COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test

FOR *IN VITRO* DIAGNOSTIC USE.

COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test	HIMCAP	48 Tests	P/N: 03542998 190
COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent	PG WR	5.1 Liters	P/N: 03587797 190

INTENDED USE

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test is an *in vitro* nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma using the COBAS® AmpliPrep Instrument for automated specimen processing and COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer for automated amplification and detection.

This 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 EDTA plasma HIV-1 RNA levels during the course of antiretroviral treatment.

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test is not intended for use as a screening test for the presence of HIV-1 in blood or blood products or as a diagnostic test to confirm the presence of HIV-1 infection.

The test can quantitate HIV-1 RNA over the range of 48 - 10,000,000 copies/mL. One copy of HIV-1 RNA is equivalent to 1.7 ± 0.1 International Units (IU) based on the WHO 1st International Standard for HIV-1 RNA for Nucleic Acid-Based Techniques (NAT) (NIBSC 97/656).

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 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 principal 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 COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test uses PCR technology to achieve high sensitivity and dynamic range for the quantitative detection of HIV-1 RNA in EDTA anti-coagulated plasma.

PRINCIPLES OF THE PROCEDURE

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test is a nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma. Specimen preparation is automated using the COBAS® AmpliPrep Instrument with amplification and detection automated using the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer.

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test is based on three major processes: (1) specimen preparation to isolate HIV-1 RNA; (2) reverse transcription of the target RNA to generate complementary DNA (cDNA), and (3) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide probe specific to the target.

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test permits automated specimen preparation followed by automated reverse transcription, PCR amplification and detection of HIV-1 target RNA and HIV-1 Quantitation Standard (QS) Armored RNA. The Master Mix reagent contains primers and probes specific for both HIV-1 RNA and HIV-1 QS RNA. The Master Mix has been developed to ensure equivalent quantitation of group M subtypes of HIV-1. The detection of amplified DNA is performed using a target-specific and a QS-specific dual-labeled oligonucleotide probe that permit independent identification of HIV-1 amplicon and HIV-1 QS amplicon.

The quantitation of HIV-1 viral RNA is performed using the HIV-1 QS. It compensates for effects of inhibition and controls the preparation and amplification processes, allowing a more accurate quantitation of HIV-1 RNA in each specimen. The HIV-1 QS is a non-infectious Armored RNA construct that contains HIV sequences with identical primer binding sites as the HIV-1 target RNA and a unique probe binding region that allows HIV-1 QS amplicon to be distinguished from HIV-1 target amplicon.

The HIV-1 QS is added to each specimen at a known copy number and is carried through the specimen preparation, reverse transcription, PCR amplification and detection steps of cleaved dual-labeled oligonucleotide detection probes. The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer calculates the HIV-1 RNA concentration in the test specimens by comparing the HIV-1 signal to the HIV-1 QS signal for each specimen and control.

Target Selection

Selection of the target RNA sequence for HIV-1 depends on identification of regions within the HIV-1 genome that show maximum sequence conservation among the various group M HIV-1 subtypes. Generic silica based specimen preparation is used to capture the HIV-1 RNA and HIV-1 QS RNA and defined oligonucleotides are used as primers in amplification of the HIV-1 RNA and HIV-1 QS RNA. A target-specific and a QS-specific dual-labeled oligonucleotide probe permit independent identification of HIV-1 amplicon and HIV-1 QS amplicon. Accordingly, the appropriate selection of the primers and the dual-labeled oligonucleotide probe is critical to the ability of the test to amplify and detect the HIV-1 subtypes. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test uses reverse transcription and PCR amplification primers that define a sequence within the highly conserved region of the HIV-1 *gag* gene[32]. The gag region encodes the group-specific antigens or core structural proteins of the virion. The nucleotide sequence of the primers has been optimized to yield comparable amplification of group M subtypes of HIV-1.

Specimen Preparation

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test utilizes automated specimen preparation on the COBAS® AmpliPrep Instrument by a generic silica-based capture technique. The procedure processes 850 µL of plasma. The HIV-1 virus particles are lysed by incubation at elevated temperature with a protease and chaotropic lysis/binding buffer that releases nucleic acids and protects the released HIV-1 RNA from RNases in plasma. Protease and a known number of HIV-1 QS Armored RNA molecules are introduced into each specimen along with the lysis reagent and magnetic glass particles. Subsequently, the mixture is incubated and the HIV-1 RNA and HIV-1 QS RNA are bound to the surface of the magnetic glass particles. Unbound substances, such as salts, proteins and other cellular impurities, are removed by washing the magnetic glass particles. After separating the magnetic glass particles and completing the washing steps, the adsorbed nucleic acids are eluted at elevated temperature with an aqueous solution. The processed specimen, containing the magnetic glass particles as well as released HIV-1 RNA and HIV-1 QS RNA, is added to the amplification mixture and transferred to the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer. The HIV-1 target RNA and the HIV-1 QS RNA are then reverse transcribed, amplified and simultaneously

detected by cleavage of a target-specific and a QS-specific dual-labeled oligonucleotide probe.

Reverse Transcription and PCR Amplification

The reverse transcription and PCR amplification reaction is performed with the thermostable recombinant enzyme *Thermus specie* DNA Polymerase (Z05). In the presence of manganese (Mn^{2+}) and under the appropriate buffer conditions, Z05 has both reverse transcriptase and DNA polymerase activity[30,31]. This allows both reverse transcription and PCR amplification to occur together with real-time detection of the amplicon.

Processed specimens are added to the amplification mixture in amplification tubes (K-tubes) in which both reverse transcription and PCR amplification occur. The reaction mixture is heated to allow a downstream primer to anneal specifically to the HIV-1 target RNA and to the HIV-1 QS RNA. In the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine and deoxythymidine triphosphates, Z05 polymerase extends the annealed primers forming a DNA strand complementary to the RNA target.

<u>Target Amplification</u>

Following reverse transcription of the HIV-1 target RNA and the HIV-1 QS RNA, the Thermal Cycler in the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer heats the reaction mixture to denature the RNA:cDNA hybrid and to expose the specific primer target sequences. As the mixture cools, the primers anneal to the target DNA. The thermostable *Thermus specie* Z05 DNA Polymerase (Z05) in the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine and deoxythymidine triphosphates, extends the annealed primers along the target template to produce a double-stranded DNA molecule termed an amplicon. The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer automatically repeats this process for a designated number of cycles, with each cycle intended to double the amount of amplicon DNA. The required number of cycles is preprogrammed into the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer. Amplification occurs only in the region of the HIV-1 genome between the primers; the entire HIV-1 genome is not amplified.

<u>Selective Amplification</u>

Selective amplification of target nucleic acid from the specimen is achieved in the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[33], but not DNA containing deoxythymidine. 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 contains deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by the AmpErase enzyme prior to amplification of the target DNA. Also, any nonspecific product formed after initial activation of the Master Mix by manganese is destroyed by the AmpErase enzyme. The AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. The AmpErase enzyme remains inactive for a prolonged period of time once exposed to temperatures above 55ºC, i.e. throughout the thermal cycling steps, and therefore does not destroy target amplicon formed after PCR.

Detection of PCR Products in a COBAS® TaqMan® Test

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test utilizes real-time[34,35] PCR technology. The use of dual-labeled fluorescent probes allows for real-time detection of PCR product accumulation by monitoring of the emission intensity of fluorescent reporter dyes released during the amplification process. The probes consist of HIV-1 and HIV-1 QS-specific oligonucleotide probes with a reporter dye and a quencher dye. In the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, the HIV-1 and HIV-1 QS probes are labeled with different fluorescent reporter dyes. When these probes are intact, the fluorescence of the reporter dye is suppressed by the proximity of the quencher dye due to Förster-type energy transfer effects. During PCR, the probe hybridizes to a target sequence and is cleaved by the 5' → 3' nuclease activity of the thermostable Z05 DNA polymerase. Once the reporter and quencher dyes are released and separated, quenching no longer occurs, and the fluorescent activity of the reporter dye is increased. The amplification of HIV-1 RNA and HIV-1 QS RNA are measured independently at different wavelengths. This process is repeated for a designated number of cycles, each cycle effectively increasing the emission intensity of the individual reporter dyes, permitting independent identification of HIV-1 RNA and HIV-1 QS RNA. The PCR cycle where a growth curve starts exponential growth is related to the amount of starting material at the beginning of the PCR.

Fundamentals of COBAS® TaqMan® Test Quantitation

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test is inherently quantitative over a very wide dynamic range since the monitoring of amplicon is performed during the exponential phase of amplification. The higher the HIV-1 titer of a specimen, the earlier the fluorescence of the reporter dye of the HIV-1 probe rises above the baseline fluorescence level (see Figure 1). Since the amount of HIV-1 QS RNA is constant between all specimens, the fluorescence of the reporter dye of the HIV-1 QS probe should appear at the same cycle for all specimens (see Figure 2). In specimens where the QS fluorescence is affected, the concentration is adjusted accordingly. The appearance of the specific fluorescent signals is reported as a critical threshold value (Ct). The Ct is defined as the fractional cycle number where reporter dye fluorescence exceeds a pre-determined threshold (the Assigned Fluorescence Level), and starts the exponential growth phase of this signal (see Figure 3). A higher Ct value indicates a lower titer of initial HIV-1 target material. A 2-fold increase in titer correlates with a decrease of 1 Ct for target HIV-1 RNA, while a 10-fold increase in titer correlates with a decrease of 3.3 Ct.

Figure 1 shows the target growth curves for a dilution series spanning a 5-$\log_{10}$ range. As the concentration of the virus increases, the growth curves shift to earlier cycles. Therefore, the leftmost growth curve corresponds to the highest viral titer level, whereas, the rightmost growth curve corresponds to the lowest viral titer level.

Figure 1
Target Growth Curves for a Dilution Series Spanning a 5-$\log_{10}$ Range
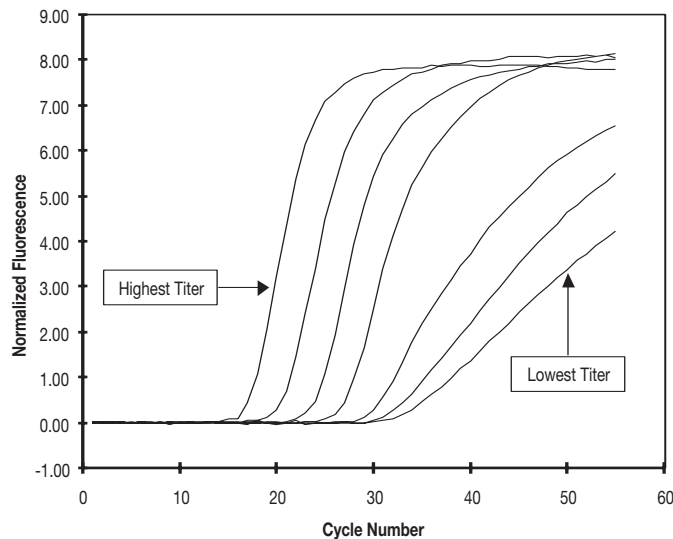

Figure 2 shows the Quantitation Standard growth curves for specimens from a viral dilution series that spans a 5-$\log_{10}$ range. The amount of Quantitation Standard added to each specimen is constant for each reaction. The Ct value of the Quantitation Standard is similar regardless of the target viral titer.

Figure 2
Quantitation Standard Growth Curves for Specimens from a Viral Dilution Series that Spans a 5-$\log_{10}$ Range
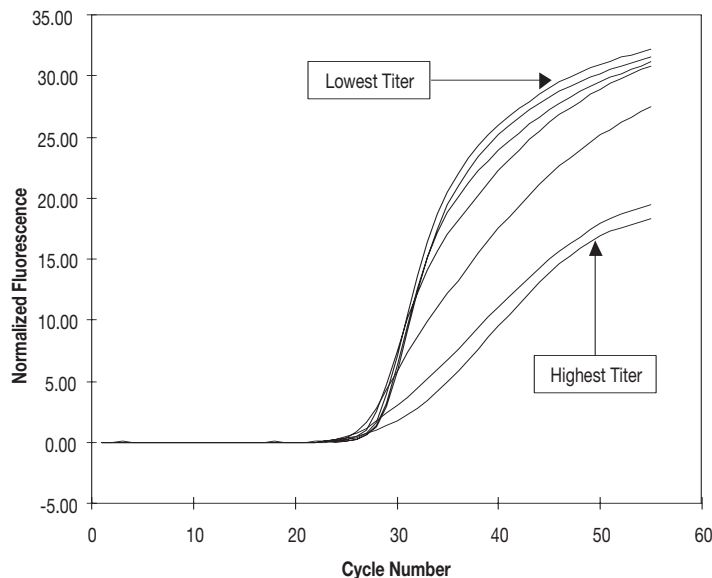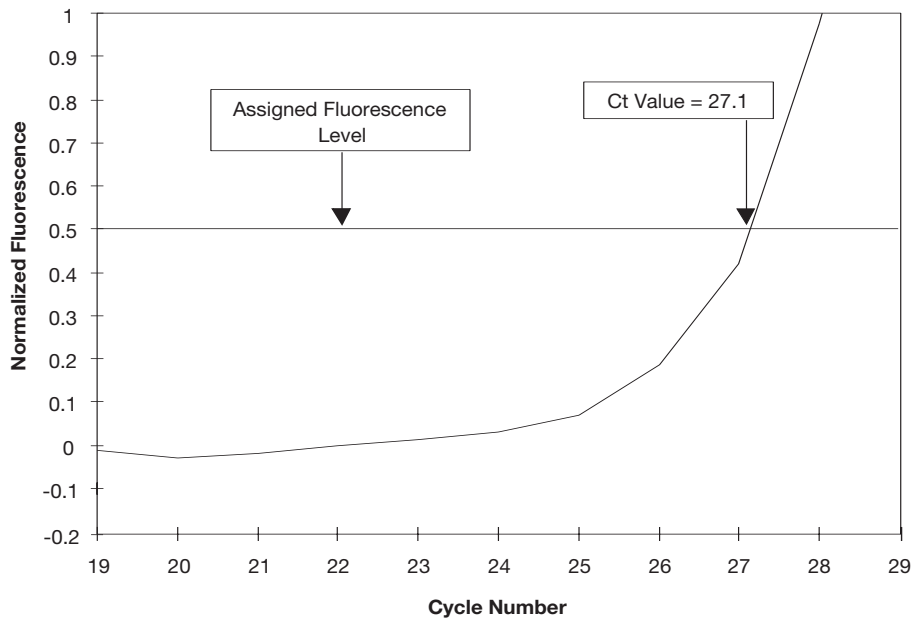

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Figure 3 provides an example of how the fluorescence values at every cycle are normalized for each growth curve. The critical threshold value (Ct) is calculated where the fluorescence signal crosses the Assigned Fluorescence Level.

Figure 3
Normalization of Fluorescence Values at Every Cycle for Each Growth Curve

HIV-1 RNA Quantitation

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test quantitates HIV-1 viral RNA by utilizing a second target sequence (HIV-1 Quantitation Standard) that is added to each test specimen at a known concentration. The HIV-1 QS is a non-infectious Armored RNA construct, containing fragments of HIV-1 sequences with primer binding regions identical to those of the HIV-1 target sequence. The HIV-1 QS contains HIV-1 primer binding regions and generates an amplification product of the same length and base composition as the HIV-1 target RNA. The detection probe binding region of the HIV-1 QS has been modified to differentiate HIV-1 QS amplicon from HIV-1 target amplicon.

During the annealing phase of the PCR on the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer, the specimens are illuminated and excited by filtered light and filtered emission fluorescence data are collected for each specimen. The readings from each specimen are then corrected for instrumental fluctuations. These fluorescence readings are sent by the instrument to the AMPLILINK software and stored in a database. Pre-Checks are used to determine if the HIV-1 RNA and HIV-1 QS RNA data represent sets that are valid, and flags are generated when the data lie outside the preset limits. After all Pre-Checks are completed and passed, the fluorescence readings are processed to generate Ct values for the HIV-1 RNA and the HIV-1 QS RNA. The lot-specific calibration constants provided with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test are used to calculate the titer value for the specimens and controls based upon the HIV-1 RNA and HIV-1 QS RNA Ct values. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test is standardized against the WHO 1st International Standard for HIV-1 RNA for Nucleic Acid-Based Techniques (NAT) (NIBSC 97/656)[36]. Titer results are reported in copies/mL (cp/mL). The conversion factor between reported HIV-1 RNA copies/mL and HIV-1 International Units/mL has been determined by Roche Molecular Systems, Inc. to be 0.6 cp/IU (1.7 IU/cp).

REAGENTS

COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test   HIMCAP   48 Tests
(P/N: 03542998 190)

HIV-1 CS1   1 x 48 Tests
(HIV-1 Magnetic Glass Particles Reagent Cassette)   1 x 7.0 mL

Magnetic glass particles
93% Isopropanol

Xi   93% (w/w) Isopropanol

Irritant

F   93% (w/w) Isopropanol

Highly
Flammable

HIV-1 CS2   1 x 48 Tests
(HIV-1 Lysis Reagent Cassette)   1 x 78 mL

Sodium citrate dihydrate
42.5% Guanidine thiocyanate
< 14% Polydocanol
0.9% Dithiothreitol

Xn   42.5% (w/w) Guanidine thiocyanate

Harmful

HIV-1 CS3   1 x 48 Tests
HIV-1 Multi-Reagent Cassette containing:

Pase   1 x 3.8 mL
(Proteinase Solution)

Tris buffer
< 0.05% EDTA
Calcium chloride
Calcium acetate
≤ 7.8% Proteinase
Glycerol

Xn   ≤ 7.8% (w/w) Proteinase

Harmful

EB   1 x 7.0 mL
(Elution Buffer)

Tris-base buffer
0.2% Methylparaben

HIV-1 CS4   1 x 48 Tests
HIV-1 Test-Specific Reagent Cassette containing:

HIV-1 QS   1 x 3.6 mL
(HIV-1 Quantitation Standard)

Tris-HCl buffer
EDTA
< 0.005% Poly rA RNA (synthetic)
< 0.001% Armored HIV-1 RNA construct containing HIV-1 primer
    binding sequences and a unique probe binding region
    (non-infectious RNA in MS2 bacteriophage)
0.05% Sodium azide

HIV-1 MMX
(HIV-1 Master Mix)
1 x 2.5 mL

    Tricine buffer
    Potassium acetate
    Potassium hydroxide
    20% Dimethylsulfoxide
    Glycerol
    < 0.04% dATP, dCTP, dGTP, dUTP, dTTP
    < 0.003% Upstream and downstream primers to the GAG
      region of HIV-1
    < 0.003% Oligonucleotide aptamer
    < 0.003% Fluorescent-labeled oligonucleotide probes
      specific for HIV-1 and the HIV-1 QS
    < 0.05% Z05 DNA Polymerase (microbial)
    < 0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial)
    0.09% Sodium azide

CAP/CTM Mn^{2+}
(CAP/CTM Manganese Solution)
1 x 19.8 mL

    < 0.5% Manganese acetate
    Glacial acetic acid
    0.09% Sodium azide

HIV-1 H(+)C
(HIV-1 High Positive Control)
4 x 1.0 mL

    < 0.001% Armored HIV-1 RNA construct containing HIV-1
      sequences (non-infectious RNA in MS2 bacteriophage)
    Negative Human Plasma, non-reactive by FDA licensed tests
      for antibody to HCV, antibody to HIV-1/2, HIV p24 antigen
      and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable
      by PCR methods
    0.1% ProClin® 300 preservative

HIV-1 L(+)C
(HIV-1 Low Positive Control)
4 x 1.0 mL

    < 0.001% Armored HIV-1 RNA construct containing HIV-1
      sequences (non-infectious RNA in MS2 bacteriophage) at a mean
      concentration at least 100 fold lower than the mean concentration
      of Armored HIV-1 RNA in HIV-1 H(+)C
    Negative Human Plasma, non-reactive by FDA licensed tests for
      antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg;
      HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods
    0.1% ProClin® 300 preservative

CTM (–) C
[COBAS® TaqMan® Negative Control (Human Plasma)]
4 x 1.0 mL

    Negative Human Plasma, non-reactive by FDA licensed tests for
      antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg;
      HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods
    0.1% ProClin® 300 preservative

HIV-1 H(+)C Clip
(HIV-1 High Positive Control Barcode Clip)
1 x 4 Clips

HIV-1 L(+)C Clip
(HIV-1 Low Positive Control Barcode Clip)
1 x 4 Clips

HIV-1 (–) C Clip
(HIV-1 Negative Control Barcode Clip)
1 x 4 Clips

PG WR

COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent
(P/N: 03587797 190)

PG WR
(COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent)
1 x 5.1 L

    Sodium citrate dihydrate
    < 0.1% N-Methylisothiazolone-HCl

WARNINGS AND PRECAUTIONS

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

B. This test is for use with human plasma collected in the anticoagulant EDTA.

C. Do not pipet by mouth.

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

E. Avoid microbial and ribonuclease contamination of reagents when removing aliquots from control vials.

F. The use of sterile disposable pipets and RNase-free pipet tips is recommended.

G. Do not pool controls from different lots or from different vials of the same lot.

H. Do not mix reagent cassettes or controls from different kits.

I. Do not allow reagent cassettes to come to ambient temperature outside the COBAS® AmpliPrep Instrument.

J. Do not open COBAS® AmpliPrep cassettes and exchange, mix, remove or add bottles.

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

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

M. Material Safety Data Sheets (MSDS) are available on request from your local Roche office.

N. Specimens and controls should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[37] and in the CLSI Document M29-A[38]. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

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. Do not autoclave bleach solution.

O. **CAUTION: CTM (–) C, HIV-1 L(+)C** and **HIV-1 H(+)C** contain Human Plasma derived from human blood. The source material has been tested by FDA licensed tests and found non-reactive for the presence of Hepatitis B Surface Antigen (HBsAg), antibodies to HIV-1/2 and HCV, and HIV p24 Antigen. Testing of Negative Human Plasma by PCR methods showed no detectable HIV-1 RNA, HCV RNA or HBV DNA. No known test methods can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all human sourced material should be considered potentially infectious. **CTM (–) C, HIV-1 L(+)C** and **HIV-1 H(+)C** should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[37] and in the CLSI Document M29-A[38]. Thoroughly clean and disinfect all work surfaces (for instrument work surfaces see COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.1.x Series) with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

P. **HIV-1 QS, CAP/CTM Mn^{2+}** and **HIV-1 MMX** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide-containing solutions down laboratory sinks, flush the drains with a large volume of water to prevent azide buildup.

Q. Wear eye protection, laboratory coats and disposable gloves when handling any reagent. 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.

R. Do not allow **HIV-1 CS2** and liquid waste from the COBAS® AmpliPrep Instrument, which contain guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. These mixtures can produce a highly toxic gas.

S. When disposing of used COBAS® AmpliPrep Sample Processing Units (SPUs), which contain guanidine thiocyanate, avoid any contact with sodium hypochlorite (bleach) solution. These mixtures can produce a highly toxic gas.

STORAGE AND HANDLING REQUIREMENTS

A. ***Do not freeze reagents or controls.***

B. Store **HIV-1 CS1, HIV-1 CS2, HIV-1 CS3** and **HIV-1 CS4** at 2-8°C. Unused, these reagents are stable until the expiration date indicated. Once used, these reagents are stable for 28 days at 2-8°C or until the expiration date, whichever comes first. **HIV-1 CS1, HIV-1 CS2, HIV-1 CS3** and **HIV-1 CS4** can be used for a maximum of 4 instrument cycles, up to a maximum of 64 hours cumulative on board the COBAS® AmpliPrep Instrument. Reagents must be stored at 2-8°C between instrument cycles.

C. Store **HIV-1 H(+)C, HIV-1 L(+)C** and **CTM (–) C** at 2-8°C. The controls are stable until the expiration date indicated. Once opened, any unused portion must be discarded.

D. Store Barcode clips [**HIV-1 H(+)C Clip, HIV-1 L(+)C Clip** and **HIV-1 (–) C Clip**] at 2-30°C.

E. Store **PG WR** at 2-30°C. **PG WR** is stable until the expiration date indicated. Once opened, this reagent is stable for 28 days at 2-30°C or until the expiration date, whichever comes first.

MATERIALS PROVIDED

A. **COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test** | HIMCAP |
(P/N: 03542998 190)

    HIV-1 CS1
    (HIV-1 Magnetic Glass Particles Reagent Cassette)

    HIV-1 CS2
    (HIV-1 Lysis Reagent Cassette)

    HIV-1 CS3
    (HIV-1 Multi-Reagent Cassette)

    HIV-1 CS4
    (HIV-1 Test-Specific Reagent Cassette)

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

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

    CTM (–) C
    [COBAS® TaqMan® Negative Control (Human Plasma)]

    HIV-1 H(+)C Clip
    (HIV-1 High Positive Control Barcode Clip)

    HIV-1 L(+)C Clip
    (HIV-1 Low Positive Control Barcode Clip)

    HIV-1 (–) C Clip
    (HIV-1 Negative Control Barcode Clip)

B. **COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent** | PG WR |
(P/N: 03587797 190)

    PG WR
    (COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent)

MATERIALS REQUIRED BUT NOT PROVIDED
Instrumentation and Software

- COBAS® AmpliPrep Instrument
- COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer
- Optional: Docking Station
- AMPLILINK Software, Version 3.1.x Series
- Data Station for the AMPLILINK software, with printer
- COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.1.x Series
- COBAS® TaqMan® Analyzer (plus optional Docking Station) Instrument Manual for use with AMPLILINK Software, Version 3.1.x Series
- COBAS® TaqMan® 48 Analyzer Instrument Manual for use with AMPLILINK Software, Version 3.1.x Series
- AMPLILINK Software Version 3.1.x Series Application Manual for use with the COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, and COBAS® TaqMan® 48 Analyzer

- COBAS® TaqMan® Analyzer HIV-1 PCR TEST FILE CD ROM
- COBAS® TaqMan® 48 Analyzer HIV-1 PCR TEST FILE CD ROM

Disposables

- Sample processing units: SPUs (P/N: 03755525001)
- Sample input tubes (S-tubes) with barcode clips (P/N: 03137040001)
- Racks of K-tips (P/N: 03287343001)
- K-tube Box of 12 x 96 (P/N: 03137082001)

OTHER MATERIALS REQUIRED BUT NOT PROVIDED

- Sample Rack (SK 24 rack) (P/N: 28122172001)
- Reagent Rack (P/N: 28122199001)
- SPU rack (P/N: 28122806001)
- K-tube capper, motorized (P/N: 03516539001)
- K-tube capper (P/N: 03339874001)
- K-carrier (P/N: 28150397001)
- K-carrier Transporter (P/N: 03517519001)
- K-carrier rack (P/N: 03286436001)
- Pipettors with aerosol barrier or positive displacement RNase-free tips (capacity 1000 µL)*
- Disposable gloves, powderless
- Vortex mixer

* 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.

**SPECIMEN COLLECTION, TRANSPORT AND STORAGE**

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

A. Specimen Collection

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test is for use with plasma specimens. Blood should be collected in sterile tubes using EDTA (lavender top) as the anticoagulant.

Store whole blood at 2-25°C for no longer than 6 hours. 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. Figures 4 and 5 show the data from specimen collection studies. These studies were performed using the COBAS® TaqMan® HIV-1 Test for use with the High Pure System (HPS), P/N: 03501752 190 and 03502295 001).

Figure 4
HIV-1 Stability in Whole Blood with EDTA Anticoagulant

10
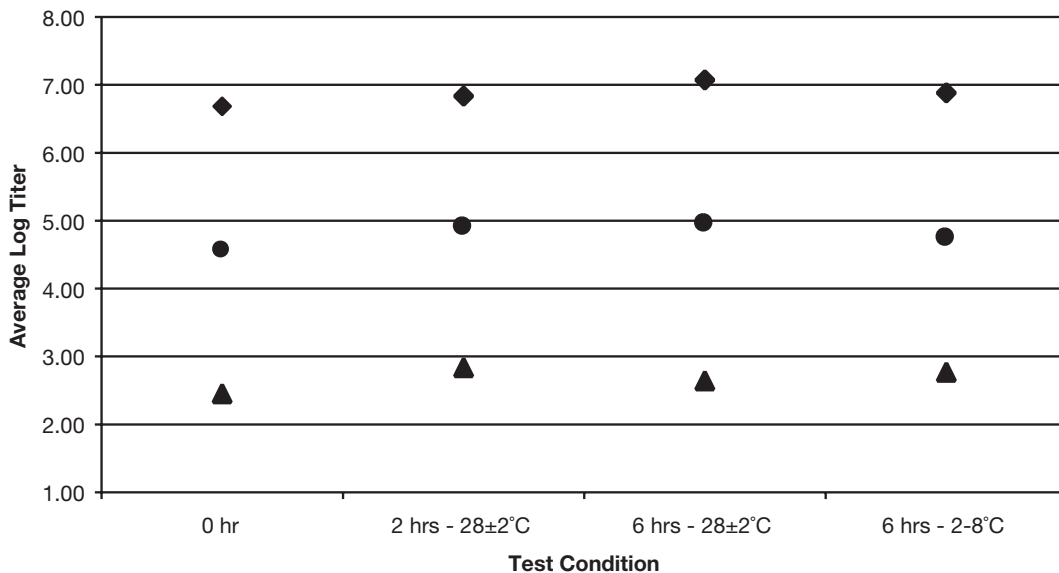
B.   Specimen Transport

Transportation of whole blood or plasma must comply with country, federal, state and local regulations for the transport of etiologic agents[39]. Whole blood must be transported at 2-25°C and centrifuged within 6 hours of collection. Plasma 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 for longer storage. Figure 5 shows the stability for HIV-1 in EDTA plasma.

C.   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. It is recommended that specimens be stored in 1100-1200 µL aliquots in sterile, 2.0 mL polypropylene screw-cap tubes (such as Sarstedt 72.694.006). Plasma specimens may be frozen and thawed up to five times without a loss of HIV-1 RNA. Figure 6 shows the data from a freeze/thaw study (study was performed using COBAS® TaqMan® HIV-1 Test for use with the High Pure System (HPS), P/N: 03501752 190 and 03502295 001).

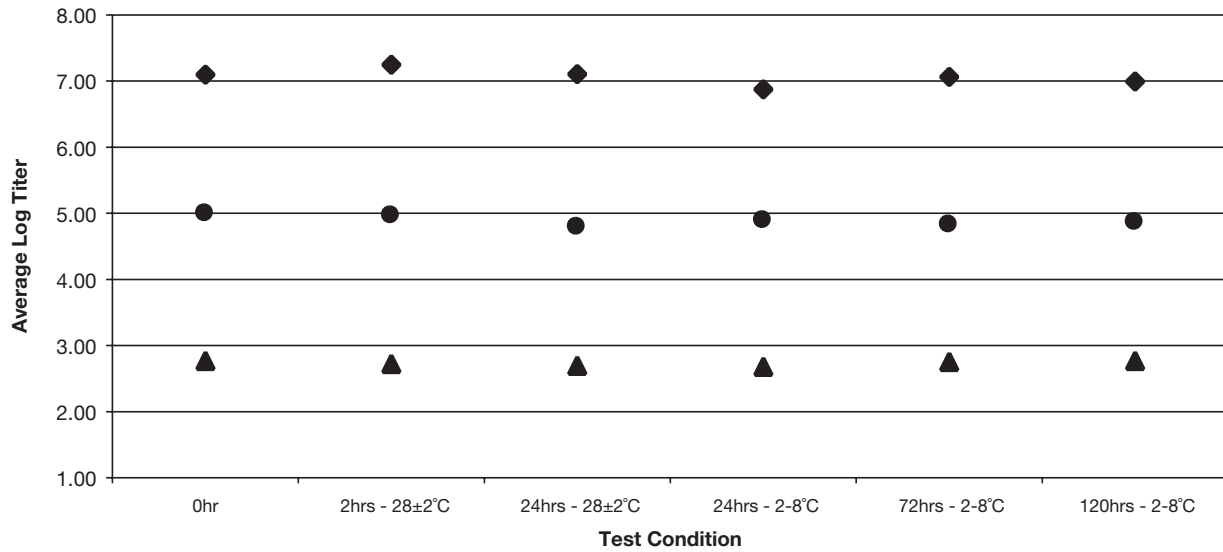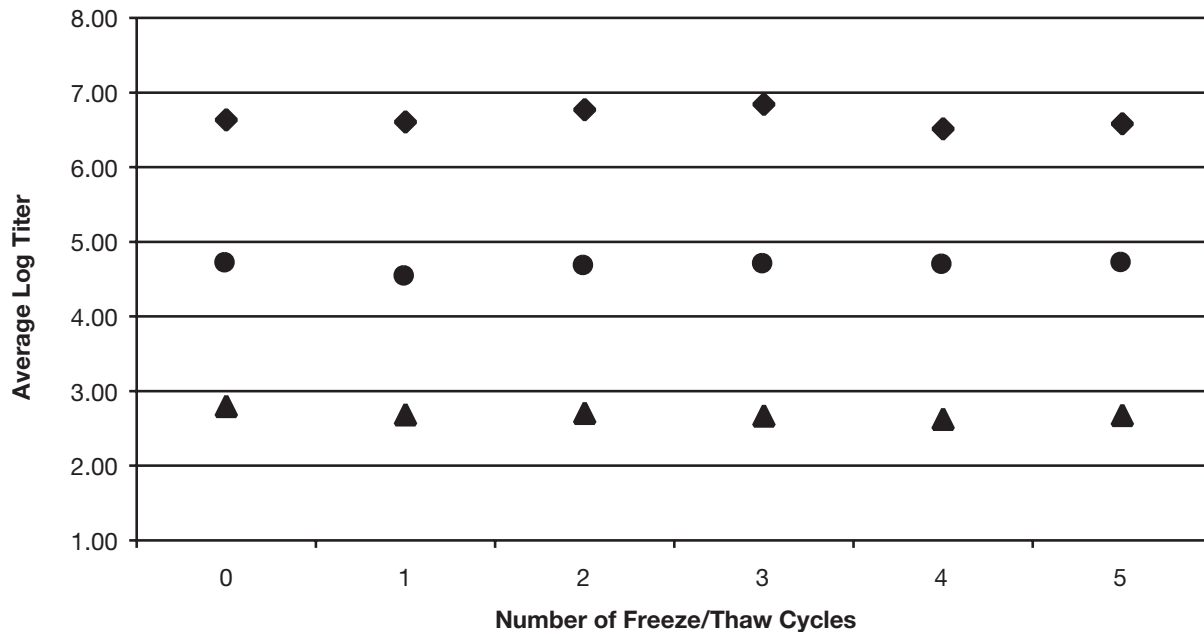### Figure 5
HIV-1 Stability in EDTA-Plasma

Figure 6
HIV-1 Results after up to Five Freeze/Thaw Cycles

INSTRUCTIONS FOR USE

NOTE: Refer to the following for detailed operating instructions: (a) the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.1.x Series; and either (b) the COBAS® TaqMan® Analyzer (plus optional Docking Station) Instrument Manual for use with AMPLILINK Software, Version 3.1.x Series or (c) the COBAS® TaqMan® 48 Analyzer Instrument Manual for use with AMPLILINK Software, Version 3.1.x Series.

Batch Size:

Each kit contains reagents sufficient for 48 tests, which may be performed in batches of 12 to 24 tests. At least one replicate each of **CTM (–) C**, **HIV-1 L(+)C** and **HIV-1 H(+)C** must be included in each batch (see "*Quality Control*" section).

Workflow:

The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run must be started within 120 minutes following completion of specimen and control preparation of a specific batch.

NOTE: If this time limit is exceeded, processed specimens and controls must be discarded.

(Processing time for one specimen on the COBAS® AmpliPrep Instrument is 216 seconds. The COBAS® AmpliPrep Instrument can process three racks of 24 specimens (n = 72) in approximately 5 hours. The amplification and detection cycle takes 3 hours, 5 minutes on both the COBAS® TaqMan® Analyzer and COBAS® TaqMan® 48 Analyzer. Completion of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test takes approximately 8 hours 10 minutes and 7 hours 35 minutes for sample processing and result generation, using the CTM 48 and CTM 96 workflows, respectively).

NOTE: DO NOT FREEZE or STORE processed specimens and controls at 2-8°C.

Specimen and Control Preparation

NOTE: If using frozen specimens, place the specimens at room temperature until completely thawed and vortex for 3-5 seconds before use. Controls should be removed from 2-8°C storage and equilibrated to ambient temperature before use.

COBAS® AmpliPrep Instrument Set-up

Part A.   Maintenance and Priming

A1.   The COBAS® AmpliPrep Instrument is ready for operation in stand-by mode.

A2.   Turn the Data Station for the AMPLILINK software **ON**. Prepare the Data Station as follows:

  a. Log onto Windows® XP.

  b. Double click the AMPLILINK software icon.

  c. Log onto AMPLILINK software by entering the assigned User ID and password.

A3.   Check the supply of **PG WR** using the **Status** Screen and replace if necessary.

A4.   Perform all Maintenance that is listed in the Due Tab, as outlined in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.1.x Series. The COBAS® AmpliPrep Instrument will automatically prime the system.

Part B. Loading of Reagent Cassettes

NOTE: All reagent cassettes should be removed from 2-8°C storage, immediately loaded onto the COBAS® AmpliPrep Instrument and allowed to equilibrate to ambient temperature on the instrument for at least 30 minutes before the first specimen is to be processed. Do not let reagent cassettes come to ambient temperature outside the instrument.

B1.　Place **HIV-1 CS1** onto a reagent rack. Place **HIV-1 CS2, HIV-1 CS3** and **HIV-1 CS4** onto a separate reagent rack.

B2.　Load the reagent rack containing **HIV-1 CS1** onto rack position **A** of the COBAS® AmpliPrep Instrument as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.1.x Series.

B3.　Load the reagent rack containing **HIV-1 CS2, HIV-1 CS3** and **HIV-1 CS4** onto rack position **B, C, D** or **E** of the COBAS® AmpliPrep Instrument as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.1.x Series (see also Table 1 for additional information).

Part C. Loading of Disposables

NOTE: Determine the number of COBAS® AmpliPrep reagent cassettes, Sample Processing Units (SPUs), Input Sample tubes (S-tubes), K-tips and K-tubes needed. One SPU, one Input S-tube, one K-tip and one K-tube are needed for each specimen or control.

Multiple configurations for use of the COBAS® AmpliPrep Instrument with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer are possible. For reference, see Table 1 below. Depending on the configuration used, load the appropriate number of reagent cassette racks, sample racks with Input S-tubes, SPU racks, K-tip racks, K-tube racks and K-carriers on K-carrier racks onto the respective rack positions of the COBAS® AmpliPrep Instrument as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.1.x Series (*also see* Table 1 for additional information).

C1.　Place the SPUs in the SPU rack(s) and load the rack(s) onto rack position **J, K** or **L** of the COBAS® AmpliPrep Instrument as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.1.x Series.

C2.　Depending on the configuration used, load full K-tube rack(s) onto rack position **M, N, O** or **P** of the COBAS® AmpliPrep Instrument as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.1.x Series.

C3.　Load full K-tip rack(s) onto rack position **M, N, O** or **P** of the COBAS® AmpliPrep Instrument as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.1.x Series.

C4.　For configurations 3 to 5 using the COBAS® TaqMan® 48 Analyzer, load K-carriers on K-carrier rack(s) onto rack position **M, N, O** or **P** of the COBAS® AmpliPrep Instrument as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.1.x Series.

Configuration	Transfer mode to COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer	Racks, carriers and disposables	Position on COBAS® AmpliPrep Instrument
1. COBAS® AmpliPrep Instrument plus Docking Station plus COBAS® TaqMan® Analyzer	Automated transfer of K-carrier	K-tubes in full K-tube racks	M-P
		K-tips in full K-tips racks	M-P
		Input S-tubes containing specimens and controls on sample racks	F-H
		SPUs in SPU racks	J-L
		CS1 on Cassette rack	A
		CS2, CS3, CS4 on Cassette rack	B-E
2 COBAS® AmpliPrep Instrument plus COBAS® TaqMan® Analyzer	Manual transfer of K-tubes via sample rack(s) onto COBAS® TaqMan® Analyzer	K-tubes in full K-tube racks	M-P
		K-tips in full K-tips racks	M-P
		Input S-tubes on sample racks	F-H
		SPUs in SPU racks	J-L
		CS1 on Cassette rack	A
		CS2, CS3, CS4 on Cassette rack	B-E
		<u>After specimen processing is finished:</u>	
		K-tubes on sample racks (ready for manual transfer)	Same as above (F-H)
3. & 4. COBAS® AmpliPrep Instrument plus 1-2 COBAS® TaqMan® 48 Analyzer(s)	Manual transfer of K-carrier via K-carrier rack(s) onto COBAS® TaqMan® 48 Analyzer	K-tubes on sample racks	F-H
		K-tips in full K-tips racks	M-P
		Input S-tubes on sample racks	F-H
		SPUs in SPU racks	J-L
		CS1 on Cassette rack	A
		CS2, CS3, CS4 on Cassette rack	B-E
		Empty barcoded K-carrier on K-carrier rack	M-P
		<u>After specimen processing is finished:</u>	
		K-tubes in K-carrier on K-carrier rack	Same as above (M-P)
5. COBAS® AmpliPrep Instrument plus COBAS® TaqMan® Analyzer plus COBAS® TaqMan® 48 Analyzer	Manual transfer of K-tubes on sample rack to COBAS® TaqMan® Analyzer. Manual transfer of K-carrier via K-carrier rack to COBAS® TaqMan® 48 Analyzer	Same as configurations 2 and 3	Same as configurations 2 and 3

Part D. Ordering and Loading of Specimens

D1. Prepare sample racks as follows: Attach a barcode label clip to each sample rack position where a specimen (S-tube) is to be placed. Attach one of the specific barcode label clips for the controls [**CTM (–) C, HIV-1 L(+)C and HIV-1 H(+)C**] to each sample rack position where the controls (S-tube) are to be placed. The barcode label clips for controls must have the same control lot number as the lot number on the control vials in the kit. Take care in assigning the correct control to the position with the appropriate control barcode clip. Place one Input S-tube into each position containing a barcode label clip.

D2. Using the AMPLILINK software, create specimen orders for each specimen and control in the **Orders** window **Sample** folder. The Test file name in the **Orders** window will be HIMAPU96 or HIMAPU48. Select the appropriate test file and complete by saving.

D3. Assign specimen and control orders to sample rack positions in the **Orders** window **Sample Rack** folder. The sample rack number must be entered for the rack prepared in Step D1.

D4. Print the **Sample Rack Order** report to use as a worksheet.

D5. Prepare specimen and control racks in the designated area for specimen and control addition as follows: Vortex each specimen and control [**CTM (–) C, HIV-1 L(+)C** and **HIV-1 H(+)C**] for 3 to 5 seconds. Avoid contaminating gloves when manipulating the specimens and controls.

D6. Transfer 1000 to 1050 µL of each specimen and control [**CTM (–) C, HIV-1 L(+)C** and **HIV-1 H(+)C**] to the appropriate barcode labeled Input S-tube using a micropipettor with an aerosol barrier or positive displacement RNase-free tip. ***Avoid transferring particulates and/or fibrin clots from the original specimen to the Input S-tube.*** Specimens and controls should be transferred to tube positions as assigned and recorded on the worksheet in step D4. The barcode label clips for controls must have the same control lot number as the lot number on the control vials in the kit. Assign the correct control to the position with the appropriate control barcode clip. ***Avoid contaminating the upper part of the S-tubes with specimens or controls.***

D7. For configurations 1 and 2 using the COBAS® TaqMan® Analyzer, load the sample rack(s) filled with Input S-tubes onto rack positions **F, G** or **H** of the COBAS® AmpliPrep Instrument.

D8. For configurations 3 to 5 using the COBAS® TaqMan® 48 Analyzer, load sample rack(s) with Input S-tubes and K-tubes (one for each Input S-tube, loaded in the correct position adjacent to Input S-tubes) onto rack position **F, G** or **H** of the COBAS® AmpliPrep Instrument as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.1.x Series.

Part E. Start of COBAS® AmpliPrep Instrument Run

E1. Start the COBAS® AmpliPrep Instrument using the AMPLILINK software as described in the AMPLILINK Software Version 3.1.x Series Application Manual for use with the COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, and COBAS® TaqMan® 48 Analyzer.

Part F. End of COBAS® AmpliPrep Instrument Run and Transfer to COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer (only for configurations 2-5)

F1. Check for flags or error messages in the system screen as described in (a) the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.1.x Series and (b) the AMPLILINK Software Version 3.1 Series Application Manual for use with the COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, and COBAS® TaqMan® 48 Analyzer.

F2. Remove processed specimens and controls from the COBAS® AmpliPrep Instrument on either sample racks (for COBAS® TaqMan® Analyzer without docking station) or K-carrier racks (for COBAS® TaqMan® 48 Analyzer), depending on the configuration (for further details see Part G).

F3. Remove waste from COBAS® AmpliPrep Instrument.

NOTE: All processed specimens and controls should not be exposed to light after completion of specimen and control preparation.

Amplification and Detection

COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Set-up

The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run must be started within 120 minutes following completion of specimen and control preparation. If this time limit is exceeded, the processed specimens and controls must be discarded.

NOTE: DO NOT FREEZE or STORE processed specimens and controls at 2-8°C.

For a detailed description of the possible configurations, refer to (a) the COBAS® TaqMan® Analyzer (plus optional Docking Station) Instrument Manual for use with AMPLILINK Software, Version 3.1.x Series; (b) the COBAS® TaqMan® 48 Analyzer Instrument Manual for use with AMPLILINK Software, Version 3.1.x Series and Part C of this Package Insert.

Part G. Loading Processed Specimens

G1. Depending on the instrument configuration, perform the appropriate steps to transfer the K-tubes to the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer:

Configuration 1:   Automated transfer of K-carrier via docking station to COBAS® TaqMan® Analyzer. Manual intervention is unnecessary.

Configuration 2 and 5:   Manual transfer of K-tubes in sample rack(s) to COBAS® TaqMan® Analyzer

Configuration 3, 4 and 5: Manual transfer of K-carrier on K-carrier rack(s) to the COBAS® TaqMan® 48 Analyzer. Manual transfer of K-carriers into COBAS® TaqMan® 48 Analyzer using the K-carrier Transporter.

Part H. Start of COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Run

H1. Start the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer by one of the options below depending on the instrument configuration used:

Configuration 1:   No intervention necessary.

Configuration 2 and 5:   Automatic start of the COBAS® TaqMan® Analyzer after insertion of sample rack(s).

Configuration 3, 4 and 5: Fill K-carrier with empty K-tubes if there are fewer than 6 K-tubes on the K-carrier. Filling is guided by the AMPLILINK software. Open thermal cycler cover, load K-carrier into thermal cycler and close lid. Start the COBAS® TaqMan® 48 Analyzer run.

Part I. End of COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Run

I1. At the completion of the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run, print Results Report. Check for flags or error messages in the Result Report as described in (a) the COBAS® TaqMan® Analyzer (plus optional Docking Station) Instrument Manual for use with AMPLILINK Software, Version 3.1.x Series or (b) the COBAS® TaqMan® 48 Analyzer Instrument Manual for use with AMPLILINK Software, Version 3.1.x Series and (c) the AMPLILINK Software Version 3.1.x Series Application Manual for use with the COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, and COBAS® TaqMan® 48 Analyzer. Specimens with flags and comments are interpreted as described in the *Results* section. After acceptance, store data in archive.

I2. Remove used K-tubes from the COBAS® TaqMan® 48 Analyzer. If using the COBAS® TaqMan® 48 Analyzer, tubes are transferred to a waste container, which should be emptied as described in the COBAS® TaqMan® Analyzer Instrument Manual.

NOTE: Caution! K-carrier may be hot after cycling.

RESULTS

The COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer automatically determines the HIV-1 RNA concentration for the specimens and controls. **The HIV-1 RNA concentration is expressed in copies (cp)/mL. The conversion factor between HIV-1 RNA copies/mL and HIV-1 International Units (IU)/mL is 0.6 cp/IU, using the WHO 1st International Standard for HIV-1 RNA for Nucleic Acid-Based Techniques (NAT) (NIBSC 97/656).**

AMPLILINK Software:

- Determines the Cycle Threshold value (Ct) for the HIV-1 RNA and the HIV-1 QS RNA.

- Determines the HIV-1 RNA concentration based upon the Ct values for the HIV-1 RNA and HIV-1 QS RNA and the lot-specific calibration coefficients provided on the cassette barcodes.

- Determines that the calculated cp/mL for **HIV-1 L(+)C** and **HIV-1 H(+)C** fall within the assigned ranges.

Batch Validation

Check AMPLILINK software results window or printout for flags and comments to ensure that the batch is valid.

NOTE: For instructions on printing results and for interpreting flags and comments, please refer to the following: (a) the AMPLILINK Software Version 3.1.x Series Application Manual for use with the COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, and COBAS® TaqMan® 48 Analyzer or (b) the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.1.x Series and (c) the COBAS® TaqMan® Analyzer (plus optional Docking Station) Instrument Manual for use with AMPLILINK Software, Version 3.1.x Series or (d) the COBAS® TaqMan® 48 Analyzer Instrument Manual for use with AMPLILINK Software, Version 3.1.x Series.

For control orders, a check is made to determine if the cp/mL value for the control is within its specified range. If the cp/mL value for the control lies outside of its range, a FLAG is generated to show the control has failed.

The batch is valid if no flags appear for any of the controls [**HIV-1 H(+)C, HIV-1 L(+)C, CTM (–) C**]. The following results are obtained for a valid batch:

Control	Result	Interpretation
Negative Control	Target Not Detected	Control within range
Low Positive Control	A numeric titer, X.XXE+XX cp/mL	Control within range
High Positive Control	A numeric titer, X.XXE+XX cp/mL	Control within range

The batch is <u>not</u> valid if any of the following flags appear for the HIV-1 Controls:

Negative Control:

Flag	Result	Interpretation
__N_NC_INVALID	Invalid	An invalid result or a "valid" result that was not negative for HIV-1 target

HIV-1 Low Positive Control:

Flag	Result	Interpretation
__L_LPCINVALID	< 4.80E+01 cp/mL	Control below range
__L_LPCINVALID	Target Not Detected	Control below range
__L_LPCINVALID	A numeric titer, X.XXE+XX cp/mL	Control out of range
__L_LPCINVALID	> 1.00E+07 cp/mL	Control above range
__L_LPCINVALID	Invalid	An invalid result

HIV-1 High Positive Control:

Flag	Result	Interpretation
__H_HPCINVALID	< 4.80E+01 cp/mL	Control below range
__H_HPCINVALID	Target Not Detected	Control below range
__H_HPCINVALID	A numeric titer, X.XXE+XX cp/mL	Control out of range
__H_HPCINVALID	> 1.00E+07 cp/mL	Control above range
__H_HPCINVALID	Invalid	An invalid result

If the batch is invalid, repeat the entire batch including specimen and control preparation, amplification and detection.

Interpretation of Results:

For a valid batch, check each individual specimen for flags or comments on the result printout. Interpret the results as follows:

⇒ A valid batch may include both valid and invalid specimen results depending on whether flags and/or comments are obtained for the individual specimens.

Specimen results are interpreted as follows:

Titer Result	Interpretation
Target Not Detected	Ct value for HIV-1 above the limit for the assay or no Ct value for HIV-1 obtained. Report results as "HIV-1 RNA not detected".
< 4.80E+01 cp/mL	Calculated cp/mL are below the Limit of Detection of the assay. Report results as "HIV-1 RNA detected, less than 48 HIV-1 RNA cp/mL".
≥ 4.80E+01 cp/mL and ≤ 1.00E+07 cp/mL	Calculated results greater than or equal to 48 cp/mL and less than or equal to 1.00E+07 cp/mL are within the Linear Range of the assay.
> 1.00E+07 cp/mL	Calculated cp/mL are above the range of the assay. Report results as "greater than 1.00E+07 HIV-1 RNA cp/mL". If quantitative results are desired, the original specimen should be diluted with HIV-1-negative human EDTA-plasma and the test repeated. Multiply the reported result by the dilution factor.

NOTE: Specimens above the range of the assay may also produce an Invalid result with a flag "QS_INVALID". If quantitative results are desired, the original specimen should be diluted with HIV-1-negative human EDTA-plasma and the test repeated. Multiply the reported result by the dilution factor.

QUALITY CONTROL

One replicate each of the COBAS® TaqMan® Negative Control, the HIV-1 Low Positive Control and the HIV-1 High Positive Control must be included in each test batch. The batch is valid if no flags appear for any of the controls [**HIV-1 H(+)C, HIV-1 L(+)C** and **CTM (–) C**].

There are no requirements regarding the position of the controls on the sample rack.

Check the batch printout for flags and comments to ensure that the batch is valid. Refer to the following manuals for detailed information on printing results and interpreting comments: (a) the AMPLILINK Software Version 3.1.x Series Application Manual for use with the COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, and COBAS® TaqMan® 48 Analyzer and either (b) the COBAS® TaqMan® Analyzer (plus optional Docking Station) Instrument Manual for use with AMPLILINK Software, Version 3.1.x Series or (c) the COBAS® TaqMan® 48 Analyzer Instrument Manual for use with AMPLILINK Software, Version 3.1.x Series.

Negative Control

The **CTM (–) C** must yield a "Target Not Detected" result. If the **CTM (–) C** is flagged as invalid, then the entire batch is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If **CTM (–) C** is consistently invalid in multiple batches, contact your local Roche office for technical assistance.

Positive Controls

The assigned range for **HIV-1 L(+)C** and **HIV-1 H(+)C** is specific for each lot of reagents, and is provided on the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test reagent cassette barcodes.

The HIV-1 RNA cp/mL for **HIV-1 L(+)C** and **HIV-1 H(+)C** should fall within their assigned ranges. If one or both of the positive controls are flagged as invalid, then the entire batch is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If the HIV-1 RNA titer of one or both of the positive controls is consistently outside the assigned ranges in multiple batches, contact your local Roche office for technical assistance.

PROCEDURAL PRECAUTIONS

1.   As with any test procedure, good laboratory technique is essential to the proper performance of this assay.

PROCEDURAL LIMITATIONS

1.   This test has been validated for use with only human plasma collected in EDTA anticoagulant. Testing of other specimen types may result in inaccurate results.

2.   The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test has neither been evaluated with specimens containing HIV-1 groups O and N, nor with specimens containing HIV-2.

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

4.   The presence of AmpErase enzyme in the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Master Mix reduces the risk of amplicon contamination. However, contamination from HIV-1 positive controls and clinical specimens can be avoided only by good laboratory practices and careful adherence to the procedures specified in this Package Insert.

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

6.   This product can only be used with the COBAS® AmpliPrep Instrument and the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.

INTERFERING SUBSTANCES

Elevated levels of triglycerides, bilirubin, albumin, hemoglobin and human DNA in specimens as well as the presence of autoimmune diseases such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA) and Antinuclear Antibody (ANA) have been shown not to interfere with the quantitation of HIV-1 RNA or impact the specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test.

The following drug compounds tested at the Peak Plasma Level (C_{max}) and at 3 times the C_{max} have been shown not to interfere with the quantitation of HIV-1 RNA or impact the specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test with the exception of Nevirapine, for which absence of interference has only been shown at the Peak Plasma Level (C_{max}):

Nucleotide DNA Polymerase Inhibitors Tenofovir Adefovir dipivoxil	**Nucleoside Reverse Transcriptase and DNA Polymerase Inhibitors** Lamivudine Zidovudine Stavudine Abacavir Didanosine
HIV Protease Inhibitors Indinavir Saquinavir Ritonavir Nelfinavir Amprenavir Lopinavir/Ritonavir	**Non-nucleoside HIV Reverse Transcriptase Inhibitors** Nevirapine Efavirenz
	HIV Fusion Inhibitor Enfuvirtide
Immune Modulators Interferon alpha-2a Interferon alpha-2b Peginterferon alpha-2a Peginterferon alpha-2a + Ribavirin Interferon alpha-2b + Ribavirin	**Antidepressants** Paroxetine HCl Fluoxetine Sertraline
Nucleoside Inhibitors Ribavirin	**Compounds for the Treatment of Herpes Viruses** Ganciclovir Valganciclovir Acyclovir

NON-CLINICAL PERFORMANCE EVALUATION

A.   Limit of Detection

The limit of detection of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test was determined by analysis of three independent dilution series of a HIV-1 Secondary Standard (prepared from the HIV-1B strain 8E5 LAV in HIV-1-negative human EDTA plasma). The concentration of the HIV-1 Secondary Standard is traceable to the WHO 1st International Standard for HIV-1 RNA for Nucleic Acid-Based Techniques (NAT) (NIBSC 97/656). A total of 108 replicates per concentration level were tested.

The concentration of HIV-1 RNA in EDTA plasma that can be detected with a positivity rate of greater than 95% as determined by probit analysis is 48 cp/mL. The study was performed for three lots of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test reagents and the combined results are shown in Table 2.

Table 2
Limit of Detection of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test
determined with HIV-1B Secondary Standard in EDTA plasma

Level No.	HIV-1B Input Conc. (copies/mL)	Total Number of Replicates Tested	Number of Positives	Hit Rate
1	100	108	108	100%
2	75	108	108	100%
3	50	108	107	99%
4	40	108	99	92%
5	30	108	98	91%
6	20	108	81	75%
7	10	108	55	51%

B.   Precision

Precision and linearity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test were determined by analysis of serial dilutions prepared from a highly concentrated cell culture stock of the HIV-1B in HIV-1-negative human EDTA plasma. The concentration assignment of the HIV-1B linearity panel was performed by a method that ensures traceability to the WHO 1st International Standard for HIV-1 RNA for Nucleic Acid-Based Techniques (NAT) (NIBSC 97/656).

Within-Run, Run-to-Run and Total Precision were evaluated in accordance with the methods defined in the CLSI Guideline EP5-A, "Evaluation of Precision Performance of Clinical Chemistry Devices".[41] A run, consisting of 5 dilution levels and 7 replicates at each level, was performed daily for 15 days. Each sample was carried through the entire COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test procedure, including specimen preparation, amplification and detection using different systems operated by multiple users. Therefore, the precision reported here represents all aspects of the test procedure. The study was performed for three lots of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test reagents, and the results are shown in Table 3.
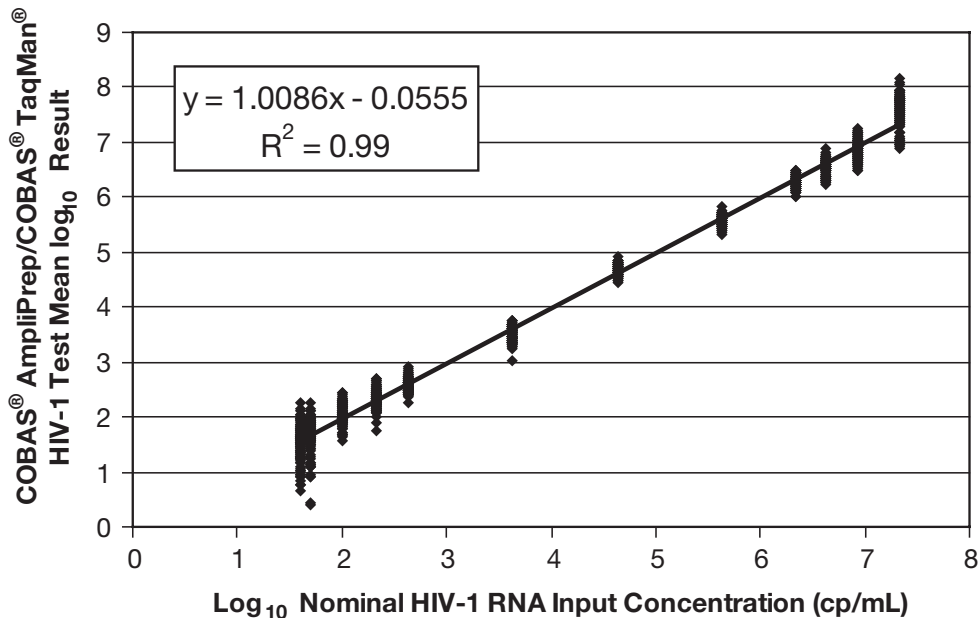
Table 3
Total Precision for three lots of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test

Nominal HIV-1 Conc. Levels [cp/mL]	Log_{10} of Nominal Conc.	Lot 1		Lot 2		Lot 3	
		Total CV [%]	Total precision as SD [log_{10}]	Total CV [%]	Total precision as SD [log_{10}]	Total CV [%]	Total precision as SD [log_{10}]
1.0E+02	2.000	39.5	0.18	41.4	0.18	41.8	0.18
4.3E+03	3.634	24.5	0.11	29.3	0.13	23.1	0.10
4.3E+04	4.634	20.0	0.09	20.6	0.09	16.9	0.08
4.3E+05	5.634	22.4	0.10	20.0	0.09	22.8	0.10
2.2E+06	6.333	24.0	0.10	23.5	0.10	24.7	0.10

C.   Linear Range

As shown in Figure 7, the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test was found to give a linear response from 48 HIV-1 RNA cp/mL to 10,000,000 HIV-1 RNA cp/mL applying the accuracy acceptance criterion of ± 0.3 $\log_{10}$ from the nominal input concentration. The study was performed using one lot of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test reagents, with 103 – 105 replicates per level.

Figure 7
Linear range of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test

The graph shows:
$$y = 1.0086x - 0.0555$$
$$R^2 = 0.99$$

Y-axis: COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test Mean $\log_{10}$ Result

X-axis: Log_{10} Nominal HIV-1 RNA Input Concentration (cp/mL)

D.   Inclusivity

Eight subtype categories have been proposed for HIV-1 group M based on nucleotide divergence. These subtypes are designated with capital alphabetical letters from A through H[40].

Similar Subtype Quantitation

The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test on HIV-1 subtypes was evaluated by analysis of cell culture stock material of representatives of each HIV-1 group M subtype A through H. The assignment of nominal concentrations of the cell culture stock solutions was performed by the COBAS® AmpliPrep/COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 assay method (Figure 8). Based on the determined stock concentrations, the HIV-1 target concentrations of 2.0E+02, 1.9 - 3.3E+04 and 1.0E+05 - 1.0E+06 cp/mL were prepared for each HIV-1 subtype by exact dilution of the cell culture stock solution in EDTA plasma. Afterwards the concentrations were determined by the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test in n=7 replicates per level of each subtype using one reagent lot. Results were compared to the input concentrations as determined by the reference method.

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test gave equivalent results for all tested representatives of the HIV-1 group M subtypes (see Figure 8). Mean observed $\log_{10}$ concentration results were within ± 0.3 $\log_{10}$ of the respective reference method input concentration.

Figure 8
Performance of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test on HIV-1 group M
Subtypes A through H compared against the reference method
COBAS® AmpliPrep/COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5
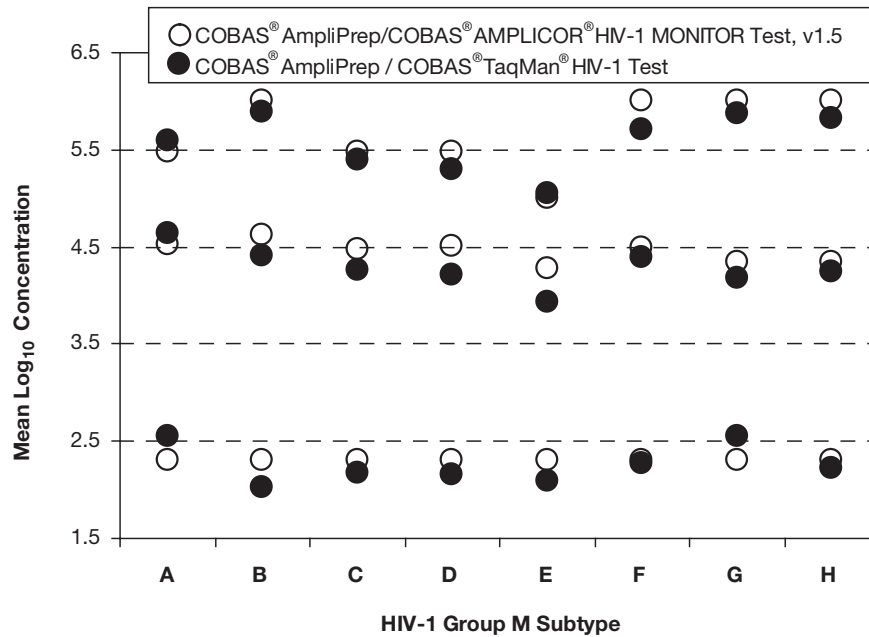
Subtype Limit of Detection

Parent solutions containing HIV-1 cell culture material representing HIV-1 subtypes A to H have been obtained as frozen stocks from German National Reference Centre for Retrovirology at University Erlangen-Nuremberg, Germany. The certified parent input concentration as determined by the VERSANT® HIV-1 RNA 3.0 Assay (bDNA) reference method was used to prepare the Subtype LOD-panels. Two independent dilution series of the different HIV-1 subtypes were evaluated on two days with one lot of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test in a total of 24 replicates per concentration level for each subtype representative. The results of the probit analyses at 95% hit rate demonstrate that the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test has a sensitivity of ≤ 50 cp/mL across all subtypes ranging from < 15 to 46 cp/mL of HIV-1 group M as shown in Table 4.

Table 4
Inclusivity as Limit of Detection of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test as determined with cell-cultured viral stocks of the different HIV-1 subtypes diluted in EDTA plasma

Subtype	Isolate Designation	95% hit rate conc. by probit model	95% Confidence Interval [cp/mL]		Lowest level with ≥ 95% hit rate
		[cp/mL]	Lower	Upper	[cp/mL]
A	92UG029	20			30
A	92UG037	52			50
A	4237A/98	< 15			15
A combined	92UG029/92UG037/ 4237A/98	**23**	**12**	**34**	40
B	HIV-1 Secondary Standard (8E5 LAV)	38			40
B	WHO 1st Int. Std. for HIV-1 RNA (97/656)	46			50
B	MVP-899-87	35			50
B combined	HIV-1 Secondary Standard (8E5 LAV)/ WHO 1st Int. Std. for HIV-1 RNA (97/656)/ MVP-899-87	**40**	**33**	**50**	50
C	92BR025	16			30
C	98TZ017	50			50
C	3777A/97	16			15
C combined	92BR025/98TZ017/ 3777A/97	**31**	**25**	**41**	40
D	92UG021	35			40
D	92UG035	< 15			15
D	92UG024	17			30
D combined	92UG021/92UG035/ 92UG024	**26**	**22**	**34**	40
E	92TH022	< 15			15
E	92TH009	44			50
E	92TH001	34			50
E combined	92TH022/92TH009/ 92TH001	**33**	**27**	**44**	50
F	93BR020	**46**	**34**	**82**	40
G	ARP173/RU570	**< 15**	NA	NA	15
H	ARP175/HIV V1557	**< 15**	NA	NA	15

NA - not applicable (no analysis possible since the statistical software does not allow calculating a confidence interval based on the observed hit rate profile)

E.   Specificity

The clinical specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test was determined by analysis of HIV-1-negative EDTA plasma specimens from blood donors. A total of 513 individual EDTA plasma specimens were tested. All specimens were negative for HIV-1 RNA. Based on these results, the clinical specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test is 100% with the confidence interval ranging from 99.3 to 100%.

F. Analytical Specificity

The analytical specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test was evaluated by adding cultured organisms (viruses, bacteria, yeast) or DNA (HTLV-2) at 5E+04 particles/mL input concentration into HIV-1-negative human EDTA plasma and into HIV-1-positive human EDTA plasma at 1E+04 cp/mL HIV-1 (see Table 5). The cultured organisms added to specimens have been shown not to interfere with the quantitation of HIV-1 RNA or impact the specificity of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test.

Table 5
Analytical Specificity Organisms

Virus	Bacteria
Adenovirus type 2	Staphylococcus aureus
Cytomegalovirus	Propionibacterium acnes
Epstein-Barr Virus	
Human Herpes Virus type 6	
Herpes simplex virus type 1	
Herpes simplex virus type 2	**Yeast**
Human T-Cell Lymphotropic virus type 1	Candida albicans
Human T-Cell Lymphotropic virus type 2	
Influenza A	
Hepatitis A virus	
Hepatitis B virus	
Hepatitis C virus	

G. Method Correlation

The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test was compared to the COBAS® AmpliPrep/COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5, to the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 and to the VERSANT HIV-1 RNA 3.0 Assay (bDNA) by analysis of n=71 undiluted clinically characterized plasma specimens from HIV-1 infected patients. Specimens were obtained from Teragenix (Fort Lauderdale, FL, USA). Correlation was determined using the specimens for which quantitative results were obtained with each method under comparison. Specimens with a concentration result above the measuring range of the COBAS® AmpliPrep/COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 using the ultrasensitive procedure (PHS) were prediluted to fall into the measuring range of the PHS. Bivariate linear regression analysis was performed on those specimens that yielded results within the linear range as shown in Figures 9, 10 and 11. The results obtained with the three methods under comparison showed high correlation.

Figure 9
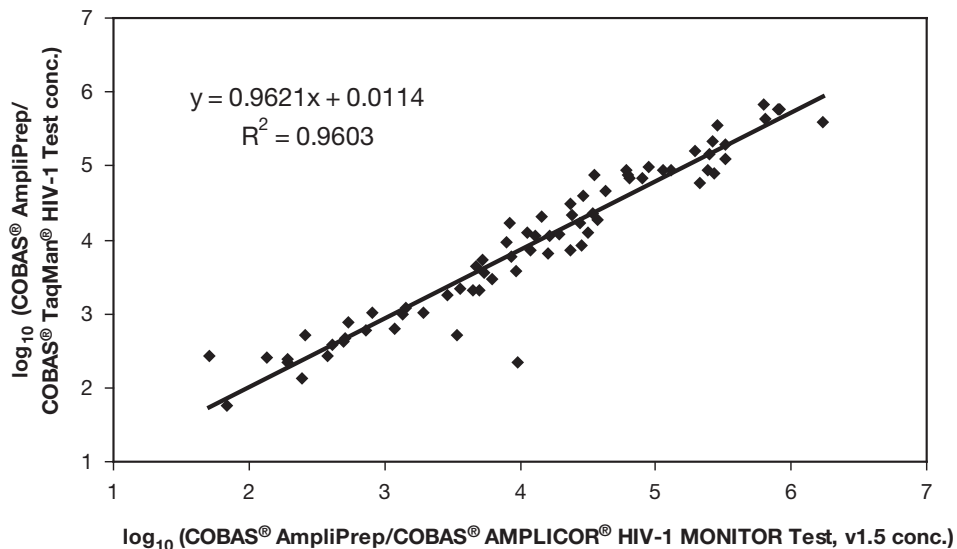**Correlation of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test and the COBAS® AmpliPrep/COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5**

$y = 0.9621x + 0.0114$

$R^2 = 0.9603$

$\log_{10}$ (COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test conc.)

$\log_{10}$ (COBAS® AmpliPrep/COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 conc.)

Figure 10
Correlation of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test and the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5
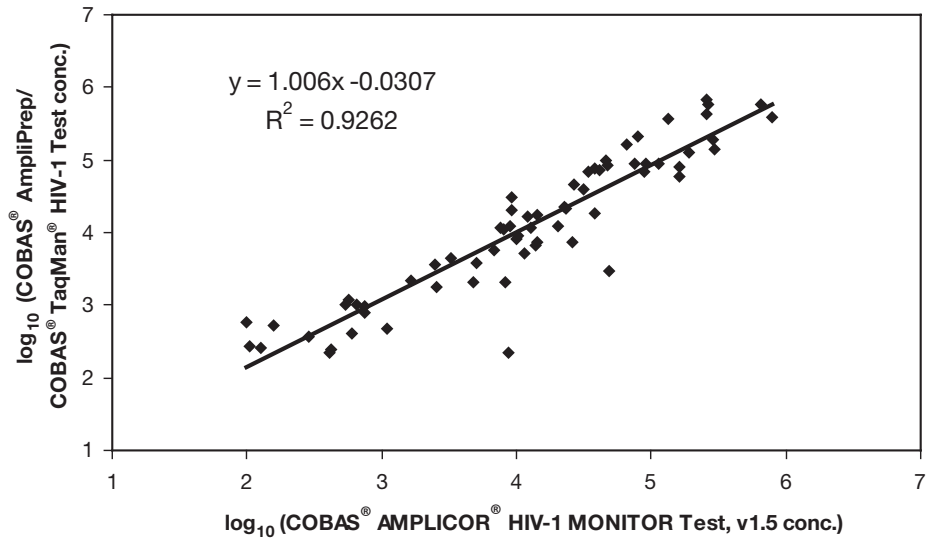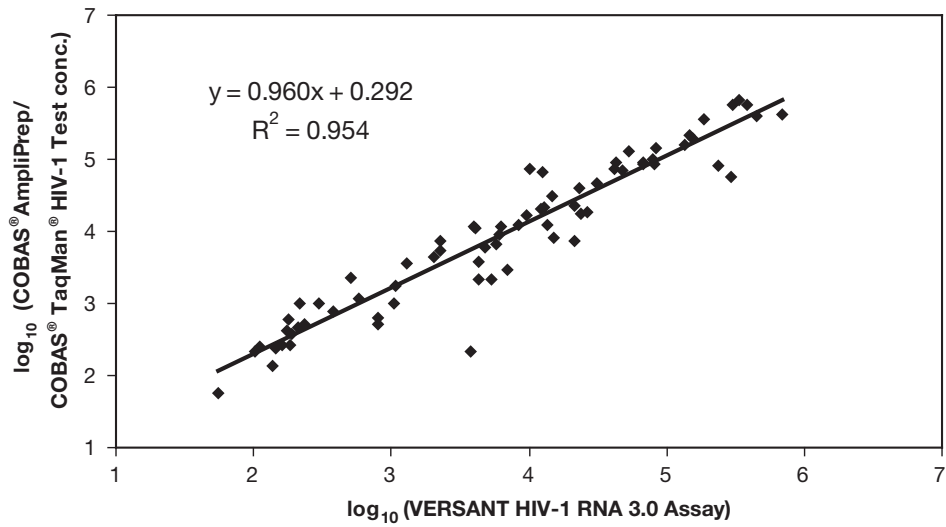
$$y = 1.006x - 0.0307$$
$$R^2 = 0.9262$$

Y-axis: $\log_{10}$ (COBAS® AmpliPrep/ COBAS® TaqMan® HIV-1 Test conc.)

X-axis: $\log_{10}$ (COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 conc.)

Figure 11
Correlation of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test and the VERSANT HIV-1 RNA 3.0 Assay (bDNA)

$$y = 0.960x + 0.292$$
$$R^2 = 0.954$$

Y-axis: $\log_{10}$ (COBAS® AmpliPrep/ COBAS® TaqMan® HIV-1 Test conc.)

X-axis: $\log_{10}$ (VERSANT HIV-1 RNA 3.0 Assay)

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CLINICAL PERFORMANCE EVALUATION

A. Reproducibility

This study was conducted to evaluate the reproducibility of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test in EDTA plasma. Well characterized HIV-1 group M, subtype B virus stock cultures and EDTA plasma that was negative for HIV-1 RNA and HIV 1/2 antibodies were used to construct a 10-member panel. The study was designed to measure inter-run, intra-run, lot to lot, day to day, site to site, and operator to operator variability. Each panel was tested by multiple operators at each of three sites; one internal Roche Molecular Systems, Inc. (RMS) site, and two sites external to RMS. Each operator performed 5 days of testing on each of 3 lots of reagents with each panel. Each operator was to complete 1 run per day. Each run comprised a single panel with each panel member tested in duplicate.

Precision was evaluated using a random effects model with terms for lot, site/instrument, operator within site, between day/run and within-run components.

Table 6 shows the total precision variance and total precision standard deviation as determined by analysis of variance. Analysis of variance provides an estimate of the total precision of the test that properly weights the between-lot, between-site/instrument, between-operator, between-day/run (day-to-day) and within-run components. The total precision reported as lognormal variance was less than 35% for all panel members except the panel member with the lowest concentration (100 copies/mL). The within-run component contributed the most variability (56% to 100%), followed by lot-to-lot variability. The site/instrument, operator and day/run components contributed less to variability.

Table 7 summarizes the results for the HIV-1 Negative Panel Member. The negative panel member was used to estimate the analytical specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test. One false positive result was observed out of 179 valid test results giving a specificity of 99% [95% Confidence Interval (CI) = (0.97, 1.00)].

Table 6
Reproducibility Results Summary

HIV-1 RNA Concentration (log10 cp/mL)			Contribution to Total Variance (%)					Total Precision
Expected	Observed (Average)	N	Lot	Site / Instrument	Operator	Day / Run	Within Run	Standard Deviation (Lognormal %CV)
2.000	2.020	147	0%	0%	0%	0%	**100%**	0.19 (46%)
2.699	2.743	173	2%	6%	4%	20%	**68%**	0.14 (34%)
3.000	2.995	178	0%	8%	0%	8%	**84%**	0.14 (32%)
3.699	3.743	178	16%	5%	3%	18%	**57%**	0.14 (34%)
4.301	4.410	179	14%	6%	2%	5%	**74%**	0.14 (32%)
4.699	4.836	178	21%	1%	0%	2%	**76%**	0.12 (29%)
5.398	5.501	178	32%	0%	0%	6%	**62%**	0.13 (31%)
5.699	5.837	178	28%	0%	0%	3%	**68%**	0.14 (34%)
6.699	6.871	108	32%	4%	0%	9%	**56%**	0.13 (30%)

Note: Within assay range results are from 4.80E+1 cp/mL to 1.00E+7 cp/mL inclusive.

Table 7
HIV-1 Negative Panel Member Summary

Total Valid Results	Target Not Detected	Target Detected	Analytical Specificity	Exact 95% CI
179	178	1	0.99	(0.97, 1.00)

Clinical Sensitivity and Specificity

Methodology

This study was designed to evaluate the clinical specificity and sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test by testing fresh and frozen samples collected from normal healthy donors and patients with HIV-1. This study compared Test results obtained with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test to those obtained with an FDA-approved test, ie, the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5. Clinical specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test was evaluated by testing 399 frozen samples and 120 fresh samples, in EDTA plasma, collected from normal healthy blood donors who were negative for HIV-1 antibodies. Frozen samples were randomly distributed across testing sites. Fresh samples were distributed to testing sites in a non-random manner. Clinical sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test was evaluated testing 351 frozen samples with HIV-1 RNA concentrations ≥50 cp/mL and 122 fresh samples in EDTA plasma, collected from HIV-1-positive blood donors. Frozen samples were randomly distributed across testing sites, stratified by CD4 count category. Fresh samples were distributed to testing sites in a non-random manner. Frozen samples used in this study were previously tested with COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 and, therefore, were not retested in this study on that platform. Collection of frozen normal donor (HIV-1-negative) samples occurred at 4 sites: 1 each in Tennessee, Florida, Pennsylvania, and California. Collection of frozen HIV-1-positive samples occurred at 6 sites: 1 each in California, New Jersey, Maryland, and Missouri, and at 2 sites in Florida. Collection of fresh samples occurred at 5 sites, as follows: HIV-1-positive samples were collected at 4 sites: 2 in Florida, 2 in California; samples from normal healthy donors were collected at 1 site in Tennessee. Fresh samples collected for this study were tested prospectively on the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 platform. Testing was performed at 3 sites, with 1 COBAS® AmpliPrep/COBAS® TaqMan® 48 Analyzer system per site, with at least 3 reagent lots.

Statistical Methods

Normal subjects were considered evaluable if they contributed both valid COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test and COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 results (where the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 result was Target Not Detected). HIV-1 subjects were considered evaluable if they contributed both valid COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test and COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 results (where the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 result was ≥50 cp/mL).

COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test clinical specificity was calculated as the percentage (95% exact confidence interval [CI]) of normal subjects with Target Not Detected COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 results, who had Target Not Detected COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test results. COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test clinical specificity was calculated overall and by sample type (fresh and frozen separately).

COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test clinical sensitivity was calculated as the percentage (95% exact CI) of HIV-1 subjects with COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 results ≥50 cp/mL, who had detectable HIV-1 viral load on the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test. COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test clinical sensitivity was calculated overall and by CD4 count category (<200, 200-500, >500 cells/μL) and sample type (fresh and frozen separately).

Results

Table 8 summarizes the number of fresh and frozen samples from evaluable normal and HIV-1 subjects in this study.

Table 8
Number of Evaluable Normal and HIV-1 Subjects by Sample Type

Sample Type	Normal Subjects	HIV-1 Subjects	Total
Fresh	120 (23.1%)	122 (25.8%)	242
Frozen	399 (76.9%)	351 (74.2%)	750
Total	519	473	992

Table 9 shows the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test clinical specificity results from the 519 evaluable normal subjects. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test clinical specificity was 99.4% (516/519; 95% CI = 98.3% to 99.9%). The HIV-1-positive COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test results were all <40 cp/mL.

Table 9
COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test Specificity - Evaluable Normal Subjects

COBAS® AMPLICOR® HIV-1 MONITOR Test, v 1.5 Result	COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test Result		Total	COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test Clinical Specificity (95% Exact CI)
	HIV-1 Positive	HIV-1 Negative		
HIV-1 Negative	3 (0.6%)	516 (99.4%)	519	99.4% (98.3%, 99.9%)

Note: CI = Confidence Interval

Table 10 shows the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test clinical sensitivity results from the 473 evaluable HIV-1 subjects. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test clinical sensitivity was 98.3% (465/473; 95% CI = 96.7% to 99.3%).

Table 10
COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test Sensitivity - Evaluable HIV-1 Subjects

COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 Result	COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test Result		Total	COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test Clinical Sensitivity (95% Exact CI)
	HIV-1 Positive	HIV-1 Negative		
HIV-1 Positive	465 (98.3%)	8 (1.7%)	473	98.3% (96.7%, 99.3%)

*All 8 samples that were determined to be positive for HIV-1 by COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 but not by COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test had a low copy number: 6 of the 8 had HIV-1 titers of < 100 cp/mL, 1 had a titer of 253 cp/mL, and the remaining sample had a titer of 479 cp/mL.

Note: CI = Confidence Interval

Table 11 shows the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test clinical sensitivity by CD4 count category (<200, 200-500, >500 cells/µL).

Table 11
COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test Sensitivity by CD4 Count Category - Evaluable HIV-1 Subjects

CD4 Count Category (cells/µL)	COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 Result	COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test Result		Total	COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test Clinical Sensitivity (95% Exact CI)
		HIV-1 Positive	HIV-1 Negative		
<200	HIV-1 Positive	141 (99.3%)	1 (0.7%)	142	99.3% (96.1%, 100.0%)
200 – 500	HIV-1 Positive	212 (98.6%)	3 (1.4%)	215	98.6% (96.0%, 99.7%)
>500	HIV-1 Positive	112 (96.6%)	4 (3.4%)	116	96.6% (91.4%, 99.1%)

Note: CI = Confidence Interval

Conclusion

When compared with results from the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5, clinical specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test was 99.4%, and clinical sensitivity was 98.3%, indicating similar performance of both tests. Furthermore, in both fresh and frozen samples, the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test showed similar specificity (>98%) and sensitivity (>98%), indicating comparable test performance for both sample types.

Selection of the HIV-1 samples included in this study was based on a single test result with the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5. For low titer samples, the results from the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 are subject to large variability (coefficient of variation = 50% at 100 cp/mL). All 8 samples that were determined to be positive for HIV-1 by the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 but not by the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test had a low copy number: 6 of the 8 had HIV-1 titers of <100 cp/mL, 1 had a titer of 253 cp/mL, and the remaining sample had a titer of 479 cp/mL. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test was able to detect 111 out of the 119 samples with titers <480 cp/mL. The observed results are consistent with what is expected with low-titer samples. Furthermore, the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test and the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 appeared to show comparable agreement across the linear range.

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test also showed similar clinical sensitivity among CD4 count categories (>96% overall), suggesting comparable performance in samples from HIV-1 patients with various CD4 counts.

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