COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, version 2.0

FOR *IN VITRO* DIAGNOSTIC USE.

COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0	**HI2CAP**	48 Tests	P/N: 05212294 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, version 2.0 (v2.0) is an *in vitro* nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human EDTA plasma or from a **cobas®** Plasma Separation Card (**PSC**) dried plasma spot using the COBAS® AmpliPrep Instrument for automated specimen processing and the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer for automated amplification and detection. The test can quantitate HIV-1 RNA over the range of 20 - 10,000,000 copies/mL (33 to 1.67 x 10^7 International Units [IU]/mL in EDTA plasma and 738 – 10,000,000 copies/mL (1230 to 1.67 x 10^7 International Units [IU]/mL) in a **PSC** dried plasma spot). One copy of HIV-1 RNA is equivalent to 1.67 IU based on the WHO 1st International Standard for HIV-1 RNA for Nucleic Acid-Based Techniques (NAT) (NIBSC 97/656)[36].

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 group M and HIV-1 group O 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 HIV-1 RNA levels during the course of antiretroviral treatment.

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 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.

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].

The World Health Organization also now recommends the use of dried spot specimens to expand the reach of viral load testing in resource limited settings without ready access to phlebotomy services or robust EDTA plasma sample transportation capabilities[27]. A dried plasma spot from the **PSC** which also stabilizes the HIV RNA in dried plasma can improve viral load testing coverage in these settings by enabling sample transportation over longer distances and harsher environmental conditions than EDTA plasma.

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)[28-30]. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 uses PCR technology to achieve maximum sensitivity and dynamic range for the quantitative detection of HIV-1 RNA in EDTA anti-coagulated plasma and in a **PSC** dried plasma spot.

PRINCIPLES OF THE PROCEDURE

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 is a nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human EDTA plasma or from a **PSC** dried plasma spot. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 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 detection probe specific to the target.

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 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 and of HIV-1 group O. The detection of amplified DNA is performed using target-specific and QS-specific dual-labeled oligonucleotide probes 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 subsequent steps of specimen preparation, reverse transcription, simultaneous PCR amplification and detection 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 HIV-1 group M subtypes and HIV-1 group O specimens. In order to address the high genetic variability of the virus, two regions of HIV genome

are simultaneously targeted for amplification and detection by the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0.

Two target-specific and one QS-specific dual-labeled oligonucleotide probes permit independent identification of the HIV-1 amplicon and of the HIV-1 QS amplicon. Accordingly, the appropriate selection of the primers and the dual-labeled oligonucleotide probes is critical to the ability of the test to amplify and detect the HIV-1 group M subtypes and HIV-1 group O. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 uses reverse transcription and PCR amplification primers that define sequences within the highly conserved regions of the HIV-1 *gag* gene[33] and of the HIV-1 LTR region.

Specimen Preparation

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 utilizes automated specimen preparation on the COBAS® AmpliPrep Instrument by a generic silica-based capture technique. The procedure processes 850 µL of plasma or SPEX-based extract from the **PSC**. 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 two target-specific and one 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* Z05 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[31,32]. 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 the downstream primers 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 DNA strands complementary to the RNA target.

Target Amplification

Processed specimens are added to the amplification mixture in amplification tubes (K-tubes) in which PCR amplification occurs. 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 hybrids and to expose the specific primer target sequences. As the mixture cools, the primers anneal to the target DNA. 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 double-stranded DNA molecules termed amplicons. 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 two regions 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 specimen is achieved in the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 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[34], 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 during amplification.

Detection of PCR Products in a COBAS® TaqMan® Test

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 utilizes real-time[35,36] 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, v2.0 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, v2.0 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 probes 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 a similar 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 predetermined 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 Over a 5-log$_{10}$ Range

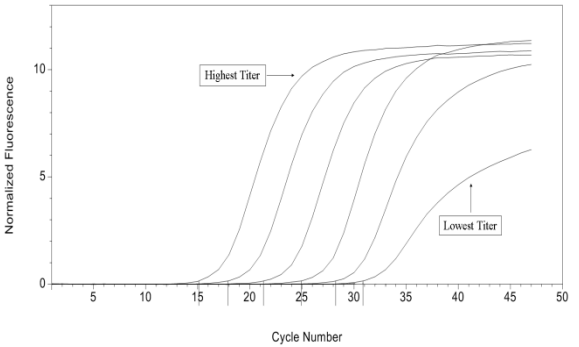


Figure 2 shows the Quantitation Standard growth curves for specimens from a viral dilution series that spans a 5-log$_{10}$ range. The amount of Quantitation Standard added to each specimen is constant for each reaction. The Ct value of the Quantitation Standard is similar regardless of the viral titer.

Figure 2
Quantitation Standard Growth Curves for a Dilution Series of Virus Spanning a 5-Log$_{10}$ Range

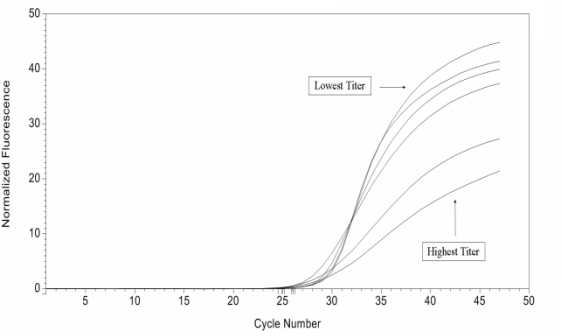
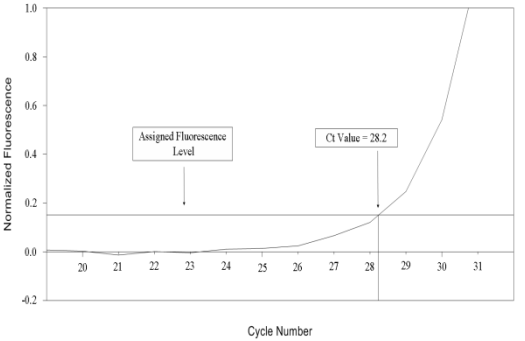


Figure 3 provides an example of how the fluorescence values at every cycle are normalized for each growth curve. The fractional cycle number (Ct) is calculated where the fluorescence signal crosses the Assigned Fluorescence Level.

Figure 3
Fluorescence Values at Every Cycle are Normalized for Each Growth Curve



HIV-1 RNA Quantitation

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 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 *gag* 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 *gag* target RNA. The detection probe binding region of the HIV-1 QS has been modified to differentiate HIV-1 QS amplicon from HIV-1 *gag* target amplicon.

During the annealing phase of the PCR in 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, v2.0 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, v2.0 is standardized against a World Health Organization International Standard for HIV-1 RNA. Titer results are reported in copies/mL (cp/mL) or International Units (IU/mL). The conversion factor between reported HIV-1 RNA cp/mL and HIV-1 IU/mL has been determined by Roche Molecular Systems, Inc. to be 0.6 cp/IU (1.67 IU/cp).

REAGENTS

COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 **HI2CAP** **48 Tests**
(P/N: 05212294 190)

HIV-1 v2.0 CS1 **1 x 48 Tests**
(HIV-1 Magnetic Glass Particles Reagent Cassette)
- Magnetic glass particles
- 93% Isopropanol

HIV-1 v2.0 CS2 **1 x 48 Tests**
(HIV-1 Lysis Reagent Cassette)
- Sodium citrate dihydrate
- 42.5% Guanidine thiocyanate
- < 14% Polydocanol
- 0.9% Dithiothreitol

HIV-1 v2.0 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

EB 1 x 7.0 mL
(Elution Buffer)
- Tris-base buffer
- 0.2% Methylparaben

HIV-1 v2.0 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 1 x 2.5 mL
(HIV-1 Master Mix)
- 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* and the LTR region of HIV-1
- < 0.003% Oligonucleotide aptamer

< 0.003% Fluorescent-labeled oligonucleotide probes specific for HIV-1 and the HIV-1 Quantitation Standard
< 0.05% Z05 DNA Polymerase (microbial)
< 0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial)
0.09% Sodium azide

CAP/CTM Mn^{2+} 1 x 19.8 mL
(CAP/CTM Manganese Solution)
< 0.5% Manganese acetate
Glacial acetic acid
0.09% Sodium azide

HIV-1 H(+)C, v2.0 4 x 1.0 mL
(HIV-1 High Positive Control, v2.0)
< 0.001% Armored HIV-1 RNA construct containing HIV-1 sequences (non-infectious RNA in MS2 bacteriophage).
Negative Human Plasma, non-reactive by 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, v2.0 4 x 1.0 mL
(HIV-1 Low Positive Control, v2.0)
< 0.001% Armored HIV-1 RNA construct containing HIV-1 sequences (non-infectious RNA in MS2 bacteriophage).
Negative Human Plasma, non-reactive by 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 4 x 1.0 mL
[COBAS® TaqMan® Negative Control (Human Plasma)]
Negative Human Plasma, non-reactive by 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, v2.0 Clip **1 x 4 Clips**
(HIV-1 High Positive Control, v2.0 Barcode Clip)

HIV-1 L(+)C, v2.0 Clip **1 x 4 Clips**
(HIV-1 Low Positive Control, v2.0 Barcode Clip)

HIV-1 (–) C Clip **1 x 4 Clips**
(HIV-1 Negative Control, v2.0 Barcode Clip)

COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent **PG WR** **1 x 5.1 L**
(P/N: 03587797 190)

PG WR
(COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent)
Sodium citrate dihydrate
< 0.1% N-Methylisothiazolone-HCl

COBAS® AmpliPrep/COBAS® TaqMan® Specimen Pre-Extraction Reagent
(P/N: 06989861190)

SPEX

5 x 78 mL

SPEX
(**cobas**® Specimen Pre-Extraction Reagent)
42.5% guanidine thiocyanate
0.79% Sodium citrate
0.01% Citric acid
3.6% Polydocanol
1.8% Dithiothreitol

Note: This reagent is optional and should only to be used in conjunction with the PSC to generate dried plasma spot samples. See PSC Method Sheet ms_07963084190.

WARNINGS AND PRECAUTIONS

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

B. This test is for use with human plasma collected in the anticoagulant EDTA or from a **PSC** dried plasma spot.

C. Do not pipette 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 pipettes and RNase-free pipette tips is recommended.**

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

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

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

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

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

L. Safety Data Sheets (SDS) are available on request from your local Roche office.

M. Specimens and controls should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[38] and in the CLSI Document M29-A3[39]. 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.

Note: If spillage of PSC dried plasma spot samples in cobas® Specimen Pre-Extraction Reagent (SPEX) (which contain guanidine thiocyanate) occurs, do not allow it to come in contact with sodium hypochlorite containing disinfectants such as bleach. This mixture can produce a highly toxic gas.

N. **CAUTION**: **CTM (–) C, HIV-1 L(+)C, v2.0** and **HIV-1 H(+)C, v2.0** contain Human Plasma derived from human blood. The source material has been tested 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, v2.0** and **HIV-1 H(+)C, v2.0** should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[38] and in the CLSI Document M29-A3[39]. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

O. **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.

P. 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.

Q. Do not allow **HIV-1 v2.0 CS2, SPEX** (used in **PSC** dried plasma spot procedure) 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.

R. **SPEX** is light sensitive and shipped in light protective bottles.

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.

T. Refer to **PSC** Method Sheet ms_07963084190 for additional warnings and precautions.

STORAGE AND HANDLING REQUIREMENTS

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

B. Before use, visually inspect each reagent cassette and vial to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.

C. Store **HIV-1 v2.0 CS1, HIV-1 v2.0 CS2, HIV-1 v2.0 CS3** and **HIV-1 v2.0 CS4** at 2-8ºC. 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 v2.0 CS1, HIV-1 v2.0 CS2, HIV-1 v2.0 CS3** and **HIV-1 v2.0 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.

D. Store **HIV-1 H(+)C, v2.0, HIV-1 L(+)C, v2.0** and **CTM (–) C** at 2-8ºC. The controls are stable until the expiration date indicated. Once opened, any unused portion must be discarded.

E. Store Barcode clips [**HIV-1 H(+)C, v2.0 Clip, HIV-1 L(+)C, v2.0 Clip** and **HIV-1 (–) C Clip**] at 2-30°C.

F. 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.

G. Store **SPEX** (used in **PSC** dried plasma spot procedure) at 2-8°C. **SPEX** 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.

H. **PSC** storage and handling requirements are specified in the **PSC** Method Sheet ms_07963084190.

MATERIALS PROVIDED

A. **COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0** **HI2CAP**
(P/N: 05212294 190)

HIV-1 v2.0 CS1
(HIV-1 Magnetic Glass Particles Reagent Cassette)

HIV-1 v2.0 CS2
(HIV-1 Lysis Reagent Cassette)

HIV-1 v2.0 CS3
(HIV-1 Multi-Reagent Cassette)

HIV-1 v2.0 CS4
(HIV-1 Test-Specific Reagent Cassette)

HIV-1 H(+)C, v2.0
(HIV-1 High Positive Control, v2.0)

HIV-1 L(+)C, v2.0
(HIV-1 Low Positive Control, v2.0)

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

HIV-1 H(+)C, v2.0 Clip
(HIV-1 High Positive Control, v2.0 Barcode Clip)

HIV-1 L(+)C, v2.0 Clip
(HIV-1 Low Positive Control, v2.0 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)

C. **COBAS® AmpliPrep/COBAS® TaqMan® Specimen Pre-Extraction Reagent** **SPEX**
(P/N: 06989861190)

MATERIALS REQUIRED BUT NOT PROVIDED

Instrumentation and Software

- COBAS® AmpliPrep Instrument
- COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer
- Optional: **cobas p** 630 Instrument
- Optional: Docking Station
- AMPLILINK Software Version 3.3 or Version 3.4 Series
- Control Unit for the AMPLILINK Software, with printer
- Instrument and Software Manuals:
  - COBAS® AmpliPrep Instrument Manual for use with the AMPLILINK Software Version 3.3 and 3.4 Series
  - COBAS® TaqMan® Analyzer Instrument Manual for use with the AMPLILINK Software Version 3.3 and 3.4 Series
  - COBAS® TaqMan® 48 Analyzer Instrument Manual for use with the AMPLILINK Software Version 3.3 and 3.4 Series
  - AMPLILINK Software Version 3.3 Series Application Manual for use with COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, COBAS® Taqman® 48 Analyzer, COBAS® AMPLICOR® Analyzer and **cobas p** 630 Instrument

    or
  - AMPLILINK Software Version 3.4 Series Application Manual
  - Optional: **cobas p** 630 Instrument Operator's Manual Software Version 2.2
- Test Definition File (TDF). See product information card, provided with the kit, for name and current version of the TDF.

Disposables

- Sample processing units: SPUs
- Sample input tubes (S-tubes) with barcode clips
- Racks of K-tips
- K-tube Box of 12 x 96

OTHER MATERIALS REQUIRED (FOR EDTA PLASMA SAMPLE APPLICATION ONLY) BUT NOT PROVIDED

- Sample Rack (SK 24 rack)
- Reagent Rack
- SPU rack
- K-tube capper, motorized
- K-tube capper
- K-carrier
- K-carrier Transporter
- K-carrier rack
- 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.*

OTHER MATERIALS AND CONSUMABLES REQUIRED (FOR PSC DRIED PLASMA SPOT SAMPLE APPLICATION ONLY) BUT NOT PROVIDED

- **cobas**® Plasma Separation Card*
- Sterile or disposable forceps or tweezers**
- 140 µL capillary (e.g., Vitrex plastic tube) with compatible dispenser (e.g., Vitrex pipette holder)*
- Single Use lancing device (e.g., Greiner Bio-one: MiniCollect® Safety Lancet penetration depth 2.00 mm)*
- Sample bag (plastic transparent resealable ziplock) and silica gel desiccant sachets (for a total of 4 grams. For **PSC** storage and delivery, see **PSC** Method Sheet ms_07963084190 for more information).
- Transport bag (e.g., Wicoseal 180 x 60 x 240 mm)
- Pipette (e.g., Multistepper pipette)
- Eppendorf Thermomixer® (e.g., model R 5355 or C or equivalent) with Thermoblock for 24 cryo tubes

* See **PSC** Method Sheet ms_07963084190 for more information about the **PSC** sample collection.

** To prevent cross-contamination, use only one pair of forceps for each patient! The usage of metal forceps that are autoclaved after single use is recommended.

EDTA PLASMA SPECIMEN COLLECTION, TRANSPORT AND STORAGE

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

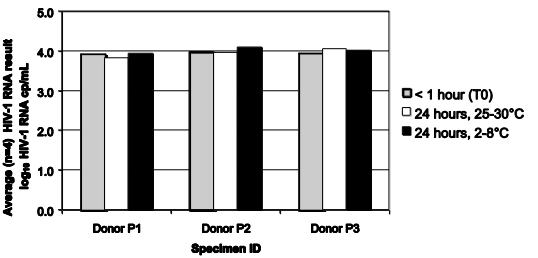
Note: This test has been validated for use with only human plasma collected in EDTA anticoagulant or from a PSC dried plasma spot. Testing of other specimen types may result in inaccurate results.

A. Specimen Collection

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 is for use with plasma specimens. Blood should be collected in sterile tubes using EDTA as the anticoagulant and mixed adequately according to the tube manufacturer's instructions.

Store whole blood at 2-25°C for no longer than 24 hours. Separate plasma from whole blood within 24 hours of collection by centrifugation at 800-1600 x g for 20 minutes at room temperature. Transfer plasma to a sterile polypropylene tube. Figure 4 shows specimen stability data from specimen stability studies performed with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test (P/N: 03543005 190).

Figure 4
HIV-1 Stability in Whole Blood (Collected in EDTA-Plasma Tubes)



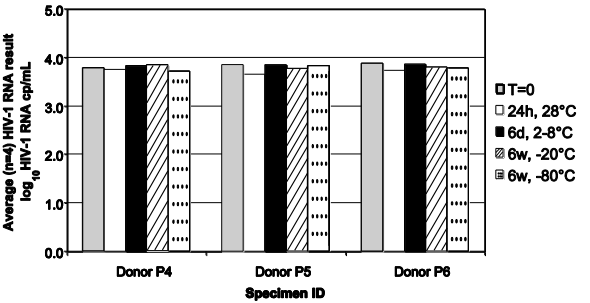
B. Specimen Transport

Transportation of whole blood or plasma must comply with country, federal, state and local regulations for the transport of etiologic agents[40]. Whole blood must be transported at 2-25°C and centrifuged within 24 hours of collection. Plasma may be transported at 2-8°C or frozen at -20°C to -80°C.

C. Specimen Storage

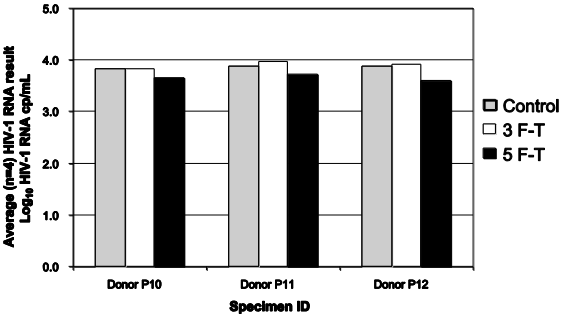
Plasma specimens may be stored at room temperature (25-30°C) for up to 1 day or at 2-8°C for up to 6 days. Plasma specimens were shown to be stable for six weeks if frozen at -20°C to -80°C. It is recommended that specimens be stored in 1100-1200 µL aliquots in sterile, 2.0 mL polypropylene screw-cap tubes (such as Sarstedt 72.694.006). Figure 5 shows the specimen stability data from specimen storage studies performed with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test (P/N: 03543005 190).

Figure 5
HIV-1 Stability in EDTA Plasma



Plasma specimens may be frozen and thawed up to five times without a significant loss of HIV-1 RNA. Figure 6 shows the data from a freeze-thaw study performed with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test (P/N: 03543005 190).

Figure 6
HIV-1 Results After Up to Five Freeze-Thaw (F-T) Cycles (EDTA-Plasma)



PSC DRIED PLASMA SPOT SAMPLE COLLECTION, TRANSPORT AND STORAGE

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

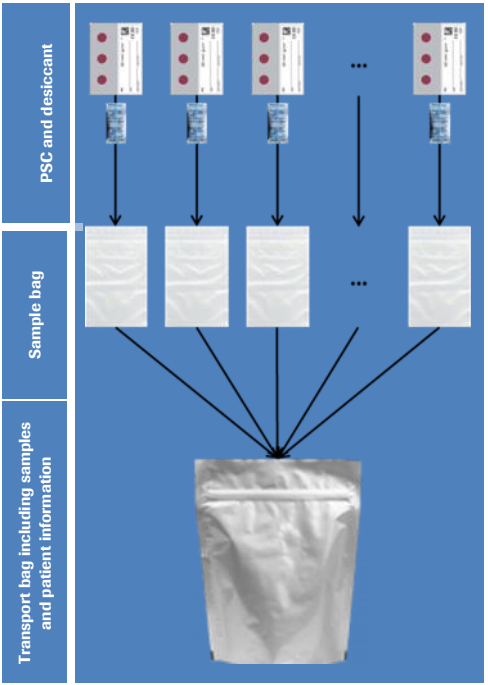
Note: This test has been validated for use with only human plasma collected in EDTA anticoagulant or from a PSC dried plasma spot. Testing of other specimen types may result in inaccurate results.

A. Sample Collection

PSC dried plasma spot samples are collected by appropriate clinical procedures. The expiry date of the **PSC** must be checked before use. Proceed only if the **PSC** has not expired yet and if the bag in which the **PSC** is sealed is completely closed and intact. Label the **PSC** with patient's name, date of birth, date and time of sample collection. Apply 140 µL of whole blood on each circle of the **PSC** membrane delineated by the spotting layer using an appropriate capillary and a dispenser. It is recommended to fill all three spots on the **PSC**, in order to allow retesting. Do not apply samples from more than one patient on the same **PSC**. Ensure that BOTH sides of the **PSC** spots (front: membrane with blood; back: spot with plasma) are saturated after 5 minutes. Check the back side through the transparent back layer. Do not allow the membranes to get in contact with gloves, tools or any potentially contaminated surfaces during this process.

Allow the **PSC** to dry at room temperature for at least 4 hours (to maximum overnight), protecting it from direct sunlight. Do not remove the spotting layer. This is done at the laboratory. After drying, store the **PSC** in an individual sample bag with 4 grams of desiccant and seal the bag (Figure 7). The collected sample bags must be packed in a transport bag together with their relative Patient Information Sheet. It is recommended to pack a maximum number of 25 **PSCs** per transport bag.

Figure 7
Overview of the Packaging Concept for PSC Transport



B. Sample Transport

If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents. Transport bags containing **PSCs** must be transported within 28 days at 18-45°C and up to 85% humidity.

C. Sample Storage

PSCs in individual sample bags with 4 grams of desiccant, within a transport bag may be stored after transportation at room temperature (18-30°C), at 2-8°C or at ≤-10°C for up to 56 days (with and without layer separation).

INSTRUCTIONS FOR USE

Note: For detailed operating instructions, a detailed description of the possible configurations, printing results and interpreting flags, comments and error messages, refer to: (1) the COBAS® AmpliPrep Instrument Manual for use with the AMPLILINK Software Version 3.3 and 3.4 Series; (2) the COBAS® TaqMan® Analyzer Instrument Manual for use with the AMPLILINK Software Version 3.3 and 3.4 Series; (3) the COBAS® TaqMan® 48 Analyzer Instrument Manual for use with the AMPLILINK Software Version 3.3 and 3.4 Series; (4) the AMPLILINK Software Version 3.3 Series Application Manual for use with COBAS® AmpliPrep Instrument,

COBAS® TaqMan® Analyzer, COBAS® Taqman® 48 Analyzer, COBAS® AMPLICOR® Analyzer and cobas p 630 Instrument or the AMPLILINK Software Version 3.4 Series Application Manual; (5) Optional: cobas p 630 Instrument Operator's Manual Software Version 2.2.

Information contained in Part A–C and F–J is general for both sample types (EDTA plasma and PSC dried plasma spot samples). Part D contains information about ordering and loading of specimens for EDTA plasma samples only. Part E contains information about ordering and loading of specimens for PSC dried plasma spot samples only.

Batch Size

Each kit contains reagents sufficient for 48 tests, which may be performed in batches of 12 to 24 tests. At least one of each control **[CTM (–) C**, **HIV-1 L(+)C, v2.0** and **HIV-1 H(+)C, v2.0]** 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.

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 Control Unit for the AMPLILINK software **ON**. Prepare the Control Unit as follows:

1. Log onto the Microsoft Windows Operating System.
2. Double click the AMPLILINK software icon.
3. 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. 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 as condensation may form on the barcode labels. Do not wipe off condensation if it appears on the barcode labels.

B1. Place **HIV-1 v2.0 CS1** onto a reagent rack. Place **HIV-1 v2.0 CS2, HIV-1 v2.0 CS3** and **HIV-1 v2.0 CS4** onto a separate reagent rack.

B2. Load the reagent rack containing **HIV-1 v2.0 CS1** onto rack position **A** of the COBAS® AmpliPrep Instrument.

B3. Load the reagent rack containing **HIV-1 v2.0 CS2, HIV-1 v2.0 CS3** and **HIV-1 v2.0 CS4** onto rack position **B, C, D** or **E** of the COBAS® AmpliPrep Instrument. (See 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 workflows 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 workflow 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 (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.

C2. Depending on the workflow used, load full K-tube rack(s) onto rack position **M, N, O** or **P** of the COBAS® AmpliPrep Instrument.

C3. Load full K-tip rack(s) onto rack position **M, N, O** or **P** of the COBAS® AmpliPrep Instrument.

C4. For workflow 3 using the COBAS® TaqMan® 48 Analyzer, load K-carriers on K-carrier rack(s) onto rack position **M** & **N,** or **O** & **P** of the COBAS® AmpliPrep Instrument.

Table 1
Possible Workflows for Using COBAS® AmpliPrep Instrument with COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer

Workflow		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-tip 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-tip 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
			After specimen processing is finished: K-tubes on sample racks (ready for manual transfer)	Same as above (F – H)
3	COBAS® AmpliPrep Instrument plus 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-tip 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
			Empty barcoded K-carrier on K-carrier rack	M - P
			After specimen processing is finished: K-tubes in K-carrier on K-carrier rack	Same as above (M – P)

Part D. Ordering and Loading of Specimens: EDTA Plasma Samples

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, v2.0** and **HIV-1 H(+)C, v2.0**] to each sample rack position where the controls (S-tube) are to be placed. The barcode label clips for controls should have the same control lot number as the lot number on the control vials in the kit. Take care in assigning the right 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, select the test definition file (HI2CAP96 for COBAS® AmpliPrep Instrument plus COBAS® TaqMan® Analyzer, HI2CAP48 for COBAS® AmpliPrep Instrument plus COBAS® TaqMan® 48 Analyzer), create specimen orders for each specimen and control in the **Orders** window **Sample** folder 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 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, v2.0** and **HIV-1 H(+)C, v2.0**] 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, v2.0** and **HIV-1 H(+)C, v2.0**] 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 should have the same control lot number as the lot number on the control vials in the kit. Assign the right control to the position with the appropriate control barcode clip. ***Avoid contaminating the upper part of the S-tubes with specimens or controls.*** If using the **cobas p** 630 Instrument for preparation of specimens, refer to the **cobas p** 630 Instrument Operator's Manual.

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

D8. For workflow 3 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 right position adjacent to Input S-tubes) onto rack position **F, G** or **H** of the COBAS® AmpliPrep Instrument.

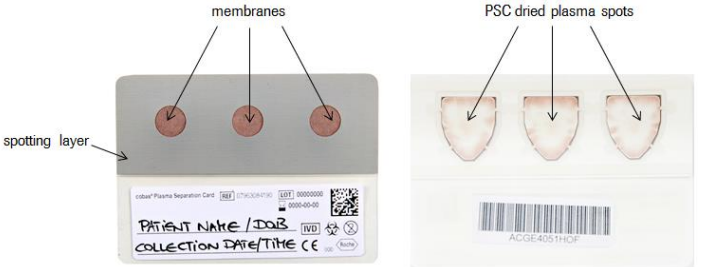
Part E. Ordering and Loading of Specimens: PSC Dried Plasma Spot Samples

E1. Check the integrity of the transport bag before opening. Proceed only if the transport bag is completely sealed.

Open the transport bag and, for each sample bag, proceed only if:

- The laboratory request form is completely filled.
- The barcode of the laboratory request form and the **PSC** match.
- The sample bag is completely closed and each of them contains a **PSC** with 4 grams of desiccant.
- The sample collection date is available, and the sample collection occurred in the past 28 days, and before the expiration date of the **PSC**.
- The **PSC** is not expired.
- The **PSC** dried plasma spot looks homogeneous on the front side and looks completely covered with plasma when observed from the back side (see Figure 8).

Figure 8
PSC Dried Plasma Spots to Be Processed (Left: Front Side; Right: Back Side)

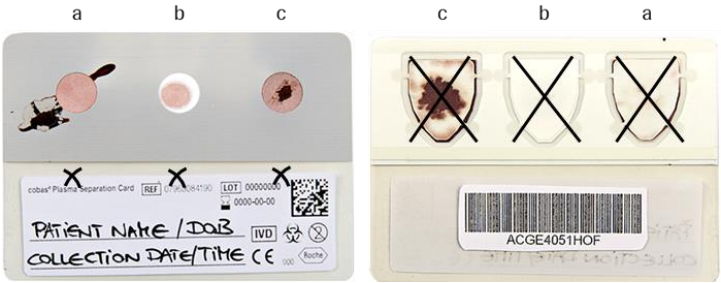


E2. **PSC** dried plasma spots should be marked and rejected if:

- blood spills are visible (Figure 9a) and/or the membrane is not completely covered with blood (Figure 9b) and therefore the **PSC** spot contains an inhomogeneous front area and/or a back side not completely covered with plasma (visible through the carrier).
- the membrane is damaged (Figure 9c) and therefore the **PSC** spot contains an inhomogeneous front area and a dark red-brownish back side (visible through the carrier).

Note: The three spots are meant to allow for retesting. A PSC could contain a bad spot, but still provides a good sample for testing. Mark properly the bad spots, in order to be recognizable. Avoid marking them on the spotting layer. Always compare the three spots between each other to evaluate their quality.

Figure 9
Rejection Criteria for PSCs (Left: Front Side; Right: Back Side).
Spots with blood spills (a) or not covered membrane (b), or visibly damaged membranes (c) should not be processed. Spots should be clearly marked when rejected.



E3. 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, v2.0** and **HIV-1 H(+)C, v2.0**] to each sample rack position where the controls (S-tube) are to be placed. The barcode label clips for controls should have the same control lot number as the lot number on the control vials in the kit. Take care in assigning the right control to the position with the appropriate control barcode clip. Place one Input S-tube into each position containing a barcode label clip.

E4. Using the AMPLILINK software, select the appropriate test definition file (HI2PSC96 for COBAS® AmpliPrep Instrument plus COBAS® TaqMan® Analyzer, HI2PSC48 for COBAS® AmpliPrep Instrument plus COBAS® TaqMan® 48 Analyzer) and create specimen orders for each specimen and control in the **Orders** window **Sample** folder and complete by saving.

E5. Assign specimen and control orders to sample rack positions in the **Orders** window **Sample Rack** folder. The sample rack number must be for the rack prepared in Step E1. The patient barcode on the back side of the **PSC** might be scanned through the sample bag (Figure 10). Do not open the sample bag at this point and complete by saving.

Figure 10
Scanning of the Sample Barcode to Order PSC Dried Plasma Spot Samples

E6. Print the **Sample Rack Order** report to use as a worksheet and verify again the use of the appropriate test file.

E7. Perform the steps E7 to E11 under a safety hood. For the **PSC** selected, open the sample bag, and remove the spotting layer (Figure 11). Slightly bend the **PSC** and remove one dried plasma spot with sterile forceps or tweezers by pulling it up. Bend the removed dried plasma spot on the **PSC** to facilitate tube insertion (Figure 12).

Figure 11
Removal of the Spotting Layer

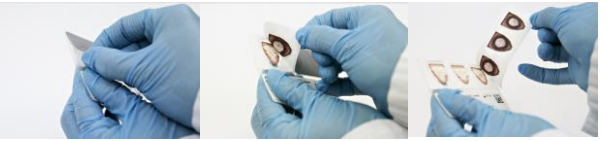


Figure 12
Removal of the PSC Dried Plasma Spot and Bending

Note: Use one pair of forceps or tweezers per patient.

E8. Transfer one pre-bent **PSC** dried plasma spot into the corresponding Input S-tube so that the lowest tip of the **PSC** dried plasma spot reaches the bottom of the tube and is attached to the tube wall to prevent pipetting errors (Figure 13 and Figure 14).

Note: Dried plasma spots may become brittle upon storage. See storage requirements for PSC. Handle them carefully while inserting into the tube.

Figure 13
Transfer of the PSC Dried Plasma Spot into the Tube

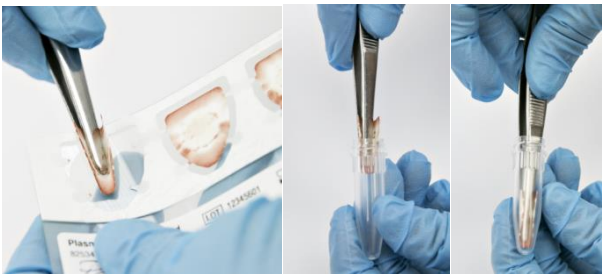
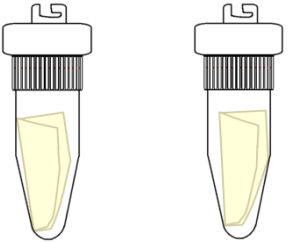


Figure 14
PSC Dried Plasma Spot Best Positioning in S-Tube



Valid Potential risk of clot

Note: Ensure that the Input S-tube position number on the Sample Rack Order Report is equal to the SK24 rack position.

E9. Place **PSC** with remaining dried plasma spots back to its original Sample Bag containing 4 grams of fresh desiccant for retesting, if required (Figure 15). **PSCs** can be stored for a period of 56 days after transport with and without spotting layer separation (at 18-30°C, or at 2-8°C, or at ≤-10°C).

Figure 15
PSC in Sample Bag for Potential Retesting

E10. Close the Input S-tube and transfer the tube containing the **PSC** dried plasma spot on the SK24 rack position 4-24 (for further details refer to Table 2). Repeat until all samples are processed.

Note: Allow SPEX to equilibrate to ambient temperature before use.

E11. Open the S-Input tubes individually, save the cap under clean conditions and add 1100 µL of **SPEX** to each S-Input tube (Figure 16). Recap the S-Input tube. Repeat until all samples are processed.

Figure 16
1100 µl SPEX Added to Each PSC Dried Plasma Spot Sample

Note: Avoid contaminating the upper part of the S-Input tubes with samples and make sure that the tubes are properly capped to prevent evaporation.

E12. Transfer the S-Input tubes to the Eppendorf Thermomixer and incubate the prepared S-Input tubes at 56°C and 1000 rpm continuous shaking for 10 minutes to extract the virus from the dried plasma (Figure 17).

Figure 17
Incubation is Performed for 10 Minutes.

Note: Start the incubation right after the addition of SPEX.

E13. During the incubation time prepare the controls as follows.

Note: Do not incubate the controls.

Place one S-Input tube into each position (1 to 3) of the prepared SK24 rack containing a control barcode label clip.

Mix 1 vial of the **CTM (–) C** thoroughly for 20 seconds by vortexing. Transfer 1000 µL of **CTM (–) C** to the S-Input tube 1 of the SK24 rack. Immediately, recap this S-Input tube.

Mix 1 vial of the **HIV-1 L(+)C, v2.0** thoroughly for 20 seconds by vortexing. Transfer 1000 µL of **HIV-1 L(+)C, v2.0** to the S-Input tube 2 of the SK24 rack. Immediately, recap this S-Input tube.

Mix 1 vial of the **HIV-1 H(+)C, v2.0** thoroughly for 20 seconds by vortexing. Transfer 1000 µL of **HIV-1 H(+)C, v2.0** to the S-Input tube 3 of the SK24 rack. Immediately, recap this S-Input tube.

Table 2
Ordering and Loading for PSC Dried Plasma Spot Samples

Sample	AMPLILINK Ordering Position	Eppendorf IsoRack Position	SK24 Rack Position
	S-Input tube	S-Input tube	S-Input tube
CTM(-)C	1	Empty	1
HIV-1 L(+)C, v2.0	2	Empty	2
HIV-1 H(+)C, v2.0	3	Empty	3
PSC dried plasma spot + 1100 µL SPEX	4-24	4-24	4-24

E14. After the incubation transfer the samples in the correct order to the SK24 rack containing the controls (Step E13) (for further details refer to Table 2).

Note: Check that the position of the PSC dried plasma spot in the tube is still correct (Figure 14) and adjust with a sterile pipette tip if necessary. In case of bubbles remove them with a sterile pipette tip.

E15. Load the sample rack(s) filled with S-Input tubes onto rack positions **F**, **G** or **H** of the COBAS® AmpliPrep Instrument.

Part F. Start of COBAS® AmpliPrep Instrument Run

F1. Start the COBAS® AmpliPrep Instrument using the AMPLILINK software.

Part G. End of COBAS® AmpliPrep Instrument Run and Transfer to COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer (for Workflow 2 and 3 in Table 1 Only)

G1. Check for flags or error messages.

G2. 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 workflow (for further details see Part G).

G3. Remove waste from the 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.

Note: Do not freeze or store processed specimens and controls at 2-8°C.

Part H. Loading Processed Specimens

H1. Depending on the workflow, perform the appropriate steps to transfer the K-tubes to the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer:

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

Workflow 2: Manual transfer of K-tubes in sample rack(s) to COBAS® TaqMan® Analyzer.

Workflow 3: 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 I. Start of COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Run

I1. Start the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer by one of the options below depending on the workflow used:

Workflow 1: No intervention necessary.

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

Workflow 3: 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 J. End of COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Run

J1. At the completion of the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run, print Results Report. Check to ensure that the appropriate test definition file was used and look for flags or error messages in the Result report. Specimens with flags and comments are interpreted as described in the Results section. After acceptance, store data in archive.

J2. Remove used K-tubes from the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.

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 cp/mL or IU/mL, depending on the used TDF. The conversion factor between HIV-1 RNA cp/mL and HIV-1 IU/mL is 0.6 cp/IU, using WHO 1st International Standard for HIV-1 RNA for Nucleic Acid-Based Techniques (NAT) (NIBSC 97/656)[37]. This conversion factor was determined using the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5, the COBAS® AmpliPrep/COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 and the COBAS® TaqMan® HIV-1 Test For Use With The High Pure System.**

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, v2.0** and **HIV-1 H(+)C, v2.0** fall within the assigned ranges.

Batch Validation – AMPLILINK Version 3.3 and Version 3.4 Series

Check AMPLILINK software results window or printout for flags and comments to ensure that the batch is valid. For control orders, a check is made to determine if the cp/mL or IU/mL value for the control is within its specified range. If the cp/mL or IU/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 L(+)C, v2.0; HIV-1 H(+)C, v2.0** and **CTM (–) C**].

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

Negative Control

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

HIV-1 Low Positive Control, v2.0

Flag	Result	Interpretation
LPCINVALID	Invalid	An invalid result or a control out of range

HIV-1 High Positive Control, v2.0

Flag	Result	Interpretation
HPCINVALID	Invalid	An invalid result or a control out of range

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".
Copies/mL	< 2.00E+01 cp/mL (Plasma) < 738 cp/ml (**PSC**)	Calculated cp/mL are below the Limit of Detection of the assay. Report results as "HIV-1 RNA detected, less than 20 HIV-1 RNA cp/mL " for Plasma and "HIV-1 RNA detected, less than 738 HIV-1 RNA cp/mL " for **PSC**.
	≥ 2.00E+01 cp/mL and ≤ 1.00E+07 cp/mL (Plasma) ≥ 738 cp/mL and ≤ 1.00E+07 cp/mL (**PSC**)	Calculated results greater than or equal to 20 cp/mL and less than or equal to 1.00E+07 cp/mL are within the Linear Range of the assay used with Plasma. Calculated results greater than or equal to 738 cp/mL and less than or equal to 1.00E+07 cp/mL are within the Linear Range of the assay used with the **PSC**.
	> 1.00E+07 cp/mL (Plasma and **PSC**)	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 1:100 with HIV-1-negative human EDTA-plasma and the test repeated. Multiply the reported result by the dilution factor.
International Units/mL	< 3.34E+01 IU/mL (Plasma)	Calculated IU/mL are below the Limit of Detection of the assay. Report results as "HIV-1 RNA detected, less than 33.4 HIV-1 RNA IU/mL " for Plasma.
	≥ 3.34E+01 IU/mL and ≤ 1.67E+07 IU/mL (Plasma)	Calculated results greater than or equal to 33.4 IU/mL and less than or equal to 1.67E+07 IU/mL are within the Linear Range of the assay used with Plasma.
	> 1.67E+07 IU/mL (Plasma)	Calculated IU/mL are above the range of the assay. Report results as "greater than 1.67E+07 HIV-1 RNA IU/mL". If quantitative results are desired, the original specimen should be diluted 1:100 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 that produce an invalid result with a flag "QS_INVALID" should not be reported as > 1.00E +07 cp/mL or 1.67E +07 IU/mL. The original specimen should be diluted 1:100 with HIV-1-negative human EDTA-plasma and the test repeated. Multiply the reported result by the dilution factor.

Note: Titer Result "Failed". Interpretation: Specimen is not correctly processed during specimen preparation on the COBAS® AmpliPrep Instrument.

Note: Titer Result "Invalid". Interpretation: An Invalid Result.

QUALITY CONTROL

One **CTM (–) C**, one **HIV-1 L(+)C, v2.0** and one **HIV-1 H(+)C, v2.0** must be included in each test batch. The batch is valid if no flags appear for any of the controls [**HIV-1 L(+)C, v2.0, HIV-1 H(+)C, v2.0** and **CTM (–) C**].

Check the batch printout for flags and comments to ensure that the batch is valid.

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 titer range for **HIV-1 L(+)C, v2.0** and **HIV-1 H(+)C, v2.0** is specific for each lot of reagents, and is provided on the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 reagent cassette barcodes.

The HIV-1 RNA cp/mL for **HIV-1 L(+)C, v2.0** and **HIV-1 H(+)C, v2.0** should fall within their assigned titer 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 ranges in multiple batches, contact your local Roche office for technical assistance.

PROCEDURAL PRECAUTIONS

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, v2.0 has neither been evaluated with specimens containing HIV-1 group 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 Test, v2.0 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.
7. Though rare, mutations within the highly conserved regions of the viral genome covered by the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 primers and/or probes may result in the under-quantitation of or failure to detect the virus.
8. Detection of HIV-1 RNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods and patient factors, (i.e., age, presence of symptoms, and/or stage of the infection). While the clinical specificity of the test is 99.3% (95% CI = 98.2% to 99.8%), some low level false positive results in HIV-negative individuals have been noted.
9. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to quantify technology differences.

INTERFERING SUBSTANCES

Elevated levels of triglycerides (up to 3500 mg/dL), bilirubin (up to 28 mg/dL), albumin (up to 8900 mg/dL), hemoglobin (up to 900 mg/dL) and human DNA (up to 0.4 mg/dL) in specimens as well as the presence of autoimmune diseases or respective markers such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA) and Antinuclear Antibody (ANA) were shown not to interfere with the quantitation of HIV-1 RNA or impact the specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0. The evaluation was performed according to CLSI Guideline EP7-A2 using one lot of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 reagents.

The following drug compounds tested at 3 times the Peak Plasma Level (Cmax) 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, v2.0:

HIV drugs:	
Protease Inhibitors Atazanavir Darunavir Fosamprenavir Lopinavir/Ritonavir Nelfinavir mesylate Ritonavir Saquinavir Tipranavir	**Nucleoside Analogue, Inhibitor of Reverse Transcriptase** Abacavir sulfate Didanosine, ddI Emtricitabine Lamivudine, 3TC Stavudine, 4dT Tenofovir DF Zidovudine
Integrase Inhibitor Raltegravir	**Non-nucleoside, Inhibitor of Reverse Transcriptase** Efavirenz Nevirapine
Entry Inhibitor Maraviroc	**Fusion Inhibitors** Enfuvirtide
HBV and / or HCV Drugs:	
Nucleotide analogue Adefovir dipivoxil	**Nucleoside analogue** Entecavir Telbivudine
Immune Modulator Peginterferon α-2a Peginterferon α-2b Ribavirin	
Compounds for Treatment of Herpes Viruses:	
Nucleotide analogue Acyclovir	**Nucleotide derivative** Ganciclovir Valganciclovir HCl

NON-CLINICAL PERFORMANCE EVALUATION

Key Performance Characteristics for EDTA Plasma Samples

A. Limit of Detection

The limit of detection of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 was determined by testing the 2nd International HIV-1 RNA WHO Standard, NIBSC Code 97/650[42], HIV-1 subtype B, diluted in HIV-1-negative human EDTA plasma. The limit of detection was determined for three reagent lots. Three dilution series were analyzed for each reagent lot. A total of approximately 126 replicates per concentration level were tested. The evaluation was performed according to CLSI Guideline EP17-A.

The concentration of HIV-1 RNA that can be detected with a positivity rate of greater than 95% as determined by PROBIT Analysis, is 20 cp/mL or 33 IU/mL. The results for the individual lots were 17.7 cp/mL (95% confidence interval: 13.7 – 26.9 cp/mL) for lot 1, 17.0 cp/mL (95% confidence interval: 14.0 – 22.6 cp/mL) for lot 2 and 14.2 cp/mL (95% confidence interval: 11.2 – 22.1 cp/mL) for lot 3. The combined results for all three reagent lots are shown in Table 3. **The conversion factor between IU/mL and cp/mL was determined using the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5, the COBAS® AmpliPrep/COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 and the COBAS® TaqMan® HIV-1 Test For Use With The High Pure System.**

Table 3
Limit of Detection of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

Nominal Input (HIV-1 RNA IU/mL)	Nominal Input (HIV-1 RNA cp/mL)	No. Replicates	No. Positives	Positivity Rate
100	60	126	126	100%
67	40	186	185	99%
50	30	126	125	99%
33	**20**	**126**	**124**	**98%**
25	15	59	53	90%
17	10	126	108	86%
8	5	125	66	53%
0	0	126	0	0%
PROBIT 95% Hit Rate		**27.5 IU/mL** (95% confidence interval: 23.8 – 33.0 IU/mL) **16.5 cp/mL** (95% confidence interval: 14.3 – 19.8 cp/mL)		

In addition, dilutions of cell culture supernatants representing HIV-1 group M subtypes A-H in HIV-1-negative human EDTA plasma were analyzed with two reagent lots. For each HIV-1 subtype isolate several concentration levels (nominal titers between 10 and 75 cp/mL) were tested in 24 replicates per reagent lot. The assignment of nominal concentrations to the cell culture stock materials was performed by averaging the titers of the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5, the VERSANT® HIV-1 RNA 3.0 Assay (bDNA) titer and the Abbott RealTi*m*e HIV-1 assay. Hit rate analysis shows a positivity rate of greater than 95% for all subtypes at 20 cp/mL or lower. The combined results for the two reagent lots are shown in Table 4.

Table 4
Limit of Detection Verification for the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

Subtype	Isolate Designation	Lowest Concentration Level ≥ 95% Hit Rate (cp/mL)
A	92UG029	10
A	4237A/98	20
B	92TH026	20
B	8E5/LAV	20
C	92BR025	20
C	3777A/97	11
D	92UG021	20
D	92UG035	11
CRF01_AE	92TH022	12
CRF01_AE	92TH009	14
F	93BR020	20
G	ARP173/RU570	13
H	HIV V1557	16

B. Precision

The Precision of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 was determined by analysis of serial dilutions of a HIV-1 cell culture supernatant specimen (HIV-1 subtype B) in HIV-1-negative human EDTA plasma. The titer assignment of the cell culture supernatant (stock concentration) was performed by a method that ensures traceability to the 1st International HIV-1 RNA WHO Standard, NIBSC Code 97/656[36]. Three reagent lots were analyzed and 15 runs per reagent lot were performed, each consisting of 6 dilution levels and 3 replicates at each level. Each specimen was taken through the entire COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 procedure, including specimen preparation, amplification and detection. Therefore, the precision reported here represents all aspects of the test procedure. The results for each reagent lot and for the three reagent lots combined are shown in Table 5.

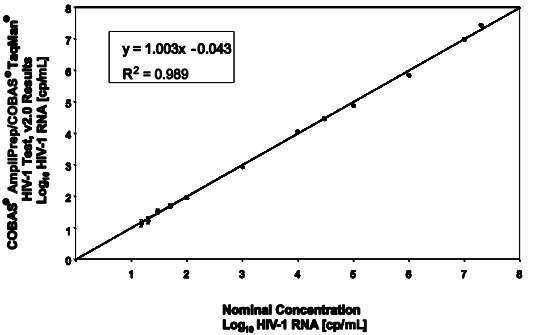
Table 5
Precision of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

Titer (cp/mL)	Lot 1	Lot 2	Lot 3	All three lots combined	
	Total SD in log	Total SD in log	Total SD in log	Total SD in log	Total Lognormal CV (%)
1.0E+02	0.19	0.16	0.17	0.17	41
1.0E+03	0.07	0.09	0.07	0.08	20
1.0E+04	0.07	0.07	0.06	0.07	16
1.0E+05	0.04	0.05	0.07	0.06	15
1.0E+06	0.10	0.09	0.10	0.10	25
1.0E+07	0.11	0.12	0.14	0.13	33

C. Linear Range

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 was found to give a linear response from 20 (log_{10} = 1.30) HIV-1 RNA cp/mL to 1.0E+07 (log_{10} = 7.00) HIV-1 RNA cp/mL. The evaluation was performed according to CLSI Guideline EP6-A using two lots of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 reagents and serial dilutions of a high titer HIV-1 RNA (+) cell culture supernatant specimen. Two reagent lots were analyzed and 15 runs per reagent lot were performed, each consisting of 12 dilution levels and 3 replicates at each level. The results for one reagent lot are shown in Figure 18.

Figure 18
Linearity for the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0



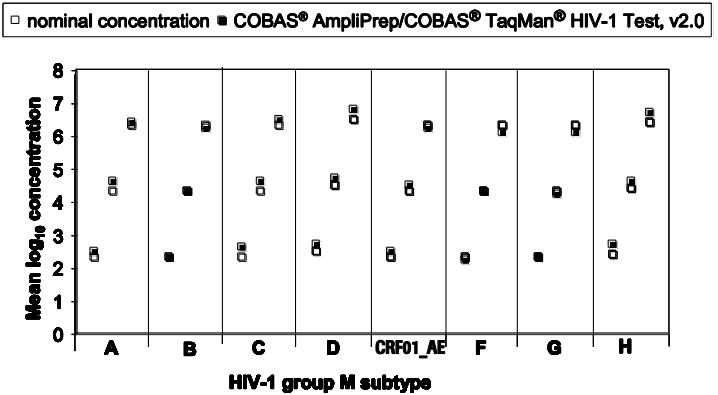
D. Inclusivity of HIV-1 Group M

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[41].

The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 on HIV-1 subtypes was evaluated by analysis of cell culture stock material of representatives for each HIV-1 group M subtype A through H. The assignment of nominal concentrations to the cell culture stock materials was performed by averaging the titers of the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5, the VERSANT® HIV-1 RNA 3.0 Assay (bDNA) titer and the Abbott RealT*ime* HIV-1 assay. Each cell culture stock material was diluted to nominal concentrations of approximately 2.00E+02, 2.00E+04 and 2.00E+06 cp/mL in EDTA plasma. The concentrations were then tested in 10 replicates by the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 using one reagent lot. The mean log_{10} titers of all concentrations and subtypes were compared to the respective log_{10} nominal titers.

The evaluation of the 8 HIV-1 subtype isolates by the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 demonstrates equivalent results for all tested representatives of the HIV-1 group M subtypes (see Figure 19). Mean log_{10} concentration results for all subtypes were within ± 0.3 log_{10} of the assigned input concentration.

Figure 19
COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

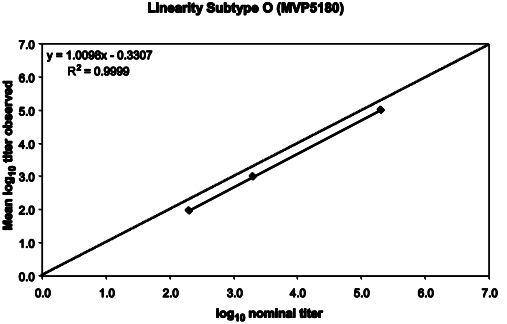


E. HIV-1 Group O Detection

Dilutions of a HIV-1 group O cell culture supernatant (isolate MVP5180) in human EDTA plasma were analyzed with two reagent lots. Several concentration levels (nominal titers between 10 and 75 cp/mL) were tested in 24 replicates per reagent lot. Assignment of the nominal concentration to the cell culture stock material was performed by the Abbott RealTi*m*e HIV-1 assay. Hit rate analysis shows a positivity rate of greater than 95% at 20 cp/mL.

The HIV-1 group O cell culture stock material was diluted to nominal concentrations of approximately 2.00E+02, 2.00E+03 and 2.00E+05 cp/mL in EDTA plasma. The concentrations were then tested in 10 replicates by the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 using one reagent lot. The mean $\log_{10}$ titers of all concentrations were linear and within ± 0.3 $\log_{10}$ of the respective $\log_{10}$ nominal titer (see Figure 20).

Figure 20
COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0



In addition, 10 cell culture materials and one diluted patient specimen (11613) representing HIV-1 group O were tested in parallel in the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 and in the Abbott RealTi*m*e HIV-1 assay. All 11 specimens were found positive with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 (see Table 6). Both tests returned a mean $\log_{10}$ titer for the 11 specimens within 0.1 $\log_{10}$.

Table 6
Recognition of HIV-1 Group O Isolates by the COBAS® AmpliPrep/ COBAS® TaqMan® HIV-1 Test, v2.0

Isolate Designation	$\log_{10}$ titer COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0	$\log_{10}$ titer Abbott RealTi*m*e HIV-1 assay
BBI PRD 301, BV-5050	3.09	2.42
BBI PRD 301, BV-5051	2.86	3.35
BBI PRD 301, BV-5003	3.00	2.71
BBI PRD 301, BV-5024	2.87	2.69
MVP5180	2.78	3.25
HIV-1 CA-9	3.31	3.08
BCF01	5.71	5.61
BCF02	5.16	5.39
BCF07	4.27	4.81
BCF011	5.57	5.26
11613	2.97	2.05
Mean $\log_{10}$ titer	**3.78**	**3.69**

F. Specificity

The specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 was determined with two reagent lots by analysis of HIV-1-negative EDTA plasma specimens from blood donors. A total of 660 individual EDTA plasma specimens showed valid results and all were negative for HIV-1 RNA in the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0. Based on these results, the specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 is 100% (one-sided lower 95% confidence limit: ≥ 99.6%).

G. Analytical Specificity

The analytical specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 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 EDTA plasma at 1.5E+02 cp/mL HIV-1 (see Table 7).

None of the organisms tested showed cross reaction with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0. HIV-1-positive specimens returned titer results that were within ± 0.5 $\log_{10}$ from a HIV-1-positive control.

Table 7
Analytical Specificity Specimens

Virus	Bacteria
Adenovirus type 5	*Staphylococcus aureus*
Cytomegalovirus	*Propionibacterium acnes*
Epstein-Barr virus	
Human Herpes Virus type 6	
Herpes simplex virus type 1	
Herpes simplex virus type 2	
Human T-Cell Lymphotropic virus type 1	**Yeast**
Human T-Cell Lymphotropic virus type 2	*Candida albicans*
Influenza A	
Hepatitis A virus	
Hepatitis B virus	
Hepatitis C virus	

H. Method Correlation

The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 was compared to the COBAS® AmpliPrep/COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5, to the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test and to the Abbott RealTime HIV-1 assay by analysis of 92 prospectively collected, undiluted HIV-1 positive clinical specimens and by analysis of 34 diluted cell culture supernatants. The specimens comprised HIV-1 group M subtypes A to H as well as circulating recombinant forms of the virus and were analyzed at two external sites. A total of 126 samples spread over the dynamic range of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 were tested with the four tests. Only valid titer pairs within the linear ranges of both assays compared were considered for Deming regression analysis (see Figure 21 to Figure 23).

Figure 21
Correlation of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

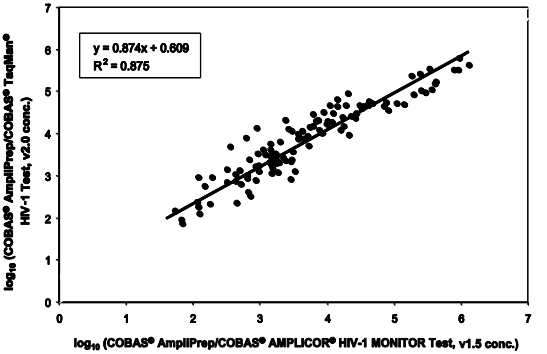


Figure 22
Correlation of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

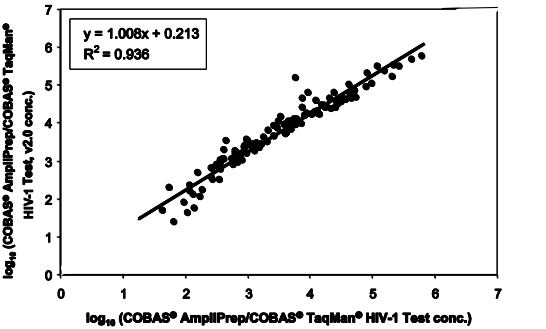
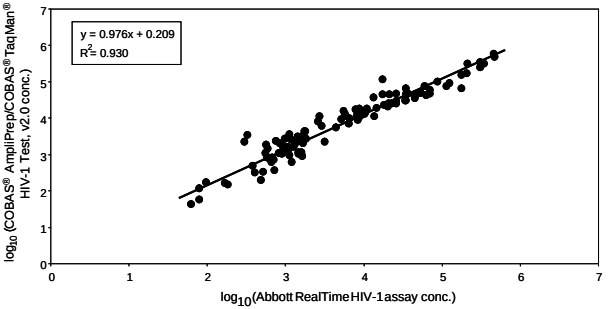


Figure 23
Correlation of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0



I. Whole System Failure

The whole system failure rate for two kit lots of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2 was determined by testing 113 replicates for lot 1 and 114 replicates for lot 2 of EDTA plasma spiked with HIV-1 group M subtype B. These samples were tested at a target concentration of approximately 3 x LoD. The combined results (lot 1 and 2) of this study determined that one replicate returned a negative result for the HIV-1 target, resulting in a whole system failure rate of 0.4%.

Key Performance Characteristics for PSC Dried Plasma Spot Samples

A. Plasma Limit of Detection Using the Plasma Separation Card

The plasma limit of detection of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 in combination with the **PSC** was determined by analysis of plasma titers assigned to serial dilutions of HIV-1 group M cell culture supernatant, in HIV-negative human whole blood. Panels of five concentration levels plus a negative were tested over three lots of **PSCs** and three lots of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test reagents, multiple runs, days, operators, and instruments. A part of the highest panel member was centrifuged and the plasma was titer assigned by Calibrator Bracketing Method (CBM) using **cobas**® HIV-1 (on **cobas**® 6800/8800 Systems) with the 3rd HIV-1 WHO International Standard, HIV-1 group M, subtype B for preparation of the high and low calibrator.

The results for **PSC** are shown in Table 8. The study demonstrates that the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 in combination with the **PSC** detected HIV-1 RNA at a concentration of 737.9 cp/mL as determined by Probit with a hit rate of 95%.

Table 8
Limit of Detection of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

HIV-1 group M assigned plasma concentration (cp/mL)	Number of Reactives	Number of Valid Replicates	% Reactive
1691.1	61	61	100%
1098.1	51	51	100%
2276.4	61	62	98.39%
845.6	59	60	98.33%
549.1	46	49	93.88%
1138.2	59	60	98.33%
563.7	54	60	90.00%
366	49	53	92.45%
758.8	56	61	91.80%
281.9	46	62	74.19%
183	36	49	73.47%
379.4	50	62	80.65%
140.9	38	63	60.32%
91.5	22	54	40.74%
189.7	37	63	58.73%
0	0	63	0.00%
0	0	54	0.00%
0	0	59	0.00%
PROBIT 95% Hit Rate		737.9 cp/mL (95% confidence interval: 614.3 - 938.5 cp/mL) 1230 IU//mL (1023.8 – 1564.2 IU/mL)	

Estimation of LOD in Whole Blood:

The corresponding titers in whole blood of the same sample cannot be exactly determined, since whole blood is not a sample type for viral load testing. Whole blood titers based on the amount of HIV-1 RNA spiked into the whole blood samples were not used for LoD estimation because the amount of spiked RNA does not necessarily correspond to the amount of RNA in plasma. Even after centrifugation, RNA can remain in the buffy coat or associated with the cellular fraction of whole blood. However, since other technologies like dried blood spots have used spiked whole blood titers to estimate their limit of detection in whole blood, an estimate for the **PSC** whole blood LoD can be provided based on an empirical factor which is assumed to be related to the average hematocrit content (45%) of the samples (Table 9).

Whole blood LOD values are estimates based on the relation:

Whole blood LOD estimate = **PSC** plasma LOD / 1.8

Table 9
Whole Blood LoD Estimate

LoD by PROBIT analysis (95% Hit Rate)	439.0 cp/mL
95% confidence interval	366.1 – 557.6 cp/mL

B. Precision Using the Plasma Separation Card

Precision of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 in combination with the **PSC** was determined by analysis of serial dilutions of an HIV-1 high positive sample (high titer HIV-1 RNA positive cell culture supernatant specimen) in HIV negative EDTA whole blood. Five dilution levels were tested in 48 replicates for each level and process volume across two lots of **PSC** and two reagent lots of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 using two CAP/CTM Systems and two operators over 12 days. Each sample was carried through the entire **PSC** workflow and COBAS® AmpliPrep/COBAS®

TaqMan® HIV-1 Test, v2.0 procedure. The precision results reported here represent all aspects of the test procedure. The results are shown in Table 10.

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 in combination with the **PSC** showed high precision for two lots of **PSC** and reagents tested across a concentration range of 7.38E+02 cp/mL to 1.00E+07 cp/mL.

Table 10
Within Laboratory Precision of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 in Combination with the PSC

Measured concentration (cp/mL)	Source material	Pooled SD
6.31E+06	Cell Culture	0.08
1.05E+06	Cell Culture	0.09
1.02E+05	Cell Culture	0.12
1.05E+04	Cell Culture	0.19
2.29E+03	Cell Culture	0.25

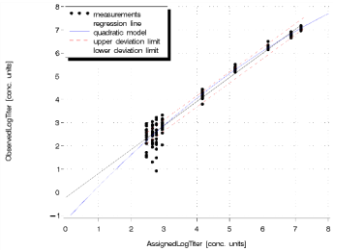
C. Linear Range Using the Plasma Separation Card

The linearity study of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 in combination with the **PSC** was performed with a dilution series consisting of 9 panel members spanning the linear range for the predominant HIV-1 group M subtype B. Panel members were prepared from a high titer HIV-1 RNA positive cell culture supernatant specimen. The evaluation was performed according to CLSI Guideline EP06-A[20]. Two **PSC** and two reagent lots were analyzed on two CAP/CTM Systems, three operators and in total 20 replicates per concentration level.

A part of one panel member was centrifuged and the plasma was titer assigned by Calibrator Bracketing Method (CBM) using **cobas®** HIV-1 (on **cobas®** 6800/8800 Systems) with the 3rd HIV-1 WHO International Standard, HIV-1 group M, subtype B for preparation of the high and low calibrator.

In combination with the **PSC**, COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 is linear from 7.38E+02 cp/mL to 1.00E+07 cp/mL and shows an absolute deviation from the better fitting non-linear regression of less than ± 0.16 $\log_{10}$ with the **PSC** (see Figure 24). Across the linear range, the accuracy of the test was within ± 0.3 $\log_{10}$.

Figure 24
Linearity for the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 in Combination with the Plasma Separation Card



D. Subtype Verification Using the Plasma Separation Card

Although HIV subtype should not affect **PSC** performance, cultured HIV-1 samples for common HIV-1M subtypes (A, C and D) were diluted to one concentration level in whole blood. The precision and accuracy determination was performed with 12 replicates for each sample. Testing was conducted with 1 lot of **PSC** and 1 lot of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 reagents.

Each whole blood sample was centrifuged and the plasma was titer assigned by Calibrator Bracketing Method (CBM) using **cobas**® HIV-1 (on **cobas**® 6800/8800 Systems) with the 3rd HIV-1 WHO International Standard, HIV-1 group M, subtype B for preparation of the high and low calibrator.

The results are shown in Table 11. These results verify that COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 in combination with the **PSC** detected HIV for HIV-1M (A, C and D).

Table 11
Verification of HIV-1 Group M Subtypes A, C and D

HIV-1 M Subtype	Number of valid replicates	Accuracy	Precision	Plasma vs. PSC-plasma Equivalency
Subtype A	12	-0.02	0.09	0.17
Subtype C	12	0.13	0.16	0.06
Subtype D	12	0.07	0.15	0.05

E. Specificity Using the Plasma Separation Card

The specificity of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 in combination with the **PSC** was determined by analyzing HIV negative EDTA whole blood samples from individual donors. 160 individual EDTA whole blood samples were tested with two lots of **PSC** and two lots of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 reagents. One sample tested positive and 159 negative for HIV-1 RNA. In the test panel the specificity of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 in combination with the **PSC** was 99.4% (95% confidence limit: ≥ 97.07%).

F. Whole System Failure Using the Plasma Separation Card

The whole system failure rate of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 in combination with the **PSC** was determined by testing 100 replicates of EDTA whole blood spiked with HIV-1 group M subtype B. These samples were tested at a target concentration of approximately 3 x LoD. The results of this study determined that no replicate returned a negative result for the HIV-1 target, resulting in a whole system failure rate of 0%.

CLINICAL PERFORMANCE EVALUATION

Key Performance Characteristics for EDTA Plasma Samples

Reproducibility

Reproducibility of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 Test was evaluated in EDTA plasma using 2 different workflows (COBAS® AmpliPrep/COBAS® TaqMan® Analyzer System and COBAS® AmpliPrep/COBAS® TaqMan® 48 Analyzer System). The study was performed using panels constructed from well-characterized HIV-1 group M, subtype B cultured virus stock and from EDTA plasma that was negative for HIV-1 RNA and HIV-1/2 antibodies. The panel covered the dynamic range of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 as well as the key medical decision points for the intended use and supported by the 2008 Department of Health and Human Services Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents[13]. The study was designed to evaluate key variables contributing to total precision variance, including lot, site/instrument, operator, day/run, and within-run. Additional analysis were conducted to compare the performance characteristics and comparative precision variability between the two workflows. Two operators at each of 3 sites performed 5 days of testing with each of 3 reagent kit lots using each workflow. Each run consisted of one set of controls (1 high positive, 1 low positive, and 1 negative) and a 7-member panel tested in triplicate (21 sample) on the COBAS® AmpliPrep Instrument. The prepared samples and controls were amplified and detected on the COBAS® TaqMan® Analyzer or on COBAS® TaqMan® 48 Analyzer.

Reproducibility was evaluated by using a random effects model with terms for (a) lot, (b) site/instrument, (c) operator nested within site/instrument, (d) day/run nested within lot, site/instrument, and operator, and (e) aliquots within-run components by using PROC MIXED and $\log_{10}$ transformed results. The percentage of variability due to each component and coefficient of variation of the $\log_{10}$ transformed HIV-1 RNA concentration were calculated. Only the Within Assay Range (2.00E+01 to 1.00E+07 cp/mL) data were investigated.

Table 12 shows the total precision variance and total precision standard deviation obtained from the COBAS® AmpliPrep/COBAS® TaqMan® Analyzer System as determined by analysis of variance. In general, the within-run component contributed more variability than other components.

Table 12
Attributable Percentage of Total Variance, Total Precision Standard Deviation, and Lognormal CV of HIV-1 RNA Concentration ($\log_{10}$ cp/mL) from Tests Within Assay Range

HIV-1 RNA Concentration ($\log_{10}$ cp/mL)			Contribution to Total Variance (%)					Total Precision
Expected	Observed (Average)	No. of Valid Tests[1]	Lot	Site/ Instrument	Operator	Day/ Run	Within-Run	Standard Deviation (Lognormal %CV)
1.699	1.832	270	5%	2%	0%	8%	85%	0.20 (48%)
2.602	2.676	275	6%	1%	0%	17%	77%	0.11 (25%)
3.000	3.067	274	16%	0%	4%	12%	69%	0.10 (24%)
3.699	3.822	273	20%	6%	0%	17%	57%	0.10 (23%)
4.699	4.746	273	27%	0%	0%	14%	59%	0.07 (17%)
5.699	5.644	274	33%	10%	0%	19%	38%	0.10 (23%)
6.699	6.751	259	27%	14%	0%	20%	39%	0.12 (27%)

Note: Within assay range results are from 20 cp/mL to 1.00E+07 cp/mL (1.30 $\log_{10}$ cp/mL to 7.00 $\log_{10}$ cp/mL), inclusive.

[1] Number of tests within assay range.

Results obtained from the COBAS® AmpliPrep/COBAS® TaqMan® 48 System Workflow are summarized in Table 13. In general, the within-run component contributed more variability than other components with the exception of the highest titer panel member.

Table 13
Attributable Percentage of Total Variance, Total Precision Standard Deviation, and Lognormal CV of HIV-1 RNA Concentration (log_{10} cp/mL) from Tests within Assay Range

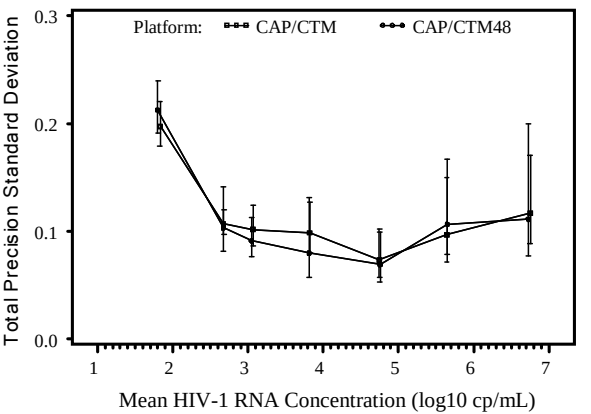
HIV-1 RNA Concentration (log_{10} cp/mL)			Contribution to Total Variance (%)					Total Precision
Expected	Observed (Average)	No. of Valid Tests[1]	Lot	Site/ Instrument	Operator	Day/ Run	Within-Run	Standard Deviation (Lognormal %CV)
1.699	1.804	266	7%	2%	0%	2%	89%	0.21 (52%)
2.602	2.672	273	26%	0%	2%	5%	68%	0.10 (24%)
3.000	3.048	272	17%	0%	0%	6%	77%	0.09 (21%)
3.699	3.814	271	39%	0%	2%	13%	46%	0.08 (19%)
4.699	4.756	272	30%	0%	0%	10%	61%	0.07 (16%)
5.699	5.647	272	35%	0%	6%	16%	43%	0.11 (25%)
6.699	6.727	269	45%	0%	4%	13%	38%	0.11 (26%)

Note: Within assay range results are from 20 cp/mL to 1.00E+07 cp/mL (1.30 log_{10} cp/mL to 7.00 log_{10} cp/mL), inclusive.

[1] Number of tests within assay range.

The results shown in Figure 25 display the plot of the total precision standard deviation with the corresponding approximate 95% Confidence Intervals against the mean log_{10} HIV-1 RNA concentrations. These results indicate a comparable precision performance between the COBAS® AmpliPrep/COBAS® TaqMan® (CAP/CTM) System and the COBAS® AmpliPrep/COBAS® TaqMan® 48 (CAP/CTM48) System configurations.

Figure 25
Total Precision Standard Deviation (approximate 95% CI)



Note: The approximate 95% CI for the total precision standard deviation was calculated by taking the square root of the 95% CI bounds of the total precision variance.

Clinical Sensitivity, Specificity and Method Comparison

Methodology

The primary objective of this study was to evaluate the clinical specificity and sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 in specimens from HIV-negative and HIV-1-positive subjects. Both fresh (never frozen) and frozen EDTA plasma samples were tested in each of the evaluations. The secondary objectives were to compare results and evaluate the positive percent agreement and negative percent agreement of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 results to those obtained with the FDA-approved tests, COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test HIV-1 Test and the COBAS® AMPLICOR HIV-1 MONITOR Test, v1.5.

Clinical specificity was evaluated with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 by testing 148 fresh (never frozen) samples and 418 frozen samples collected from blood donors who were negative for HIV-1/2 antibodies. Clinical sensitivity of the test was evaluated with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 by testing 117 fresh samples and 301 frozen samples in EDTA plasma collected from HIV-1-infected subjects (frozen samples were randomly distributed across test sites by CD4 cell count category). Test results from the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 were compared to those obtained with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test and COBAS® AMAPLICOR HIV-1 MONITOR Test, v1.5. Testing was conducted at 3 test sites, with 1 COBAS® AmpliPrep/COBAS® TaqMan® System per site. Three COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 reagent lots were used.

Statistical Methods

Fresh and frozen samples from HIV-negative and HIV-1–positive subjects were tested with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0, the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, and the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5. HIV-negative subjects were evaluable for statistical analyses of the specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 if they generated valid COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 results. HIV-1-positive subjects were evaluable for statistical analyses of the sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 if they generated valid COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 results and had valid COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test results within the linear range of the assay.

The clinical specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 was calculated as the percentage of evaluable HIV-negative subjects who had Target Not Detected COBAS® AmpliPrep/COBAS® TaqMan®HIV-1 Test, v2.0 results. The associated 95% exact confidence interval (CI) was also provided. The clinical sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 was calculated as the percentage of evaluable HIV-1-positive subjects who had detectable HIV-1 viral load on the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0. The associated 95% exact confidence interval (CI) was also provided. The method comparison evaluated COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 results separately with both comparative platforms (COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test and the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5). Positive and negative percent agreements were calculated between the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 and each comparative platform. Paired samples from HIV-1-positive subjects contributing within linear range results for both the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 and the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test were compared using scatter plots and analyzed using the Deming regression.

Results

A total of 566 evaluable HIV-negative and 418 HIV-1-positive patient specimens were included in clinical specificity and sensitivity analyses. Approximately 75% of the patient specimens were frozen and 25% were fresh. The specific distribution of each platform is summarized in Table 14.

Table 14
Evaluable HIV-1 Negative and Positive Subjects by Sample Type

Sample Type	HIV-Negative Specimens	HIV-1-Positive Specimens
Fresh	148 (26.1%)	117 (28.0%)
Frozen	418 (73.9%)	301 (72.0%)
Total	566	418

The demographic characteristics of the 418 evaluable HIV-1-positive specimens are summarized in Table 15. The CD4 cell counts of the subjects distributed approximately evenly across CD4 cell count categories (<200, 200-500, >500 cells/µL). Most of the subjects were male (74.2%) and between 30 to 49 years of age (72.5%). The ethnic distribution is comparable to that observed in the HIV-1 population of the United States[32].

Table 15
Demographic Characteristics of Evaluable HIV-1-Positive Subjects

Demographic Characteristic	Category	HIV-1-Positive Subjects
Overall	Total	418
CD4 Cell Count (cells/µL)	< 200	130 (31.1%)
	200 - 500	152 (36.4%)
	> 500	136 (32.5%)
Sample Type	Fresh	117 (28.0%)
	Frozen	301 (72.0%)
Sex	Male	310 (74.2%)
	Female	108 (25.8%)
Age (Years)	18-29	23 (5.5%)
	30-39	100 (23.9%)
	40-49	203 (48.6%)
	50-59	74 (17.7%)
	≥ 60	18 (4.3%)
Ethnicity	Caucasian	129 (30.9%)
	Hispanic	46 (11.0%)
	Black	223 (53.3%)
	Asian / Pacific Islander	3 (0.7%)
	Other	17 (4.1%)
On Antiretroviral Medication	Yes	240 (57.4%)
	No	178 (42.6%)

The clinical specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 (Table 16) was 99.3% (562/566; 95% CI = 98.2% to 99.8%), with 4 specimens classified as false positives. Three of these specimens were reported at < 20 cp/mL, below the LLoQ of the assay. The remaining single specimen out of the 566 tested was within the linear range but at a very low titer (28.8 cp/mL). The clinical specificity of

the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 was similar for both fresh specimens (99.3% [147/148; 95% CI = 96.3% to 100%]) and frozen specimens (99.3% [415/418; 95% CI = 97.9% to 99.9%]).

Table 16
Clinical Specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

Subject Group	CAP/CTM HIV-1 Test, v2.0			
	Positive	Negative	Total N	Clinical Specificity (95% exact CI)
HIV-Negative	4 (0.7%)	562 (99.3%)	566	99.3% (98.2%, 99.8%)

The clinical sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 was defined as the percentage of evaluable HIV-1-positive subjects who had a positive COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 result and is summarized in Table 17. The clinical sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 was 100% (418/418; 95% CI = 99.1% to 100%). There were no subjects that had false negative COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 results. The clinical sensitivity was tested in an HIV-patient population reflective of that in the United States with regards to gender, age, ethnicity and exposure to antiretroviral therapy[29]. The test demonstrated 100% clinical sensitivity independent of the above listed demographics, CD4 cell count, or sample type (fresh versus frozen).

Table 17
Clinical Sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

Subject Group	CAP/CTM HIV-1 Test, v2.0			
	Positive	Negative	Total N	Clinical Sensitivity (95% exact CI)
HIV-1-Positive	418 (100.0%)	0 (0.0%)	418	100.0% (99.1%, 100.0%)

Clinical Method Comparison

COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 versus the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test

The comparison of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 and COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test results for the 950 subjects eligible for the analysis is summarized in Table 18. The positive percent agreement of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 with respect to the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test was 99.5% (427/429; 95% CI = 98.3% to 99.9%). The negative percent agreement of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 with respect to the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test was 98.1% (511/521; 95% CI = 96.5% to 99.1%). There were 10 samples with positive COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 results and negative COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test results. Three samples were at titers below the LLoQ of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, most likely a reflection of the increased sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0. Three samples were false positive COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test v2.0 results from HIV-negative subjects identified in the clinical specificity analysis that again were below the LLoQ. Four samples had titers ranging from 24.9 cp/mL to 158 cp/mL and are likely reflective of the known variability associated with low titer quantitation.

Table 18
Comparison of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 versus the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test

CAP/CTM HIV-1 Test, v2.0	CAP/CTM HIV-1 Test		
	Positive	Negative	Total
Positive	427	10	437
Negative	2	511	513
Total	429	521	950
Positive Percent Agreement (95% exact CI)	99.5% (98.3%, 99.9%)		
Negative Percent Agreement (95% exact CI)		98.1% (96.5%, 99.1%)	

CI=confidence interval; CAP/CTM HIV-1 Test = COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test; CAP/CTM HIV-1 Test, v2.0 = COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0.

Note: HIV-negative and HIV-1-positive subjects contributing both valid CAP/CTM HIV-1 Test, v2.0 and CAP/CTM HIV-1 Test results were included in this summary table.

A total of 417 paired HIV-1-positive samples had results within the linear range of both assays and were evaluable for the method comparison analysis. Table 19 shows the mean paired difference and 95% CI for the bias between the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 and the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 returns higher titers than the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, except at both the higher range (> 5 $\log_{10}$ cp/mL) and the lower range (< 2 $\log_{10}$ cp/mL) where it returns titers that are lower (see Table 19). The overall systematic bias is estimated as 0.2591 $\log_{10}$ cp/mL.

Table 19
Mean Paired Difference and 95% CI for the Bias Between the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 and the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test

Number of Paired HIV-1-Positive Samples Within Linear Range of Both Assays = 417		
Mean Difference ($\log_{10}$ cp/mL)	**Standard Error**	**95% CI**
0.2591	0.0122	(0.235, 0.283)

CI = confidence interval; CAP/CTM HIV-1 Test = COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test; CAP/CTM HIV-1 Test, v2.0 = COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0.

Note: HIV-1-positive subjects contributing both valid CAP/CTM HIV-1 Test and CAP/CTM HIV-1 Test, v2.0 results within the linear range of each assay were included in this summary table.

The results of the Deming regression analysis between COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 and COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test results for paired HIV-1-positive specimens within the linear range of both assays are tabulated in Table 20 and displayed graphically in Figure 26 (in this figure, the dashed line indicates perfect agreement between the two test methods, i.e., y = x).

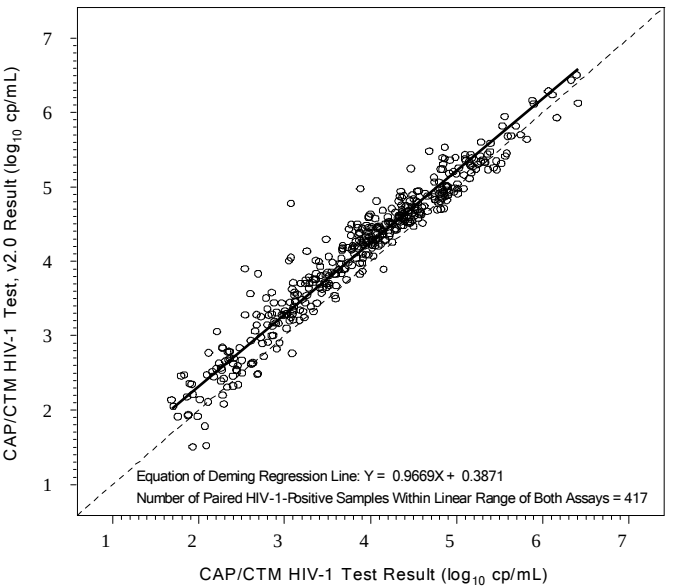
Table 20
Parameter Estimates from Deming Regression Analysis Between the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 and the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test

Number of Paired HIV-1-Positive Samples Within Linear Range of Both Assays = 417				
Parameter	**Parameter Estimate log_{10} cp/mL**	**Standard Error**	**95% CI**	**r^2**
Intercept	0.3871	0.0488	(0.291, 0.483)	0.9375
Slope	0.9669	0.0122	(0.943, 0.991)	

CI = confidence interval; CAP/CTM HIV-1 Test = COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test; CAP/CTM HIV-1 Test, v2.0 = COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0.

Note: HIV-1-positive subjects contributing both valid CAP/CTM HIV-1 Test and CAP/CTM HIV-1 Test, v2.0 results within the linear range of each assay were included in this summary table.

Figure 26
Deming Regression Analysis Between the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 and the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test



COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 versus COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5

Table 21 shows the comparison of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 and CA HIV-1 MONITOR Test, v1.5 results for 991 subjects eligible for the analysis. The positive percent agreement of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 with respect to the COBAS® AMPLICOR (CA) HIV-1 MONITOR Test, v1.5 was 100% (419/419; 95% CI = 99.1% to 100%). The negative percent agreement of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 with respect to the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 was 97.4% (557/572; 95% CI = 95.7% to 98.5%). Of the 15 subjects with positive COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test v2.0 results and negative COBAS® AMPLICOR HIV-1 MONITOR Test, v1.5 results, 4 were false positive COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test v2.0 results from HIV-negative subjects identified in the clinical specificity analysis that again were below the LLoQ. Eleven were from HIV-1-positive subjects with COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test v2.0 results ranging from below the LLoQ to 223 cp/mL and negative COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 results.

Table 21
Comparison of the CAP/CTM HIV-1 Test, v2.0 with the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5

CAP/CTM HIV-1 Test, v2.0	CA HIV-1 MONITOR Test, v1.5		
	Positive	Negative	Total
Positive	419	15	434
Negative	0	557	557
Total	419	572	991
Positive Percent Agreement (95% exact CI)	100.0% (99.1%, 100.0%)		
Negative Percent Agreement (95% exact CI)		97.4% (95.7%, 98.5%)	

CI = confidence interval; CAP/CTM HIV-1 Test = COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test; CAP/CTM HIV-1 Test, v2.0 = COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0.

Note: HIV-negative and HIV-1-positive subjects contributing both valid CAP/CTM HIV-1 Test, v2.0 and CA HIV-1 MONITOR Test, v1.5 results were included in this summary table.

Conclusion

EDTA plasma

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 exhibits high levels of agreement with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test in quantitative analyses (r^2 = 0.9375) and in concordance analyses (positive percent agreement = 99.5%; negative percent agreement = 98.1%). It quantifies clinical specimens 0.2591 $\log_{10}$ cp/mL higher overall than the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, with lower quantitation at the higher range (> 5 $\log_{10}$ cp/mL) and the lower range (< 2 $\log_{10}$ cp/mL).

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 also shows high levels of agreement with the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5 in concordance analyses (positive percent agreement = 100.0%; negative percent agreement = 97.4%).

These test results support the utility of the test for the intended use of assessing disease progression and monitoring antiretroviral therapy in HIV-1 infected patients.

Performance of PSC Dried Plasma Spot Samples Compared to EDTA Plasma Samples

The performance of **PSC** dried plasma spot samples were compared to centrifuged EDTA plasma samples by testing 325 samples from patients infected with HIV-1 and 2 HIV negative whole blood spiked with cell culture supernatants. Eighty-five specimens that had a measurable titer on both plasma and **PSC** were analyzed. The specimens comprised of HIV-1 M samples (FP= prospective collection by venipuncture and from capillary blood, VL= routine viral load testing, CD=CD4+ cell count leftover samples, SP = spiked samples) and were tested one replicate each (**PSC** and plasma) at an external site. Titers below the quantitation range were excluded from analysis. The Deming regression was performed considering log-transformed titers.

The Deming regression results are shown in Figure 27. The symbols ✳ and ● in Figure 27 show single determinations.

Figure 27
Deming Regression

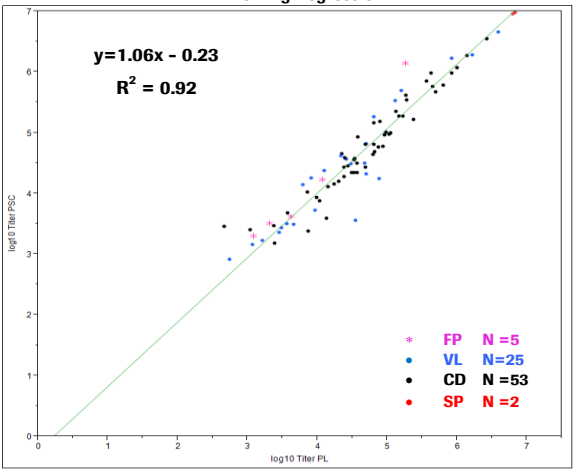


Table 22
Summary of Results for Matrix Equivalency

Matrix (Plasma Type) Equivalency	Number of specimens with valid titer	Bland Altman Analysis		Deming Regression Analysis		
		Mean Log_{10} difference	95% CI [lower/upper]	Slope	Intercept	R-squared
PSC vs liquid plasma	85	0.05	[-0.01; 0.11]	1.06	-0.23	0.92

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Doc Rev. 17.0 02/2019	Updated the Notified Body Number under the CE mark. Please contact your local Roche Representative if you have any questions.
Doc Rev. 18.0 09/2019	Updated **STORAGE AND HANDLING REQUIREMENTS** section to instruct the user to visually inspect the product for signs of leakage before use and to not use the product if there is any evidence of leakage. Updated the harmonized symbol page. Please contact your local Roche Representative if you have any questions.

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