et al., 2022). Thus, our platform maintains low per-test costs while enabling quantitative multiplex RT-PCR detection with significantly lower instrument costs compared to benchtop systems, supporting practical deployment in low-resource settings. The system achieves minimized user steps and reduced contamination risk by combining RNA extraction with amplification for an efficient self-testing process.

The system features four independent detection channels that enable viral load testing for four users at the same time during each testing session. The feature optimizes performance while preserving the necessary compact structure for self-testing mechanisms. Multiple units working together can scale up testing capabilities for self-testing or decentralized applications. The semi-automated process enables people with any level of technical skill to perform tests using minimal guidance which simplifies and optimizes self-testing. This system revolutionizes self-testing for HIV and HCV through the combined use of RNA Extraction and Multiplex RT-PCR. This system enables people to manage their health by tracking viral suppression levels to make educated treatment choices. This system measures viral load quantitatively unlike rapid antigen tests which only determine infection status. The system's closed-loop design and automation enable rapid results, demonstrating potential for self-testing applications by addressing key challenges in HIV and HCV management and improving access to reliable viral load monitoring.

3.2. Multiplex PCR assay specificity

For the multiplexed assay developed to detect HIV-1, HCV, and

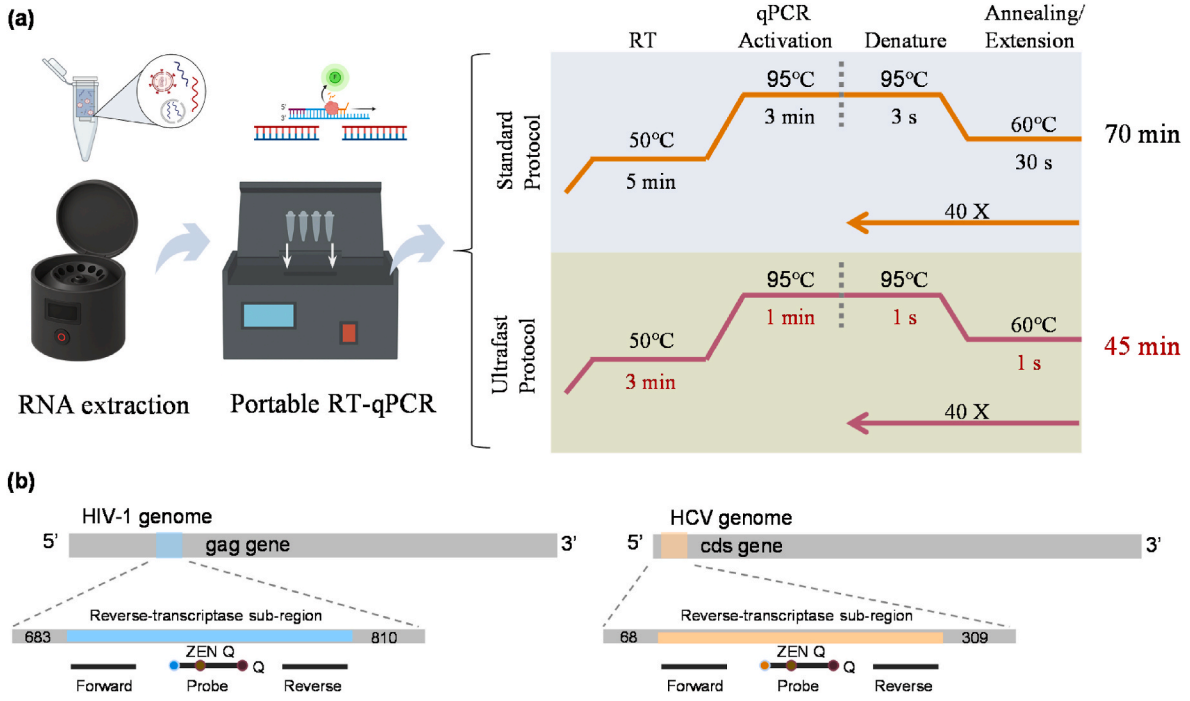

Fig. 1. Overall device workflow and assay design. (a) Workflow comparison of the portable rapid qRT-PCR versus conventional qRT-PCR for HIV and HCV detection, from plasma samples to results. (b) Primer and probe design targeting the HIV-1 gag gene and HCV cds gene, using two primers and one probe per virus.

RNase P, we constructed unique primer-probe sets for all three targets. Our assay design was based on two reverse transcription PCR primers and a distinct for each virus, targeting the early part of the gag gene. The sub-part of reverse transcriptase specific for both HIV-1 and HCV is presented in Supplementary Tables S6-7. This exact region was chosen due to its high level of sequence conservation across different strains making it universally suitable for the purpose of detection. The primers and probes in this study target conserved regions of HIV-1 and HCV, theoretically covering major subtypes and genotypes (Fig. 1b). Future work will include systematic validation using clinical samples from HIV-1 subtypes B, C, D and HCV genotypes 1–6, as well as evaluation of potential cross-reactivity with other common RNA viruses such as HBV and influenza to ensure assay specificity and broad clinical applicability. In addition, a separate set of primers and a probe for RNase P was designed to serve as an internal control, indicating that RNA extraction was successful, and the assay is functional. We made sure that for each set of primers and probes, the thermal cycling conditions were the same. As a result, amplification of all targets could be carried out in a single mixture and on a concurrent basis, making the entire assay more efficient. A number of precautions were made to enable the creation of separate signals for each target RNA. First, signals for all of the patients were assigned exclusively to their own channels. As a result of this minimization, their potential localization to the adjacent channels and the following crosstalk was sufficiently reduced. Making sure that the multiplex RT-PCR assay is as specific as possible is crucial for the accuracy of testing methods. A decrease in the level of cross-reactivity can result in a better diagnostic tool.

In order to test the specific and multiplexing capacities, we did several experiments with eight synthetic RNA samples representing HIV-1, HIV-2, and RNase P. These samples were in different RNA concentrations ranging from, 0 or 1000 copies of RNA concentration per reaction, as depicted in Fig. 2a. The assay was then post amplified, and the results analyzed through agarose gel electrophoresis as shown in Fig. 2b. In the implemented 60 PCR cycles, there were clear bands at a specific amount of base pairs which proof that the amplification was successful. In all the lanes 2, 3, and 5, there were single bands for each of the respective target representing HIV-1, HIV-2, and RNase P. The dual bands in the lanes 4, 6, and 7 are responsible for confirming the co-amplification of the targets. The multiple bands in the lane 8 are proof that this assay can also do multiplex detections. The last lane is the no template control which proof no contamination. These results are collaborated by real-time PCR done over 60 cycles, and the results are shown in Fig. 2c, which also proves that the amplified RNA can be detected through fluorescence. Both results have proved the assay is reliable dynamic capabilities. They are proof that the assay is of high specificity, multiplex correctly, and can be loaded and run in one step. The capabilities make it an excellent candidate for deployment in portable diagnostics platforms for quick and precise detection in low-resource settings.

Overall, the developed multiplexed assay for HIV-1, HCV, and RNase P is a highly efficient diagnostic method in terms of fast processing, high specificity, and multiplexing capacity. The specifically developed primer-probe sets ensure an accurate approach to target detection and compatibility under uniform thermal cycling conditions. As such, DNA amplification of target regions can be conducted simultaneously, which not only enhances the overall efficiency but also supports the portability of the assay. The conducted experimental analyses both through agarose gel electrophoresis and real-time PCR indicate the high reliability and uniqueness of the designed sets when utilized for target detection. The outcomes obtained through the conducted research also confirm that the key practical implications of the assay can potentially lead to its extensive use in the management and control of HIV and HCV worldwide.

3.3. Rapid PCR speed limitation

We explored the upper limit of rapid PCR processing speed by assuming that the duration of the incubation period plays a major role in determining reaction efficiency. The activity of essential enzymes like reverse transcriptase and DNA polymerase in this process demonstrates high sensitivity to both temperature changes and reaction time. When activation times for RT and qPCR are reduced they risk incomplete template synthesis or primer annealing which could lower amplification efficiency. The development of self-testing diagnostics depends on the accurate identification of speed limits because rapid detection needs to be precise. Shortening the reaction time without sacrificing accuracy

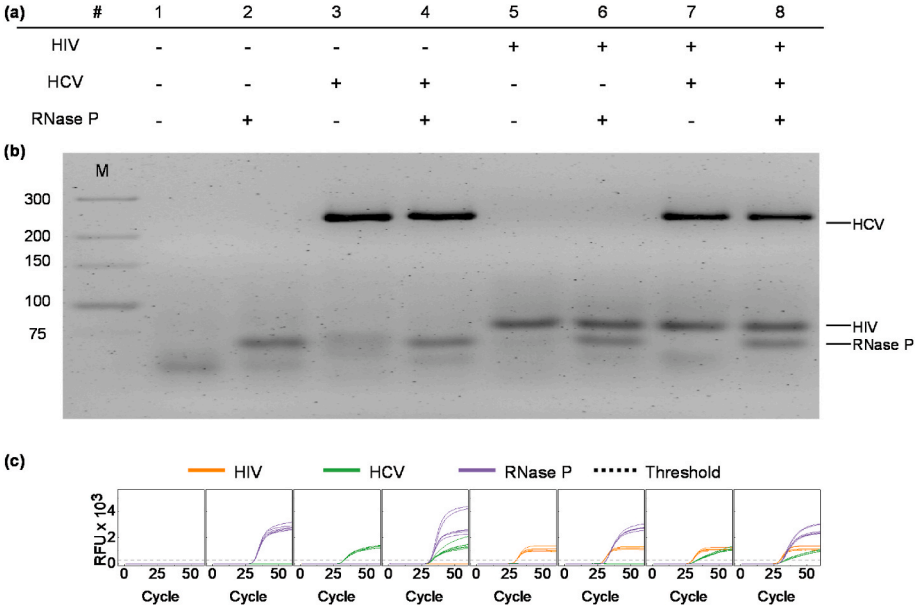

Fig. 2. Evaluation of multiplex RT-PCR specificity. (a) Samples 1–8 contained synthetic HIV, HCV, and RNase P RNA with varying concentrations (0–1000 copies/reaction) and designated positive or negative ("+" or "–"). (b) Agarose gel electrophoresis showed amplification results for Samples 1–8, each tested in six replicates. (c) Real-time multiplex RT-PCR data for Samples 1–8 were obtained using a Bio-Rad PCR analyzer, with HIV, HCV, and RNase P signals captured from three optical channels and combined into one image.

would greatly improve both the practicality and efficiency of self-testing methods. The acceleration of the process demands careful optimization since we must prevent increases in both false negatives and false positives.

We conducted a systematic investigation into how thermal speed influences PCR efficiency to answer our research questions and confirm our hypothesis. RT-PCR was conducted using the TaqMan® Fast Virus 1-Step Master Mix (Thermo Fisher), which contains a thermostable reverse transcriptase and a fast DNA polymerase optimized for high-speed cycling. This enzyme system enabled the use of 1-s denaturation and extension steps while maintaining amplification efficiency. We tested three experimental sets by changing the incubation periods for RT, qPCR activation, and denaturation/extension phases. During the initial experiment we maintained RT at 5 min and qPCR activation at 3 min while we simultaneously decreased denaturation/extension times from 3s/3s–2s/2s and then to 1s/1s. While keeping qPCR activation and denaturation/extension conditions stable, this set reduced RT time to find its minimum necessary duration. The third experimental set maintained RT duration and qPCR activation timing fixed to find the optimal qPCR activation period. The reactions each consisted of 1000 RNA copies with all experiments conducted three times.

Fig. 3 shows how the amplification curves for HIV-1, HCV, and RNase P respond when researchers vary one experimental parameter at a time while maintaining all other parameters as constant. The signals from three optical channels combined show which parameter combinations achieve successful target detection. Supplementary Table S8 combines the experimental results to show that RT activation time can be decreased to 3 min while qPCR activation time can be decreased to 1 min before both HIV-1 and RNase P become undetectable. HCV detection continued to be the main bottleneck because its amplification efficiency dropped under fast-tracking conditions. The findings show that practical speed limits exist even though many sources claim PCR cycles can be accelerated to under 1 min.

The reduced RT-PCR reaction time was enabled by the use of TaqMan® Fast Virus 1-Step Master Mix, whose thermostable enzymes support efficient amplification under 1-s denaturation and extension steps, making the rapid cycling protocol feasible without compromising performance. The optimized protocol achieves maximum speed with RT at 3 min and qPCR activation at 2 min while maintaining detection through 1 s each for denaturation and extension. Future enhancements could be

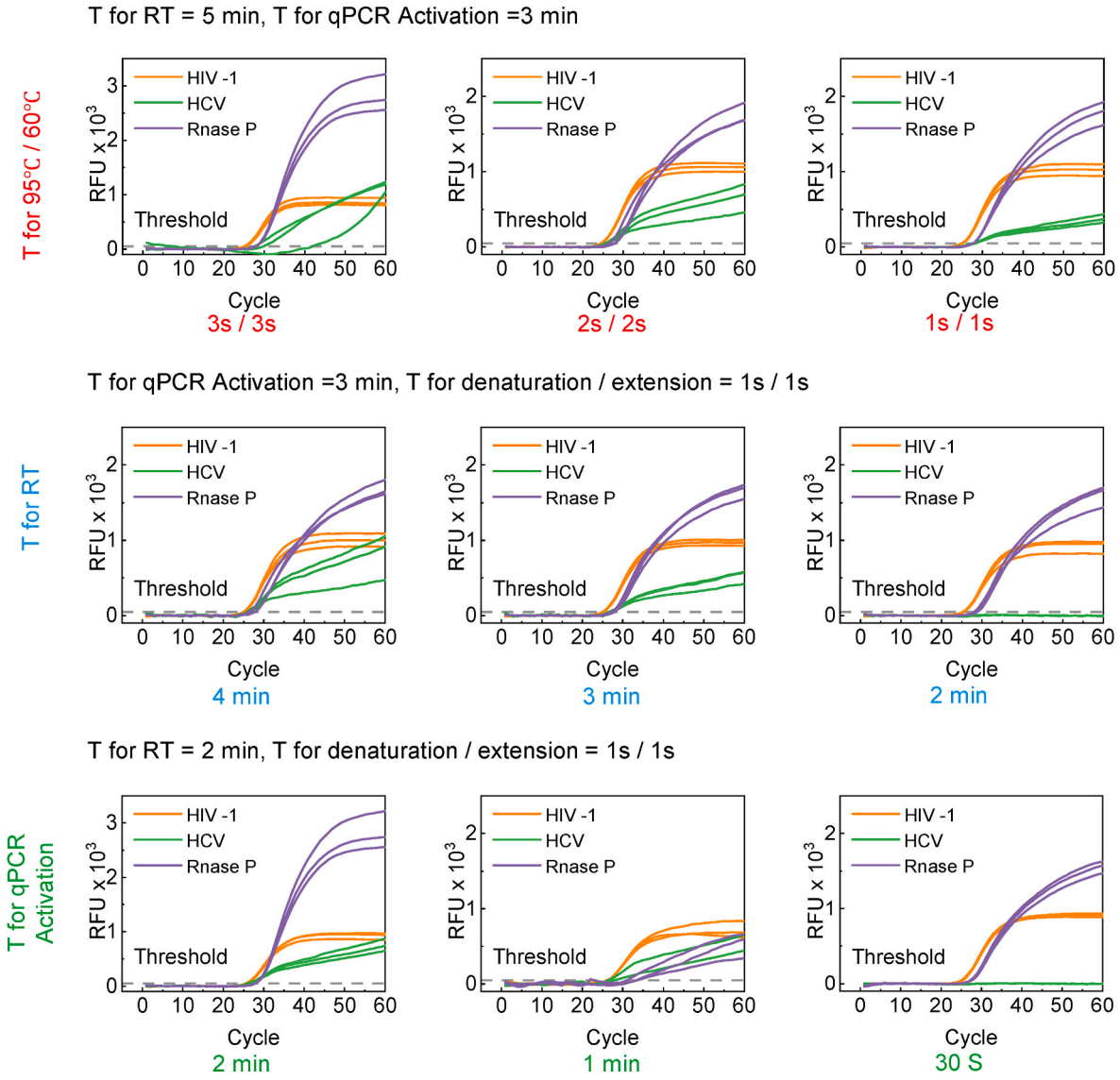

Fig. 3. This figure shows the amplification curves for HIV-1, HCV, and RNase P under different experimental conditions. Each curve represents varying one parameter—RT time, qPCR activation time, or denaturation/extension duration—while keeping the others constant. Integrated signals from three optical channels visualize detection efficiency across conditions and highlight optimal parameter combinations.

realized through the utilization of high-efficiency enzymes combined with rapid thermal cycling technology. These research outcomes establish the performance limits for advanced rapid PCR technologies necessary to fulfill self-testing requirements. Speed determines disease detection speed while PCR optimization for self-testing provides expanded access to correct diagnostics which are convenient and timely.

3.4. Rapid PCR assay performance validation

Then we performed a detailed sensitivity analysis to contrast the portable multiplex PCR detection method against the traditional benchtop PCR system while assessing both its analytical precision and detection performance. The multiplex PCR assay aims to detect HIV-1, HCV, and RNase P at the same time while optimizing detection protocols for various viral loads to maintain consistent results in clinical and self-testing applications. Evaluating this system is essential because it involves two crucial aspects. The multiplex PCR kit evaluation against a benchtop PCR system demonstrates its compatibility with laboratory-grade detection standards which improves its medical credibility and acceptance. The system's practical effectiveness depends directly on its ability to detect samples with low viral loads. We analyzed duplicate samples with standard laboratory devices to address this matter.

The first step in the examination process was the establishment of the examined assay's ability to properly measure given amounts of specific RNA throughout a significant concentration range, from 1 to 10^5 copies/reaction. The latter was subsequently determined in terms of multiplex RT-PCR experiments conducted in real time with the use of the Bio-Rad benchtop PCR detector. Six repetitions of each dilution of a particular RNA standard were performed to ensure the required level of statistical reliability. The results of the measuring as exhibited in Fig. 4a demonstrate the ability to determine the amounts of HIV-1, HCV and RNase P through three distinct optical channels, thus allowing a more detailed view of the examined assay's performance across given ranges of concentrations.

In conducting a comprehensive evaluation of the quantitative performance of our multiplex assay, its ability to establish highly reliable linear correlations between known RNA concentrations and those detected is evident. Each concentration was tested six times, and a concentration was considered detectable if at least four out of the three replicates yielded positive results. The limits of detection for both viruses were set at 5 copies/reaction, with their estimate being corroborated by the compelling character of the produced correlation. The limit of detection (LoD) was defined as the lowest concentration at which all three replicate reactions yielded successful detection results, corresponding to a $\geq$95 % detection rate criterion. More specifically, this correlation is reflected in the calculated coefficients, which fall between R^2 0.98 and R^2 0.99. It is evident that this result serves as the most powerful indicator of the quantitative capability of the developed multiplex assay. Additionally, the absence of the necessity for the use of an additional system that would allow assessing the volume of originally present plasma is noteworthy in this regard. Furthermore, this result is captured in Fig. 4b, which reveals the cycle threshold values required in analyzing each target assessed in the study's scope. The time required for the fluorescence units to exceed the 100 units threshold is considered to be an important distinguishing factor for the calculation of these values, indicated by a dashed line on the graph. As can be seen, their values are highly similar, displaying the quantitative precision of the results generated by the desktop PCR system. Therefore, the study provides evidence that the developed assay is both accurate and highly sensitive to the wide range of RNA concentrations. Its powerful performance included its ability to examine the instances of lower viral load with a high degree of success, highlighting its applicability to multiple clinical settings. Thus, the study's findings can be seen as recognizing the future of the development and provision of a combined high-speed and precise PCR tool, associated with the possibility of conducting diagnosing on desktop machines.

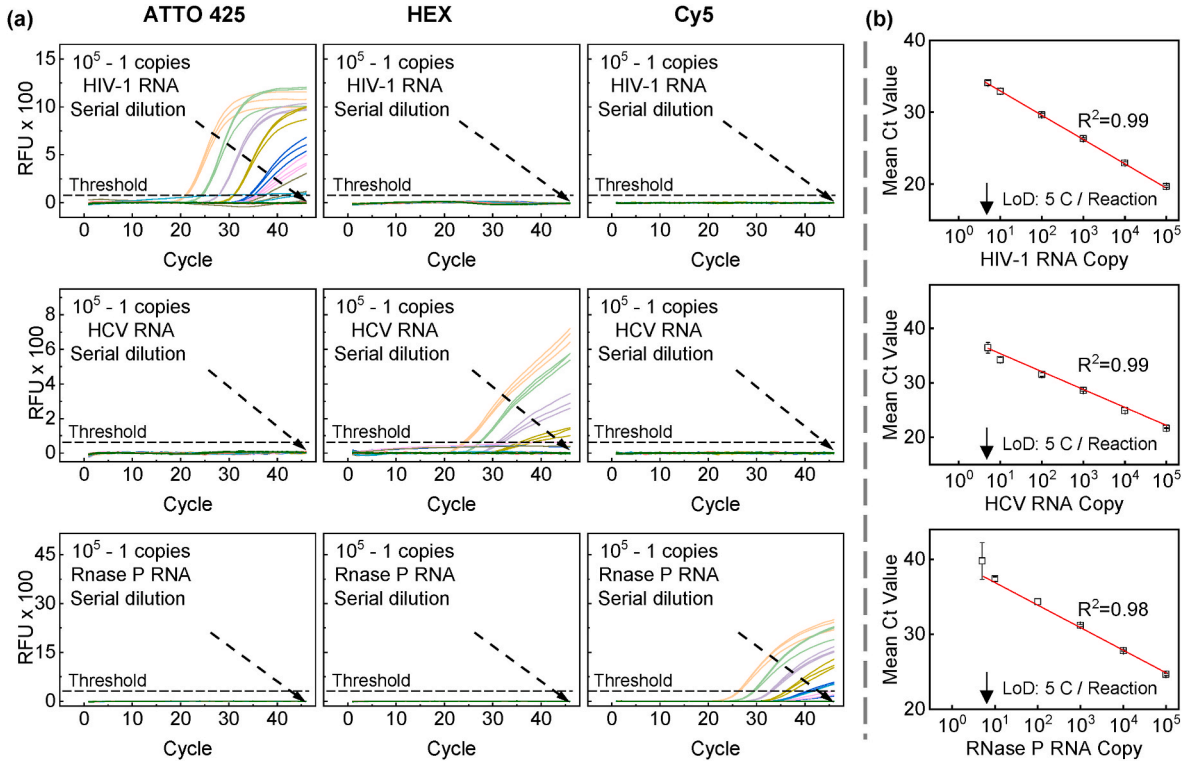

Fig. 4. Evaluation of multiplex RT-PCR sensitivity and quantitative accuracy using Bio-Rad instruments. (a) Real-time multiplex RT-PCR results from serially diluted RNA standards (10^5 to 0 copies/reaction), tested in six replicates, showing HIV-1, HCV, and RNase P detection across three optical channels. (b) Ct values plotted against RNA concentrations for each target. Cq values were defined as the cycle when RFU exceeded 100 (dashed line). Data points represent means of three replicates with standard deviation error bars.

3.5. Analyzer analytical evaluation

In addition to testing clinical sensitivity, we have extensively validated the quantitative accuracy of our testing system. As such, we subjected a panel displaying real-time RT-PCR data for various serially diluted RNA samples to a systematic, intense investigation. The purpose of this validation is related to the need to establish the quantitative accuracy and reliability of our testing system to an even greater extent, as it should function properly in the most challenging, extreme testing environment. As a rule, laboratory-based analyzers are seen as the epitome of precision and accuracy in RT-PCR testing. Meanwhile, in the case of resource-limited regions, the availability of the equipment will be limited. As a result, it is crucial to confirm that the testing system will be capable of providing the same capacity for analysis and reliability in testing the viral load as a conventional benchtop analyzer does. This study focused on demonstrating the feasibility and analytical performance of the device. Future work will include systematic evaluation of storage stability under different environmental conditions and extended field trials in resource-limited settings to ensure reliable performance without refrigeration or regular maintenance.

These tests, shown in Fig. 5a, were performed in triplicate for each RNA concentration ranging from 10^5 to 0 copies per reaction. The purpose of these tests was to demonstrate the repeatability and reliability of the test in a wider range of concentrations. The threshold was determined by using baseline fluorescence from negative controls to distinguish true amplification signals from background noise, ensuring reliable Ct value identification. Although minor signal fluctuations are observed in the amplification curves, they do not impact the precision of viral load quantification. The determination of viral load is based on the cycle threshold (Ct) value, which is identified when the signal crosses a predefined threshold. These fluctuations remain within an acceptable range and do not interfere with Ct value determination. It can also be seen from Fig. 5b that the performance of the testing system and the conventional Bio-Rad analyzer is effectively identical. For each type of RNA, the amplification curves were the same on both systems. Using the same HIV, HCV, and RNase P primer-probe design and the same experimental methods, the correlation coefficients for all tests were 0.97–0.99, further demonstrating that the portable system achieves accuracy and consistency comparable to stationary equipment. The use of our testing system with the multiplex RT-PCR test confirmed the detection limit at 10 copies per reaction. At the same time, the combination of the multiplex RT-PCR test with the traditional PCR demonstrated similar performance, which also underlines the repeatability of the testing system compared to the laboratory equipment and its conceptual power in any environment, including those with the lowest resources available. It should be added that the testing system may be effectively used with the multiplex test in any setting where it is needed. The importance of this circumstance cannot be overstated due to the necessity to carry out HIV/HCV detection in a variety of environments with no access to laboratories.

This assay provides highly sensitive, specific, and accurate results in a compact and portable format, making it a major advantage for on-site diagnostics and real-time viral load monitoring. Currently, the rapid detection and effective management of HIV and HCV are challenges that thousands of laboratory professionals face daily. In this context, the development and promotion of this tool for use in the expanding network of professional laboratories are highly valuable. Given the high genetic diversity of HIV and HCV, a comprehensive diagnostic approach that combines DNA or RNA sequencing with the testing system remains one of the most effective strategies. This study focuses on the preliminary development and analytical evaluation of the HIV/HCV coinfection analyzer without using clinical samples. Clinical validation will be conducted in future work, including testing on a wide range of viral loads and samples from patients with different ages, comorbidities, and geographic backgrounds. Overall, the ability of this assay to deliver fast, accurate, and easy-to-use results in a portable format is of great

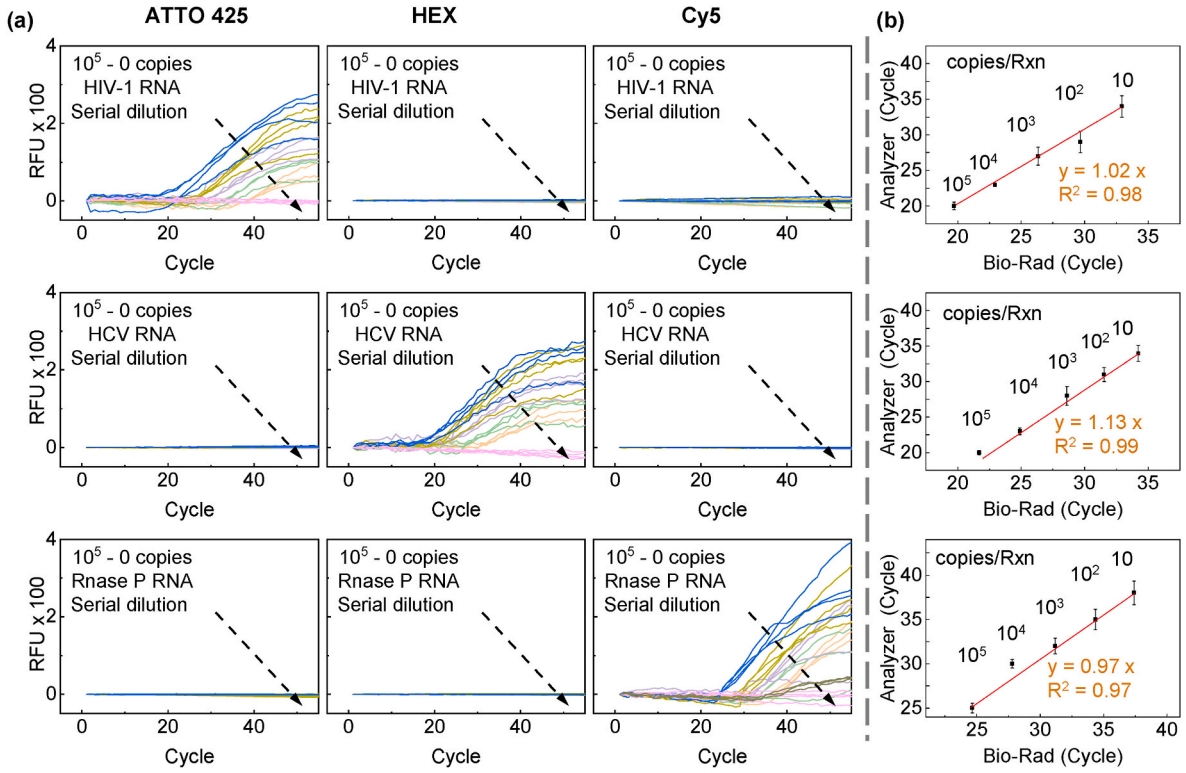

Fig. 5. (a) Real-time RT-PCR results for serially diluted RNA (10^5 to 0 copies/reaction), tested in triplicate, with signals for each target shown in separate columns. (b) Comparison of Cq values for all three targets measured by the Bio-Rad benchtop PCR analyzer and the portable analyzer. Each data point represents the mean of three replicates (N = 3), with error bars indicating standard deviation.

significance to drug developers, healthcare institutions, and government agencies in combating the spread of HIV and HCV.

4. Conclusion

In this study, we developed a handheld viral load testing device that combines RNA extraction with rapid multiplex RT-PCR to provide HIV and HCV results in under 60 min. The system extracted RNA with 80 % efficiency and detected as few as 5 copies per reaction. The validation studies demonstrated a strong correlation with Bio-Rad benchtop PCR systems (R^2 = 0.97–0.99) which supported its accuracy and demonstrated comparability to standard laboratory testing methods. The system processes four samples simultaneously and improves testing efficiency through its user-friendly design which makes it suitable for non-professional use in areas with limited resources. The novel method integrates precise viral load measurement with portable design to enable dependable self-monitoring for patients with HIV/HCV co-infection and those at high risk. Through early disease detection and timely treatment modifications the system lowers transmission risks and helps patients take control of their healthcare management. This study was validated by laboratory personnel. Future work will include evaluating the impact of common user errors (e.g., insufficient blood collection, improper extraction procedures) and conducting real-world self-testing studies to assess failure rates and robustness, followed by optimization of the user interface and incorporation of error indication systems. Future studies will involve patient-derived samples to further validate clinical applicability and compare results with commercial qPCR assays. Upcoming developments could extend the system's abilities to identify more pathogens while including wireless data transmission which benefits remote healthcare monitoring and supports worldwide public health efforts.

CRediT authorship contribution statement

Tianyi Liu: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Anthony J. Politza:** Writing – original draft, Visualization, Investigation, Formal analysis. **Aneesh Kshirsagar:** Methodology, Investigation, Formal analysis. **Md Ahasan Ahamed:** Software, Methodology, Formal analysis. **Weihua Guan:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of generative AI in scientific writing

During the preparation of this work, the authors used ChatGPT (powered by OpenAI's language model, GPT-4; http://openai.com) in order to refine the manuscript text for grammatical correctness and to improve readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

A provisional patent application has been submitted concerning the technology discussed in this document.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2025.117843.

Data availability

Data will be made available on request.

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