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A point-of-care PCR test for HIV-1 detection in resource-limited settings

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ABSTRACT

A low-cost, fully integrated sample-to-answer, quantitative PCR (qPCR) system that can be used for detection of HIV-1 proviral DNA in infants at the point-of-care in resource-limited settings has been developed and tested. The system is based on a novel DNA extraction method, which uses a glass fiber membrane, a disposable assay card that includes on-board reagent storage, provisions for thermal cycling and fluorescence detection, and a battery-operated portable analyzer. The system is capable of automated PCR mix assembly using a novel reagent delivery system and performing qPCR. HIV-1 and internal control targets are detected using two spectrally separated fluorophores, FAM and Quasar 670. In this report, a proof-of-concept of the platform is demonstrated. Initial results with whole blood demonstrate that the test is capable of detecting HIV-1 in blood samples containing greater than 5000 copies of HIV-1. In resource-limited settings, a point-of-care HIV-1 qPCR test would greatly increase the number of test results that reach the infants caregivers, allowing them to pursue anti-retroviral therapy.

1. Introduction

DNA PCR is currently a gold standard for screening infants for HIV-1. In rural clinics, blood samples are often collected onto filter paper, dried, and transported to urban laboratories for testing (Sherman et al., 2005), where genomic DNA is extracted and PCR is performed in large batches. Due to logistical problems, results are often not returned to the caregiver in a timely manner, resulting in a large number of patients not knowing their disease status and not enrolling for therapy. In Uganda, nearly 43% of HIV-1 test results are never delivered to the patients and caregivers (Kiyaga et al., 2010). Early infant diagnosis by PCR coupled with timely treatment has the potential to drastically reduce infant mortality from HIV-1(Violari et al., 2007). Point-of-care technologies deployed in rural clinics can provide results to the patients and caregivers in the same visit. Such technologies must operate within many constraints such as (1) relatively simple sample preparation and extraction, (2) sample-to-answer capability, (3) lack of skilled personnel and highly complex equipment, (4) irregular power supply, (5) minimal lab bench space, (6) no refrigeration, and (7) low cost.

Many platforms are under development for providing sample-to-answer solutions (Brennan et al., 2009; Mairhofer et al., 2009). However, few have demonstrated sample-in-answer-out capability starting with whole blood (Buchan et al., 2011; Cho et al., 2007; Easley et al., 2006; Jobbagy et al., 2007; Raja et al., 2005; Tanriverdi et al., 2010). Typically in such platforms during complete sample-to-answer analysis, the following steps are performed

(1) Blood samples are mixed with chaotropic agents such as guandinium-HCl and isopropanol or sonicated in the presence of functionalized particles or beads to lyse leukocytes and capture nucleic acids. Another method involves heat induced cell lysis by irradiating magnetic beads with a laser (Cho et al., 2007). DNA capture is followed by one or two rounds of washing and elution. However, the above steps require microfabricated pumps such as diaphragm pumps or syringe pumps to enable fluid and bead flow from one chamber to another. Furthermore, to prevent inhibitory PCR reagents such as Gu-HCl from entering the PCR chamber, valves are used to direct flow (Chen et al., 2005).
(2) PCR is performed in serpentine channels (Kopp et al., 1998) or single chambers using microfabricated single-use or re-usable heating elements and temperature sensors (Beyor et al., 2009; Easley et al., 2006; Legendre et al., 2006; Liu et al., 2007; Toriello et al., 2008). Digital PCR is increasingly being used to improve quantification accuracy with a much higher dynamic range (Mazutis et al., 2009) by partitioning the PCR mix into smaller droplets before thermocycling.
(3) For detecting amplified product, fluorescence and capillary electrophoresis have commonly been used.

We previously reported a novel method, Fast Isolation of Nucleic Acid (FINA), for preparation of qPCR ready DNA in 2 min. FINA, when

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used with the Stratagene Mx3005p qPCR system (Agilent Technologies, Santa Clara, CA), can detect as few as 20 copies of HIV-1 in 100 μL blood with a clinical sensitivity and specificity of 99% and 100%, respectively (Jangam et al., 2009). In this report, we use FINA to develop a sample-in-answer-out platform that is different from most technologies described above in two respects:

(1) DNA capture has been separated from the rest of the system and involves (a) blood sample collection in an off-the-shelf microtube (b) DNA capture on separation membrane (c) a single wash with a stable PCR compatible reagent provided in a dropper bottle. The membrane immobilized DNA serves as a template for PCR. FINA does not use functionalized particles and therefore, does not require any active pumping or valves for DNA purification. This dramatically simplifies the fabrication and assembly process for our assay card.
(2) The simplicity of the extraction process enables our assay card to have a single chamber for PCR with two flow channels for entry of pre-packaged PCR reagent and exit of air. The PCR reagents are stored in a reservoir using foil laminate packaging and are assembled automatically prior to PCR utilizing a single re-usable DC motor.

In our integrated sample-to-answer platform, the operator steps are similar to those in adult HIV-1 testing and hence require minimal operator re-training. An overview of the test is shown in Fig. 1. The test requires the operator to collect 100 μL blood via heel prick. Blood can be collected in traditional collection devices such as microtainers (BD, Franklin Lakes, NJ). The collection device is pre-coated with a lysing agent, Triton-X-100 which lyses blood cells releasing cellular DNA. The lysate is added to a disposable separator module (Fig. 2) that captures DNA on a glass fiber membrane bonded to a sample introduction module (SIM) and separates it via capillary action from other components of blood that are potent PCR inhibitors. A single wash step ensures no PCR inhibitors remain on the SIM, which is subsequently inserted into the assay card that stores necessary PCR reagents on-board. This assay card is inserted into a portable analyzer that performs PCR mix assembly and qPCR. In this paper, we describe the building blocks for the various components of the point-of-care PCR test and present preliminary results on the performance of the various sub-systems of the analyzer and HIV-1 testing using blood samples.

2. Materials and methods

2.1. Sample Introduction Module (SIM) fabrication

The SIM consists of two main parts: the DNA capture membrane and plastic membrane holder. The membrane holder is a

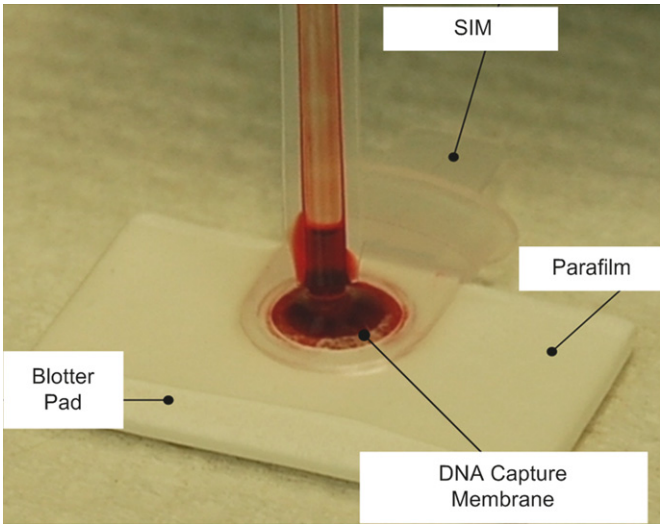

Fig. 2. Separator module used to isolate cellular DNA from whole blood. The SIM is in intimate contact with the blotter pad for capillary action. Parafilm makes an air tight seal around the SIM preventing lysed blood from coming in contact with the blotter pad directly.

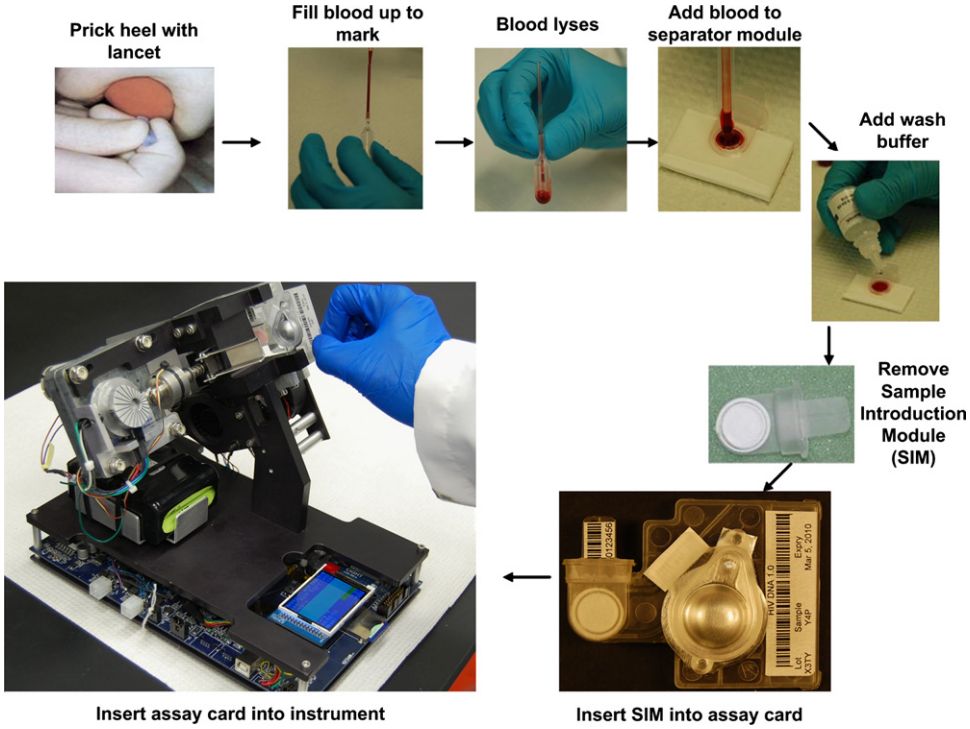

Fig. 1. Schematic of the workflow of the qPCR based point-of-care HIV-1 test.