COBAS AmpliScreen HIV-1 Test, version 1.5

FOR *IN VITRO* DIAGNOSTIC USE.

COBAS AmpliScreen HIV-1 Test, version 1.5	HIV-1	96 Tests	P/N: 03577066 190
COBAS AmpliScreen MultiPrep Specimen Preparation and Control Kit	MULTIPREP/CTL	96 Tests	P/N: 03272885 123
COBAS AMPLICOR Wash Buffer	WB	500 Tests	P/N: 20759899 123 ART: 07 5989 9 US: 83314

INTENDED USE

The COBAS AmpliScreen HIV-1 Test, version 1.5 (v1.5) is a qualitative *in vitro* test for the direct detection of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma from donations of whole blood, blood components, plasma intended for transfusion or further manufacture, and organ and tissue donors.

The COBAS AmpliScreen HIV-1 Test, v1.5 is intended for use in screening of individual donor samples of human plasma, or pools of human plasma comprised of equal aliquots of individual donations. It is also intended for use to screen individual organ donors when plasma specimens are obtained while the donor's heart is still beating and to detect HIV-1 RNA in blood specimens from cadaveric (non-heart beating) organ and tissue donors.

This assay may be used as an alternative to licensed HIV-1 p24 antigen tests for screening human plasma from donations of whole blood and blood components.

This assay is not intended for use as an aid in diagnosis.

SUMMARY AND EXPLANATION OF THE TEST

Human Immunodeficiency Virus (HIV-1) is the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS)[1-3]. HIV-1 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-1, 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-1-specific immune response and a decline of plasma viremia, usually within four to six weeks of the onset of symptoms[9,10]. The prevalence of HIV-1 infection is 1.1% overall in the world, 0.56% in North America and 0.25% in West Europe[11].

Serological screening assays have greatly reduced, but not completely eliminated, the risk of transmitting viral infections by transfusion of blood products[12-15]. HIV-1 p24 antigen is the principal core protein of HIV-1 and is found in serum or plasma either free or bound by anti-p24 antibody.

HIV-1 p24 antigen can be measured with commercially available enzyme immunoassays (EIA), which reduce the seroconversion window period, i.e., the time between infection and the rise of antibodies to the virus[16], by approximately 5 to 6 days[17,18]. Recent studies indicate that nucleic acid based amplification tests for HIV-1 RNA will further reduce the residual transmission risk by detecting HIV-1 RNA in donations made during the seroconversion window period. Nucleic acid-based tests can detect viremic units donated by carriers who do not seroconvert or who lack antibodies to serological markers normally detected by immunological assays[16,19,20].

HIV-1 RNA in plasma can be detected by nucleic acid amplification technologies, such as the PCR[21-23]. The COBAS AmpliScreen HIV-1 Test, v1.5 uses PCR technology to achieve maximum sensitivity for the detection of HIV-1 RNA in plasma samples[24].

A number of proposals have been made for performing nucleic acid tests on mini-pools comprised of small aliquots from many individual samples. The high sensitivity of PCR has demonstrated that potentially infectious donations contained within mini-pools can be detected even if the mini-pool contains a single viremic donor.[13,25,26]

The assay incorporates an Internal Control for monitoring assay performance in each individual test as well as the AmpErase® (uracil-N-glycosylase) enzyme to reduce potential contamination by previously amplified material (amplicon).

NOTE: The COBAS AmpliScreen HIV-1 Test, v1.5 has been validated by Roche using the components and protocols listed within this package insert. The user takes responsibility to validate any changes to components other than those listed. User should refer to manufacturers' guidelines for the substituted components.

PRINCIPLES OF THE PROCEDURE

The COBAS AmpliScreen HIV-1 Test, v1.5 is based on five major processes:

1. Sample Processing
2. Reverse transcription of target RNA to generate complementary DNA (cDNA)[27]
3. PCR amplification[27] of target cDNA using HIV-specific complementary primers
4. Hybridization of the amplified products to oligonucleotide probes specific to the target(s)
5. Detection of the probe-bound amplified products by colorimetric determination.

Sample Processing

Two Roche specimen processing procedures are used with the COBAS AmpliScreen HIV-1 Test, v1.5 as follows:

- MultiPrep Specimen Processing Procedure is recommended for use in the preparation of specimens in the mini-pool format and individual specimens from cadaveric donors
- Standard Sample Processing Procedure is recommended for use in the preparation of individual non-cadaveric donor samples

NOTE: For testing of cadaveric specimens, the specimen should be first diluted 1:5 in MultiPrep Specimen Diluent (MP DIL) prior to processing using the MultiPrep Specimen Processing Procedure.

In the Standard Specimen Processing Procedure, HIV-1 RNA is isolated directly from plasma by lysis of the virus particles with MultiPrep Lysis Reagent followed by precipitation of the RNA with alcohol. In the MultiPrep Specimen Processing Procedure, HIV-1 viral particles are first pelleted from the plasma sample by high speed centrifugation, followed by lysis of the pelleted virus with a chaotropic agent (MultiPrep Lysis Reagent) and precipitation of the RNA with alcohol.

The MultiPrep Internal Control (**MP IC**), containing the HIV-1 Internal Control, is introduced into each sample with the MultiPrep Lysis Reagent and serves as an extraction and amplification control for each processed specimen and control. The HIV-1 Internal Control is an RNA transcript with primer binding regions identical to those of the HIV-1 target sequence, a randomized internal sequence of similar length and base composition as the HIV-1 target sequence, and a unique probe binding region that differentiates the HIV-1 Internal Control amplicon from target amplicon. These features were selected to ensure equivalent amplification of the HIV-1 Internal Control and the HIV-1 target RNA.

Reverse Transcription

The reverse transcription and amplification reactions are performed with the thermostable recombinant enzyme *Thermus thermophilus* DNA Polymerase (r*Tth* pol). In the presence of manganese (Mn^{2+}) and under the appropriate buffer conditions, r*Tth* pol has both reverse transcriptase and DNA polymerase activity[28]. This allows both reverse transcription and PCR amplification to occur in the same reaction mixture. Reverse transcription using r*Tth* pol produces a cDNA copy of the HIV-1 target and the HIV-1 Internal Control RNA.

PCR Amplification

Following reverse transcription using r*Tth* pol, a second DNA strand is produced from the cDNA copy, thereby yielding a double-stranded DNA copy of the HIV-1 target and HIV-1 Internal Control RNA. The reaction mixture is heated to separate the resulting double-stranded DNA. As the mixture cools, primers anneal to the target DNA, in the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), the r*Tth* pol extends the annealed primers along the target templates to produce a double-stranded DNA molecule termed an amplicon. The COBAS AMPLICOR Analyzer automatically repeats this process for a designated number of cycles, each cycle effectively doubling the amount of amplicon DNA. The required number of cycles is preprogrammed in the COBAS AMPLICOR Analyzer.

Selective Amplification

To ensure selective amplification of nucleic acid target in the sample and prevent amplification of pre-existing amplicon, the AmpErase (uracil-N-glycosylase) enzyme is added to the COBAS AmpliScreen HIV-1 Test, v1.5. The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[29], but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon because of the use of deoxyuridine triphosphate in place of deoxythymidine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contain deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by the AmpErase enzyme before amplification of the target DNA. The AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of DNA, thereby rendering the DNA non-amplifiable. The AmpErase enzyme is inactive at temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. Following amplification, any residual enzyme is denatured by the addition of the Denaturation Solution, thereby preventing the degradation of any target amplicon.

Hybridization Reaction

Following PCR amplification, the COBAS AMPLICOR Analyzer automatically adds Denaturation Solution to the A-tubes to chemically denature the HIV-1 target amplicon and the HIV-1 Internal Control amplicon to form single-stranded DNA. Aliquots of denatured amplicon are then transferred to two detection cups (D-cups). A suspension of magnetic particles coated with an oligonucleotide probe specific for HIV-1 target amplicon or HIV-1 Internal Control amplicon is added to the individual D-cups. The biotin-labeled HIV-1 target and HIV-1 Internal Control amplicon are hybridized to the target-specific oligonucleotide probes bound to the magnetic particles. This hybridization of amplicon to the target-specific probe increases the overall specificity of the COBAS AmpliScreen HIV-1 Test, v1.5.

Detection Reaction

Following the hybridization reaction, the COBAS AMPLICOR Analyzer washes the magnetic particles in the D-cups to remove unbound material, and then adds avidin-horseradish peroxidase conjugate. The avidin-horseradish peroxidase conjugate binds to the hybridized biotin-labeled amplicon. The COBAS AMPLICOR Analyzer removes unbound conjugate by washing the magnetic particles and then adds a substrate solution containing hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine (TMB) to each D-cup. In the presence of hydrogen peroxide, the particle-bound horseradish peroxidase catalyzes the oxidation of TMB to form a colored complex. The absorbance is measured by the COBAS AMPLICOR Analyzer at a wavelength of 660 nm.

MATERIALS PROVIDED BY ROCHE

The COBAS AmpliScreen MultiPrep Specimen Preparation and Control Kit and the COBAS AMPLICOR Wash Buffer kit are provided as stand-alone kits to be used in conjunction with the COBAS AmpliScreen HIV-1 Test, v1.5, as well as with the COBAS AmpliScreen HCV Test, v2.0 and the COBAS AmpliScreen HBV Test.

NOTE: For cadaveric blood specimens, there is not sufficient MP DIL (MultiPrep Specimen Diluent) in the COBAS AmpliScreen MultiPrep Specimen Preparation and Control Kit to run 96 tests as an additional 800 µL MP DIL is used to prepare each specimen and control. Additional COBAS AmpliScreen MultiPrep Specimen Preparation and Control Kits must be ordered to run 96 tests.

COBAS AmpliScreen MultiPrep Specimen Preparation and Control Kit MULTIPREP/CTL **96 Tests**
(P/N: 03272885 123)

MP (–) C
[MultiPrep Negative (–) Control]

MP (+) C
[MultiPrep Positive (+) Control]

MP LYS
(MultiPrep Lysis Reagent)

MP DIL
(MultiPrep Specimen Diluent)

MP IC
(MultiPrep Internal Control)

NHP
[Negative Plasma (Human)]

COBAS AmpliScreen HIV-1 Test, version 1.5 HIV-1 **96 Tests**
(P/N: 03577066 190)

COBAS AmpliScreen HIV-1 Amplification Reagents, version 1.5 HIV AMP

HIV-1 MMX, v1.5
(HIV-1 Master Mix, version 1.5)

HIV-1 Mn^{2+}, v1.5
(HIV-1 Manganese Solution, version 1.5)

COBAS AmpliScreen HIV-1 Detection Reagents, version 1.5 HIV DK

IH PS1, v1.5
(HIV-1 Probe Suspension 1, version 1.5)

IH4, v1.5
(HIV-1 Probe Suspension 2, version 1.5)

II PS1, v1.5
(HIV-1 IC Probe Suspension 1, version 1.5)

II4, v1.5
(HIV-1 IC Probe Suspension 2, version 1.5)

DN4
(Denaturation Solution)

CN4
(Avidin-Horseradish Peroxidase Conjugate)

SB3
(Substrate A)

SB
(Substrate B)

COBAS AMPLICOR Wash Buffer WB **500 Tests**
(P/N: 20759899 123; ART: 07 5989 9; US: 83314)

WB
(10X-Wash Concentrate)

OTHER MATERIALS REQUIRED BUT SOLD SEPARATELY (MAY BE PURCHASED FROM ROCHE)

- COBAS AMPLICOR Analyzer, Printer, and *Operator's Manual* for the COBAS AMPLICOR Analyzer
- COBAS AMPLICOR A-rings
- COBAS AMPLICOR D-cups
- AMPLILINK Software and *Operator's Manual* for the AMPLILINK software
- Sarstedt 1.5-mL tube Barcode Labels
- Hamilton Archive and Intermediate Plate Barcode Labels

MATERIALS REQUIRED BUT NOT PROVIDED BY ROCHE

- Hamilton MICROLAB® AT plus 2 Pipettor or equivalent
- Refrigerated high speed centrifuge with fixed angle rotor (45 degrees, capacity for at least 24 x 1.5-mL tubes) with an RCF of 23,600 x g (Heraeus Centrifuge 17RS or Biofuge 28RS with HFA 22.1 rotor, Heraeus Biofuge Stratos with the 3331 rotor or equivalent).
- Microcentrifuge, (max. RCF 16,000 x g, min. RCF 12,500 x g) (Eppendorf® 5415C, HERMLE Z230M, or equivalent)

- Eppendorf 1.25 mL Combitip® Reservoir (sterile) or equivalent
- Eppendorf Multipette® pipette or equivalent
- Ethanol, 90% or 95%, reagent grade for Molecular Biology or Histology use
- Distilled or deionized water
- Powderless, disposable gloves
- Isopropyl alcohol, reagent grade
- Disposable, Sterile, Polystyrene pipettes (5 mL, 10 mL and 25 mL)
- Sterile, RNase-free, fine-tip transfer pipettes
- Pipettors (capacity 20 µL to 1000 µL, capable of providing ± 3% accuracy and precision ≤ 5%) with aerosol barrier or positive displacement RNase-free tips
- Tube racks (Sarstedt P/N 93.1428 or equivalent)
- 1.5 mL sterile, non-siliconized, conical polypropylene screw-cap tubes, (Sarstedt 72.692.105 or equivalent)
- Vortex mixer
- Hamilton Slotted Deepwell Archive Plate, 2.2 mL and Sealing Capmat
- Hamilton Slotted Intermediate Plate

REAGENTS

COBAS AmpliScreen MultiPrep Specimen Preparation and Control Kit MULTIPREP **96 Tests**

MP (–) C 8 x 0.1 mL
[MultiPrep Negative (–) Control]

< 0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

MP (+) C 8 x 0.1 mL
[MultiPrep Positive (+) Contro]

Tris-HCl buffer
< 0.001% Non-infectious linearized plasmid DNA (microbial) containing HBV sequences
< 0.001% Non-infectious *in vitro* transcribed RNA (microbial) containing HCV sequences
< 0.001% Non-infectious *in vitro* transcribed RNA (microbial) containing HIV-1 sequences
< 0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

MP LYS 8 x 9.0 mL
(MultiPrep Lysis Reagent)

Tris-HCl buffer
60% Guanidine thiocyanate
3% Dithiothreitol
< 1% Glycogen

Xn 60% (w/w) Guanidine thiocyanate

Harmful

MP DIL 8 x 4.8 mL
(MultiPrep Specimen Diluent)

Tris-HCl buffer
< 0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

MP IC 8 x 0.1 mL
(MultiPrep Internal Control)

Tris-HCl buffer
< 0.001% Non-infectious plasmid DNA containing HBV primer binding sequences and a unique probe binding region
< 0.001% Non-infectious *in vitro* transcribed RNA (microbial) containing HCV primer binding sequences and a unique probe binding region
< 0.001% Non-infectious *in vitro* transcribed RNA (microbial) containing HIV-1 primer binding sequences and a unique probe binding region
< 0.005% Poly rA RNA (synthetic)
EDTA
< 0.1% Amaranth dye
0.05% Sodium azide

NHP 16 x 1.6 mL
[Negative Plasma (Human)]

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

COBAS AmpliScreen HIV-1 Test, version 1.5 HIV-1 **96 Tests**

COBAS AmpliScreen HIV-1 Amplification Reagents, version 1.5 HIV AMP

HIV-1 MMX, v1.5 8 x 0.7 mL
(HIV-1 Master Mix, version 1.5)

Bicine buffer
Glycerol
< 0.01% r*Tth* DNA Polymerase (r*Tth* pol, microbial)
Potassium acetate
< 0.07% dATP, dCTP, dGTP, dUTP, dTTP
< 0.001% SKCC1B and SK145 biotinylated primers
< 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial)
0.05% Sodium azide

HIV-1 Mn^{2+}, v1.5 8 x 0.1 mL
(HIV-1 Manganese Solution, version 1.5)

< 2% Manganese
Acetic acid
Amaranth dye
0.05% Sodium azide

COBAS AmpliScreen HIV-1 Detection Reagents, version 1.5 HIV DK

IH PS1, v1.5 1 x 100 Tests
(HIV-1 Probe Suspension 1, version 1.5)

MES buffer
< 0.01% Suspension of Dynabeads® (paramagnetic particles) coated with HIV-1-specific oligonucleotide capture probe SK102
0.09% Sodium azide

IH4, v1.5 1 x 100 Tests
(HIV-1 Probe Suspension 2, version 1.5)

Sodium phosphate buffer
24.9% Sodium thiocyanate
< 0.2% Solubilizer

II PS1, v1.5 1 x 100 Tests
(HIV-1 IC Probe Suspension 1, version 1.5)

MES buffer
< 0.01% Suspension of Dynabeads (paramagnetic particles) coated with HIV-1 IC-specific oligonucleotide capture probe CP35
0.09% Sodium azide

II4, v1.5 1 x 100 Tests
(HIV-1 IC Probe Suspension 2, version 1.5)

Sodium phosphate buffer
24.9% Sodium thiocyanate
< 0.2% Solubilizer

DN4 1 x 100 Tests
(Denaturation Solution)

1.6% Sodium hydroxide
EDTA
Thymol blue

Xi 1.6% (w/w) Sodium hydroxide

Irritant

CN4 2 x 100 Tests
(Avidin-Horseradish Peroxidase Conjugate)

Tris-HCl buffer
< 0.001% Avidin-horseradish peroxidase conjugate
Bovine serum albumin (mammalian)
Emulsit 25 (Dai-ichi Kogyo Seiyaku Co., Ltd.)
0.1% Phenol
1% ProClin® 150

SB3 10 x 75 Tests
(Substrate A)

Citrate solution
0.01% Hydrogen peroxide
0.1% ProClin 150

SB 10 x 75 Tests (10 x 5 mL)
(Substrate B)

0.1% 3,3',5,5'-Tetramethylbenzidine (TMB)
40% Dimethylformamide (DMF)

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

Toxic

R: 61-20/21-36 May cause harm to the unborn child. Harmful by inhalation and in contact with skin. Irritating to eyes.

S: 53-45 Avoid exposure – obtain special instructions before use. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

COBAS AMPLICOR Wash Buffer WB **500 Tests**

WB 2 x 250 Tests
(10X-Wash Concentrate)

< 2% Phosphate buffer
< 9% Sodium chloride
EDTA
< 2% Detergent
0.5% ProClin® 300

STORAGE INSTRUCTIONS

A. Room Temperature is defined as 15-30ºC.

B. Do not freeze reagents.

C. Store the following reagents at 2-8°C. Unopened, these reagents are stable until the expiration date indicated.

MP LYS, **MP IC**, **MP (+) C**, **MP (–) C**, **MP DIL** and **NHP**

HIV-1 MMX, v1.5 and **HIV-1 Mn^{2+}, v1.5**

IH PS1, **v1.5**, **IH4**, **v1.5**, **II PS1**, **v1.5** and **II4**, **v1.5**

CN4, **SB3** and **SB**

D. Store **DN4** and **WB** at 2-25°C. **DN4** and **WB** are stable until the expiration dates indicated.

E Do not expose **SB3**, **SB** or Working Substrate to metals, oxidizing agents or direct sunlight.

F. The following reagents are one time use. Discard any unused portion.

MP IC, **MP (+) C**, **MP (–) C**, **MP DIL** and **NHP**

HIV-1 Mn^{2+}, **v1.5**; **SB, IH PS1, v1.5** and **II PS1, v1.5.**

PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE.

A. Specimens may be infectious. Use Universal Precautions when performing the assay.[30-31] Only personnel proficient in the use of the COBAS AmpliScreen Test System and trained in handling infectious materials should perform this procedure. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water. Follow by wiping down the surface with 70%

ethanol.

B. **CAUTION**: **The Negative Human Plasma (NHP) of this kit contains human blood products non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2, HIV-1 p24 antigen and HBsAg. Testing of Negative Human Plasma by PCR methods showed no detectable HIV-1 RNA, HCV RNA or HBV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All human blood-sourced materials should be considered potentially infectious and should be handled with Universal Precautions.** If spillage occurs, immediately disinfect, then wipe up with a 0.5% (final concentration) sodium hypochlorite solution (diluted bleach) or follow appropriate site procedures.

C. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

D. This product contains sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing azide compounds are disposed of in a plumbing system, they should be diluted and flushed with generous amounts of running water. These precautions are recommended to avoid accumulation of deposits in metal piping in which explosive conditions could develop.

E. ***Heparin has been shown to inhibit PCR. Do not use heparinized plasma with this procedure.***

F. Use only supplied or specified required disposables to ensure optimal assay performance.

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

H. Adequately vortex, where specified, to ensure optimal assay performance.

I. Handle all materials containing specimens or controls according to Good Laboratory Practices in order to prevent cross-contamination of specimens or controls.

J. Before use, visually inspect each reagent bottle to ensure that there are no signs of leakage and/or abnormal color. If there is any evidence of leakage and/or abnormal color, do not use that bottle for testing.

K. Dispose of all materials that have come in contact with specimens and reagents in accordance with country, federal, state and local regulations.

L. Do not use a kit after its expiration date. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers. Do not use expired reagents.

M. Material Safety Data Sheets (MSDS) are available on request.

N. Supplies and equipment must be dedicated to each pre-amplification activity and should not be used for other activities or moved between areas. **Fresh, clean gloves must be worn in each area and must be changed before leaving that area.** Equipment and supplies used for reagent preparation must not be used for specimen preparation activities or for pipetting or processing amplified DNA or other sources of target DNA. Post-amplification supplies and equipment must remain in the Post-Amplification Area at all times.

O. Avoid contact of **MP LYS, HIV-1 MMX, v1.5, HIV-1 Mn^{2+}, v1.5, IH4, v1.5, II4, v1.5, DN4, CN4, SB3, SB** and Working Substrate (mixed **SB3** and **SB** reagent) with the skin, eyes or mucous membranes. **If contact does occur, immediately wash with large amounts of water, otherwise burns can occur.** If these reagents are spilled, dilute with water before wiping dry. **Do not allow MP LYS**, **which contains guanidine thiocyanate, or IH4**, **v1.5 and II4, v1.5**, **which contain sodium thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.**

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

Q. Refer to the *Operator's Manuals* for the AMPLILINK software and COBAS AMPLICOR Analyzer for use with the COBAS AmpliScreen Tests.

R. Closely follow procedures and guidelines provided to ensure that the specimen and control preparation is performed correctly. Any deviation from the given procedures and guidelines may affect optimal assay performance. COBAS AmpliScreen has been validated by Roche using the components and protocols listed within this package insert. The user takes responsibility to validate any changes to components other than those listed. User should refer to manufacturers' guidelines for the substituted components.

S. The use of excessively hemolyzed cadaveric specimens should be avoided.

REAGENT PREPARATION

A. **MP IC**, **MP (+) C**, **MP (–) C**, **MP DIL** and **NHP**

1. Warm **MP IC**, **MP (+) C**, **MP (–) C**, **MP DIL** and **NHP** to room temperature before use by using a 37°C incubator or on the laboratory bench top.

B. **Working Lysis Reagent**

1. Warm **MP LYS** to 25-37°C to dissolve precipitate (maximum 30 minutes). Mix thoroughly until the crystals are dissolved. ***Prior to use, examine each bottle of MP LYS against a white background for appearance of a yellow color or signs of leakage. If there is any yellow color or signs of leakage, do not use that bottle for testing. Contact your local Roche office for replacement.***
2. Vortex **MP IC** briefly before use. Tap vial to collect the solution in the base. Pipette 100 µL **MP IC** into 1 bottle **MP LYS.** Cap the **MP LYS** bottle and vortex briefly. The pink color confirms that the **MP IC** has been added to the **MP LYS**. Discard the remaining **MP IC**.
3. Store Working Lysis Reagent at room temperature. Use within 4 hours of preparation.

C. **Working Amplification Master Mix**

1. Prepare Working Master Mix in a template-free area (e.g., in a dead air box). **Reagent preparation area must be clean and disinfected in accordance with methods outlined in *Precautions* (Item A). Failure to do so may result in reagent contamination.**
2. Pipette 100 µL **HIV-1 Mn^{2+}**, **v1.5** into 1 bottle **HIV-1 MMX**, **v1.5**. Recap **HIV-1 MMX**, **v1.5** bottle and mix well by inverting 10-15 times. The pink color confirms that the **HIV-1 Mn^{2+}**, **v1.5** has been added to the **HIV-1 MMX**, **v1.5**. Discard the remaining **HIV-1 Mn^{2+}**, **v1.5**. Do not vortex the Working Master Mix. These reagents do not need to be at room temperature before use.
3. Store at 2-8°C and use within 4 hours of preparation.

D. **Working Probe Suspension Detection Reagents**

1. Prepare Working HIV-1 Probe Suspension: Mix **IH PS1**, **v1.5** well by vortexing briefly to suspend the microparticles. Pipette 2.5 mL **IH PS1**, **v1.5** into one **IH4**, **v1.5** cassette.
2. Prepare Working IC Probe Suspension: Mix **II PS1, v1.5** well by vortexing briefly to suspend the microparticles. Pipette 2.5 mL **II PS1, v1.5** into one **II4, v1.5** cassette.
3. Both Working Probe Suspension Detection Reagents are stable for 30 days at 2-8°C. Working Reagents can be used for a maximum of ten instrument cycles (12 hours per cycle). Mixing occurs automatically on the COBAS AMPLICOR Analyzer.
4. Store Working Probe Suspension Detection Reagents at 2-8°C between instrument cycles. Remove from refrigerator 30 minutes before use on the COBAS AMPLICOR Analyzer.

E. **DN4 – Denaturation Reagent and CN4 Conjugate Reagent**

1. Once opened, **DN4** and **CN4** are stable for 30 days at 2-8°C, or until the expiration date, whichever comes first. Both **DN4** and **CN4** can be used for a maximum of ten instrument cycles (12 hours per cycle).
2. Store **DN4** and **CN4** at 2-8°C between instrument cycles. Remove from refrigerator 30 minutes before use on the COBAS AMPLICOR Analyzer.

F. **Working Substrate Reagent**

1. Working Substrate must be prepared each day by pipetting 5 mL **SB** into one **SB3** cassette. Pipette up and down at least 5 times to mix.
2. Working Substrate is stable on the COBAS AMPLICOR Analyzer for a maximum of 16 hours.
3. Do not expose **SB3**, **SB** or Working Substrate to metals, oxidizing agents, or direct light.

G. **Wash Buffer Reagent**

1. Examine **WB** before dilution and if necessary, warm at 30-37°C to dissolve any precipitate. Add 1 volume of **WB** to 9 volumes of distilled or deionized water. Mix well. Keep a minimum of 3-4 liters of Working Wash Buffer (1X) in the Wash Buffer Reservoir of the COBAS AMPLICOR Analyzer at all times.
2. Working Wash Buffer (1X) should be stored at 2-25°C in the COBAS AMPLICOR Wash Buffer Reservoir and is stable for 2 weeks from the date of preparation.

H. **70% Ethanol**

1. Prepare 70% ethanol fresh daily.
2. One mL 70% ethanol is needed for each specimen and control processed. For example, mix 11.7 mL 90% ethanol and 3.3 mL of distilled or deionized water for every 12 specimens and controls to be processed.

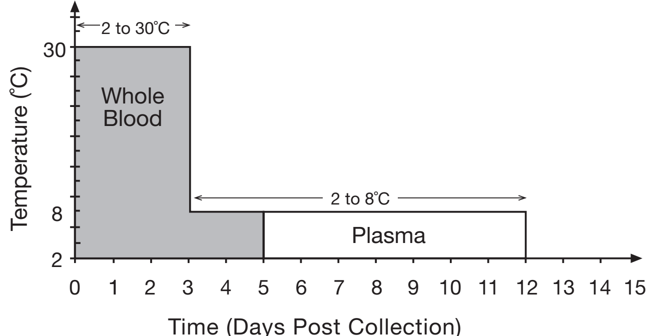
SPECIMEN COLLECTION, STORAGE AND POOLING

NOTE: Handle all specimens as if they are potentially infectious agents.

Living (Heart Beating) Blood Donor Specimens

A. EDTA, CPD, CPDA-1, CP2D, ACD-A and 4% Sodium Citrate may be used with the COBAS AmpliScreen HIV-1 Test, v1.5. Follow sample tube manufacturer's instructions.

B. Blood collected in EDTA may be stored at 2-30°C for up to 72 hours from time of draw, followed by an additional two days at 2-8ºC. For storage longer than five days, remove the plasma from the red blood cells by centrifugation at 800-1600 x g for 20 minutes. Following removal, plasma may be stored at 2-8°C for an additional seven days. Alternatively, plasma may be stored at ≤ -18°C for up to one month.



C. Blood collected in CPD, CPDA-1, or CP2D may be stored for up to 72 hours at 1-24ºC. Following centrifugation of the CPD, CPDA-1, or CP2D samples at 800-1600 x g for 20 minutes, plasma may be stored at 1-6°C for an additional 7 days from the date the plasma was removed from the red blood cells. Plasma separated from the cells may be stored at ≤ -18ºC for up to one month.

D. ACD-A or 4% sodium citrate anticoagulated apheresis plasma can be stored at 1-6ºC for up to 6 hours, followed by subsequent storage at ≤ -18ºC for up to one month.

E. Do not freeze whole blood.

F. ***Heparin has been shown to inhibit PCR. Use of heparinized specimens is not recommended.***

G. Warm pooled or individual donor specimens to room temperature before using.

H. Covered Archive Plates may be stored at 2-8°C for up to 7 days from the date the plasma was removed from the red blood cells.

I. No adverse effect on assay performance was observed when plasma specimens were subjected to three freeze-thaw cycles.

J. Thaw frozen specimens at room temperature before using.

K. The user should validate other collection and storage conditions. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.[32]

L. **False positive results may occur if cross contamination of specimens is not adequately controlled during specimen handling and processing.**

M. **<u>SPECIMEN POOLING</u>:**

1. The COBAS AmpliScreen HIV-1 Test, v1.5 may be used in screening individual donor samples of human plasma, or pools of human plasma comprised of equal aliqouts of individual donations.
2. Pooling algorithms may require the preparation and testing of smaller Secondary Pools and/or individual specimens for follow-up testing in the event a Primary Pool tests positive.

Cadaveric Blood Specimens

N. Cadaveric blood specimens can be collected in serum or EDTA anticoagulant tubes.

O. For collection, storage and handling of specimens from deceased donor, follow general standards and/or regulations. Cadaveric samples may be stored for up to 72 hours at refrigerated conditions (2-8°C), or up to 48 hours at ambient temperature (15-30°C). Other storage and handling conditions must be validated by the user.

P. Cadaveric blood specimens should be briefly (e.g. pulse) centrifuged prior to use to remove particulate matter.

NOTE: Cadaveric specimens should be placed at 2-8°C as soon as possible after collection. The use of excessively hemolyzed cadaveric specimens should be avoided.

PROCEDURAL NOTES

A. **Run Size**

1. Each kit contains reagents sufficient for eight 12-specimen runs, which may be performed separately or simultaneously. At least one preparation of the COBAS AmpliScreen MultiPrep Negative (–) Control and one preparation of the COBAS AmpliScreen MultiPrep Positive (+) Control must be included in each A-ring (see "*Quality Control*" section).
2. The Specimen Preparation and Amplification Reagents are packaged in eight single-use bottles. The MultiPrep Negative (–) and MultiPrep Positive (+) Controls are packaged in single-use vials. For the most efficient use of reagents, specimens and controls should be processed in batches that are multiples of 12.
3. The use of sterile gauze, when uncapping sample tubes, may reduce the potential for cross contamination between specimens.

B. **Equipment**

1. Prepare the COBAS AMPLICOR Analyzer and the Data Station or equivalent for the AMPLILINK Software for use according to instructions in the *Operator's Manual* for the AMPLILINK software and the *Operator's Manual* for the COBAS AMPLICOR Analyzer.
2. Prepare the Hamilton MICROLAB AT plus 2 System and SUNPLUS Data Station or equivalent for use according to instructions in the *Operator's Manuals*.
3. Pre-cool the high-speed centrifuge and rotor to 2-8°C. See operating instructions for the high speed centrifuge for details.
4. Perform manufacturer recommended maintenance and calibration on all instruments, including pipettors, to ensure proper functioning.

C. **Reagents**

1. All reagents, **except HIV-1 MMX**, **v1.5** and **HIV-1 Mn^{2+}**, **v1.5**, must be at room temperature before use. Visually examine reagents for sufficient volume before beginning the test procedure. See section "*Reagent Preparation*" for specific reagent storage conditions.
2. Add all reagents using a pipettor capable of delivering specified volume with ± 3% accuracy and a precision of ≤ 5% CV. Check pipettor functionality and calibrate as recommended by pipettor manufacturer.
3. Prepare Working Master Mix in a template-free area (e.g., in a dead air box). **Reagent preparation area must be clean and disinfected in accordance with methods outlined in "*Precautions*" (Item A). Failure to do so may result in reagent contamination.**
4. Prepare 70% ethanol fresh each day.
5. Check expiration date of opened or Working Reagents before loading on the COBAS AMPLICOR Analyzer.

6. Check to ensure that all reagents used are of the same master lot of kit reagents.

D. **Workflow**

1. To minimize the possibility of laboratory areas becoming contaminated with amplicon, the laboratory area should be separated into several distinct areas organized around Pre-Amplification and Post-Amplification. Personnel should use proper anti-contamination safeguards when moving between areas.
2. The Pre-Amplification Area should have a template-free area for preparation of Working Master Mix and an amplicon free area for specimen and control preparation.
3. The Post-Amplification Area should have a COBAS AMPLICOR Analyzer(s) and AMPLILINK Data Station(s) with additional area for preparing Working Amplification and Detection Reagents.
4. Pipettors and other supplies should be dedicated to a specific area. Samples, equipment and reagents should not be returned to the area where a previous step was performed.

E. **Temperature**

Room temperature is defined as 15° to 30ºC.

F. **Vortexing**

Proper vortexing during sample preparation is important to ensure homogeneous mixture after additions of reagents.

G. **Pipetting**

1. Pooled or individual plasma specimens must be at room temperature before pipetting.
2. Use a clean pipette tip or disposable transfer pipette with each specimen or control. Use aerosol barrier or positive displacement RNase-free tips.
3. Confirm that all pipettors are correctly set to dispense the specified volumes in accordance with the specimen preparation procedures and guidelines.

H. **Specimen Processing**

1. Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens and controls. Do not use snap cap tubes.
2. Avoid contaminating gloves when manipulating specimens.
3. Specimens and controls should be prepared in a laminar flow hood. ***Failure to do so may result in sample contamination.*** Specimen and control preparation area must be cleaned and disinfected in accordance with methods outlined in "*Precautions*" (Item A).

I. **Decontamination**

Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water. Follow by wiping down the surface with 70% ethanol.

INSTRUCTIONS FOR USE

The MultiPrep Specimen Processing Procedure is used for extracting nucleic acid from pooled specimens and from individual cadaveric specimens. The Standard Specimen Processing Procedure is used for extracting nucleic acid from individual non-cadaveric specimens as well as for tertiary resolution.

The MultiPrep and the Standard Specimen Processing Procedures are generic nucleic acid extraction procedures and can be used for the extraction of HIV-1 RNA, HCV RNA, and/or HBV DNA. A single extraction is sufficient for multiple assays. Workflow can be performed on the same day or over multiple days under the following conditions:

NOTE: The performance data for the Multiprep Specimen Processing Procedure presented in the "PERFORMANCE CHARACTERISTICS" section should be taken into consideration when determining pool size. For the Roche COBAS AmpliScreen System, a pool size of 24 has been validated. The user must validate other pool sizes or configurations.

Amplification, Hybridization and Detection of Stored Processed Specimens

Amplification, hybridization and detection can occur on the same day as specimen processing or on a subsequent day. If amplification, hybridization and detection are to be done on a subsequent day, perform the MultiPrep Specimen Processing Procedure described in steps B1 through B21 or the Standard Specimen Processing Procedure described in steps B22 through B38. Store the processed specimens and controls as indicated. On the subsequent day, begin with Step A (Reagent Preparation - Working Master Mix), thaw processed specimens and controls at room temperature, and continue with Step B39.

Hybridization and Detection of Stored Denatured Amplicon

Hybridization and detection of the denatured amplicon may occur on the same day as amplification or on a subsequent day. If hybridization and detection are to be done on a subsequent day, the denatured amplicon may be left on-board the COBAS AMPLICOR Analyzer for not more than 24 hours before starting the hybridization and detection steps. Alternatively, the denatured amplicon may be stored at 2-8°C for not more than five days before starting the hybridization and detection steps.

A. Reagent Preparation – Working Master Mix

Performed in: Pre-Amplification – Reagent Preparation Area (e.g., dead air box)

A1. Determine the appropriate number of A-ring(s) needed for specimen and control testing.

A2. Place the A-ring(s) on the A-ring holder(s).

A3. For each A-ring, prepare one Working Master Mix.

A4. Pipette 50 µL Working Master Mix into each A-tube. Discard unused Working Master Mix. Do not close the covers of the A-tubes at this time.

A5. Place the A-ring containing Working Master Mix in a sealable bag and seal the plastic bag. Record the assay name (HIV-1) and the time the Working Master Mix was prepared.

A6. Store the A-ring(s) containing Working Master Mix at 2-8°C until specimen and control preparation is completed. The A-rings with Working Master Mix must be used within 4 hours of preparation.

A7. Decontaminate area. See "*Procedural Notes*", Item I.

B. Specimen and Control Preparation

Performed in Pre-Amplification – Specimen and Control Preparation Area

MultiPrep Specimen Processing Procedure (Pooled Specimens and Individual Cadaveric Specimens)

B1. **For pooled specimens**, pipette 1000 µL of each pool into an appropriately labeled screw-cap tube using the COBAS AmpliScreen Pooling System, a hand-held pipettor or other user-validated method. Cap the tubes. Proceed to Step B2.

For individual cadaveric specimens, pipette 200 µL into an appropriately labeled screw-cap tube and add 800 µL MultiPrep Diluent (**MP DIL**) using a hand-held pipettor or other user-validated method. Cap the tubes. Vortex each specimen tube briefly. Proceed to Step B2.

B2. Vortex **NHP** briefly.

B3. For each Negative and Positive Control, pipette 1000 µL **NHP** into an appropriately labeled screw-cap tube when testing pooled specimens. Cap the tubes.

For cadaveric testing, pipette 200 µL **NHP** into an appropriately labeled screw-cap tube and add 800 µL MultiPrep Diluent (**MP DIL**) using a hand-held pipettor or other user-validated method. Cap the tubes. Vortex each specimen tube briefly.

B4. Use a permanent marker to make an orientation mark on each tube.

B5. Place the specimen and control tubes into the pre-cooled high-speed centrifuge with the orientation marks facing outward, so that the orientation marks will align with the pellets formed during centrifugation.

B6. Centrifuge specimens and control tubes at 23,000 - 24,000 x g for 60 ± 4 minutes at 2-8°C. The pellet will form on the outer wall as indicated by the orientation mark.

***NOTE: The 60 ± 4 minutes begins when the centrifuge reaches 23,000 - 24,000 x g*.**

B7. Remove the tubes from the centrifuge and remove the caps. Slowly aspirate 900 µL of the supernatant from each centrifuged tube leaving approximately 100 µL of supernatant. Avoid contact with the pellet. Discard the supernatant and pipette tip appropriately. Use a fresh

pipette tip for each tube.

B8. Prepare a Working Lysis Reagent bottle for every batch of 12 specimens and controls to be processed.

B9. Pipette 600 µL Working Lysis Reagent into each specimen and control tube. Cap and vortex tubes briefly.

B10. Prepare Controls as follows:

a. Negative Control

Vortex **MP (–) C** briefly. Tap vial to collect the solution in the base. Pipette 20 µL **MP (–) C** to the tube labeled "MP (–) C" containing Working Lysis Reagent and **NHP**. Cap the tube and vortex briefly.

b. Positive Control

Vortex **MP (+) C** briefly. Tap vial to collect the solution in the base. Pipette 20 µL **MP (+) C** to the tube labeled "MP (+) C" containing Working Lysis Reagent and **NHP**. Cap the tube and vortex briefly.

B11. Incubate all tubes for 10 to 15 minutes at room temperature after adding Working Lysis Reagent to the last tube. After the incubation period, briefly vortex all tubes.

B12. Pipette 700 µL of isopropanol into each tube. Cap the tubes and vortex briefly.

B13. Place the tubes into a microcentrifuge with the orientation marks facing outward to align with the pellets that will form. Centrifuge at 14,250 ± 1750 x g for 15-20 minutes at room temperature.

B14. Slowly aspirate the supernatant from each tube. Remove as much liquid as possible without disturbing the pellet.

B15. Pipette 1.0 mL of 70% ethanol into each tube. Cap the tubes and vortex briefly.

B16. Place the tubes into a microcentrifuge with the orientation marks facing outward to align with the pellets that will form. Centrifuge at 14,250 ± 1750 x g for 5-10 minutes at room temperature.

B17. Slowly aspirate the supernatant from each tube using a fine-tip disposable transfer pipette. Remove as much liquid as possible without disturbing the pellet. Use a new transfer pipette for each tube.

B18. Using a new transfer pipette for each tube, repeat Step B17 to remove as much of the remaining supernatant as possible without disturbing the pellet. ***Residual ethanol can inhibit amplification.***

B19. Pipette 200 µL **MP DIL** into each tube. Use a pipette tip to break apart the pellet. This can be done by aspirating 30-40 µL of the diluent in the tip and scraping the sides and base of the tube in an up/down motion for at least 10 seconds and dispensing 30-40 µL. Cap the tubes and vortex briefly to resuspend the extracted RNA. Note that some insoluble material may remain.

B20. At this point amplification of the processed specimens and controls must be started within 2 hours. If not, the processed specimens and controls can be stored at -70°C or colder for up to one month. ***Thawing should be completed within one hour at room temperature.***

B21. Proceed to step ***B39, Loading the A-ring***

Standard Specimen Processing Procedure (Individual Specimens [Non-Cadaveric])

B22. Pipette 200 µL of each specimen into an appropriately labeled screw-cap tube using the COBAS AmpliScreen Pooling System, a hand-held pipettor or other user-validated method. Cap the tubes.

B23. Vortex **NHP** briefly.

B24. For each Negative and Positive Control pipette 200 µL **NHP** into appropriately labeled screw-cap tubes. Cap the tubes.

B25. Use a permanent marker to make an orientation mark on each tube.

B26. Prepare a Working Lysis Reagent bottle for every 12 specimens and controls to be processed.

B27. Pipette 600 µL Working Lysis Reagent into each tube. Cap and vortex tubes briefly.

B28. Prepare Controls as follows:

a. Negative Control

Vortex **MP (–) C** briefly. Tap vial to collect the solution in the base. Pipette 20 µL **MP (–) C** into the tube labeled "MP (–) C" containing Working Lysis Reagent and **NHP**. Cap the tube and vortex briefly.

b. Positive Control

Vortex **MP (+) C** briefly. Tap vial to collect the solution in the base. Pipette 20 µL **MP (+) C** to the tube labeled "MP (+) C" containing Working Lysis Reagent and **NHP**. Cap the tube and vortex briefly.

B29. Incubate all tubes for 10-15 minutes at room temperature after adding Working Lysis Reagent to the last tube. After the incubation period, briefly vortex all tubes.

B30. Pipette 800 µL of isopropanol into each tube. Cap the tubes and vortex briefly.

B31. Place the tubes into a microcentrifuge with the orientation marks facing outward to align with the pellets that will form. Centrifuge at 14,250 ± 1750 x g for 15-20 minutes at room temperature.

B32. Slowly aspirate the supernatant from each tube. Remove as much liquid as possible without disturbing the pellet.

B33. Pipette 1.0 mL of 70% ethanol into each tube. Cap the tubes and vortex briefly.

B34. Place the tubes into a microcentrifuge with the orientation marks facing outward to align with the pellets that will form. Centrifuge at 14,250 ± 1750 x g for 5-10 minutes at room temperature.

B35. Slowly aspirate the supernatant from each tube using a fine-tip disposable transfer pipette. Remove as much liquid as possible without disturbing the pellet. Use a new transfer pipette for each tube.

B36. Using a new transfer pipette for each tube, repeat Step B35 to remove as much of the remaining supernatant as possible without disturbing the pellet. ***Residual ethanol can inhibit amplification.***

B37. Pipette 200 µL **MP DIL** into each tube. Use a pipette tip to break apart the pellet. This can be done by aspirating 30-40 µL of the diluent in the tip and scraping the sides and base of the tube in an up/down motion for at least 10 seconds and dispensing 30-40 µL. Cap the tubes and vortex briefly to resuspend the extracted RNA. Note that some insoluble material may remain.

B38. At this point amplification of the processed specimens and controls must be started within 2 hours. If not, the processed specimens and controls can be stored at -70°C or colder for up to one month. ***Thawing should be completed within one hour at room temperature.***

Loading the A-ring

B39. Create an A-ring worklist record for each A-ring to identify the A-tube with the appropriate control or specimen to be pipetted.

B40. If processed specimens and controls were stored frozen, thaw at room temperature before proceeding. Briefly vortex the processed specimens and controls.

B41. Pipette 50 µL of each processed specimen and control into the appropriate A-tube containing HIV-1 Working Master Mix. Immediately cap the A-tube and repeat this step for all 12 A-tubes to complete the A-ring loading. Use the A-ring worklist record to ensure the appropriate specimen or control is added to the correct A-tube position for each A-ring.

B42. Transfer the A-ring with sealed tubes containing the processed specimens and controls in Working Master Mix to the Amplification/Detection Area. Proceed to Part C.

NOTE: Amplification must begin within 45 minutes from when the first specimen or control in the A-ring is added to the Working Master Mix.

C. Reverse Transcription, Amplification and Detection

Performed in Post-Amplification – Amplification/Detection Area

C1. Perform Daily Instrument Maintenance as outlined in the *Operator's Manual* for the COBAS AMPLICOR Analyzer including:

a. Wipe D-cup handler tip with a lint-free moist cloth and dry.

b. Wipe initialization post with a lint-free moist cloth and dry.

C2. Before each run:

a. Check waste container and empty if necessary.

b. Check Wash Buffer Reservoir and add prepared Wash Buffer if necessary.

c. Replace used D-cup racks.

d. Prime the COBAS AMPLICOR Analyzer.

C3. Instrument Loading and System Operation

a. Prepare enough of the following detection reagent cassettes to complete the workload: Working HIV-1 Probe Suspension Reagent (**IH4**, **v1.5**), Working IC Probe Suspension Reagent (**II4**, **v1.5**), Working Substrate (**SB3**), Denaturation Reagent (**DN4**), and Conjugate Reagent (**CN4**).

b. Place the **IH4**, **v1.5** and **II4**, **v1.5** cassettes in the test-specific reagent rack.

c. Place **DN4**, **CN4** and **SB3** cassettes in the generic reagent rack. Record on the cassette the date when each cassette was opened.

d. Identify the reagent racks as generic or test specific using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software.

e. Configure the reagent racks by entering the reagent positions and lots using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software.

f. Load the reagent racks onto the Analyzer using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software. Make sure that each reagent cassette is in its assigned position and that each cassette fits tightly into its rack.

g. Place the D-cup rack on the D-cup platform. Two D-cups are required for each A-tube and two D-cups are required for each Working Substrate cassette to allow for blanking by the COBAS AMPLICOR Analyzer, as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer.

h. Place the A-ring into the thermal cycler segment of the COBAS AMPLICOR Analyzer and close the cover on the thermal cycler segment.

i. Load the A-ring into the COBAS AMPLICOR Analyzer using the Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software.

j. Create an A-ring order, using the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software. Use the A-ring worklist record created for specimen processing to assist in entering the A-ring order.

k. Repeat steps h. through j. above to load a second A-ring on the COBAS AMPLICOR Analyzer.

l. Start the COBAS AMPLICOR Analyzer as described in the *Operator's Manual* for AMPLILINK software.

m. Wait for the COBAS AMPLICOR Analyzer to indicate that the load check has passed.

NOTE: The required quantity of each detection reagent is automatically calculated by the COBAS AMPLICOR Analyzer during the Load Check to determine if sufficient reagents are available for the requested tests.

n. The COBAS AMPLICOR Analyzer automatically performs reverse transcription, amplification and detection. Results are expressed as absorbance values at 660 nm and as positive or negative.

o. As a Quality Control measure, the AMPLILINK A-ring Results Report and the Run Log may be printed (e.g., daily, weekly or monthly) and retained along with the respective A-ring worklist. A selection of A-ring worklist records should be periodically compared with the AMPLILINK A-ring Results Report to verify that the A-ring ID, instrument serial number, and specimen IDs are identical. Reconcile the Run Log with the selected A-ring worklist to account for all A-ring IDs associated with the run. If there are discrepancies, perform follow-up investigation.

QUALITY CONTROL PROCEDURES

1. At least one MultiPrep (–) Control and one MultiPrep (+) Control must be processed with each A-ring.

a. Negative Control

The absorbance for the **MP (–) C** should be less than 0.2 at 660 nm and its associated **MP IC** should be greater than or equal to 0.2 for the Negative Control to be valid. If the absorbance value for the **MP (–) C** is greater than or equal to 0.2 at 660 nm and/or its associated **MP IC** is less than 0.2, the entire A-ring is invalid, and the entire test procedure for that A-ring (specimen and control preparation, amplification and detection) must be repeated.

b. Positive Control

The absorbance for the **MP (+) C,** should be greater than or equal to 1.0 at 660 nm and its associated **MP IC** should be greater than or equal to 0.2 at 660 nm for the Positive Control to be valid. If the absorbance value for the **MP (+) C,** is less than 1.0 and/or its associated **MP IC** is less than 0.2, the entire A-ring is invalid, and the entire test procedure for that A-ring (specimen and control preparation, amplification and detection) must be repeated.

Summary of Control Acceptance Criteria

	HIV-1 Result		IC Result	
	A_{660}	Comment	A_{660}	Comment
Negative Control	< 0.2	Negative	≥ 0.2	Valid
Positive Control	≥ 1.0	Positive	≥ 0.2	Valid

2. Flags and comments may be generated by the COBAS AMPLICOR Analyzer during a run. The Operator must check the run printout(s) for flags and comments to verify that the run is valid. Refer to the *Operator's Manual* for the AMPLILINK software and the *Operator's Manual* for the COBAS AMPLICOR Analyzer for interpretation of flags and comments.

3. External Control

If required by the laboratory or by a regulatory agency, an External Run Control must be processed in the same manner as a sample. The External Control should contain a defined number of target sequence copies and the level of this control should be a multiple of the cut-off value of the test system. The absorbance of the HIV-1 External Run Control should be equal to or greater than 0.2 at 660 nm. Any absorbance value for **MP IC** is acceptable. If the absorbance of the HIV-1 External Run Control does not meet the above criteria, the negative samples may be in question. Therefore, the laboratory should follow their established Standard Operating Procedure for the appropriate action.

INTERPRETATION OF RESULTS

1. Flags and comments may be generated by the COBAS AMPLICOR Analyzer during a run. The Operator must check the run printout(s) for flags and comments to verify that the run is valid. Refer to the *Operator's Manual* for the AMPLILINK software and the *Operator's Manual* for the COBAS AMPLICOR Analyzer for interpretation of flags and comments.

2. Specimen Results

Two absorbance values are obtained for each specimen: one for the HIV-1 target and one for the internal control (**MP IC**). For a sample with an absorbance less than 0.2, the **MP IC** absorbance for that specimen must be greater than or equal to 0.2 at 660 nm for a valid negative specimen test result. If the absorbance for the HIV-1 target is greater than or equal to 0.2, the **MP IC** result is disregarded and the test result is valid and positive.

3. For a valid run, results are interpreted as follows:

HIV-1 Result		IC Result		Interpretation
A_{660}	Comment	A_{660}	Comment	
< 0.2	NEGATIVE	≥ 0.2	VALID	Specimen is negative for HIV-1 RNA.
< 0.2	NEGATIVE	< 0.2	INVALID	Invalid result. Repeat entire test procedure for invalid specimen.
≥ 0.2	POSITIVE	ANY	VALID	Specimen is positive for HIV-1 RNA.

Invalid Test Runs

When invalid Positive or Negative Control results are obtained on an A-ring, that A-ring is invalid. Repeat the entire test procedure for the associated specimens (including specimen and control preparation, amplification and detection) in the A-ring by processing another aliquot of the original plasma specimens.

With the exception of instrument failures subsequent to denaturation of amplicon, an instrument failure during a test run, as indicated by system error messages, also constitutes an invalid test run. In such instances, repeat the test procedure for the associated controls and specimens (amplification and detection) in the run by processing another aliquot of the processed specimen.

For instrument failures subsequent to successful denaturation of amplicon, it is not necessary to repeat the entire test procedure for the associated specimens. In such instances, the denatured amplicon may be redetected by the COBAS AMPLICOR Analyzer. The denatured amplicon may be left on the COBAS AMPLICOR Analyzer for not more than 24 hours before continuing with the hybridization and detection steps. Alternatively, the denatured amplicon may be stored at 2-8°C for not more than five days before continuing with the hybridization and detection steps.

Invalid Specimen Results

For non-cadaveric plasma specimen(s) that are invalid, perform repeat testing in single on the remaining replicate tube(s). The test result for the pool or individual donor specimen is based only on the repeat valid test result. If the last available replicate of a pooled specimen gives an invalid result, each individual donor specimen in that pool should be tested. If an individual donor specimen gives an invalid result, the test result for that individual donor specimen should be considered invalid for HIV-1 RNA.

For cadaveric specimens that are invalid, additional cadaveric specimen is diluted 1:5 with **MP DIL** reagent and retested in duplicate using the MultiPrep Specimen Processing Procedure. The test result for the cadaveric specimen is based on the repeat valid test results.

Results of Pooled Donor Specimens

The COBAS AmpliScreen HIV-1 Test, v1.5 may be used in screening individual donor samples of human plasma, or pools of human plasma comprised of equal aliqouts of individual donations.

The testing algorithm for testing of pooled samples for the COBAS AmpliScreen HIV-1 Test, v1.5 requires a single level of testing for Primary Pools that are negative for HIV-1 RNA. Depending on the pooling algorithm used, a positive Primary Pool may require deconstruction into smaller intermediate Secondary Pools for testing or go to immediate testing of individual donations. If using intermediate pools, a positive Secondary Pool may require further Tertiary Resolution Testing of individual donations. An example is given below.

Negative Primary Pools

When the Primary Pool is negative, report the results for all associated individual donor specimens in that Primary Pool as "HIV-1 RNA Negative".

Positive Primary Pools - Utilizing Secondary Pool Testing

When the Primary Pool is positive, prepare smaller Secondary Pools containing the associated donor specimens. The Secondary Pools must be processed using the MultiPrep Specimen Processing Procedure.

- If one or more of the Secondary Pools tests positive, report the results for the donor specimens in the negative Secondary Pools as "HIV-1 RNA Negative". For positive Secondary Pools, proceed to the section entitled "***Positive Primary Pool, Positive Secondary Pools - Utilizing Tertiary Resolution Testing.***"
- If all four Secondary Pools are negative, the individual donor specimens in that Primary Pool may be reported as "HIV-1 RNA Negative."
- As part of an overall Quality Assurance program, you may wish to conduct additional testing to determine the cause of the initial positivity of the Primary Pool, for which the four associated secondary pools were negative. Positive pools resolved as negative at the secondary resolution testing level is usually a result of a contamination event to the primary pool, but theoretically may be due to a viral load below the limit of detection.

Positive Primary Pool, Positive Secondary Pools - Utilizing Tertiary Resolution Testing

For a positive Secondary Pool, test each of the individual donor specimens in that Secondary Pool. The individual donor specimens can be processed using the Standard Specimen Processing procedure.

- If one or more of the individual donor specimens is positive, the positive donor specimen(s) is (are) reported as "HIV-1 RNA Positive" and the remaining negative donor specimens associated with the positive Secondary Pool are reported as "HIV-1 RNA Negative."
- If all of the individual donor specimens in that Secondary Pool test negative, the donor specimens in the Secondary Pool may be reported as "HIV-1 RNA Negative."
- As part of an overall Quality Assurance program, you may wish to conduct additional testing to determine the cause of the positivity of the Primary and Secondary Pools, for which the associated individual samples were negative. Positive pools resolved as negative at the tertiary resolution testing level is usually a result of a contamination event to the secondary pool, but theoretically may be due to a viral load below the limit of detection.

Results of Individual Donor Samples

If an individual donor specimen is positive, the positive donor specimen is reported as "HIV-1 RNA Positive."

If an individual donor specimen is negative, the negative donor specimen is reported as "HIV-1 RNA Negative."

Results of Individual Cadaveric Specimens

If an individual cadaveric specimen is positive, the positive cadaveric specimen is reported as "HIV-1 RNA Positive."

If an individual cadaveric specimen is negative, the cadaveric specimen is reported as "HIV RNA Negative."

For cadaveric specimens that had an initial invalid result and were repeated in duplicate, if either or both the duplicate samples are positive, the specimen is reported as "HIV RNA Positive." If both duplicate specimens are negative or if one duplicate repeat is negative and one is invalid, the specimen is reported as "HIV RNA Negative." If both replicates are invalid, it is most likely due to inhibitory substances in the specimen and results should be marked as invalid or unresolved.

PROCEDURAL LIMITATIONS

1. This test has been evaluated only for use in combination with the COBAS AmpliScreen MultiPrep Specimen Preparation and Control Kit, COBAS AMPLICOR Analyzer, and the Hamilton MICROLAB AT plus 2 Pipettor for the automated preparation of plasma pools. The user takes responsibility to validate any changes to components other than those listed. User should refer to manufacturers' guidelines for the substituted components.
2. Eight Group O culture specimens were only evaluated as diluted samples due to limited specimen volume. All HIV-1 Group O specimens tested were found to be HIV-1 p24 antigen positive, however, only five (63%) were detected by the COBAS AmpliScreen HIV-1 Test, v1.5.
3. This COBAS AmpliScreen HIV-1 Test, v1.5 is intended to be used in conjunction with licensed tests for detecting antibodies to HIV-1. The COBAS AmpliScreen HIV-1 Test, v1.5 may not be used to replace HIV-1 antibody detection tests such as EIA or Western Blot (See Performance Characteristics section, Tables 12 and 13).
4. ***Heparin inhibits PCR; specimens collected using heparin as the anticoagulant should not be used with the COBAS AmpliScreen HIV-1 Test, v1.5.***
5. Reliable results are dependent on adequate specimen collection and proper transport procedures.
6. 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, patient factors (i.e., age, presence of symptoms), and/or stage of infection and pool size.
7. Only the Hamilton MICROLAB AT plus 2 Pipettor has been validated for use with the COBAS AmpliScreen HIV-1 Test, v1.5 for the automated preparation of plasma pools. Adhere to the hardware instructions and safety precautions outlined in the *User Manual* for the Hamilton MICROLAB AT plus 2 Pipettor.

PERFORMANCE CHARACTERISTICS

Reproducibility

The reproducibility of the COBAS AmpliScreen HIV-1 Test, v1.5 was established by testing two six-member EDTA plasma panels with known concentrations of HIV-1. Panel One was tested using the MultiPrep Specimen Processing Procedure. Panel One was comprised of HIV-1 RNA positive samples at concentrations of 10, 25, 50, 75, and 25,000 copies/mL and one HIV-1-negative sample. Panel Two was tested using the Standard Specimen Processing Procedure. Panel Two was comprised of HIV-1 positive samples at concentrations of 50, 100, 150, 250, and 25,000 copies/mL and one HIV-1 negative sample.

Testing was performed at three sites with two operators at each site using five COBAS AmpliScreen HIV-1 Test, v1.5 kit lots. Each operator used a dedicated COBAS AMPLICOR Analyzer throughout the study. Each operator was provided panel sets that had been randomized and labeled in blinded fashion.

All valid reproducibility data were evaluated by calculating the percentage of correct results for each panel member. The data were analyzed by site, lot, testing day, run, and operator for each Specimen Processing Procedure (MultiPrep and Standard).

The reproducibility study for the COBAS AmpliScreen HIV-1 Test, version 1.5 demonstrated consistency by lot and site for both the MultiPrep and

Standard Specimen Processing Procedures as seen in Tables 1 and 2 below:

Table 1
Reproducibility Results - MultiPrep Specimen Processing Procedure

Results By Lot (# Positive / # Tested)						
	Negative	**10 c/mL**	**25 c/mL**	**50 c/mL**	**75 c/mL**	**25,000 c/mL**
Lot #1 (%)	1/88 (1%)	51/90 (57%)	77/90 (86%)	86/90 (96%)	89/89 (100%)	90/90 (100%)
Lot #2 (%)	0/89 (0%)	47/90 (52%)	72/90 (80%)	83/90 (92%)	88/90 (98%)	90/90 (100%)
Lot #3 (%)	2/90 (2%)	50/89 (56%)	80/89 (90%)	88/89 (99%)	88/90 (98%)	90/90 (100%)
Lot #4 (%)	0/90 (0%)	45/90 (50%)	78/90 (87%)	84/90 (93%)	90/90 (100%)	90/90 (100%)
Lot #5 (%)	0/89 (0%)	51/89 (57%)	73/89 (82%)	83/90 (92%)	90/90 (100%)	90/90 (100%)
Results By Site (# Positive / # Tested)						
Site #1 (%)	3/150 (2%)	72/150 (48%)	133/150 (89%)	142/150 (95%)	149/150 (99%)	150/150 (100%)
Site #2 (%)	0/147 (0%)	82/148 (55%)	108/148 (73%)	136/149 (91%)	146/149 (98%)	150/150 (100%)
Site #3 (%)	0/149 (0%)	90/150 (60%)	139/150 (93%)	146/150 (97%)	150/150 (100%)	150/150 (100%)

Table 2
Reproducibility Results - Standard Specimen Processing Procedure

Results By Lot (# Positive / # Tested)						
	Negative	**50 c/mL**	**100 c/mL**	**150 c/mL**	**250 c/mL**	**25,000 c/mL**
Lot #1 (%)	0/90 (0%)	44/90 (49%)	75/89 (84%)	83/89 (93%)	85/88 (97%)	90/90 (100%)
Lot #2 (%)	0/89 (0%)	49/88 (56%)	72/88 (82%)	83/89 (93%)	86/89 (97%)	90/90 (100%)
Lot #3 (%)	0/89 (0%)	39/88 (44%)	72/89 (81%)	74/87 (85%)	86/90 (96%)	90/90 (100%)
Lot #4 (%)	1/87 (1%)	49/90 (54%)	59/88 (67%)	71/89 (80%)	85/90 (94%)	90/90 (100%)
Lot #5 (%)	0/89 (0%)	37/90 (41%)	65/89 (73%)	76/88 (86%)	85/89 (96%)	89/89 (100%)
Results By Site (# Positive / # Tested)						
Site #1 (%)	0/150 (0%)	73/149 (49%)	117/150 (78%)	134/150 (89%)	145/150 (97%)	150/150 (100%)
Site #2 (%)	0/144 (0%)	63/147 (43%)	109/144 (76%)	118/142 (83%)	138/146 (95%)	150/150 (100%)
Site #3 (%)	1/150 (1%)	82/150 (55%)	117/149 (79%)	135/150 (90%)	144/150 (96%)	149/149 (100%)

Analytical Sensitivity - Dilutional Panels

The analytical sensitivity of the COBAS AmpliScreen HIV-1 Test, v1.5 was determined by testing 10 HIV-1 seropositive clinical specimens. The titer of each specimen was quantitated with a commercially available assay using a secondary standard calibrated against the WHO International Standard. These specimens were diluted in normal human plasma to 150, 50, and 16.7 copies/mL for the MultiPrep Specimen Processing Procedure and 300, 100, and 33.3 copies/mL for the Standard Specimen Processing Procedure.

The COBAS AmpliScreen HIV-1 Test, v1.5 detected 50 copies/mL HIV-1 RNA at a frequency greater than 98% with a lower 95% confidence limit of 96.5% using the MultiPrep Specimen Processing Procedure. The assay detected 100 copies/mL HIV-1 RNA at a frequency greater than 98% with a lower 95% confidence limit of 96.5% using the Standard Specimen Processing Procedure. The data are presented in Tables 3 and 4.

When evaluated using PROBIT analysis, the combined data for all samples processed by the MultiPrep Specimen Processing Procedure indicate an average 95% Limit of Detection (LOD) of 39.2 copies/mL, with the lower and upper 95% confidence limits of 34.0 copies/mL and 48.3 copies/mL, respectively. The LOD of 39.2 copies/mL corresponds to approximately 61.25 IU/mL. This equates to an LOD of 940.8 copies/mL or 1470 IU/mL for an individual donor specimen tested in a pool of 24 donors.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Standard Specimen Processing Procedure indicate an average 95% LOD of 96.2 copies/mL with the lower and upper 95% confidence limit of 83.3 copies/mL and 116.7 copies/mL, respectively. The LOD of 96.2 copies/mL corresponds to approximately 150.3 IU/mL.

A conversion factor of 0.64 RNA copies/IU was used to convert copies/mL to IU/mL. This conversion factor was obtained from a collaborative study organized by NIBSC.

Table 3
MultiPrep Procedure Testing Summary for All Clinical Samples
Combined Input Values with 95% One-tailed Lower Confidence Limit

MultiPrep Sample Processing Procedure				
HIV-1 RNA Concentration (c/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit – One-Tailed
150	220	220	100.0%	98.6%
50	214	217	98.6%	96.5%
16.7	116	219	53.0%	47.2%

Table 4
Standard Procedure Testing Summary for All Clinical Samples
Combined Input Values with 95% One-tailed Lower Confidence Limit

Standard Sample Processing Procedure				
HIV-1 RNA Concentration (c/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit – One-Tailed
300	216	218	99.1%	97.1%
100	216	219	98.6%	96.5%
33.3	97	217	44.7%	39.0%

Analytical Sensitivity - WHO HIV-1 International Standard

The analytical sensitivity of the COBAS AmpliScreen HIV-1 Test, v1.5 was also determined using the WHO HIV-1 International Standard (97/656). The WHO HIV-1 International Standard was serially diluted in HIV-1- negative plasma to final concentrations of 140, 100, 70, 50, 35, and 25 IU/mL for the MultiPrep Specimen Processing Procedure and 800, 560, 400, 280, 200, and 140 IU/mL for the Standard Specimen Processing Procedure. Each dilution was tested using two lots of COBAS AmpliScreen HIV-1 Test, v1.5.

When evaluated using PROBIT analysis, the combined data from all samples using the MultiPrep Sample Processing Procedure indicate an average 95% LOD of 78.4 IU/mL, with lower and upper 95% confidence limits of 68.4 IU/mL and 94.4 IU/mL, respectively. This equates to an LOD of 1882 IU/mL for an individual donor specimen tested in a pool of 24 donors.

When evaluated using PROBIT analysis, the combined data from all samples tested using the Standard Sample Processing Procedure indicate an average 95% LOD of 323.4 IU/mL, with lower and upper 95% confidence limits of 284.9 IU/mL and 387.3 IU/mL, respectively. A significant difference was observed in the LOD determination between testing performed with the WHO HIV-1 International Standard and the dilutional panels above. Variation in the preparation of the sample dilutions evaluated and the conversion factor utilized to convert copies/mL to IU/mL may may be potential contributors to the observed difference.

Tables 5 and 6 summarize the overall results for the MultiPrep and Standard Specimen Processing Procedures, respectively.

Table 5
Serial Dilution Testing Summary for MultiPrep Method with HIV-1 RNA WHO International Standard (97/656)
Combined Input Values with Lower 95% Confidence Limit (One-Sided)

HIV-1 RNA Concentration (IU/mL)	Number of Positives	Number of Individual Tests	% Positive	95% Lower Confidence Limit (One-sided)
140	128	130	98.5%	95.2%
100	115	120	95.8%	91.4%
70	128	130	98.5%	95.2%
50	103	120	85.8%	79.5%
35	79	118	66.9%	59.1%
25	70	120	58.3%	50.4%

Table 6
Serial Dilution Testing Summary for Standard Method with HIV-1 RNA WHO International Standard (97/656)
Combined Input Values with Lower 95% Confidence Limit (One-Sided)

HIV-1 RNA Concentration (IU/mL)	Number of Positives	Number of Individual Tests	% Positive	95% Lower Confidence Limit (One-sided)
800	119	120	99.2%	96.1%
560	119	120	99.2%	96.1%
400	118	119	99.2%	96.1%
280	126	137	92.0%	87.1%
200	100	119	84.0%	77.5%
140	82	120	68.3%	60.6%

Analytical Sensitivity - CBER HIV-1 Panel

The FDA CBER HIV-1 Panel Members were processed using the MultiPrep and Standard Specimen Processing Procedures. The MultiPrep Specimen Processing Procedure detected 100% of all positive members ranging from 10 - 250,000 copies/mL. The Standard Specimen Processing Procedure detected 100% of all positive members ranging from 100 - 250,000 copies/mL. The data are shown in Table 7.

Table 7
FDA CBER HIV-1 RNA Panel Results

CBER HIV-1 (Copies/mL)	**CBER HIV-1 Panel Test Results**												
	A1 250,000	**A2 25,000**	**A3 1,000**	**A4 100**	**A5 0**	**B1 2,500**	**B2 10**	**B3 250,000**	**B4 0**	**B5 100**	**B6 50**	**B7 25,000**	**B8 0**
MultiPrep Method	100%	100%	100%	100%	0%	100%	100%	100%	0%	100%	100%	100%	0%
Standard Prep Method	100%	100%	100%	100%	0%	100%	0%	100%	0%	100%	75%	100%	0%

Group / Subtype Detectability

One hundred culture specimens representing 20 each of HIV-1 Group M, subtypes A through E, 3 culture specimens of Subtype F, 4 culture specimens of Subtype G, 8 culture specimens of Group O, and 1 culture specimen of Group N were tested. The Group M specimens were tested at 400 copies/mL using the Standard Specimen Processing Procedure, and at 200 copies/mL using the MultiPrep Specimen Processing Procedure. The Group O and N specimens were diluted 5-, 25-, 125-, 625-, and 3125-fold and tested using the MultiPrep and Standard Specimen Processing Procedures. Data are provided in Table 8. Group O specimens were only evaluated as diluted samples due to limited specimen volume.

Table 8
HIV-1 Group/Subtype Tested

Group	Subtype	Quantity	Reactive Total (MultiPrep)	Reactive Total (Standard Prep)
M	A	20	20/20	20/20
	B	20	20/20	20/20
	C	20	20/20	20/20
	D	20	20/20	20/20
	E	20	20/20	20/20
	F	3	3/3	3/3
	G	4	4/4	4/4
O*	N/A	8	5/8	5/8
N*	N/A	1	1/1	1/1

* *Due to limited volume, specimens were only tested diluted and the actual HIV-1 RNA Group O and Group N copy numbers were not determined. Non-reactive diluted specimens may have been below the Limit of Detection.*

Seroconversion Panels

Forty-one commercially available anti-HIV seroconversion panels were tested undiluted using the Standard Specimen Processing Procedure and diluted 1:24 using the MultiPrep Specimen Processing Procedure. The COBAS AmpliScreen HIV-1 Test, v1.5 detected HIV-1 RNA earlier than Abbott

HIV 1/2 antibody test in 39 of the 41 panels, using both the MultiPrep and Standard Specimen Processing Procedures.

The COBAS AmpliScreen HIV-1 Test, v1.5 detected HIV-1 RNA a mean of 12.8 days (median 11 days, minimum 0 days and maximum of 89 days) before HIV 1/2 antibody using the MultiPrep Specimen Processing procedure and a mean of 14.2 days (median 12 days, minimum 0 days and maximum of 89 days) before HIV 1/2 antibody when using the Standard Specimen Processing Procedure. The data are presented in Tables 9 and 10.

The COBAS AmpliScreen HIV-1 Test, v1.5 was also compared to the licensed HIV-1 p24 antigen assays (Abbott and Coulter). Forty of the 41 panels contained specimens collected before the antigenemia "ramp up" phase, and were used to assess the effectiveness of the COBAS AmpliScreen HIV-1 Test, v1.5 in closing the pre-seroconversion window period, as compared to licensed Abbott HIV-1 p24 antigen assays (due to limited volume, only 38 panels were tested with the licensed Coulter HIV-1 p24 antigen test). In every instance where HIV-1 p24 antigen is detected, HIV-1 RNA was also detected in the same specimen time point. In some panels, HIV-1 RNA was detected before HIV-1 p24 antigen.

COBAS AmpliScreen HIV-1 Test, v1.5 detected HIV-1 RNA a mean of 4.4 to 6.8 days before the licensed HIV-1 p24 antigen tests using the MultiPrep Specimen Processing procedure and a mean of 5.8 to 8.3 days before the licensed HIV-1 p24 antigen tests when using the Standard Specimen Processing Procedure. The data are presented in Tables 9 and 10.

Table 9
Summary of the Pre-Seroconversion Detection of
HIV-1 RNA vs. HIV-1/2 Antibody and HIV-1 p24 Antigen Assays - MultiPrep Specimen Processing Procedure

	Days Before HIV-1/2 Antibody (41 Panels Tested)	Days Before Abbott p24 Antigen (40 Panels Tested)	Days Before Coulter p24 Antigen (38 Panels Tested)
Mean	12.8	6.8	4.4
Median	11	5	3.5
Maximum	89*	32	28
Minimum	0	0	0

** For one panel, the time interval between sampling was 80 days.*

Table 10
Summary of the Pre-Seroconversion Detection of
HIV-1 RNA vs. HIV-1/2 Antibody and HIV-1 p24 Antigen Assays - Standard Specimen Processing Procedure

	Days Before HIV-1/2 Antibody (41 Panels Tested)	Days Before Abbott p24 Antigen (40 Panels Tested)	Days Before Coulter p24 Antigen (38 Panels Tested)
Mean	14.2	8.3	5.8
Median	12	7	5
Maximum	89*	32	28
Minimum	0	0	0

** For one panel, the time interval between sampling was 80 days*

Dilutional Sensitivity with Weakly Reactive HIV-1 p24 Antigen Samples

Twenty-five HIV-1 p24 antigen weakly positive (S/CO 1.00 to 3.7 using a licensed HIV-1 p24 EIA) samples were evaluated. These were diluted with HIV-1 negative plasma to 5,000 copies/mL and further diluted 1:24 to represent the Primary Pool. The HIV-1 RNA copy numbers were determined by a commercially available HIV-1 quantitative assay (Roche's AMPLICOR HIV-1 MONITOR™ Test). The final viral concentration was approximately 208 copies/mL. In addition, another set was diluted to 100 copies/mL. All 25 samples tested at 5,000 copies/mL were negative for HIV-1 p24 antigen. All 25 samples tested with COBAS AmpliScreen HIV-1 Test, v1.5 at the 1:24 dilution of the 5,000 copies/mL (208 copies/mL) and all 25 samples tested at 100 copies/mL were positive for HIV-1 RNA.

Dilutional Sensitivity with Weakly Reactive HIV-1 Antibody Positive Samples

Twenty-five known HIV-1 seropositive specimens were diluted to Signal/Cutoff (S/CO) levels between 1 and 5 and tested using a licensed HIV-1 antibody assay (Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA). These weakly reactive, seropositive samples were then singly introduced into pools with 23 negative plasma samples in random fashion. An additional 144 negative plasma tubes were used to make six negative pools and randomly distributed as discrete sets among the 25 positive pools for testing. A total of 744 samples were tested according to the COBAS AmpliScreen test algorithm. NAT-positive specimens were deconstructed and resolved to the individual sample. Of the 25 weakly-reactive serologically positive samples, a total of 19 were concordant positive and six were discordant negative in the COBAS AmpliScreen HIV-1 Test, v1.5.

Each of the six discordant NAT-negative samples was subject to viral load determination by Roche's quantitative PCR assay, AMPLICOR HIV-1 MONITOR Test, v1.5. Five of the six discordant NAT negative samples were observed to have less than 100 copies/mL HIV-1 RNA, and one had a mean titer of 100 copies/mL. Because each of these samples, when diluted 24-fold, would not be expected to be reliably detected in 24-membered minipools, they were removed from the sensitivity calculation. Therefore, the overall observed sensitivity of the COBAS AmpliScreen HIV-1 Test, v1.5, in this study was 100.0%.

Analytical Specificity - Potentially Cross Reactive and Interfering Microorganisms

The analytical specificity of the COBAS AmpliScreen HIV-1 Test, v1.5 was evaluated by testing a panel of microorganisms and other disease states, including 21 viral isolates, five bacterial strains and one yeast isolate. No-cross reactivity was observed with the COBAS AmpliScreen HIV-1 Test, v1.5. Table 11, below summarizes the microorganisms studied.

Table 11
Analytical Specificity - Microorganisms and Disease States Tested

Adenovirus type 2	Epstein Barr Virus	Human Papilloma Virus, Type 16
Adenovirus type 3	Hepatitis A Virus	Human Papilloma Virus, Type 18
Adenovirus type 7	Hepatitis B Virus	HTLV-I
Autoimmune samples	Hepatitis C Virus	HTLV-II
Burkitt's Lymphoma	Herpes Simplex type 1	*Neisseria gonorrhoeae*
Candida albicans	Herpes Simplex type 2	*Propionibacterium acnes*
Chlamydia trachomatis	HIV-2	*Staphylococcus aureus*
Coxsackievirus B1	Human Herpes Virus 6	*Staphylococcus epidermidis*
Cytomegalovirus	Human Herpes Virus 7	Varicella-Zoster
Echovirus 1, 5	Human Papilloma Virus, Type 6a	

Up to 25 individual patient plasma specimens from each of the following disease categories were spiked with low levels of HIV-1 positive plasma: HAV, HBV, HCV, HIV-2, autoimmune disease, EBV, CMV, and *Candida albicans*. No false negative test results were observed.

Analytical Specificity - Non-HIV-1 Samples

Up to 25 individual patient plasma specimens (all HIV-1 negative) from each of the following disease categories: HAV, HBV, HCV, HIV-2, autoimmune disease, EBV, CMV, and *Candida albicans*, were tested with COBAS AmpliScreen HIV-1 Test, v1.5 by using both MultiPrep and Standard Specimen Processing Procedures. All samples were found to be negative. No false positive test results were observed.

Potentially Interfering Substances

Endogenous Interfering Substances

HIV-1 spiked and non-spiked plasma samples derived from whole blood containing abnormally high concentrations of bilirubin (up to 20 mg/mL), triglycerides (up to 3000 mg/dL), hemoglobin (up to 1.0 g/dL), and albumin (up to 6 g/dL) were tested. These endogenous substances did not interfere with the sensitivity or specificity of the COBAS AmpliScreen HIV-1 Test, v1.5 using either the MultiPrep or Standard Specimen Processing Procedures.

Exogenous Interfering Substances

HIV-1 spiked and non-spiked plasma samples derived from whole blood containing abnormally high concentrations of aspirin (up to 50 mg/mL), pseudoephedrine-HCl (up to 3 mg/dL), ascorbic acid (up to 20 mg/dL), acetaminophen (up to 40 mg/dL), or ibuprofen (up to 40 mg/dL) were tested. These exogenous substances did not interfere with the sensitivity or specificity of the COBAS AmpliScreen HIV-1 Test, v1.5 using either the MultiPrep or Standard Specimen Processing Procedures.

PERFORMANCE CHARACTERISTICS FOR CADAVERIC SPECIMENS

Sensitivity Study

Sixty pre-mortem EDTA plasma and 58 cadaveric specimens non-reactive for HIV-1 were divided into 5 groups. Specimens within each group were spiked with HIV-1 viral target to a concentration of 3X the LOD using a different clinical viral isolate for each group. The spiked specimens were equally divided and tested between three COBAS AmpliScreen HIV-1 Test v1.5 Kit lots.

The COBAS AmpliScreen HIV-1 Test, v1.5, using samples diluted 1:5 and the MultiPrep Specimen Processing Procedure, correctly detected 100% (60/60) pre-mortem EDTA plasma specimens and 94.8% (55/58) of cadaveric specimens spiked with HIV-1 RNA at 3X the LOD of the COBAS AmpliScreen HIV-1 Test, v1.5. The results reflect retesting one cadaveric specimen that was inhibited on initial testing. In repeat testing, the specimen resolved negative. The summary of the final test results of this study is presented in Table 12 below.

Table 12
Summary of Sensitivity Test Results

		Pre-Mortem EDTA Plasma Specimen	Post-Mortem EDTA Plasma Specimen
	Replicates	60	58
Test Results	+	60	55
	-	0	3
	Inhib.	0	0
	Sensitivity	100%	94.8%
95% Confidence Interval	Upper	100%	98.9%
	Lower	94.0%	85.6%

Specificity Study

Sixty pre-mortem and 58 post-mortem specimens that were negative for HIV-1 RNA were divided into three groups, diluted 1:5 in MP DIL, processed using the MultiPrep Specimen Processing Procedure, and tested between 3 lots of the COBAS AmpliScreen HIV-1 Test, v1.5.

The MultiPrep Specimen Processing Procedure detected 96.7% (58/60) of all negative specimens in pre-mortem EDTA plasma specimens, and 100% (57/57) in post-mortem EDTA plasma specimens. One post-mortem EDTA plasma specimen exhibited inhibition on initial and repeat testing. The summary of results of this test is presented in Table 13 below.

Table 13
Summary of Specificity Test Results

		Pre-Mortem EDTA Plasma Specimen	Post-Mortem EDTA Plasma Specimen
	Total Specimens Tested	60	58
Test Results	+	2*	0
	-	58	57
	Inhib.	0	1
	Final Specificity	96.7%	100%
95% Confidence Interval	Upper	100%	100%
	Lower	94.0%	90.8%

* Two pre-mortem specimens found initially reactive were negative upon repeat testing.

Reproducibility Study

Twenty pre-mortem EDTA plasma and 20 individual cadaveric specimens were spiked with HIV-1 viral target using a secondary standard to a final concentration of 3X the LOD. Each of the 20 pre- and post-mortem specimens were tested using three different COBAS AmpliScreen HIV-1 v1.5 Test kit lots at three different testing sites in this study. At each testing site, each specimen was tested singly in two separate runs using each of the three different kit lots (total of six valid test results for each specimen at each site). There were a total of 18 valid test results (six results per site x 3 testing sites) for each specimen.

All valid reproducibility data for post-mortem and pre-mortem specimens were evaluated by calculating the percentage of correct results for each assay. The data were analyzed by lot and by testing site. The summary of results of the reproducibility study test is presented in Table 14 below.

Table 14
Summary of Reproducibility Study Test Results — Post-Mortem versus Pre-Mortem

	Post-Mortem	Pre-Mortem
Results by Lot (# Positive / # Tested, Percent Hit Rate)		
Lot # 1	118/120 98.3%	119/120 99.2%
Lot # 2	120/120 100%	119/119 100%
Lot # 3	116/120 96.7%	119/120 99.2%
Results by Site (# Positive / # Tested, Percent Hit Rate)		
Site # 1	114/120 95.0%	118/120 98.3%
Site # 2	120/120 100%	120/120 100%
Site # 3	120/120 100%	119/119 100%

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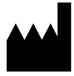

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