COBAS® AmpliScreen HIV-1 Test, version 1.5

FOR IN VITRO DIAGNOSTIC USE.

COBAS® AmpliScreen HIV-1 Test, version 1.5
96 Tests P/N: 03322114 018

COBAS® AmpliScreen Multiprep Specimen Preparation and Control Kit
96 Tests P/N: 03302555 018

COBAS® AMPLICOR® Wash Buffer
500 Tests P/N: 20759899 123
ART: 07 5999 9
US: 8314

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.

The COBAS® AmpliScreen HIV-1 Test, version 1.5 (v1.5) is intended to be used for detection of HIV-1 RNA in conjunction with licensed tests for detecting antibodies to HIV-1. This product is intended for use as a donor screening test to detect HIV-1 RNA in plasma samples from individual human donors, including donors of Whole Blood and blood components. Source Plasma and other living donors. It is also intended for use to screen organ donors when 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 test is not intended for use on samples of cord blood. This test is not intended for use as an aid in diagnosis. Plasma from all donors may be screened as individual samples. For donations of Whole Blood and blood components, plasma may be tested in pools comprised of equal aliquots of not more than 24 individual donations. For donations of Source Plasma, plasma may be tested in pools comprised of equal aliquots of not more than 96 individual donations.

This assay may be used as an alternative to licensed HIV-1 p24 antigen tests for screening human plasma from donors. For testing of cadaveric specimens, the specimen should be first diluted 1:5 in Multiprep Specimen Diluent (MP DIL) prior to

NOTE: For testing of cadaveric specimens, the specimen should be first diluted 1:5 in Multiprep Specimen Diluent (MP DIL) prior to

PRINCIPLES OF THE PROCEDURE

The COBAS® AmpliScreen HIV-1 Test, version 1.5 is based on five major processes:

1. Sample Processing
2. Reverse transcription of target RNA to generate complementary DNA (cDNA)
3. PCR amplification 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 specimen processing procedures are used with the COBAS® AmpliScreen HIV-1 Test, v1.5 as follows:

- Multiprep Specimen Processing Procedure for preparation of mini-pool specimens and individual cadaveric specimens
- Standard Sample Processing for preparation of individual 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 a 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 (Tth pol). In the presence of manganese (Mn²⁺) and under the appropriate buffer conditions, rTth pol has both reverse transcriptase and DNA polymerase activity. This allows both reverse transcription and PCR amplification to occur in the same reaction mixture. Reverse transcription using rTth pol produces a cDNA copy of the HIV-1 target copy of the 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 Mn²⁺ and excess deoxynucleotide triphosphates (dNTPs), the rTth 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 (ucr-1-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, but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always pre-
sent in ampiclon because of the use of deoxyuridine triphosphate in place of deoxothymidine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only ampiclon contain deoxyuridine. Deoxyuridine renders contaminating ampiclon 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 ampiclon. Following amplification, any residual enzyme is denatured by the addition of the Denaturation Solution, thereby preventing the degradation of any target ampiclon.

Hybridization Reaction
Following PCR amplification, the COBAS® AMPLICOR® Analyzer automatically adds Denaturation Solution to the A-tubes to chemically denature the HIV-1 target ampiclon and the HIV-1 Internal Control ampiclon to form single-stranded DNA. Aliquots of denatured ampiclon are then transferred to two detection cups (D-cups). A suspension of magnetic particles coated with an oligonucleotide probe specific for HIV-1 target ampiclon or HIV-1 Internal Control ampiclon is added to the individual D-cups. The biotin-labeled HIV-1 target and HIV-1 Internal Control ampiclon are hybridized to the target-specific oligonucleotide probes bound to the magnetic particles. This hybridization of ampiclon 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 ampiclon. 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 the COBAS® AmpliScreen HCV Test, v2.0, and the COBAS® AmpliScreen HBV HIV Test.

MATERIALS REQUIRED BUT NOT PROVIDED BY ROCHE

Other materials required but sold separately (may be purchased from Roche).

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

- COBAS® AMPLICOR® Analyzer with software version 0022B, Printer, and Operator’s Manual for the COBAS® AMPLICOR® Analyzer
- COBAS® AMPLICOR® Master Mix, version 1.5
- AMPULINK Software, version 1.4 and Operator’s Manual for the AMPULINK software
- Hamilton MICROLAB® AT plus 2 Pipettor (with Hamilton SUNPLUS and RUNENDE Software, and the Roche Pooling Methods Software, version 1.3), the COBAS® AmpliScreen Pooling System Guide (Roche Pooling Methods Software, version 1.3) and the COBAS® AmpliScreen Pooling System Guide are validated to prepare pools of equal aliquots of not more than 24 individual plasma donations using Hamilton MICROLAB AT plus pipettor with Hamilton SUNPLUS and RUNENDE Software
- Additional MP Dil from the COBAS® AmpliScreen Multiprep Specimen Preparation and Control Kit is required for testing of cadaveric specimens

NOTE: The user must validate all pooling algorithms and equipment other than those supplied by Roche.

OTHER M ETHODS REQUESTED BUT NOT PROVIDED BY ROCHE

- 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

MATERIALS REQUIRED BUT NOT PROVIDED BY ROCHE
- 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

<table>
<thead>
<tr>
<th>Kit</th>
<th>Description</th>
<th>Volume</th>
<th>Test</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP (-) C</td>
<td>[Multiprep Negative (-) Control] &lt; 0.005% Poly rA RNA (synthetic) EDTA</td>
<td>8 x 0.1 mL</td>
<td>96 Tests</td>
<td>0.05% Sodium azide</td>
</tr>
</tbody>
</table>
| MP (+) C | [Multiprep Positive (+) Control] Tris-HCl buffer                           | 8 x 0.1 mL | 96 Tests | < 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 |

#### COBAS® AmpliScreen HIV-1 Test, version 1.5

<table>
<thead>
<tr>
<th>Kit</th>
<th>Description</th>
<th>Volume</th>
<th>Test</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 MMX, v1.5</td>
<td>(HIV-1 Master Mix, version 1.5) Bicine buffer Glycerol</td>
<td>8 x 0.7 mL</td>
<td>96 Tests</td>
<td>&lt; 0.01% rTth DNA Polymerase (rTth pol, microbial) Potassium acetate &lt; 0.07% dATP, dCTP, dGTP, dUTP dTTP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.001% SKCC1B and SK145 biotinylated primers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial) 0.05% Sodium azide</td>
</tr>
</tbody>
</table>

#### COBAS® AmpliScreen HIV-1 Detection Reagents, version 1.5

<table>
<thead>
<tr>
<th>Kit</th>
<th>Description</th>
<th>Volume</th>
<th>Test</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV DK</td>
<td>(HIV-1 Probe Suspension 1, version 1.5) MES buffer</td>
<td>8 x 0.1 mL</td>
<td>100 Tests</td>
<td>&lt; 0.01% Suspension of Dynabeads® (paramagnetic particles) coated with HIV-1-specific oligonucleotide capture probe SK102 0.09% Sodium azide</td>
</tr>
</tbody>
</table>

#### COBAS® AmpliScreen Multiprep Specimen Diluent

<table>
<thead>
<tr>
<th>Kit</th>
<th>Description</th>
<th>Volume</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-HCl buffer</td>
<td>&lt; 0.005% Poly rA RNA (synthetic) EDTA</td>
<td>8 x 4.8 mL</td>
<td>0.05% Sodium azide</td>
</tr>
</tbody>
</table>

#### COBAS® AmpliScreen Multiprep Internal Control

<table>
<thead>
<tr>
<th>Kit</th>
<th>Description</th>
<th>Volume</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-HCl buffer</td>
<td>&lt; 0.001% Non-infectious plasmid DNA containing HBV primer binding sequences and a unique probe binding region</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 0.001% Non-infectious in vitro transcribed RNA containing HCV primer binding sequences and a unique probe binding region</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 0.001% Non-infectious in vitro transcribed RNA containing HIV-1 primer binding sequences and a unique probe binding region</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 0.005% Poly rA RNA (synthetic) EDTA</td>
<td>8 x 0.1 mL</td>
<td>0.05% Sodium azide</td>
</tr>
</tbody>
</table>

#### COBAS® AmpliScreen Multiprep Lysis Reagent

<table>
<thead>
<tr>
<th>Kit</th>
<th>Description</th>
<th>Volume</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-HCl buffer</td>
<td>60% Guanidine thiocyanate 3% Dithiothreitol 1% Glycogen Xn</td>
<td>8 x 9.0 mL</td>
<td>Harmful</td>
</tr>
</tbody>
</table>

#### COBAS® AmpliScreen Multiprep Lysis Reagent

<table>
<thead>
<tr>
<th>Kit</th>
<th>Description</th>
<th>Volume</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% ProClin® 300 preservative</td>
<td>&lt; 0.005% Poly rA RNA (synthetic) EDTA</td>
<td>8 x 0.1 mL</td>
<td>0.05% Sodium azide</td>
</tr>
</tbody>
</table>

#### COBAS® AmpliScreen Multiprep Lysis Reagent

<table>
<thead>
<tr>
<th>Kit</th>
<th>Description</th>
<th>Volume</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05% Sodium azide</td>
<td>&lt; 0.01% Suspension of Dynabeads® (paramagnetic particles) coated with HIV-1-specific oligonucleotide capture probe SK102</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### COBAS® AmpliScreen Multiprep Lysis Reagent

<table>
<thead>
<tr>
<th>Kit</th>
<th>Description</th>
<th>Volume</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.09% Sodium azide</td>
<td>&lt; 0.01% Suspension of Dynabeads® (paramagnetic particles) coated with HIV-1-specific oligonucleotide capture probe SK102</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
IH4, v1.5
(HIV-1 Probe Suspension 2, version 1.5)
- Sodium phosphate buffer
- 24.9% Sodium thiocyanate
- 0.2% Solubilizer

II PS1, v1.5
(IC Probe Suspension 1)
- MES buffer
- < 0.01% Suspension of Dynabeads (paramagnetic particles) coated with HIV-1 IC-specific oligonucleotide capture probe CP35
- 0.09% Sodium azide

II 4, v1.5
(IC Probe Suspension 2)
- Sodium phosphate buffer
- 24.9% Sodium thiocyanate
- < 0.2% Solubilizer

DN4
(Denaturation Solution)
- 1.6% Sodium hydroxide
- EDTA
- Thymol blue
- XI 1.6% (w/w) Sodium hydroxide

CN4
(Avidin-Horseradish Peroxidase Conjugate)
- Tris-HCl buffer
- < 0.001% Avidin-horseradish peroxidase conjugate
- Bovine serum albumin (mammalian)
- Emulsit 25 (Da-i-chi Kogyo Selyaku Co., Ltd.)
- 0.1% Phenol
- 1% ProClin® 150 preservative

SB3
(Substrate A)
- Citrate solution
- 0.01% Hydrogen peroxide
- 0.1% ProClin® 150 preservative

SB
(Substrate B)
- 0.1% 3,3’,5,5’-Tetramethylbenzidine (TMB)
- 40% Dimethylformamide (DMF)

SB3 and SB

DN4 and WB

PRECAUTIONS
FOR IN VITRO DIAGNOSTIC USE.

A. Specimens may be infectious. Use Universal Precautions when performing the assay. Only personnel proficient in the use of the COBAS® AmpliScreen 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.

CAUTION: The Negative Human Plasma (NHP) of this kit contains human blood products non-reactive by US FDA licensed tests for antibody to HIV-1/2, antibody to HCV, HIV-1 p24 antigen and HBsAg. 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.
B. Working Lysis Reagent

Working Probe Suspension Detection Reagents

Wash Buffer Reagent

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

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.

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

SPECIMEN COLLECTION, STORAGE AND POOLING

H. 70% Ethanol

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 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.

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.

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

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.

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

H. 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.

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

E. DN4 – Denaturation Reagent and CN4 Conjugate Reagent

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.

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

B. Working Lysis Reagent

A. 70% Ethanol

1. Prepare 70% ethanol fresh daily.

2. Store at 2-8°C and use within 4 hours of preparation.

3. Do not expose Working Lysis Reagent to room temperature before use by using a 37°C incubator or on the laboratory bench top.

A. 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.

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.


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.

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.


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 into one IH PS1, v1.5 cassette and store in the refrigerator at 2-8°C. If these reagents are spilled, dilute with water before use and use within 4 hours of preparation.

2. Prepare Working HIV-1 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 IH PS1, v1.5 cassette and store in the refrigerator at 2-8°C. If these reagents are spilled, dilute with water before use and use within 4 hours of preparation.

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 six 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, DH and CN4 are stable for 30 days at 2-8°C, or until the expiration date, whichever comes first. Both DH and CN4 can be used for a maximum of six instrument cycles (12 hours per cycle).

2. Store DH 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, and store Working Substrate in the refrigerator at 2-8°C for a maximum of 16 hours.

2. 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 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 2-6°C. 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 1-24°C. 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 ≤-18°C for up to one month.

D. ACD-A or 4% sodium citrate anticoagulated apheresis plasma can be stored at 1-6°C for up to 6 hours, followed by subsequent storage at ≤-18°C 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. SPECIMEN POOLING:

NOTE: Pooling of specimens should only be performed on individual whole blood and source plasma donations. Blood specimens and cadaveric specimens collected to screen organ and tissue donors must be tested individually and not as part of a pool.

1. The Pooling System for use with the COBAS® AmpliScreen test performs barcode scanning and pooling operations that combine aliquots from 24 individual samples into a single Primary Pool that is used for testing. The pooling algorithm requires preparation of Secondary Pools as well as individual specimens for follow-up testing in the event a Primary Pool tests positive. If less than 24 specimens are available, testing is performed using the individual specimens.

2. For Source Plasma, the Hamilton performs barcode scanning and pooling operations that combine aliquots from 96 individual samples into a single Primary Pool that is used for testing. Positive Primary Pools are traced to the positive individual using an overlapping pooling matrix. Minipools are prepared from the eight individual donations for columns 1 - 12 and from the 12 individual donations for rows 1 - 8. The positive unit is identified by the intersection of the positive column and positive row. Confirmatory testing is conducted on the implicated unit using Standard Specimen Processing Procedure. (Hamilton MICROLAB® AT plus 2 Pipettor with SUNRISE PLUS v3.3 software was used to prepare pools of up to 96 equal aliquots of plasma during clinical trials).

NOTE: The user must validate other pooling algorithms and equipment other than those supplied by Roche.

Cadaveric Blood Specimens

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

NOTE: You may test either a serum or EDTA plasma specimen collected pre-mortem from a cadaveric organ/tissue donor using test procedures for cadaveric donors.

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

NOTE: Cadaveric samples 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 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 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 MinP², 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. All reagents for use with the pipettor capable of delivering sufficient 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.
INSTRUCTIONS FOR USE

3. Confirm that all pipettors are correctly set to dispense the specified volumes in accordance with the specimen preparation procedures and guidelines.

2. Use a clean pipette tip or disposable transfer pipette with each specimen or control. Use aerosol barrier or positive displacement RNase-free tips.

1. Pooled or individual plasma specimens must be at room temperature before pipetting.

1. 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.

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, begin with Step A (Reagent Preparation – Working Master Mix), thaw processed specimens and controls at room temperature, the subsequent day, begin with Step A (Reagent Preparation - Working Master Mix), thaw processed specimens and controls at room temperature, and control preparation area must be cleaned and disinfected in accordance with methods outlined in "Precautions" (Item A).

Amplification, Hybridization and Detection of 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.


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.


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.


B3. For each Negative and Positive Control pipette 1000 µL NHP into an appropriately labeled screw-cap tube. 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. Proceed to Step B2.

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.


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.
C. Reverse Transcription, Amplification and Detection

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.

b. Positive 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.

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

B15. 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.

B16. 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. Use a new transfer pipette for each tube.

B17. Place the tubes into a microcentrifuge and 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.

B18. 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 10-15 minutes at room temperature.

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. Use a new transfer pipette for each tube.

B20. 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.

B21. 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. Note that some insoluble material may remain.

B22. Place the tubes into a microcentrifuge and 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.

B23. 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.

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.


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 into 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 to resuspend the extracted RNA. Note that some insoluble material may remain.

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. Use a new transfer pipette for each tube.

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.

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 the 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 (II PS1, v1.5), Working Substrate (SB3), Denaturation Reagent (DN4), and Conjugate Reagent (CN4).

b. Place the IH4, v1.5 and II PS1, 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. 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 COBAS® AMPLICOR® Analyzer barcode scanner for 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.
j. Repeat steps h. through i. 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.

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 an External Control (i.e., an additional run control other than the Multiprep (-) Control or Multiprep (+) Control) is required by the laboratory, the External Control should meet regulatory requirements for such controls. The absorbance of the HIV-1 External Control should be equal to 0.2 at 660 nm and its associated MP IC should be less than or equal to 0.2, the entire A-ring is invalid, and the entire test procedure for that A-ring (sample and control preparation, amplification and detection) must be repeated.

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 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 (sample 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 (sample and control preparation, amplification and detection) must be repeated.

   HIV-1 Result IC Result
<table>
<thead>
<tr>
<th>A660 Comment</th>
<th>A660 Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.2</td>
<td>Negative</td>
</tr>
<tr>
<td>≥ 0.2</td>
<td>Positive</td>
</tr>
</tbody>
</table>

   Interpretation
   - < 0.2 NEGATIVE ≥ 0.2 VALID Specimen is negative for HIV-1 RNA.
   - < 0.2 NEGATIVE < 0.2 INVALID Ininvalid 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 failure, an aliquot obtained 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.

Summary of Control Acceptance Criteria

<table>
<thead>
<tr>
<th>HIV-1 Result</th>
<th>IC Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>A660 Comment</td>
<td>A660 Comment</td>
</tr>
<tr>
<td>&lt; 0.2</td>
<td>Negative</td>
</tr>
<tr>
<td>≥ 0.2</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Interpretation
- < 0.2 NEGATIVE ≥ 0.2 VALID Specimen is negative for HIV-1 RNA.
- < 0.2 NEGATIVE < 0.2 INVALID Ininvalid result. Repeat entire test procedure for invalid specimen.
- ≥ 0.2 POSITIVE ANY VALID Specimen is positive for HIV-1 RNA.
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 and one HIV-1 negative sample. Panel Two was tested using the Standard concentrations of HIV-1. Panel One was tested using the Multiprep Specimen Processing Procedure. Panel One was comprised of HIV-1 RNA positive blinded fashion.

Though rare, mutations within the highly conserved region of the viral genome covered by the COBAS® AmpliScreen HIV-1 Test, v1.5 primers is reported as "HIV-1 RNA Negative." If both replicates are invalid, it is most likely due to inhibitory substances in the specimen, and the results should be marked as invalid or unresolved.

For 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 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.

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 and three levels of testing (Primary Pool, Secondary Pool and tertiary resolution) for Primary Pools that are positive for HIV-1 RNA.

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.

The samples are released as negative if the results of the duplicate testing are negative.

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."
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 Table 1 and 2 below:

### Table 1
Reproducibility Results - Multiprep Specimen Processing Procedure

<table>
<thead>
<tr>
<th>Results By Lot (# Positive / # Tested)</th>
<th>Negative 10 c/mL</th>
<th>25 c/mL</th>
<th>50 c/mL</th>
<th>75 c/mL</th>
<th>25,000 c/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot #1</td>
<td>1/88 (1%)</td>
<td>51/90 (57%)</td>
<td>77/90 (86%)</td>
<td>86/90 (96%)</td>
<td>89/90 (100%)</td>
</tr>
<tr>
<td>Lot #2</td>
<td>0/89 (0%)</td>
<td>47/90 (52%)</td>
<td>72/90 (80%)</td>
<td>83/90 (92%)</td>
<td>86/90 (96%)</td>
</tr>
<tr>
<td>Lot #3</td>
<td>2/90 (2%)</td>
<td>50/89 (56%)</td>
<td>80/89 (90%)</td>
<td>88/89 (99%)</td>
<td>88/90 (98%)</td>
</tr>
<tr>
<td>Lot #4</td>
<td>0/90 (0%)</td>
<td>45/90 (50%)</td>
<td>78/90 (87%)</td>
<td>84/90 (93%)</td>
<td>90/90 (100%)</td>
</tr>
<tr>
<td>Lot #5</td>
<td>0/89 (0%)</td>
<td>51/89 (57%)</td>
<td>73/89 (82%)</td>
<td>83/90 (92%)</td>
<td>90/90 (100%)</td>
</tr>
</tbody>
</table>

### Table 2
Reproducibility Results - Standard Specimen Processing Procedure

<table>
<thead>
<tr>
<th>Results By Site (# Positive / # Tested)</th>
<th>Negative 10 c/mL</th>
<th>25 c/mL</th>
<th>50 c/mL</th>
<th>75 c/mL</th>
<th>25,000 c/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site #1</td>
<td>3/150 (2%)</td>
<td>72/150 (48%)</td>
<td>133/150 (89%)</td>
<td>142/150 (95%)</td>
<td>149/150 (99%)</td>
</tr>
<tr>
<td>Site #2</td>
<td>0/147 (0%)</td>
<td>82/148 (55%)</td>
<td>108/148 (73%)</td>
<td>136/149 (91%)</td>
<td>146/149 (98%)</td>
</tr>
<tr>
<td>Site #3</td>
<td>0/149 (0%)</td>
<td>90/150 (60%)</td>
<td>139/150 (83%)</td>
<td>146/150 (97%)</td>
<td>150/150 (100%)</td>
</tr>
</tbody>
</table>

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 titers of each specimen were quantitated with 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 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% 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.

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.

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

<table>
<thead>
<tr>
<th>HIV-1 RNA Concentration (c/mL)</th>
<th>Number of Positives</th>
<th>Number of Individual Tests</th>
<th>% Positive</th>
<th>95% Lower Confidence Limit – One-Tailed</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>220</td>
<td>220</td>
<td>100.0%</td>
<td>98.6%</td>
</tr>
<tr>
<td>50</td>
<td>214</td>
<td>217</td>
<td>98.6%</td>
<td>96.5%</td>
</tr>
<tr>
<td>16.7</td>
<td>116</td>
<td>219</td>
<td>53.0%</td>
<td>47.2%</td>
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</tbody>
</table>
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. 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 284.3 IU/mL and 387.3 IU/mL, respectively.

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

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

<table>
<thead>
<tr>
<th>HIV-1 RNA Concentration (c/mL)</th>
<th>Number of Positives</th>
<th>Number of Individual Tests</th>
<th>% Positive</th>
<th>95% Lower Confidence Limit – One-Tailed</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>216</td>
<td>218</td>
<td>99.1%</td>
<td>97.1%</td>
</tr>
<tr>
<td>100</td>
<td>216</td>
<td>219</td>
<td>98.6%</td>
<td>96.5%</td>
</tr>
<tr>
<td>33.3</td>
<td>97</td>
<td>217</td>
<td>44.7%</td>
<td>39.0%</td>
</tr>
</tbody>
</table>

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)

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

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)

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

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

<table>
<thead>
<tr>
<th>Group / Subtype Detectability</th>
<th>A1 250,000</th>
<th>A2 25,000</th>
<th>A3 1,000</th>
<th>A4 100</th>
<th>A5 0</th>
<th>B1 2,500</th>
<th>B2 10</th>
<th>B3 250,000</th>
<th>B4 0</th>
<th>B5 100</th>
<th>B6 50</th>
<th>B7 25,000</th>
<th>B8 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiprep Method</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Standard Prep Method</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>0%</td>
<td>100%</td>
<td>75%</td>
<td>100%</td>
<td>0%</td>
</tr>
</tbody>
</table>

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.
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 assay 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 8

<table>
<thead>
<tr>
<th>Group</th>
<th>Subtype</th>
<th>Quantity</th>
<th>Reactive Total (Multiprep)</th>
<th>Reactive Total (Standard Prep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>A</td>
<td>20</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>20</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>20</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>20</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>20</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>4</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>O†</td>
<td>N/A</td>
<td>8</td>
<td>5/8</td>
<td>5/8</td>
</tr>
<tr>
<td>N*</td>
<td>N/A</td>
<td>1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
</tbody>
</table>

* 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.

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

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>12.8</td>
<td>6.8</td>
</tr>
<tr>
<td>Median</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Maximum</td>
<td>89†</td>
<td>32</td>
</tr>
<tr>
<td>Minimum</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>14.2</td>
<td>8.3</td>
</tr>
<tr>
<td>Median</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Maximum</td>
<td>89†</td>
<td>32</td>
</tr>
<tr>
<td>Minimum</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* 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 tubes 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 mini-pools, 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.
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 Sample 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/dL), 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 relative to antibody-negative status in this study.

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), acetic acid (up to 20 mg/dL), acetylsalicylic acid (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 Sample Processing Procedures.

CLINICAL PERFORMANCE

AIDS and HIV-1 Asymptomatic Populations

Seropositive samples from 217 patients diagnosed with AIDS and seropositive samples from 764 HIV-1 asymptomatic patients were randomly intermixed with 1,399 negative plasma samples. These 2,400 samples were used to create 100 Primary Pools that contained on average 10 positive and 14 negative samples. In addition, 600 negative samples were used to create 25 negative Primary Pools. This resulted in 125 panels, each representing a Primary Pool comprised of 24 sample tubes, (20 panels containing AIDS samples, 80 panels containing asymptomatic samples, and 25 negative panels). These panels were pooled using the Hamilton MICROLAB AT plus and tested with the COBAS® AmpliScreen HIV-1 Test, v1.5. Primary, Secondary and Tertiary testing were performed at the clinical sites. If discordant results between the Primary Pool test result and either the Secondary or Tertiary testing were observed at the sites, resolution testing was performed at Roche.

A summary of the testing performed at the clinical sites is provided in Tables 12 and 13. There was a total of 23 HIV-1 antibody positive specimens that resulted in one or more HIV-1 RNA positive primary pools. All were found to be negative in either the secondary or tertiary testing at the clinical sites. Of these 23 specimens, 9 tested negative at the secondary pool level in 5 different secondary pools at a single clinical site and 14 tested negative by tertiary testing. The results of the resolution testing performed at Roche yielded 21 of 23 specimens that were resolved as HIV-1 RNA positive with the COBAS® AmpliScreen HIV-1 Test, v1.5. A summary of the testing after resolution at Roche is provided in Table 13. Sensitivity and specificity were based upon the final resolution status of all samples. Individual specimens known to contain less than 100 copies/mL were not included in the sensitivity calculation. The sensitivity of the COBAS® AmpliScreen HIV-1 Test, v1.5 relative to antibody-negative status in this study was determined to be 99.7% with the 95% confidence interval ranging from 99.1% to 100%. The specificity of the COBAS® AmpliScreen HIV-1 Test, v1.5 relative to antibody-negative status in this study was determined to be 98.9% with the 95% confidence interval ranging from 98.3% to 99.3%.

Table 11

<table>
<thead>
<tr>
<th>Analytical Specificity - Microorganisms and Disease States Tested</th>
<th>HIV-1 Antibody</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus type 2</td>
<td>Positive</td>
<td>733</td>
</tr>
<tr>
<td>Epstein Barr Virus</td>
<td>Negative</td>
<td>22</td>
</tr>
<tr>
<td>Human Papilloma Virus, Type 16</td>
<td>Total</td>
<td>755</td>
</tr>
<tr>
<td>Adenovirus type 3</td>
<td>Positive</td>
<td>754</td>
</tr>
<tr>
<td>Hepatitis A Virus</td>
<td>Negative</td>
<td>22</td>
</tr>
<tr>
<td>Human Papilloma Virus, Type 18</td>
<td>Total</td>
<td>776</td>
</tr>
<tr>
<td>Adenovirus type 7</td>
<td>Positive</td>
<td>756</td>
</tr>
<tr>
<td>Hepatitis B Virus</td>
<td>Negative</td>
<td>1977</td>
</tr>
<tr>
<td>Total</td>
<td>1,999</td>
<td>2,755</td>
</tr>
</tbody>
</table>

Table 12

<table>
<thead>
<tr>
<th>Results of HIV-1 Seropositive Specimens Tested at the Clinical Sites (Discordant Specimens &lt; 100 Copies/mL Removed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 Antibody</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>COBAS® AmpliScreen HIV-1 Test, v1.5 Result</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Table 13

<table>
<thead>
<tr>
<th>Results of HIV-1 Seropositive Specimens (Discordant Specimens &lt; 100 Copies/mL Removed) Following Resolution Testing at Roche</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 Antibody</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>COBAS® AmpliScreen HIV-1 Test, v1.5 Result</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

High Risk Population

Specimens were prospectively collected from a population of patients being evaluated at AIDS clinics. Specimens were tested in a blinded fashion in order to identify at least 50 HIV-1 RNA positives with the COBAS® AmpliScreen HIV-1 Test, v1.5 using both the Standard Sample Processing Procedure and the Multiprep Sample Processing Procedure. Specimens for the Multiprep Procedure were diluted 1:24 with Normal Human Plasma. Samples for the Standard Procedure were tested without dilution. Of 374 specimens tested, 55 were found positive for HIV-1 RNA when tested using the Standard procedure and 54 were found positive when tested using the Multiprep procedure. One sample was found to be positive when tested using the Standard Sample Processing Procedure but negative when diluted 1:24 with NHP and tested using the Multiprep Sample Processing Procedure. This sample was confirmed positive by using both HIV-1 p24 antigen and HIV-1 antibody tests, indicating that this sample may be a window period specimen. There were 54 of the 55 specimens that were confirmed positive for HIV-1 antibody by Western Blot. Samples were judged to be NAT/serology concordant if the NAT result was: 1) positive and at least one serologic assay is positive; or 2) negative and serologic assays are both negative. A total of 316 of the 374 samples were negative for HIV-1 antibody. There were three antibody-positive specimens that were negative for HIV-1 RNA using the COBAS® AmpliScreen HIV-1 Test, v1.5. However, these specimens were negative by HIV-1 p24 antigen ELISA, and when tested with a quantitative assay (AMPLICOR® HIV-1 MONITOR Test, v1.5), the titer was below the assay detectable limit. The data are presented in Tables 14 and 15.
A total of 792,055 specimens were selected from geographically divergent sites. The results from these specimens were used to determine the specificity and sensitivity of COBAS® AmpliScreen HIV-1 Test, v1.5. Using the antibody and antigen results, the HIV-1 status of each specimen was determined. HIV-1 status-negative included either: 1) anti-HIV-1 EIA negative and HIV-1 RNA negative (EIA nonreactive or neutralization negative) unless the subject was enrolled in the follow-up study and had test results that changed this assessment, or 2) anti-HIV EIA repeatedly reactive, WB/IFA negative and HIV-1 p24 antigen negative or indeterminate.

HIV-1 status-positive included either: 1) anti-HIV-1 EIA repeatedly reactive, WB/IFA positive, or 2) follow-up study test results of anti-HIV-1 repeatedly reactive or HIV-1 RNA positive. The detection rate of such window period cases was 0.0000002% (1 in 4,000,000). There was one additional confirmed window period case detected. A confirmed window period case is defined as an enrolled individual from whom the index donation was positive in the COBAS® AmpliScreen HIV-1 Test, v1.5 but non-reactive by EIA for HIV-1/2 and a follow-up specimen was shown to be anti-HIV-1 EIA repeatedly reactive and/or HIV-1 RNA positive. The detection rate of such window period cases was 0.0000002% (1 in 4,000,000). There was one additional specimen that was anti-HIV-1 EIA negative, HIV-1 p24 antigen positive and HIV-1 RNA positive; however, this donor was not enrolled in the follow-up study.

A total of 104,448 donations from 35,905 donors were tested in the 96-member minipool format in 1,088 pools. Two donations from 2 donors were reactive with the COBAS® AmpliScreen HIV-1 Test, v1.5 but non-reactive by EIA for HIV-1/2 and a follow-up specimen was shown to be anti-HIV-1 EIA repeatedly reactive and/or HIV-1 RNA positive. The detection rate of such window period cases was 0.0000002% (1 in 4,000,000). There was one additional specimen that was anti-HIV-1 EIA negative, HIV-1 p24 antigen positive and HIV-1 RNA positive; however, this donor was not enrolled in the follow-up study.

Single Donation Testing Performance
A random selection of 10,727 primary pools revealed that 26 primary pools were reactive with the COBAS® AmpliScreen HIV-1 Test, v1.5 for an initially reactive rate of 0.24%. There were 11 reactive pools with at least 1 confirmed anti-HIV-positive specimen and 0 pools were positive due to confirmed window period cases. A total of 15 pools were reactive but were not confirmed. Results are summarized in Table 16.

A total of 792,055 specimens were selected from geographically divergent sites. The results from these specimens were used to determine the specificity and sensitivity of COBAS® AmpliScreen HIV-1 Test, v1.5. Using the antibody and antigen results, the HIV-1 status of each specimen was determined. HIV-1 status-negative included either: 1) anti-HIV-1 EIA negative and HIV-1 RNA negative (EIA nonreactive or neutralization negative) unless the subject was enrolled in the follow-up study and had test results that changed this assessment, or 2) anti-HIV EIA repeatedly reactive, WB/IFA negative and HIV-1 p24 antigen negative or indeterminate.

HIV-1 status-positive included either: 1) anti-HIV-1 EIA repeatedly reactive, WB/IFA positive, or 2) follow-up study test results of anti-HIV-1 repeatedly reactive or HIV-1 RNA positive. The detection rate of such window period cases was 0.0000002% (1 in 4,000,000). There was one additional confirmed window period case detected. A confirmed window period case is defined as an enrolled individual from whom the index donation was positive in the COBAS® AmpliScreen HIV-1 Test, v1.5 but non-reactive by EIA for HIV-1/2 and a follow-up specimen was shown to be anti-HIV-1 EIA repeatedly reactive and/or HIV-1 RNA positive. The detection rate of such window period cases was 0.0000002% (1 in 4,000,000). There was one additional specimen that was anti-HIV-1 EIA negative, HIV-1 p24 antigen positive and HIV-1 RNA positive; however, this donor was not enrolled in the follow-up study.
There were 1085 pools that were used to determine the specificity of HIV-1 RNA. Of these pools, 1082 were HIV-1 RNA-negative. The specificity of the COBAS® AmpliScreen HIV-1 Test, v1.5 in this study was 1082/1085 or 99.7235% with 95% confidence level of 99.19% to 99.94%.

NON-CLINICAL PERFORMANCE

Analysis of HIV-1 p24 Positive, Antibody Negative Samples

Twenty samples were selected from commercially available seroconversion panels that met the criterion of positive for HIV-1 p24 antigen and negative for anti-HIV-1/2 using licensed tests. The selected samples were diluted 1:96 in Normal Human Plasma that was found negative for HIV-1 RNA using the COBAS® AmpliScreen HIV-1 Test, v1.5. Each sample was processed diluted 1:96 using the Multiprep Specimen Processing Procedure to simulate Primary Plasma Pools. The COBAS® AmpliScreen HIV-1 Test, v1.5 successfully detected HIV-1 RNA in all 20 samples that were positive by HIV-1 p24 antigen and negative for anti-HIV-1/2. The results demonstrate that the test has sufficient sensitivity to detect HIV-1 yield samples in a 96-sample minipool format. Results are summarized in Table 18.

Table 18
Summary of Test Results

<table>
<thead>
<tr>
<th>US FDA Licensed HIV-1 p24 Antigen</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>COBAS® AmpliScreen HIV-1 Test, v1.5</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>HIV-1 Serocconversion Panels</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

Ten commercially available HIV-1 seroconversion panels were tested using the Multiprep Specimen Processing Procedure. Blinded panel members were diluted 1:96 with HIV-1 negative human plasma. COBAS® AmpliScreen HIV-1 Test, v1.5 results were compared to HIV 1/2 antibody and HIV-1 p24 antigen results. In two panels, the COBAS® AmpliScreen HIV-1 Test, v1.5 detected HIV-1 RNA on the same bleed as HIV-1 p24 antigen. In the remaining 8 panels COBAS® AmpliScreen HIV-1 Test, v1.5 detected HIV-1 RNA 2 to 12 days earlier. Data are presented in Table 19.

Table 19
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

<table>
<thead>
<tr>
<th>Days Before HIV-1/2 Antibody (10 panels tested)</th>
<th>Days Before Abbott HIV-1 p24 Antigen (10 panels tested)</th>
<th>Days Before Coulter HIV-1 p24 Antigen (10 panels tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>12</td>
<td>7.5</td>
</tr>
<tr>
<td>Median</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Maximum</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>Minimum</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

In 100% of the HIV-1 seroconversion panels tested, COBAS® AmpliScreen HIV-1 Test, v1.5 detected HIV-1 RNA prior to anti-HIV-1/2 reactivity (range 9 to 15 days).

Positive Pooled Deconstruction

This study was performed with 10 pools. Each pool had 1 to 10 positive sample(s) intermixed among the 96 members and was used to evaluate the ability of the resolution algorithm to correctly identify the positive member or members and to evaluate the pooling dilution effect. The algorithm includes the following three testing levels: Level 1: 96 Member Mini-pool Testing, Level 2: Column and Row Mini-pool Testing, and Level 3: Single sample testing. The results of this study demonstrate that the 96-sample pooling strategy, in combination with the COBAS® AmpliScreen HIV-1 Test, v1.5 is capable of detecting and identifying HIV-1-positive donations in plasma minipools of 96 samples.
Sensitivity Study
Sixty pre-mortem EDTA plasma and fifty-eight cadaveric EDTA plasma 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 with three COBAS® AmpliScreen HIV-1, v1.5 Test kit lots.

The COBAS® AmpliScreen HIV-1, v1.5 Test 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. Two post-mortem specimens negative on the initial test were repeated and found to be positive. One specimen that exhibited inhibition on initial testing was negative on repeat testing. The summary of the test results of this study is presented in Table 20 below.

<table>
<thead>
<tr>
<th>Table 20</th>
<th>Summary of Sensitivity Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Mortem EDTA Plasma Specimen</td>
</tr>
<tr>
<td>Total Specimens Tested</td>
<td>60</td>
</tr>
<tr>
<td>Test Results</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>60</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Inhib.</td>
<td>0</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
</tr>
<tr>
<td>95% Confidence Interval</td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>100%</td>
</tr>
<tr>
<td>Lower</td>
<td>94%</td>
</tr>
</tbody>
</table>

Specificity Study
Sixty pre-mortem and 60 post-mortem specimens which 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 using 3 lots of the COBAS® AmpliScreen HIV-1 Test, v1.5.

The COBAS® AmpliScreen HIV-1 Test, v1.5 using samples diluted 1:5 and the Multiprep Specimen Processing Procedure yielded negative results on 96.7% (58/60) of the pre-mortem EDTA plasma specimens, and 100% (57/57) of the post-mortem EDTA plasma specimens. One post-mortem EDTA plasma specimen exhibited inhibition on initial and repeat testing. The summary of the specificity study results is presented in Table 21 below.

<table>
<thead>
<tr>
<th>Table 21</th>
<th>Summary of Specificity Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Mortem EDTA Plasma Specimen</td>
</tr>
<tr>
<td>Total Specimens Tested</td>
<td>60</td>
</tr>
<tr>
<td>Test Results</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>2*</td>
</tr>
<tr>
<td>-</td>
<td>58</td>
</tr>
<tr>
<td>Inhib.</td>
<td>0</td>
</tr>
<tr>
<td>Final Specificity</td>
<td>96.7%</td>
</tr>
<tr>
<td>95% Confidence Interval</td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>100%</td>
</tr>
<tr>
<td>Lower</td>
<td>94.0%</td>
</tr>
</tbody>
</table>

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 22 below.

<table>
<thead>
<tr>
<th>Table 22</th>
<th>Summary of Reproducibility Study Test Results – Post-Mortem versus Pre-Mortem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Post-Mortem</td>
</tr>
<tr>
<td>Results by Lot (# Positive / # Tested, Percent Hit Rate)</td>
<td></td>
</tr>
<tr>
<td>Lot #1</td>
<td>118/120 (98.3%)</td>
</tr>
<tr>
<td>Lot #2</td>
<td>120/120 (100%)</td>
</tr>
<tr>
<td>Lot #3</td>
<td>116/120 (96.7%)</td>
</tr>
<tr>
<td>Results by Site (# Positive / # Tested, Percent Hit Rate)</td>
<td></td>
</tr>
<tr>
<td>Site #1</td>
<td>114/120 (95%)</td>
</tr>
<tr>
<td>Site #2</td>
<td>120/120 (100%)</td>
</tr>
<tr>
<td>Site #3</td>
<td>120/120 (100%)</td>
</tr>
</tbody>
</table>

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


