Abstract

- **Objective:** To compare HIV-1 RNA levels obtained with reverse transcriptase polymerase chain reaction (RT-PCR) assay and branched DNA (bDNA) assay.
- **Design:** Retrospective chart review.
- **Setting and participants:** 194 HIV+ patients who had an HIV viral load measured by bDNA and by RT-PCR.
- **Results:** 127 (65.5%) had HIV RNA detectable by RT-PCR and bDNA. 53 (27.3%) had undetectable HIV RNA by both assays. 10 (5.1%) had an undetectable HIV RNA level by bDNA but a detectable level by RT-PCR, and 4 (2.1%) had an undetectable HIV RNA level by RT-PCR but a detectable level by bDNA. There was no statistically significant difference between the 2 assays in detecting viral copies (McNemar test for paired samples, 3.27; \( P = 0.0707 \)). There was good correlation between the assays with a Pearson correlation of 0.97 (\( P < 0.001 \)) and a Spearman rank correlation of 0.96 (\( P < 0.001 \)). 2 of the 194 patients had additional testing done. Clinical decision making was not affected by the change from RT-PCR to bDNA assay. A Bland-Altman analysis of the 127 patients with detectable viral copies by both assays showed RT-PCR assay had a mean bias of 0.321 log\(_{10}\) viral copies/mL with limits of agreement of −0.452 to +1.098 log\(_{10}\) viral copies/mL.
- **Conclusion:** Switching from RT-PCR HIV viral load assay to bDNA assay did not affect clinical decision making and led to cost savings associated with HIV RNA testing. The 2 assays cannot be used interchangeably, as the RT-PCR gives higher viral load copies than the bDNA.

Since the mid 1990s, quantitative HIV-1 RNA testing in plasma has become the standard of care in predicting progression of HIV disease and for the therapeutic monitoring of individuals on antiretroviral drug treatment regimens [1,2]. According to the U.S. Department of Health and Human Services (DHHS), the objective of antiretroviral therapy (ART) is maximal suppression of viral replication to levels below the limit of detection by a sensitive viral load assay [1]. Viral load is a useful biologic marker for disease progression, and reducing viral replication is associated with improved immune function and a decrease in opportunistic infections and mortality. It is believed that suppression of plasma viral load to the lowest possible levels is important for prevention of viral rebound and development of HIV drug resistance [1–3].

There are currently 3 U.S. Food and Drug Administration–approved commercially available assays for the detection and quantitation HIV-1 RNA from plasma samples. These assays are:

1. The Amplicor HIV Monitor Test, version 1.5 (Roche Diagnostics), which uses reverse transcriptase polymerase chain reaction (RT-PCR) to quantitate HIV-1 RNA. This test is available as a standard and ultrasensitive assay. The standard assay has a lower limit of detection of 400 copies/mL and an upper limit of 750,000 copies/mL. The ultrasensitive assay has a lower limit of detection of 50 copies/mL and an upper limit of detection of 100,000 copies/mL [4].
2. Versant HIV RNA 3.0 Assay (Siemens) is a branched DNA (bDNA) assay. The bDNA test detects HIV RNA in plasma by hybridization and signal amplification. The lower limit of detection is 75 copies/mL and the upper limit of detection is 500,000 copies/mL [5–7].
3. The NucliSens HIV RNA QT (BioMerieux) is a nucleic acid sequence–based amplification assay. The lower limit of detection is 80 copies/mL and the upper limit of detection is 8,000,000 copies/mL [8].

Although the DHHS guidelines do not recommend a specific HIV viral load assay, the guideline recommendations are based on studies in which most patients had HIV RNA measured by RT-PCR [9,10]. There are differences in the HIV-1 RNA viral load assays that could have potential

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clinical implications. The RT-PCR assay has a lower limit of detection of 50 copies/mL versus 75 copies/mL with bDNA. Within the detectable range of the RT-PCR assay, HIV viral load measurements made by RT-PCR are reported to be as much as 2 times higher than measurements made by bDNA [4,5]. A subset of patients may have HIV-1 RNA levels between the lower limits of the 2 assays (> 50 and < 75 copies/mL). One might expect that the assay with the lower limit of detection (RT-PCR) would be more likely to pick up these cases. This may lead to a change in HIV therapy. Furthermore, DHHS guidelines recommend different treatment courses based on a viral load measure greater than 100,000 copies/mL. If the bDNA assay gives viral load measurements that are consistently lower than measurements made by RT-PCR, then therapy may be delayed with bDNA results when it would have been initiated with RT-PCR results. The only previous studies comparing bDNA to RT-PCR in clinical settings found good correlation between bDNA and RT-PCR, but these studies did not look at clinical endpoints [7,11]. In addition, 1 of these studies compared the standard RT-PCR assay, with a lower limit of detection of 400 copies/mL, to bDNA (lower limit of detection, 75 copies/mL) [11].

In January 2007, we began measuring HIV-1 RNA levels using bDNA assay. Prior to that, our HIV RNA levels were done by an outside laboratory using an RT-PCR assay. The primary motive in making this change was to reduce hospital costs associated with sending out HIV-1 RNA tests [12]. Although financial considerations were the initial motive for the change, it was felt that there were several advantages to making this switch, including a faster turnaround time to obtaining results, less laboratory handling and processing, and direct entry of results into the hospital’s electronic medical records. Furthermore, it was felt that there would be little or no clinical impact of this switch. Nevertheless, because of the inherent differences in the assays, our laboratory agreed to do paired testing on samples during the transition phase. This paired testing allowed us to establish a new baseline (“rebaseline”) HIV RNA measure for our patients using bDNA.

The goal of this study was to compare RT-PCR viral load measurements with bDNA viral load measurements and to determine if discrepancies between RT-PCR and bDNA results lead to changes in clinical care of patients and resource utilization.

Methods

A retrospective chart review of patients seen at the Hospital of Saint Raphael HIV clinic between January and July 2007 who had an HIV viral load measured by bDNA (Versant HIV-1 RNA 3.0 Assay, Siemens) and by RT-PCR (Amplicor HIV Monitor Test, version 1.5, Roche Diagnostics) on the same blood sample (rebaseline) was done. Patients seen between January and July 2007 who had a bDNA assay ordered had a portion of the same blood sample sent to an outside laboratory for RT-PCR. One rebaseline sample was sent per patient. If the patient had 2 bDNA assays ordered during this transition period, only the first sample was sent for rebaseline. We did not perform a rebaseline RT-PCR on patients who had multiple sequential undetectable RT-PCR and whose first bDNA was also undetectable. Six months or more after the rebaselined assays were done, charts were reviewed to evaluate the clinical impact of the change from RT-PCR to bDNA HIV RNA assays.

A brief description of the viral load assay methods follows. For the bDNA assay, viral RNA is released from the specimen and captured in microwells. The RNA is then hybridized to multiple DNA probes that recognize multiple sequences spanning almost the entire length of the 2700 base pair HIV pol gene. The probes are derived from multiple HIV subtypes. A signal probe is added, and the signal is amplified and detected. The viral load is calculated using a standard curve. For the RT-PCR assay, viral RNA is extracted from the patient specimen. Using a single probe and 2 primers, RT-PCR amplifies a 142 base pair conserved region of the HIV gag gene, derived from HIV subtype B. The amplified DNA is detected, and the viral load is calculated using a standard curve.

All rebaselined viral load assays were reviewed to compare HIV RNA level obtained by the 2 tests. Quantitative differences in RNA levels were determined. A difference of 0.5 log_{10} viral copies/mL between the 2 assays was considered clinically significant [1]. We also compared results of rebaselined assays in which viral RNA was detectable by 1 assay but not by the other assay. Medical records of patients with a rebaselined HIV viral load were reviewed and the following data was collected: demographics (date of birth, sex, race, HIV risk factor), CD4 measure from time of rebaseline until 1 January 2008, HIV viral load measures from time of rebaseline until 1 January 2008, and ART at time of rebaseline through 1 January 2008. Clinic charts were also reviewed for clinical impressions to determine if additional HIV RNA or CD4 tests were ordered as a result of the change from RT-PCR to bDNA testing, and to determine if discrepancies in the 2 test results led to changes in clinical management of patients. Paired HIV RNA measures were categorized into 4 groups: (A) HIV RNA was detectable by both assays, (B) HIV RNA was undetectable by RT-PCR and detectable by bDNA, (C) HIV RNA was undetectable by bDNA and detectable by RT-PCR, and (D) HIV RNA was undetectable by both assays.

Statistical Analysis

Assuming a clinically significant difference of 0.5 log_{10} viral copies/mL between the 2 assays, a level of significance of
5%, and a power of 80%, 102 patient samples are required at the 2-sided 5% significance level. The proportion of patients who are detectable by each test was assessed for significant differences using the McNemar test for paired samples. Because the detection limits of the assays might affect normality assumptions, both Pearson and Spearman correlation coefficients were calculated, and a regression equation with $\log_{10}$ bDNA as dependent and $\log_{10}$ RT-PCR as explanatory variable was determined. A Bland-Altman plot [13,14] of difference in $\log_{10}$ viral copies/mL of the 2 assays against the average $\log_{10}$ viral copies/mL of the 2 assays was used to explore level of agreement between the 2 assays for the 127 patients in whom both assays detected viral copies within the reportable results of the 2 assays. The primary utility of the Bland-Altman plot is the visual aid it provides, allowing for evaluation of whether correlation between the 2 assays is the same over the range of measured viral loads. If the points at 1 end of the plot are significantly further away from the mean line than at the other end, there is the suggestion that the 2 assays correlate differently at low and high viral loads. This suggestion is tested statistically with the Pitman test of difference of variances [14,15], which assesses the null hypothesis of no association between variance and mean of the 2 assay assessments. All statistical analysis was done using Intercooled STATA version 9.2 (Stata Corp, Texas Station, TX).

**Results**

194 patients had paired HIV RNA measures obtained. Of these, 62% were male, 55% were African American, 28% were white, and 17% were Hispanic. HIV risk factors were heterosexual contact (43%), male sex with men (20%), intravenous drug use (28%), unknown (6%), and transfusion (1%).

127 patients (65.5%) had detectable HIV RNA by both RT-PCR and bDNA. 53 patients (27.3%) had undetectable HIV RNA by both assays. For the remaining patients, 10 (5.1%) had an undetectable bDNA result but a detectable RT-PCR result, and 4 (2.1%) had an undetectable RT-PCR result but a detectable bDNA result. There was no statistically significant difference between the 2 assays in detecting presence or absence of viral copies (Table). McNemar test for paired samples gave a chi-square value of 3.27 ($P = 0.07$).

**Comparison of Viral Load Values by the 2 Assays**

The viral load in all samples ranged from 2 to 429,540 viral copies/mL for the bDNA and < 50 to 750,000 viral copies/mL for the RT-PCR assays. The bDNA assay detected viral copies/mL that were below the cut-off limit of the test (< 75 copies/mL) in 63 patients, with estimated values ranging from 2 to 70 viral copies/mL.

There was good correlation between the 2 assays with a Pearson correlation of 0.97 ($P < 0.001$) and a Spearman rank correlation of 0.96 ($P < 0.001$) (Figure 1). The Deming regression equation for $\log_{10}$ bDNA viral copies/mL with $\log_{10}$ RT-PCR viral copies/mL as predictor is given by $\log_{10}$ bDNA $= 0.8267 \times \log_{10}$ RT-PCR + 0.3987. The slope is 0.8267 with a standard error of 0.0163 ($P < 0.05$).

The correlation coefficients measure the strength of the association between these 2 assays, but the Bland-Altman plot measures the agreement between them. Figure 2 shows the Bland-Altman plot of the difference in the $\log_{10}$ bDNA viral copies/mL versus the average assay results ($\log_{10}$ bDNA/2) of the 127 patients who had detectable viral loads within the limits of detection of the 2 assays. The mean difference (bias) was 0.321 log$_{10}$ viral copies/mL (95% confidence interval [CI], 0.253 – 0.392 log$_{10}$ viral copies/mL). The lower limit of agreement was -0.452 log$_{10}$ viral copies/mL (95% CI, -0.571 to -0.333 log$_{10}$ viral copies/mL) and the upper limit of agreement was 1.098 log$_{10}$ viral copies/mL (95% CI, 0.979–1.217 log$_{10}$ viral copies/mL). Spearman’s rho was 0.2141, suggesting that the difference between the 2 assays increased as the values increased. The Pitman test of difference in variance was not significant ($r = 0.169$, $P = 0.057$).

The range of the difference in $\log_{10}$ viral copies/mL between the 2 assays was -0.429 to 3.643. The 3.643 log$_{10}$ viral copies/mL was an outlier, the next largest difference being 1.293 log$_{10}$ viral copies/mL. When the Bland-Altman plot was repeated with the outlier removed (graph and data not shown), the mean difference (bias) was 0.295 log$_{10}$ viral copies/mL (95% CI, 0.249–0.342 log$_{10}$ viral copies/mL), and the upper and lower limits of agreement respectively were 0.811 log$_{10}$ viral copies/mL (95% CI, 0.732–0.892 log$_{10}$ viral copies/mL) and -0.221 log$_{10}$ viral copies/mL (95% CI, -0.3 to -0.142 log$_{10}$ viral copies/mL). The boundaries of the limits of agreement again exceed the clinically significant number of 0.5 log$_{10}$ viral copies/mL.

**Clinical Decisions**

Two (1.03%) of the 194 patients had additional testing based on rebaseline data. In 1 case, bDNA detected 77 copies/mL and RT-PCR gave a negative result. In the other case, bDNA detected 250 copies/mL and RT-PCR gave a negative result. No additional testing was done for these cases.

**Table. HIV Viral DNA Detection by the 2 Assays**

<table>
<thead>
<tr>
<th></th>
<th>Detectable</th>
<th>Undetectable</th>
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</thead>
<tbody>
<tr>
<td>bDNA (LLD, 75 copies/mL)</td>
<td>127 (65.5%)</td>
<td>4 (2.1%)</td>
</tr>
<tr>
<td>RT-PCR (LLD, 50 copies/mL)</td>
<td>10 (5.1%)</td>
<td>53 (27.3%)</td>
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Note: McNemar's $\chi^2 = 3.27$ ($P = 0.0707$). LLD = lower limit of detection.
and the RT-PCR showed < 50 copies/mL. An additional bDNA test was obtained that measured < 75 copies/mL. No change in ART was made. At last follow-up, the patient’s HIV RNA was < 75 copies/mL. The second patient had a rebaseline HIV RNA level of < 75 copies/mL by bDNA and 151 copies/mL by RT-PCR. An additional bDNA measure was obtained 4 weeks after rebaseline. The bDNA HIV RNA measured at 5085 copies/mL. ART was not changed. Six months later the bDNA HIV RNA measured at < 75 copies/mL. ART was not changed. Six months later the bDNA HIV RNA level of < 75 copies/mL and RT-PCR level of 103 copies/mL. This patient was lost to follow-up for 10 months at which point he was off ART and his bDNA HIV RNA level subsequently measured > 100,000 copies/mL.

At rebaseline, 134 (69.1%) patients were on ART. Of these, 84 (62.7%) had no change in ART. For the remaining 50 patients who were on ART at rebaseline, 42 (31.3%) had their ART changed, 5 (3.7%) stopped ART, and 3 (2.2%) had no follow-up visits. The reasons for changing ART were drug resistance (19 patients), adverse drug effects (16 patients), drug interaction (1 patient), failed therapy with high HIV RNA levels (5 patients), low CD4 count (2 patients), poor adherence (2 patients), pregnancy (1 patient), and unknown (2 patients). The 5 patients who stopped ART all stopped therapy on their own accord. Fifty-nine (30.4%) patients were not on ART at rebaseline. One patient’s ART status was unknown. Of the 59 patients not on ART at rebaseline, 27 (45.8%) patients started ART. Reasons for starting ART were meeting the DHHS recommendations (13 patients), patient ready (7 patients), improved adherence (10 patients), restarting after treatment interruptions (1 patient), and unknown (1 patient). In no cases were ART changes made as a result of discrepancies between RT-PCR and bDNA HIV RNA results.

Discussion

In 2007, our hospital laboratory changed its HIV RNA assay method from RT-PCR (Amplicor HIV Monitor Test, version 1.5, Roche Diagnostics) to bDNA (Versant HIV-1 RNA 3.0 Assay, Siemens). During the transition phase, 194 paired tests were done on clinical samples. We found strong correlation between bDNA and RT-PCR and an excellent discriminatory ability of the bDNA assay to detect presence or absence of HIV RNA viral copies. There was not a significant difference between the assays in detecting HIV RNA. We found that the mean HIV RNA level was on average 0.321 log_{10} viral copies lower with the bDNA assay compared with the RT-PCR assay. This result is consistent with prior studies comparing the 2 assays [4,5]. Although previous studies have shown that on average bDNA measurements are one third lower than RT-PCR measurements, this is not consistent across all viral load measures nor is this difference likely to be clinically significant. In our study, the limits of agreement from the Bland-Altman plot show that the difference in the log_{10} viral copies/mL between the assays was greater than 0.5 log_{10} and the 2 assays cannot be used interchangeably. When switching from 1 assay to another, the clinician must be aware of the differences in the assay measurements.

Despite the difference in absolute HIV RNA levels detected, we did not find a clinical impact. None of the 74 patients
who had a change in ART did so as a result of discrepancies between the RT-PCR and bDNA results. Although bDNA consistently detected lower HIV RNA levels than RT-PCR, this did not impact clinical decision making. At the time that our laboratory started using the bDNA assay, the DHHS guidelines recommended considering starting ART if the HIV RNA level was greater than 100,000 copies/mL. Since then, the DHHS guidelines have been updated (December 2007 and January 2008). These guidelines no longer recommend different treatment courses based on HIV RNA levels [1]. These updated guidelines are consistent with the International AIDS Society guidelines [2] and reflect our clinical practice at the time.

Only 2 patients had additional testing as a result of the transition from the RT-PCR to bDNA. One other patient was referred for additional testing but never followed through with the testing. In neither case did the additional testing lead to changes in clinical decision making.

Our primary motive for switching from RT-PCR to bDNA HIV RNA testing was to reduce hospital costs associated with HIV-1 RNA testing [12]. At our hospital, the cost per reportable test result for bDNA is $57. The cost per reportable test result for RT-PCR, if performed at our hospital, is $98 (personal communication). This price includes the cost of reagents but does not include technician time, disposables, or biohazard waste management. Elbeik et al [12] did a cost comparison between RT-PCR (Amplicor HIV Monitor Test, version 1.5, Roche Diagnostics) and bDNA (Versant HIV RNA 3.0 Assay, Siemens). In their study, the cost of reagents per reportable test result was similar for the 2 assays. The costs of labor, disposables, and biohazard waste handling were significantly less for bDNA than for RT-PCR. Compared with RT-PCR, bDNA showed 60% less labor costs, 30% less disposables costs, and 50% less biohazard waste handling costs. The result was an overall lower cost of performing bDNA [12].

In addition to the cost associated with the test, there were several advantages to making the switch from RT-PCR (a send out) to in-house bDNA, including less laboratory handling and processing, faster turnaround time to obtain results, no need to reflex from a standard to an ultrasensitive assay as for RT-PCR, and direct entry of results into the computerized medical records. We have now eliminated the added expense associated with sending specimens for RT-PCR to an outside laboratory. Preparation of samples for packing and shipping is expensive, time-consuming, requires additional laboratory personnel time, lengthens turnaround time for laboratory results, and allows for additional opportunities for laboratory errors. Since we began doing bDNA HIV viral load testing at the hospital, the turnaround time (time from ordering the test to obtaining results) has become more predictable. At our hospital, the bDNA test is run every Wednesday. Results are predictably back and

Figure 2. Bland-Altman plot of difference versus mean of the 2 assays. CI = confidence interval; LOA = limits of agreement (mean difference ± 1.96 SD).
entered into the electronic medical record by Thursday afternoon. Previously, when we were sending HIV viral load tests to an outside laboratory, the turnaround time varied from 5 to 10 days. Finally, direct entry of results into the hospital computerized medical records has allowed for easier access to and tracking of results.

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