

Switch from Signal Amplification to COBAS® AmpliPrep/COBAS® TaqMan 48: Is There a Need to Re-Baseline?

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ABSTRACT

Background: Accurate quantitation of plasma Human immunodeficiency virus-1 (HIV) RNA levels is required for clinical management of HIV-1-infected patients. Several assays are used to quantify HIV RNA, and prior to implementing a change in viral load method, continuity in reported values is addressed by the laboratory and communicated to clinicians.

Methods: We initially compared COBAS® AmpliPrep-COBAS® TaqMan 48 (TaqMan) v.1.0 (Roche) to prior methodology, branched-chain DNA (bDNA) v.3.0 (Siemens) to determine if establishment of new patient baselines were necessary. Study data from 81 specimens run by both assays were compared using nonparametric tests, e.g., Wilcoxon signed-rank, Spearman. Subsets for comparison included only those that fell into overlapping ranges for both assays.

Results: The methods correlated (Spearman correlation, $r_s = 0.91$), but TaqMan values were significantly higher than those of bDNA ($p < 0.0001$). Based upon this, new baselines ($n=768$) collected over 6-months were determined by running bDNA on all specimens that could be quantitated by TaqMan ($n=308$). Of those with sufficient quantity to establish new baselines with the TaqMan, 308 and 272 were quantifiable by TaqMan and bDNA, respectively. Parallel data within overlapping ranges ($n=262$) were again highly correlated ($r_s = 0.89$), but still were statistically different ($p = 0.0044$). Additional analyses for regression and pair differences by range were run on combined (study and parallel) data.

Conclusion: Our data demonstrate non-equivalence in HIV-1 RNA values of TaqMan v.1.0 as compared to bDNA v.3.0, and that new baselines for HIV viral load RNA HIV-1 should be re-established. New baselines for those patients missed by parallel testing can be calculated using regression analysis.

INDEX TERMS: Viral Load, HIV, polymerase chain

reaction, branched DNA signal amplification assay, baseline survey

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INTRODUCTION

Human immunodeficiency virus-1 (HIV) is major cause of morbidity and mortality throughout the world. The use of reverse transcriptase polymerase chain reaction (RT PCR) to detect HIV was first described in 1988. Accurate, precise quantitation of plasma HIV RNA levels is required as it helps inform treatment initiation and therapeutic drug choices. It is used as a marker for appropriate response to therapy. Detectable viremia in a patient on therapy prompts concerns of therapeutic failure and drug resistance. Specific interventions for mother to child HIV transmission prevention such as intrapartum zidovudine and cesarean section are dictated by viral load copy numbers. In the developed world there exist a number of platforms in use for measuring viral load, such as branched-chain

DNA (bDNA), transcription mediated amplification, nucleic acid sequence-based amplification and RT PCR. The sensitivity of bDNA methods is achieved by releasing viral RNA from the specimen which is captured in microwells. The RNA is then hybridized to multiple DNA probes that recognize multiple sequences spanning almost the entire length of 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 following a series of hybridization reaction through the use of chemiluminescence technology.¹ For RT PCR assay, viral RNA is extracted from the specimen and using a single probe and 2 primers, a 142 base pair conserved region of the HIV *gag* gene derived from HIV subtype B is amplified. The amplified DNA is detected, and the viral load is calculated using a standard curve.²

Analysis of variance between specimen pairs tested in parallel by different methods usually reveals the greatest differences when comparing two platforms that differ vastly in their chemistry, e.g., the signal amplification assay, VERSANT® HIV-1 RNA 3.0 bDNA (Siemens Diagnostics, Tarrytown, NY, USA) versus a target amplification such as the real time RT PCR, marketed as COBAS® AmpliPrep-COBAS® TaqMan 48 (TaqMan) (Roche Diagnostics, Indianapolis, IN) or the Amplicor Monitor end point (conventional) RT PCR assays (Roche).³ On the other hand, one would expect less variance between the two real time RT PCR methods, even if produced by different companies.

The decision to switch HIV-1 viral load method often is based upon availability of a new instrument with better performance or automation, vendor service, poor performance of the current assay, or price. Once a new test is evaluated and verified, it must be determined if parallel testing using both methods for established patients to document a new baseline is required. Comparison data from verification studies are weighed carefully as re-defining baselines is both a great expense and disruption for everyone involved. However, although there are numerous references describing assay verification and method comparisons, few specifically address this very practical issue of the need to rebaseline.^{4,5,6,7} Some state the need for using only one assay for longitudinal patient monitoring.⁸ Certainly no consensus of when one should or should not rebaseline is available.

The Diagnostic Virology Laboratory (DVL) at Louisiana State University Health Sciences Center at Shreveport (LSUHSC-S) evaluated the TaqMan for HIV-1 viral load measurement, comparing it to the prior bDNA platform. As part of the decision to switch methods, the DVL was faced with the proposition of setting new baselines for approximately 1000 HIV-infected LSUHSC-S clinic and hospital patients. We compared COBAS® AmpliPrep-COBAS® TaqMan 48 (TaqMan) (Roche) to prior methodology, branched-chain DNA (bDNA) (VERSANT® HIV-1 RNA 3.0 bDNA, Siemens) to determine if establishment of new patient baselines were necessary. This was done both by parallel testing patients for 6 months, then by calculation using linear regression. This comparison was reviewed and approved by the IRB for ethical human research at LSUHSC-S.

Data on log₁₀ viral load measurement using the two assay methods were obtained for each patient in two non-overlapping cohorts. The measurements obtained by the two methods were correlated and within patient (pair) differences were analyzed. Nonparametric methods such as the Spearman rank correlation analysis and the Wilcoxon signed rank test were used due to the observed non-normality of the log measurements and pair differences between the two assays. Significant average pair difference indicated non-equivalence between the two assays and consequently, the need to determine new baselines for the new assay to be implemented (TaqMan). Given significant correlation between the two measurements, linear regression analysis was performed to estimate a patient's baseline TaqMan value given his bDNA value.

These findings emphasize the importance in checking carefully for differences in assays prior to instituting a new platform and what should be done when statistical differences are found. Our data demonstrate non-equivalence in HIV-1 RNA values of TaqMan v.1.0 as compared to bDNA v.3.0, and suggest need for re-baselining. However, new baselines for those patients missed by parallel testing can be calculated using regression analysis.

MATERIALS AND METHODS

COBAS® Ampliprep/COBAS® TaqMan 48 v.1.0 (Roche Diagnostics, Indianapolis, IN). This viral load method combines automated extraction and sample

preparation in the AmpliPrep with reverse transcription of RNA into double stranded DNA, amplification and detection in the TaqMan using hydrolysis probes. This target amplification method is referred to as real-time because detection occurs during amplification. The PCR cycle (denaturation, annealing and extension) number at which fluorescence becomes detectable is referred to as the T_c and is inversely proportional to the amount of target present in the sample. The test can quantitate HIV-1 RNA over the range of 48 - 10,000,000 copies/mL. (One copy of HIV-1 RNA is equivalent to 1.7 ± 0.1 International Units).⁹ All testing was performed according to manufacturer's instructions.

VERSANT® HIV-1 RNA 3.0 bDNA (Siemens Diagnostics, Tarrytown, NY, USA). This signal amplification platform, a sandwich nucleic acid hybridization assay, was run on the semi-automated 340 bDNA Analyzer. HIV-1 RNA is hybridized to a set of capture probes complementary to different regions of the *pol* gene.^{8,10,11} The detection probes are enzyme-labeled, and with the addition of substrate, a chemiluminescent signal is generated that correlates to the concentration of the target in the original specimen.

OptiQuant HIV RNA Quantitation Panel. The OptiQuant HIV RNA Quantification Panel from AcroMetrix (Benicia, CA) is a commercially-available standard containing HIV-1 RNA (genotype B). This was used to determine the intra-run (both assays) and inter-run (TaqMan only) coefficients of variation (CV), as well as instrument ranges. Ranges for these standards are provided in Table 1.

Specimens. Viral load testing was run on batched specimens collected from patients seen at the LSUHSC-S hospital and clinics. Ethylenediaminetetraacetic acid (EDTA)-whole blood was centrifuged at 3000 rpm (1500g) for 20 minutes within 4 hours of venipuncture; the plasma was then divided into aliquots and frozen at -80°C until use. No aliquot was freeze-thawed beyond this initial storage. Extra aliquots were stored for at least such time as the viral load results had been reported to the chart and the clinician had several days to review the results.

Patient Cohorts. Preliminary data required to determine the size of our study set were obtained by comparing

TaqMan HIV-1 viral loads on a convenience sample of 28 archived patient specimens, collected within the prior six months and previously quantitated for HIV-1 RNA by bDNA.

Table 1. Reproducibility of Assays^a

| STD Copies/mL (Log ₁₀) | Variable ^b | TaqMan Mean | | | bDNA Mean | | |
|--|-----------------------|-------------------|------|-------|-------------------|------|------|
| | | Log ₁₀ | SD | CV | Log ₁₀ | SD | CV |
| 95 (1.98) | Intra-run | 2.00 | 0.11 | 5.5% | 1.90 | 0.05 | 2.6% |
| | Intra-run | 2.55 | 0.04 | 1.41% | | | |
| | Inter-run | 2.28 | 0.31 | 13.6% | | | |
| 492 (2.69) | Intra-run | 2.94 | 0.01 | 3.4% | 2.63 | 0.12 | 4.6% |
| | Intra-run | 2.94 | 0.17 | 5.72 | | | |
| | Inter-run | 2.94 | 0.12 | 4.16% | | | |
| 6810 (3.83) | Intra-run | 3.93 | 0.15 | 3.8% | 3.64 | 0.04 | 1.1% |
| | Intra-run | 4.03 | 0.16 | 4.1% | | | |
| | Inter-run | 4.07 | 0.21 | 4.9% | | | |
| 77,200 (4.89) | Intra-run | 4.91 | 0.05 | 1.0% | 4.63 | 0.04 | 0.1% |
| | Intra-run | 4.78 | 0.15 | 3.1% | | | |
| | Inter-run | 4.84 | 0.13 | 2.6% | | | |
| 710,000 (5.85) | Intra-run | 5.87 | 0.05 | 0.9% | ND ^c | ND | ND |
| | Intra-run | 5.84 | 0.26 | 4.4% | | | |
| | Inter-run | 5.86 | 0.17 | 2.8% | | | |
| 6,750,000 (6.83) | Intra-run | 7.0 | 0.0 | 0% | ND ^c | ND | ND |
| | Intra-run | 6.96 | 0.06 | 0.9% | | | |
| | Inter-run | 6.98 | 0.04 | 0.6% | | | |

^aAnalysis by MiniTab 15 Stat Software. CV in % is calculated as (SD/Mean) x 100.

^bThree replicates were assayed per run on separate days. Inter-run compares assays from both days (n=6).

^cThis standard concentration is out of range for the bDNA.

Cohort A. The actual study cohort, Cohort A, used to determine significance of variation between paired samples run by both methods, was comprised of 81 specimens, including the preliminary dataset of 28, plus additional archived HIV-1-positive plasmas. For comparison of pairs and correlation analysis, all data outside the range of the reporting instrument were omitted, i.e. TaqMan values less than 48 or greater than 10 million and bDNA values less than 75 or greater than 500,000. To further reduce bias, another cohort, Trimmed Cohort A was defined by omission of all data points (obtained from either assay) that fell outside the range of the bDNA (75-500,000 copies/mL). This smaller, narrower Trimmed Cohort A had 53 specimens.

Cohort B. For purposes of determining new (TaqMan) baselines, specimens collected prospectively from established adult HIV-1 patients were measured for viremia levels. For a time span lasting 6 ½ months (mid-Dec. 2007 – July 2008), clinicians were asked to provide duplicate samples for parallel testing by both methods. The TaqMan assay was run first. If HIV quantitated (>48 copies/mL), then the bDNA was run (within a week) on a frozen aliquot of the same specimen and both values were reported to the chart. If RNA was not detected by TaqMan, no bDNA was run on that sample. Of the approximately 1000 HIV-1 patients currently served by the DVL, 869 had blood submitted during this period; 768 had sufficient plasma to run by both assays, but only 308 (40%) had detectable RNA by TaqMan (Cohort B). Of these 308 patient specimens, 262 had viral load values that were also within the range of the bDNA and could be used for non-biased comparison (Trimmed Cohort B). Cohorts A and B contained no common patients.

Statistics. To measure reproducibility, the coefficient of variation (CV) was calculated on logarithm (base 10) of viral loads using 3 replicates that were assayed at 6 different standard concentrations. Summary statistics (mean, standard deviation and median) and statistical analysis to correlate and compare TaqMan and bDNA were calculated on the \log_{10} viral load measurements in trimmed Cohorts A and B. Nonparametric methods such as the Spearman rank correlation coefficient and the Wilcoxon signed rank test were used due to the observed lack of normality of the log measurements (Shapiro-Wilk) and differences in values between the two assays. As there were no common patients in cohorts A and B, viral load data for the trimmed cohorts were combined ($n=315$) and used to determine significance of pair differences for specified ranges (Table 2) and by regression analysis to determine a formula for calculations of future HIV-1 baselines. In addition to statistically comparing \log_{10} viral load for the bDNA and TaqMan assays for the different cohorts with the Wilcoxon signed rank test, the Bland-Altman plot was constructed for each cohort to demonstrate the amount of agreement/disagreement between the two assays. Given a sample of n each of which has 2 values for log HIV RNA level (for bDNA and TaqMan), the Bland-Altman plot is a scatter plot of the differences between the two values (plotted on y-axis) against the means of the two values (plotted on the x-axis) to see

how the amount of disagreement relates to the magnitude of the measurement.

RESULTS

Observed data from the quantification panels (7 standard concentrations run in triplicate) yielded alternate trends with consistent results whereas 81% of the TaqMan results were above the mean, almost all of the bDNA results (93%) were below the mean. Table 1 shows coefficients of variation (CV's) for the two assays, calculated by dividing the standard deviation (SD) by the mean and multiplying by 100. While TaqMan has a larger CV at most of the lower concentrations, it is able to detect with small CV's viral loads at the higher concentrations which are non-detectable by bDNA.

A verification/validation study cohort of 81 specimens (Cohort A) was measured for viral load by both the TaqMan and bDNA assays. None of these samples had TaqMan RNA values greater than that of the TaqMan cutoff (10,000,000 copies/mL), whereas 2 samples measured by the TaqMan were above the upper range of bDNA (greater than 500,000). Twenty-four samples had no detectable RNA by both methods, and one sample quantitated by TaqMan (84 copies/mL), but not by bDNA (<75 copies/mL). Three samples were detectable by TaqMan at 48-75 copies/mL and undetectable by bDNA. From Cohort A, a subset of 53 specimens (Trimmed Cohort A) was formed using only those sample pairs with viral loads that fell within the range of bDNA (75 – 500,000 copies/mL) as measured by either assay. These viral load data were log-transformed and used to statistically compare the two assays. Although use of values obtained from overlapping ranges reduced the sample size, it allowed for a more accurate comparison of those numbers. The median TaqMan result was 48% higher than bDNA in this trimmed subset of Cohort A, 30,038 vs 17,221 copies/mL.

Table 2 shows significant correlation (r_s 0.92) and significant pair difference between the TaqMan and bDNA \log_{10} viral load for Trimmed Cohort A (Wilcoxon signed-rank test $p < 0.001$). Pair differences showed an average difference of 0.165 between the log viral load values for the two assays which was highly significant. The standard deviation for differences in pairs was 0.29. Twenty-five percent and 9% of the values fell outside of 2 and 3 standard deviations,

respectively. The significant average pair difference indicated non-equivalence between the two assays and consequently, the need to determine new baselines for the new platform to be implemented (TaqMan).

Table 2. Differences between TaqMan and bDNA HIV-1 RNA Viral Load Values for Paired Samples

| Trimmed Cohort | n | Pair Difference Mean \pm SD | Median | Wilcoxon p-value | Spearman R_s (p-value) |
|----------------|-----|-------------------------------|--------|------------------|--------------------------|
| A | 53 | 0.165 \pm 0.29 | 0.21 | <0.001 | 0.92 (<0.001) |
| B | 262 | 0.029 \pm 0.33 | 0.05 | <0.001 | 0.95 (<0.001) |

It was arranged by consultation with HIV clinicians that parallel testing would be performed on patients 15-years of age and above, using parallel testing of samples collected over a 6 ½ -month period. During the time frame allowed for submission of sample for determining new baselines, 753 specimens were obtained of which 308 had detectable RNA. These samples constituted Cohort B. The TaqMan median for this dataset was only 13% higher than the bDNA median. Further evaluation of Cohort B obtained by parallel testing allowed us to readdress the decision to re-baseline based on the earlier study of Cohort A. Thirty samples were below the lower limit of detection by bDNA. Eleven were between 48-75 copies/mL by TaqMan and yielded a quantitative result. Nineteen were quantifiable at >75 copies/mL but undetectable by bDNA. Of the 308 samples in Cohort B, a trimmed cohort of 262 specimens that fell within the range of bDNA was used to re-examine significance and correlation between the two assays. The median TaqMan for Trimmed Cohort B, was lower than that of the bDNA (8432 vs 8463 copies/mL, respectively). The standard deviation for differences in pairs was 0.33 (Table 2). Twenty-nine percent and 2% of the values fell outside of 2 and 3 standard deviations, respectively. Again the two assays were highly correlated (r_s 0.95), but were significantly different in viral load assay measurements (Wilcoxon signed-rank $p<0.001$); thus confirming the results from Trimmed Cohort A and the decision to re-baseline.

Combining data from both cohorts, Trimmed Cohort A added to Trimmed Cohort B, \log_{10} values for the two assays were compared further by viral load range: less than 1000, 1000-5000, 5000-10,000, 10,000-50,000, 50,000-100,000, and 100,000-500,000 copies/mL. Only at higher ranges of viral loads (above 1000 and

especially above 5000 copies/mL) were the two assays significantly different. This is illustrated on a Bland-Altman graph (Figure 1) by tighter grouping of data at high concentrations.

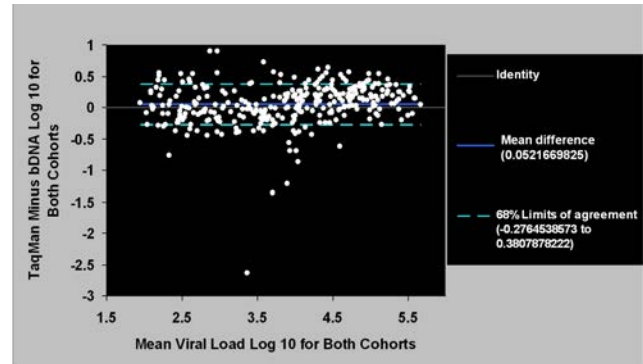


Figure 1. Bland-Altman Plot of \log_{10} difference for Combined Trimmed cohorts A and B

At least two hundred returning HIV-1 clinic patients did not have new TaqMan baseline values because they were not sampled during the time-frame allowed. However, they had previous bDNA values. Given the significant correlation ($R^2 = 0.89$, $p<0.001$) between TaqMan and bDNA \log_{10} viral load measurements, it can be assumed that TaqMan \log_{10} viral load baselines could be estimated from bDNA \log_{10} viral load baseline with linear regression analysis using the combined (trimmed) cohorts data. The fitted regression line to the plotted value can be used to estimate a missing TaqMan baseline value as:

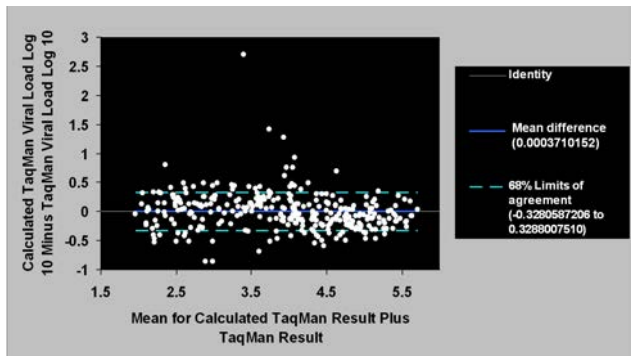
$$\text{Estimated TaqMan Baseline} = 0.006227 + 1.012 \times \text{bDNA Baseline}$$

Table 3 shows standard errors and confidence intervals for the intercept and slope of the estimated regression line. Given the highly significant regression coefficient, a patient's TaqMan baseline can be estimated confidently from his bDNA baseline. A comparison of the differences between estimated (calculated) TaqMan viral loads and actual measured TaqMan results by Bland-Altman plot is shown in Fig.2. There was no significant difference between viral loads by calculation and by measurement (Wilcoxon $p=0.298$); thus, calculated TaqMan values from the combined cohort are equivalent to measured values obtained by parallel testing.

Table 3. Parameter Estimates for the Fitted Regression Line

| Parameter | Estimate | Stand Error | 95% Confidence Interval | p-value ^a |
|-----------|----------|-------------|-------------------------|----------------------|
| Intercept | 0.00623 | 0.0784 | -0.148 to 0.1604 | 0.94 |
| Slope | 1.0119 | 0.0197 | 0.973 to 1.051 | <0.01 |

^aFor testing null hypothesis of zero value of parameter

**Figure 2.** Bland-Altman Plot of differences between Measured TaqMan and Calculated TaqMan Values.

DISCUSSION

We have shown that when bringing in a new quantitative platform with extremely different chemistry from the laboratory's current platform, an initial comparison study should be scrutinized statistically. If a statistical difference between paired results run by each method is found, any patient who is being followed clinically should be re-baselined. However, it may not be necessary to obtain extra blood samples to determine the new baseline with the new platform. It is possible to re-baseline using regression analysis to estimate the conversion of the old platform result to the new platform viral load, as long as the two assays are significantly correlated.

Precision and accuracy in HIV viral load testing are paramount and should take priority in any diagnostic laboratory for a variety of reasons. Necessity for new baselines in a cohort of established HIV-1 patients when a laboratory undergoes a platform change from signal amplification to target amplification is complicated and controversial. Since the mid-1990s, a difference in viral load of 0.5 log₁₀, or threefold change in magnitude, has been considered clinically significant and is used as guidance for interpreting sequential HIV-1 viral load specimens from individual patients.^{12,13} Thus, inter-assay plus intra-assay test variability must

remain low enough to allow for biologic variability between sequential blood draws on an individual patient, even in the event of a platform change. The proportion of variability that is biological is reported to be greater than 50% of the total variability.^{14,15,16,17,18,19} Therefore, unless the variability between assays is negligible, it is suggested that laboratories undergoing a platform change consider re-establishing baseline viremia levels for all HIV-1 patients currently being monitored.^{6,20,21,22,23,24} Others caution against changing assay methods during serial measurements.²¹

Our findings between signal and target amplification platforms are similar to those reported previously and include wide differences among results with an almost constant lower quantitation by the bDNA assay.^{21,25,26, 27} In 1998 Hodinka reported conventional RT PCR to yield results 2 to 2.5 fold greater than those obtained by an early version of bDNA.³ Such significant differences between the paired results of signal and target amplification methods are reported to fall throughout the analytical range.⁷ Others report viremia levels estimated by bDNA and conventional PCR to be statistically significant ($P < 0.001$),^{21,28,29} but like our study good correlation is found between bDNA and conventional RT-PCR with R² values of 0.97.^{21, 29}

In contrast to our average mean pair differences of 0.3 log₁₀, others report differences between the same methods, bDNA and TaqMan, to be less than 0.2 log₁₀ and in a multicenter study these differences were considered non-significant.^{30,31} Other platform comparisons, albeit with earlier versions of both tests found mean differences of 0.14 log₁₀, 0.1 log₁₀ throughout their analytical ranges of assay ranges, 0.072 log₁₀ throughout the assay range for bDNA and 0.0365 log₁₀.^{5,11, 32, 33, 34}

Other authors report similar results, but with different conclusions. Some claim two specimens measuring within < 0.5 log₁₀ by two assays or a sequential change of >0.3 log₁₀ should be considered concordant or clinically significant.^{5,35,36} However, discordance defined as > 0.5 log difference in pairs, is the recommended definition for clinicians to distinguish clinical significance in sequential values for an individual patient, and as mentioned above, the use of this cut-off for paired sample significance just because it is the clinical cutoff is erroneous. In sequential samples,

biologic variation accounts for >50%, whereas when considering variance between paired samples, biologic variation is non-contributory for a single blood sample.

Several limitations should be placed on the interpretation of our study results. We assume all of our HIV-1 strains are M, subtype B. Outlier values could represent non-B subtypes, but this is unlikely given the information collected on the patient population. If this assumption is incorrect, then the data differences would be expected to be even greater.³⁶ Another limitation is the use of subsets (trimmed) for our statistical analysis, however if the entire range for the TaqMan were included, differences would have seemed even higher. Another limitation was the inability to find statistical agreement or even data distribution similar to our study of significant differences between these two current platform versions. It appears that most current studies have parametric data allowing for comparison of standard deviations and means. In retrospect, new baselines were not necessary for 59 patients with viral loads less than 1000, as the two assays were not significantly different in this range.

Our work corroborates that of Gleaves et al. who found the relationship between Roche RT PCR and bDNA to be consistent, and that with some exceptions, a conversion factor might be used to convert between the two.⁶ Our results from the regression analysis and the comparison of the data estimated for TaqMan versus that actually measured by TaqMan, support this assumption. These estimates can be used on patients lacking parallel measurements for TaqMan baselines provided they have previous bDNA values.

We maintain that if one allows for variance of 2-3 fold after a method change, and normal variance for the next patient draw is 3-fold, the reported values become unreliable for patient monitoring with an analyte such as HIV-1 RNA where an accepted value for clinically significant change is already defined. Inherent in this decision is the importance of accuracy in viral load determination, i.e. these measures can directly affect decisions on patient care such as therapy. Even without statistical differences, some authors agree that re-baselining in this case is necessary or at least recommended.^{6,23} A later version (2.0) of the real time RT PCR assay (Roche) that we implemented is reported to correlate better with other methods due to possible

primer-probe mismatches in the v. 1.0.³⁶ This may explain some of the differences we found and reiterates the need for careful comparison of instrument results.

Re-baselining by the laboratory provides useful information to the clinician about magnitude of change in viral load that is attributable to change in platform versus biological difference and changes due to blip or viral escape. For instance, 4 individuals in cohort A were detectable between 48 and 75 copies/mL due to change in platform. One by bDNA and 3 by PCR would have been construed as detectable/treatment failures (3/81 or 3.7%). When the analysis was performed with a larger cohort, cohort B (n=308), we found 30 individuals below the lower limit of detection by bDNA - 11 of which were quantifiable by TaqMan between 48 and 75 copies/mL and 19 which were detectable by TaqMan at >75 copies/mL. These would have been interpreted as detectable when the difference was solely due to a platform change, not recognized without parallel runs.

In conclusion, our data demonstrate non-equivalence in HIV-1 RNA values of TaqMan v.1.0 as compared to bDNA v. 3.0, and that new baselines for HIV-1 viral load RNA should be re-established when switching between these assays. New baselines for those patients missed by parallel testing can be calculated using regression analysis. The problem we faced in our laboratory validation with statistical differences between assay results is not unique to these two methods. Laboratorians must be diligent when bringing on a new platform to ensure that only negligible variation in patient results is due to the switch in assays. If the difference is significant, re-baselining is required. However, if the results are different, but still correlated, there is the possibility of using regression analysis instead of re-drawing blood from each patient under surveillance.

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