

Detection of Proviral HIV-1 DNA in Whole Blood using Recombinase Polymerase Amplification (RPA)

Brittany Rohrman¹, Rebecca Richards-Kortum¹

¹Rice University, 6100 Main St, MS-142, Houston, TX 77005

Introduction: More than 2 million children are currently living with HIV-1, and over 90% of all pediatric HIV-1 infections occur in sub-Saharan Africa. HIV-1 may be managed effectively using anti-retroviral therapy (ART), but identifying children in need of therapy is challenging in low resource settings. The gold standard method for pediatric HIV-1 diagnosis is based on polymerase chain reaction (PCR) and detection of HIV-1 viral genomic RNA or proviral DNA present in peripheral blood mononuclear cells. PCR requires trained technicians, costly reagents, electrically powered equipment, and dedicated laboratory space, which are often unavailable in areas where HIV-1 prevalence is highest. Efforts to improve access to pediatric HIV-1 diagnosis in developing countries have resulted in dried blood spot (DBS) PCR programs, through which district clinics mail dried blood samples to centralized labs for DNA PCR testing. However, because results may take several weeks to reach patients, many never return to the clinic to learn their HIV-1 status. An HIV-1 test based on nucleic acid detection is needed to provide results rapidly at the point of care, where patients may receive counseling and initiate treatment.

Methods: We designed an HIV-1 diagnostic assay that costs less than \$10 and provides a result within one hour. First, HIV-1 proviral DNA in 100 μ L of whole blood is released from cells by incubating the sample with a lysis solution. A region of the HIV-1 *pol* gene is then amplified using recombinase polymerase amplification, an isothermal enzymatic method that is capable of rapidly amplifying samples containing PCR inhibitors at a low amplification temperature. This method only requires a simple heater, eliminating the need for thermal cycling equipment. Dual-labeled amplified DNA is visualized using gold nanoparticles on lateral flow strips, which are easy to use and familiar to health care workers. This assay was performed using HIV-negative blood spiked with known concentrations of cultured 8E5 lymphoblasts, which contain one copy of the HIV-1 genome. The limit of detection and dynamic range of the assay were characterized to determine whether the assay needs further modification for detecting HIV-1 in clinical samples.

Results and Discussion: Amplification occurs in the presence of lysed blood products without requiring further purification and tolerates up to 200 ng of background DNA. The assay detects 10^3 to 10^5 8E5 cells, which correspond to HIV-1 proviral loads between 10^4 to 10^6 HIV-1 DNA copies/mL. The clinical range of proviral HIV-1 DNA concentrations is 10^2 to 10^6 HIV-1 DNA copies/mL; thus, the diagnostic assay is capable of detecting HIV-1 DNA in spiked samples at HIV-1 concentrations found in clinical samples. However, the assay must be modified to include enrichment of target DNA to expand the dynamic range of the assay. These results suggest that after further modification, this assay may be a feasible approach for detecting HIV-1 DNA in low resource settings.