COBAS AmpliScreen™ HIV-1 Test, v1.5

Summary of Basis for Approval

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Trade Name	COBAS AmpliScreen HIV-1 Test, v1.5
Proper Name (Licensed Name)	Human Immunodeficiency Virus Type 1 / Polymerase Chain Reaction / Blood Cell Derived [COBAS AmpliScreen™]
Applicant / Manufacturer	Roche Molecular Systems, Inc. 4300 Hacienda Drive Pleasanton, CA 94588 FDA Registration No: 2243471
Biological License Application (BLA) Reference Number(s)	STN 125059/0
Report Date	December 6, 2002

I. 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 (HIV-1) RNA in human plasma from donations of whole blood and blood components for transfusion.

The test is intended for use in screening of individual donor samples of human plasma, or pools of human plasma comprised of equal aliquots of not more than 24 individual donations. The test is intended to be used for detecting HIV-1 RNA in conjunction with licensed tests for detecting antibodies to HIV-1.

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.

II. BRIEF DESCRIPTION OF DEVICE AND PRINCIPLES

A. Summary and Explanation of the Test

The COBAS AmpliScreen HIV-1 Test, v1.5, uses a generic sample preparation technique in a mini-pool testing format along with automated amplification and detection using PCR on the COBAS AMPLICOR™ Analyzer for the detection of HIV RNA in blood donations. The assay incorporates an Internal Control for monitoring assay performance in each individual test as well as AmpErase® to reduce potential contamination by previously amplified material (amplicon).

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

Sample processing, reverse transcription of target RNA to generate complementary DNA (cDNA), PCR amplification of target cDNA using HIV-specific complementary primers, hybridization of the amplified products to oligonucleotide probes specific to the target(s), and detection of the probe-bound amplified products by colorimetric determination.

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

- Multiprep Specimen Processing procedure for preparation of mini-pool specimens
- Standard Specimen Processing for preparation of individual donor samples

In the Standard Specimen Processing procedure, HIV-1 RNA is isolated directly from plasma by lysis of the virus particles with a chaotropic agent 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 and precipitation of the RNA with alcohol.

The Multiprep Internal Control, containing the HIV-1 Internal Control, is introduced into each sample 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.

The reverse transcription and amplification reactions are performed with the thermostable recombinant enzyme *Thermus thermophilus* DNA Polymerase (r*Tth* pol). r*Tth* 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 r*Tth* pol produces a cDNA copy of the HIV-1 target and the HIV-1 Internal Control RNA.

Following reverse transcription, 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 again to separate the resulting double-stranded DNA, and 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.

Following PCR amplification, the COBAS AMPLICOR Analyzer automatically adds denaturation solution to chemically denature the HIV-1 amplicon and the HIV-1 Internal Control amplicon to form single-stranded DNA. A suspension of magnetic particles coated with an oligonucleotide probe specific for HIV-1 amplicon or HIV-1 Internal Control amplicon is added. 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.

Following the hybridization reaction, the COBAS AMPLICOR Analyzer washes the magnetic particles 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 adds a substrate solution containing hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine (TMB). 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.

B. Description of Kit and Component Formulations

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.

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

Amplification Reagents

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

Bicine buffered solution with DMSO, glycerol, r*Tth* DNA Polymerase, potassium acetate, primers, dNTPs, AmpErase and sodium azide as a preservative

HIV-1 Mn2+ v1.5 (HIV-1 Manganese Solution, version 1.5) 8 x 0.1 mL

Manganese solution with acetic acid, indicator dye and sodium azide as a preservative

COBAS AmpliScreen HIV-1 Detection Reagents, version 1.5

DN4 (Denaturation Solution) 1 x 100 Tests

EDTA
Thymol blue
Sodium hydroxide

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

MES buffer solution containing capture oligonucleotides and magnetic microparticles with sodium aside as a preservative

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

Sodium phosphate buffer
Sodium thiocyanate
Solubilizer

II PS1 (IC Probe Suspension 1) 1 x 100 Tests

MES buffer solution containing magnetic microparticles with capture oligonucleotides and sodium azide as a preservative

II4 (IC Probe Suspension 2) 1 x 100 Tests

Sodium phosphate buffer containing sodium thiocyanate

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

Tris-HCl buffer solution containing Avidin-horseradish peroxidase conjugate, bovine serum albumin, Emulsit 25 and phenol with ProClin® 150 as a preservative

SB3 (Substrate A) 10 x 75 Tests

Citrate solution containing hydrogen peroxide with ProClin® 150 as a preservative

SB (Substrate B) 10 x 75 Test

3,3′,5,5′-Tetramethylbenzidine (TMB)
Dimethylformamide (DMF)

COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit 96 Tests

MP (+) C (Multiprep Positive (+) Control) 8 x 0.1 mL

Tris-HCl buffered solution containing noninfectious RNA transcripts for HCV and HIV-1 and noninfectious HBV DNA plasmid with EDTA and sodium azide as a preservative.

MP LYS (Multiprep Lysis Reagent) 8 x 9.0 mL

Tris-HCl buffered solution with Dithiothreitol, Glycogen and Guanidine thiocyanate

MP DIL (Multiprep Specimen Diluent) 8 x 4.8 mL

Tris-HCl buffered solution with EDTA and sodium azide as a preservative

MP IC (Multiprep Internal Control) 8 x 0.1 mL

Tris-HCl buffered solution with non-infectious internal control RNA transcripts for HCV and HIV-1 and DNA plasmid for HBV, Poly rA RNA, EDTA, indicator dye and sodium azide as a preservative.

MP (–) C (Multiprep Negative (-) Control) 8 x 0.1 mL

Poly rA RNA, EDTA and sodium azide as a preservative

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

Human plasma, non-reactive by US FDA licensed tests for antibody to HIV-1/2, antibody to HCV, HIV-1 p24 antigen and HBsAg, with ProClin® 300 as a preservative.

COBAS AMPLICOR Wash Buffer 500 Tests

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

Phosphate buffer solution containing detergent with ProClin® 300 as a preservative

III. MANUFACTURING AND CONTROLS

A. Description of Manufacturing Facilities

The COBAS AmpliScreen HIV-1 Test, v1.5 is manufactured by Roche Molecular Systems, Inc. (RMS) and prepared under U.S. License 1636. The corporate headquarters is located at 4300 Hacienda Drive, Pleasanton, California. The primary RMS manufacturing facility is located at ----------. One component of the COBAS AmpliScreen HIV-1 Test, v1.5 (the COBAS AmpliScreen Multiprep Internal Control) is produced in manufacturing laboratories in the RMS facility located at ---------- (referred to as the ----------). The Multiprep Positive Control is one of the Positive Controls manufactured for RMS by ----------, located at ----------. The buffers used in the manufacturing of the Positive Controls are supplied by the RMS ---------- facility and RMS Alameda facility (----------). Final product is stored and distributed from the RMS --------- warehouse located at ----------, ----------. Finished, approved product is distributed in the United States from the Roche Diagnostic distribution center located in ----------.

The DNA oligonucleotide primers (----------) and probes (----------) used in the COBAS AmpliScreen HIV-1 Test, version 1.5 are ----------

The *Thermus thermopilus* (r*Tth*) and Uracil-N-Gylcosylase (rUNG) enzymes used in the Test are manufactured at RMS. ----------.

The RNA Multiprep Positive and Internal Controls include ----------. The HIV and HCV RNA Multiprep Positive Controls (pSYC35 HIV and pHCVIIA HCV) and Internal Controls (----------), and the HBV Plasmid Multiprep Positive Control (----------) and Internal Control DNA (----------), are ---------- --

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The Negative Control is prepared from Human Plasma pools, which are tested and found to be negative for anti-HCV, HBV and HIV.

The raw materials used in this product are subjected to appropriate quality control evaluations before they are accepted for use in manufacturing. Acceptance criteria and performance specifications have been established for all test kit components. Components are assembled into test kits, each lot of which is subjected to a final performance test.

Each COBAS AmpliScreen HIV-1 Test, version 1.5 kit lot is tested with in-house panels of samples with varying HIV-1 copy numbers/mL, as well as the CBER HIV-1 Reference Panel, and must meet the performance requirements of both panels.

B. Stability Program

Components of the COBAS AmpliScreen HIV-1 Test, v1.5 were entered into the stability program in order to define the recommended storage conditions and to establish the expiration dating period (i.e., shelf-life) for each component. The expiration date of the complete Test kit is defined on a lot-by-lot basis as the expiration date of the component lot with the shortest expiration date. In addition, components from the reserve and stability inventory that were at, near or beyond shelf life were assembled into "virtual kits" and functional testing was performed.

The results of stability studies completed to date support the shelf-life claims summarized in the table below.

COBAS AmpliScreen HIV-1 Test, v1.5
Quality Control Component Shelf-Life, Storage Temperature and Stability Tests

Component	Code	Proposed Shelf Life (months)	Storage Temperature	Real Time Stability Tests
AmpliScreen Multiprep Lysis Reagent	----------	---	2-8°C	----------
AmpliScreen Multiprep Internal Control	----------	---	2-8°C	----------
AmpliScreen Multiprep Specimen Diluent	----------	---	2-8°C	----------
AmpliScreen HIV-1 Master Mix, v1.5	----------	---	2-8°C	----------
AmpliScreen HIV-1 Manganese Solution, v1.5	----------	---	2-8°C	----------
AmpliScreen Multiprep Negative Control	----------	---	2-8°C	----------
AmpliScreen Multiprep Positive Control	----------	---	2-8°C	----------
Negative Plasma (Human)	----------	---	2-8°C	----------
COBAS AMPLICOR Denaturation Solution	----------	---	2-25°C	----------
COBAS AmpliScreen HIV-1 Probe Suspension 1, v1.5	----------	---	2-8°C	----------
COBAS AmpliScreen HIV-1 Probe Suspension 2, v1.5	----------	---	2-8°C	----------
COBAS AmpliScreen HIV-1 IC Probe Suspension 1	----------	---	2-8°C	----------
COBAS AmpliScreen HIV-1 IC Probe Suspension 2	----------	---	2-8°C	----------
COBAS AMPLICOR Avidin- HRP Conjugate	----------	---	2-8°C	----------
COBAS AMPLICOR Substrate A	----------	---	2-8°C	----------
COBAS AMPLICOR Substrate B	----------	---	2-8°C	----------
COBAS AMPLICOR 10XWash Concentrate	----------	---	2-25°C	----------

C. Methods of Validation

All test kit components are monitored by in-process testing. Product purity and potency are assured through the evaluation of the product appearance, chemical testing, and performance testing. Product performance is assessed through quality release evaluations of the final test kit against an in-house panel containing negative control specimens and specimens that are known to be positive for HIV-1 virus. A CBER HIV-1 Panel is also tested in this evaluation and the test kit must meet all performance requirements. The COBAS AmpliScreen HIV-1 Test, version 1.5 meets the FDA release requirements.

D Labeling

The product labeling, including immediate container labels, box or package labels, and package insert have been reviewed for compliance with 21 CFR§610.60, 610.61, 610.62 and 809.10 and were found acceptable. The package insert clearly states the intended use as a qualitative *in vitro* test for the direct detection of Human Immunodeficiency Virus Type 1 RNA in human plasma from donations of whole blood and blood components for transfusion. The test is intended for use in screening of individual donor samples of human plasma or pools of human plasma comprised of equal aliquots of not more than 24 individual donations. The test is intended to be used for detecting HIV-1 RNA in conjunction with licensed tests for detecting antibodies to HIV-1. 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. The product tradename, COBAS AmpliScreen HIV-1 Test, version 1.5 is not known to conflict with any other biologic or device tradename.

E. Establishment Inspection

A Pre-license Inspection of the manufacturing facilities where the COBAS AmpliScreen product lines are manufactured, tested, stored and shipped was conducted from July 8, 2002 through July 19, 2002.

F. Environmental Impact Analysis, Claims for a Categorical Exclusion

Roche Molecular Systems, Inc. claimed a Categorical Exclusion from the submission of an Environmental Impact Statement with the COBAS AmpliScreen HIV-1 Test, version 1.5, Biologics License Application. This claim for a Categorical Exclusion was made pursuant to 21 CFR 25.24(e)(4). The manufacture of the COBAS AmpliScreen HIV-1 Test, version 1.5, is performed under controlled conditions and in compliance with the appropriate federal, state, and local environmental regulations. The disposal of waste from the use of this product is performed in compliance with appropriate federal, state, and local environmental regulations. Based on the materials, concentration, volumes used in this product, the method(s) of product disposal, it is unlikely that the release of any of the substances of this product at the expected level of exposure will be harmful to the environment or toxic to organisms in the environment.

IV. PERFORMANCE CHARACTERISTICS

A. Pre-clinical Studies Summary

Pre-clinical performance studies include the determination of the following:

1. Assay Cutoff
2. Analytical Sensitivity
    a. Determination of Limit of Detection (LOD), Using the WHO HIV-1 International Standard, 97/656
    b. Analytical Sensitivity — CBER HIV-1 Panel
    c. Analytical Sensitivity — Dilutional Panels
    d. Group / Subtype Detectability
    e. Sensitivity with Seroconversion Panels
    f. Dilutional Sensitivity with Weakly-Reactive HIV-1 p24 Antigen Samples
    g. Dilutional Sensitivity with Weakly-Reactive HIV-1 p24 Antibody Positive Samples
3. Analytical Specificity
    a. Analytical Specificity — Potentially Cross Reactive and Interfering Microorganisms
    b. Analytical Specificity — Non-HIV-1 Samples
4. Potentially Interfering Substances
    a. Endogenous Interfering Substances
    b. Exogenous Interfering Substances
5. Uracil-N-glycosylase (UNG) Performance
6. Reproducibility Studies

B. Clinical Trials Summary

Clinical studies include the determination of the following:

1. Pool Reactivity in Volunteer Blood Donors
2. Single Donation Testing Performance
3. AIDS and HIV-1 Asymptomatic Population
4. Detection of Window Period Cases
5. Sensitivity in a High Risk Population

A. Pre-clinical Studies Summary

1. Assay Cutoff

Study Description. The COBAS AmpliScreen HIV-1 Test, v1.5 cutoff value was determined by testing seronegative plasma specimens drawn from ------ blood donors, and --- HIV-1 seropositive specimens drawn from plasma donors. In addition, --- HIV-1 RNA-positive seroconversion panel specimens were tested by both the Multiprep and Standard Specimen Processing procedures. The complete description of all samples included in the determination of the cutoff value is provided in the following table.

Table 1: Determination of the Cutoff Data Summary

Results

The data show a bimodal distribution of the A_{660} values for true positive and true negative specimens for both the Multiprep and Standard procedures. This is expected for amplified nucleic acid tests where the goal of the procedure is to achieve large absorbance signals in the presence of low target levels. A cutoff value of =0.20 A_{660} was determined appropriate for the COBAS AmpliScreen HIV-1 Test, v1.5, for both the Multiprep and Standard Sample Processing Procedures. Based on study results, a cutoff of =0.20 A_{660} would provide optimal clinical performance for either specimen processing procedure. The following table summarizes the test result validity criteria for Primary Pools, Secondary Pools, and Individual Donor Samples.

Table 2: Cutoff Summary and Test Result Validity Criteria

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

2. Analytical Sensitivity

a. Determination of Limit of Detection (LOD) Using the WHO HIV-1 International Standard, 97/656

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 284.9 IU/mL and 387.3 IU/mL, respectively.

Tables 3 and 4 summarize the overall results for the Multiprep and Standard Specimen Processing Procedures, respectively.

Table 3: : 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	% Positives	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 4: 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	% Positives	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%

b. 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 5.

Table 5: 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%

c. 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 6 and 7.

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.

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 6: Multiprep Procedure Testing Summary for All Clinical Samples, Combined Input Values with 95% One-tailed Lower Confidence Limits

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 7: 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%

d. 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.*

e. Sensitivity with 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. 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.

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 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.*

f. 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.

g. 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 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%.

3. Analytical Specificity

a. 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.

b. 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.

4. Potentially Interfering Substances

a. 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.

b. 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.

5. Uracil-N-Glycosylase (UNG) Performance

AmpErase (uracil-N-glycosylase, UNG) catalyzes the degradation of DNA containing deoxyuridine, but not DNA containing thymidine or RNA containing uridine. Deoxyuridine is not a constituent of the HIV-1 Target RNA, but is always present in amplicon. In the AmpliScreen HIV-1 Master Mix reagent, version 1.5 deoxyuridine triphosphate replaces thymidine triphosphate as one of the dNTPs. Only target amplicon contain deoxyuridine is susceptible to UNG-mediated degradation prior to amplification of the target DNA. Therefore, AmpErase is an effective countermeasure against inadvertent amplicon carryover.

6. Reproducibility Studies

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 Table 12 and 13 below.

Table 12: 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 13: 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%)

B. Clinical Trials Summary

1. Pool Reactivity in Volunteer Blood Donors

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 14.

Table 14: Pool Reactivity in Volunteer Blood Donors

Category	Pools	Percentage
Pools Tested	10,727	100
Non- reactive pools	10,701	99.75
Initially reactive pools	26	0.24
Initial pools with concordant positive serology	11	0.1
Positive pools due to window case	0	0
Initially reactive pools with negativeserology and negative individual donation AmpliScreen testing (false positive)	15	0.14

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 status of each specimen was determined. HIV status-negative included either: 1) anti-HIV EIA negative and HIV-1 p24 antigen 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 status-positive included either: 1) anti-HIV EIA repeatedly reactive, WB/IFA positive, or 2) follow-up study test results of anti-HIV repeatedly reactive or HIV-1 RNA positive. HIV status unknown included anti-HIV EIA repeat reactive, WB/IFA indeterminate or unknown, HIV-1 p24 antigen negative or indeterminate.

There were 791,733 specimens that were determined to be HIV status-negative. Of these, 791,732 were also HIV-1 RNA-negative. The specificity of the COBAS AmpliScreen HIV-1 Test, v1.5 in this study was 791,732/791,733 or 99.9999% with 95% confidence limits of 99.99% to 100.00%.

There were 42 specimens that were determined as HIV status-positive. Of these, 38 were also HIV-1 RNA positive. The sensitivity of the COBAS AmpliScreen HIV-1 Test, v1.5 in this study was 38/42 or 90.48% with 95% confidence limits of 77.38% to 97.34%.

2. Single Donation Testing Performance

A total of 587 specimens were tested individually in the COBAS AmpliScreen HIV-1 Test, v1.5 clinical trial. The HIV-1 status of these samples was based upon EIA and supplemental test results as described above.

Of the 587 specimens, 271 specimens had available HIV-1 antibody test data. Of these 271 specimens, 271 were classified as HIV-1 status-negative; there were no HIV-1 status- positive donors. The specificity of the COBAS AmpliScreen HIV-1 Test, 1.5 in this study was 100%(271/271) with 95% confidence interval of 98.65% to 100%. There were no HIV-1 RNA positive specimens detected by individual donation testing using the COBAS AmpliScreen HIV-1 Test, v1.5.

3. AIDS and HIV-1 Asymptomatic Population

Seropositive samples from 217 patients diagnosed with AIDS and seropositive samples from 784 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 was 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 15 and 16. There were 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 16. 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 15: Results of HIV-1 Seropositive Specimens Tested at the Clinical Sites (Discordant Specimens < 100 Copies/mL Removed)

		HIV-1 Antibody		Total
		Positive	Negative	
COBAS AmpliScreen HIV-1 Test, v1.5 Result	Positive	733	22	755
	Negative	23	1977	2000
Total		**756**	**1,999**	**2,755**

Table 16: Results of HIV-1 Seropositive Specimens (Discordant Specimens < 100 Copies/mL Removed) Following Resolution Testing at Roche

		HIV-1 Antibody		Total
		Positive	Negative	
COBAS AmpliScreen HIV-1 Test, v1.5 Result	Positive	754	22	776
	Negative	2	1977	1979
Total		**756**	**1,999**	**2,755**

4. Detection of Window Period Cases

From November 11, 1999 to December 31, 2001, approximately 8 million donations were tested. During this period there were 2 confirmed window period cases 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 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 EIA negative, HIV-1 p24 antigen positive and HIV-1 RNA positive; however, this donor was not enrolled in the follow-up study.

5. Sensitivity in a 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 negative when tested by both 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 p24 antigen EIA, 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 17 and 18.

Table 17: Clinical Sensitivity in a High Risk Population with Standard Prep

			HIV-1 Antibody Reactive by EIA			HIV-1 Antibody Negative	HIV-1 Antibody Reactive by EIA, HIV-1 RNA [3] 100 c/mL			HIV-1 Antibody Negative, RNA [3] 100 c/mL
			Western Blot				Western Blot			
Standard Prep	Total Tested		NT	Neg / Ind	Pos		NT	Neg / Ind	Pos	
	+	55	54	0	0	1	54	0	0	0
	–	319	0	7	3	309	0	0	0	307

Table 18: Clinical Sensitivity in a High Risk Population with Multiprep

			HIV-1 Antibody Reactive by EIA			HIV-1 Antibody Negative	HIV-1 Antibody Reactive by EIA, HIV-1 RNA [3] 100 c/mL			HIV-1 Antibody Negative, RNA [3] 100 c/mL
			Western Blot				Western Blot			
Multiprep	Total Tested		NT	Neg / Ind	Pos		NT	Neg / Ind	Pos	
	+	54	54	0	0	0	54	0	0	0
	–	320	0	7	3	310	0	0	0	307

V. PACKAGE INSERT

A copy of the Package Insert (directions for use) is attached.