REVOLUTIONARY DIAGNOSTICS – NONTRADITIONAL APPROACHES FOR DEVELOPING BREAKTHROUGH CAPABILITIES AGAINST EMERGING THREATS

Fingerpick Blood-based Hiv Self-nucleic Acid Testing On Usb At The Point Of Need

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Background: Uptake of HIV self-testing has gained increasing acceptance now days. The Rapid Antigen Self-Test for HIV Antigens provides a quick cheap test less than 15 minutes, $10. However, antigen tests are 30% to 40% less sensitive than nucleic acid tests. On the other hand, many NAT devices for HIV testing require extensive training for technicians to operate in centralized labs.

Purpose: Filling the still-existing gap in accurate, fast, and inexpensive HIV self-testing.

Objective: In this work, we present a fully integrated nucleic acid testing (NAT) device for streamlined HIV self-testing.

Rationale of the research: The test requires a single step from the user to load the 100µl finger-prick blood sample, and the testing result can be easily read out by a GUI in less than 1 hour. This will be the prototype of POC HIV nucleic acid self-testing.

Relationship to other areas of study: This instrument can not only be used in the rapid and accurate detection of HIV virus, but also can detect different viruses/diseases by changing the reagent cartridge

Materials and Methods: The HIV NAT-on-USB device consists of a highly portable palm-sized analyzer and a ready-to-use disposable reagent cartridge (Fig.1a). Fig. 1b-d shows the exposed view and the assembled view of the analyzer, respectively. The USB-interfaced analyzer integrates the optical modules (excitation/detection), thermal modules (actuation/sensing), and mechanical modules (PCB coil electromagnet driver). These modules are controlled by a microcontroller unit (MCU) to fully automate the sample-toanswer process on the disposable cartridge.These modules are controlled by a microcontroller unit (MCU) to fully automate the sample-to-answer process including sample preparation, purification, and real-time reverse-transcription loop-mediated isothermal amplification (RT-LAMP) on the disposable cartridge.

Results and Discussion: The multiple USB-interfaced analyzers can be used simultaneously and independently in a plug-and-play (PnP) fashion (Fig. 1e). For the intra-device verification, we tested a series of mock samples with different HIV-1 RNA concentrations. Fig. 1f shows the real-time data obtained from testing a triplicate panel of these samples with a single USB interfaced analyzer. HIV-1 RNA concentrations at 500 copies/mL of whole blood were all amplified successfully. For the inter-device verification, we tested four independent devices with multiple triplicated mock samples. As shown in Fig. 1g, the device to device showed Pearson correlation coefficients ranging from 0.79 to 0.92, suggesting a good quantitative agreement between these devices. The sensitivity, and specificity of the test was 96.2% (95% CI=90.9%-100%) and 88.5% (95% CI= 79.8%-97.1%). The tests performed with all four different devices showed excellent accuracy (93%) in differentiating the clinically relevant viral load threshold at 1000 copies/mL.

Conclusions: In summary, we present a novel device that can extract RNA using magnetic beads and amplify the extracted nucleic acid material using RT-LAMP. In ~60 min, we achieved a limit of detection (LoD) of 500 viral RNA copies/mL of whole blood at a 95% confidence level. Due to its ease of use and high sensitivity, the HIV NAT-on-USB device would be beneficial for the high-risk populations seeking private self-testing at the early stages of exposure.

This work was supported by the National Institutes of Health (R61AI147419), and National Science Foundation (1912410 & 1902503).
Liu, Tianyi, et al. "Fingerpick Blood-Based Nucleic Acid Testing on A USB Interfaced Device towards HIV self-testing." Biosensors and Bioelectronics 209 (2022): 114255.