AMPLICOR HIV-1 MONITOR™ TEST
Standard/UltraSensitive

AMPLICOR HIV-1 MONITOR Test US:83088 24 Tests

INTENDED 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.

SUMMARY AND EXPLANATION OF THE TEST

Human Immunodeficiency Virus (HIV) is the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS)[1-3]. HIV infection can be transmitted by sexual contact, exposure to infected blood or blood products, or by an infected mother to the fetus[4]. Within three to six weeks of exposure to HIV, infected individuals generally develop a brief, acute syndrome characterized by flu-like symptoms and associated with high levels of viremia in the peripheral blood[5-8]. In most infected individuals this is followed by an HIV-specific immune response and a decline of plasma viremia, usually within four to six weeks of the onset of symptoms[9,10]. After seroconversion, infected individuals typically enter a clinically stable, asymptomatic phase that can last for years[11-13]. The asymptomatic period is characterized by persistent, low level plasma viremia[14] and by a gradual depletion of CD4+ T lymphocytes, leading to severe immunodeficiency, multiple opportunistic infections, malignancies and death[15]. Although virus levels in the peripheral blood are relatively low during the asymptomatic phase of the infection, virus replication and clearance appear to be dynamic processes in which high rates of virus production and infection of CD4+ cells are balanced by equally high rates of virus clearance, death of infected cells and replenishment of CD4+ cells, resulting in relatively stable levels of both plasma viremia and CD4+ cells[16-18].

Quantitative measurements of HIV viremia in the peripheral blood have shown that higher virus levels may be correlated with increased risk of clinical progression of HIV disease, and that reductions in plasma virus levels may be associated with decreased risk of clinical progression[19-21]. Virus levels in the peripheral blood can be quantitated by measurement of the HIV p24 antigen in serum, by quantitative culture of HIV from plasma, or by direct measurement of viral RNA in plasma using nucleic acid amplification or signal amplification technologies [22-26].

p24 antigen is the principle core protein of HIV and is found in serum either free or bound by anti-p24 antibody. Free p24 antigen can be measured with commercially available enzyme immunoassays (EIA), although the usefulness of p24 antigen as a marker of viral load is limited since the antigen is detectable in only 20% of asymptomatic patients and 40-50% of symptomatic patients. Procedures to dissociate antigen-antibody complexes improve the sensitivity of the p24 antigen tests, but the viral protein remains undetectable in most asymptomatic patients[22].

Infectious HIV in plasma can be cultured by inoculation into activated peripheral blood mononuclear cells (PBMC) from normal donors. Quantitation is achieved by inoculating PBMC with serial dilutions of the plasma specimen. Quantitative culture has limited utility for monitoring virus levels in infected individuals since only a small fraction of virus particles is infectious *in vitro*. Infectious virus is often undetectable in asymptomatic individuals[22].

HIV RNA in plasma can be quantitated by nucleic acid amplification technologies, such as the Polymerase Chain Reaction (PCR)[27-29]. The AMPLICOR HIV-1 MONITOR Test uses PCR technology to achieve maximum sensitivity and dynamic range for the quantitative detection of HIV-1 RNA in ACD or EDTA anti-coagulated plasma[24].

PRINCIPLES OF THE PROCEDURE

The AMPLICOR HIV-1 MONITOR Test is based on five major processes: specimen preparation: reverse transcription (RT) of target RNA to generate complementary DNA (cDNA); PCR amplification of target cDNA using HIV-1 specific complimentary primers; hybridization of the amplified products to oligonucleotide probes specific to the target(s); and detection of the probe-bound amplified products by colorimetric determination.

The AMPLICOR HIV-1 MONITOR Test permits the reverse transcription and amplification of HIV-1 and Quantitation Standard (QS) RNA to occur simultaneously. The Master Mix reagent contains a biotinylated primer pair specific for HIV-1 and QS target nucleic acid.

The quantitation of HIV-1 viral RNA is performed using a Quantitation Standard (QS). The QS is a non-infectious RNA transcript that contains the identical primer binding sites as the HIV-1 target and a unique probe binding region that allows QS amplicon to be distinguished from HIV-1 amplicon. The QS is incorporated into each individual specimen at known copy number and is carried through the specimen preparation, reverse transcription, PCR amplification, hybridization and detection steps along with the HIV-1 target and is amplified along with the HIV-1 target. HIV-1 RNA levels in the test specimens are determined by comparing the absorbance of the specimen to the absorbance obtained for the Quantitation Standard. Therefore, the QS compensates for any effects of inhibition and controls for the amplification process to allow the accurate quantitation of each specimen.

Specimen Preparation

The AMPLICOR HIV-1 MONITOR Test can be used with either of two specimen processing procedures, the Standard procedure and the UltraSensitive procedure. With the Standard specimen processing procedure, HIV-1 RNA can be quantitated over the range of 400 - 750,000 copies/mL. With the UltraSensitive procedure, HIV-1 RNA can be quantitated over the range of 50 - 75,000 copies/mL.

In the Standard specimen processing procedure, HIV-1 RNA is isolated directly from plasma by lysis of virus particles with a chaotropic agent followed by precipitation of the RNA with alcohol. With the UltraSensitive specimen processing procedure, HIV-1 viral particles are first pelleted from

the plasma specimen by high speed ultracentrifugation followed by lysis of the pelleted virus particles with a chaotropic agent and precipitation of the HIV-1 RNA with alcohol. A known number of QS RNA molecules is introduced into each specimen with the lysis reagent. The QS is carried through the specimen preparation, amplification and detection steps and is used for the quantitation of HIV-1 RNA in the test specimen. The QS compensates for any effects of inhibition to permit the accurate quantitation of HIV-1 RNA in each specimen.

Reverse Transcription and PCR Amplification

The AMPLICOR HIV-1 MONITOR Test amplifies and detects a 142 base target sequence located in a highly conserved region of the HIV-1 *gag* gene[30], defined by the primers SK431 and SK462. 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 reverse transcription and amplification reactions are performed with the thermostable recombinant 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 (RT) and DNA polymerase activity[31]. This allows both reverse transcription and PCR amplification to occur in the same reaction mixture.

Reverse Transcription

The processed specimens are added to the amplification mixture in reaction tubes in which both reverse transcription and PCR amplification occur. The downstream or antisense primer (SK431) and the upstream or sense primer (SK462) are biotinylated at the 5' ends. The reaction mixture is heated to allow the downstream primer to anneal specifically to the HIV-1 and QS target RNA. In the presence of excess deoxynucleoside triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine and thymidine triphosphates, r*Tth* pol extends the annealed primer forming a complementary (cDNA) strand.

PCR Amplification

Following reverse transcription of the HIV-1 and QS target RNA, the reaction mixture is heated to denature the RNA:cDNA hybrid and expose the HIV-1 and QS target sequences. As the mixture cools, the upstream primer anneals to the cDNA strand, the r*Tth* pol catalyzes the extension reaction, and a second DNA strand is synthesized. 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 heated again to separate the resulting double-stranded DNA and expose the primer target sequences. As the mixture cools, the primers anneal to the target DNA. r*Tth* pol, in the presence of excess dNTPs, extends the annealed primers along the target templates to produce a 142 base pair sequence termed an **amplicon**. This process is repeated for a number of cycles, each cycle effectively doubling the amount of amplicon. Amplification occurs only in the region of the HIV-1 genome between the primers. The entire HIV-1 genome is not amplified.

Selective Amplification

Selective amplification of target nucleic acid from the clinical specimen in the AMPLICOR HIV-1 MONITOR Test is achieved by the use of AmpErase® and deoxyuridine triphosphate (dUTP). AmpErase (uracil-N-glycosylase, UNG) recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[32], but not DNA containing thymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon due to the use of deoxyuridine

triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contain deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase prior to amplification of the target DNA. AmpErase, which is included in the Master Mix Reagent, catalyzes the cleavage of deoxyuridine containing DNA 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 Master Mix, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering 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.

Hybridization Reaction

Following PCR amplification, the HIV-1 and QS amplicons are chemically denatured to form single stranded DNA by the addition of denaturation solution, and aliquots are added to separate wells of a microwell plate (MWP) coated with HIV-1-specific (SK102) and QS-specific (CP35) oligonucleotide probes. HIV-1 and QS amplicons are bound to HIV-1 and QS wells, respectively, by hybridization to the MWP-bound probes. To achieve quantitative results over a large dynamic range, serial dilutions of the denatured amplicon are analyzed in the MWP.

Detection Reaction

Following the hybridization reaction, the MWP is washed to remove any unbound material, and an avidin-horseradish peroxidase conjugate (Av-HRP) is added to each well of the MWP. The Av-HRP binds to the biotin-labeled amplicon captured by the plate-bound oligonucleotide probes. The MWP is washed again to remove unbound Av-HRP and a substrate solution containing hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine (TMB) is added to the wells. In the presence of hydrogen peroxide, the bound horseradish peroxidase catalyzes the oxidation of TMB to form a color complex. The reaction is stopped by addition of a weak acid, and the optical density at 450 nm is measured using an automated microwell plate reader.

HIV-1 RNA QUANTITATION

The AMPLICOR HIV-1 MONITOR Test quantitates viral load by utilizing a second target sequence (QS) that is added to the amplification mixture at a known concentration. The QS is a non-infectious 219 base *in vitro* transcribed RNA molecule with primer binding regions identical to those of the HIV-1 target sequence. The QS, therefore, contains SK431 and SK462 primer binding sites and generates an amplification product of the same length (142 bp) and base composition as the HIV-1 target. The probe binding region of the QS has been modified to differentiate QS-specific amplicon from HIV-1 target amplicon.

Within the linear range of the assay, the optical density (OD) 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 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 using the following equation:

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

Where:

Total HIV-1 OD =	calculated total OD for HIV-1 amplicon
Total QS OD =	calculated total OD for QS amplicon
Input HIV-1 QS copies/PCR =	the number of copies of QS in each reaction; this information is lot specific and is entered by user
Dilution Factor =	factor to convert copies/PCR to copies/mL • Dilution Factor = 40 for the Standard Procedure • Dilution Factor = 4 for the UltraSensitive Procedure

REAGENTS

AMPLICOR HIV-1 MONITOR Test **24 Tests**
(US:83088)

HIV-1 MONITOR Specimen Preparation Reagents

HIV-1 LYS 2 x 9.0 mL
(HIV-1 MONITOR Lysis Reagent)
Tris HCl buffer
68% Guanidine thiocyanate
3% Dithiothreitol
< 1% Glycogen

Xn ✖ 68% (w/w) guanidine thiocyanate
Harmful

HIV-1 QS 2 x 0.1 mL
(HIV-1 MONITOR Quantitation Standard)
Tris-HCl buffer
< 0.001% Non-infectious *in vitro* transcribed RNA (microbial) containing HIV-1 primer binding sequences and a unique probe binding region
< 0.005 % Poly rA RNA (synthetic)
EDTA
Amaranth dye
0.05% Sodium azide

HIV-1 DIL 2 x 4.8 mL
(HIV-1 MONITOR Specimen Diluent)
Tris-HCl buffer
< 0.005 % Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

AMPLICOR HIV-1 MONITOR Control Reagents

NHP — 2 x 1.6 mL
[Negative Plasma (Human)]
Human plasma, non-reactive by FDA licensed tests for antibody to HCV, antibody to HIV-1/2, and HBsAg
0.1% ProClin® 300

HIV-1 (–) C — 2 x 0.05 mL
(HIV-1 MONITOR (–) Control)
Tris-HCl buffer
< 0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

HIV-1 L(+)C — 2 x 0.05 mL
(HIV-1 MONITOR Low (+) Control)
Tris-HCl buffer
<0.001% Non-infectious *in vitro* transcribed RNA (microbial) containing HIV-1 sequences
<0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

HIV-1 H(+)C — 2 x 0.05 mL
(HIV-1 MONITOR High (+) Control)
Tris-HCl buffer
<0.001% Non-infectious *in vitro* transcribed RNA (microbial) containing HIV-1 sequences
<0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

AMPLICOR HIV-1 MONITOR Amplification Reagents

HIV-1 MMX — 2 x 0.5 mL
(HIV-1 MONITOR Master Mix)
Bicine buffer
Glycerol
<0.01% r*Tth* DNA Polymerase (r*Tth* pol, microbial)
Potassium acetate
<0.07% dATP, dCTP, dGTP, TTP, dUTP
<0.001% SK431 and SK462 primers, biotinylated
<0.01% AmpErase® (microbial)
0.05% Sodium azide

HIV-1 Mn^{2+} 2 x 0.1 mL
(HIV-1 MONITOR Manganese Solution)
2% Manganese acetate
Acetic acid
Amaranth dye
0.05% Sodium azide

AMPLICOR HIV-1 MONITOR Detection Reagents

HIV-1 MONITOR MWP 2 x 12 Tests
(HIV-1 MONITOR Microwell Plate)
MWP coated with HIV-1 specific DNA probe (Rows A to F) and
QS-specific probe (Rows G to H)
Twelve, 8-well strips in one resealable pouch with desiccant

[1] MONITOR DN 1 x 12 mL
(MONITOR Denaturation Solution)
1.6% sodium hydroxide
EDTA
Amaranth dye

Xi  1.6% (w/w) sodium hydroxide
Irritant

[2] MONITOR HYB 2 x 12 mL
(MONITOR Hybridization Buffer)
Sodium phosphate solution
< 25% Sodium thiocyanate
< 0.2% solubilizer

[3] AV-HRP 2 x 12 mL
(Avidin-Horseradish Peroxidase Conjugate)
Tris HCl buffer
<0.001% Avidin-horseradish peroxidase conjugate
Bovine gamma globulin (mammalian)
Emulsit 25 (Dai-ichi Kogyo Seiyaku Co., Ltd)
0.1% Phenol
1% ProClin 150

[4A] SUB A 2 x 12 mL
(Substrate A)
Citrate solution
0.01% Hydrogen peroxide
0.1% ProClin 150

[4B] SUB B 2 x 3 mL
(Substrate B)
0.1% 3,3',5,5'-Tetramethylbenzidine (TMB)
40% Dimethylformamide (DMF)

T  40% (w/w) Dimethylformamide (DMF)
Toxic

R: 61-20/21-36 May cause harm to the unborn child. Harmful by inhalation and in contact with the skin. Irritating to eyes
S: 53-46 Avoid exposure - obtain special instructions before use. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

[5] STOP 2 x 12 mL
(Stop Reagent)
4.9% sulfuric acid

10X WB 3 x 90 mL
(10X-Wash Concentrate)
< 2% Phosphate buffer
< 9% Sodium chloride
EDTA
< 2% Detergent
0.5% ProClin

WARNINGS AND PRECAUTIONS

A. **FOR *IN VITRO* DIAGNOSTIC USE.**

B. This test is for use with human plasma collected in EDTA or ACD anticoagulants only. ***Heparin has been shown to inhibit PCR and must not be used with this procedure.***

C. Do not pipette by mouth.

D. Do not eat, drink or smoke in laboratory work areas. Wear disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

E. Avoid microbial and ribonuclease contamination of reagents when removing aliquots from reagent bottles. The use of sterile disposable pipettes and pipette tips is recommended.

F. Do not pool reagents from different lots or from different bottles of the same lot.

G. Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

H. Do not use a kit after its expiration date.

I. Material Safety Data Sheets (MSDS) are available on request from the Roche Response Center® or your local Roche office. See telephone numbers listed on the last page of this insert.

J. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Pre-Amplification Area and moving to the Post-Amplification (Amplification/Detection) Area. Pre-amplification activities must begin with reagent preparation and proceed to specimen preparation. Supplies and equipment must be dedicated to each activity and not used for other activities or moved between areas. Gloves must be worn in each area and must be changed before leaving that area. Equipment and supplies used for reagent preparation must not be used for specimen preparation activities or for pipetting or processing amplified DNA or other sources of target DNA. Post-Amplification supplies and equipment must be confined to the Post-Amplification Area at all times.

K. Specimens should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[33] and in the NCCLS Document M29-T[34]. Thoroughly clean and disinfect all work surfaces with 10% bleach.

L. **CAUTION:** This kit contains a component (**NHP**) derived from human blood. The source material has been assayed by US FDA approved tests and found non-reactive for the presence of Hepatitis B Surface Antigen (HBsAg) and antibodies to HIV-1-1/2 and HCV. No known test methods can offer complete assurance that product derived from human blood will not transmit infectious agents. Therefore all human sourced material should be considered potentially infectious. **NHP** should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[33] and in the NCCLS Document M29-T[34]. Thoroughly clean and disinfect all work surfaces with 10% bleach.

Note: ***Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.***

M. **HIV-1 QS, HIV-1 DIL, HIV-1 MMX, HIV-1 Mn^{2+}, HIV-1 (–) C, HIV-1 L(+)C,** and **HIV-1 H(+)C** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. After disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of water to prevent azide buildup.

N. Wear eye protection, laboratory coats and disposable gloves when handling **HIV-1 LYS, HIV-1 MMX, [1] MONITOR DN, [2] MONITOR HYB, [3] AV-HRP, [4A] SUB A, [4B] SUB B,** Working Substrate (mixed **[4A] SUB A and [4B] SUB B**) and **[5] STOP**. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills of these reagents occur, dilute with water before wiping dry.

O. Avoid contact between the skin or mucous membranes and **[4B] SUB B** or Working Substrate. If skin contact occurs wash immediately with large amounts of water.

P. **[4B] SUB B** and Working Substrate contain dimethylformamide which has been reported to be toxic in high oral doses and may be harmful to the unborn child. Skin contact, inhalation of fumes and ingestion should be avoided. If skin contact occurs, wash thoroughly with soap and water and seek medical advice immediately.

Q. Do not allow **HIV-1 LYS**, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.

R. Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens. ***Do not use snap cap tubes.***

STORAGE AND HANDLING REQUIREMENTS

A. ***Do not freeze reagents.***

B. Store **HIV-1 LYS, HIV-1 QS,** and **HIV-1 DIL** at 2-8°C. Unopened, these reagents are stable until the expiration date indicated. Once opened, any unused portion should be discarded.

C. A precipitate forms in **HIV-1 LYS** during storage at 2-8°C . Prior to use, warm to 25-37°C and mix thoroughly to dissolve the precipitated material. Once opened, any unused portion should be discarded. Working Lysis Reagent (prepared by addition of **HIV-1 QS** to **HIV-1 LYS**) should be stored at room temperature and used within 4 hours of preparation.

D. Store **HIV-1 MMX** and **HIV-1 Mn^{2+}** at 2-8°C. These reagents are stable until the expiration date indicated. Working Master Mix (prepared by the addition of **HIV-1 Mn^{2+}** to **HIV-1 MMX**) must be stored at 2-8°C and is stable for 4 hours at 2-8°C.

E. Store **NHP, HIV-1 (–) C, HIV-1 L(+)C** and **HIV-1 H(+)**C at 2-8°C. These reagents are stable until the expiration date indicated.

F. Store **[1] MONITOR DN, [2] MONITOR HYB** and **[5] STOP** at 2-25°C. These reagents are stable until the expiration date indicated.

G. Store **HIV-1 MONITOR MWP** at 2-8°C in the foil pouch provided. **HIV-1 MONITOR MWP** is stable in the unopened pouch until the expiration date indicated. Once opened, **HIV-1 MONITOR MWP** is stable for 3 months (or until the expiration date, whichever comes first) in the resealed pouch containing desiccant.

H. Store **[3] AV-HRP, [4A] SUB A** and **[4B] SUB B** at 2-8°C. Unopened, these reagents are stable until the expiration date indicated. Once opened, these reagents are stable for 3 months (or until the expiration date, whichever comes first).

I. Working Substrate must be freshly prepared by mixing **[4A] SUB A** with **[4B] SUB B** and is stable at ambient temperature for 3 hours when protected from light. Do not expose **[4A] SUB A, [4B] SUB B** or Working Substrate (mixed **[4A] SUB A** and **[4B] SUB B**) to metals, oxidizing agents or direct light.

J. Store **10X WB** at 2-25°C. The solution is stable until the expiration date indicated. Working Wash Solution (1X) should be stored at 2-25°C in a clean, closed plastic container and is stable for 2 weeks.

MATERIALS PROVIDED

AMPLICOR HIV-1 MONITOR Test
(US:83088)

A. AMPLICOR HIV-1 MONITOR Specimen Preparation Reagents

HIV-1 LYS
(HIV-1 MONITOR Lysis Reagent)
HIV-1 QS

(HIV-1 MONITOR Quantitation Standard)

HIV-1 DIL
(HIV-1 MONITOR Specimen Diluent)

B. AMPLICOR HIV-1 MONITOR Control Reagents

NHP
[Negative Plasma (Human)]

HIV-1 (–) C
(HIV-1 MONITOR (–) Control)

HIV-1 L(+)C
(HIV-1 MONITOR Low (+) Control)

HIV-1 H(+)C
(HIV-1 MONITOR High (+) Control)

C. AMPLICOR HIV-1 MONITOR Amplification Reagents

HIV-1 MMX
(HIV-1 MONITOR Master Mix)

HIV-1 Mn^{2+}
(HIV-1 MONITOR Manganese Solution)

D. AMPLICOR HIV-1 MONITOR Detection Reagents

HIV-1 MONITOR MWP
(HIV-1 MONITOR Microwell Plate)

[1] MONITOR DN
(MONITOR Denaturation Solution)

[2] MONITOR HYB
(MONITOR Hybridization Buffer)

[3] AV-HRP
(Avidin-Horseradish Peroxidase Conjugate)

[4A] SUB A
(Substrate A)

[4B] SUB B
(Substrate B)

[5] STOP
(Stop Reagent)

10X WB
(10X-Wash Concentrate)

MATERIALS REQUIRED BUT NOT PROVIDED:

Pre-Amplification Area - Reagent Preparation

- For PE Applied Biosystems GeneAmp® PCR System 9600 thermal cycler, use MicroAmp® Reaction Tubes (PE #N801-0533), Caps (PE #N801-0535), Tray/Retainers (PE #N801-0530) and Base (PE #N801-0531)
- For PE Applied Biosystems GeneAmp PCR System 2400 thermal cycler, use MicroAmp Reaction Tubes (PE #N801-0533), Caps (PE #N801-0535), Tray/Retainers (PE #N801-5530) and Base (PE #N801-5531)
- Plastic resealable bag
- Eppendorf® Repeater® pipet with 1.25 mL Combitip® Reservoir (sterile, individually wrapped)
- Pipettors (capacity 50 µL and 200 µL)* with aerosol barrier or positive displacement RNase-free tips
- Vortex mixer
- Latex gloves, powderless

Pre-Amplification Area - Specimen and Control Preparation

- 2.0 mL screw-cap tubes, sterile (Sarstedt 72.693.005, 72.694.005, 72.694.006, or equivalent) **
- 1.5 mL screw-cap tubes, sterile (Sarstedt 72.692.105, or equivalent) **
- Tube racks (Sarstedt 93.1428)
- Ethyl alcohol, absolute or 95% (freshly diluted to 70% using deionized water)
- Isopropyl alcohol, reagent grade (Fisher A416-500)
- Fine tip, sterile transfer pipets, RNase-free
- Sterile disposable, polystyrene pipets (5 mL, 10 mL and 25 mL)
- Micropipettes (capacity 12.5 µL, 25 µL, 50 µL, 100 µL, 200 µL, 400 µL, 500 µL, 600 µL, 800 µL and 1000 µL)* with aerosol barrier or positive displacement tips
- Microcentrifuge (max. RCF 16,000 x g, min. RCF 12,500 x g); Eppendorf 5415C, HERMLE Z230M, or equivalent
- Refrigerated centrifuge and fixed angle rotor (45 degrees, capacity for at least 24 1.5 mL tubes) with a RCF of 23,600 x g: Heraeus 17RS with HFA 22.1 rotor, or equivalent..
- Vortex mixer
- Latex gloves, powderless

Post Amplification Area - Amplification/Detection

- Multichannel pipettor (capacity 25 µL and 100 µL) or electronic pipettor (Impact® or AMPLICOR®)
- Aerosol barrier or positive displacement pipettor tips (25 µL and 100 µL) and barrier-free tips (100 µL)*
- PE Applied Biosystems GeneAmp PCR System 9600 or GeneAmp PCR System 2400 thermal cycler
- MicroAmp base and cap installing tool for PE Applied Biosystems GeneAmp PCR System 9600 or GeneAmp PCR System 2400
- Microwell Plate Washer***
- Microwell Plate Reader****
- Disposable Reagent Reservoirs
- Microwell plate lid (Fisher catalog no. 07-200-376)
- Incubator 37°C ± 2°C
- Distilled or deionized water
- Graduated vessels
- Latex gloves, powderless

* Pipettors should be accurate within 3% of stated volume. Aerosol barrier or positive displacement RNase-free tips must be used where specified to prevent specimen and amplicon cross-contamination.

** Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens. ***Do not use snap cap tubes.***

*** Capable of washing 12 x 8 microwell format with 250-300 µL of Wash Solution per well at 30 second time intervals.

**** Microwell Reader Specifications: Bandwidth = 10 ± 3 nm; Absorbance Range = 0 to ≥ 3.00 A_{450}; Repeatability ≤1%; Accuracy ≤ 3% from 0 to 2.00 A_{450}; Drift ≤ 0.01 A_{450} per hour.

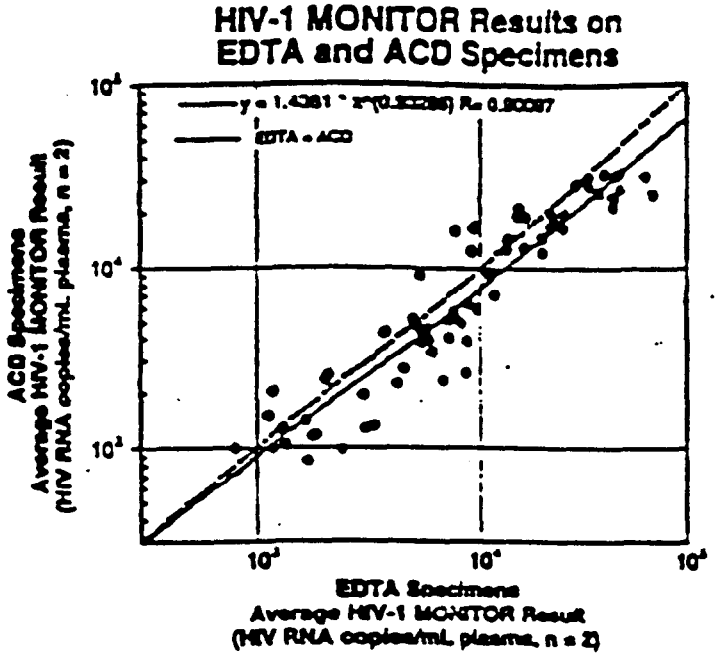
SPECIMEN COLLECTION, STORAGE and TRANSPORT

NOTE: Handle all specimens as if they are capable of transmitting infectious agents.

A. Specimen Collection

The AMPLICOR HIV-1 MONITOR Test is for use with plasma specimens only. Blood should be collected in sterile tubes using EDTA (lavender top, Becton-Dickinson #6454 or equivalent) or ACD (yellow top, Becton-Dickinson #4606 or equivalent) as the anticoagulant. ***Specimens anticoagulated with heparin are unsuitable for this test.*** The use of ACD anticoagulated specimens will yield test results that are approximately 15% lower than test results obtained from EDTA anticoagulated specimens due to the dilution effect of the 1.5 mL of ACD anticoagulant present in the blood collection tube. Figure I shows the effect of ACD and EDTA anticoagulants on HIV RNA results from plasma specimens.

Figure 1
Comparison of ACD and EDTA Anticoagulants



Store whole blood at 2-25°C for no longer than 6 hours. Do not refrigerate. Separate plasma from whole blood within 6 hours of collection by centrifugation at 800-1600 x g for 20 minutes at room temperature. Transfer plasma to a sterile polypropylene tube.

B. Specimen Storage

Plasma specimens may be stored at room temperature for up to 1 day, at 2-8°C for up to 5 days, or frozen at -20°C to -80°C.

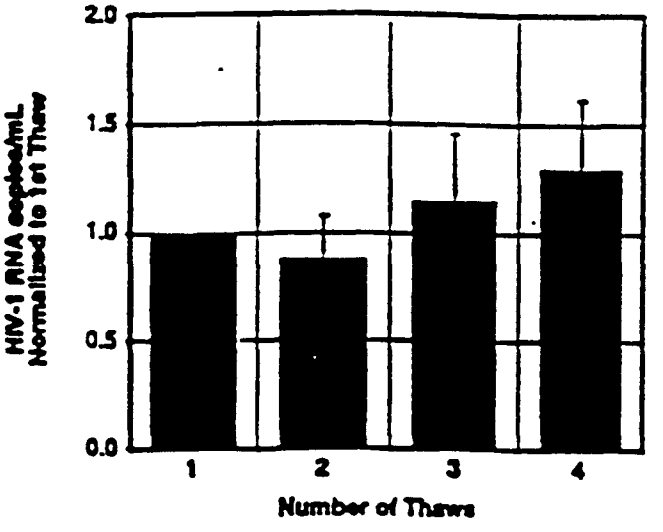
Virus pellets obtained from step 9 of the UltraSensitive Specimen Preparation Procedure are stable for up to 6 hours at room temperature and at least 14 days at -20°C or colder.

It is recommended that specimens be stored in 600-700 µL aliquots in sterile, 2 mL polypropylene screw cap tubes (such as Sarstedt catalog no. 782.694.006). In-house studies have shown that plasma specimens may be frozen and thawed up to 3 times. Figure 2 shows the data from these freeze-thaw studies.

C. Specimen Transport

Transportation of whole blood or plasma must comply with country, federal, state and local regulations for the transport of etiologic agents[35]. Whole blood must be transported at 2-25°C and processed within 6 hours of collection. Plasma may be transported at 2-8°C or frozen.

Figure 2
Average HIV-1 Results from Three Pools of HIV-1 Positive Plasma
After 2, 3 and 4 Freeze-Thaw Cycles



INSTRUCTIONS FOR USE

Note: ***Visually examine reagents for sufficient reagent volume before beginning the test procedure.***

Note: ***Serum and plasma specimens must be at ambient temperature before use. Use pipettors with aerosol barrier or positive displacement tips where specified. Use extreme care to ensure selective amplification.***

Note: ***Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens. Do not use snap cap tubes.***

Run Size:

Each kit contains reagents sufficient for two 12-test batches, which may be performed separately or simultaneously. It is recommended that one replicate of the HIV-1 MONITOR (-) Control, the HIV-1 MONITOR Low (+) Control and the HIV-1 MONITOR High (+) Control be included in each test run (see *"Quality Control"* section).

The Specimen Preparation and Amplification Reagents are packaged in 12-test, single-use bottles. For the most efficient use of reagents, specimens and controls should be processed in batches of 12 or 24.

Workflow:

The AMPLICOR HIV-1 MONITOR Test can be completed in one day or over two days. If the testing is to be completed in a single work day, follow the instructions in Parts A through D in order. If the testing is to be completed over 2 days, the procedure may be stopped after Specimen Preparation (Part B) or after Amplification (Part C).

- To perform specimen processing on Day 1 and amplification/detection on Day 2, perform "Standard Specimen and Control Preparation", Steps B1.1 through B1.17, or "UltraSensitive

Specimen and Control Preparation". Steps B2.1 through B2.22, on Day 1 and store the processed specimens as indicated in Step B1.17 or Step B2.22, respectively. On Day 2 begin with Part A, thaw the processed specimens at room temperature and then continue with either Step B1.18 (for Standard Specimen Processing) or Step B2.23 (for UltraSensitive Specimen Processing).

- To complete specimen processing and amplification on Day 1 and detection on Day 2, perform Parts A, B and C on Day 1 and store the denatured amplicon as indicated in Step C.5. Continue with Part D on Day 2.

A. Reagent Preparation.
Performed in: Pre-Amplification - Reagent Preparation Area

Note: ***Store HIV-1 MMX and HIV-1 Mn^{2+} at 2 - 8°C until use.***

1. Place each batch of up to 12 MicroAmp reaction tubes in a single row of a MicroAmp tray. Lock tubes in place with a tube retainer.
2. Prepare Working Master Mix by adding 100 µL **HIV-1 Mn^{2+}** to one tube of **HIV-1 MMX** (the mixture is sufficient for 12 reactions). *It is not necessary to measure the volume of Master Mix. Add 100 µL **HIV-1 Mn^{2+}** to the entire tube of **HIV-1 MMX**.* **Recap the HIV-1 MMX tube and mix well by inverting the tube 10-15 times** The pink dye in **HIV-1 Mn^{2+}** is used for visual confirmation that **HIV-1 Mn^{2+}** has been added to **HIV-1 MMX**. ***Discard remaining HIV-1 Mn^{2+}.***
3. Pipette 50 µL of Working Master Mix into each reaction tube using a repeat pipettor or a pipettor with an aerosol barrier or positive displacement tip. Confirm that **HIV-1 Mn^{2+}** was added to **HIV-1 MMX** by checking for the pink color. ***Discard unused Working Master Mix.***
4. Place the MicroAmp tray in a plastic, resealable bag, and move to the Pre-Amplification - Specimen Preparation Area. ***Store the MicroAmp Tray at 2-8°C until specimen preparation is completed. Amplification must begin within 4 hours of preparation of the Working Master Mix.***
5. Remove gloves and dispose of them properly. Remove labcoat and/or any other protective garments and leave them in the Reagent Preparation Area.

B. Specimen and Control Preparation
Performed in Pre-Amplification-Specimen and Control Preparation Area

Note: ***All reagents must be at ambient temperature before use.***

Note: ***To amplify previously prepared specimens, perform the steps in Part A, thaw prepared specimens at room temperature and continue with Part B1, "Standard Specimen and Control Preparation", Step B1.18, or with Part B2, "UltraSensitive Specimen and Control Preparation", Step B2.23.***

Note: ***A precipitate forms in HIV-1 LYS upon storage at 2-8°C. Prior to use, warm at 25-37°C and mix to dissolve the precipitated material.***

B1. Standard Specimen and Control Preparation

1. Prepare 70% ethanol. For 12 tests, mix 11.0 mL 95% ethanol and 4.0 mL of deionized or distilled water or mix 14.0 mL absolute ethanol with 6.0 mL of deionized or distilled water.
2. Label a 2.0 mL screw cap microcentrifuge tube for each specimen and control. ***DO NOT USE SNAP CAP TUBES***. See "*Warnings and Precautions*" section.
3. Prepare Working Lysis Reagent. Vortex **HIV-1 QS** for at least 10 seconds prior to use to ensure that it is thoroughly mixed. For each batch of up to 12 specimens and controls, add 100 µL **HIV-1 QS** to one bottle of **HIV-1 LYS** and mix well. *It is not necessary to measure the volume of **HIV-1 LYS**. Add 100 µL **HIV-1 QS** to the entire bottle of **HIV-1 LYS***. The pink dye in **HIV-1 QS** is used for visual confirmation that **HIV-1 QS** has been added to **HIV-1 LYS**. ***Discard remaining HIV-1 QS.***
4. Thaw plasma specimens at room temperature and vortex for 3 - 5 seconds.
5. Spin tube briefly to collect specimen in base of tube. Take care to avoid contaminating gloves when manipulating specimens.
6. Dispense 600 µL Working Lysis Reagent into each tube. Check that Working Lysis Reagent is pink to confirm that **HIV-1 QS** was added to **HIV-1 LYS**.
7. Add 200 µL of each patient specimen to appropriately labeled tubes. Cap the tubes and vortex for 3 - 5 seconds.
8. For each negative, low and high positive control, add 200 µL **NHP** to the appropriate tubes. Cap the tubes and vortex for 3 - 5 seconds, then add 50 µL of **HIV-1 (–)C, HIV-1 L(+)C** and **HIV-1 H(+)C** to the appropriately labeled tubes. Cap the tubes and mix.
9. Incubate the tubes for 10 minutes at room temperature.
10. Add 800 µL room temperature 100% isopropanol to each tube. Re-cap the tube and vortex for 3 - 5 seconds.
11. Put an orientation mark on each tube and place the tubes into the microcentrifuge with the orientation mark facing outward, so that the pellet will align with the orientation mark. Centrifuge specimens at maximum speed (at least 12,500 x g) for 15 minutes at room temperature.
12. Using a new, fine tip disposable transfer pipette for each tube, carefully remove and discard the supernatant from each tube, being careful not to disrupt the pellet (which may not be visible). Remove as much liquid as possible without disturbing the pellet. Withdraw the supernatant slowly, allowing the liquid to drain completely off the sides of the tube. ***Do not use vacuum aspiration***.
13. Add 1.0 mL room temperature 70% ethanol to each tube, re-cap the tube, and vortex for 3 - 5 seconds.
14. Place the tubes into a microcentrifuge with the orientation marks facing outward and centrifuge the tubes for 5 minutes at maximum speed (at least 12,500 x g) at room temperature.
15. Carefully remove the supernatant using a new, fine tip disposable transfer pipette for each tube as before without disturbing the pellet. The pellet should be clearly visible at this step. ***Remove as much of the supernatant as possible! Residual ethanol can inhibit the amplification***.
16. Add 400 µL **HIV-1 DIL** to each tube. Re-cap the tube and vortex vigorously for 10 seconds to resuspend the extracted RNA. Note that insoluble material often remains.

17. Amplify the processed specimens (Step B.18) within 2 hours of preparation or store frozen at -20°C or colder for up to one week.

18. Pipette 50 µL of each prepared control and patient specimen to the appropriate MicroAmp reaction tubes using a micropipettor with plugged tips. Use a new tip for each specimen and control. Cap the tubes. Seal the caps using the MicroAmp cap installing tool. Record the positions of the controls and specimens.

19. ***Transfer the MicroAmp Tray with sealed tubes containing the processed specimens and controls in Working Master Mix to the Amplification/Detection Area.***

B2. Specimen and Control Preparation - UltraSensitive Procedure

1. Pre-cool the ultracentrifuge and rotor to 2 - 8°C. See the operating instructions for the ultracentrifuge for details.

2. Prepare 70% ethanol. For 12 tests, mix 11.0 mL of 95% ethanol and 4.0 mL of deionized or distilled water or mix 14.0 mL absolute ethanol and 6.0 mL of deionized or distilled water.

3. Prepare Working Lysis Reagent. Vortex **HIV-1 QS** for at least 10 seconds prior to use to ensure that it is thoroughly mixed. For each batch of up to 12 specimens and controls, add 25 µL **HIV-1 QS** to one bottle of **HIV-1 LYS** and mix well. *It is not necessary to measure the volume of **HIV-1 LYS***. Add 25 µL **HIV-1 QS** to the entire bottle of **HIV-1 LYS.** The pink dye in **HIV-1 QS** is used for visual confirmation that **HIV-1 QS** has been added to **HIV-1 LYS**. ***Discard the remaining HIV-1 QS.***

4. Label a 1.5 mL screw cap microcentrifuge tube for each specimen and control. ***DO NOT USE SNAP CAP TUBES.*** See *"Warnings and Precautions"* section.

5. Thaw plasma specimens at room temperature and vortex for 3 - 5 seconds.

6. Spin tubes briefly to collect specimens in base of the tubes. Take care to avoid contaminating gloves when manipulating specimens.

7. Add 500 µL of each patient specimen to the appropriately labeled tube.

8. For each negative and positive control, add 500 µL NHP to each appropriately labeled tube.

9. Put an orientation mark on each tube and place the tubes into the ultracentrifuge with the orientation mark facing outward, so that the pellets will align with the orientation marks. Centrifuge specimens and control tubes containing NHP at 23,600 x g at 2 - 8° C for 60 minutes.

10. Using a new, fine tip disposable transfer pipette for each tube, carefully remove and discard the supernatant from each tube, being careful not to disrupt the pellet. ***The pellet may not be visible at this step. Remove as much liquid as possible without disturbing the pellet. Withdraw the supernatant slowly, allowing the liquid to drain completely off the sides of the tube. Do not use vacuum aspiration.***

11. Dispense 600 µL Working Lysis Reagent into each tube. Check that Working Lysis Reagent is pink to confirm that **HIV-1 QS** was added to **HIV-1 LYS**.

12. For each negative and positive control, add 12.5 µL of **HIV-1 (-)C, HIV-1 L(+)C**, and **HIV-1 H(+)C** to the appropriately labeled tubes. Cap the tubes and mix by vortexing for 3 - 5 seconds.

13. Incubate the tubes for 10 minutes at room temperature.

14. Add 600 µL room temperature 100% isopropanol to each tube. Re-cap the tube and vortex for 3 - 5 seconds

15. Place the tubes into the microcentrifuge with the orientation marks facing outward, so that the pellets will align with the orientation marks. Centrifuge specimens at maximum speed (at least 12,500 x g) for 15 minutes at room temperature.

16. Using a new, fine tip disposable transfer pipette for each tube, carefully remove and discard the supernatant from each tube, being careful not to disrupt the pellet. ***The pellet may not be visible at this step. Remove as much liquid as possible without disturbing the pellet. Withdraw the supernatant slowly, allowing the liquid to drain completely off the sides of the tube. Do not use vacuum aspiration.***

17. Add 1.0 mL room temperature 70% ethanol to each tube, re-cap the tube, and vortex for 3 - 5 seconds.

18. Place the tubes into the microcentrifuge with the orientation marks facing outward and centrifuge the tubes for 5 minutes at maximum speed (at least 12,500 x g) at room temperature.

19. Using a new, fine tip disposable transfer pipette for each tube, carefully remove and discard the supernatant from each tube, being careful not to disrupt the pellet. ***The pellet should be clearly visible at this step. Remove as much liquid as possible without disturbing the pellet. Withdraw the supernatant slowly, allowing the liquid to drain completely off the sides of the tube. Do not use vacuum aspiration.***

20. Repeat Step B2.19 to remove as much of the remaining supernatant as possible. Residual ethanol can inhibit the amplification.

21. Add 100 µL **HIV-1 DIL** to each tube. Re-cap the tube and vortex vigorously for 10 seconds to resuspend the extracted RNA. Note that insoluble material often remains.

22. Amplify the processed specimens (Step B.42) within 2 hours of preparation or store frozen at - 20°C or colder for up to one week.

23. Pipette 50 µL of each processed control and patient specimen to appropriate MicroAmp reaction tubes using a micropipettor with plugged tips. Use a new tip for each specimen and control. Cap the tubes. Seal the caps using the MicroAmp cap installing tool. Record the positions of the controls and specimens.

24. ***Transfer the MicroAmp tray with sealed tubes containing the processed specimens and controls in Working Master Mix to the Amplicfication/Detection Area.***

C. Reverse Transcription and Amplification
Performed in Post-Amplification - Amplification/Detection Area

Note: Warm the MONITOR DN to ambient temperature before use.

Note: Turn on the GeneAmp PCR System 9600 or GeneAmp PCR System 2400 thermal cycler at least 30 minutes prior to beginning the amplification.

1. Place the Tray/Retainer assembly into the thermal cycler block.

2. Program the Perkin-Elmer GeneAmp PCR System 9600 or GeneAmp PCR System 2400 thermal cycler as follows.

HOLD Program	2 minutes at 50°C
HOLD Program	30 minutes at 60°C
CYCLE Program (4 Cycles)	10 sec at 95°C, 10 sec at 55°C, 10 sec at 72°C
CYCLE Program (26 Cycles)	10 sec at 90°C, 10 sec at 60°C, 10sec at 72°C
HOLD Program	15 minutes at 72°C

In the CYCLE programs, the ramp time and allowed setpoint error should be left at the default settings of 0:00 (which is the maximum rate) and 2°C, respectively. Link the 5 programs together into a METHOD program.

Consult the PE Applied Biosystems GeneAmp PCR System 9600 or GeneAmp PCR System 2400 User's Manual for additional information on programming and operation of the thermal cycler.

3. Start the METHOD program. The program runs approximately one hour and 30 minutes.

4. Remove the tray from the thermal cycler at any time during the final HOLD program, place in the MicroAmp Base and continue immediately with Step C.5. Do not allow the reaction tubes to remain in the thermal cycler beyond the end of the final HOLD program and do not extend the final HOLD program beyond 15 minutes. ***DO NOT BRING AMPLIFIED SAMPLES INTO THE PRE-AMPLIFICATION AREA. AMPLIFIED CONTROLS AND SPECIMENS SHOULD BE CONSIDERED A MAJOR SOURCE OF CONTAMINATION.***

5. Remove the caps from the reaction tubes carefully to avoid creating aerosols of the amplification products. Immediately pipette 100 µL **[1] MONITOR DN** into each reaction tube using a multichannel pipettor and mix by pipetting up and down 5 times. (Program 1, AMPLICOR Electronic Impact Pipettor; see instructions for the AMPLICOR Electronic Impact Pipettor).

   Denatured amplicon can be held at room temperature no more than 2 hours before proceeding to the detection reaction (Part D). If the detection reaction can not be performed within 2 hours, re-cap the tubes and store the denatured amplicon at 2-8°C for up to one week.

D. Detection

Performed in: Amplification/Detection Area

Note: Warm all reagents to room temperature prior to use.

1. Prepare Working Wash Solution as follows. Examine **10X WB**, and, if necessary, warm at 30 - 37°C to redissolve any precipitate. Add 1 volume **10X WB** to 9 volumes of distilled or deionized water. Mix well. The volume of Working Wash Solution required depends on model of MWP washer being used. Store Working Wash Solution in a clean, closed plastic container at 2 - 25°C for up to 2 weeks.

2. Allow **HIV-1 MONITOR MWP** to warm to room temperature before removing from the foil pouch. Add 100 µL **[2] MONITOR HYB** to each well (Program 2, AMPLICOR Electronic Impact Pipettor; see instructions for the AMPLICOR Electronic Impact Pipettor). Rows A through F of the **HIV-1 MONITOR MWP** are coated with the HIV-specific oligonucleotide probe; rows G and H are coated with the QS-specific oligonucleotide probe.

3. Add 25 µL of the denatured amplicon to the HIV wells in row A of the MWP and mix up and down 10 times with a 12-channel pipettor with plugged tips. Make serial 5-fold dilutions in the HIV wells in rows B through F as follows. Transfer 25 µL from row A to row B and mix as before. Continue through row F. Mix row F as before, then remove and discard 25 µL. Discard pipet tips. (Addition of the denatured amplicon to the MWP and the serial dilutions may be done with Program 3 of the AMPLICOR Electronic Impact Pipettor. This program transfers 25 µL, mixes by pipetting 60 µL up and down 5 times, and aspirates 25 µL; see instructions for the AMPLICOR Electronic Impact Pipettor.)

4. Add 25 µL of the denatured amplicon to the QS wells in row G of the MWP and mix up and down 10 times with a 12-channel pipettor with plugged tips. Using a 12-channel pipettor with plugged tips, pipette 25 µL of denatured amplicon into row G of the MWP and mix by pipetting up and down 10 times (or use Program 3, AMPLICOR Electronic Impact Pipettor). Transfer 25 µL from row G to row H. Mix as before, then remove 25 µL from row H and discard.
5. Cover the MWP with MWP lid and incubate for 1 hour at 37°C ± 2°C.
6. Wash the MWP 5 times with the Working Wash Solution using an automated MWP washer. Program the MWP washer as follows:
   (a) Fill each well to top (250-300 µL). Let soak for 30 seconds. Aspirate.
   (b) Repeat Step (a) four additional times.
   (c) Tap the plate dry.
7. Add 100 µL **[3] AV-HRP** conjugate to each well (Program 2, AMPLICOR Electronic Impact Pipettor). Cover the MWP and incubate for 15 minutes at 37°C ± 2°C.
8. Wash the MWP as described in Step D.7.
9. Prepare the Working Substrate Solution. For each MWP, mix 12 mL **[4A] SUB A** with 3 mL **[4B] SUB B**. Protect Working Substrate from direct light. Working Substrate must be at room temperature and used within 3 hours of preparation.
10. Pipette 100 µL Working Substrate Solution into each well (Program 2, AMPLICOR Electronic Impact Pipettor).
11. Allow color to develop for 10 minutes at room temperature in the dark.
12. Add 100 µL **[5] STOP** to each well (Program 2, AMPLICOR Electronic Impact Pipettor).
13. Measure the optical density at 450 nm (single wavelength) within 10 minutes of **[5] STOP** addition.

RESULTS

For each specimen and control, calculate the HIV-1 RNA level as follows:

1. Choose the appropriate HIV well as follows:
   a. The HIV wells in rows A through F represent neat and 1:5, 1:25, 1:125, 1:625 and 1:3125 serial dilutions, respectively, of the HIV-1 amplicon. The absorbance values should decrease with the serial dilutions, with the highest OD_{450} for each specimen or control in row A and the lowest OD_{450} in row F.
   b. Choose the well with the lowest OD_{450} that is ≥ 0.20 and ≤ 2.00 OD units.
   c. If any of the following conditions exist, see ***Unexpected Results***:
      - all HIV OD values < 0.20
      - all HIV OD values > 2.0
      - HIV OD values not in sequence (OD values do not decrease from well A to well F)
2. Subtract background from the selected HIV OD value (background = 0.07 OD units).
3. Calculate the **Total HIV OD** by multiplying the **background-corrected OD** value of the selected HIV well by the dilution factor associated with that well.

Row	Dilution Factor
A	1
B	5
C	25
D	125
E	625
F	3125

4. Choose the appropriate QS well as follows:

   a. The QS wells in rows G and H represent neat and 1:5 dilutions, respectively, of the QS amplicon. The absorbance value in row G should be greater than the value in row H.

   b. Choose the well with the lowest OD_{450} that is ≥ 0.30 and ≤ 2.0 OD units.

   c. If the following conditions exist, see ***Unexpected Results:***

   - both QS OD values < 0.30
   - both QS OD values > 2.0
   - QS OD values not in sequence (well H has a higher OD than well G)

NOTE: *All patient specimens and controls should yield QS OD values that meet the criteria described in Step 4, demonstrating that the specimen processing, reverse transcription, amplification and detection steps were performed correctly. If any control has a QS OD value that does not meet the criteria described in Step 4,* ***the entire run is invalid.*** *If any specimen has a QS OD value that does not meet the criteria described in Step 4,* ***the result for that specimen is invalid, but the run is still acceptable.***

5. Subtract background from the selected QS OD values (background = 0.07 OD units).

6. Calculate the ***Total QS OD*** by multiplying the ***background-corrected OD*** value of the selected QS well by the dilution factor associated with that well.

Row	Dilution Factor
G	1
H	5

7A. Determination of Standard Test Results

Calculate HIV-1 RNA copies/mL plasma as follows:

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

Where:

Total HIV-1 OD	=	calculated Total HIV-1 OD (from Step 3)
Total QS OD	=	calculated Total QS OD (from Step 6)
Input HIV-1 QS copies/PCR	=	the number of copies of QS in each reaction; this information is lot specific. See AMPLICOR HIV-1 MONITOR Test Data Card.
40	=	factor to convert copies per PCR to copies per mL of plasma

NOTE: Test results greater than 750,000 should be reported as "Greater than 750,000 copies/mL". If quantitative results are desired for such specimens, the original plasma specimen should be diluted with HIV negative human plasma and retested. Test results less than 400 should be reported as "HIV-1 RNA detected; Less than 400 copies/mL".

7B. Determination of UlraSensitive Test Results

Calculate HIV-1 RNA copies/mL plasma as follows:

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

Where:

Total HIV-1 OD	=	calculated Total HIV-1 OD (from Step 3)
Total QS OD	=	calculated Total QS OD (from Step 6)
Input HIV-1 QS copies/PCR	=	the number of copies of QS in each reaction; this information is lot specific. See AMPLICOR HIV-1 MONITOR Test Data Card.
4	=	factor to convert copies per PCR to copies per mL of plasma

NOTE: Test results greater than 75,000 should be reported as "Greater than 75,000 copies/mL". If quantitative results are desired for such specimens, the original plasma specimen should be retested using the Standard Specimen Processing Procedure. Test results less than 50 should be reported as "HIV-1 RNA detected; Less than 50 copies/mL".

8. Unexpected Results

a. ***All HIV OD values < 0.20.*** Do not calculate these results. Report these results as: "HIV-1 RNA not detected (Less than 400 copies/mL)" for the Standard procedure and "HIV-1 RNA not detected (Less than 50 copies/mL)" for the UltraSensitive procedure.

b. ***All HIV OD values > 2.0.*** If all HIV-1 wells have OD values greater than 2.0, the HIV-1 copy number is above the linear range of the assay. Do not calculate these results. Report the result as "Not determined". If quantitative results are desired, retest the specimen as follows:

- If the test result was generated using the Standard specimen processing procedure, prepare a 1:50 dilution of the original specimen with HIV negative human plasma and repeat the test using the Standard procedure. Calculate the HIV-1 result as above, then multiply the final result by 50.
- If the test result was generated using the UltraSensitive specimen processing procedure, retest the original specimen undiluted using the Standard specimen processing procedure.

c. ***HIV OD values out of sequence.*** If HIV wells do not follow the general pattern of decreasing OD values from well A to well F, an error in dilution may have occurred. Examine the data using the criteria shown in (g) below to determine if an error occurred. If an error occurred, the result for that specimen is invalid and the entire test procedure for that specimen (including specimen preparation) should be repeated.

d. ***Both QS OD values < 0.30.*** If both QS wells have OD values less than 0.30 then either the processed specimen was inhibitory to the amplification or the RNA was not recovered during specimen processing. The result for that specimen is invalid. Repeat the entire test procedure (including specimen processing) for that specimen.

e. ***Both QS OD values > 2.0.*** If both QS wells have OD values greater than 2.0, an error occurred. The result for that specimen is invalid. Repeat the entire test procedure (including specimen processing) for that specimen.

f. ***QS OD values out of sequence.*** QS OD values out of sequence. If the absorbance of well H is greater than the absorbance in well G, then an error occurred. The result for that specimen is invalid. Repeat the entire test procedure (including specimen processing) for that specimen.

g. ***Examples of unexpected results.***

- The OD values for HIV wells should follow a pattern of decreasing OD values with increasing Dilution Factor (i.e., from well A to well F), except for wells that are saturated and wells with background OD values.
- In reactions containing high HIV-1 RNA copies per mL, wells A, B and C can become saturated, turning a greenish-brown color prior to the addition of Stop Solution and a brown color after the addition of Stop Solution, resulting in lower OD_{450} (see examples 1 and 2 in Table 1). These results are valid even though the HIV wells do not have decreasing OD values from well A through well F.
- In reactions containing low HIV-1 RNA copies/mL, wells B through F may contain background OD values (see example 3 in Table 1). Such tests are valid even though the HIV wells do not have decreasing OD values from well A through well F. Wells with OD values > 2.3 may be saturated and wells with very low OD values (OD < 0.1) are close to background. These wells may not follow a pattern of decreasing OD values from well A to well F (examples 1, 2 and 3).
- All wells with OD values ≤ 2.3 and ≥ 0.1 should follow a pattern of decreasing OD values from well A to well F. If OD values ≤ 2.3 and ≥ 0.1 do not follow a pattern of decreasing OD values from well A to well F, then an error occurred (example 4). The results for this specimen are invalid. Repeat the entire test procedure (including specimen processing) for that specimen.

Table 1
Examples of Unexpected Results

Specimen Results (OD_{450})					
Row	Dilution Factor	Example 1	Example 2	Example 3	Example 4
A	1	2.610	2.564	0.812	3.126
B	5	2.461	2.684	0.161	0.857
C	25	3.112	2.432	0.055	1.432
D	125	2.668	1.032	0.064	0.292
E	625	2.984	0.287	0.079	0.074
F	3125	1.568	0.074	0.052	0.066
Interpretation:		Very high titer specimen. Not an error	High titer specimen. Not an error	Low titer specimen. Not an error	Error

QUALITY CONTROL

It is recommended that one replicate of the AMPLICOR HIV-1 (–) Control, the AMPLICOR HIV-1 MONITOR Low (+) Control, and the AMPLICOR HIV-1 MONITOR High (+) Control be included in each test run. As with any new laboratory procedure, new operators should consider the use of additional controls each time the test is performed until such time as a high degree of confidence is reached in their ability to perform the test correctly. There are no requirements regarding the position of the controls in the MicroAmp tray.

All controls should yield QS OD values that meet the criteria described in Step 4 of the ***RESULTS*** section, demonstrating that the specimen processing, reverse transcription, amplification and detection steps were performed correctly. ***If any control has a QS OD value that does not meet the criteria described in RESULTS, Step 4, the entire run is invalid.*** Repeat the entire process (specimen and control preparation, reverse transcription, amplification and detection).

Negative Control

The AMPLICOR HIV-1 (–) Control should yield a "Not Detected" result, i.e., all HIV-1 OD values less than 0.20. If the HIV-1 (–) Control does not meet this criteria, the entire run is invalid. Repeat the entire process (specimen and control preparation, reverse transcription, amplification and detection). If the OD values of the HIV-1 (–) Control are consistently greater than 0.20, contact the Roche Response Center (or your local Roche office, see the last page of this insert) for technical assistance.

Positive Controls

The assigned range for the AMPLICOR HIV-1 MONITOR Low (+) and High (+) Controls **in both the Standard and UltraSensitive procedures** is specific for each lot of control and is provided on the AMPLICOR HIV-1 MONITOR Test Data Card supplied with the kit. The HIV-1 RNA copy number/mL for both the HIV-1 MONITOR Low (+) Control and the HIV-1 MONITOR High (+) Control should fall within the range indicated on the Data Card. If one or both HIV-1 MONITOR (+) Controls does not meet this criteria, the entire run is invalid. Repeat the entire process (specimen and control preparation, reverse transcription, amplification and detection). If the HIV-1 RNA copy number/mL of one or both AMPLICOR HIV-1 MONITOR (+) Controls is consistently outside the assigned range, contact the Roche Response Center (or your local Roche office, see the last page of this insert) for technical assistance.

PROCEDURAL PRECAUTIONS

1. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Pre-Amplification Area and moving to the Post-Amplification (Amplification/Detection) Area. Pre-amplification activities must begin with reagent preparation and proceed to specimen preparation. Supplies and equipment must be dedicated to each activity and not used for other activities or moved between areas. Gloves must be worn in each area and must be changed before leaving that area. Equipment and supplies used for reagent preparation must not be used for specimen preparation activities or for pipetting or processing amplified DNA or other sources of target DNA. Post-Amplification supplies and equipment must be confined to the Post-Amplification Area at all times.

2. As with any test procedure, good laboratory technique is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test and the potential for contamination, extreme care should be taken to preserve the purity of kit reagents or amplification mixtures. All reagents should be closely monitored for purity. Discard any reagents that may be suspect.

PROCEDURE LIMITATIONS

- 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 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 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 quantitate HIV-1 RNA over the range of 50 - 750,000 copies/mL by using a combination of two specimen processing procedures, the Standard and UltraSensitive procedures. With the Standard procedure, the Test 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.

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

3. ***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 <u>not</u> be used with the AMPLICOR HIV-1 MONITOR Test.***

4. 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.

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

6. Only the PE Applied Biosystems GeneAmp PCR System 9600 or GeneAmp PCR System 2400 thermal cyclers can be used with this product.

7. As with any diagnostic test, results from the AMPLICOR HIV-1 MONITOR test should be interpreted with consideration of all clinical and laboratory findings.

PROCEDURAL GUIDELINES

The decision of when to use the UltraSensitive specimen processing procedure will be dependent upon the laboratory's specific patient population. It is recommended that each laboratory evaluate the characteristics of the patient population (types of treatments, previous viral load results, routine or clinical trial patients) to establish the best laboratory procedure for initial testing and when to reflex to the alternate method. For example, if the patient population does not include treatment naïve patients and if all patients are known to be on antiretroviral therapy, the laboratory may elect to use the UltraSensitive specimen processing procedure as the routine method and reflex to the Standard method only when viral load results are greater than 75,000 copies/mL. If the patient population is unknown as it relates to therapy or baseline viral load results, the laboratory may elect to use the Standard specimen processing procedure as the routine method and reflex to the UltraSensitive specimen processing procedure only after the Standard specimen preparation yields a viral load below 400 copies/mL.

INTERFERING SUBSTANCES

- ***Heparin inhibits PCR. Do not use specimens collected in heparin.***

- The following endogenous substances have been shown not to interfere with the quantitation of HIV-1-RNA by this test: triglycerides up to 1024 mg/dL, bilirubin up to 21 mg/dL and hemoglobin up to 19.5 mg/dL.

- 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.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The analytical sensitivity of the AMPLICOR HIV-1 MONITOR Test 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 AMPLICOR HIV-1 MONITOR 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.

Limit of Detection

The lower limit of detection of the AMPLICOR HIV MONITOR Test 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).

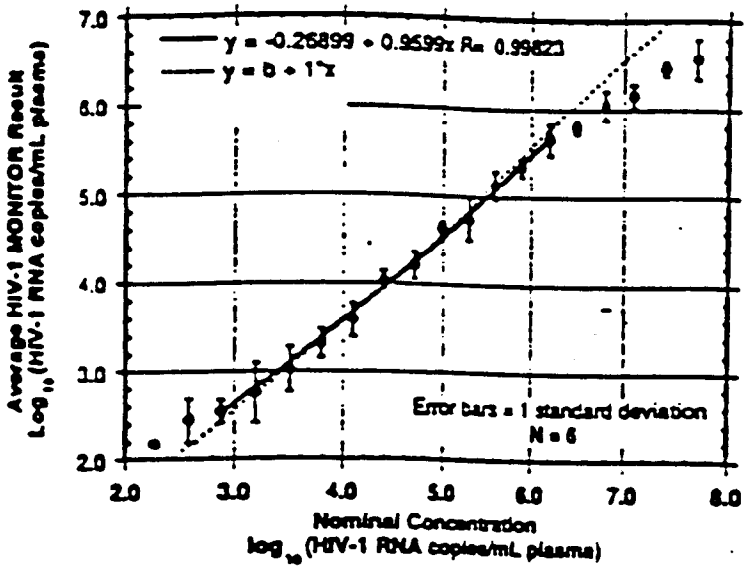
Linear Range

The linear range of the AMPLICOR HIV-1 MONITOR Test 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. As shown in Figure 3, 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.

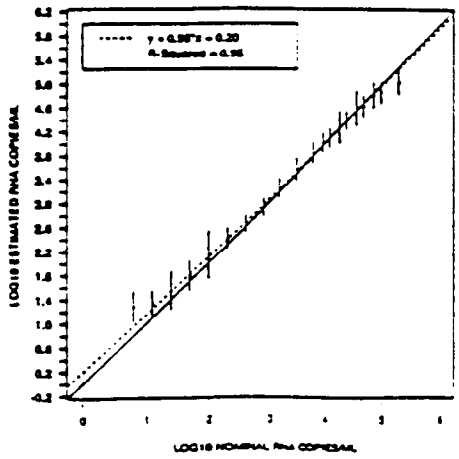
Figure 3
AMPLICOR HIV-1 MONITOR Test
with Standard Specimen Processing
Linearity



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. As shown in Figure 4, 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.

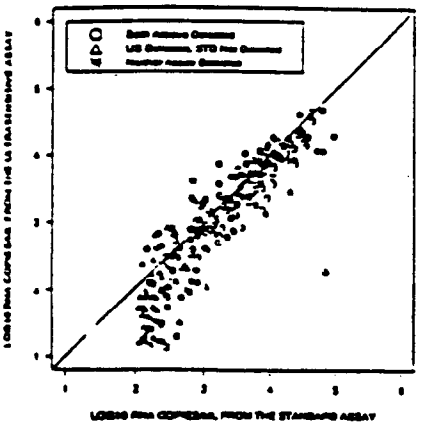
Figure 4
AMPLICOR HIV-1 MONITOR Test
with UltraSensitive Specimen Processing
Linearity



Correlation of Results for Standard and Ultrasensitive 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.

Figure 5
AMPLICOR HIV-1 MONITOR Test
Correlation of Standard and UltraSensitive Specimen Processing Procedures



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 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.

Clinical Specificity

The clinical specificity of the AMPLICOR HIV-1 MONITOR Test was determined by analysis of 495 anti-HIV-1 negative blood donors. None of these specimens was reactive with the AMPLICOR HIV-1 MONITOR Test. Assuming a zero prevalence of HIV-1 infection in the seronegative blood donors, the specificity of the test was 100%.

Anti-HIV-1 Negative Blood Donors

N	HIV RNA copies/mL		HIV OD_{450} (in row A of the Microwell Plate)				
	Not Detected	Positive	Minimum	Average	Maximum	SD	% CV
495	495	0	0.033	0.053	0.130	0.012	22.6

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 four of the specimens and contributed to less than 10% of the apparent HIV-1 RNA test result in the fifth specimen.

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 2a.

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 2b.

Table 2a
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	2,043	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	325,561	2,209	16,169	524,528
CV (%)	-	32.3	42.3	104.8**	41.1	45.3

Table 2b
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

Reproducibility

Assay reproducibility 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 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 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 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 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 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 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

Clinical Performance

Patient Prognosis

The use of the AMPLICOR HIV-1 MONITOR Test to predict the risk of disease progression in HIV infected individuals was evaluated in ACTG studies 116A and 116B/117. The data from these studies were analyzed by the Cox Proportional Hazards Model to evaluate the frequency of disease progression based upon HIV-1 RNA level. ACTG Study 116A was a double-blinded study that compared the clinical efficacy of zidovudine (ZDV) in combination with two doses of 2',3'-dideoxyinosine (ddI) in patients with advanced HIV disease who had up to 16 weeks of prior treatment with zidovudine. ACTG Study 116B/117 was an efficacy study which compared 2',3'-dideoxyinosine (ddI) and zidovudine therapy of patients with HIV infection who had been on zidovudine treatment for more than 16 weeks. The patient population in each of these studies included patients with a diagnosis of AIDS at study baseline, ARC at baseline and asymptomatic patients at baseline. Disease progression was defined as progression to AIDS, new AIDS defining event, or death.

The ACTG 116A HIV-1 RNA sub-study included 186 patients from the clinical study who had had plasma and PBMC specimens collected upon entrance into the study and whose specimens had been stored appropriately and were available for testing in the sub-study. The ACTG 116B/117 HIV-1 RNA sub-study included 99 randomly selected patients from the drug study who had plasma specimens collected on entrance into the study and whose specimens had been stored appropriately and were available for testing in the sub-study.

The unadjusted and adjusted relative hazards for disease progression as measured by baseline HIV-1 RNA levels, change in HIV-1 RNA levels over 8 weeks, and CD4+ cell counts were evaluated using Cox Proportional Hazards Models. The unadjusted Relative Hazard represents the risk conferred by the variable alone, whereas the Adjusted Relative Hazard represents the risk conferred by that variable after controlling for the other variables in the model. These models give the increased risk (if any) of disease progression associated with the variables entered into the model. The analyses were performed by assessing the relative hazards of a 5-fold difference in the study variable. The results of these analyses are presented in Tables 5 through 8. These data show that in a population of patients with advanced HIV disease and undergoing specific anti-reverse transcriptase therapies, 5-fold higher baseline HIV-1 RNA levels are associated with increased risk of disease progression. For patients who have had greater than 16 weeks of prior ZDV therapy (patients in ACTG Study 116B/117), 5-fold higher baseline HIV-1 RNA levels have not proven to be of significant prognostic value. For patients who have had either no prior ZDV therapy or up to 16 weeks or less of ZDV therapy, 5-fold changes between baseline and Week 8 RNA levels have significant prognostic value. For patients who have had greater than 16 weeks of prior ZDV therapy, 5-fold changes between baseline and Week 8 RNA levels have not proven to be of significant prognostic value.

The frequency of disease progression was also analyzed for each study by dividing each study population into deciles by rank order of baseline HIV-1 RNA. The deciles were evaluated for the frequency of disease progression. For each study, a frequency of disease progression of $\geq$ 60% was found for all patients with baseline HIV-1 RNA levels above 250,000 copies/mL. In Study 116A, an approximate 35% frequency of disease progression was found for patients in the first four deciles (<11912, <34661, <72438 and <103806 HIV-1 RNA copies/mL). The frequency of disease progression was between 40% and 50% in the next three deciles (<15695, <194312 and <247229). In the last three deciles, where the HIV-1 RNA levels were >250000 HIV-1 RNA copies/mL, the frequency of disease progression was greater than 60%. In Study 116B/117 the frequency of disease progression was found to be more variable, but still showed the general trend of higher

rates of progression with increased HIV-1 RNA levels. An average 30% disease progression rate (range = 10% - 60%) was found for the first six deciles in this study (<11571, <31292 and <49743, <62132, <97781 and <150866). The rate of disease progression increased to 50% and 60% for the next two deciles (<251627 and <403146, respectively). The disease progression rates were greater than 80% for the last two deciles (<794027 and <1456302) where the HIV-1 RNA levels were greater than 403,000. Summary tables and bar charts of these analyses are presented in Figures 4 and 5.

Table 5
Association of Study Variables at Baseline with Disease Progression
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 in HIV-1-RNA or decrease in CD4 cell count
2 - p values are for Adjusted Relative Hazards

Table 6
Association of Change in HIV-1-1 RNA from Baseline to Week 8 with Disease Progression
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-1-1 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 in HIV-1-RNA or decrease in CD4 cell count
2 - p values are for Adjusted Relative Hazards

Table 7
Association of Study Variables at Baseline with Disease Progression
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

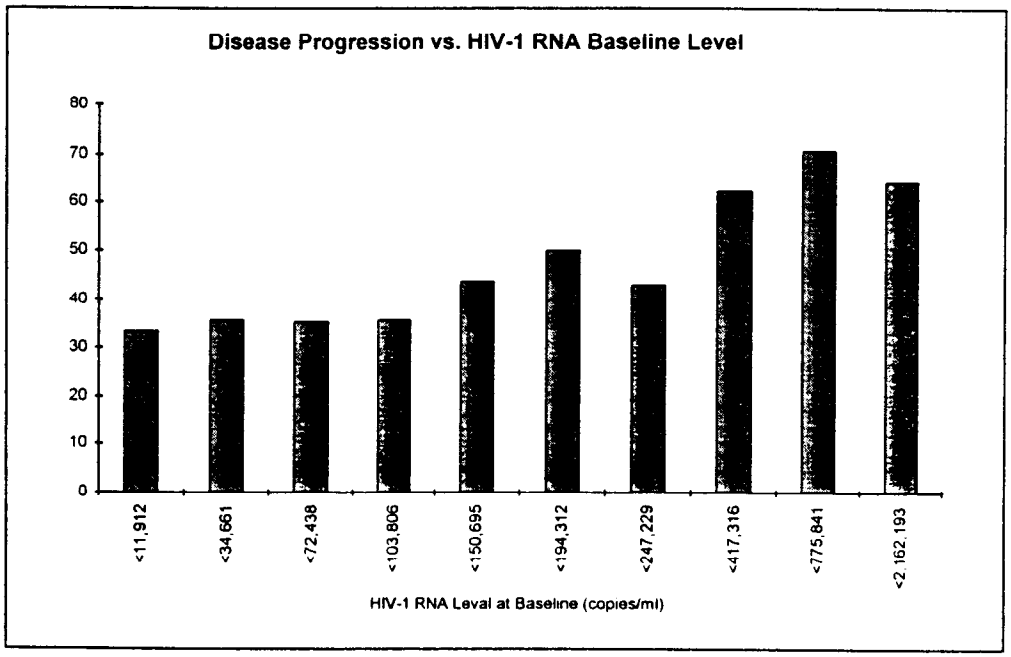
1 - The Relative Hazard is the hazard ratio resulting from a 5-fold increase in HIV-1-RNA or decrease in CD4 cell count
2 - p values are for Adjusted Relative Hazards

Table 8
Association of Change in HIV-1-1 RNA from Baseline to Week 8 with Disease Progression
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-1-1 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

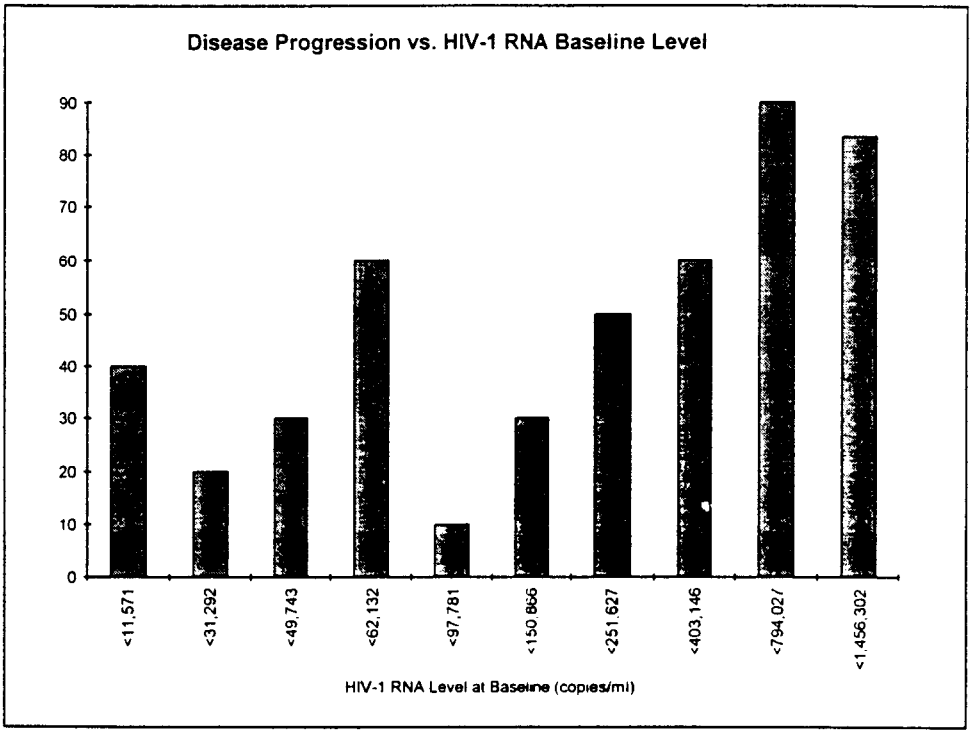
1 - The Relative Hazard is the hazard ratio resulting from a 5-fold increase in HIV-1-RNA or decrease in CD4 cell count
2 - p values are for Adjusted Relative Hazards

Figure 4
Frequency of Disease Progression by Baseline HIV-1 RNA level
Study 116A



Record #	Decile	n	# Progressions	# 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 5
Frequency of Disease Progression by Baseline HIV-1 RNA level
Study 116B/117



Record #	Decile	n	# Progressions	# 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

Measuring Response to Antiretroviral Therapy

The use of the AMPLICOR HIV-1 MONITOR Test to measure the effects of antiretroviral therapy was evaluated in a clinical study of antiretroviral compounds including the reverse transcriptase inhibitor zalcitabine (ddC, tradename HIVID®), the protease inhibitor drug Saquinavir (SAQ, trade name INVIRASE®), and combinations of these two drugs. The study (Study NV14256) was a double–blind, phase III randomized study whose primary objective was to evaluate the safety, tolerability and efficacy of three treatments (ddC, SAQ, and SAQ in combination with ddC) based on clinical endpoints in patients discontinuing (or unable to take) zidovudine (ZDV) therapy and to compare survival among the three treatment groups (including death after an AIDS Defining Event (ADE), or dose limiting toxicity).

For Study NV14256, prior ZDV treatment was an inclusion criterion with the majority of patients having over one year of prior ZDV treatment. A total of 970 patients were entered into Study NV14256 at forty nine clinical sites. The study had the following three treatment arms (patients): ddC (325), Saquinavir (327), and Saquinavir + ddC (318). The demographic and baseline disease characteristics of the patients in Study NV14256 represented a diverse population of patients with advanced HIV-1 infection and with a wide range of prior antiretroviral treatment regimes.

The utility of the AMPLICOR HIV-1 MONITOR Test to measure the effects of drug treatment over time was evaluated by analyzing the median change from baseline and the DAVGt (difference averaged over time - mean change from baseline over t weeks) in the HIV-1 RNA level. To evaluate the ability of the AMPLICOR HIV-1 MONITOR Test to detect changes in HIV-1 RNA levels as a result of therapy, the median change from baseline was analyzed over time for each treatment arm of the study. Figure 6 shows the median change from baseline over a 48 week time period for each treatment arm in Study NV14256. Measurable and sustained decreases in HIV-1 RNA levels as determined by the AMPLICOR HIV-1 MONITOR Test were seen. The largest and the most sustained median change in HIV-1 RNA levels at each time point was seen in patients in the combination treatment arm (SAQ+ddC).

The utility of serial HIV-1 RNA measurements to assess viral response to antiretroviral treatment was also 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 9, 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 7, 8 and 9.

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 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 10 and 11 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.

The data presented here regarding the clinical utility and the use of the AMPLICOR HIV-1 MONITOR Test to monitor the effects of antiretroviral therapy were derived from a single clinical study that compared two drugs and three therapeutic regimens for a specific patient group. Because a single clinical study may not adequately demonstrate the clinical utility of quantitative HIV RNA testing in all patient populations, in all clinical situations, or with all antiretroviral therapies, sufficient care should be taken before extending the interpretation of the data from the study presented here to any individual patient case. As with any diagnostic test, results from the AMPLICOR HIV-1 MONITOR Test should be interpreted with consideration of all relevant clinical and laboratory findings for each patient.

Figure 6
HIV-1 RNA Medians of Change from Baseline Over 48 Weeks
Study NV14256

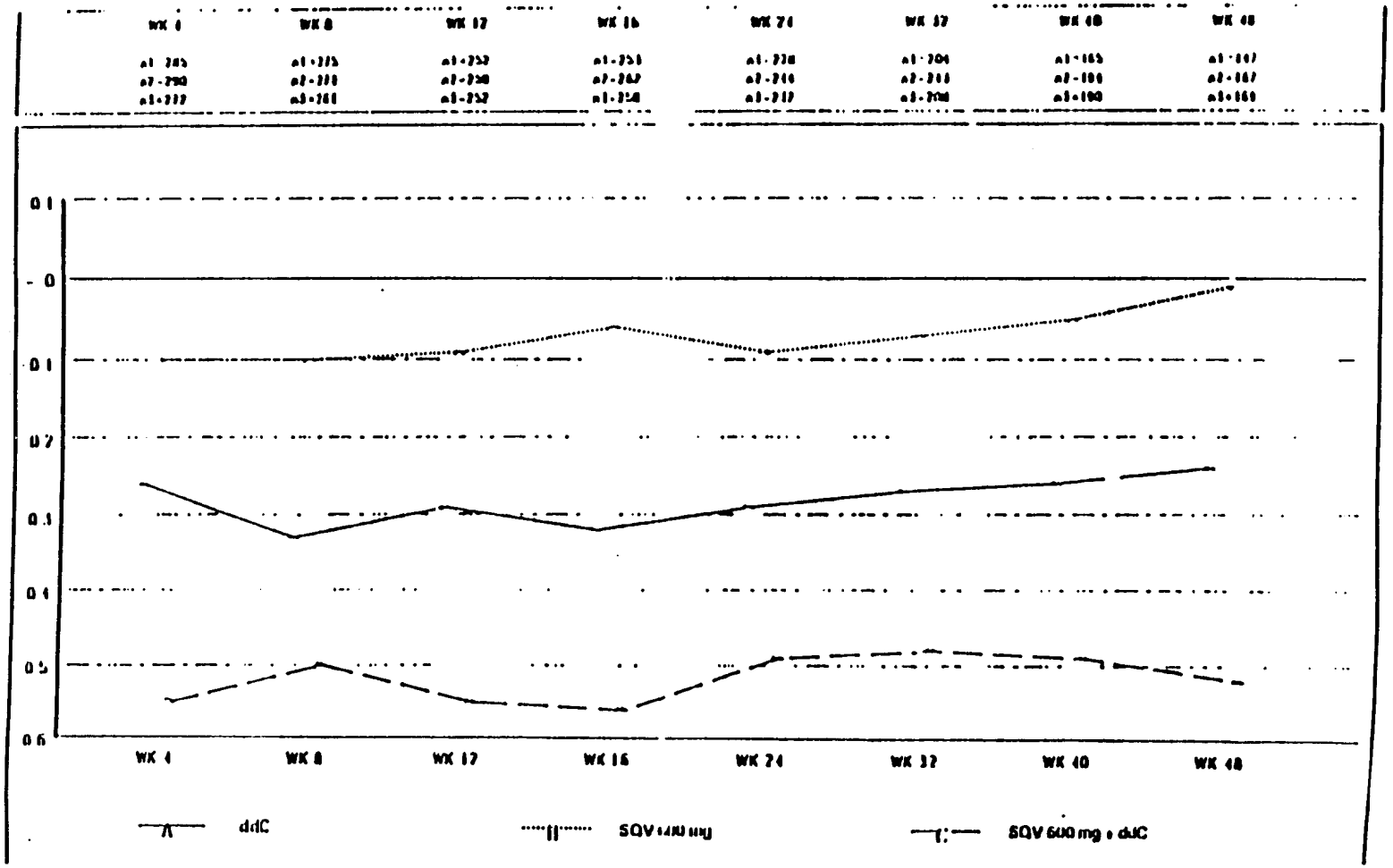


Table 9
ADE Risk versus Log10(Last RNA) and Other Covariates
Based on Time from the Given Study Week for Patients Surviving Through that Week
Summary of Cox Model Results - Model Stratified by Treatment Group
Study NV14256

Study Week	Variable	Cox Model Coefficient	Hazard Ratio*	p Value
	log10(Baseline RNA)	0.798	2.22	0.0012
4	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
	log10(Baseline RNA)	0.8096	2.25	0.0004
8	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
	log10(Baseline RNA)	0.8714	2.39	0.005
16	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
	log10(Baseline RNA)	0.6910	2.00	0.0136
24	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
	log10(Baseline RNA)	0.5926	1.81	0.0492
32	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
	log10(Baseline RNA)	0.8003	2.23	0.0159
40	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
	log10(Baseline RNA)	0.5993	1.82	0.0978
48	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
	log10(Baseline RNA)	1.0917	2.98	0.0127
56	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
	log10(Baseline RNA)	1.2803	3.60	0.0135
64	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 7
Kaplan Meier Estimated Survivals
Study NV14256 Treatment Group: SAQ 600mg
Patients Surviving ADE Free Through Week 8

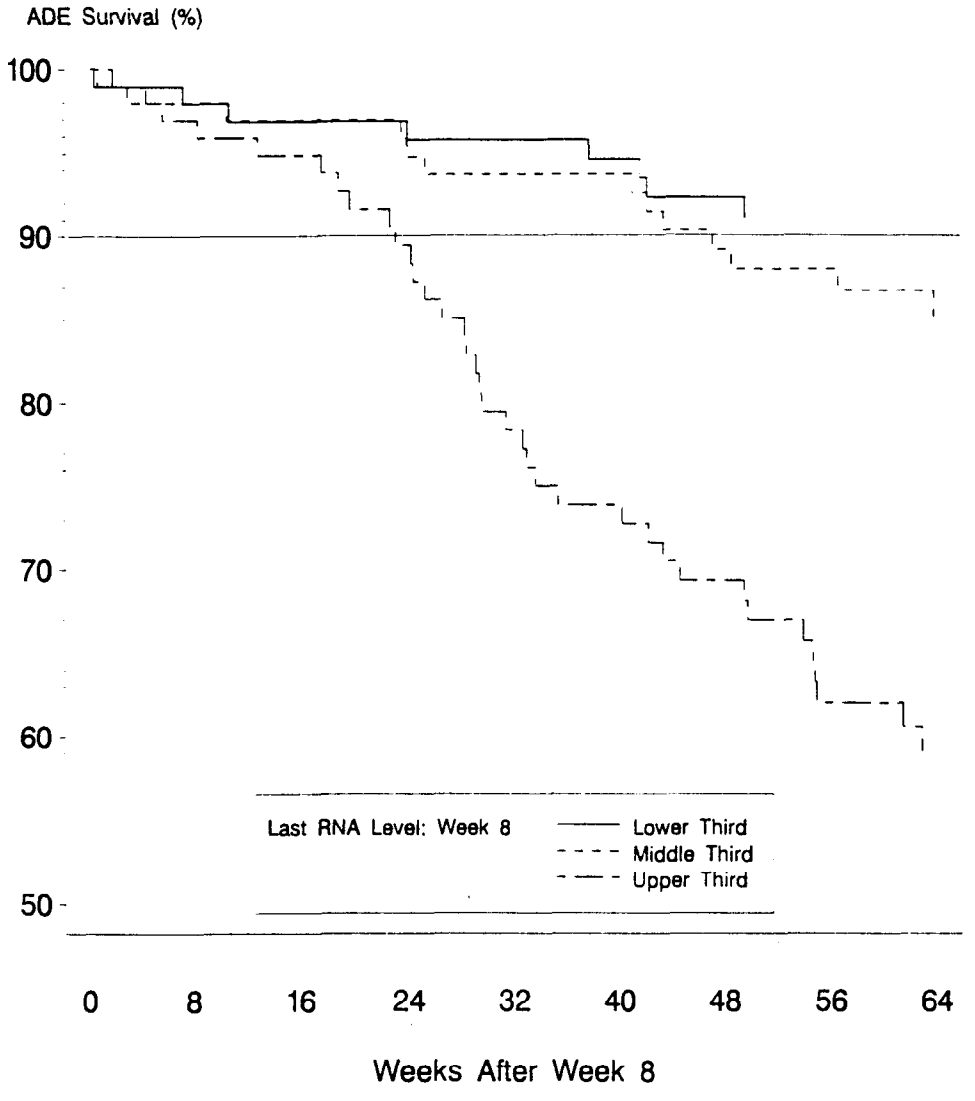


Figure 8
Kaplan Meier Estimated Survivals
Study NV14256 Treatment Group: ddC
Patients Surviving ADE Free Through Week 8

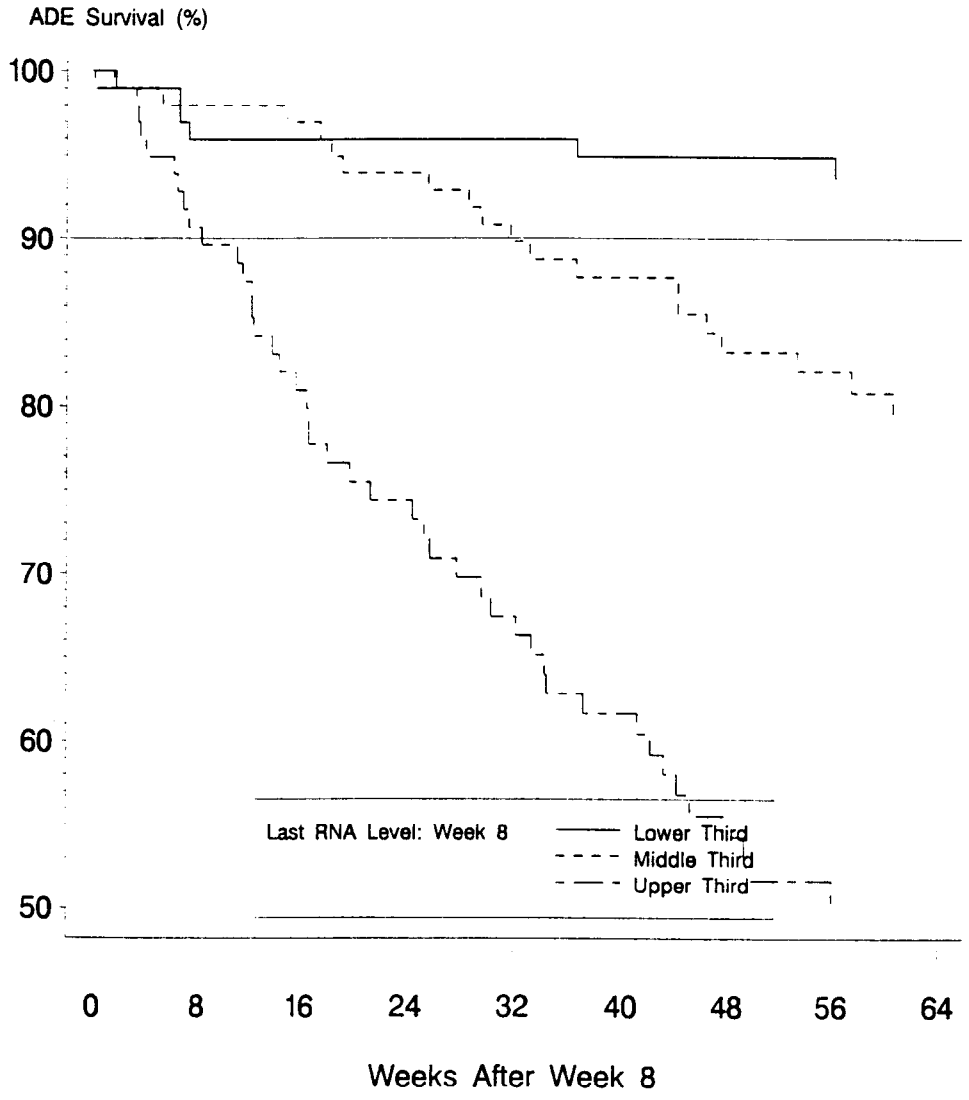


Figure 9
Kaplan Meier Estimated Survivals
Study NV14256 Treatment Group: ddC + SAQ 600mg
Patients Surviving ADE Free Through Week 8

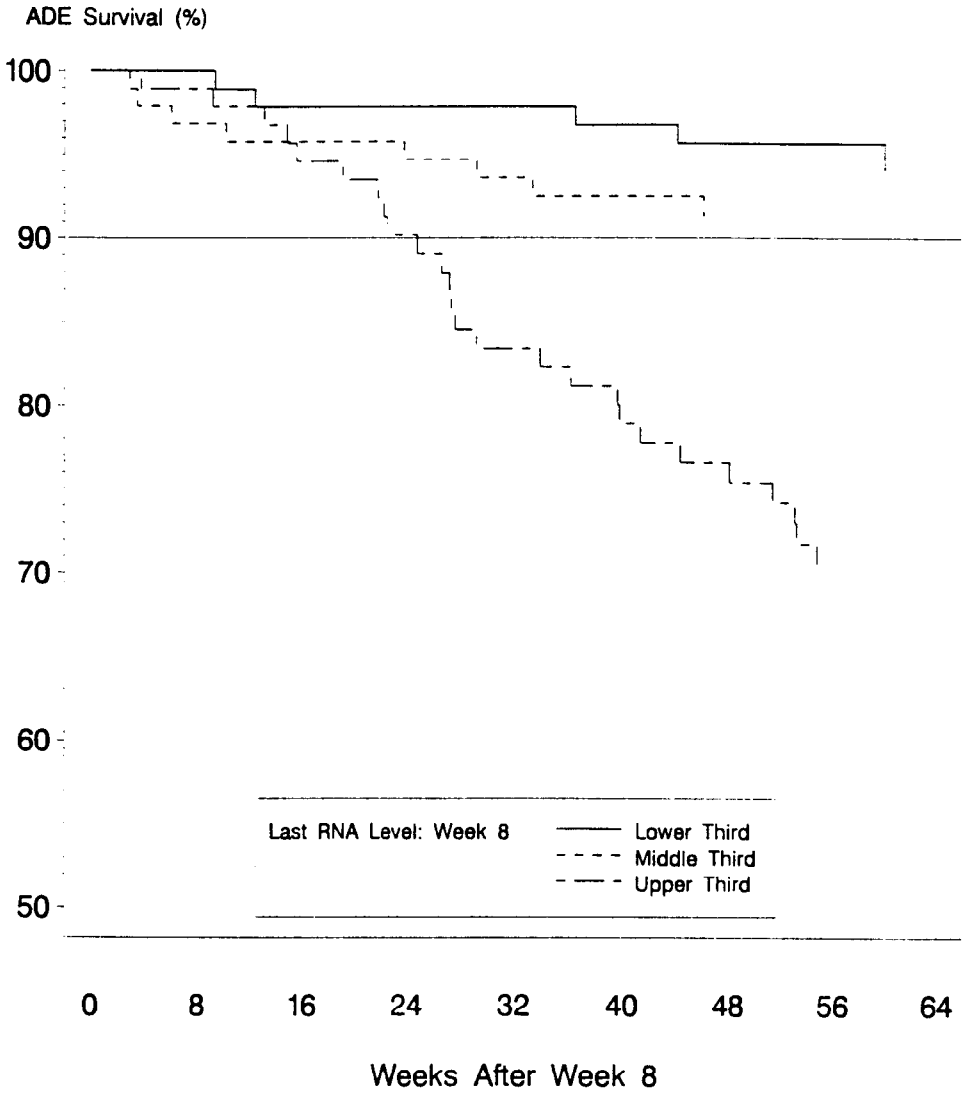


Figure 10
Hazard Ratio for Time to First ADE
Patients Surviving ADE Free Through Week 4
Study NV14256

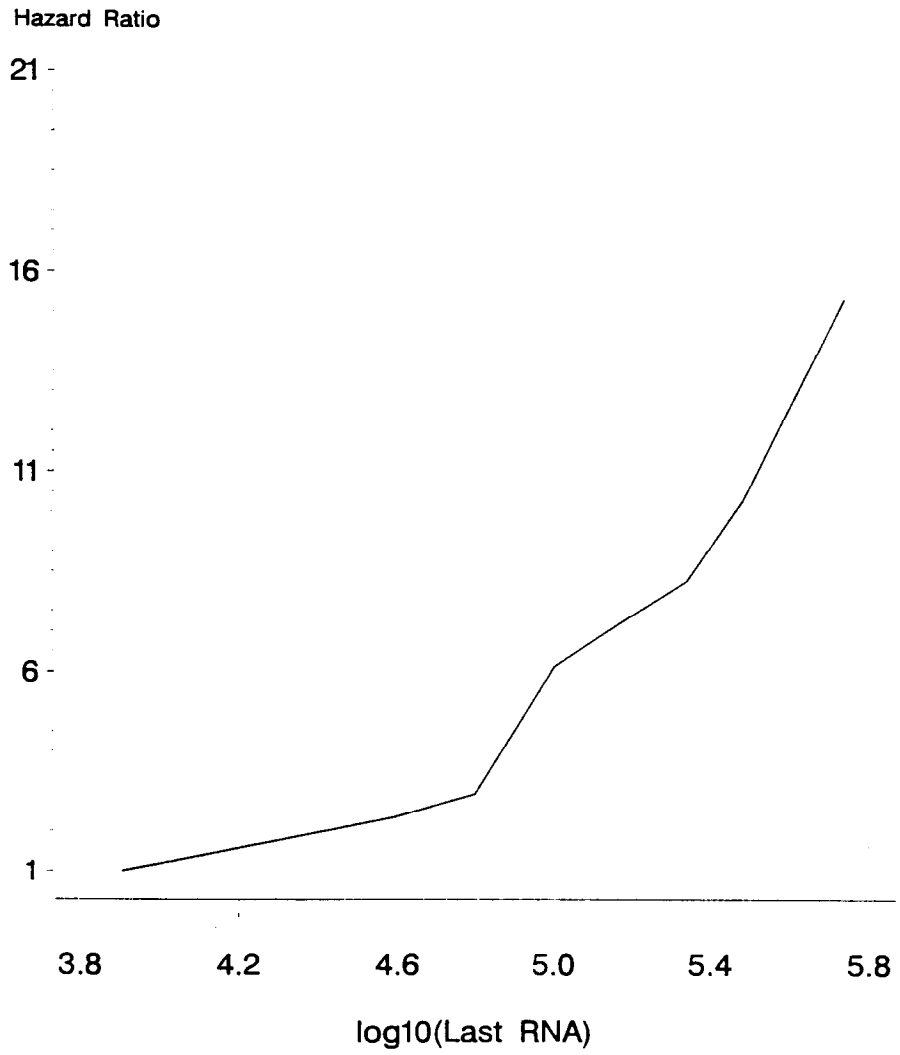
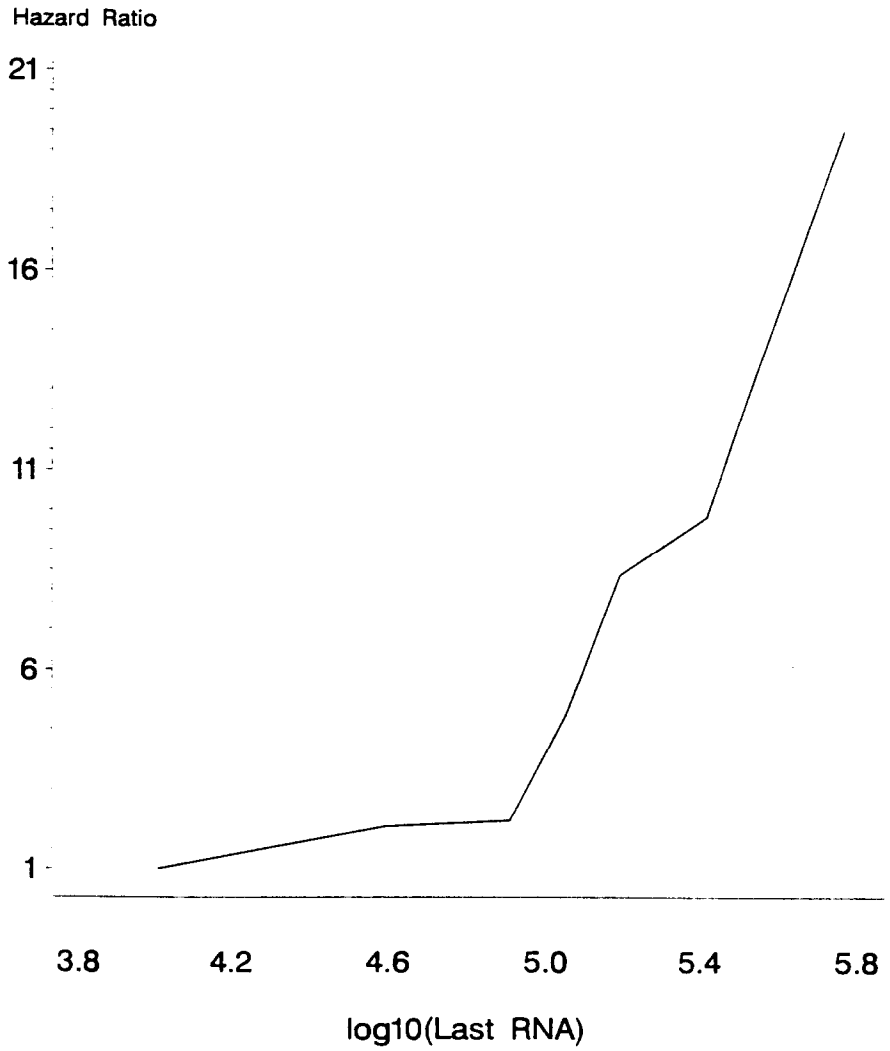


Figure 11
Hazard Ratio for Time to First ADE
Patients Surviving ADE Free Through Week 16
Study NV14256



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