Multicenter Evaluation of the VERSANT® HIV-1 RNA 3.0 Assay [bDNA] with the VERSANT™ 440 Molecular System

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Abstract

Background and Aim: The VERSANT® HIV-1 RNA 3.0 Assay [bDNA] (HIV 3.0) is a signal amplification nucleic acid probe assay for the direct quantification of HIV-1 RNA in human plasma using the Siemens System 340 bDNA Analyzer. The VERSANT™ 440 Molecular System is an upgraded system designed to more fully automate the VERSANT bDNA assay steps. This study examined the analytical sensitivity, specificity, precision, and linearity of HIV 3.0 using the VERSANT 440.* Results obtained on clinical specimens were also compared for correlation between VERSANT 440 and System 340.

Methods: Three testing sites participated in this study using three HIV 3.0 kit lots and two operators at each site. A 7-member dilution panel of HIV-1 RNA standards ranging in concentration from 59 to 589,756 copies/mL in plasma was tested in 108 or 216 replicates for each standard at all three sites to determine the lower limit of detection (LoD), linearity, and reproducibility, and to verify the lower and upper limits of quantification (LLoQ and ULoQ). Analytical specificity was derived from testing 300 HIV-1 antibody-negative plasma specimens (ProMedDx, Norton, MA) collected from healthy blood donors. Matched ACD- and EDTA-anticoagulated plasma specimens containing HIV-1 RNA levels between 108 and 334,583 copies/mL were tested to evaluate effects of anticoagulants on quantification of HIV-1 RNA. Plasma specimens from 183 HIV-infected patients were tested using a single HIV 3.0 kit lot on both VERSANT 440 and System 340 for correlation of results. These clinical specimens included three circulating recombinant form strains of HIV-1 and the following numbers of HIV-1 group M subtypes: 3 A, 153 B, 5 C, 4 D, 5 E, 5 F, and 5 G.

Results: For HIV 3.0 used with VERSANT 440, the LoD (95% detection rate of >35 $\log_{10}$ copies/mL) was 68 copies/mL (95% CI, 62 to 71 copies/mL). The LLoQ and ULoQ were verified as 75 and 500,000 copies/mL, respectively, the same as those for HIV 3.0 with System 340. Within the VERSANT 440 quantification range, combined intra- and inter-assay %CV ranged from 10.1% to 24.4%, with excellent linear correlation (slope = 1.005, r = 0.999) between expected and observed mean $\log_{10}$ copies/mL results for the HIV-1 RNA standards, and differences between linearized and observed mean results varied from –0.02 to 0.04 $\log_{10}$ copies/mL. Analytical specificity relative to the LLoQ (75 copies/mL) was 100%. Paired results for ACD-plasma vs. EDTA-plasma showed a mean titer difference of –0.03 $\log_{10}$ copies/mL, with a range of –0.27 to 0.20 $\log_{10}$ copies/mL. HIV 3.0 quantification results (ranging from 80 to 427,999 copies/mL by VERSANT 440) for 156 of the 183 clinical plasma specimens showed good correlation (slope = 1.004, R^2 = 0.985; Deming regression) between VERSANT 440 and System 340, with a mean difference of 0.01 $\log_{10}$ copies/mL, range of –0.27 to 0.46 $\log_{10}$ copies/mL (all differences were <0.50 $\log_{10}$ copies/mL), and 95% of the differences falling between –0.21 and 0.23 $\log_{10}$ copies/mL.

Conclusions: HIV 3.0 used with VERSANT 440 is a reliable and accurate quantitative assay, with analytical sensitivity, specificity, reproducibility, and range of quantification equivalent to those of HIV 3.0 with System 340 for the measurement of HIV-1 RNA levels in plasma. VERSANT 440 has the advantage of more fully automated bDNA processing compared to System 340.

* The VERSANT HIV-1 RNA 3.0 Assay [bDNA] is CE-marked for use with both the System 340 and VERSANT 440, but it is commercially available for use in the US only with the System 340 at this time.

Introduction

The VERSANT 440 Molecular System (VERSANT 440; Siemens Healthcare Diagnostics Inc., Tarrytown, NY) is a walk-away, automated diagnostic instrument designed for reagent addition, incubation, washing, plate sealing, agitation, and luminescence reading processes of the VERSANT branched-DNA (bDNA) assays. This integrated system allows the operator to prepare and place assay reagents and clinical serum or plasma specimens onboard the instrument at testing set-up, and the instrument automatically completes the bDNA assay steps. The VERSANT 440 provides onboard temperature control of the assay reagents and automated preparation and delivery of all assay reagents to the microtiter well plates, while processing 12 to 168 samples per assay run. A touch-screen computer user interface and bidirectional interface between the VERSANT 440 and laboratory information system also improve laboratory workflow and efficiency (Figure 1).

Figure 1. VERSANT 440 Molecular System.

Materials and Methods

1. VERSANT 440 performance characteristics — accuracy, linearity, reproducibility, analytical sensitivity and analytical specificity

- A panel of 7 HIV-1 standards (QC1 to QC7) ranging from 59 to 589,756 copies/mL were prepared from serial dilution of whole HIV-1 particles from 8E5 LAV viral stock in HIV-1 RNA-negative human plasma. Each panel member was assigned an HIV-1 RNA target concentration by Siemens Healthcare Diagnostics Inc. after testing of replicates on multiple assay plates by multiple operators using the HIV 3.0 assay on the System 340 analyzer. For accuracy, linearity, reproducibility and analytical sensitivity, 2 operators at each of the 3 study sites performed the testing using 3 different HIV 3.0 kit lots. Each of the 7 panel members were tested in replicates of 6 or 12 on each bDNA assay plate for a total of 108 (QC1 to QC4) or 216 (QC5 to QC7) replicates, respectively.

  Detection cutoff (DC) = The HIV-1 RNA level below which 95% of the negative specimens do not yield quantitative results

  Limit of detection (LoD) = The lowest HIV-1 RNA concentration for which the assay can yield a quantitative result 95% of the time, relative to the DC

  Lower limit of quantification (LLoQ) = The lowest HIV-1 RNA concentration for which the assay can yield a quantitative result 95% of the time with coefficient of variation (CV) of <45%, and falling within ±0.1 $\log_{10}$ copies/mL from the linearized expected concentration

  Upper limit of quantification (ULoQ) = The highest HIV-1 RNA concentration quantifiable by the assay with CV of <45% and falling within ±0.1 $\log_{10}$ copies/mL from the linearized expected concentration

- Analytical specificity was determined only at one testing site (Siemens Clinical Laboratory) using 2 different HIV 3.0 kit lots to test 300 unique EDTA-plasma specimens collected from healthy blood donors who were negative for HIV-1/-2 antibodies.

2. Effects of ACD and EDTA anticoagulants

Matched ACD- and EDTA-anticoagulated plasma specimens (VIRx, San Francisco, CA; ProMedDx LLC, Norton, MA) collected from 40 HIV-1-infected subjects were tested at Siemens Clinical Laboratory using one HIV 3.0 kit lot. These specimens contained HIV-1 RNA levels varying from 100 to 330,000 copies/mL. Matched plasma specimens from each subject were tested in the same assay run for quantitative comparison.

3. Correlation between VERSANT 440 and System 340 in clinical specimens

Plasma specimens collected from 183 HIV-1-infected patients were divided across and tested by the 3 testing sites all using a single HIV 3.0 kit lot on the VERSANT 440. Quantification results on these specimens using the same HIV 3.0 kit lot on the System 340 were obtained only at one testing site (Siemens Clinical Laboratory). These specimens, with HIV-1 RNA levels spanning the range of HIV 3.0, included 153 specimens of HIV-1 group M, subtype B, and 30 specimens of group M, subtypes A, C to G, and circulating recombinant forms AG and B/D.

Results

1. VERSANT 440 performance characteristics — accuracy, linearity, reproducibility, analytical sensitivity and analytical specificity

- With a DC of 35 copies/mL for HIV 3.0 (the same for both VERSANT 440 and System 340), the LoD was determined to be 68 copies/mL (95% CI, 62 to 71 copies/mL).
- Both the LLoQ and ULoQ were estimated to be 75 copies/mL and 500,000 copies/mL, respectively, by interpolation of the assay linearity and precision (Table 1).
- Differences between corresponding assigned titer and mean observed titer ranged from –0.02 $\log_{10}$ copies/mL (QC7) to 0.04 $\log_{10}$ copies/mL (QC4).
- Deming regression analysis showed good linearity (r = 0.999) and correlation (EDTA > 0.999) between the mean observed titers and assigned titers across the quantification range of HIV 3.0 using VERSANT 440 (Figure 2).
- Within the quantification range of HIV 3.0 using VERSANT 440, total %CV ranged from 10.1% to 24.4% (Table 1).
- All 300 HIV-1/-2 antibody-negative plasma specimens collected from healthy blood donors yielded results of <75 copies/mL, resulting in an analytical specificity of 99.3% (lower 95% confidence limit of 99.0%).

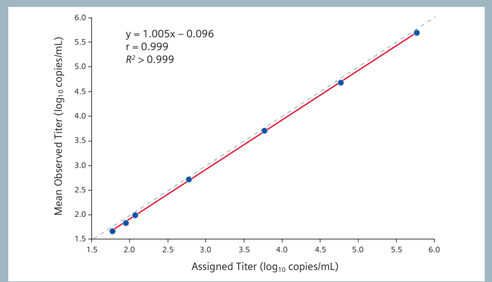
Table 1. Accuracy, linearity, and reproducibility of HIV 3.0 using VERSANT 440 for HIV-1 standards.

HIV-1 standard	No. of replicates	Assigned titer (copies/mL)	Mean linearized (copies/mL)	Mean observed (copies/mL)	Mean observed vs. mean ($\log_{10}$ copies/mL)	% Detected	Total % CV
QC1	108	589,756	484,962	499,355	0.01	100.0	12.7
QC2	108	58,976	48,497	48,114	0.00	100.0	14.3
QC3	108	5,898	4,850	5,124	0.02	100.0	10.1
QC4	107†	590	485	534	0.04	100.0	13.9
QC5	216	118	97	97	0.00	100.0	24.1
QC6	216	88	72	70	–0.01	100.0	22.9
LLoQ*	—	75	—	—	–0.02	95.0	24.4
QC7	216	59	49	46	–0.02	88.0	26.3

* The % detected and %CV values were interpolated for LLoQ.

† One replicate of QC4 did not yield a result due to an instrument error for the reaction well.

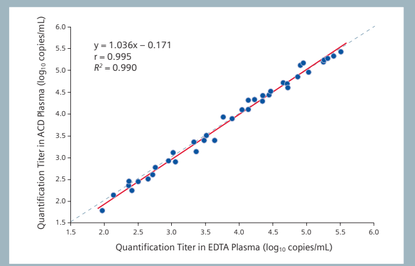
Figure 2. Correlation between mean observed and assigned (expected) titers of HIV-1 standards.



2. Effects of ACD and EDTA anticoagulants on HIV 3.0 with VERSANT 440

- Deming regression analysis (Figure 2) of quantification titers among the 40 matched pairs of ACD- and EDTA-anticoagulated plasma specimens showed good linearity (r = 0.995) and correlation (R^2 = 0.990), with mean titer difference of –0.03 $\log_{10}$ copies/mL and a range of –0.27 to 0.20 $\log_{10}$ copies/mL.

Figure 3. Correlation of HIV-1 RNA quantification titers between ACD- and EDTA-anticoagulated plasma specimens.



3. Correlation between VERSANT 440 and System 340 with clinical specimens

- Of the 183 clinical plasma specimens tested, 27 specimens yielded results beyond the quantification range of HIV 3.0 using VERSANT 440 or System 340 and were not included for data analyses.
- For the remaining 156 specimens that yielded quantifiable titers by both VERSANT 440 and System 340, Deming regression analysis (Figure 4) showed good linearity (r = 0.992) and correlation (R^2 = 0.985) across the quantification ranges of both assay systems.
- A Bland-Altman plot (Figure 5) demonstrated a mean titer difference of 0.01 $\log_{10}$ copies/mL between VERSANT 440 and System 340, with a range of –0.27 to 0.46 $\log_{10}$ copies/mL and 95% of the titer differences falling between –0.21 and 0.23 $\log_{10}$ copies/mL.

Figure 4. Correlation of quantitative HIV-1 RNA titers in clinical plasma specimens between VERSANT 440 and System 340.

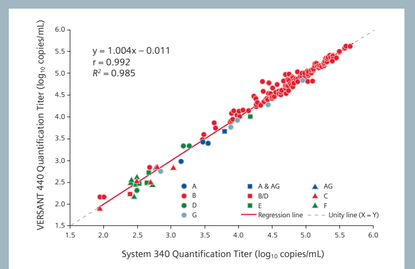
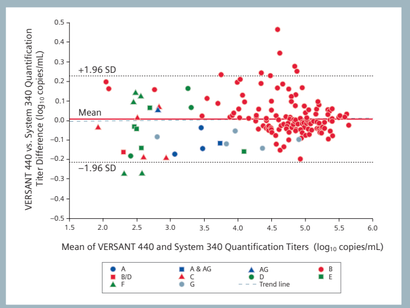


Figure 5. Bland-Altman plot of HIV-1 RNA titer differences in clinical plasma specimens between VERSANT 440 and System 340.



Conclusions

- HIV 3.0 used with VERSANT 440 showed analytical sensitivity, specificity, reproducibility, and range of quantification equivalent to those of HIV 3.0 with System 340 for the measurement of HIV-1 RNA levels in plasma.
- Compared to System 340, VERSANT 440 has the advantage of automated bDNA processing for high-throughput testing.

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