A Rapid and Automated Sample-to-Result HIV Load Test for Near-Patient Application

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Background. Current viral load tests for human immunodeficiency virus (HIV) can only be performed in laboratory environments, require highly trained operators and expensive equipment, and have a turnaround time of several hours to days. The Liat HIV Quant Assay proves that such nucleic acid testing can be performed rapidly and easily, allowing application at the point of care.

Methods. The Liat Analyzer automates the entire assay, including sample preparation, amplification, and detection, in a self-contained and closed-tube system. Dynamic range, limit of detection, and subtype specificity were evaluated. Clinical samples were tested retrospectively to correlate the result of this assay with those of commercial HIV load assays.

Results. The Liat assay demonstrated linearity of 6 logs ($R^2, 0.98$) and a limit of detection of 57 copies/mL. HIV-1 group M (clades A–H), group O, and HIV-2 were detected. Testing of clinical samples showed a high degree of concordance between the copy numbers detected by the Liat assay and those detected by the Siemens and Roche assays (92.0% and 88% correlation coefficient of the log copy number, respectively). The time from sample collection to result was 88 min.

Conclusions. Results suggest that the Liat HIV Quant Assay has performance equivalent to that of commercial HIV load assays and significantly reduces assay time, simplifies test operation, and provides biocontainment to allow operation in nonlaboratory settings.

Human immunodeficiency virus type 1 (HIV-1) load quantitation has been shown to be a critical predictor for HIV disease progression and clinical outcome. Tarwater et al [1] found that an HIV RNA level >1000 copies/mL attained after highly active antiretroviral therapy initiation was a strong predictor of the rate of subsequent CD4+ T cell count decreases and of AIDS development in patients. Likewise, analysis of data from antiretroviral treatment trials demonstrated that the risk of developing an AIDS-defining illness or death was reduced proportionally to the magnitude of the reduction of the HIV-1 RNA level; this finding suggests that maintaining maximal suppression of plasma HIV RNA is a rigorous benchmark for assessing the efficacy of antiretroviral therapy regimens [2–4] and that plasma viral load is a better predictor of progression to AIDS and death than is CD4+ T cell count.

Current clinical guidelines recommend obtaining plasma viral load measurements before initiation of therapy and repeat viral load measurement at 4–8-week intervals until the level decreases below the assay’s limit of detection [5]. Thereafter, repeat viral load testing is recommended every 3–4 months to monitor treatment efficacy. Virological failure is defined by repeated, detectable viremia after successful viral suppression or detection of viremia above an HIV load of 1000–5000 copies/mL. Because such results indicate ongoing viral replication during antiretroviral therapy, changes in drug regimen are typically indicated to prevent the selection of drug resistance mutations, which may limit future treatment options.

Because viral load monitoring has become an integral part of HIV disease management and therapeutic mon-
monitoring, multiple manufacturers have produced quantitative nucleic acid tests for HIV-1. Such tests include the Amplicor HIV-1 Monitor (version 1.5; Roche Diagnostics), the Versant HIV-1 RNA test (version 3.0; Siemens AG/Bayer), the Abbott Realtime HIV-1 assay (Abbott Laboratories), and the NucliSENS HIV-1 QT assay (bioMérieux). The tests offer varying degrees of automation. For example, Roche offers 3 Amplicor viral load monitoring assays: the Amplicor HIV-1 Monitor consists of independent steps for RNA isolation, reverse-transcription, polymerase chain reaction (RT-PCR), and detection; the COBAS Amplicor HIV-1 Monitor requires manual RNA extraction but uses the COBAS Amplicor analyzer for RT-PCR amplification and detection; and the COBAS AmpliPrep/COBAS Amplicor HIV-1 Monitor further automates the assay with the COBAS AmpliPrep for sample processing. The tests typically use 0.5–1.0 mL of plasma and have a dynamic range >5 logs. Roche’s Amplicor tests are offered in standard and ultrasensitive varieties, which have sensitivities of 400 copies/mL and 40 copies/mL, respectively [6]. The tests are offered on large high-throughput platforms that typically process 48 or 96 samples in one batch. The turnaround time varies from a few hours to 2 days.

These commercial assays are widely used in centralized laboratories for high throughput testing. The limitations of these laboratory tests for resource-limited environments are well known. In such settings and in many hospital laboratories and smaller clinical laboratories, the high assay complexity, stringent process requirements, sensitivity to contamination, high equipment cost, and availability of trained personnel make the implementation of such nucleic acid tests highly prohibitive. In addition, the assays have a long turnaround time, and thus, a result cannot be provided during the same office visit, leading to missed opportunities for appropriate therapeutic intervention. This is particularly problematic in resource-limited settings, where there is a high rate of patient loss to follow-up. Failure to access effective viral load tests and delayed changes in antiretroviral therapy regimens can rapidly lead to virological and/or immunological failure and to clinical progression.

Currently, the World Health Organization (WHO) does not recommend the routine use of HIV load testing to assist clinical decisions in resource-constrained settings, specifically because of its cost and complexity [7]. However, the WHO recognizes that, by relying solely on clinical and CD4+ T cell count criteria, treatment failure will be recognized later in the developing world, thus leading to greater opportunity for drug resistance to emerge before a change in treatment regimen and a higher possibility of developing drug class cross-resistance. The WHO further recommends better methods of determining viral load in resource-limited settings to allow this treatment monitoring method to be used more widely.

Here, we describe an automated, rapid, easy-to-use, and sensitive assay for the quantitation of HIV RNA in plasma samples that is based on the novel lab-in-a-tube (Liat) technology platform. With the Liat Analyzer (IQuum), the Liat HIV Quant Assay integrates raw sample processing and detection, including target enrichment, inhibitor removal, nucleic acid extraction, reverse transcription, and real-time PCR, in a single closed-tube format and provides an interpreted result in 88 min. The performance characteristics of the Liat assay were evaluated using spiked samples. Clinical sample testing was also conducted to compare the performance of the Liat assay with that of the commercial HIV quantitation assays.

**MATERIALS AND METHODS**

**Liat Analyzer and Liat Tube.** The Liat platform comprises 2 components, the Liat Analyzer and the Liat Tube (IQuum). With the Liat system, an operator can perform sophisticated nucleic acid testing in 3 simple steps: (1) loading a raw sample into the Liat Tube, (2) scanning the tube barcode to identify the test and sample, and (3) inserting the Liat Tube into the Liat Analyzer (Figure 1). By enabling the random access pro-
cessing of a single specimen at a time, the Liat Analyzer automates all nucleic acid test processes, including reagent preparation, target enrichment, inhibitor removal, nucleic acid extraction, amplification, and real-time detection. The Liat Tube uses a pencil-sized flexible tube as a sample vessel and contains all assay reagents prepacked in the tube segments in unit-dose volumes separated by peelable seals in the order in which they are used in a given assay. In the Liat Analyzer, multiple sample processors are aligned perpendicular to the Liat Tube; and an actuator to compress the Liat Tube to manipulate the sample, to sequentially move the sample from one segment to another, and to burst peelable seals to selectively release reagents from tube segments. An embedded microprocessor controls the action of these sample processors to enable the system to perform all required assay processes in the Liat Tube (Figure 2). The internal optical system provides 6 independent detection channels for real-time monitoring, allowing for multiplex detection in each test.

The modular architecture and fluid processing capabilities of the Liat systems readily allows the adaptation of the gold standard nucleic acid test chemistries typically used in the laboratory. The system not only automates such assay chemistries from the sample to result while maintaining the quality of results, but also significantly simplifies test operations and reduces the turnaround time. Therefore, the Liat Analyzer can be operated in a nonlaboratory environment, such as a physician’s office.

**Liat HIV Quant Assay chemistry.** In collaboration with a major diagnostic company, we adapted its commercially available HIV assay chemistry to the Liat system. This assay uses chaotropic lysis and magnetic beads–based technology, to isolate viral RNA from plasma samples, and real-time RT-PCR, to detect HIV RNA. An armored RNA was provided as an internal control. The armored RNA comprised an encapsulated RNA sequence with a unique internal control probe–binding site flanked by the HIV-1 primer binding sites and, thus, serve as the competitive amplification template for HIV target quantitation. An HIV-1 armored RNA–positive control comprising an assembly of a specific HIV-1 RNA sequence and viral coat proteins in pseudoviral particles was also provided.

A unit dose of assay reagents was packed into segments of the Liat HIV assay tube in the following order: internal control

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**Figure 2.** Sample processing in the Liat Tube. The flexible Liat Tube contains reagent package in tube segments and separated by peelable seals. The Liat Analyzer comprises actuators and clamps, which compress tube segments in coordinated manner, and blocks, which are heated (gray shading) to incubate the sample. A simplified 7-actuator system is shown to illustrate the sample processing principle. Magnets and photometer are also shown. A, The Liat Tube is inserted in the Liat Analyzer. B, A volume adjustment step removes excess sample. A lysis buffer, stored in the segment 2, is released by the actuator of the Liat Analyzer, compressing the flexible tube segment to open the peelable seal. C, After a reagent is released, it can be mixed with the sample by alternatively compressing 2 adjacent segments of the Liat Tube. The nucleic acids released by the lysis buffer are captured on the surface of silica-coated magnetic beads. D, The magnetic beads are then captured by a magnetic field, and the lysis solution is transferred to a waste chamber in the cap of the Liat Tube. E and F, The magnetic beads are washed in 2 different wash buffers. The wash process resuspends the beads in the wash solution, immobilizes the beads, and transfers the solution to a waste chamber to remove polymerase chain reaction (PCR) inhibitors. Nucleic acids are then eluted from the beads by mixing the beads with elution buffer. G, The eluate is transferred to the segment containing PCR reagents. H and I, A rapid PCR is conducted by alternatively moving the reaction mixture between 2 different temperature zones. By using real-time detection chemistries, target nucleic acids are detected by the 6-channel photometer module when the exponential increase in fluorescence exceeds a cycle number crossing threshold (Ct) during PCR. Quantitation is performed against a known quantity of internal competitive control, which is processed along with the sample, amplified in the same tube as the HIV target, and detected in a different fluorescence channel of the photometer module as the HIV target.
Figure 3. The Liat HIV Quant Assay amplification plot. The assay was run as triplicate with 1000 copies/mL of HIV-1 armored RNA and 125 copies/mL of internal control armored RNA. Amplification plots of the HIV fluorescent signal by cycle number are shown as solid markers, and corresponding amplification plots of internal control fluorescent signal by cycle number are shown as open markers. Marker style represents the same run.

Figure 4. Linearity of the Liat HIV Quant Assay. A dilution series of HIV-1 armored RNA (range, 100–1 × 10^8 copies/mL) in plasma samples were tested in the presence of 125 copies/mL internal control armored RNA. With use of linear regression, an equation y = −3.53x + 43.9 was derived, in which y represents the crossing threshold (Ct) and x represents log_{10} HIV copies/mL (R^2, 0.996).

Liat assay operation. Two hundred microliters of plasma was directly added to a Liat HIV assay tube. The tube was then capped and inserted into the Liat Analyzer. The Liat Analyzer automatically conducted all sample preparation, amplification, and detection steps without any user intervention.

Sample preparation was performed by first mixing the plasma sample with a chaotropic lysis and/or binding buffer, proteinase K, and magnetic glass beads. After incubation, magnetic glass beads in the mixture were captured with a magnet in the Liat Analyzer, and 2 washing steps were performed. Nucleic acid was eluted, and the eluate was transferred to the RT-PCR segment, where it was mixed with RT-PCR reagents.

Amplification and detection were performed using uracil-DNA glycosylase inactivation, reverse transcription, and PCR amplification. All these processes were conducted automatically in the closed Liat Tube by the Liat Analyzer. While running the assay, the Liat Analyzer indicated each sample processing step and, when selected, displayed 2 real-time PCR curves for HIV and internal control detection on its touch screen. The remaining assay time was also indicated on the screen in real time. Turnaround time for all steps, from tube insertion to result reporting, was 88 min.

Linearity and dynamic range. Samples were prepared by serial dilution of HIV-1 armored RNA–positive control in normal human plasma (SeraCare Life Sciences). Three replicates for each concentration over a range of 6 log concentrations (18 total replicates) were tested over 3 days. Negative normal human plasma samples were also tested. The efficiency of the assay was calculated using the following formula: efficiency = ((10^{−1/slope}) − 1), with efficiency given as a percentage. Linear regression was used to obtain the standard curve for HIV-1 RNA quantitation.

Limit of detection. A preliminary limit of detection was determined using diluted HIV-1 armored RNA–positive control in normal human plasma samples at 0, 50, 63, 78, 125, and 250 copies/mL with 8, 8, 9, 5, 5, and 4 replicates, respectively. Three lots of Liat HIV assay tubes and 3 Liat Analyzers were used over 3 days to conduct the limit of detection test. SPSS software (SPSS) was used to determine the limit of detection at the 95% confidence level. The determined limit of detection was further confirmed by testing the samples with known viral loads from the SeraCare HIV RNA Linearity Panel (PRD801).

Subtype specificity. Subtype Infectivity Panel (PRD320; SeraCare Life Sciences) was used to determine the subtype specificity of the Liat HIV Quant Assay. This panel was composed of highly characterized, cultured HIV isolates from different geographic regions, including isolates from HIV-1 group M (clades A–H and 1 circulating form), group O, and HIV-2. Each subtype was tested after diluting with normal human plasma (SeraCare Life Sciences) in duplicate.

Retrospective clinical HIV samples. A total of 20 positive and 5 negative clinical plasma samples were tested. Twenty positive samples were provided by Dr. Chris Pilcher from HIV/AIDS Division, San Francisco General Hospital, University of California, San Francisco. The samples were collected in the...
Table 1. Limit of Detection of Liat HIV Quant Assay

<table>
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<tr>
<th>HIV-1 armored RNA level, copies/mL</th>
<th>No. of replicates</th>
<th>No. of replicates detected</th>
<th>Hit rate, %</th>
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**NOTE.** Data were combined from 3 lots of reagents for 3 days and with 3 machines. The analytical limit of detection at a 95% detection rate was determined to be 57 copies/mL of HIV-1 armored RNA with use of SPSS probit analysis.

United States during the period 2000–2001 and were originally tested within 6 h after blood samples were obtained with use of the Roche COBAS Amplicor Ultrasensitive HIV-1 test and/or the Siemens Versant HIV-1 RNA bDNA test (version 3.0). The range of HIV RNA loads in the samples was $1 \times 10^2$–$1 \times 10^3$ copies/mL. After testing, the samples were stored at $-80^\circ$C. These archived plasma samples were sent to IQuum in 2008 at a volume of $\sim 200 \mu$L and were tested using the Liat HIV Quant Assay. IQuum was blinded to the original test results during the testing. Negative samples were obtained from SeraCare.

**RESULTS**

Figure 3 shows real-time PCR curves for 3 runs of the Liat HIV Quant Assay on samples with an HIV load of 1000 copies/mL. Both HIV and internal control real-time PCR curves showed amplification reactions, resulting in a mean crossing threshold (Ct) value (± standard deviation) of $33.7 \pm 0.5$ for HIV and $34.9 \pm 0.7$ for internal control. The total assay time was $\sim 88$ min.

The linearity and the standard curve of the assay were determined using serial dilutions of HIV-1 armored RNA of $1 \times 10^{-5}$–$1 \times 10^2$ copies/mL. All negative control runs were detected as negative. A total of 21 replicates of the dilutions were detected and included in the standard curve (Figure 4). The Liat HIV Quant Assay was shown to be linear over the 6 log dynamic range tested, with a correlation coefficient of 0.996. The efficiency of the assay was calculated as 92%.

To estimate the analytical limit of detection of the Liat HIV Quant Assay, normal human plasma samples spiked with HIV-1 armored RNA in the range of 50–250 copies/mL were tested (Table 1). We also tested negative human plasma samples from 5 healthy individuals who were negative for HIV RNA and antibodies. At an input amount of 63 copies/mL of HIV-1 armored RNA, 100% of reactions were detected as positive. Using SPSS probit analysis, the analytical limit of detection at a 95% detection rate was determined to be 57 copies/mL of HIV-1 armored RNA.

Detection of HIV-1 groups and subtypes was further evaluated by testing the SeraCare Subtype Infectivity Panel (PRD332). Eight HIV-1 group M isolates (clades A–H and 1 circulating form), 1 group O isolate, and 1 HIV-2 isolate were tested, and results of the Liat HIV Quant Assay were positive for all (Table 2).

Archived clinical samples were then tested retrospectively to preliminarily compare the Liat assay with other commercial quantitative nucleic acid tests. All 20 clinical samples from University of California, San Francisco, were detected as positive. In a comparison of the Liat assay results for frozen retrospective samples with commercial assay results obtained when the corresponding samples were fresh, the correlation coefficient of the log copy number between the Liat and Siemens Versant HIV-1 RNA bDNA test (version 3.0) was 0.92 and that between the Liat and Roche COBAS Amplicor HIV-1 tests was 0.88. Linear regression analysis of the log copy number detected by the Liat and COBAS assays produced a slope of 0.87 and an
Figure 5. Comparison of the Liat HIV Quant Assay with other assays, with use of 20 retrospective plasma samples from patients with HIV infection. A, Comparison with the Roche COBAS TaqMan HIV-1 Ultrasensitive test. A linear regression equation (\(y = 0.752x + 0.894\)) was derived, in which \(y\) represents \(\log_{10}\) HIV copies/mL detected by the Liat assay and \(x\) represents \(\log_{10}\) HIV copies/mL detected by the COBAS assay (\(R^2, 0.782\)). B, Comparison with the Siemens Versant HIV-1 RNA bDNA assay (version 3.0). A linear regression equation was derived (\(y = 0.941x + 0.003\)), in which \(y\) represents \(\log_{10}\) HIV copies/mL detected by the Liat assay and \(x\) represents \(\log_{10}\) HIV copies/mL detected by the Versant assay (\(R^2, 0.947\)).

intercept of 0.76 (\(R^2, 0.78\)) (Figure 5A). Likewise, for the Liat and Versant assays, regression analysis yielded a slope of 1.02 and an intercept of 0.28 (\(R^2, 0.88\)) (Figure 5B).

DISCUSSION

Our results revealed that the Liat HIV Quant Assay performed with the Liat Analyzer has comparable sensitivity and specificity to gold standard laboratory HIV assays on the market. Specificity testing further showed that the assay detects all HIV-1 group M (clades A-H), group O, and HIV-2 strains. Linearity over 6 logs (\(R^2, 0.98\)) and a limit of detection of \(\sim 57\) copies/mL was also demonstrated. The assay gave repeatable results, with a mean Ct value (± standard deviation) of 33.7 ± 0.5 (coefficient of variation, 0.7%) at an HIV RNA load of \(1 \times 10^6\) copies/mL. This limit of detection is highly comparable to that of the COBAS Amplicor HIV-1 Ultrasensitive test and the Versant HIV-1 RNA bDNA test (version 3.0), which are considered to be among the most highly sensitive tests on the market. Although these commercial assays are capable of detecting an HIV RNA load of \(\sim 40\) copies/mL [8], they use 0.7 mL and 1.0 mL of plasma, respectively, compared with the 0.2 mL used in the Liat assay. Tang et al [9] evaluated the effect of using different sample volumes on limit of detection with the Abbott Realtime HIV-1 assay. The results showed that, although the assay sensitivity was 25 copies/mL with 1.0 mL of plasma, it decreased to 119 copies/mL with 0.2 mL of plasma. This suggests that, if the Liat assay were to use the same sample volume as the COBAS and Versant assays, the limit of detection of the Liat assay would be 11–16 copies/mL.

Retrospective testing of clinical samples further showed a high degree of concordance between the IQuum Liat HIV Quant Assay and the Roche COBAS HIV-1 test and Siemens Versant HIV RNA bDNA test (version 3.0). The correlation coefficient of the log copy number detected was 0.92 between the Liat and the Versant assay and 0.88 between the Liat and the COBAS assay, whereas that between the COBAS and Versant tests was 0.94. Of the 20 samples tested, there was only one outlier result that differed by >1 log copies/mL with the COBAS assay; if this result is excluded, the correlation coefficients between the Liat and Versant assays and the Liat and COBAS assays would be 0.94 and 0.90, respectively. The COBAS and Versant assays were performed for the samples concurrently at the time of collection, and the Liat assay was performed 7–8 years later on the archived samples. Because all 3 assays were not run concurrently, we cannot directly compare the data. However, the data suggest that the Liat assay results correlate very well with those of the commercial assays.

In addition to maintaining sensitivity and specificity similar to those of the commercial assays, the Liat HIV Quant Assay simplifies the test to an “insert-and-test” process without operator intervention or the use of other laboratory equipment. All processing steps occur in the self-contained Liat Tube, which provides closed containment to minimize amplicon cross-contamination and reduces the infectious material risks to the operator. Furthermore, the integrated process controls and built-in self-corrective and error diagnostics functions of the Liat system decrease the possibility of an erroneous result when operated by minimally trained users.

Of equal significance, the time to result for the Liat HIV Quant Assay is only 88 min. To our knowledge, there is no quantitative nucleic acid test on the market today that can
match this turnaround time. Versant and COBAS assay times are 7 h and 2 days, respectively. The fastest Food and Drug Administration–approved HIV system is perhaps the Abbott RealTime HIV-1 test, which has a time to result of ~5 h—still >3 times longer than that of the Liat assay.

The Liat HIV Quant Assay has shown the potential of overcoming the limitations of current HIV tests and providing viral load tests to point-of-care and resource-limited settings. The sample-to-result automation, ease of use, biocontainment, and fail-safe design make the Liat system ideally suitable for use by minimally trained operators in nonlaboratory environments. The random access, rapid turnaround time, and on-site testing capability allow the patients to wait at the clinic while testing is performed, thus providing single-visit viral load monitoring and timely therapeutic intervention. For point-of-care settings, such a test will provide the physician with immediate actionable information to make critical treatment decisions more efficiently. Likewise, patients will benefit from the greater convenience of single-visit therapeutic monitoring, especially considering the number of repeat tests required. Ongoing developments to adapt the assay to whole blood samples and to enable the Liat Analyzer to run on battery power will further enhance the ability to use the test in resource-limited settings. Indeed, we expect that the Liat assay will enable viral load testing to be performed easily, reliably, and at low capital cost, thus enabling its wider use to avoid drug resistance, to slow disease progression, and to affect better patient outcomes.

Acknowledgments

We thank Dr Chris Pilcher for kindly providing the clinical samples.

References