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Novel application of Locked Nucleic Acid chemistry for a TaqMan assay for measuring diverse Human Immunodeficiency Virus type 1 subtypes

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Summary

There remains a need for sensitive and cost-effective assays to monitor therapy in human immunodeficiency virus type-1 (HIV-1) infection. However, the genetic diversity of HIV poses difficulties for traditional real-time PCR assays that require long oligonucleotides probes. LNATM probes may be useful in overcoming these limits to traditional probe design. A new application of LNATM chemistry in a Taqman assay applicable to a wide range of HIV-1 subtypes is described. This assay, based on a 13-mer LNATM probe that matches the majority of HIV-1 sequences in the Los Alamos database, exhibited a wide dynamic range (10^1 to 10^7 copies of HIV DNA), high sensitivity (limit of detection of 1 copy of HIV DNA in 10^5 cells), and broad applicability to a range of HIV-1 subtypes (including A, B, C, D, F, H, B/C, and A/E CRFs). Using the LNATM probe assay, HIV-1 DNA was detected in all dried blood spots (DBS) from treatment naïve HIV-1 positive Ugandan children, and HIV DNA levels significantly correlated with viral RNA levels in plasma ($r=0.765$, $p<0.0001$). This approach to Taqman probe design should be explored further for use in diagnosis and monitoring of HIV in resource-limited settings, especially where several subtypes co-circulate.

Keywords

HIV; Taqman PCR; HIV subtypes; Dried Blood Spots; Locked Nucleic Acid

1. Introduction

Measurements of HIV-1 DNA and RNA in blood are used to assess viral reservoir size and viral replication and have utility for prognosis and optimal management of HIV-infected patients (Mellors et al., 1996; Montoya et al., 1993). While commercial viral load (VL)

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assays are widely available for patient management in resource-rich countries, they remain largely unavailable for use in resource-limited settings due to cost and the scarcity of laboratory resources needed to perform both the initial specimen processing (plasma or PBMC preparation) and the molecular assays. More affordable in-house VL assays could be developed and explored as an alternative, especially if they could be combined with simplified sample collection and processing. One attractive platform is the Taqman real-time PCR system.

Taqman PCR is a quantitative fluorescent-hydrolysis probe-based PCR procedure that can be employed for both sensitive and specific nucleic acid detection (Valasek and Repa, 2005). However, Taqman assays are especially intolerant of target/probe mismatches (Higuchi et al., 1992; Higuchi et al., 1993; Holland et al., 1991; Valasek and Repa, 2005; Zhao et al., 2002); this represents a major limitation in using this method for quantitation of divergent HIV-1 variants (Gardner et al., 2003; Geelen et al., 2003; Swanson et al., 2005; Tang et al., 2007).

An extreme example of the problem that HIV-1 genetic diversity poses for many PCR based assays is the complexity of the group M HIV-1 subtypes. Within group M, HIV-1 has been classified into at least nine recognized subtypes and sixteen circulating recombinant forms (CRFs). (Osmanov et al., 2002; Peeters and Sharp, 2000; Swanson et al., 2005) (Los Alamos HIV-1 sequence data Base (www.hiv.lanl.gov)). In many areas of the world, subtypes co-circulate so that two or more very divergent subtypes can be encountered within a geographic area. Currently, most Taqman PCR assays designed to quantify HIV-1 DNA are optimized for HIV-1 subtype B (Debiaggi et al., 2000; Desire et al., 2001; Palmer et al., 2003; Zhao et al., 2002) and may not be suitable for non-B subtypes. Subtype-specific probes have been designed for non-B subtypes (Kamat et al., 2007), but these do not resolve a potential sensitivity problem when the subtype identity is unknown. In order to achieve optimal probe annealing temperatures, traditional nucleic acid probes for Taqman PCR comprise 25 to 35 nucleotides. Given the natural heterogeneity of HIV-1, it is challenging to find three well-conserved regions within 200 base pairs (two for the primers and one for the internal probe homology sequence) that encompass all or even most HIV-1 group M subtypes. A recent report from Rouet described the improved performance of a real time PCR assay for non-B subtypes using a 15mer probe targeting the Long terminal repeat region (LTR) used for quantifying plasma viral load (Rouet et al., 2007). However, there is a theoretical limit to the design of shorter primers and probes using standard nucleotide chemistries.

Previously, a novel nucleic acid analogue was developed by introduction of a 2'-O, 4'-C-methylene bridge that restricts flexibility of the ribofuranose ring, locking it into a rigid C3'-endo conformation (Exiqon). Such bases, called Locked Nucleic Acids, (LNATM) exhibit superior hybridization affinity and enhanced biostability, effectively raising the T_m of an oligonucleotide by 3 to 8 degrees Celsius for each LNATM base (Kumar et al., 1998).

A new application of the LNATM chemistry for designing shorter Taqman PCR probes (Mouritzen et al., 2003; Simeonov and Nikiforov, 2002; You et al., 2006) that facilitate the targeting of very short, cross-subtype-conserved sequences within the HIV-1 genome is described. In addition to validating the assay using well-characterized standards, the assay was applied to clinical samples consisting of dried blood spots (DBS) collected from untreated HIV-positive Ugandan children. HIV DNA was detectable in 38 of 38 these DBS and the HIV DNA levels correlated significantly with levels of HIV-1 RNA in plasma tested using a commercial VL assay.

2. Methods

2.1. Samples from treatment naïve HIV-positive Ugandan patients

DBS specimens were collected from May of 2006 to October of 2007 in Uganda as a part of an Institutional Review Board-approved cohort study of HIV+ children at risk for malaria. Informed consent was obtained from the parents or guardians of all participants prior to study enrollment. DBS were obtained by pricking the finger and blotting drops of whole blood onto Whatman #3 filter paper (Whatman, Dassel Germany). Spots were dried and stored at room temperature in sealed plastic bags with desiccant.

2.2. HIV-1 plasmid DNA and HIV-1 isolates

The following samples were obtained from the NIH AIDS Research and Reference Reagent Program (catalogue numbers in parentheses). HIV-1 DNA plasmids: subtype A: p92UG037.1 (3605); subtype B: pNL4-3 (114); pWT/Bal (11414); Subtype B/C: pSG3.1 (2003); subtype C: p98TZ013.10 (6187); subtype D: p94UG114.1 (4001); subtype A/E: p90CF402.1.8 (4007); subtype F: p93BR020.1 (4004); subtype H: p90CF056.1 (4005); A panel of 50 International HIV-1 isolates (11412), containing 10 Clade A; 10 Clade B; 10 Clade C; 10 Clade D; and 10 Clade A/E virus stocks.

2.3. HIV-1 isolation and HIV RNA measurement using commercial p24 and HIV RNA assays

Each isolate of HIV-1 was grown in peripheral blood mononuclear cells (PBMCs) obtained from two healthy donors. Briefly, PBMCs were isolated by Ficoll density gradient centrifugation, stimulated with 5 µg/ml of phytohemagglutinin (PHA)(Sigma, St. Louis, MO) and recombinant interleukin 2 (IL2) at 5 U/ml for one day, and then washed prior to infection. 10⁷ stimulated, pooled-donor PBMCs were inoculated with each HIV-1 isolate for 5 hours, then washed with complete medium (RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 mg/ml streptomycin and 2mM glutamine), resuspended with 10 ml fresh complete medium with 20 U/ml of IL2, and incubated at 37°C with 5% CO₂ in T25 flasks. Supernatants, collected at day 15, were passed through a 0.22 µm filter. Production of HIV-1 was verified by detection of HIV-1 p24 using a commercial ELISA (PerkinElmer, Boston, MA). 47 successfully propagated isolates were available for testing. Isolates were diluted in phosphate buffered saline 1:100 for HIV-1 p24 testing and 1:20000 prior to HIV RNA testing.

HIV RNA in diluted supernatants was measured using the Abbott RealTime HIV-1 Viral Load Assay per manufacturer's specifications on the Abbott M2000 instrument (Abbott, North Chicago, IL).

2.4. Purification of HIV-1 DNA and RNA

All HIV-1 DNA plasmids were propagated in Stbl2 competent cells (Invitrogen, Carlsbad CA) under antibiotic selection at 30°C and purified using a QIAfilter plasmid purification kit (QIAGEN, Valencia, CA). Plasmids were verified by restriction enzyme digestion.

Virion RNA from virus stocks and from the HIV-1 Virology Quality Assurance (VQA) RNA Quantification standards (3443, NIH AIDS Research and Reference Reagent Program) was purified using the QIAamp Viral RNA mini kit (Qiagen, Valencia, CA).

Proviral HIV-1 DNA was purified from a ten-fold dilution series of 10¹ to 10⁵ 8E5 cells (NIH AIDS Research and Reference Reagent Program) (95) diluted in 10⁶ healthy donor PBMCs using the QIAamp DNA blood mini kit (Qiagen, Valencia, CA). 8E5 cells are chronically-infected with HIV-1 LAV and contain a single copy of integrated HIV-1 proviral DNA that contains a frameshift mutation rendering progeny virions non-infectious,

thereby preventing the accumulation of multiply-infected cells and providing a fixed 1:1 ratio of HIV-1 DNA copy/cell (Folks et al., 1986).

2.5. Preparation of control DBS samples

Two DBS panels were prepared for study. For the first panel, 10^1 to 10^4 8E5 cells were spotted onto Whatman #3 filter paper (Whatman, Dassel, Germany), allowed to dry, overlaid with 10 μ l of healthy control whole blood, and allowed to dry again at 25°C overnight. For the second panel, 10^3 to 10^6 8E5 cells were mixed with 1 ml of whole blood from healthy control volunteers, and 10 μ l of the mixture was spotted onto filter paper and allowed to dry at 25°C overnight.

2.6. DNA extraction from DBS

DBS were immersed in 260 μ l of extraction buffer (10 mM TrisHCl, pH 8.0, 50 mM KCl, 0.45% Tween 20 and 0.45% NP-40) and incubated at 56°C for 30 minutes, treated with 40 μ l of Qiagen proteinase at 56°C for 60 minutes, heated to 90°C for 20 minutes, and vortexed twice during heating. Following addition of 300 μ l of buffer AL, samples were incubated at 70°C for an additional 10 minutes. Contents (lysate and filter paper) were loaded onto a QIAshredder column (Qiagen, Chatsworth, CA), and centrifuged at 18500g for 1 minute. The QIA shredder column was discarded and 300 μ l of 100% ethanol was added to the pass-through and vortexed to mix well. The mixture was loaded onto a QIAamp blood Mini spin column (Qiagen, Chatsworth, CA) and purified according to the manufacturer's instructions, except that the final DNA elution was performed with 50 μ l of water. Samples were either stored at -20°C or immediately assayed by real-time PCR.

2.7. LNATM-Taqman assay

Real-time PCR was performed on an ABI 7300 Real-time system with the following primers and probes: forward primer: G19-2-F-7Y (5'-AGCAGCYATGCAAATGTTA-3') (1374-1392); reverse primer: G-20-R (5'-AGAGAACCAAGGGGAAGTGA-3') (1474-1493); dual-labeled fluorescent LNATM probe with 6-FAMTM at the 5' end and a Black hole quencher BHQTM-1 at the 3' end: G-probe (5'-CCATCAATGAGGA-3') (1400-1412) (bases underlined are LNATM). Assays were run at a final volume of 25 μ l consisting of 12.5 μ l of TaqMan® Gene Expression Master mix (ABI, Foster city, CA), 7.5 μ l of primers (400 nM both forward and reverse) and probe (200 nM) (final concentration), 5 μ l of input target DNA with cycling conditions: 50°C for 2 min, 95°C for 5 min, then 50 cycles of 95°C for 15sec, 59°C for 1 min. Real time RT PCR for HIV-1 RNA was performed using 12.5 μ l of TaqMan® RNA-to-CTTM-1STEP KIT (ABI), 7.5 μ l of primers (400 nM both forward and reverse) and probe (200 nM), and 5 μ l of RNA at cycling conditions: 48°C for 20 min, 95°C for 5 min, then 50 cycles of 95°C for 15sec, 59°C for 1 min.

2.8. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA); Normality of data was tested with the D'Agostino and Pearson Omnibus test. Significance of associations was tested by calculating a Pearson r correlation coefficient.

3. Results

3.1. LNATM Probe homology to available group M HIV-1 sequences

A conserved region was identified between nucleotides 1374 and 1493 (HXB2 numbering) of the p24 domain of the *gag* gene. Within this region, a 13 base sequence (HXB2 coordinates 1400-1412) was identified for the LNATM probe. This probe is an exact match to ~70% of all full length HIV-1 sequences in the Los Alamos HIV-1 Sequence Database. By

subtype, the LNATM probe sequence was a perfect match for 88.5% of subtype A, 87.4% of B, 87.5% of B/C, 54.4% of C, 87.8% of D, 90.8% of A/E, 66.5% of F and 100% of H. The frequency of either perfect matches or single mismatches by subtype was 90.5% overall, 98% for subtype A (n=86), 95% for B (n=204), 87% for C (n=366), 98% for D (n=49), 98% for CRFs A/E (n=87), 90% for F (n=18) and 100% for H (n=3).

3.2. Assay efficiency and dynamic range

To evaluate the dynamic range of the LNATM-Taqman assay, a dilution series of HIV-1 DNA (pNL4-3 plasmid) from 10^1 to 10^7 copies per reaction mixture was tested in three independent experiments with triplicate samples at each dilution. Threshold cycle (C_T) values inversely correlated with template input ($R^2=0.999$). The average coefficient of variation for the assays was 0.075. For each 10-fold dilution, there was an increase in C_T value of approximately three, consistent with excellent amplification and detection efficiency across the entire 6 $\log_{10}$ dynamic range.

3.3. Detection limits of the LNATM-Taqman assay for HIV-1 DNA and RNA

First, HIV-1 DNA template standards of defined copy number (10, 5, and 1) obtained from the NIH AIDS Research and Reference Reagent Program were used to determine assay sensitivity. Frequencies of detection were 95% (19/20) for 10 copies, 83% (10/12) for 5 copies, and 42% (5/12) for 1 copy. Mean copy numbers (calculated by extrapolation from the plasmid standard curve) were 9.46 ± 1.74 , 4.75 ± 1.85 , and 0.47 ± 0.4 , respectively. Negative controls (n=30) were uniformly negative.

HIV-1 proviral DNA was measured for a dilution series from 1 to 10^4 8E5 cells. HIV-1 proviral DNA was detected across the entire dilution range. 8E5 cellular input correlated inversely with C_T (data not shown). No HIV-1 was detected in any samples of PBMC DNA alone (n=16).

Assay performance for subtype B HIV-1 RNA was assessed using the HIV-1 Virology Quality Assurance (VQA) RNA quantitation standards (NIH AIDS Research and Reference Reagent Program). Detection frequencies were 100% (16/16) for 1000 copies, 94% (15/16) for 100 copies, and 87% (14/16) for 10 copies. Extrapolated copy numbers were 996 ± 96.18 , 98.51 ± 19.12 , and 9.12 ± 1.92 , respectively. All negative controls (n=16) were negative.

3.4. LNATM-Taqman Assay performance for group M HIV-1 DNA

A panel of group M HIV-1 plasmids of different subtypes (A, B, B/C, C, D, A/E, F and H) was tested in three independent experiments at inputs of 1000 copies per sample. The assay performed reliably for every subtype tested (Table 1).

3.5. Assay performance for virion RNA from HIV-1 subtypes A, B, C, D and A/E

47 HIV-1 isolates, comprising 5 HIV-1 subtypes, were assayed. For each isolate, clarified day 15 culture supernatants were tested in parallel using the Abbott RealTime HIV Viral Load assay, the Perkin Elmer p24 Elisa and with the LNATM Taqman assay following reverse transcription. Due to differences in the dynamic range of these assays, the p24 assays were conducted on 1:100 dilutions while HIV RNA assays were conducted on 1/20000 dilutions but results are expressed as the concentrations for the original culture supernatants. All samples had detectable HIV-1 RNA by both assays. One subtype D sample gave discrepant results with the two assays, nevertheless, the correlation between HIV RNA concentrations measured by the two assays was good (Pearson r of 0.81, $p<0.0001$ for all datapoints; Pearson r of 0.99, $p<0.0001$ with omission of outlier) (Fig 1).

3.6. Measurement of HIV-1 proviral DNA in DBS

The ability of the LNATM Taqman assay to measure HIV-1 DNA present in DBS prepared by: 1) sequentially spotting 8E5 cells and then whole blood; and 2) spotting 8E5 cells pre-mixed with whole blood was studied. Using cellular DNA corresponding to 1 to 1000 8E5 cell equivalents (10% of each extraction), HIV-1 DNA was detected in all samples and results from both DBS preparation methods were comparable (Figure 2).

3.7. Assay performance using samples from a pediatric HIV-1 cohort study in Kampala, Uganda

DBS were studied from 38 ART-naïve HIV-1 -positive children with subtypes of A, D, AC and ACD. Plasma HIV-1 viral loads (measured using Amplicor HIV-1 Monitor v1.5 (Roche, Piscataway NJ)) in parallel plasma samples ranged from 9896 copies per ml to 595476 copies per ml. HIV-1 DNA was detected from all 38 samples. Calculated HIV-1 DNA copy numbers per μ l of blood (mean 28 copies/ μ l) correlated significantly with HIV-1 RNA measured using plasma samples collected on the same day ($r = 0.76$, $p < 0.00001$) (Figure 3).

4. Discussion

A new Taqman assay based on LNATM chemistry was developed for measuring levels of diverse HIV-1 variants, including non-B subtype HIV-1. The assay demonstrated excellent sensitivity and consistent performance using well-defined subtype B and non-subtype B HIV-1 standards. Furthermore, in a pilot study using clinical samples of DBS from HIV-1 infected Ugandan children, levels of HIV-1 DNA correlated well with levels of HIV-1 RNA in parallel plasma tested using a commercial HIV-1 RNA assay.

The diversity of HIV-1 limits the ability of traditionally longer Taqman probes to perform reliably across multiple HIV-1 subtypes (Gardner et al., 2003; Geelen et al., 2003; Swanson et al., 2005; Tang et al., 2007). Several published real-time Taqman PCR assays for HIV-1 proviral DNA that utilize traditional Taqman probes ranging from 21bp to 33bp in length were surveyed. When compared against full-length sequences available in the Los Alamos HIV-1 database, the probe with the highest exact match-frequency was only a perfect match to 39.4% of all 1240 sequences and 61.8% to subtype B sequences (Desire et al., 2001). Others fared worse, with exact-match frequencies of 38% (Zhao et al., 2002), 19% (Palmer et al., 2003) and 1% (Drosten et al., 2001) for the 1240 HIV-1 sequences. One probe specifically designed for subtype C HIV-1 was a perfect match to only 45.4% of C subtype sequences (Kamat et al., 2007). In contrast, the 13-mer LNATM probe designed for the current assay was a perfect match to 70% of 1240 full-length sequences representing all major subtypes.

The LNATM-Taqman assay performed well with both laboratory standards and with patient specimens. Accurate quantitation was observed across a wide dynamic range (10^1 to 10^7 copies of pNL4-3). The limit of detection for the assay, as judged by quantitation standards obtained from the NIH AIDS Reagent Program and dilutions of 8E5 cells, was 10 copies of HIV-1 RNA and between 1 and 5 copies of HIV-1 DNA. The sensitivity of this assay meets or exceeds the sensitivity of other quantitative nucleic acid assays published to date (Desire et al., 2001; Luo et al., 2005; Ou et al., 2007; Palmer et al., 2003; Zhao et al., 2002).

A major challenge to any quantitative HIV-1 nucleic acid detection assay is performance across HIV-1 subtypes. The LNATM-Taqman assay performed well for detection of both viral RNA (from culture supernatants) and proviral DNA (from culture pellets) from HIV-1 subtypes A, B, C, D, and CRF AE. In one culture supernatant sample, the LNATM-Taqman assay yielded a result that was lower than the result from the Abbott RealTime Viral Load assay. The sequence of this virus is a perfect match to the LNATM containing probe but

showed a mismatch to the 3' non- LNATM primer (data not shown). Future iterations of the assay could utilize LNATM in the primers as well to further reduce primer template mismatches. Because the greatest diversity of HIV-1 is present in resource-limited countries where commercial VL testing is frequently impractical, the performance of the assay was assessed using DBS from HIV+ Ugandan children, who are principally infected with HIV-1 subtypes A, D, C and by A/C/D recombinants (Osmanov et al., 2002). HIV DNA was detectable in 38/38 samples and correlated well with conventional plasma VL measurements. Such correlations between HIV DNA in PBMC and DBS with plasma viral load have been described by others (Leelawiat et al., 2009; Uttayamakul et al., 2005). Furthermore, Gottlieb (Gottlieb et al., 2002) and Kostrikis (Kostrikis et al., 2002) have shown that HIV DNA levels independently predict disease progression. Together, these data suggest that HIV-1 DNA (and/or HIV-1 RNA) measurements from DBS, already shown to be useful for diagnosis and drug resistance testing (Buckton et al., 2008; Cachafeiro et al., 2009; Ikomey et al., 2009; Kerr et al., 2009), should be further explored as a marker of disease progression- risk in situations where plasma VL assays are impractical because of cost and resource limitations.

In addition to its sensitivity and ability to measure diverse HIV-1 subtypes in DBS, the LNATM-Taqman assay can be performed at a relatively low cost. Several commercial assays are capable of measuring HIV-1 VL across a range of diverse HIV-1 variants (Valasek and Repa, 2005) (Roche Amplicor), but these assays require plasma (typically requiring a "cold chain" of storage) and reagent costs of approximately US \$50.00 per sample. These requirements have restricted their use in resource-limited settings (Creek et al., 2007; Leelawiat et al., 2009; Ou et al., 2007; Sherman et al., 2005; Uttayamakul et al., 2005). Less costly PCR-based in-house assays have been described, but these do not all contend with the genetic diversity of HIV-1, especially given the complex and evolving subtype composition in many regions of the world. The reagent and assay costs of the LNATM-Taqman assay, exclusive of technician time, are approximately US \$4.00 per sample, including nucleic extraction kits, primers, LNATM-probes and Taqman reaction mixes. This is comparable to the estimated costs of other published "in-house" assays (Luo et al., 2005). While no assay is likely to accommodate every genetic variant of HIV-1, the LNATM-Taqman assay performed well across a wide range of HIV-1 subtypes yet is conducted using a single set of reagents, and can be performed at relatively low cost.

LNATM oligonucleotides have been used to advantage before for quantitative fluorescent-hydrolysis probe-based PCR assays. The high thermostability of LNATM probes has led to the development of ready-to-use 9 and 11 mer LNATM probe libraries suitable for a nearly limitless array of target sequences (Mouritzen et al., 2005). Many assays take advantage of the exaggerated destabilization of the primer-template that occurs when critical oligo positions containing LNATM bases are mismatched. This represents an excellent strategy for discriminatory PCR assays (Latorra et al., 2003) (Simeonov and Nikiforov, 2002). LNATM probes have been used in viral diagnostics for influenza, another RNA virus exhibiting sequence diversity, including typing (A/B) and subtyping (H1/H3/H5) of influenza A viruses by multiplex real-time RT-PCR assays (Suwannakarn et al., 2008) and for HCV quantitation ((Morandi et al., 2007)). Most recently, Althaus and colleagues examined the utility of a new primer probe design approach to quantitative assays to measure HIV-1. Their study also included an analysis of LNATM oligos and these authors also concluded that LNATM based probes provide advantages that can accommodate HIV diversity (Althaus et al., 2010).

5. Conclusions

LNATM chemistry can be used to overcome problems that HIV-1 diversity places on Taqman based real-time quantitation of HIV-1 RNA and DNA by permitting the use of shorter probes that are homologous to better but shorter regions of conservation in the HIV genome. A prototypic assay is described with excellent performance characteristics when tested against a range of HIV-1 subtypes, and a limit of detection of approximately 1 copy of HIV DNA and approximately 10 copies of HIV RNA. Performance was robust even with non-standard samples such as DBS. The very good correlation between HIV-1 DNA measured in patients' DBS using the LNATM-Taqman assay and plasma HIV-1 RNA measured using standard commercial assays invites the further study and development of this assay for potential applications in resource-poor settings. Finally, the high sensitivity of this assay suggests additional applications such as characterizing residual HIV-1 DNA reservoirs in patients under suppressive ART.

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FIG. 1.

Comparison of HIV-1 levels representing diverse subtypes and CRFs measured with the test assay and commercial assays for p24 antigen and HIV RNA. A. The log₁₀ HIV RNA copies/ml measured using the LNATM-Taqman assay (with RT step) plotted against log₁₀ p24 pg/ml. (Pearson) $r=0.57$, $p<0.0001$. B. The log₁₀ HIV RNA copies/ml measured using the LNATM-Taqman assay (with RT step) against log₁₀ HIV RNA copies/ml by the Abbott RealTime HIV-1 viral load assay. (Pearson) $r=0.81$, $p<0.0001$ for all values and $r=0.99$, $p<0.0001$ with censoring of the single outlier datapoint. The plotted values obtained using the LNATM-Taqman assay are the mean of duplicate assays.

FIG. 2.

Quantitation of HIV-1 proviral DNA from DBS. A: 8E5 cells spotted on filter paper, dried then followed by whole blood. B: 8E5 cells mixed with whole blood then spotted onto filter paper. Each data point is the mean $\pm$ SD of eight values obtained using blood from 2 donors (duplicate extractions from each donor with each extraction assayed in duplicate) (n=16).

FIG. 3.

Measurement and correlation of HIV-1 proviral DNA in DBS with parallel plasma viral load in HIV-1 -positive Ugandan children. Plasma RNA was measured as described in methods using a commercial RNA assay. HIV-1 DNA was measured using the LNATM-Taqman assay with total DNA extracted from DBS with result shown as the mean of two DBS from each patient. The HIV-1 DNA copy number per μ l of blood is significantly correlated with the plasma HIV-1 RNA level ($r=0.76$, and $p<0.0001$).

TABLE 1

Taqman assays for HIV-1 plasmid of different subtypes/CRFs

Subtype/CRFs	NIH catalog No.	Reagent	C _T value ^a	HIV-1 Copies. ^b
A	3605	p92UG037.1	32 ± 1.2	1×10 ³
B	114	pNL4-3	32 ± 0.8	1×10 ³
B	11414	pWT/Bal	32 ± 1.3	1×10 ³
B/C	2003	pSG3.1	33 ± 1.5	1×10 ³
C	6187	P98TZ013.10	34 ± 1.9	1×10 ³
D	4001	p94UG114.1	34 ± 1.8	1×10 ³
A/E	4007	p90CF402.1.8	33 ± 1.8	1×10 ³
F	4004	p93BR020.1	32 ± 1.2	1×10 ³
H	4005	p90CF056.1	32 ± 1.3	1×10 ³

^a C_T shown was the mean ± SD from 3 independent assays each representing the mean of results from triplicate wells (n=9).

^b HIV-1 Copy number was calculated based on plasmid size and the concentration of the plasmid by UV spectrophotometry.