SUMMARY OF SAFETY AND EFFECTIVENESS

INDICATIONS FOR USE

The VERSANT® HIV-1 RNA 3.0 Assay (bDNA) is an *in vitro* signal amplification nucleic acid probe assay for the direct quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in plasma of HIV-1 infected individuals using the Bayer® System 340 bDNA Analyzer. The test can quantitate HIV-1 RNA over the range of 75 – 500,000 HIV-1 RNA copies/mL. Plasma samples containing Group M Subtypes A – G have been validated for use in the assay.

This test is intended for use in conjunction with clinical presentation and other laboratory markers of disease status as an aid in management of individuals infected with HIV-1. HIV-1 RNA results from the assay can be used to assess prognosis of disease progression and to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of therapy. Monitoring the effects of antiretroviral therapy by serial measurements of plasma HIV-1 RNA has been validated for patients with viral loads ≥ 25,000 copies/mL.

The VERSANT HIV-1 RNA 3.0 Assay (bDNA) is not intended for use as a screening assay for HIV infection or as a diagnostic test to confirm the diagnosis of HIV infection.

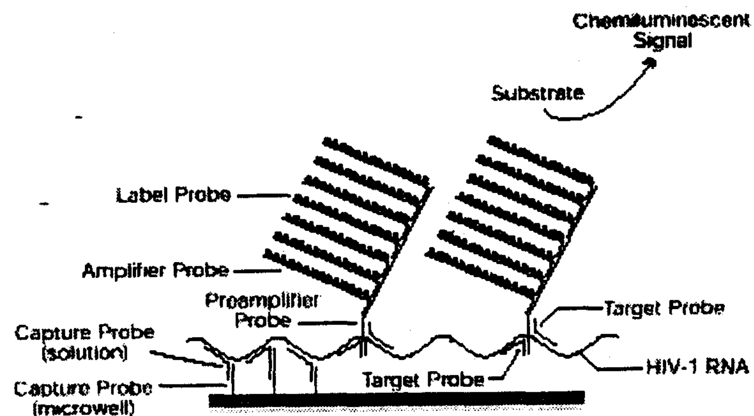
DEVICE DESCRIPTION

The VERSANT HIV-1 RNA 3.0 Assay (bDNA) is a sandwich nucleic acid hybridization procedure for the direct quantitation of HIV-1 RNA in human plasma.

Test Priniciples

HIV-1 is first concentrated from plasma by centrifugation. After HIV-1 genomic RNA is released from the virions, the RNA is captured to a microwell by a set of

specific, synthetic oligonucleotide capture probes. A set of target probes hybridizes to both the viral RNA and the pre-amplifier probes. The capture probes, comprising 17 individual capture extenders, and the target probes, comprised of 81 individual target extenders, bind to different regions of the pol gene of the viral RNA. The amplifier probe hybridizes to the pre-amplifier forming a branched DNA (bDNA) complex. Multiple copies of an alkaline phosphatase



(AP) labeled probe are then hybridized to this immobilized complex.

Figure 1 Scheme of assay design.

Detection is achieved by incubating the complex with a chemiluminescent substrate. Light emission is directly related to the amount of HIV-1 RNA present in each sample, and results are recorded as relative light units (RLUs) by the analyzer. A standard curve is defined by light emission from standards containing known concentrations of beta propiolactone (BPL)-treated virus. Concentrations of HIV-1 RNA in specimens are determined from this standard curve.

Kit Configuration and Components

Assay Kit Components

The VERSANT HIV-1 RNA 3.0 Assay (bDNA) reagents are supplied in a 2 box kit. Box 1 contains Bead Suspension, Lysis Diluent and Reagent, Capture and Target Probes, Wash Buffers, Capture Wells, Barrier Film, Pre-Amplifier/Amplifier

and Label Diluent, Dextran Sulfate, Pre-Amplifier and Amplifier Probes and Chemiluminescent Substrate and Substrate Enhancer. Box 2 contains Label Probe, High Positive, Low Positive and Negative Controls, as well as Standards A through F and Product Insert Supplement. Component lot numbers are matched to the stated kit lot and cannot be mixed. The Product Insert Supplement contains important information, including kit and component lot numbers, kit expiration date, a list of lot-specific concentrations (copies/mL) of Standards A through F, as well as the lot-specific mean concentrations of the Positive Controls and their acceptable ranges.

Instrument and Software

The VERSANT bDNA assay reagents are designed to be used with the Bayer System 340 Analyzer and DMS software.

The analyzer can simultaneously process two 96-well plates and automates the following functions:

- seals the wells during incubation to minimize evaporation
- heats the wells to a precisely controlled temperature
- cools the wells after each incubation in preparation for the wash
- washes unbound material from the wells
- quantitation of the luminescence readings
- exports the luminescence data and the corresponding events, errors, and warnings form the event log to the DMS

The Bayer Data Management Software (DMS) is designed to collect and analyze luminescence data from the Bayer System 340 bDNA Analyzer and performs the following tasks:

- prints a plate map of the standards, controls, and sample IDs
- collects luminescence data

- generates a standard curve
- calculates results for controls and samples based upon the standard curve
- calculates quality control parameters such as the standard curve fit and the coefficient of variation (CV) of replicate values
- evaluates control results against a programmed range
- prints a report of the results and the result event log
- creates a text file from the results and event log that can be exported for use in other software programs.

Quality Control

The VERSANT HIV-1 RNA 3.0 Assay (bDNA) contains Standards A through F and three controls, the High Positive Control, the Low Positive Control and the Negative Control. An assay is considered valid if the following conditions occur:

- The values determined for the HIV-1 positive controls are within the specified range defined in the Product Insert Supplement.
- The relative light units (RLUs) for the standards are geomean RLU Std A > RLU Std B > RLU Std C > RLU Std D > geomean RLU Std E > geomean RLU Std F
- The HIV-1 Negative Control reads below its acceptable value as pre-programmed into the DMS.

Manufacturing

The kits, instruments and software are manufactured in compliance with Current Good Manufacturing Practices. In-process quality control of assay reagents, instruments and software are performed to ensure that each kit lot and instruments meets acceptable criteria to support performance claims set out in the Product Insert and Product Insert Supplement.

ALTERNATIVE PRACTICES AND PROCEDURES

Currently, there are several direct and indirect methods for the detection and quantitation of human immunodeficiency virus type I (HIV-1) in human specimens. These methods provide some means of measuring the progression of HIV disease and for monitoring a patient's response to therapy. The methods include: nucleic acid technologies for direct detection and quantitation of circulating viral particles; laboratory tests for CD4+ T-cell counts, which may provide an indirect assessment of the patient's immune system status; quantitation of HIV p24 antigen and electron microscopic procedures for the quantitation of circulating viral particles.

MARKETING HISTORY

The VERSANT HIV-1 RNA 3.0 Assay and System 340 instrument and DMS software is currently approved in several countries, including France, Canada, Australia, Spain, and Brazil. The product has not been withdrawn from the market in any country.

POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

HIV-1 infected patients should not experience adverse effects when the VERSANT HIV-1 RNA 3.0 Assay (bDNA) is used according to its directions for use and when used as an aid in the management of individuals infected with HIV-1. The test is not indicated for use in patients who have not been diagnosed with HIV-1 infection.

As an aid in the medical management of individuals infected with HIV-1, the device is intended to provide the clinician with viral load information for use in addition to other available patient medical information and laboratory test results. In the event of an overestimation of HIV-1 RNA, there is a possibility that a patient could have antiretroviral therapy initiated or changed when such an action

was not needed. In the event of an underestimation of HIV-1 RNA, the physician could delay the initiation or change in antiretroviral therapy drug regimen when it could have been helpful to the patient.

Based on an evaluation of the product's performance in clinical and nonclinical studies, reviewers believe that the probable benefit to the patient from use of the product outweighs the probable risk of injury or illness to the patient from its use.

SUMMARY OF THE PERFORMANCE CHARACTERISTICS

This section summarizes the nonclinical and clinical studies performed using the VERSANT HIV-1 RNA 3.0 Assay (bDNA) and System 340 instrument and DMS software. Section 0 describes the results of nonclinical laboratory studies, including studies performed to characterize the synthetic DNA used in the Assay and studies conducted to determine the performance characteristics of the Assay. Section 4.6.2 summarizes the results from well-controlled clinical studies performed to support the safety and effectiveness of the VERSANT HIV-1 RNA 3.0 Assay (bDNA).

Nonclinical Laboratory Studies

DNA Characterization

Characterization studies were performed on certain DNA-containing assay materials. Analytical test methods that demonstrated the quality (e.g., identity and purity) of the DNA components were developed and validated. The characterization studies indicated that the validated DNA synthesis and purification process results in the production of DNA oligonucleotides with the

correct sequence, including the correct order of all bases and the correct representation of bases at the degenerate base positions.

Assay Standardization

The VERSANT HIV-1 RNA 3.0 Assay (bDNA) is anchored to a Reference Standard ( ), which is evaluated by independent, validated analytical methods (. The quantity of RNA in the transcript is determined from its The assay utilizes dilutions of the United States National Institute of Standards and Technology (NIST) standard reference material and is, therefore, directly traceable to the US NIST.

kit lots were used to determine the conversion factor between the VERSANT HIV-1 RNA 3.0 Assay copies/mL and International Units (IU). Dilutions of the WHO International Standard for Human Immunodeficiency Virus-1 (HIV-1) RNA (NIBSC code 97/656) were tested in this evaluation. The conversion factor was consistent between lots, with a mean VERSANT HIV-1 RNA copies/mL to IU factor of 0.306.

Device Stability

The stability of the VERSANT HIV-1 RNA 3.0 Assay (bDNA) was assessed on manufactured kit lots. Evaluations of stability included testing the kits periodically through for all lots. Assay results were compared to established specification ranges to evaluate potential assay deterioration. Results of these real time stability studies indicate that the VERSANT HIV-1 RNA 3.0 Assay (bDNA) is stable for at least 12 months when it is stored at its labeled storage conditions of 2° to 8° C (Box 1) and -60° to -80° C (Box 2). Stability of the kit lots is monitored every kit not to exceed kits per year to verify performance claims.

Assay Performance Characteristics: Reproducibility, Linearity, Limit of Detection and Limit of Quantitation

The performance characteristics of the VERSANT HIV-1 RNA 3.0 Assay (bDNA) were assessed using a – member HIV-1 positive EDTA plasma panel and a member HIV-1 positive ACD plasma panel. The panels were created by

The member EDTA panel ranged from 620,282 to 62 HIV-1 RNA copies/mL. The ACD panel members had HIV-1 RNA at levels of 5,640 and 94 HIV-1 RNA copies/mL. Two operators at each of 3 external trial sites performed the testing, with each operator performing 4 assay runs with each of the clinical reagent lots. The results were used to establish reproducibility, linearity, limit of detection and limit of quantitation.

Reproducibility

Table 1 shows, for each panel member, the percent CV for the overall between-run, within-run and total variation, where "overall between run" incorporates lot, site and operator components.

Table 1 **Coefficient of Variation (%CV) for Overall Between-Run, Within-Run and Total Variation**

Panel Member	N	Value Assignment (HIV-1 RNA copies/mL)	Overall Between Run	Within Run	Total %CV
1	214	620,282	20.3%	14.1%	24.9%
2	216	62,028	21.6%	12.9%	25.3%
3	216	6,203	18.7%	13.6%	23.2%
4	215	620	19.4%	15.5%	25.0%
5	216	124	18.8%	18.7%	26.7%
6	216	93	18.7%	23.1%	30.0%
7	215	62*	19.8%	25.2%	32.4%
3A	214	5,640	23.5%	16.0%	28.7%
5A	214	94	30.1%	24.2%	39.3%

*Below the limit of quantitation of the assay.

The total percent coefficient of variation (%CV) was less than 29% over most of the range (124 to 620,282 HIV-1 RNA copies/mL). For the panel members below 100 HIV-1 RNA copies/mL, the total %CV was less than 40%.

Linearity

The assay was linear across the reportable range, with the maximum deviation from linearity less than 0.05 $\log_{10}$. The linearity of the assay was confirmed using serially diluted individual patient specimens collected into either EDTA or ACD anticoagulants.

Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Table 2 shows the percent of each panel member's determinations that was detected (i.e., quantitated) with respect to the reporting threshold of 35 HIV-1 RNA copies/mL. The reporting threshold (RT) is chosen so that 95% of negative

specimens read below this number. The table indicates that the LoD, at which detection is required to be at least 95%, fell between 62 and 93 HIV-1 RNA copies/mL.

Table 2 **Percent Detection for each Panel Member**

Panel Member	Value Assignment (HIV-1 RNA copies/mL)	Percent Detected
1	620,282	100
2	62,028	100
3	6,203	100
4	620	100
5	124	100
6	93	98
7	62	83

The estimated LoD was 68 HIV-1 RNA copies/mL with the upper one-sided 95% confidence limit of 73 HIV-1 RNA copies/mL. Because the reproducibility of quantitation was acceptable in this range (a total imprecision of less than 40% CV -corresponding to less than 0.17 log10 sd) the upper one-sided 95% confidence limit for the LoD was rounded up to the nearest 5 HIV-1 RNA copies/mL to obtain the LoQ of 75 HIV-1 RNA copies/mL.

Potentially Interfering Substances and Conditions

The following endogenous and exogenous substances and conditions were studied in HIV-1 positive and HIV-negative specimens to determine if any had an effect on assay specificity or HIV-1 quantitation. The various substances or conditions were evaluated by adding these substances to HIV negative EDTA

plasma specimens (N=25) and to specimens spiked with a low concentration of BPL-treated HIV-1 viral stock solution (N=25).

Potentially Interfering Exogenous Substances

The drugs were pooled as shown in Table 3 and tested to final concentrations of five times the reported peak serum or plasma concentrations in the therapeutic range. Pathogens were also pooled. Each pathogen was diluted to a final concentration of 10^3 CFU/mL or PFU/mL unless otherwise noted. If a drug or pathogen pool gave statistically different results than the control pool, the individual drugs or pathogens in that pool were tested at the final concentrations stated above. The drugs and pathogens listed in Table 3 were found to have no effect on the VERSANT™ HIV-1 RNA 3.0 Assay (bDNA) at the target concentrations.

Table 3 summarizes the substances and conditions tested and the effect on assay performance.

Table 3 Effect of Potentially Interfering Exogenous Substances or Conditions on Quantitation of HIV-1

Drugs Tested	Pathogens Tested
AZT, 3TC, ritonavir, nevirapine, ganciclovir	HSV-2, CMV, HBV, HCV, EBV*
ddI, d4T, indinavir, trimethoprim/sulfamethoxazole, foscarnet	*E. coli, E. cloacae, P. aeruginosa, K. pneumoniae*
AZT, ddC, nelfinavir, fluconazole	*S. aureus, S. epidermidis, H. influenzae, C. albicans*
saquinavir, azithromycin dihydrate, acyclovir	*P. mirabilis, S. marcescens*, Streptococcus group B

ritonavir, saquinavir, cidofovir, ciprofloxacin, delavirdine	*S. pneumoniae, M. avium, C. neoformans*
rifampin, rifabutin, ethambutol, INH, clarithromycin, hydroxyurea	

*EBV was tested at ~10^3 PFU/mL. CMV, HBV and HCV quantitations were performed using Bayer bDNA assays and were tested at final concentrations of approximately 6 x 10^1, 1.0 x 10^6, and 1.0 x 10^8 copies/mL, respectively.

Potentially Interfering Endogenous Substances

Specimens with levels of triglycerides up to 2000 mg/dL, bilirubin up to 20mg/dL or hemoglobin up to 200 mg/dL showed no clinically significant interference with the HIV-1 RNA quantitation by this assay. HIV-1 positive specimens with bilirubin concentrations of 20 mg/dL showed 28% lower quantitation or a difference of 0.14 logs when compared to the reference. HIV-1 positive specimens with 2000 mg/dL of triglycerides showed 32% lower quantitation or a difference of 0.17 logs when compared to the reference. HIV-1 positive specimens with 200 mg/dL of hemoglobin showed 9% lower quantitation or a difference of 0.04 logs when compared to the reference. These differences in quantitation are well within the noise level of the assay. The official guidelines from the DHHS[1] state that "A minimally significant change in plasma viremia is considered to be a 3-fold or 0.5 $\log_{10}$ increase or decrease." Changes in viral load of less than 0.5 log should be ignored as assay noise given the inherent variability of the assay plus biologic variation. Therefore the change in quantitation based on these interfering substances is of such small magnitude that is not deemed clinically relevant.

Multiple Freeze-Thaw Cycles

The effect of 1, 2 and 3 freeze-thaw cycles was tested in 25 HIV negative EDTA plasma specimens and 25 specimens spiked with a low concentration of BPL-

treated HIV-1 viral stock solution (N = 25). EDTA plasma samples were also collected from 25 HIV-1 infected individuals, aliquoted, and tested after 1, 2 and 3 freeze thaw cycles. There was no effect of up to 3 freeze-thaw cycles on HIV-negative or HIV-1 positive (real or spiked) specimens.

Specimen Collection and Handling

Studies were performed to establish the types of collection tubes that can be used with the VERSANT HIV-1 RNA 3.0 Assay (bDNA) and to determine the handling and storage conditions that can be used with each tube type.

Glass K_3 EDTA Vacutainer™ tubes, plastic K_2 EDTA Vacutainer tubes, K_2 Plasma Preparation tubes (PPT), and ACD Solution A Vacutainer tubes were evaluated. All of the EDTA types provided statistically equivalent HIV-1 quantitations. Matched ACD-A and K_3 EDTA tubes were drawn from 126 HIV-negative individuals. There was no difference in RLUs between theses tube types. Within the HIV-1 positive group of 99 subjects, the ACD-A readings were, on the average, 15% lower (0.07 $\log_{10}$) than the readings using the K_3 EDTA tubes. This difference was consistent across the reportable range of the assay. Because the volume of the acid-citrate-dextrose solution creates a 17.6% dilution of the total blood volume, this decrease in signal was expected. Both EDTA and ACD anticoagulants can be used in the VERSANT HIV-1 RNA 3.0 Assay (bDNA).

HIV-1 positive plasma collected in K_3 EDTA and ACD Solution A tubes was either frozen immediately after centrifugation or held at 2 to 8°C for either 8, 24, or 48 hours, then frozen at -60 to -80°C. HIV-1 quantitations at all time points were

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[1] Department of Health and Human Services (DHHS) and the Henry J. Kaiser Family Foundation. Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents, Feb 4, 2002.

statistically equivalent to those of the plasma specimens that were immediately frozen.

Plasma collected in K_2 EDTA PPT was either frozen immediately at -60° to -80° C (reference procedure) after centrifugation, held at room temperature for 24 hours, then frozen, or held at 2 to 8°C for 24 hours, then frozen. Storage at room temperature for 24 hours had no effect on HIV-1 quantitation. However, storage at 2-8°C resulted in a lower quantitation than the reference condition.

Table 4 summarizes the results of the specimen collection and handling studies.

Table 4 **Summary Of Anticoagulant And Specimen Handling Studies**

Tube Type	Time to Centrifugation	Plasma Handling	Storage Temp	Duration of Storage Prior to Freezing
K_3 EDTA Vacutainer	≤ 4 hours	Plasma removed from cells	2-8 °C	48 hours after collection
K_2 EDTA* Vacutainer	≤ 4 hours	Plasma removed from cells	2-8 °C	48 hours after collection
ACD (Solution A) Vacutainer	≤ 4 hours	Plasma removed from cells	2-8 °C	48 hours after collection
K_2 EDTA PPT	≤ 2 hours	Plasma left on separator gel	Room temperature	24 hours after collection

HIV Subtypes

The following numbers of distinct isolates were used to evaluate assay performance with the HIV-1 Group M subtypes: Subtype A – 10; Subtype B – 12; Subtype C – 10; Subtype D – 10; Subtype E – 12; Subtype F – 7; and Subtype G – 7.

Two studies were performed that compared the quantitations obtained using the VERSANT HIV-1 RNA 3.0 Assay (bDNA) to concentrations obtained by independent methods. In the first study, transcripts were prepared from – HIV-1 Group M subtypes (A through G), from 2 isolates in Group O (O1 and O2), and from an HIV-2 isolate, using cloned *pol* gene sequences inserted into a transcription vector (T7). The transcripts were purified and analyzed using agarose gel electrophoresis. The concentration of each transcript was determined using phosphate analysis, and confirmed using hyperchromicity and

OD_{260}. Each Group M transcript was diluted to target concentrations of 3,000 and 300,000 HIV-1 RNA copies/mL. The two Group O transcripts and the HIV-2 transcripts were tested at a concentration of approximately 250,000 copies/mL.

The second study evaluated specimens derived from several different culture isolates from each of HIV-1 Group M Subtypes A-G. These isolates had concentrations assigned using viral particle counts by electron microscopy and were diluted to 25,000 EM particles/mL. Additionally, 23 of these isolates were serially diluted to 4 or 6 concentrations and tested.

Table 5 shows the efficiency of quantitation for Subtypes A, C-G, compared to Subtype B, using these two sets of data.

Table 5 Relative Efficiency of Quantitation Estimates for Each Subtype

Subtype	Efficiency of Quantitation Based on:									Overall Relative Efficiency of Quantitation
	Transcripts	EM Viral Particle Count for Distinct Isolates								
A	42.2%	63.2%	39.4%	48.9%						47.6%
B	100.0%	117.9%	136.3%	161.7%	47.2%	58.8%	104.7%	132.6%		100.0%
C	25.8%	36.6%	47.7%	27.8%	25.1%	63.3%				35.5%
D	83.0%	35.3%	73.6%	45.3%						55.9%
E	57.8%	30.3%	46.8%	40.0%	46.4%	53.1%	51.8%	59.0%	65.4%	48.9%
F	46.8%	47.6%	71.8%	68.4%						57.5%
G	36.5%	33.3%								34.9%

The results show that Subtypes A, C-G had VERSANT HIV-1 RNA 3.0 Assay (bDNA) quantitations that were within 3-fold of Subtype B.

While there was some detection of the Group O transcripts, the mean assay results of both were less than 2% of the input concentrations. The HIV-2 transcript was not detected at the concentration tested.

Additional studies were performed to determine if reproducibility and linearity was equivalent for the Group M Subtypes A-G. Analyses for reproducibility were performed using specimens at four levels—high (> 50,000 copies/mL), medium-low (2000-5000 copies/mL), low (200 copies/mL), and very low (100 copies/mL). The number of specimens per subtype varied from 36 to 90. Statistical tests for homogeneity of variance across subtypes at each level were not statistically significant, indicating that there is no evidence for differences in assay reproducibility due to subtype.

Analyses for linearity were performed at five ranges in different experiments—300,000 to 3,000; 50,000 to 2,000; 5,000 to 200; 2,000 to 200; and 200 to 100. There were no statistically significant differences seen across any of the five ranges studied. Thus, there was no evidence that the assay is non-linear for non-B subtypes.

The VERSANT HIV-1 RNA 3.0 Assay (bDNA) has been validated for use with HIV-1 Group M Subtypes A-G.

Clinical Studies

Testing for the clinical studies were carried out at 7 trial sites across the U.S. An additional 8 sites provided specimens for use in the trial.

Clinical Sensitivity

The clinical sensitivity of the VERSANT HIV-1 RNA 3.0 Assay (bDNA) was evaluated in HIV-1 infected individuals, stratified by $CD4^+$ cell count and by gender: The CD4+ groups were:

- $CD4^+$ <200 cells/mm^3
- $CD4^+$ between 200 and 500 cells/mm^3 inclusive
- $CD4^+$ >500 cells/mm^3

Specimens from a total of 984 subjects were tested in this study. Table 6 summarizes the study population demographics and risk factors for acquiring HIV infection.

Table 6 **Study Population Characteristics**

Characteristic	Mean	Range
Age	39	15-77
	Number	**Percent**
Gender		
Male	698	71.0
Female	286	29.0
Race		
Asian	4	0.4
African-American	408	41.5
Caucasian	502	51.0
Hispanic	45	4.6
Other	25	2.6
Risk Factors		
Homosexual Activity and IV Drug Use	64	6.5
Homosexual Activity (No IV Drug Use)	487	49.5
IV Drug Use (No Homosexual Activity)	201	20.4
Blood Products Exposure	13	1.3
Occupational Exposure	6	0.6
None of the Above	213	21.7

Data were analyzed to determine if assay performance was different in males and females. Because differences were not statistically significant, all data were combined to establish performance in the 3 CD4+ T-cell categories. These data are presented in Table 7.

Table 7 **Percent of All Specimens with VERSANT HIV-1 RNA 3.0 Assay (bDNA) Quantitations At or Above the LoQ, by CD4+ Category**

CD4+ Category	Gender	N	% with Results ≥ LoQ (75 HIV-1 RNA Copies/mL)	95% Lower Confidence Level	95% Upper Confidence Level
CD4+ < 200	Combined	302	98.7%	96.7%	99.6%
CD4+ 200-500	Combined	371	88.4%	83.2%	94.7%
CD4+ > 500	Combined	311	65.3%	59.7%	70.6%

A laboratory testing specimens from HIV- positive patients can expect to detect virus in approximately 99% of patients with CD4+ counts of less than 200 cells/mm^3, in approximately 89% of patients with CD4+ counts between 200 and 500 cells/mm^3, and in approximately 65% of patients with CD4+ counts of greater than 500 cells/mm^3.

Clinical Specificity: Specimens from Low Risk Populations

EDTA plasma specimens from 912 unlinked repeat volunteer male and female blood donors were tested using – reagent lots of the VERSANT HIV-1 RNA Assay (bDNA). All specimens were negative for HBsAg, HBcAb, HCV antibody, HIV-1/HIV-2 antibody (Abbott HIV-1/HIV-2 EIA) and for p24 antigen. The collection centers were the Sacramento Center for Blood Research and the New York Blood Center. Because these subjects were repeat donors, they had a very low risk of being HIV-1 positive.

Data were analyzed to determine if assay performance was different in males and females. Because performance was not statistically different, all data were

combined for the analysis of assay specificity. Table 8 summarizes the assay specificity and 95% confidence intervals for the combined data.

Table 8 **Percent of Specimens with Non- Detectable Virus (Result < LoQ) by the VERSANT HIV-1 RNA 3.0 Assay (bDNA)**

Gender	N	% With Results < LoQ by the VERSANT HIV-1 RNA 3.0 Assay (bDNA)	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Combined	912	97.6%	96.4%	98.5%

The assay specificity of 97.6% is based on an LoQ of 75 copies/mL

Clinical Specificity: Specimens from Individuals with Other Diseases

EDTA plasma specimens from HIV-negative patients diagnosed with the following infections or having the following antibodies/antigens were tested using the VERSANT HIV-1 RNA 3.0 Assay (bDNA):

- cytomegalovirus (CMV)
- hepatitis C virus (HCV)
- hepatitis B virus (HBV)
- human T-lymphotropic virus (HTLV)
- anti-nuclear antigen (ANA)
- rheumatoid factor (RF)

Ten subjects diagnosed with each of the 6 disease states were evaluated, for a total of 60 HIV-negative clinical specimens. The RLU values of specimens collected from volunteer repeat blood donors in the specificity study were used as reference values. For each blood donor specimen and every "other disease" specimen, the ratio of the specimen RLU to the RLU of the LoQ for that particular assay run was calculated.

Table 9 summarizes the geometric mean ratio of the specimen RLUs to the RLUs of the LoQ for the donors and disease states, the ratio between the 2 results, and the corresponding confidence interval.

Table 9 Geomean Specimen RLUs / RLU of the LoQ and the Comparison of RLUs from Donor Specimens and Disease State Specimens

Group	N	Geomean Specimen RLUs/ RLU of the LoQ	Ratio Compared to Normal Donors	Upper 95% Confidence Limit of the Ratio
Normal Donor	912	0.32		
Disease	60	0.29	0.91	0.97

The specificity of the VERSANT HIV-1 RNA 3.0 Assay (bDNA) in specimens from subjects with other diseases commonly encountered in HIV infection is equivalent to the specificity in specimens from volunteer repeat blood donors.

Within Patient Variability

The within patient variability of HIV-1 quantitation was evaluated in 24 presumably clinically stable men and 9 presumably stable women. EDTA plasma samples and health status information were collected from each subject weekly for 8 weeks.

Enrollment criteria for the study were:

- No initiation or change in retroviral therapy within 3 months of the start of the study
- No clinical events within 3 months of the start of the study
- No initiation or changes in retroviral therapy during the 8-week study period

- No clinical events during the 8-week study

Subjects were required to have had at least 6 of the 8 weekly draws to remain eligible for the study.

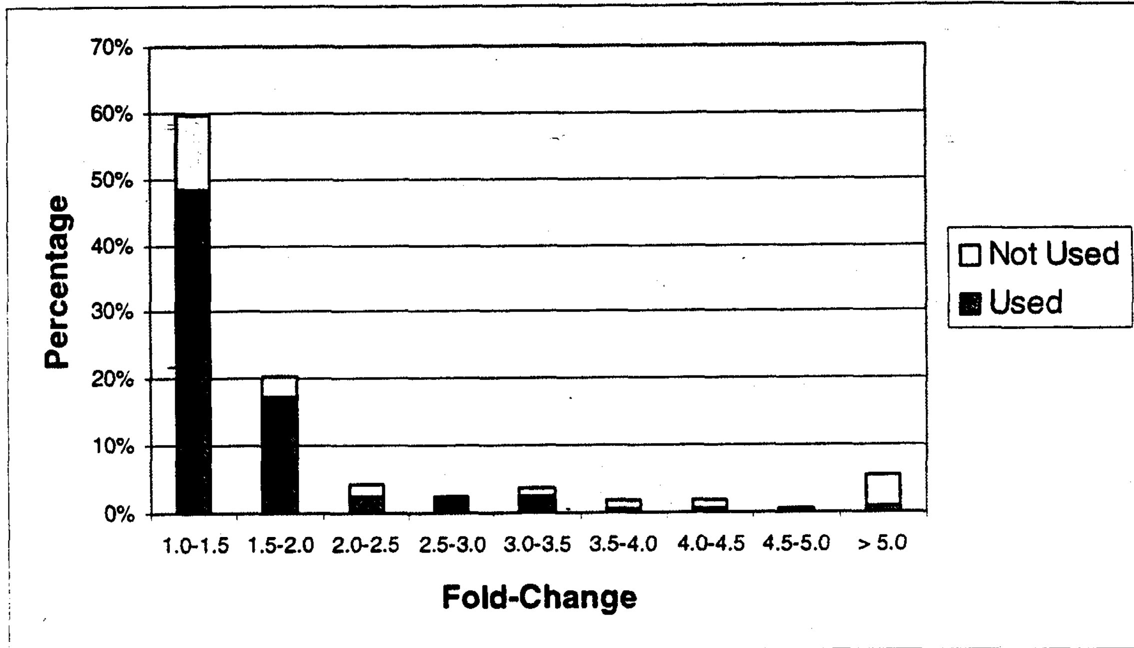
Each successive specimen from a given subject was tested in a different run (but within the same kit lot) to mimic the between run results that would be obtained if testing were done in real time.

The ultimate objective of the study was to determine the minimum fold-change between 2 successive measurements that would signal a potential change in a subject's clinical status. The data from this study were designed to provide information on biological variability. The biological variability estimate was then combined with the estimates of assay variability (site, operator, lot, between-run and within-run) from the Reproducibility Study to estimate the minimum fold-change number.

Five subjects were eliminated from the analysis because they had a 20-fold or greater change in assay quantitation by both assays during the 8-week study period. Four of these 5 subjects were intravenous drug users, so use of illicit drugs or lack of compliance with medication might have caused these large fluctuations in viral load. In addition, the results of all specimens from 3 subjects were below the limit of quantitation in the VERSANT HIV-1 RNA 3.0 Assay (bDNA); these results were not used in the calculations for assay variance.

Figure 2 shows the distribution of observed fold-changes for the serial measurements by the VERSANT HIV-1 RNA 3.0 Assay (bDNA). All subjects are included in these figures. The fold-changes represented by the horizontally striped portion of the bars in the bar graphs are the ones excluded from the estimation of variance.

Figure 2 **Distribution of Fold-Changes Between Serial Measurements in the VERSANT HIV-1 RNA 3.0 Assay (bDNA), Showing Fold-Changes Which Were Used and Not Used in the Variance Calculations**



A total of 160 pairs of serial measurements were used in the variance calculations for determining within patient variability. These measurements were used to estimate the variance in quantitation due to biological, run, and within sources of variation. The variance estimate was 0.05224 for the VERSANT HIV-1 RNA 3.0 Assay (bDNA). This variance estimate was combined with variance estimates for site, operator, and lot from the Reproducibility study (Section 0) to estimate the total assay + biological variability for the assay. These estimates were then used to calculate the maximum change between 2 successive measurements that could be attributed to chance. Larger changes in viral load would then be used to signal potential changes clinical status.

Based on the combined variance estimates, the upper 95% confidence interval of the minimum fold change between 2 successive measurements of HIV-1 using the VERSANT HIV-1 RNA 3.0 Assay (bDNA) that is unlikely to have happened

by chance was estimated to be 3.2-fold (0.51 $\log_{10}$) across the entire range of the assay. Therefore, the VERSANT HIV-1 RNA 3.0 Assay (bDNA) can be used in accordance with the guidelines provided in the *Report of the NIH Panel to Define Principles of Therapy of HIV Infection*[2], which states that "Changes greater than 0.5 $\log_{10}$ usually cannot be explained by inherent biological or assay variability and probably reflect a biologically and clinically relevant change...." (A 0.5 $\log_{10}$ change = 3.16 fold-change.).

Clinical Utility: Prognosis of Disease Progression

The relationship between HIV-1 quantitation and the prognosis of disease progression was studied in a subset of the 1,485 subjects enrolled in the Community Programs for Clinical Research on AIDS protocol 036 (CPCRA 036). This cohort study was designed as a 2-year study of HIV-1 infected subjects with < 300 CD4+ cells/mm^3 and less than Stage 2 AIDS Dementia Complex.

Specimens for this protocol were collected between October 1995 and February 1997. Baseline specimens from 201 subjects who had disease progression during follow-up and 201 matched controls (no disease progression during follow-up) were selected for testing by the VERSANT HIV-1 RNA 3.0 Assay (bDNA) and the Roche AMPLICOR Test (CPCRA Study Cohort). Selection and matching was performed by the CPCRA statistical center and was based on clinical enrollment site and randomization date.

The characteristics of the study population are shown in Table 10.

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[2] Ann Int Med 1998, 128: 1057-1078.

Table 10 Characteristics of the CPCRA 036 Study Population

Characteristic	Case (201)	Control (201)
Age (mean years)	39.5	40.0
Race (%)		
African-American	35.3%	34.8%
Caucasian	56.2%	55.7%
Latino	7.0%	8.5%
Other	1.5%	1.0%
Female (%)	12.4%	10.0%
CD4+ (mean cells/mm^3)	61.0	161.4
Average log HIV-1 RNA	4.5	3.9
Risk Factors		
Homosexual activity history only (%)	60.2%	62.7%
IV drug use history only (%)	11.4%	15.4%
History of homosexual activity and IV drug use (%)	7.0%	5.5%
Other risk factors	21.4%	16.4%
History of AIDS-defining event at baseline	58.7%	29.9%
No baseline antiretroviral (%)	10.0%	9.5%
Baseline nucleoside use (%)	90.0%	90.0%
Baseline protease inhibitor use (%)	11.4%	10.4%

Two methods were used to analyze the data.

- Logistic regression analysis was used to estimate the effect of baseline HIV-1 quantitations on disease progression, both unadjusted and adjusted for CD4+

cell count and other confounders. These effects were estimated as odds ratios.

- The baseline HIV-1 quantitations, CD4+ cell counts and other confounders were used in Cox proportional-hazards models to estimate the association of baseline HIV-1 RNA with disease progression. Relative risks of viral load measurements were calculated, both unadjusted and adjusted for CD4+ cell count and other confounders.

Survival curves were obtained for each HIV-1 RNA tertile. These curves were adjusted for the bias induced by the case-control sampling scheme using weights that are inversely proportional to the sampling probability.

In both of these models, the event of interest was the first AIDS defining event during the study period (excluding CD4 < 200). The follow-up time (days) was calculated from the date of the baseline measurement to the first study AIDS event. The covariates were defined as follows:

- Baseline HIV-1 RNA was divided into 3 groups based on tertiles rounded up to the nearest thousand. Grouping into tertiles facilitates the interpretation of results as it pertains to the practical use of the assay. Tertiles were defined separately for the VERSANT HIV-1 RNA 3.0 Assay (bDNA) and AMPLICOR Test according to their respective test result distributions.
- Baseline CD4+ cell count was included in the models as a binary variable. The CD4+ cell count median was used to define this variable taking a value of zero when CD4+ cell count was lower than the median and a value of one when CD4+ cell count was equal or higher than the median.
- Other confounders, such as gender, race and history of an AIDS-defining event at baseline, were included as indicator variables.
- An additional analysis was performed using the VERSANT HIV-1 RNA 3.0 Assay (bDNA) baseline HIV-1 RNA as a continuous variable instead of using tertiles.

Table 11 shows the odds ratios of an AIDS-defining event or death, with associated 95% confidence intervals, adjusted for differences in baseline CD4+ cells counts. Results are shown for the VERSANT HIV-1 RNA 3.0 Assay (bDNA) and the AMPLICOR Test tertiles.

Table 11 Association of Baseline HIV-1 RNA Tertile With Disease Progression, Including Death, Adjusted for Baseline CD4+ Cell Count (Odds Ratios)

VERSANT HIV-1 RNA 3.0 Assay				AMPLICOR Test			
Variable	Odds Ratio	95% CI	p-value	Variable	Odds Ratio	95% CI	p-value
HIV-1 RNA Tertile (copies/mL)				HIV-1 RNA Tertile (copies/mL)			
< 25,000	1.0 (Ref)			< 50,000	1.0 (Ref)		
25,000-150,000	2.1	1.1 , 3.9	0.03	50,000-250,000	1.9	1.0 , 3.6	0.04
>150,000	3.3	1.7 , 6.4	< 0.001	> 250,000	3.8	1.9 , 7.5	< 0.001
CD4+ Cells/mm^3				CD4+ Cells/mm^3			
> 100	1.0 (Ref)			> 100	1.0 (Ref)		
< 100	5.6	3.2 , 9.5	< 0.001	< 100	5.4	3.2 , 9.3	< 0.001

The adjusted odds ratios represent the odds of an AIDS-defining event or death for baseline HIV-1 RNA tertiles, controlling for the effect of baseline CD4+ cell counts. The adjusted odds ratios for the VERSANT HIV-1 RNA 3.0 Assay (bDNA) and the AMPLICOR test were both significantly higher than 1.00.

The odds ratios indicate that higher baseline HIV-1 RNA is associated with increased risk of disease progression or death, independent of baseline CD4+ cell counts, using either the VERSANT HIV-1 RNA 3.0 Assay (bDNA) or the AMPLICOR Test to measure viral load. Baseline CD4+ cell counts were also prognostic of disease progression, controlling for effect of baseline HIV-1 RNA.

Table 12 shows the risk ratios based on the Cox Proportional Hazards analysis.

Table 12 Association of Baseline HIV-1 RNA Tertiles With Disease Progression, Including Death, Adjusted for Baseline CD4+ Cell Count (Risk Ratios)

VERSANT HIV-1 RNA 3.0 Assay				AMPLICOR Test			
Variable	Risk Ratio	95% CI	p-value	Variable	Risk Ratio	95% CI	p-value
HIV-1 RNA Tertile (copies/mL)				HIV-1 RNA Tertile (copies/mL)			
< 25,000	1.0 (Ref)			< 50,000	1.0 (Ref)		
25,000-150,000	2.2	1.4 , 3.4	< 0.01	50,000-250,000	2.0	1.3 , 3.1	< 0.01
>150,000	2.9	1.8 , 4.7	< 0.01	> 250,000	3.5	2.1 , 5.6	< 0.01
CD4+ Cells/mm^3				CD4+ Cells/mm^3			
> 100	1.0 (Ref)			> 100	1.0 (Ref)		
< 100	4.7	3.3 , 6.8	< 0.01	< 100	4.6	3.2 , 6.6	< 0.01

The adjusted hazard for subjects with a high VERSANT HIV-1 RNA 3.0 Assay (bDNA) result or AMPLICOR Test result at baseline was significantly higher than the estimated hazard for subjects with a low HIV-1 RNA level at baseline. These findings indicate that higher baseline viral load, as measured by either the VERSANT HIV-1 RNA 3.0 Assay (bDNA) or the AMPLICOR Test, is associated with increased risk of disease progression or death, independent of baseline CD4+ cells counts. The Cox proportional hazards model yielded the same conclusions as those from the logistic model presented above.

Table 13 shows the Cox relative hazards or risk ratios for disease progression, as measured by continuous baseline HIV-1 RNA levels in the VERSANT HIV-1 3.0 Assay, adjusted for differences in baseline CD4+ cell counts. The risk ratio represents the estimated risk associated with an increase of one $\log_{10}$ copies/mL.

Table 13 **Association (Risk Ratio) of Baseline HIV-1 RNA with Disease Progression, Including Death, Adjusted for Baseline CD4+ Cell Count Using the VERSANT HIV-1 RNA 3.0 Assay (bDNA)**

Variable		Risk Ratio	95% Confidence Interval	p-value
Baseline HIV-1 RNA †		1.45	(1.1 - 1.9)	0.01
CD4+ (Cells/mm^3)	> 100	1.0 (Ref)		
	< 100	5.3	(3.7 - 7.6)	<0.01

† Relative risks for 10-fold (1 log10)–higher HIV-1 RNA level.

These data show that, regardless of baseline level of HIV-1 RNA and baseline CD4+ cell count, the risk of disease progression was increased by 45% for each one $\log_{10}$ copies/mL increase in viral load at baseline. The analysis using the logistical regression model showed similar results, as shown in Table 14, with a significance level of $p = 0.02$.

Table 14 **Association of Baseline HIV-1 RNA with Disease Progression, Including Death, Adjusted for Baseline CD4+ Cell Count (Odds Ratios) Using the VERSANT HIV-1 RNA 3.0 Assay (bDNA)**

Variable		Odds Ratio	95% Confidence Interval	p-value
Baseline HIV-1 RNA †		1.5	(1.1 - 2.1)	0.02
CD4+ (Cells/mm^3)	> 100	1.0 (Ref)		
	< 100	6.1	(3.6 - 10.4)	<0.01

† Relative risks for 10-fold (1 log10)–higher HIV-1 RNA level.

To illustrate the association between baseline VERSANT HIV-1 RNA 3.0 Assay (bDNA) results and baseline AMPLICOR Test results with disease progression,

while controlling for baseline CD4+ counts, Figures 3 through 6 show the survival curves by baseline HIV-1 RNA tertiles for subjects with baseline CD4+ counts <100 cells/mm^3 and with baseline CD4+ counts ≥ 100 cells/mm^3, separately. Figures 3 and 4 show the results using the VERSANT HIV-1 RNA 3.0 Assay (bDNA), and Figures 5 and 6 show the same analysis using the AMPLICOR Test.

Figure 3 **Progression of Disease by Baseline HIV-1 RNA Tertile Using the VERSANT HIV-1 RNA 3.0 Assay (bDNA), in Subjects with < 100 CD4+ Cells/mm^3**

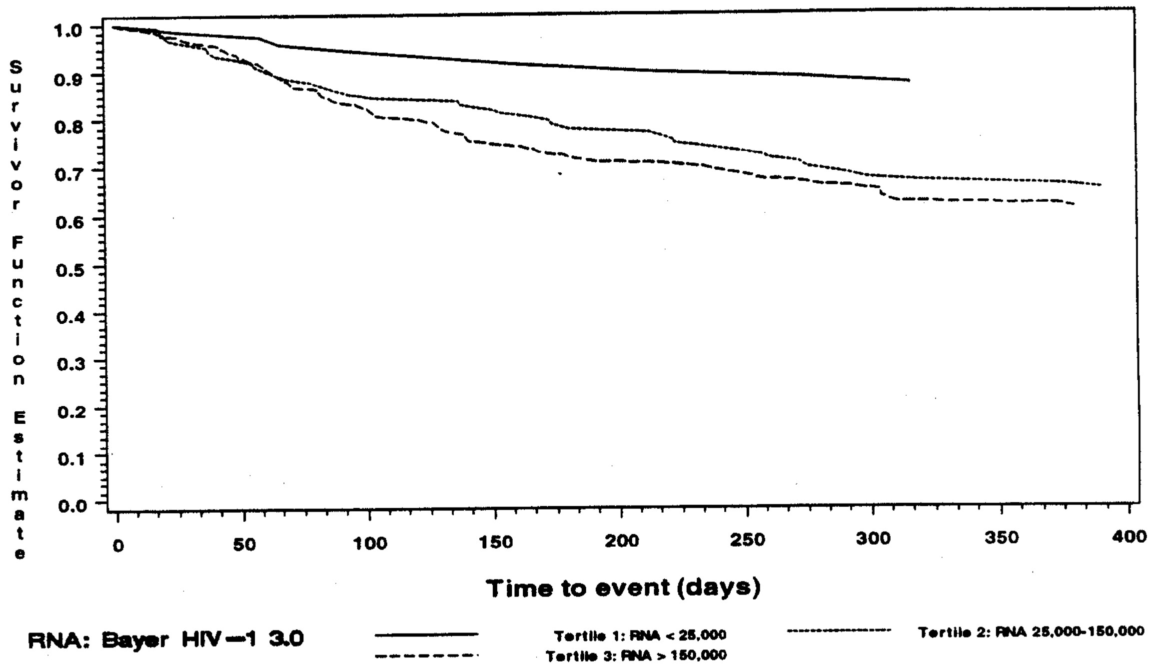


Figure 4 **Progression of Disease by Baseline HIV-1 RNA Tertile Using the VERSANT HIV-1 RNA 3.0 Assay (bDNA), in Subjects with ≥ 100 CD4+ Cells/mm^3**

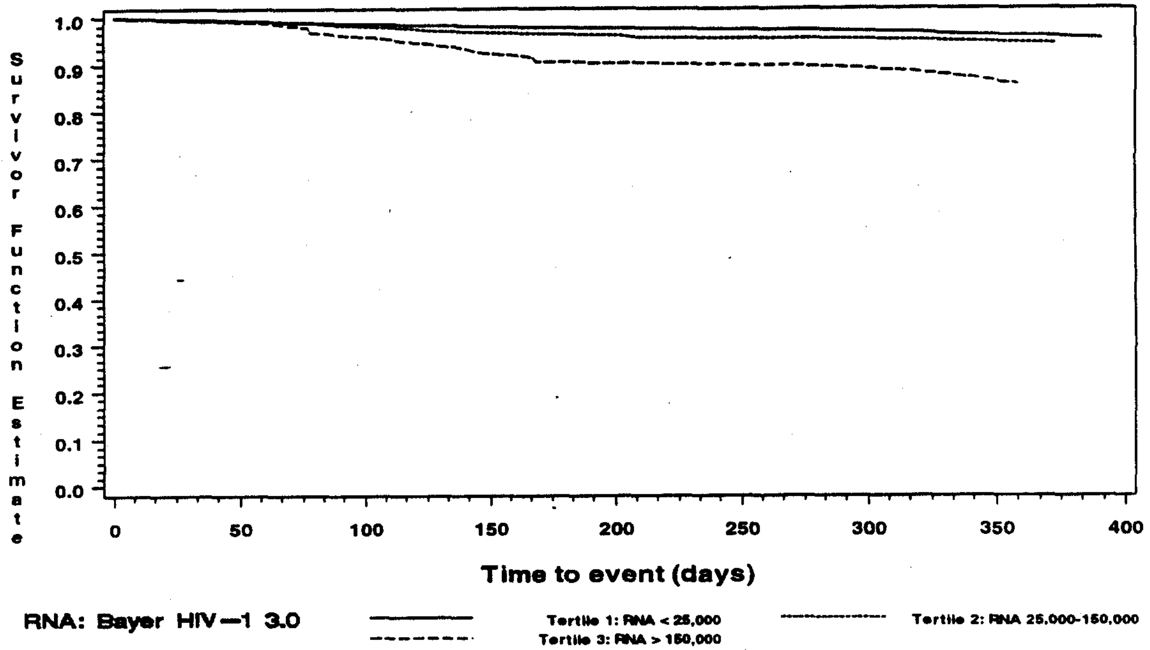


Figure 5 **Progression of Disease by Baseline HIV-1 RNA Tertile Using the AMPLICOR Test, in Subjects with <100 CD4+ Cells/mm^3**

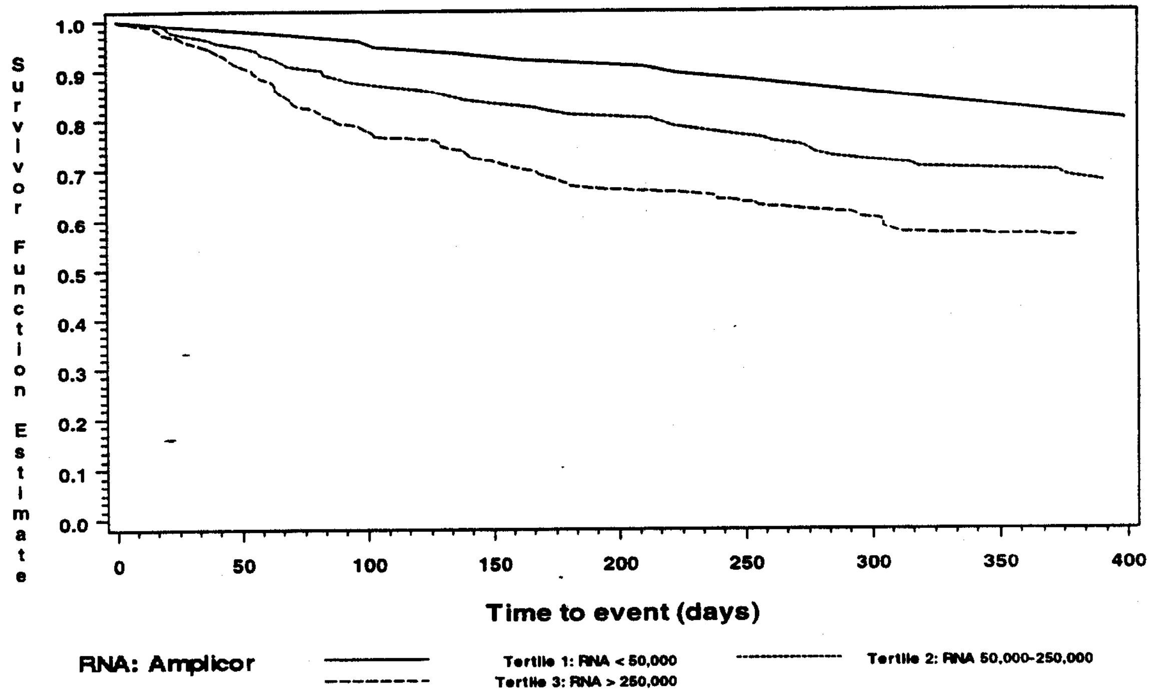
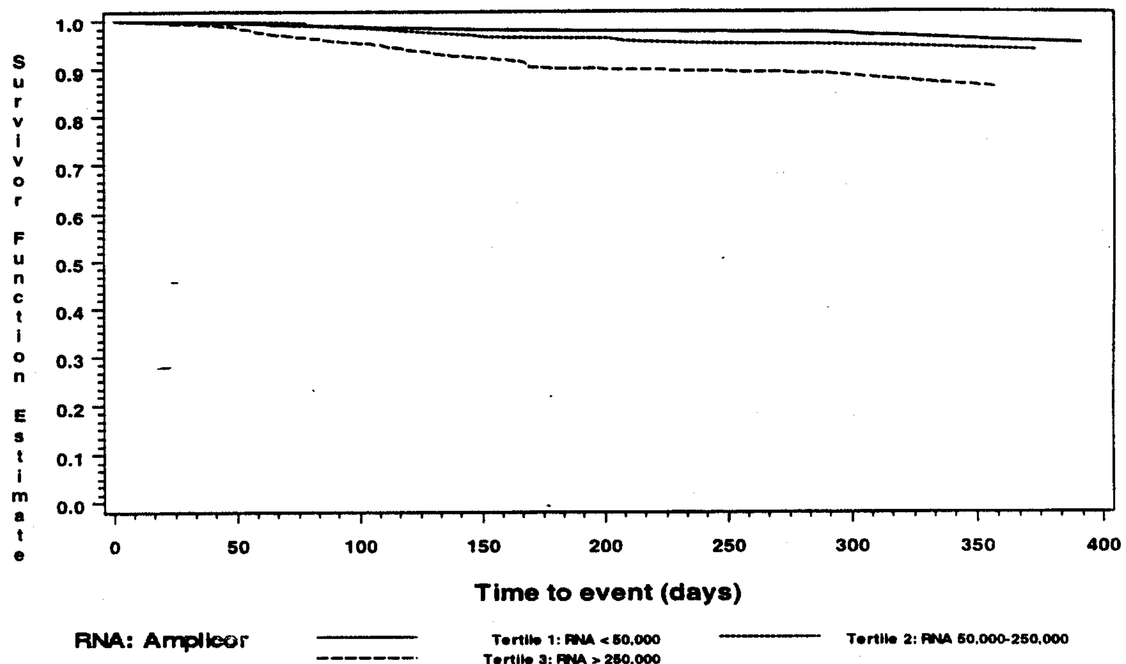


Figure 6 **Progression of Disease by Baseline HIV-1 RNA Tertile Using the AMPLICOR Test, in Subjects with ≥ 100 CD4+ Cells/mm^3**



These figures show that the subjects with a higher baseline viral load measure had a lower survival rate than subjects with a lower baseline, independent of their baseline CD4+ cell count. This effect was more apparent among patients with a low baseline CD4+ count.

Clinical Utility: Monitoring Study

Retrospectively collected serial specimens from 60 HIV-1 infected subjects were tested by the VERSANT HIV-1 RNA 3.0 Assay (bDNA) and the AMPLICOR Test. Data on clinical events, drug therapy and CD4+ cell counts were also collected through chart review. Data from the serial specimens collected in the Within Patient Variability Study were also used in the analyses. Sequential HIV-1 RNA

determinations from subjects who started antiretroviral therapy within the interval of 2 sequential blood draws were compared to determinations from subjects with no antiretroviral therapy or no change in antiretroviral therapy within the interval between 2 blood draws. The mean $\log_{10}$ quantitation decrease between sequential measures was significantly higher for sequential measures where antiretroviral therapy was started than for sequential measures where there was no antiretroviral therapy or continued therapy. These data are shown in Table 15. The Clinical Utility: Monitoring Study specimens used in the VERSANT analysis were drawn from 93 subjects with a total of 565[3] draws.

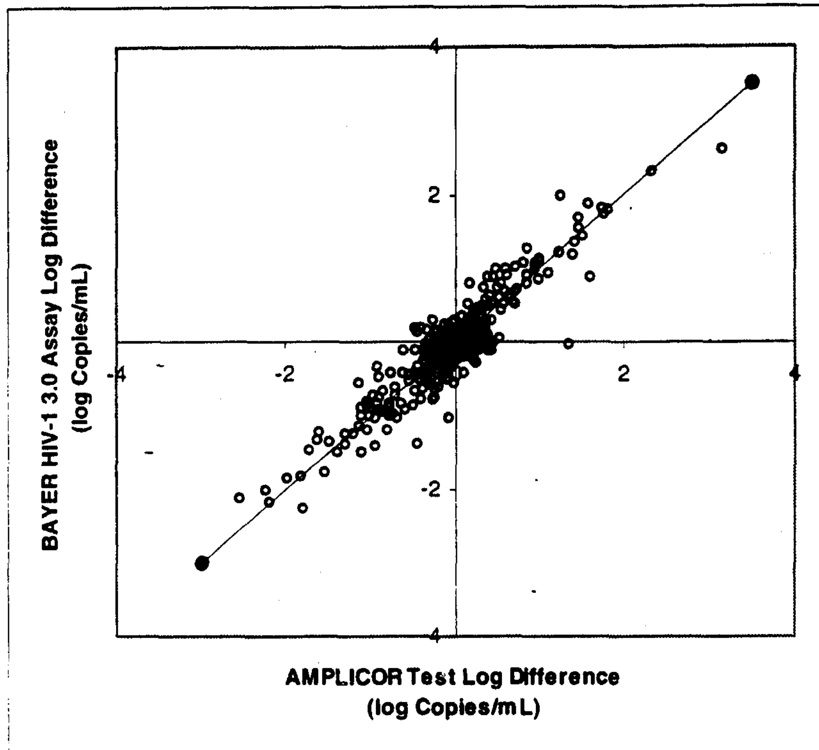
Table 15 — Mean Log_{10} Quantitation Decrease Between Sequential VERSANT Assay Measures With Lower and Upper 95% Confidence Limits, by Therapy

Drug Administration	N	Mean Log_{10} Quantitation Decrease Between 2 Sequential Measures	p-value
Continued drug therapy, no drugs, or non-antiretroviral drugs	398	0.13	<0.001
Started or changed antiretroviral drug	73	0.75	

The log changes between sequential measurements by the VERSANT Assay and AMPLICOR Test were evaluated. There was no evidence of statistically significant differences in the measurement of log changes between the two assays. Figure 7 is a scatter plot of all log changes as measured by the VERSANT HIV-1 RNA 3.0 Assay (bDNA) and the AMPLICOR Test. The line plotted is the 45-degree line through 0.

[3] The analysis is based on sequential results. The initial result for each patient cannot be used because it has no previous specimen with which to pair. Thus, the possible number of sequential

Figure 7 **Scatter Plot of VERSANT HIV-1 RNA 3.0 Assay (bDNA) Log Differences and AMPLICOR Test Log Differences with 45-Degree Line**



Comparison of the VERSANT HIV-1 RNA 3.0 Assay (bDNA) and the AMPLICOR Test in Clinical Specimens

Matched results from 936 clinical specimens were used to compare the HIV-1 RNA quantitations between the two assay methods. The scatter plot results are shown in Figure 8.

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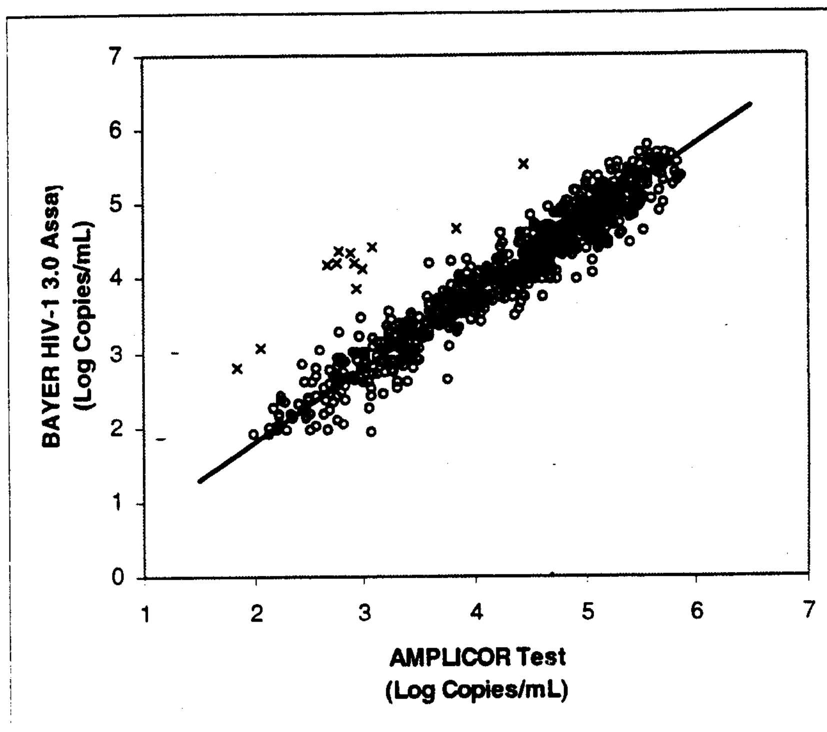
measures is 565 – 93 or 472. One result was missing, so the total number used in the analysis was 471.

Figure 8 **Scatterplot of VERSANT HIV-1 RNA 3.0 Assay (bDNA) Results and AMPLICOR Test Results with Best Fitting Best-Fitting 45-Degree Line**



Regression analysis showed that the VERSANT HIV-1 RNA 3.0 Assay (bDNA) quantitated specimens 39% lower than the AMPLICOR Test across the common reportable range of the two assays.

CONCLUSIONS DRAWN FROM STUDIES

Nonclinical Laboratory Studies

The nonclinical laboratory studies showed that the VERSANT HIV-1 RNA 3.0 Assay (bDNA) performance is consistent with the intended use for the assay.

- The quantitation limit was set appropriately, as shown in the Specificity and Reproducibility Studies, and the assay is linear and reproducible throughout the reportable range of 75 to 500,000 HIV-1 RNA copies/mL.

- Specimens with levels of triglycerides up to 2000 mg/dL, bilirubin up to 20mg/dL or hemoglobin up to 200 mg/dL showed no clinically significant interference with the HIV-1 RNA quantitation by this assay.

- Specimens undergoing up to 3 freeze-thaw cycles may be used in the assay.

- None of the therapeutic drugs tested interfered with assay performance, nor did viruses (at 10^3 PFU/mL or higher) or bacteria or fungi (at 10^2 CFU/mL).

- K_2 and K_3 EDTA and ACD Solution A anticoagulants can be used for specimen collection for the VERSANT HIV-1 RNA 3.0 Assay (bDNA). Plasma from EDTA and ACD Vacutainer tubes can be held at 2-8 °C for up to 48 hours before freezing. Plasma in K_2 Plasma Preparation Tubes can be held at room temperature for 24 hours prior to freezing.

- HIV-1 subtypes A-G quantitated within 3-fold of subtype B.

Clinical Studies

The clinical studies also showed that the assay performance is consistent with its intended use.

- The Assay is useful as an aid in the prognosis of disease progression. Using baseline specimens from subjects enrolled in the CPCRA 036 protocol, there was a statistically significant relationship between HIV-1 baseline quantitation and disease progression using 2 statistical models (logistic regression and Cox Proportional Hazards Analysis).

- The utility of HIV-1 RNA quantitations for monitoring patients was shown in 60 case studies where HIV-1 infected subjects were followed over time. The data showed how viral load determinations, when used with other clinical and laboratory information, can be used to aid in the decision of whether and when to initiate or change antiretroviral therapy.

- The Within Patient Variability data, when combined with estimates of variance from the Reproducibility Study, showed that a fold change of 3.2 or greater was unlikely to occur by chance and could be used as an indicator of potential change in clinical status. This number was consistent with the current treatment guidelines reported in the *Report of the NIH Panel to Define Principles of Therapy of HIV Infection*[4], which states that "Changes greater than 0.5 $\log_{10}$ usually cannot be explained by inherent biological or assay variability and probably reflect a biologically and clinically relevant change...." (A 0.5 $\log_{10}$ change = 3.16 fold-change.)

- The sensitivity, specificity, and nonspecificity study results all supported the safety and effectiveness of the assay.

- Specimens in the two Clinical Utility Studies (Prognosis of Disease Progression, Patient Monitoring), and a subset of the Clinical Sensitivity Study were tested by both the VERSANT HIV-1 RNA 3.0 Assay (bDNA) and the AMPLICOR Test.
    - In the Prognosis of Disease Progression analysis, the AMPLICOR Test provided statistically significant odds ratios and risk ratios for progression, which were equivalent to those for the VERSANT HIV-1 RNA 3.0 Assay (bDNA).

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[4] Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents. Department of Health & Human Services and the Henry J. Kaiser Foundation, 2001. Available at: http://hivatis.org/trtgdlns.html#AdultAdolescent.

- In the Monitoring Study, the two assays consistently "tracked" with each other; i.e., changes or lack of changes between serial determinations were consistent between the two assays. Based on the average log changes, the regression related to log changes and the concordance analysis, log changes as measured between the two assays were not significantly different.
- When matched results from all clinical specimens were combined into a single analysis, the scatter plot results were similar to those reported in the nonclinical Concordance and Precision Study. The VERSANT HIV-1 RNA 3.0 Assay (bDNA) quantitated specimens 39% lower than the AMPLICOR Test across the common reportable range of the two assays.

The results of these studies show that the VERSANT HIV RNA Assay is safe and effective for its intended use as an aid in the management of HIV-1 infected individuals.

Safety and Benefit/Risk Analysis

The VERSANT® HIV-1 RNA 3.0 Assay (bDNA) is an *in vitro* signal amplification nucleic acid probe assay for the direct quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in plasma of HIV-1 infected individuals. This test is intended for use in conjunction with clinical presentation and other laboratory markers as an indicator of disease prognosis by measuring baseline HIV-1 RNA levels and to monitor the effects of anti-retroviral therapy of HIV-1 RNA levels. The VERSANT HIV-1 RNA 3.0 Assay (bDNA) is not intended for use as a screening test for HIV infection or as a diagnostic test to confirm the presence of HIV infection.

The ability to measure viral load is a valuable tool in the management of HIV-1 infected patients. An increase in viral load has been shown to correlate with

progression of HIV-1 disease.[5,6,7] Studies have shown that viral load is useful in predicting outcome or survival time ,in determining the need to initiate, change or assess response to an antiretroviral regimen.[5,8,7,9] The performance of the product as described in the clinical and non-clinical studies above, indicate that this assay is useful in measuring baseline HIV-1 RNA for disease progression and to monitor the effects of anti-retroviral drug therapy, both of which would significantly benefit patients and outweigh any probable risk of injury or illness to the patient from its use.

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[9] Swanson B. HIV plasma viral load in the clinical setting: measurement and interpretation. J Assoc Nurses AIDS Care. 1997;8:21–2.