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Cost effective diagnosis and monitoring of HIV-1 in a resource poor setting

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ABSTRACT

The South African National Antiretroviral Treatment Guidelines recommend the use of HIV-1 viral load assays for routine monitoring of HIV-1 positive patients receiving highly active antiretroviral therapy (HAART). This thesis describes the innovative approaches to developing more affordable HIV-1 diagnostics and monitoring assays for South Africa, which take into account the tiered laboratory infrastructure of this country. An in-house HIV-1 viral load assay – the LUX assay, was developed and evaluated with a view of implementing this more affordable option in high tier laboratories. The LUX assay represents quantitative real-time RT-PCR that utilizes the LightCycler® technology (Roche) in a novel combination with a LUX™ primer. The assay showed good analytical sensitivity, specificity and reproducibility of its linear dynamic range of $4x10^2$ to $4x10^6$ RNA copies/ml. Preliminary clinical evaluation (n = 458) of the LUX assay showed good agreement with the COBAS Amplicor assay, and demonstrated its usefulness for long term monitoring of HAART patients.

ELISA based viral load testing approaches were investigated as low cost and less technically complex alternatives for medium tier laboratories. The HiSens HIV-1 p24 Ag Ultra (Perkin Elmer) and the ExaVir™ Load Quantitative HIV-RT kits (CAVIDI) were compared with the Roche Amplicor assay. Both assays showed strong association with the Roche Amplicor assay, with $R^2 = 0.686$ and $R^2 = 0.810$, respectively (n = 117). These alternative assays seemed most useful in the serial monitoring of patients on HAART. Major drawbacks included the wide variability of both assays, insufficient sensitivity of the p24 antigen assay and low throughput of the RT assay.

Development of a point-of-care HIV-1 RNA assay could address issues related to early and cost effective diagnosis of acute HIV infection. A novel isothermal amplification technique termed the Reverse Transcription Loop Dependant Amplification (RT-LDA) was developed as one component for a potential point-of-care HIV-1 RNA assay. The RT-LDA converted RNA into partially looped ssDNA amplicons, over a wide RNA concentration range ($4x10^3$ to $4x10^8$ copies/ml) using a 1 hour incubation at 53ºC. The RT-LDA technology is fully compatible with a lateral flow detection system using dipsticks and highly suitable for point-of-care testing.

Overall, this study demonstrates the feasibility of developing novel, more affordable HIV-1 testing options that would be appropriate for the tiered laboratory infrastructure present in South Africa. Evaluation of commercially available, less expensive alternative HIV viral load assays in local settings facilitates their implementation.