SUMMARY OF SAFETY AND EFFECTIVENESS DATA
AMPLICOR HIV-1 MONITOR™ TEST
(Standard/UltraSensitive)

I. GENERAL INFORMATION

Device Generic Name:	*In vitro* nucleic acid amplification test for the quantitation of HIV-1 RNA in human plasma.
Device Trade Name:	AMPLICOR HIV-1 MONITOR™ Test Standard/UltraSensitive
Applicant's Name and Address	Roche Molecular Systems, Inc. 1080 US Highway 202 Somerville, New Jersey 08876
Premarket Approval Application (PMA) Number:	BP950005/4
Date of Notice of Approval:	March 2, 1999

II. INDICATIONS FOR USE

The AMPLICOR HIV-1 MONITOR™ Test is an *in vitro* nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma. The Test can be used with either the Standard or UltraSensitive Specimen Processing Procedure. When the Standard Specimen Processing Procedure is used, the Test can quantitate HIV-1 RNA over the range of 400 - 750,000 copies/mL. When the UltraSensitive Specimen Processing Procedure is used, the Test can quantitate HIV-1 RNA over the range of 50 - 75,000 copies/mL.

The Test is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management of HIV-1 infected patients. The Test can be used to assess patient prognosis by measuring the baseline HIV-1 RNA level or to monitor the effects of antiretroviral therapy by measuring changes in plasma HIV-1 RNA levels during the course of antiretroviral treatment. Monitoring the effects of antiretroviral therapy by serial measurement of plasma HIV-1 RNA has been validated for patients with baseline viral loads ≥ 25,000 copies/mL.

The AMPLICOR HIV-1 MONITOR Test is not intended to be used as a screening test for HIV or as a diagnostic test to confirm the presence of HIV infection.

Quantitative measurements of HIV viremia in the peripheral blood have shown that higher viral levels may be correlated with a higher risk of disease progression and that reduction in viral load may be associated with decreased risk of clinical progression to acquired Immunodeficiency syndrome (AIDS).[1,2] HIV viral load measurement has been used to monitor the effect of antiretroviral therapeutic agents. The results of these measurements have shown that reduction in disease progression correlated with effective antiretroviral therapies and may be associated with decreased viral load.[3-5] Other studies suggest that higher viral burdens in HIV-infected pregnant women at the time of delivery were associated with greater risk of transmission of HIV to newborn infants.[6]

Viral load in the peripheral blood can be quantified by the measurement of HIV p24 antigen in serum or by quantitative culture of HIV isolated from plasma or peripheral blood mononuclear cells (PBMC).[7,8] The p24 antigen is the principle core protein of HIV; this antigen can either circulate freely in plasma or be bound by anti-p24 antibody. Although circulating p24 antigen can be measured using commercially

available enzyme immunoassays (EIA), the clinical utility of p24 as a marker of viral load is limited since the antigen is detectable in only 20% of asymptomatic and 40-50% of symptomatic patients. Plasma or PBMC-associated HIV virus can be cultured in activated PBMCs obtained from normal donors. However, quantitative culture is of limited utility since it is labor intensive, expensive, highly variable, and insensitive in asymptomatic patients.[9,10]

Unlike the methods described above, the AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) accurately and directly quantifies HIV-1 RNA levels in human plasma. In addition, the AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) provides accurate quantitative results in both symptomatic and asymptomatic patients and is less labor intensive than quantitative culture procedures.

III. DEVICE DESCRIPTION

(a) Test Principles

The AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) is intended for the quantitation of HIV-1 RNA in human plasma collected in standard blood collection tubes containing the anticoagulants acid citrate dextrose (ACD) or ethylenediaminetetraacetic acid (EDTA). Because heparin has been shown to inhibit the polymerase chain reaction (PCR), specimens anticoagulated with heparin are unsuitable for use with this test without further processing to remove heparin. The Test is based on the five major processes described below.

Specimen Preparation:

HIV-1 RNA is isolated from plasma by lysis of virus particles with a chaotropic agent, followed by precipitation of the RNA with alcohol. A known number of Quantitation Standard (QS) RNA molecules is introduced into each sample with the Lysis Reagent and the QS RNA is precipitated together with the HIV-1 RNA. The QS is carried through the specimen processing, amplification and detection steps and is used for the quantitation of HIV-1 RNA in the test specimen. The AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) can be used with either the Standard or UltraSensitive Specimen Processing Procedures. When the Standard Specimen Processing Procedure is used, the Test can quantitate HIV-1 RNA over the range of 400 - 750,000 copies/mL. When the UltraSensitive Specimen Processing Procedure is used, the Test can quantitate HIV-1 RNA over the range of 50 - 75,000 copies/mL.

Reverse Transcription of HIV-1 and QS Target RNA:

The reaction is performed with the recombinant thermostable enzyme *Thermus thermophilus* DNA polymerase (r*Tth* pol). In the presence of manganese and under the appropriate buffer conditions, r*Tth* pol has both reverse transcriptase and DNA polymerase activity. This allows reverse transcription and PCR amplification to occur in the same reaction mixture.

The region of HIV-1 RNA that is amplified is a 142 base sequence located in a highly conserved region of the HIV-1 *gag* gene, defined by the primers SK431 and SK462. These primers also amplify the QS transcript RNA. During the initial thermal cycling step, the reaction mixture is heated to allow the biotinylated downstream primer (SK431) to anneal specifically to the target region of both the HIV-1 and QS target RNA. The annealed primer is then extended by r*Tth* DNA polymerase using the target RNA as a template to produce biotinylated, DNA strands (cDNA) complementary to both the HIV-1 and QS RNA.

PCR Amplification of HIV-1 and QS Target DNA:

Following reverse transcription, the reaction mixture is heated to denature any remaining double- stranded RNA:cDNA hybrids and then cooled to allow the biotinylated upstream primer (SK462) to anneal to the

HIV-1 and QS cDNA. The temperature is raised and the upstream primer is extended by r*Tth* pol using the cDNA as a template to produce biotin-labeled DNA strands with the same sequences as the target HIV-1 and QS RNA. This completes the first cycle of PCR yielding a double stranded DNA copy (or amplicon) of each HIV-1 or QS RNA.

The reaction mixture is again heated to denature the DNA and expose the primer binding sites, cooled to allow the upstream and downstream primers to anneal to the separate DNA strands, and then heated for extension of the biotinylated primers by r*Tth* pol to generate new biotin-labeled amplicons. This process is repeated for a number of cycles, each cycle effectively doubling the amount of amplicon.

Hybridization of Amplified Products to Target-Specific DNA Probes:

After amplification, the HIV-1 and QS amplicons are chemically denatured to form single stranded DNA. The denatured amplicon preparations are added to designated wells of a microwell plate coated with HIV-1 specific oligonucleotide probe (SK102 coated on wells in Rows A through F) and QS-specific oligonucleotide probe (CP35 coated on wells in Rows G and H) and a series of 5-fold dilutions of the amplicons is made down the plate. The biotin-labeled HIV-1 and QS amplicons bind (hybridize) to the plate-bound HIV-1 specific and QS-specific probes and are thus captured onto the plate. This specific hybridization of the amplicons and probe thus increases the overall specificity of the reaction.

Detection of Amplified Product:

Following the hybridization reaction, the microwell plate is washed to remove unbound material and an Avidin-horseradish peroxidase (Av-HRP) conjugate is added to the plate. The avidin binds to the biotin-labeled amplicons captured by the plate-bound oligonucleotide probes. The microwell plate is washed again to remove unbound Av-HRP and a substrate mixture containing hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine (TMB) is added to each well. The substrate is converted to a colored complex by the horseradish peroxidase. The reaction is stopped by the addition of dilute sulfuric acid and the absorbance of each well is read at 450 nm using a microwell plate reader.

(b) HIV-1 Target Description

The region of HIV-1 RNA targeted for amplification by the AMPLICOR HIV-1 MONITOR Test is a highly conserved region of the *gag* gene. The *gag* region encodes the group-specific antigens or core structural proteins of the virion. The HIV-1 gag genes are generally about 1500 nucleotides in length and are located at the approximate positions 789-2290 in the HIV genome. The sequence targeted for amplification is a 142 bp sequence located within the *gag* gene.

(c) HIV-1 RNA Quantitation

The AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) was designed to quantitate viral load by utilizing a second target sequence (QS) added to the amplification mixture at a known concentration. The optical density in each well of the MWP is proportional to the amount of HIV-1 or QS amplicon in the well, and the total optical density, i.e., the product of the optical density of the well times the dilution factor for that well, is proportional to the amount of HIV-1 or QS RNA, respectively, input into each reverse transcription/PCR amplification reaction. The amount of HIV-1 RNA in each specimen is calculated from the ratio of the total optical density for the HIV-1 specific well to the total optical density for the QS-specific well and the input number of QS RNA molecules.

When the Standard Specimen Processing Procedure is used to prepare the specimen(s) for testing, the following equation is used for calculating the amount of HIV-1 RNA in each specimen.

$$\frac{\text{Total HIV-1 OD}}{\text{Total QS OD}} * \text{Input QS Copies per PCR reaction} * 40 = \text{HIV-1 RNA copies/mL}$$

The Total HIV-1 OD of the specimen is calculated by multiplying the optical density (OD) of the HIV well with the lowest OD in the range of 0.20 to 2.0 OD units by the dilution factor associated with that well. Similarly, the Total QS OD for the specimen is calculated by multiplying the OD of the QS well with the lowest OD in the range of 0.30 to 2.0 OD units by the dilution factor associated with that well. The copy level of the QS is lot specific and assigned by an analytical assay based on Poisson Analysis. The factor "40" is used to convert the HIV-1 RNA copies per PCR to HIV-1 RNA copies per mL of plasma.

When the UltraSensitive Specimen Processing Procedure is used to prepare the specimen(s) for testing, the following equation is used for calculating the amount of HIV-1 RNA in each specimen.

$$\frac{\text{Total HIV-1 OD}}{\text{Total QS OD}} * \text{Input QS Copies per PCR reaction} * 4 = \text{HIV-1 RNA copies/mL}$$

The Total HIV-1 OD of the specimen is calculated by multiplying the optical density (OD) of the HIV well with the lowest OD in the range of 0.20 to 2.0 OD units by the dilution factor associated with that well. Similarly, the Total QS OD for the specimen is calculated by multiplying the OD of the QS well with the lowest OD in the range of 0.30 to 2.0 OD units by the dilution factor associated with that well. The copy level of the QS is lot specific and assigned by an analytical assay based on Poisson Analysis. The factor "4" is used to convert the HIV-1 RNA copies per PCR to HIV-1 RNA copies per mL of plasma.

The HIV-1 MONITOR Quantitation Standard (QS) is an *in vitro* transcribed RNA molecule derived from a recombinant DNA plasmid designated pNAS2. To increase the likelihood of equivalent amplification efficiency between HIV target and the QS template, the primer-binding regions of the QS were designed to be identical to those of the HIV-1 target sequence. The QS therefore contains the SK431 and SK462 primer binding sites and generates a product of the same length (142 bases) and base composition as the HIV-1 target when reverse transcribed and amplified with the SK431 and SK462 primers. The probe binding region of the QS was modified to differentiate QS-specific amplicon from HIV-1 target specific amplicon.

(d) Primers and Probes

The AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) uses one primer set for the amplification of both the HIV-1 and QS targets. The two primers, SK462 (upstream) and SK431 (downstream), are single stranded oligonucleotides containing 30 and 27 bases, respectively. Each primer is biotinylated at the 5' end during synthesis. The SK431 and SK462 primers show greater than 90% homology with the target sequences. There is also significant homology between these primers and HIV-2 and Simian Immunodeficiency Virus (SIV) viral genomic sequences since these primers were initally designed to amplify both HIV-1 and HIV-2 viral targets.

The AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) uses two probes, an HIV-specific probe (SK102) for the detection of the HIV-1 target and a QS-specific probe (CP35) for the detection of the QS. Both probes are single-stranded DNA oligonucleotides containing 33 and 26 bases, respectively, and both are chemically conjugated to bovine serum albumin (BSA).

The HIV-specific detection probe, SK102, is a 33 base oligonucleotide derived from a highly conserved region of the HIV-1 *gag* gene with a sequence specific for positions 1402 -1435. Specificity of the SK102 probe for the HIV-1 target is demonstrated by greater than 93% identity for the 33 base pair overlap of the SK102 probe. There is also significant homology between the SK102 probe and HIV-2, SIV and non-HIV nucleic acid sequences. The apparent homology with the non-HIV-1 nucleic acid sequences will not compromise the specificity of the test because these sequences will not be amplified by the SK431 and SK462 primers due to lack of sequence homology between the non-HIV-1 sequences and the oligonucleotide primers.

The QS -specific detection probe, CP35, is a 26 base oligonucleotide that is specific for positions 8 - 33 of the pNAS2 transcript RNA. No significant homology was identified between the CP35 probe and any of the HIV sequences contained within the database.

(e) Specific and Selective Amplification

Selective amplification of HIV-1 and QS target RNA is achieved by the use of AmpErase® (uracil-N-glycosylase, UNG) and deoxyuridine triphosphate (dUTP). UNG catalyzes the degradation of DNA containing deoxyuridine, but not DNA containing thymidine. Deoxyuridine is not present in DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate as one of the dNTPs in the Master Mix; thus, only amplicon contain deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to degradation by UNG prior to amplification of the target DNA. AmpErase, which is included in the Master Mix, catalyzes the cleavage of oligonucleotides at deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step at the alkaline pH of the Master Mix, the opened chain causes the DNA to break at the position of the former deoxyuridine monophosphate (dUMP), thereby making the DNA non-amplifiable. AmpErase is inactive at temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. Following amplification, any residual enzyme is denatured by the addition of the Denaturation Solution, thereby preventing the degradation of target amplicon. AmpErase in the AMPLICOR HIV-1 MONITOR Test has been demonstrated to inactivate at least 10^3 copies of deoxyuridine-containing HIV-1 amplicon per PCR.

(f) Quality Control

The AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) contains three controls, the HIV-1 MONITOR High (+) Control, the HIV-1 MONITOR Low (+) Control, and the HIV-1 MONITOR (-) Control. When used in the Standard Specimen Preparation Procedure, the Low and High (+) Controls are equivalent to approximately 2500 copies/mL and 250,000 copies/mL, respectively, of non-infectious HIV RNA transcript, which is used to mimic the HIV-1 viral RNA target in the clinical specimen. When used in the UltraSensitive Specimen Preparation Procedure, the Low and High (+) Controls are equivalent to approximately 250 copies/mL and 25,000 copies/mL, respectively, of non-infectious HIV RNA transcript, which is used to mimic the HIV-1 viral RNA target in the clinical specimen. An expected range is assigned to each HIV-1 MONITOR (+) Control at the time of manufacture.

Non-infectious plasma-based controls that simulate patient specimens are prepared at the time of use by spiking the HIV-1 MONITOR Kit controls into negative human plasma. These controls are carried through the entire process and therefore monitor all aspects of the Test including specimen preparation, reverse transcription, amplification and detection. In this manner, the controls ensure that the entire test system is functioning properly and reliably. Because both the HIV-1 MONITOR High and Low (+) Controls and the HIV-1 MONITOR (-) Control are run with each test, effective monitoring of reagent stability, nucleic acid recovery during specimen preparation, the efficiency of the reverse transcription and PCR amplification reactions, and nucleic acid contamination is achieved.

(g) Kit Components

A list of the components of the AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) is contained in Table III-1

TABLE III-1

Components of the AMPLICOR HIV-1 MONITOR Test

AMPLICOR HIV-1 MONITOR KIT COMPONENT	*FORMULATION*
(1) HIV-1 MONITOR LYSIS REAGENT	Buffer containing guanidinium thiocyanate, dithiothreitol and glycogen, 2 x 9.0 mL
(2) HIV-1 MONITOR QS	TRIS buffer containing QS RNA, carrier RNA, EDTA and 0.05% sodium azide, 2 x 9.0 mL
(3) HIV-1 MONITOR SPECIMEN DILUENT	TRIS buffer containing EDTA, carrier RNA and 0.05% sodium azide as a preservative, 2 x 4.8 mL
(4) NEGATIVE HUMAN PLASMA	Negative human plasma (non-reactive by FDA licensed tests for antibody to HCV, HIV 1/2 and HBsAg) containing 0.1% ProClin 300™ as a preservative, 2 x 0.7 mL
(5) HIV-1 MONITOR (-) CONTROL	TRIS buffer containing carrier RNA, EDTA and 0.05% sodium azide as a preservative, 2 x 0.05 mL
(6) HIV-1 MONITOR LOW (+) CONTROL (2500 Copies HIV RNA/mL - Standard) (250 Copies HIV RNA/mL - UltraSensitive)	TRIS buffer containing EDTA, non-infectious HIV RNA transcript, carrier RNA and 0.05% sodium azide as a preservative, 2 x 0.05 mL
(7) HIV-1 MONITOR HIGH (+) CONTROL (250,000 Copies HIV RNA/mL - Standard) (25,000 Copies HIV RNA/mL - UltraSensitive)	TRIS buffer containing EDTA, non-infectious HIV RNA transcript, carrier RNA and 0.05% sodium azide as a preservative, 2 x 0.05 mL
(8) HIV-1 MONITOR MASTER MIX	Buffer containing <30% glycerol, potassium acetate, <0.001% DTP, DTP, DTP, dUTP, biotinylated SK431 and SK462 primers, <0.01% r*Tth* pol, <0.01% AmpErase and 0.05% sodium azide as a preservative, 2 x 0.5 mL
(9) HIV-1 MONITOR MANGANESE SOLUTION	Manganese acetate solution with 0.05% sodium azide as a preservative., 2 x 0.1 mL
(10) MONITOR DENATURATION SOLUTION	EDTA solution containing 1.6% sodium hydroxide and amaranth dye, 1 x 12 mL
(11) MONITOR HYBRIDIZATION BUFFER	Sodium phosphate buffer containing <0.02% solubilizer and <25% chaotrope, 2 x 12 mL
(12) AVIDIN-HRP CONJUGATE	Avidin-horseradish peroxidase conjugate in a buffered solution containing 1% ProClin 150™ as a preservative, emulsifier, BSA and 0.1% phenol, 2 x 12 mL
(13) SUBSTRATE A	Citrate buffer containing 0.01% H_2O_2 and 0.1%ProClin 150™ as a preservative, 2 x 12 mL
(14) SUBSTRATE B	0.1% 3,3',5,5'-Tetramethylbenzidine (TMB) in 40% dimethylformamide (DMF), 2 x 3 mL
(15) STOP REAGENT	4.9% Sulfuric acid, 2 x 12 mL
(16) 10X-WASH CONCENTRATE	Sodium phosphate and sodium salt solution containing EDTA, <2% detergent and 0.5% ProClin 300™ as a preservative, 3 x 90 mL
(17) HIV-1 MONITOR MICROWELL PLATE	Oligonucleotide probe coated microwell plate. Twelve, 8-well strips in one resealable pouch with desiccant. HIV-1 specific DNA Probe (SK102) coated on Rows A-F and QS-specific DNA Probe (CP35) coated on Rows G-H., 2 plates (12 x 8 wells each)

IV. WARNINGS AND LIMITATIONS OF USE

- Monitoring the effects of antiretroviral therapy by serial measurement of plasma HIV-1 RNA has only been validated for patients with baseline viral loads ≥ 25,000 copies/mL.
- The performance of the AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) has only been validated with HIV subtype B specimens.
- When testing specimens with viral load <200 copies/mL, the user should consider the use of well-characterized reference materials titered from 50 copies/mL to 200 copies/mL, inclusive.

1. The AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) is not intended to be used as a screening test for HIV or as a diagnostic test to confirm the presence of HIV infection.

2. With the Standard procedure, the AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) can accurately detect a 0.5 $\log_{10}$ (3-fold) or greater change in HIV-1 RNA if the viral load is greater than 1000 copies/mL and a 0.78 $\log_{10}$ (6-fold) or greater change when the viral load is 400 - 1000 copies/mL. With the UltraSensitive procedure, the Test can accurately detect a 0.39 $\log_{10}$ (2.5-fold) or greater change in HIV-1 RNA when the viral load is 75 - 75,000 copies/mL, a 0.44 $\log_{10}$ (2.8-fold) or greater change when the viral load is approximately 75 copies/mL, and a 0.68 log10 (5-fold) or greater change in HIV-1 RNA for patients whose viral load is approximately 50 copies/mL. Changes in viral load below these levels may not be clinically relevant.

3. The utility of plasma HIV-1 RNA in surrogate endpoint determinations has not been fully established.

4. Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.

5. This test has been validated for use only with human plasma anticoagulated with EDTA or ACD. Heparin inhibits PCR; specimen collected using heparin as the anticoagulant should not be used with the AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive).

6. The presence of AmpErase in the AMPLICOR HIV-1 MONITOR Master Mix reduces the risk of amplicon contamination. However, contamination from HIV positive controls and HIV positive clinical specimens can be avoided only by good laboratory practices and careful adherence to the procedures specified in this insert.

7. Use of this product should be limited to personnel trained in the techniques of PCR.

8. As with any diagnostic test, results from the AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) should be interpreted with consideration of all clinical and laboratory findings.

9. Elevated levels of lipids, bilirubin and hemoglobin in specimens have been shown not to interfere with the quantitation of HIV-1-RNA by this test.

10. The following drug compounds have been shown not to interfere with the quantitation of HIV-1-RNA by this test: AZT, ddI, ddC, d4T, HBY 097, nevirapine, saquinavir, isoniazid, foscarnet and ganciclovir.

V. *ALTERNATIVE PRACTICES AND PROCEDURES*

There are currently a variety of direct and indirect methods for the detection and quantitation of human immunodeficiency virus in clinical specimens. These methods provide a means of measuring the progression of disease associated with HIV infection and a patient's response to antiretroviral therapy. These are listed below:

- *surrogate markers including CD4 and CD8 cell-surface receptors*
- *ELISA, EIA and immunoblot procedures for measuring HIV antibody production*
- *HIV antigen assays including p24 Ag and ICD p24 Ag tests*
- *co-culture isolation and quantitation procedures with core antigen detection*
- *nucleic acid probe technologies for direct detection and quantitation of circulating viral particles*

VI. *POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH*

The possibility of erroneous results exists due to test malfunction or operator error. An erroneously high test results, indicating therapeutic failure and/or a higher likelihood of progression to AIDS or death, may result in unnecessary treatment and/or psychological trauma to a patient. An erroneously low test result may lead to the lack of appropriate treatment and/or a false sense of security by a patient which could lead to a worsening of the patient's condition. The risks of erroneous test results are inherent in all *in vitro* diagnostic products.

Based upon the performance of the product in the clinical studies, the probable benefit to the patient from the use of the product greatly outweighs any probable risk of injury or illness to the patient from its use.

VII. *MARKETING HISTORY*

The AMPLICOR HIV-1 MONITOR Test is currently being commercially marketed in the following countries. The AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) has not been commercially marketed in these countries.

Argentina	Cyprus	Indonesia	Netherlands	Spain	United Kingdom
Australia	Denmark	Israel	Philippines	Switzerland	United States
Belgium	France	Italy	Portugal	Taiwan	Venezuela
Brazil	Germany	Japan	Russia	Thailand	
Canada	Greece	Korea	South Africa	Turkey	

The AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) has not been withdrawn from marketing in any country for reasons related to safety or effectiveness or for any other reason.

VIII. *SUMMARY OF STUDIES*

1. Summary of Non-Clinical Studies

(a) Analytical Sensitivity

The analytical sensitivity of the AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) was established by determining the minimum number of copies of HIV-1 RNA that can be reproducibly detected by the test procedure. This was accomplished by analyzing serial dilutions of purified HIV RNA

transcript. The results demonstrate that the Test could detect less than 2 copies of HIV RNA per reaction, and that 7.5 or more copies of HIV RNA per reaction were detected 100% of the time.

(b) Limit of Detection/Quantitation

The lower limit of detection of the AMPLICOR HIV MONITOR Test (Standard/UltraSensitive) was determined by analysis of reconstructed HIV-positive plasma specimens, prepared by serial dilution of well characterized stocks of cultured HIV into HIV negative human plasma.

Standard procedure

Independent studies with blinded specimens were conducted in Europe and the United States. In the United States the performance of the Test was evaluated at RMS and in several academic laboratories with blinded specimens provided by the Virology Quality Assurance (VQA) Laboratory of the AIDS Clinical Trials Group (ACTG), Division of AIDS, National Institutes of Health. The concentration of viral RNA in the virus stocks was estimated by electron microscopy, p24 antigen, HIV-1 MONITOR Testing, and branched chain DNA analysis. The limit of detection was assessed by tabulating the number and percentage of assays for which HIV-1 RNA could not be determined for each specimen in the panels. These studies demonstrated that the HIV-1 MONITOR Test can quantitate virion associated HIV-1 RNA in plasma at concentrations as low as 400 RNA copies/mL plasma provided that the OD of the selected microwell is within the specified OD range (0.2 to 2.0).

UltraSensitive procedure

Studies were conducted at three independent laboratories in the United States using two panels of 32 blinded specimens each, prepared using well characterized stocks of HIV spiked into HIV-negative human plasma. The concentration of viral RNA in the blinded specimens was determined prior to the study by multi-site analysis. These studies demonstrated that the AMPLICOR HIV-1 MONITOR Test can quantitate virion associated HIV-1 RNA in plasma at concentrations as low as 50 RNA copies/mL plasma provided that the OD of the selected microwell is within the specified OD range (0.2 to 2.0).

(c) Linear Range

The linear range of the AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) was determined based on the analysis of serial dilutions of well characterized stocks of cultured HIV-1 prepared in HIV-negative human plasma.

Standard procedure

An HIV-1 virus stock, characterized by electron microscopy and p24 antigen analysis, was serially diluted in HIV negative plasma and the dilutions were then tested in duplicate by three separate laboratories. The test results from the study sites were combined and then plotted on a log-log scale. The AMPLICOR HIV-1 MONITOR Test with Standard Specimen Processing was found to give a linear response between 400 and 750,000 HIV-1 RNA copies/mL. Specimens with results greater than 750,000 HIV-1 RNA copies/mL must be diluted with HIV-negative human plasma and retested.

UltraSensitive procedure

Serial dilutions of two well characterized viral stocks were tested in replicates of six by three separate laboratories. The test results from the study sites were combined and then plotted on a log-log scale. The AMPLICOR HIV-1 MONITOR Test with UltraSensitive Specimen Processing was found to give a linear response between 50 and 75,000 HIV-1 RNA copies/mL. Samples with results greater than 75,000 HIV-1 RNA copies/mL should be retested using the AMPLICOR HIV-1 MONITOR Test with Standard Specimen Processing.

RNA copies/mL should be retested using the AMPLICOR HIV-1 MONITOR Test with Standard Specimen Processing.

(d) Correlation Between Standard and UltraSenstive Specimen Processing Procedures

In studies conducted at three separate laboratories, 310 patient specimens were tested by the AMPLICOR HIV-1 MONITOR Test using both the Standard and the UltraSensitive Specimen Processing procedures. The overall correlation for results combined across laboratories was 0.91 with a bias towards lower values (0.1 - 0.2 $\log_{10}$ lower) by the UltraSensitive procedure.

(e) Precision

Within-Run and Total Precision were evaluated according to the methods defined in the NCCLS Guideline (EP5-T2), "Evaluation of Precision Performance of Clinical Chemistry Devices". This procedure permits the determination of both Within-Run and Total Precision through the performance of a multiple day and multiple operator study.

Standard procedure

The precision of the AMPLICOR HIV-1 MONITOR Test with Standard Specimen Processing was evaluated using 6 specimens at different HIV-1 RNA concentrations. The study was performed in the Roche Molecular Systems Diagnostic Development laboratories. A run consisting of 24 tests was performed daily for 10 days by each of 2 operators. Since only 12 specimens can be run on a single HIV-1 MONITOR MWP, this required each operator to run the test on 2 separate microwell plates each day. The order of specimens in each run was changed each day. Each run included 4 replicates of each of 6 specimens. The six specimens were taken through the entire HIV-1 MONITOR Test procedure, including sample preparation using the Standard Specimen Preparation procedure, reverse transcription, PCR amplification and detection. Therefore, the precision reported here takes into account all aspects of the test procedure. The calculation of Within-Run and Total Precision was performed according to the methods defined in the EP5-T2 Guideline. The results from this study are shown in Table VIII-1.

UltraSensitive Procedure

The precision of the AMPLICOR HIV-1 MONITOR Test with UltraSensitive Specimen Processing was evaluated by testing 6 specimens prepared by spiking basematrix plasma with HIV-1 virus stock to the following concentrations (75, 500, 2500, 25,000 and 50,000 copies/mL). Testing was performed at 3 different laboratories. All specimens were tested in replicates of 4 on each of 10 days at each laboratory. The 6 specimens were taken through the entire HIV-1 MONITOR Test procedure, including sample preparation using the UltraSensitive Specimen Preparation procedure, reverse transcription, PCR amplification and detection. Therefore, the precision reported here takes into account all aspects of the test procedure. The calculation of Within-Run and Total Precision was performed according to the methods defined in the EP5-T2 Guideline. The results from this study are shown in Table VIII-2.

Table VIII-1
Precision of the AMPLICOR HIV-1 MONITOR Test
with Standard Specimen Processing

Sample	(–) Control	Low (+) Control*	High (+) Control*	1†	2†	3†
Total replicates	80	80	80	80	80	80
Mean copies/mL	none	2,042	768,767	2,108	39,386	1,156,875
WITHIN-RUN						
Standard Deviation	-	4	229,499	2,071	12,937	398,522
CV (%)	-	30.8	29.8	98.2	32.8	34.4
TOTAL						
Standard Deviation	-	660	325,361	2,209	16,169	524,528
CV (%)	-	32.3	42.3	104.8**	41.1	45.3

Table VIII-2
Precision of the AMPLICOR HIV-1 MONITOR Test
with UltraSensitive Specimen Processing

Sample	1†	2†	3†	4†	5†
Nominal copies/mL	75	500	2,500	25,000	50,000
Mean copies/mL	86.9	536	2,425	21,715	41,282
Total replicates	120	120	120	120	120
WITHIN-RUN					
Standard Deviation	27.4	120	502	6,130	11,846
CV (%)	31.6	22.5	20.7	28.2	28.7
TOTAL					
Standard Deviation	29.1	162	623	8,902	17,313
CV (%)	33.5	30.2	25.7	41.0	41.9

* - RNA Transcript
† - Dilutions of viral stocks in human plasma

(f) Reproducibility Studies

The reproducibility of the AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) was determined by assessing intra-assay, inter-assay and inter-lab variability.

Standard procedure

Reproducibility data were generated by testing four panels of specimens at multiple laboratories over a one year period. The study was performed by 49 laboratories participating in the AIDS Clinical Trial Group (ACTG) Virology Quality Assurance (VQA) Program. In total, the panels included 22 separate specimens (9 patient specimens and 13 spiked plasma specimens). The patient specimens ranged in concentration from 500 to 364,000 copies/mL; the spiked specimens ranged in concentration from 500 to 275,000 copies/mL. A total of 2,294 test results were generated for the specimens from the four panels.

The analysis of assay variability included separate assessments of Intra-Assay, Inter-Assay, Inter-Laboratory and Total variance for specimens with HIV-1 RNA viral loads ≥ 1000 copies/mL and for specimens with HIV-1 RNA viral loads of approximately 500 copies/mL. The results from the analysis of the data for specimens with HIV-1 RNA levels of approximately 500 copies/mL and ≥1000 copies/mL are shown in Table VIII 3. The analyses were performed after the test results were log10 transformed.

To assess assay reproducibility at approximately 500 copies/mL and at ≥ 1000 copies/mL level, the standard deviation of the sum of the two important sources of assay variance (Intra-Assay and Inter-Assay) was determined and the 95% confidence interval for the standard deviation was calculated. Table VIII-4 contains the results of these calculations. These data show that the AMPLICOR HIV-1 MONITOR™ Test can accurately detect a 0.5 $\log_{10}$ (3-fold) or greater change in HIV-1 RNA for patients whose viral load is ≥ 1000 copies/mL and accurately detect a 0.78 $\log_{10}$ (6-fold) or greater change in HIV-1 RNA for patients whose viral load is below 1000 copies/mL.

UltraSensitive procedure

Reproducibility data were generated by testing 5 samples at three laboratories over a period of 10 days. The samples were prepared by dilution of viral stocks in HIV-negative human plasma and ranged in concentration from 75 to 50,000 copies/mL. The analysis of assay variability included separate assessments of Intra-Assay, Inter-Assay, Inter-Laboratory and Total variance for specimens with HIV-1 RNA viral loads ≥ 75 copies/mL and for specimens with HIV-1 RNA viral loads of approximately 75 copies/mL and viral loads of approximately 50 copies/mL. The results from the analysis of these specimens are shown in Table VIII-3. The analyses were performed after the test results were log10 transformed.

To assess assay reproducibility at approximately 50 copies/mL, 75 copies/mL and ≥ 75 copies/mL, the standard deviation of the sum of the two important sources of assay variance (Intra-Assay and Inter-Assay) was determined and the 95% confidence interval for the standard deviation was calculated. Table VIII-4 contains the results of these calculations. These data show that the AMPLICOR HIV-1 MONITOR™ Test with UltraSensitive Specimen Processing can accurately detect a 0.39 $\log_{10}$ (2.5-fold) or greater change in HIV-1 RNA for patients whose viral load is ≥ 75 copies/mL and a 0.44 $\log_{10}$ (2.8-fold) or greater change in HIV-1 RNA for patients whose viral load is approximately 75 copies/mL, and a 0.68 log10 (5-fold) or greater change in HIV-1 RNA for patients whose viral load is approximately 50 copies/mL.

Table VIII-3
Components of Variance - AMPLICOR HIV-1 MONITOR Test:
Log10 Estimated RNA

RNA Level	# Labs	Variance Component	Standard Deviation	Variance	% of Total Variance
50 copies/mL (UltraSensitive Procedure)	3	Total	0.2482	0.0616	100
		Inter-Lab	0.0316	0.0010	1.6
		Inter-Assay	0	0	0
		Intra-Assay	0.2462	0.0606	98.4
75 copies/mL (UltraSensitive Procedure)	3	Total	0.2045	0.0422	100
		Inter-Lab	0.1288	0.0166	39.3
		Inter-Assay	0.0265	0.0007	1.7
		Intra-Assay	0.1579	0.0249	59.0
≥75 copies/mL (UltraSensitive Procedure)	3	Total	0.1661	0.0276	100
		Inter-Lab	0.0906	0.0082	29.8
		Inter-Assay	0.0814	0.0066	24.0
		Intra-Assay	0.1130	0.0128	46.2
500 copies/mL (Standard Procedure)	18	Total	0.2966	0.0880	100
		Inter-Lab	0.1131	0.0128	14.5
		Inter-Assay	0.0470	0.0022	2.5
		Intra-Assay	0.2702	0.0730	83.0
≥1000 copies/mL (Standard Procedure)	29	Total	0.1861	0.0346	100
		Inter-Lab	0.0453	0.0021	5.9
		Inter-Assay	0.1021	0.0104	30.1
		Intra-Assay	0.1489	0.0222	64.0

Table VIII-4
Assay Reproducibility - AMPLICOR HIV-1 MONITOR Test

RNA Level	Σ of Variance*	SD of Variance	95% CI
50 copies/mL (UltraSensitive Procedure)	0.0606	0.3481	± 0.68 log10
75 copies/mL (UltraSensitive Procedure)	0.0256	0.2263	± 0.44 log10
≥ 75 copies/mL (UltraSensitive Procedure)	0.0194	0.1970	± 0.39 log10
500 copies/mL (Standard Procedure)	0.0752	0.3878	± 0.76 log10
≥ 1000 copies/mL (Standard Procedure)	0.0326	0.2553	± 0.50 log10

* for Inter-Assay and Intra-Assay Variance

(g)– Validation of Assay Specificity

Analytical Specificity:

The analytical specificity of the AMPLICOR HIV-1 MONITOR Test was evaluated by adding cultured cells, cultured virus or purified nucleic acid from the following organisms and viruses to HIV negative human plasma (after step B.7 of the Instructions for Use) then analyzing these spiked samples. None of the non-HIV organisms, viruses or purified nucleic acids tested showed reactivity in the AMPLICOR HIV-1 MONITOR Test. Three of the four HIV-2 isolates that were tested gave positive results. Only HIV-2 subtype A (isolate 7824A) was not detected by the AMPLICOR HIV-1 MONITOR Test, however no specific claims can be made for the ability of this test to amplify HIV-2 isolates.

Adenovirus type 2	*Hepatitis B Virus*	*Human papilloma virus 11*
Adenovirus type 3	*Hepatitis C Virus*	*Human papilloma virus 18*
Adenovirus type 7	*HIV-2BEN*	*HTLV I/II*
Cytomegalovirus (AD-169)	*HIV-2, subtype A/B (isolate 7312A)*	*Mycobacterium avium*
Cytomegalovirus Davis	*HIV-2 subtype A (isolate 60415K)*	*Propionibacterium acnes*
Epstein-Barr virus P-3	*HIV-2 subtype A (isolate 7824A)*	*Pneumocystis carinii*
Epstein-Barr virus HR1	*Herpes simplex type I*	*Staphylococcus aureus*
Epstein-Barr virus	*Herpes simplex type II*	*Staphylococcus epidermidis*

Limited testing has been performed on HIV-1 subtypes other than Subtype B. Preliminary experimental data in which synthetic nucleic acid constructs were used to represent the HIV-1 subtype mismatches with primers SK462 and SK431 indicate that non-B subtypes of HIV-1 will be amplified by the AMPLICOR HIV-1 MONITOR Test with reduced efficiencies. The reduced amplification efficiency depends upon the number of mismatches with the SK462 and SK431 primers. Group O specimens will not be amplified by this test.

The AMPLICOR HIV-1 MONITOR Test will amplify HIV-1 DNA if it is present in the processed specimen. In-house testing using a modification of the AMPLICOR HIV-1 MONITOR Test which made the test specific and more sensitive for the qualitative detection of HIV-1 DNA was performed on fifty five HIV positive plasma specimens. This testing showed that fourteen specimens had plasma DNA present. Five of the fourteen specimens that were shown to contain plasma DNA were retested by the AMPLICOR HIV-1 MONITOR Test but without the reverse transcription step so that only plasma DNA would be amplified. The results from these tests showed that DNA was undetectable by the AMPLICOR HIV-1 MONITOR Test in 4 of the specimens and contributed to less than 10% of the apparent HIV-1 RNA test result in the other specimen.

Analysis of Seronegative Donors:

To determine the clinical specificity of the AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive), 495 HIV seronegative blood donors were tested. None of these specimens was reactive with the Test. Assuming a zero prevalence of HIV-1 infection in the seronegative blood donors, the specificity was 100%.

(h) Interference Studies

Interference studies have been performed and show that elevated levels of bilirubin (21 mg/dL), triglycerides (1024 mg/dL) and hemoglobin (19.5 mg/dL) do not interfere with the quantitation of HIV-RNA by the AMPLICOR HIV-1 MONITOR Test when using either the Standard or UltraSensitive Specimen Processing Procedures. Current antiretroviral and OI (opportunistic infection) therapeutic agents have also been evaluated for interfering effects on the AMPLICOR HIV-1 MONITOR Test. These studies found that the following drug compounds did not interfere with the quantitation of HIV-1 RNA in the AMPLICOR HIV-1 MONITOR Test: AZT, ddI, ddC, d4T, HBY097, Nevirapine, INVIRASE (saquinavir), foscarnet, ganciclovir and isoniazid.

(i) Validation of Blood Collection and Processing

Studies on the collection and storage of specimens for use with the AMPLICOR HIV-1 MONITOR Test have been performed to validate the collection and storage instructions contained in the product labeling. A summary of the findings from these studies is contained in Table VIII-5

Table VIII-5
Specimen Collection and Storage Conditions

Collection/Processing Condition	Validated Conditions
Anticoagulant	ACD or EDTA only
Whole Blood Storage	Up to 6 hours
Plasma Separation from Cells	Within 6 hours of collection
Virus Pellet Storage	Up to 6 hours at 25°C Up to 14 days at -20°C or colder
Plasma Storage	Up to 1 day at 25°C Up to 5 days at 2-8°C Long Term Storage at -20 to -80°C
Freeze/Thaw Cycles	No Effect for 3 freeze/thaw cycles

(i) AmpErase Effectiveness

AmpErase (uracil-N-glycosylase, UNG) recognizes and catalyzes the degradation of DNA containing deoxyuridine, but not DNA containing thymidine. Since deoxyuridine is not present in naturally occurring DNA but is always present in amplicon due to the use of deoxyuridine triphosphate in place of thymidine triphosphate as one of the dNTPs in the Master Mix reagent, only target amplicon contain deoxyuridine and therefore are susceptible to UNG-mediated degradation prior to amplification of the target DNA.

HIV-1 MONITOR Master Mix is formulated to contain 10 units of AmpErase per amplification reaction. In order to evaluate the performance of the AmpErase in the HIV-1 MONITOR Master Mix, HIV amplicon was spiked into each of sixteen replicate aliquots of each of two lots of Master Mix so that the final amplicon concentration was 10^4 copies per amplification reaction. The absorbance of all replicates of the HIV amplicon amplified in the presence of AmpErase was less than 0.12 OD units demonstrating that the HIV-1 MONITOR Master Mix can consistently eliminate HIV amplicon contamination at input levels of at least 10^3 copies per amplification reaction.

2. *Clinical Investigations - Prognosis (ACTG 116A and ACTG 116B/117)*

(a) Clinical Study Objectives

The AMPLICOR HIV-1 MONITOR Test was evaluated in an IND approved clinical study which was performed as part of the ACTG (AIDS Clinical Trial Group) Studies 116A and 116B/117 to evaluate the safety and efficacy of a new drug substance dideoxyinosine (ddI). In a virology sub-study to that clinical investigation, the clinical utility of the AMPLICOR HIV-1 MONITOR Test was evaluated. The objectives of the sub-study evaluation were:

- *to assess the association, if any, of decreased HIV-1 RNA level with a change in patient therapy; and*
- *to evaluate the utility of HIV-1 RNA measurements as a prognostic indicator of disease progression*

(b) Clinical Sites

Eleven sites participated in ACTG 116A and eight sites participated in ACTG 116B/117. In each study, the sites participated by collecting patient information and samples as well as performing some of the diagnostic testing. However, all AMPLICOR HIV-1 MONITOR testing was performed by Roche Molecular Systems, Inc.

(c) Study Population

A total of 286 patients were entered into the ACTG 116A and 116B/117 HIV-1 RNA sub-studies. ACTG 116A had 187 patients entered and ACTG 116B/117 had 99 patients entered. For ACTG 116A, the first ten patients enrolled at each clinical site were selected for the HIV-1 RNA sub-study. For ACTG 116B/117, patient selection for the HIV-1 RNA sub-study was based upon the availability of plasma samples at study baseline. Of the 913 patients entered into the ACTG 116B/117 study, 99 patients who met the criteria for the HIV-1 RNA sub-study were selected at random and included into the study.

(d) Study Period

ACTG Study 116 received FDA IND approval on July 26, 1989. The first patient was accrued October 20, 1989 and the study was closed to new patient accrual on March 31, 1991. The study completion date was October 27, 1992. ACTG Study 117 received FDA IND approval on July 26, 1989 and was completed on March 27, 1992.

(e) Clinical Study Results and Statistical Analyses

For ACTG Study 116A, there were a total of 187 patients from whom specimens were collected, however of the 187 only 183 could be evaluated for the Cox model. The remaining 4 patients could not be evaluated because some data needed for the analysis was not available. Of these patients, 153 (83.6%) had samples collected at Baseline; 73 (47.7%) of these patients progressed to AIDS or death. Of the 153 patients who had samples collected at Baseline, 114 (74.5%) also had samples collected at week 8. Of these patients, 62 (54%) progressed to AIDS or death. These patients/samples were used for the Cox model proportional hazards analysis of the ACTG 116A data.

The strongest evidence of the prognostic value of HIV-1 RNA came from this study, where both the Baseline HIV-1 RNA and the change from Baseline to Week 8 were statistically significant after adjusting for the other factors in the Cox model. The analyses for this study show that a 5-fold or greater increase of HIV-1 RNA copy number at baseline was associated with a 44% (95% CI; 7-93%) increase in the

Adjusted Relative Hazard for disease progression and that a 5-fold or greater increase of HIV-1 RNA copy number calculated for the change from Baseline to Week 8 was associated with a 54% (95% CI; 9-116%) increase in the Adjusted Relative Hazard for disease progression.

For ACTG Study 116B/117, there were a total of 99 patients from whom samples were collected and entered into the study. Of these patients. 86 (86.9%) had samples collected at Baseline; 39 (45.3%) of these patients progressed to AIDS or death. Of the 86 patients who had samples collected at Baseline, 65 (75.6%) also had samples collected at week 8. Of these patients, 29 (44.6%) progressed to AIDS or death. These patients/samples were used for the Cox model proportional hazards analysis of the ACTG 116B/117 data.

The analyses for this study show that a 5-fold or greater increase of HIV-1 RNA copy number at baseline was associated with a 25% (95% CI; 0-94%) increase in the Adjusted Relative Hazard for disease progression. However, the calculated Adjusted Relative Hazard was found to be not statistically significant ($p = 0.32$). The analysis also shows that a 5-fold or greater increase of HIV-1 RNA copy number calculated for the change from Baseline to Week 8 was associated with a 58% (95% CI; 0-268%) increase in the Adjusted Relative Hazard for disease progression with the data approaching statistical significance ($p = 0.09$).

Analyses using the Cox proportional hazards model show that the Baseline level of HIV-1 RNA, when viewed as an independent variable, was associated with the risk of disease progression. In Study 116A, the Baseline level of HIV-1 RNA was associated with the risk of disease progression even after all other variables in the analyses were controlled. In Study 116B/117, the Baseline level of HIV-1 RNA was associated with the risk of disease progression after all other variables in the analyses were controlled; however, the data were not statistically significant due to a number of factors including the low number of patients and progression events.

For the change in HIV-1 RNA levels from Baseline to Week 8, the Cox proportional hazards model again shows that the change in HIV-1 RNA level, when viewed as an independent variable, was associated with the risk of disease progression. In Study 116A, the change in the level of HIV-1 RNA from Baseline to Week 8 was associated with the risk of disease progression even after the relative hazard was adjusted for the other variables in the analyses. In Study 116B/117, the change in the level of HIV-1 RNA from Baseline to Week 8 was again associated with the risk of disease progression (with the analysis approaching statistical significance) even after adjustment of the relative hazard for the other variables in the analyses.

In a separate analysis, the frequency of disease progression as a function of baseline HIV-1 RNA was evaluated. For each study, a rank order by baseline HIV-1 RNA was created and then the population was divided into deciles. The frequency of disease progression for each decile was calculated. For both study populations, the data show an association of increased risk of disease progression with increased baseline HIV-1 RNA. These data also show that very high baseline HIV-1 RNA levels (>250,000 copies/mL) are associated with a greater than 60% chance of disease progression.

The results of the Cox model proportional hazards analyses for Studies 116A and 116B/117 can be found in Tables VIII-6 through VIII-9 The frequency distribution analyses for Studies 116A and 116B/117 are shown in Figure VIII-1 and Figure VIII-2.

Table VIII-6
Cox Proportional Hazards Analysis
Association of Study Variables at Baseline with Disease Progression
for ACTG Study 116A

(N = 153 Patients, 73 Progression Events)

Variable	Unadjusted Relative Hazard (95% CI)	Adjusted Relative Hazard (95% CI)	p value[2]
Log HIV-1 RNA copy number[1]	1.58 (1.20 - 2.09)	1.44 (1.07 -1.93)	0.02
Log CD4+ cell count[1]	0.39 (0.28 - 0.54)	0.45 (0.31 -0.64)	0.0001
Dx of AIDS at Baseline	2.00 (1.22 - 3.27)	1.39 (0.82 - 2.37)	0.22
ddI Treatment	0.95 (0.59 - 1.53)	1.12 (0.68 - 1.84)	0.66

1 - The Relative Hazard is the hazard ratio resulting from a 5-fold increase
2 - p values are for Adjusted Relative Hazards

Table VIII-7
Cox Proportional Hazards Analysis
Association of Change in HIV-1 RNA from Baseline to Week 8 and CD4+ with Disease Progression
for ACTG Study 116A

(N = 114 Patients, 62 Progression Events)

Variable	Unadjusted Relative Hazard (95% CI)	Adjusted Relative Hazard (95% CI)	p value[2]
Log HIV-1 RNA copy number[1]	1.46 (1.11 - 1.93)	1.63 (1.16 -2.28)	0.0005
Log Change in HIV-1 RNA from Baseline to Week 8[1]	1.18 (0.93 - 1.48)	1.54 (1.09 - 2.16)	0.013
Log CD4+ cell count[1]	0.43 (0.30 - 0.62)	0.50 (0.34 -0.73)	0.0004
Dx of AIDS at Baseline	1.83 (1.09 - 3.09)	1.28 (0.74 - 2.21)	0.38
ddI Treatment	0.76 (0.45 - 1.27)	0.87 (0.51 - 1.49)	0.61

1 - The Relative Hazard is the hazard ratio resulting from a 5-fold increase
2 - p values are for Adjusted Relative Hazards

Table VIII-8
Cox Proportional Hazards Analysis
Association of Study Variables at Baseline with Disease Progression
for ACTG Study 116B/117

(N = 86 Patients, 39 Progression Events)

Variable	Unadjusted Relative Hazard (95% CI)	Adjusted Relative Hazard (95% CI)	p value[2]
Log HIV-1 RNA copy number[1]	1.90 (1.28 - 2.82)	1.25 (0.81 -1.94)	0.32
Log CD4+ cell count[1]	0.28 (0.16 - 0.48)	0.33 (0.18 -0.62)	0.0006
Dx of AIDS at Baseline	3.13 (1.66 - 5.92)	2.38 (1.24 - 4.58)	0.01
ddI Treatment	0.89 (0.47 - 1.69)	0.88 (0.46 - 1.71)	0.71

1 - The Relative Hazard is the hazard ratio resulting from a 5-fold increase
2 - p values are for Adjusted Relative Hazards

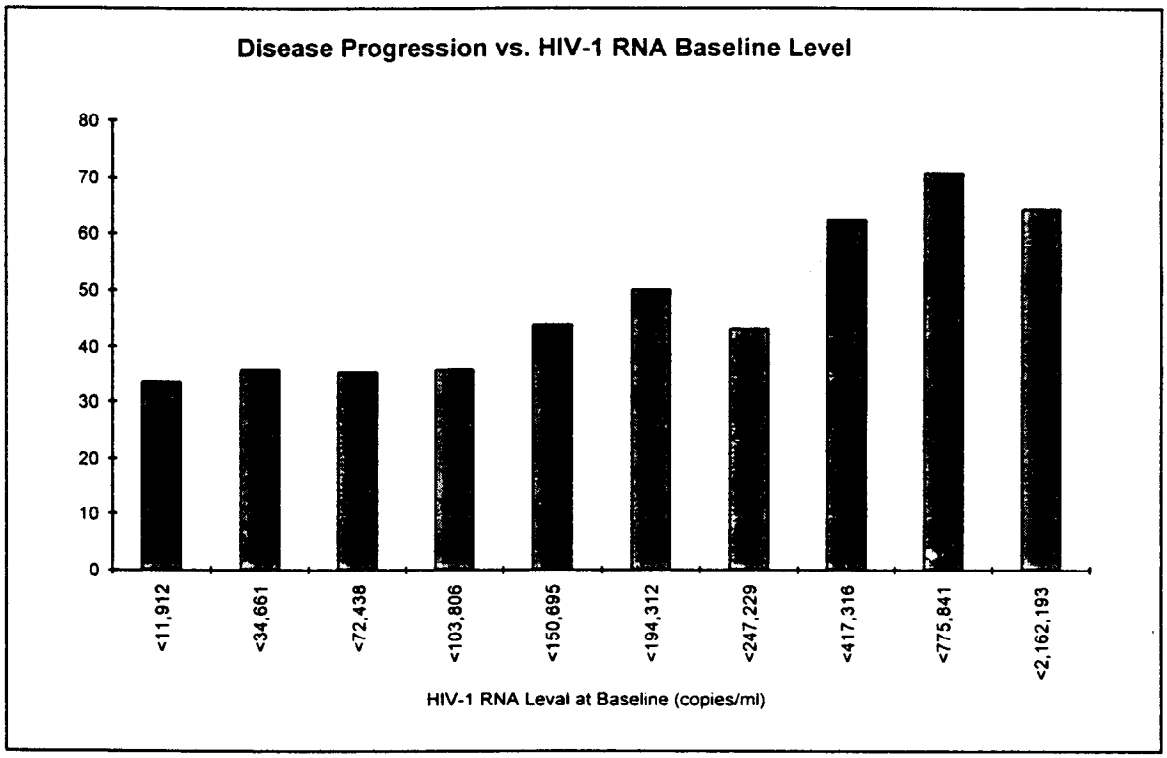
Table VIII-9
Cox Proportional Hazards Analysis
Association of Change in HIV-1 RNA from Baseline to Week 8 and CD4+ with Disease Progression
for ACTG Study 116B/117

(N = 65 Patients, 29 Progression Events)

Variable	Unadjusted Relative Hazard (95% CI)	Adjusted Relative Hazard (95% CI)	p value[2]
Log HIV-1 RNA copy number[1]	2.10 (1.32 - 3.34)	1.58 (0.93 -2.69)	0.09
Log Change in HIV-1 RNA from Baseline to Week 8[1]	1.41 (0.71 - 2.79)	1.58 (0.68 - 3.68)	0.29
Log CD4+ cell count[1]	0.25 (0.13 - 0.46)	0.29 (0.14 -0.60)	0.001
Dx of AIDS at Baseline	2.43 (1.17 - 5.05)	1.87 (0.88 - 3.97)	0.10
ddI Treatment	1.07 (0.51 - 2.27)	0.98 (0.39 - 2.48)	0.96

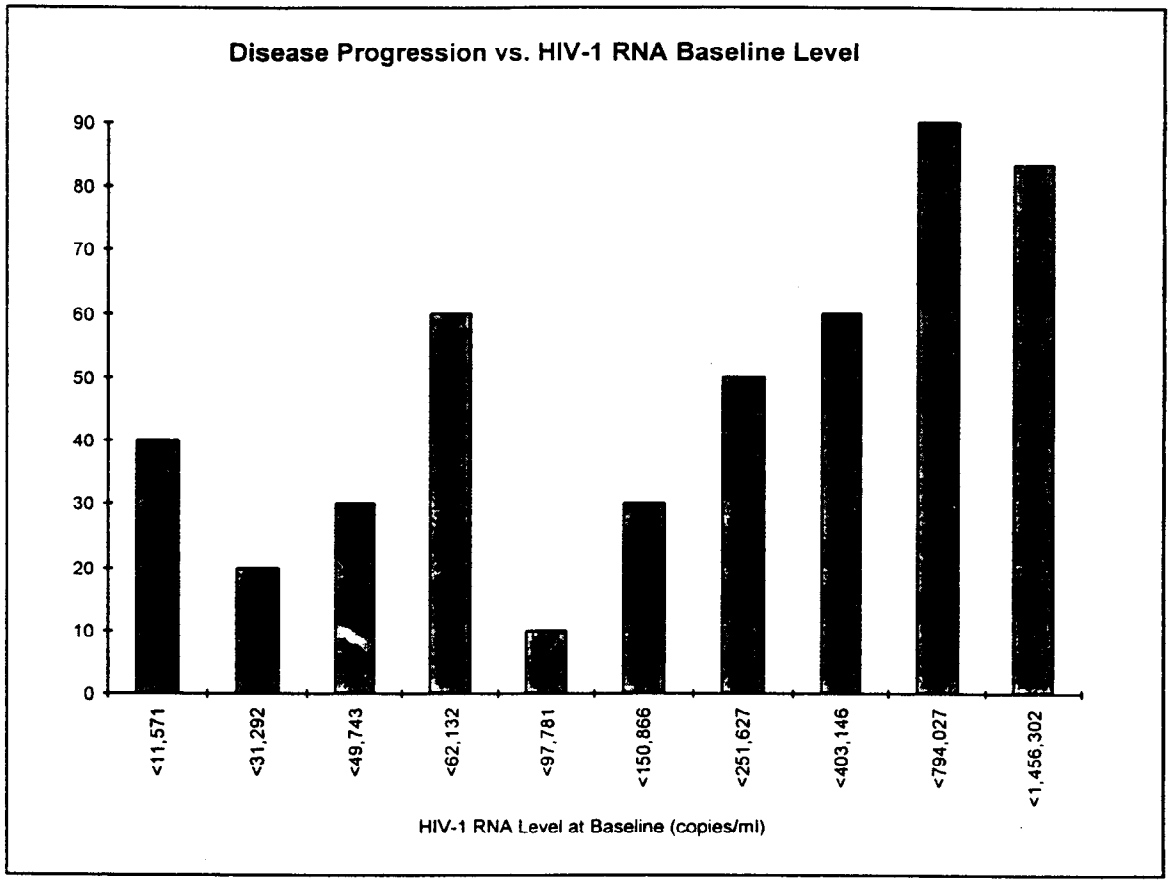
1 - The Relative Hazard is the hazard ratio resulting from a 5-fold increase
2 - p values are for Adjusted Relative Hazards

Figure VIII-1
Association of Baseline HIV-1 RNA with Disease Progression
ACTG Study 116A



Record #	Decile	n	Total	# Progressing to AIDS/Death	%
18	<11912	18	15	5	33.33
36	<34661	18	14	5	35.71
54	<72438	18	17	6	35.29
72	<103806	18	14	5	35.71
90	<150695	18	16	7	43.75
108	<194312	18	16	8	50.00
126	<247229	18	14	6	42.86
144	<417316	18	16	10	62.50
162	<775841	18	17	12	70.59
179	<2162193	17	14	9	64.29

Figure VIII-2
Association of Baseline HIV-1 RNA with Disease Progression
ACTG Study 116B/117



Record #	Decile	n	Total	# Progressing to AIDS/Death	%
10	<11571	10	10	4	40.00
20	<31292	10	10	2	20.00
30	<49743	10	10	3	30.00
40	<62132	10	10	6	60.00
50	<97781	10	10	1	10.00
60	<150866	10	10	3	30.00
70	<251627	10	10	5	50.00
80	<403146	10	10	6	60.00
90	<794027	10	10	9	90.00
96	<1456302	6	6	5	83.33

3. *Measuring Response to Antiretroviral Therapy - Study NV14256*

(a) Clinical Study Objectives

The primary objectives of the NV14256 study were to prove the clinical benefit of Saquinavir 600 mg given in combination with ddC over ddC monotherapy and to compare the clinical effects of the two monotherapy arms. The secondary objectives were to demonstrate the superiority of combination therapy over both monotherapies with respect to the surrogate markers (HIV-1 RNA and CD4 cell count), to validate the use of HIV-1 RNA and CD4 cell count as surrogates for time to first AIDS Defining Event or Death, to demonstrate the good safety and tolerability of Saquinavir alone and in combination with ddC, and to assess the treatment effect on the quality of life.

(b) Clinical Sites

Forty nine (49) clinical sites were involved in Study NV14256. In each study, the sites participated by collecting patient information and samples as well as performing some of the diagnostic testing. All AMPLICOR HIV-1 MONITOR testing was performed by [redacted] in their molecular biology testing facility located in [redacted]

(c) Study Population

A total of 970 patients were entered into study NV14256. The study had the following three treatment arms/patients: ddC/325, Saquinavir/327, and Saquinavir + ddC/318.

(d) Study Period

Study NV14256 began in February, 1994 and the study was closed on April 5, 1996.

(e) Clinical Study Information

The utility of serial HIV-1 RNA measurements to assess viral response to antiretroviral treatment was examined using the Cox Proportional Hazards Model. In this analysis, the three treatment groups were combined and a Cox model, stratified by treatment group, was fit using the following covariates as linear terms: log10(baseline HIV-1 RNA), log10(last HIV-1 RNA), baseline CD4 and last CD4. The hazard ratio in this model was defined as a 10-fold increase in HIV-1 RNA or a decrease of 100 in CD4 count. As shown in Table VIII-10, the coefficient of the log10(last HIV-1 RNA) term for this model is statistically significant and positive through week 40. Accordingly, the HIV-1 RNA Hazard Ratios through Week 40 indicate that the HIV-1 RNA levels at each time point have statistically significant and continuous prognostic value.

In a related analysis of the data, the association between HIV-1 RNA levels and survival time was assessed. For this analysis, survival time was defined as time on study in which a patient survived free of an AIDS Defining Event (ADE) or death. The analyses were performed by constructing Kaplan-Meier Survival Curves for each treatment arm by dividing the patient population within the treatment arm into thirds based on HIV-1 RNA levels, nominally the Lower, Middle, and Upper thirds. The survival curves were plotted as a function of the percent ADE Survival for each patient group (Lower, Middle and Upper) for the number of weeks following Week 8. The Kaplan Meier estimated survival analyses show that patients with low HIV-1 RNA levels (Lower Third) are more likely to survive ADE-free for a longer period of time than patients with high HIV-1 RNA levels. The Kaplan-Meier survival curves for each study treatment arm are shown in Figures VIII-3, VIII-4 and VIII-5.

In a separate analysis of the data from Study NV14256, a patient's risk of ADE or death was assessed as a function of the last (most recent) HIV-1 RNA level at various study weeks. In particular, the impact of the last HIV-1 RNA measurement on the hazard for the development of a first ADE or death (based upon actual clinical events seen later in the study) was assessed. The analysis was performed using a Cox model survival analysis using the covariates log10(baseline HIV-1 RNA), log10(last HIV-1 RNA), baseline CD4 and last CD4. The model was a linear function of the covariates predicting the log of the hazard (ratio) for an ADE. The linear function was then used to determine a Risk Index for all patients in the study. Patients who survived ADE free through Week 4 of the study were sorted by their Risk Index with the first 25% of the patients forming the "low risk" group and the remaining patients equally divided into six separate groups according to their Risk Index. Each of these six groups was then compared to the low risk group using a survival model, and the mean HIV-1 RNA level of each group was plotted versus an ADE hazard ratio calculated using a piecewise exponential model. Figures VIII-6 and VIII-7 show the Hazard Ratios for the time to first ADE versus the log10(last HIV-1 RNA) for patients surviving ADE free through Week 4 and patients surviving ADE free through Week 16, respectively. These data show that a patient in this study at Week 4 with an HIV-1 RNA level of 100,000 copies/mL (log10 =5.0) was 6 times more likely to develop an ADE or to die than was patient with an HIV-1 RNA level of 8000 copies/mL. A patient in this study at Week 16 with an HIV-1 RNA level of 1,000,000 (log10 = 6.0) was 20 times more likely to develop an ADE or to die than was a patient with an HIV-1 RNA level of 8,000 copies/mL.

Table VIII-10
ADE Risk versus Log10(Last RNA) and Other Covariates
Based on Time from the Given Study Week for Patients Surviving Through that Week
Summary of Cox Model Results - Model Stratified by Treatment Group
Study NV14256

Study Week	Variable	Cox Model Coefficient	Hazard Ratio*	p Value
4	log10(Baseline RNA)	0.798	2.22	0.0012
	log10(Last RNA)	0.4197	1.52	0.0219
	Baseline CD4	-0.0009	0.91	0.5806
	Last CD4	-0.0040	0.67	0.0084
8	log10(Baseline RNA)	0.8096	2.25	0.0004
	log10(Last RNA)	0.4186	1.52	0.0138
	Baseline CD4	-0.0019	0.83	0.2107
	Last CD4	-0.0049	0.62	0.0006
16	log10(Baseline RNA)	0.8714	2.39	0.005
	log10(Last RNA)	0.5385	1.71	0.0046
	Baseline CD4	-0.0015	0.87	0.3510
	Last CD4	-0.0049	0.62	0.0010
24	log10(Baseline RNA)	0.6910	2.00	0.0136
	log10(Last RNA)	0.7135	2.04	0.0017
	Baseline CD4	-0.0003	0.97	0.8667
	Last CD4	-0.0064	0.53	0.0001
32	log10(Baseline RNA)	0.5926	1.81	0.0492
	log10(Last RNA)	0.9000	2.46	0.0003
	Baseline CD4	-0.0040	0.67	0.0252
	Last CD4	-0.0023	0.79	0.1523
40	log10(Baseline RNA)	0.8003	2.23	0.0159
	log10(Last RNA)	0.7086	2.03	0.0096
	Baseline CD4	-0.0030	0.74	0.1282
	Last CD4	-0.0031	0.73	0.0955
48	log10(Baseline RNA)	0.5993	1.82	0.0978
	log10(Last RNA)	0.5631	1.76	0.0629
	Baseline CD4	-0.0011	0.89	0.6168
	Last CD4	-0.0079	0.45	0.0016
56	log10(Baseline RNA)	1.0917	2.98	0.0127
	log10(Last RNA)	0.0835	1.09	0.8025
	Baseline CD4	-0.0022	0.80	0.4560
	Last CD4	-0.0072	0.49	0.0201
64	log10(Baseline RNA)	1.2803	3.60	0.0135
	log10(Last RNA)	-0.2500	0.78	0.4988
	Baseline CD4	0.0006	1.07	0.8531
	Last CD4	-0.0085	0.43	0.0213

* Hazard Ratio due to a 10 fold increase in HIV-1 RNA or a decrease of 100 in CD4 count

Figure VIII-3
Kaplan Meier Estimated Survivals
Study NV14256 Treatment Group: SAQ 600mg
Patients Surviving ADE Free Through Week 8

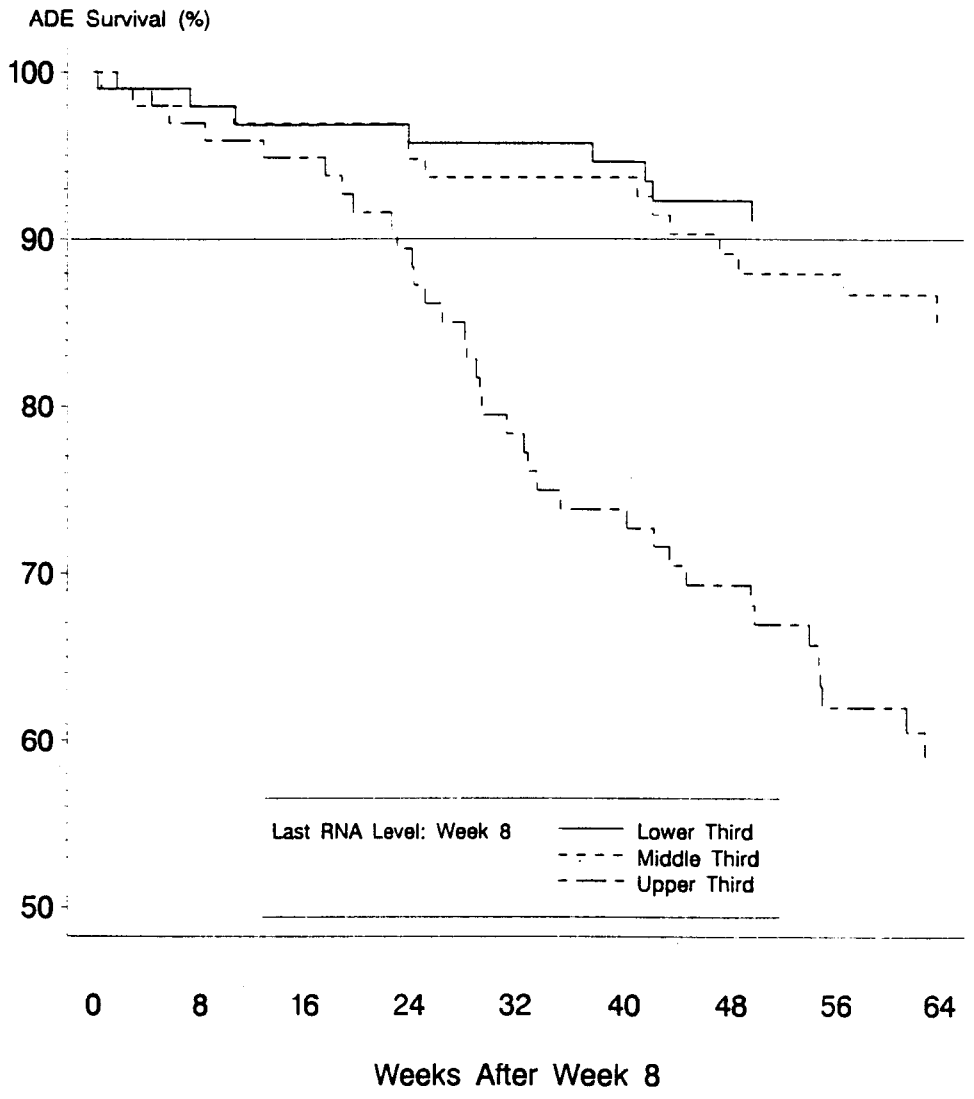


Figure VIII-4
Kaplan Meier Estimated Survivals
Study NV14256 Treatment Group: ddC
Patients Surviving ADE Free Through Week 8

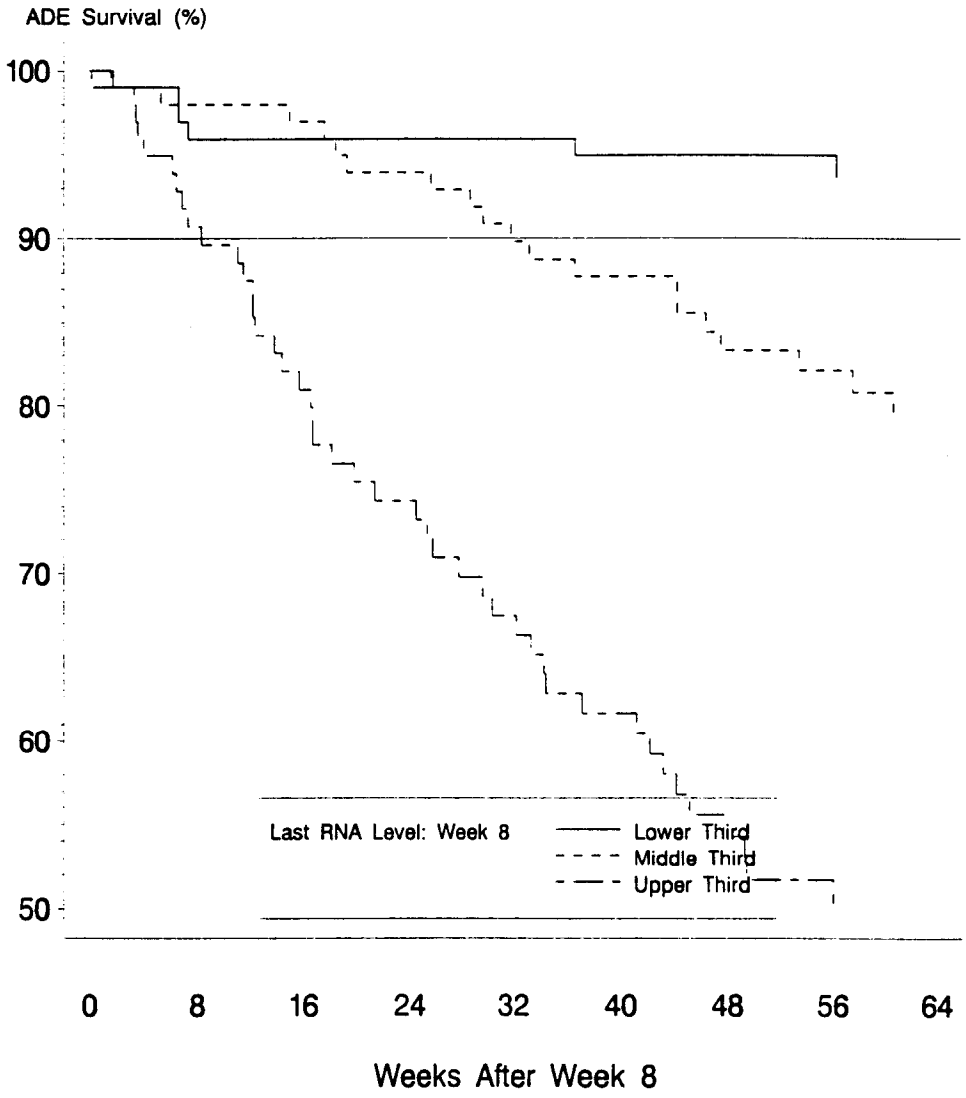


Figure VIII-5
Kaplan Meier Estimated Survivals
Study NV14256 Treatment Group: ddC + SAQ 600mg
Patients Surviving ADE Free Through Week 8

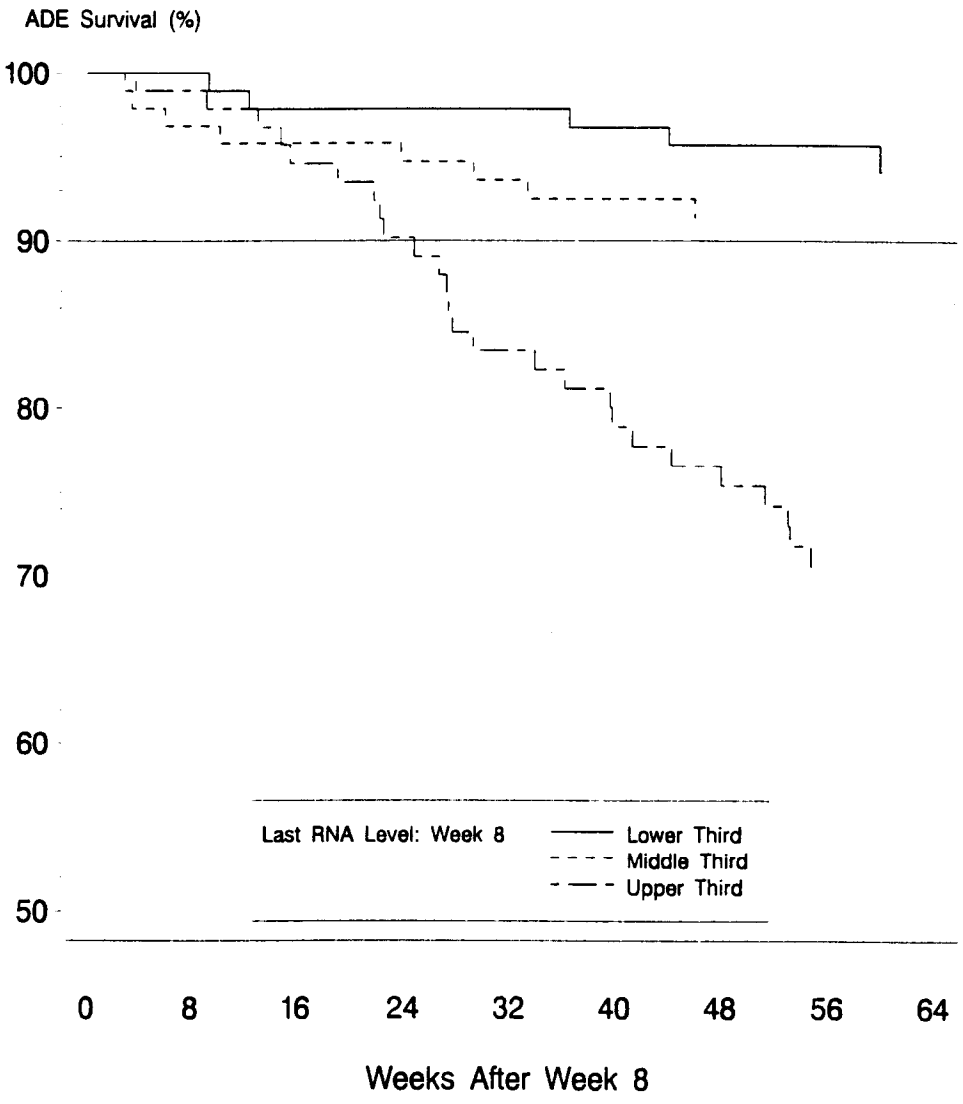


Figure VIII-6
Hazard Ratio for Time to First ADE
Patients Surviving ADE Free Through Week 4
Study NV14256

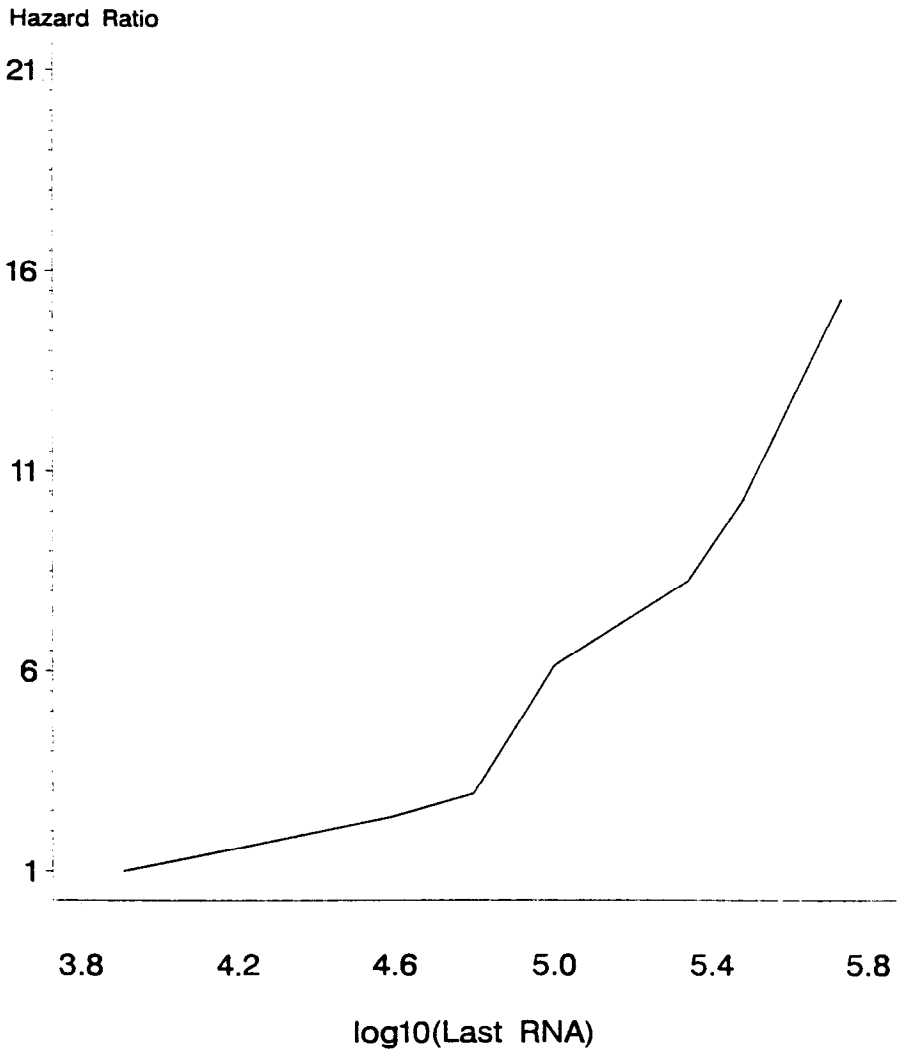
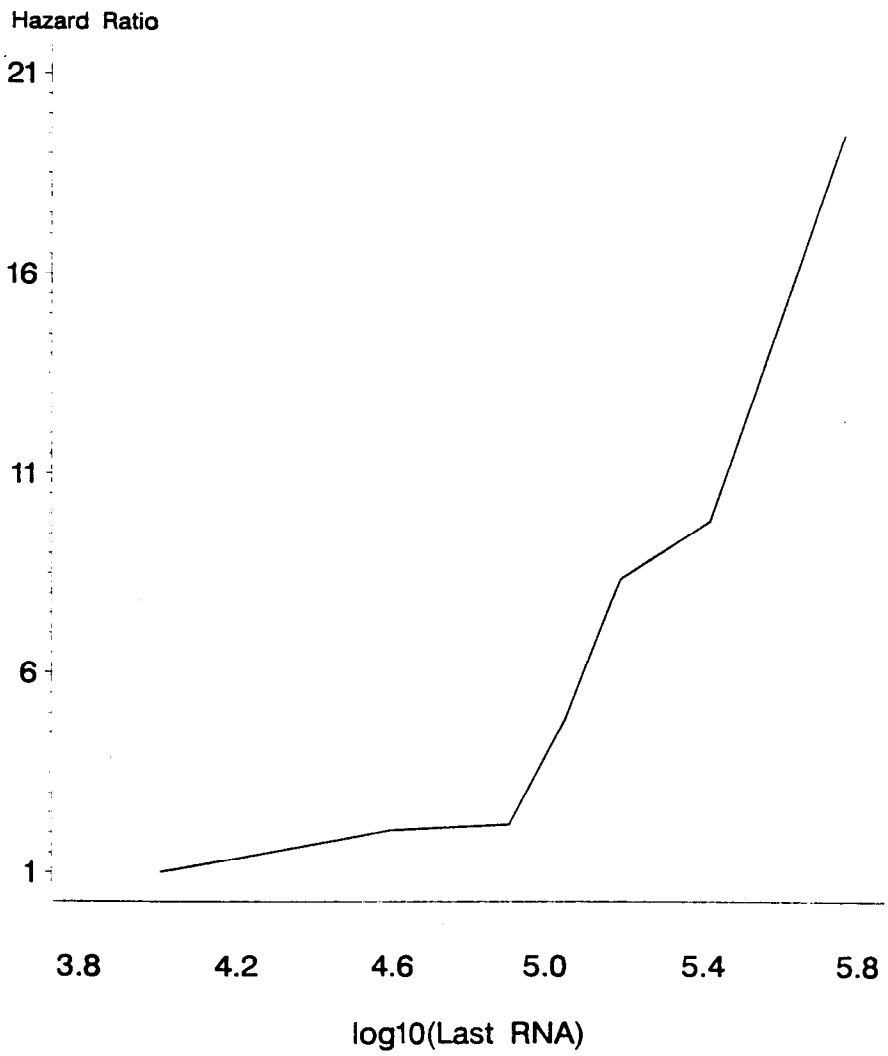


Figure VIII-7
Hazard Ratio for Time to First ADE
Patients Surviving ADE Free Through Week 16
Study NV14256



IX. CONCLUSIONS DRAWN FROM THE STUDIES

ACTG 116A and ACTG 116B/117

The Unadjusted Relative Hazards for the baseline data in both the ACTG 116A and 116B/117 Virology Sub-Studies were statistically significant ($p < 0.05$) and demonstrate that HIV-1 RNA measurements as an independent variable have prognostic value. Analyses using the Cox proportional hazards model show that the baseline level of HIV-1 RNA, when viewed as an adjusted variable, was again associated with the risk of disease progression. In ACTG Study 116A, the baseline level of HIV-1 RNA was associated with the risk of disease progression after all other variables in the analyses were controlled. In ACTG Study 116B/117, the baseline level of HIV-1 RNA was associated with the risk of disease progression after all other variables in the analyses were controlled; however, the results did not reach a high level of statistical significance ($p = 0.32$).

When the change in HIV-1 RNA level from baseline to Week 8 was evaluated, the Cox proportional hazards model shows that the change in HIV-1 RNA level, when adjusted for the other study variables, was associated with the risk of disease progression. In ACTG Study 116A, the change in the level of HIV-1 RNA from baseline to Week 8 was associated with the risk of disease progression after the relative hazard was adjusted for the other variables in the analysis. In ACTG Study 116B/117, the change in the level of HIV-1 RNA from baseline to Week 8 was again associated with the risk of disease progression after adjustment of the relative hazard for the other variables in the analyses; however, the results did not reach a high level of statistical significance ($p = 0.29$).

The Cox Proportional Hazard Analyses show the baseline HIV-1 RNA and that the HIV-1 RNA change from baseline to Week 8 have Adjusted Relative Hazards that were statistically significant in the ACTG 116A study analyses ($p = 0.0005$ and $p = 0.013$, respectively), but not statistically significant in the ACTG 116B/117 analyses. The lack of statistical significance in the ACTG 116B/117 study may have been caused by the lower number of patients included in the study (99 vs. 183 in study 116A) and/or more importantly, by the lower number of patients progressing to AIDS or death (39 in ACTG 116B/117 vs. 73 in study ACTG 116A).

The analyses of the association of baseline HIV-1 RNA levels with the frequency of disease progression also provided evidence of the prognostic value of baseline HIV-1 RNA levels. These data show that very high baseline HIV-1 RNA levels (> 250,000 copies/mL) are associated with a greater than 60% chance of disease progression.

The data from ACTG 116A and 116B/117 clinical trials that are contained in this application support the prognostic value of HIV-1 RNA measurements. In fact, the prognostic value of HIV-1 RNA measurements may be underestimated since the data were generated from retrospective studies in which the specimens were collected for purposes other than the measurement of HIV-1 RNA.

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The value of continued HIV-1 RNA assessments were, in this analysis, an evaluation of the prognostic value of the ratio: (last/baseline HIV-1 RNA) or last HIV-1 RNA assessment for future ADEs. This evaluation was carried out on the cohort of patients surviving ADE free through this specified time period and was adjusted for other possible strong predictors of ADE risk, so that the ratio (last/baseline HIV-1 RNA) or (last HIV-1 RNA) level could be one of possibly several assessments making up a prognostic determination of a patient's future ADE risk. This evaluation also took into account the three drug treatment groups represented in the patient data, and found that, in this analysis, the prognostic value of continued HIV-1 RNA assessment possibly depends on the effectiveness of drug treatment.

This analysis has found that the log10(ratio: last/baseline HIV-1 RNA) or equivalently log10(last HIV-1 RNA) should be taken into account when assessing future ADE risk. Generally, high values of HIV-1 RNA within a cohort of similarly treated patients indicated a statistically significant higher risk for the development of an ADE through Week 40.

These data show that serial measurement of HIV-1 RNA levels during the course of drug therapy should be used in addition to the baseline levels to assess the patient's viral response to drug therapy and that those serial HIV-1 RNA levels are significantly associated with a patient's risk of death or the development of AIDS Defining Events. These analyses show that antiretroviral drug therapy that decreases the HIV-1 RNA levels and increases CD4 cell count is indicative of successful therapy and will result in longer patient survival.

These analyses provide evidence for the clinical utility of HIV-1 RNA levels as a tool for monitoring the effects of antiretroviral therapy. Increases or decreases in HIV-1 RNA levels in response to antiretroviral drug therapy can be measured by the AMPLICOR HIV-1 MONITOR Test. The analyses presented here show that the last (most recent) HIV-1 RNA level is predictive of a patient's risk of death or for progression to an AIDS Defining Event. Therefore, the serial measurement of HIV-1 RNA levels for patients on antiretroviral drug therapy may be used to monitor the effects of therapy by continually assessing the patient's risk of progression to an AIDS Defining Event. When HIV-1 RNA levels are used to assess patient prognosis and to monitor the effects of antiretroviral therapy, the clinical management of HIV-1 infected patients may be achieved.

X. *BENEFIT ANALYSIS*

The AMPLICOR HIV-1 MONITOR™ TEST is an *in vitro* nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma. The 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 or to monitor the effects of antiretroviral drug therapy on HIV-1 RNA levels. The AMPLICOR HIV-1 MONITOR Test is not intended to be used as a screening test for HIV or as a diagnostic test to confirm the presence of HIV infection.

Quantitative measurements of HIV viremia in the peripheral blood have shown that high levels of viral load correlate with a higher risk of clinical progression to Acquired Immunodeficiency Syndrome (AIDS) or death. Therefore, the measurement of plasma HIV-1 RNA may be an effective tool for early prognosis in HIV infected patients. In addition, changes in viral load have been shown to be an effective measure of response to antiretroviral drug therapy or lack of response to therapy. Accordingly, the measurement of changes in plasma HIV-1 RNA levels due to antiretroviral drug therapy can be used to monitor a patient's response to the therapy. With the current availability of Highly Active Anti-Retroviral Treatment (HAART), many HIV-infected individuals have HIV-1 RNA levels that are not detectable by the currently approved AMPLICOR HIV-1 MONITOR Test. Therefore, there is a demonstrated need for the lower level quantitation of HIV-1 RNA made possible by the AMPLICOR HIV-1 MONITOR Test with UltraSensitive specimen preparation.

The studies presented in this PMA supplement show that the AMPLICOR HIV-1 MONITOR Test (Standard/UltraSensitive) is an accurate, reproducible and rapid test for the quantitation of HIV-1 RNA and should be made available to the medical community.

XII. FDA DECISION

FDA concluded that the device is safe and effective for its intended use based on the data submitted by the applicant. An approval letter was issued to Roche Molecular Systems, Inc. on March 2, 1999.

The shelf-life of the Roche AMPLICOR HIV-1 MONITOR Test has been established at 9 months at a storage temperature of 2-8°C.

XIII. REFERENCES

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