Evaluation of a single round polymerase chain reaction assay using dried blood spots for diagnosis of HIV-1 infection in infants in an African setting

Abstract:

The aim of this study was to develop an economical 'in-house' single round polymerase chain reaction (PCR) assay using filter paper-dried blood spots (FP-DBS) for early infant HIV-1 diagnosis and to evaluate its performance in an African setting. Methods An 'in-house' single round PCR assay that targets conserved regions in the HIV-1 polymerase (pol) gene was validated for use with FP-DBS; first we validated this assay using FP-DBS spiked with cell standards of known HIV-1 copy numbers. Next, we validated the assay by testing the archived FP-DBS (N = 115) from infants of known HIV-1 infection status. Subsequently this 'in-house' HIV-1 pol PCR FP-DBS assay was then established in Nairobi, Kenya for further evaluation on freshly collected FP-DBS (N = 186) from infants, and compared with findings from a reference laboratory using the Roche Amplicor® HIV-1 DNA Test, version 1.5 assay. Results The HIV-1 pol PCR FP-DBS assay could detect one HIV-1 proviral copy in 38.7% of tests, 2 copies in 46.9% of tests, 5 copies in 72.5% of tests and 10 copies in 98.1% of tests performed with spiked samples. Using the archived FP-DBS samples from infants of known infection status, this assay was 92.8% sensitive and 98.3% specific for HIV-1 infant diagnosis. Using 186 FP-DBS collected from infants recently defined as HIV-1 positive using the commercially available Roche Amplicor v1.5 assay, 178 FP-DBS tested positive by this 'in-house' single-round HIV-1 pol PCR FP-DBS PCR assay. Upon subsequent retesting, the 8 infant FP-DBS samples that were discordant were confirmed as HIV-1 negative by both assays using a second blood sample. Conclusions HIV-1 was detected with high sensitivity and specificity using both archived and more recently collected samples. This suggests that this 'in-house' HIV-1 pol FP-DBS PCR assay can provide an alternative cost-effective, reliable and rapid method for early detection of HIV-1 infection in infants.