COBAS® AmpliScreen HCV Test, version 2.0

FOR *IN VITRO* DIAGNOSTIC USE.

COBAS® AmpliScreen HCV Test, version 2.0	HCV	96 Tests	P/N: 03302563 018
COBAS® AmpliScreen Multiprep Specimen Preparation and Control Kit	MULTIPREP/CTL	96 Tests	P/N: 03302555 018
COBAS® AMPLICOR® Wash Buffer	WB	500 Tests	P/N: 20759899 123 ART: 07 5989 9 US: 83314

INTENDED USE

The COBAS® AmpliScreen HCV Test, version 2.0 (v2.0) is a qualitative *in vitro* test for the direct detection of Hepatitis C Virus (HCV) RNA in human plasma.

The COBAS® AmpliScreen HCV Test, v2.0 is intended to be used for the detection of HCV RNA in conjunction with licensed tests for detecting antibodies to HCV. This product is intended for use as a donor screening test to detect HCV in plasma specimens 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 HCV 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.

Plasma from all donors may be screened as individual specimens. 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 hematopoietic stem/progenitor cells (HPCs) sourced from bone marrow, peripheral blood or cord blood, and donor lymphocytes for infusion (DLI), plasma may be tested in pools comprised of equal aliquots of not more than 24 individual donor specimens. For donations of source plasma, plasma may be tested in pools comprised of equal aliquots of not more than 96 individual donations.

The COBAS® AmpliScreen HCV Test, v2.0 can be considered a supplemental test that confirms HCV infection for specimens that are repeatedly reactive on a licensed donor screening test for antibodies to HCV, and reactive on the COBAS® AmpliScreen HCV Test, v2.0.

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

SUMMARY AND EXPLANATION OF THE TEST

Hepatitis C Virus is considered to be the principal etiologic agent responsible for 90-95% of the cases of post-transfusion non-A and non-B hepatitis.[1,2] HCV is a single-stranded, positive sense RNA virus with a genome of approximately 10,000 nucleotides coding for 3,000 amino acids.[1] As a bloodborne virus, HCV can be transmitted by blood and blood products. The global prevalence of HCV infection, as determined by immunoserology, ranges from 0.6% in Canada to 1.5% in Japan.[2]

Serological screening assays have greatly reduced, but not completely eliminated, the risk of transmitting viral infections by transfusion of blood products.[3-6] Recent studies indicate that nucleic acid-based amplification tests for HCV RNA will allow detection of HCV infection earlier than the current antibody based tests. Nucleic acid testing (NAT) of whole blood donations has been in place in the United States since 1999 under Investigational New Drug Application (IND). Nucleic acid-based tests can detect viremic units donated by carriers who do not seroconvert or who lack antibodies to serological markers normally detected by immunological assays.[7-9]

The COBAS® AmpliScreen HCV Test, v2.0, uses a generic sample preparation technique in a mini-pool testing format along with automated amplification and detection using Polymerase Chain Reaction (PCR) on the COBAS® AMPLICOR® Analyzer for the detection of HCV RNA in blood donations. The assay incorporates an Internal Control for monitoring assay performance in each individual test as well as the AmpErase (uracil-N-glycosylase) enzyme to reduce potential contamination by previously amplified material (amplicon).

PRINCIPLES OF THE PROCEDURE

The COBAS® AmpliScreen HCV Test, v2.0 is based on five major processes:

1. Sample Processing
2. Reverse transcription of target RNA to generate complementary DNA (cDNA)[10]
3. PCR amplification[10] of target cDNA using HCV-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 HCV Test, v2.0 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, HCV 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, HCV 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 HCV 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 HCV Internal Control is an RNA transcript with primer binding regions identical to those of the HCV target sequence, a randomized internal sequence of similar length and base composition as the HCV target sequence, and a unique probe binding region that differentiates the HCV Internal Control amplicon from target amplicon. These features were selected to ensure equivalent amplification of the HCV Internal Control and the HCV target RNA.

Reverse Transcription

The reverse transcription and amplification reactions are performed with the thermostable recombinant enzyme *Thermus thermophilus* DNA Polymerase (r*Tth* pol). In the presence of manganese (Mn^{2+}) and under the appropriate buffer conditions, r*Tth* pol has both reverse transcriptase and DNA polymerase activity.[10] 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 HCV target and the HCV Internal Control RNA.

PCR Amplification

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

Selective Amplification

To ensure selective amplification of nucleic acid target in the sample and prevent amplification of pre-existing amplicon, AmpErase (uracil-N-glycosylase) enzyme is added to the COBAS® AmpliScreen HCV Test, v2.0. The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[11], but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon because of the use of deoxyuridine triphosphate in place of deoxythymidine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contain deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by the AmpErase enzyme before amplification of the target DNA. The AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of DNA, thereby rendering the DNA non-amplifiable. The AmpErase enzyme is inactive at temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. Following amplification, any residual enzyme is denatured by the addition of the Denaturation Solution, thereby preventing the degradation of any target amplicon.

Hybridization Reaction

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

Detection Reaction

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

MATERIALS PROVIDED BY ROCHE

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

COBAS® AmpliScreen Multiprep Specimen Preparation and Control Kit MULTIPREP/CTL **96 Tests**
(P/N: 03302555 018)

MP (–) C
(Multiprep Negative (–) Control)
MP (+) C
(Multiprep Positive (+) Control)
MP LYS
(Multiprep Lysis Reagent)
MP DIL
(Multiprep Specimen Diluent)
MP IC
(Multiprep Internal Control)
NHP
(Negative Plasma (Human))

COBAS® AmpliScreen HCV Test, version 2.0 HCV **96 Tests**
(P/N: 03302563 018)

COBAS® AmpliScreen HCV Amplification Reagents, version 2.0 HCV AMP

HCV MMX, v2.0
(HCV Master Mix, version 2.0)
HCV Mn^{2+}, v2.0
(HCV Manganese Solution, version 2.0)

COBAS® AmpliScreen HCV Detection Reagents, version 2.0 HCV DK

CH PS1, v2.0
(HCV Probe Suspension 1, version 2.0)
CH4, v2.0
(HCV Probe Suspension 2, version 2.0)
CI PS1
(IC Probe Suspension 1)
CI4
(IC Probe Suspension 2)
DN4
(Denaturation Solution)
CN4
(Avidin - Horseradish Peroxidase Conjugate)
SB3
(Substrate A)
SB
(Substrate B)

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

WB
(10X-Wash Concentrate)

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

- COBAS® AMPLICOR® Analyzer with software version 0022B, Printer, and *Operator's Manual* for the COBAS® AMPLICOR® Analyzer
- COBAS® AMPLICOR® A-rings
- COBAS® AMPLICOR® D-cups
- AMPLILINK Software, version 1.4 and *Operator's Manual* for the AMPLILINK 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 2 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.

- Sarstedt 1.5-mL tube Barcode Labels
- Hamilton Archive and Intermediate Plate Barcode Labels
- Refrigerated high speed centrifuge with fixed angle rotor (45 degrees, capacity for at least 24 x 1.5-mL tubes) with an RCF of 23,600 x g (Heraeus Centrifuge 17RS or Biofuge 28RS with HFA 22.1 rotor, Heraeus Biofuge Stratos with the 3331 rotor or equivalent).

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

REAGENTS

COBAS® AmpliScreen Multiprep Specimen Preparation and Control Kit MULTIPREP/CTL **96 Tests**

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

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

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

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

MP LYS 8 x 9.0 mL
(Multiprep Lysis Reagent)

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

Xn  60% (w/w) Guanidine thiocyanate

Harmful

MP DIL 8 x 4.8 mL
(Multiprep Specimen Diluent)

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

MP IC 8 x 0.1 mL
(Multiprep Internal Control)

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

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

Human plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg
0.1% ProClin® 300 preservative

COBAS® AmpliScreen HCV Test, version 2.0 HCV **96 Tests**

COBAS® AmpliScreen HCV Amplification Reagents HCV AMP

HCV MMX, v2.0 8 x 0.7 mL
(HCV Master Mix, version 2.0)

Bicine buffer
16% DMSO
Glycerol
< 0.01% *rTth* DNA Polymerase (r*Tth* pol, microbial)
Potassium acetate
< 0.001% dATP, dCTP, dGTP, dUTP
< 0.005% KY78 and KY80 primers (KY78 is biotinylated)
< 0.01%AmpErase (uracil-N-glycosylase) enzyme (microbial)
0.05% Sodium azide

HCV Mn^{2+}, v2.0 8 x 0.1 mL
(HCV Manganese Solution, version 2.0)

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

COBAS® AmpliScreen HCV Detection Reagents, version 2.0 HCV DK

CH PS1 v2.0 1 x 100 Tests
(HCV Probe Suspension 1, version 2.0)

MES buffer
< 0.4% Suspension of Dynabeads® (paramagnetic particles) coated with HCV-specific oligonucleotide capture probe KY150
0.09% Sodium azide

CH4, v2.0 1 x 100 Tests
(HCV Probe Suspension 2, version 2.0)

Sodium phosphate buffer
34.7% Sodium thiocyanate
0.2% Solubilizer

Xn  34.7% (w/w) Sodium thiocyanate
Harmful

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

MES buffer
< 0.4% Suspension of Dynabeads (paramagnetic particles) coated with IC-specific oligonucleotide capture probe SK535
0.09% Sodium azide

CI4 1 x 100 Tests
(IC Probe Suspension 2)

Sodium phosphate buffer
24.9% Sodium thiocyanate
0.2% Solubilizer

DN4 1 x 100 Tests
(Denaturation Solution)

1.6% Sodium hydroxide
EDTA
Thymol blue

Xi  1.6% (w/w) Sodium hydroxide
Irritant

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

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

SB3 10 x 75 Tests
(Substrate A)

Citrate solution
0.01% Hydrogen peroxide
0.1% ProClin® 150 preservative

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

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

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

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

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

COBAS® AMPLICOR® Wash Buffer WB **500 Tests**

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

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

STORAGE INSTRUCTIONS

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

B. Do not freeze reagents.

C. Store the following reagents at 2 - 8°C. Unopened, these reagents are stable until the expiration date indicated.
MP LYS, **MP IC**, **MP (+) C**, **MP (–) C**, **MP DIL** and **NHP**
HCV MMX, v2.0 and **HCV Mn^{2+}, v2.0**
CH PS1, **v2.0**, **CH4**, **v2.0, CI PS1** and **CI4**
CN4, **SB3** and **SB**

D. Store **DN4** at 2 - 25°C. Store **WB** at 2 - 30°C. **DN4** and **WB** are stable until the expiration dates indicated.

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

F. The following reagents are one time use. Discard any unused portion.
MP IC, **MP (+) C**, **MP (–) C**, **MP DIL** and **NHP**
HCV Mn^{2+}, **v2.0** and **SB**

PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE.

A. Specimens may be infectious. Use Universal Precautions when performing the assay.[12-13]. 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.

B. **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.

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

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

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

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

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

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

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

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

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

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

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

O. Avoid contact of **MP LYS**, **HCV MMX**, **v2.0**, **HCV Mn^{2+}, v2.0**, **CH4**, **v2.0**, **CI4**, **DN4**, **CN4**, **SB3**, **SB** and Working Substrate (mixed **SB3** and **SB** reagent) with the skin, eyes or mucous membranes. **If contact does occur, immediately wash with large amounts of water, otherwise burns can occur.** If these reagents are spilled, dilute with water before wiping dry. **Do not allow MP LYS**, **which contains guanidine thiocyanate, or CH4**, **v2.0 and CI4**, **which contain sodium thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.**

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

Q. Refer to "*Precautions*" in the package inserts accompanying other COBAS® AmpliScreen products, the COBAS® AmpliScreen Pooling System Guide, and the *Operator's Manuals* for the AMPLILINK software and COBAS® AMPLICOR® Analyzer.

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.

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

REAGENT PREPARATION

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

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

B. **Working Lysis Reagent**

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

C. **Working Amplification Master Mix**

1. Prepare Working Master Mix in a template-free area (e.g., in a dead air box). **Reagent preparation area must be clean and disinfected in accordance with methods outlined in Precautions (Item A). Failure to do so may result in reagent contamination.**
2. Pipette 100 µL **HCV Mn^{2+}**, **v2.0** into 1 bottle **HCV MMX**, **v2.0**. Recap **HCV MMX**, **v2.0** bottle and mix well by inverting 10-15 times. The pink color confirms that the **HCV Mn^{2+}**, **v2.0** has been added to the **HCV MMX**, **v2.0**. Discard the remaining **HCV Mn^{2+}**, **v2.0**. Do not vortex the Working Master Mix. These reagents do not need to be at room temperature before use.
3. Store at 2 - 8°C and use within 4 hours of preparation.

D. **Working Probe Suspension Detection Reagents**

1. Prepare Working HCV Probe Suspension: Mix **CH PS1**, **v2.0** well by vortexing briefly to suspend the microparticles. Pipette 2.5 mL **CH PS1**, **v2.0** into one **CH4**, **v2.0** cassette.
2. Prepare Working IC Probe Suspension: Mix **CI PS1** well by vortexing briefly to suspend the microparticles. Pipette 2.5 mL **CI PS1** into one **CI4** cassette.
3. Both Working Probe Suspension Detection Reagents are stable for 30 days at 2 - 8°C. Working Reagents can be used for a maximum of ten instrument cycles (12 hours per cycle). Mixing occurs automatically on the COBAS® AMPLICOR® Analyzer.
4. Store Working Probe Suspension Detection Reagents at 2 - 8°C between instrument cycles. Remove from refrigerator 30 minutes before use on the COBAS® AMPLICOR® Analyzer.

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

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

F. **Working Substrate Reagent**

1. Working Substrate must be prepared each day by pipetting 5 mL **SB** into one **SB3** cassette. Pipette up and down at least 5 times to mix.
2. Working Substrate is stable on the COBAS® AMPLICOR® Analyzer for a maximum of 16 hours.

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

G. **Wash Buffer Reagent**

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

H. **70% Ethanol**

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

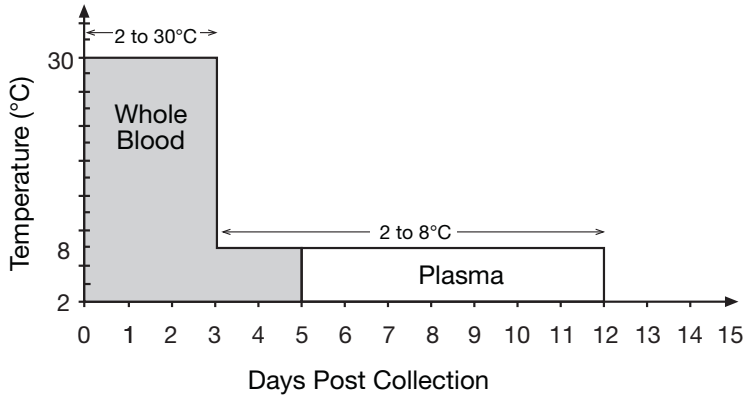
SPECIMEN COLLECTION, STORAGE AND POOLING

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

Living Donor Specimens

A. EDTA, CPD, CPDA-1, CP2D, ACD-A and 4% Sodium Citrate may be used with the COBAS® AmpliScreen HCV Test, v2.0. Follow sample tube manufacturer's instructions.

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



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

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

E. Do not freeze whole blood.

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

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

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

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

J. Thaw frozen specimens at room temperature before using.

K. The user should validate other collection and storage conditions. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.[14]

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, or on plasma specimens from donors of hematopoietic progenitor cells or donor lymphocytes for infusion. Cadaveric specimens must be tested individually and not as part of a pool.

1. The COBAS® AmpliScreen Pooling System 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 fewer than 24 specimens are available, testing is performed using the individual specimens.
2. For Source Plasma, the Hamilton MICROLAB AT Plus 2 Pipettor 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 donor using an overlapping pool testing 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 the 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 all 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: A serum or plasma specimen collected from a donor prior to death may be tested instead of a cadaveric blood specimen using either the instructions for cadaveric donor specimens or the instructions for living donor blood specimens.

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 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 HCV MMX**, **v2.0** and **HCV Mn^{2+}**, **v2.0**, must be at room temperature before use. Visually examine reagents for sufficient volume before beginning the test procedure. See section "Reagent Preparation" for specific reagent storage conditions.
2. Add all reagents using a pipettor capable of delivering specified volume with ± 3% accuracy and a precision of ≤ 5% CV. Check pipettor functionality and calibrate as recommended by pipettor manufacturer.
3. Prepare Working Master Mix in a template-free area (e.g., in a dead air box). **Reagent preparation area must be clean and disinfected in accordance with methods outlined in *"Precautions"* (Item A). Failure to do so may result in reagent contamination.**
4. Prepare 70% ethanol fresh each day.
5. Check expiration date of opened or Working Reagents before loading on the COBAS® AMPLICOR® Analyzer.
6. Check to ensure that all reagents used are of the same master lot of kit reagents.

D. **Workflow**

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

E. **Temperature**

Room temperature is defined as 15° to 30°C.

F. **Vortexing**

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

G. **Pipetting**

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

H. **Specimen Processing**

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

I. **Decontamination**

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

INSTRUCTIONS FOR USE

The Multiprep Specimen Processing Procedure is used for extracting nucleic acid from pooled specimens and from individual cadaveric specimens. The Standard Specimen Processing Procedure is used for extracting nucleic acid from individual specimens. The Multiprep Specimen Processing Procedure is also used for testing minipools of source plasma.

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

Amplification, Hybridization and Detection of Stored Processed Specimens

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

Hybridization and Detection of Stored Denatured Amplicon

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

A. Reagent Preparation - Working Master Mix

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

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

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

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

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

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

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

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

B. Specimen and Control Preparation

Performed in Pre-Amplification - Specimen and Control Preparation Area

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

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

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

B2. Vortex **NHP** briefly.

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

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

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

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

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

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

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

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

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

B10. Prepare Controls as follows:

a. Negative Control

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

b. Positive Control

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

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

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

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

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

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

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

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

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

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

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

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

Standard Specimen Processing Procedure (Individual Specimens (Non-Cadaveric) and Source Plasma Minipools)

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

B23. Vortex **NHP** briefly.

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

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

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

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

B28. Prepare Controls as follows:

a. Negative Control

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

b. Positive Control

Vortex **MP (+) C** briefly. Tap vial to collect the solution in the base. Pipette 20 µL **MP (+) C** 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.

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

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

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

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

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

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

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

and vortex briefly to resuspend the extracted RNA. Note that some insoluble material may remain.

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

Loading the A-ring

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

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

B41. Pipette 50 µL of each processed specimen and control into the appropriate A-tube containing HCV 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 HCV Probe Suspension Reagent (**CH4**, **v2.0**), Working IC Probe Suspension Reagent (**CI PS1**), Working Substrate (**SB3**), Denaturation Reagent (**DN4**), and Conjugate Reagent (**CN4**).

b. Place the **CH4**, **v2.0** and **CI PS1** cassettes in the test-specific reagent rack.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

QUALITY CONTROL PROCEDURES

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

a. Negative Control

The absorbance for the **MP (–) C** should be less than 0.1 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 0.1 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 (specimen and control preparation, amplification and detection) must be repeated.

Summary of Control Acceptance Criteria

	HCV Result		IC Result	
	A_{660}	Comment	A_{660}	Comment
Negative Control	< 0.1	Negative	≥ 0.2	Valid
Positive Control	≥ 1.0	Positive	≥ 0.2	Valid

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

3. External Control

   If 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 HCV External Control should be equal to or greater than 0.2 at 660 nm, irrespective of the **MP IC** absorbance. If the absorbance of the HCV External Control does not meet the above criterion, the negative results for specimens in the associated run may be invalidated. However, positive results for specimens in such a run should not be invalidated solely on the basis of the results obtained for an External Control; those positive results should remain the test of record. The laboratory should follow its established Standard Operating Procedure for the appropriate action.

INTERPRETATION OF RESULTS

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

2. Specimen Results

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

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

HCV Result		IC Result		
A_{660}	Comment	A_{660}	Comment	Interpretation
< 0.2	NEGATIVE	≥ 0.2	VALID	Specimen is negative for HCV 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 HCV RNA.

Invalid Test Runs

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

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

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

Invalid Specimen Results

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

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

Results of Pooled Donor Specimens (Pools of up to 24 Individual Donations)

Testing of pooled samples for the COBAS® AmpliScreen HCV Test, v2.0 requires a single level of testing for Primary Pools that are negative for HCV RNA and three levels of testing (Primary Pool, Secondary Pool and tertiary resolution) for Primary Pools that are positive for HCV RNA.

Negative Primary Pools

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

Positive Primary Pools - Secondary Pool Testing

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

- If one or more of the Secondary Pools tests positive, report the results for the donor specimens in the negative Secondary Pools as "HCV RNA Negative". For positive Secondary Pools, proceed to the section entitled "***Positive Primary Pool, Positive Secondary Pools - Tertiary Resolution Testing.***"
- If all four Secondary Pools are negative, the individual donor specimens in that Primary Pool may be reported as "HCV RNA Negative."
- As part of an overall Quality Assurance program, you may wish to conduct additional testing to determine the cause of the initial positivity of the Primary Pool.

Positive Primary Pool, Positive Secondary Pools - Tertiary Resolution Testing

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

- If one or more of the individual donor specimens is positive, the positive donor specimen(s) is (are) reported as "HCV RNA Positive" and the remaining negative donor specimens associated with the positive Secondary Pool are reported as "HCV RNA Negative."
- If all of the individual donor specimens in that Secondary Pool test negative, the donor specimens in the Secondary Pool may be reported as "HCV RNA Negative."
- As part of an overall Quality Assurance program, you may wish to conduct additional testing to determine the cause of the positivity of the Primary and Secondary Pools.

Results of Individual Donor Samples

If an individual donor specimen is positive, the positive donor specimen is reported as "HCV RNA Positive."

If an individual donor specimen is negative, the negative donor specimen is reported as "HCV RNA Negative."

Results of Pooled Source Plasma Specimens (Pools of up to 96 Individual Donations)

The testing algorithm for testing of pooled samples for the COBAS® AmpliScreen HCV Test, v2.0 requires a single level of testing for Primary Pools that are negative for HCV RNA and three levels of testing (Primary Pool, Minipool and confirmatory testing) for Primary Pools that are positive for HCV RNA.

Negative Primary Pools

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

Positive Primary Pools - Minipool Testing

Positive Primary pools are traced to the positive individual using an overlapping pool testing matrix. Minipools are prepared from the eight individual donations for columns 1-12 and from the 12 individual donations for rows 1-8. The 20 minipools are tested using the Standard Specimen Processing Procedure. 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.

Results of Individual Cadaveric Specimens

If an individual cadaveric specimen is positive, the positive cadaveric specimen is reported as "HCV RNA Positive."

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

For cadaveric specimens that had an initial invalid result and were repeated in duplicate, if either or both the duplicate samples are positive, the specimen is reported as "HCV RNA Positive." If both duplicate specimens are negative, or if one duplicate is negative and one is invalid, the specimen is reported as "HCV 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.

PROCEDURAL LIMITATIONS

1. This test has been evaluated only for use in combination with the COBAS® AmpliScreen Multiprep Specimen Preparation and Control Kit, COBAS® AMPLICOR® Analyzer and the Hamilton MICROLAB AT Plus 2 Pipettor for the automated preparation of plasma pools.
2. ***Heparin inhibits PCR; specimens collected using heparin as the anticoagulant should not be used with the COBAS® AmpliScreen HCV Test, v2.0.***
3. Reliable results are dependent on adequate specimen collection and proper transport procedures.
4. Detection of HCV RNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection and pool size.
5. Only the Hamilton MICROLAB AT Plus 2 Pipettor has been validated for use with the COBAS® AmpliScreen HCV Test, v2.0 for the automated preparation of plasma pools. Adhere to the hardware instructions and safety precautions outlined in the User Manual for the Hamilton MICROLAB AT Plus 2 Pipettor.

PERFORMANCE CHARACTERISTICS

Reproducibility

The reproducibility of the COBAS® AmpliScreen HCV Test, v2.0 was established by testing two six-member EDTA plasma panels with known concentrations of HCV. Panel One, which was tested using the Multiprep Specimen Processing Procedure, contained one HCV-negative sample and HCV-positive samples with HCV RNA concentrations of 10, 25, 50, and 50,000 IU/mL. Panel Two, which was tested using the Standard Specimen Processing Procedure, contained one HCV-negative sample and HCV-positive samples with concentrations of 25, 50, 100 and 50,000 IU/mL.

Testing was performed at three sites with two operators at each site using three COBAS® AmpliScreen HCV Test, v2.0 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 HCV Test, version 2.0 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

Results By Lot (Number Positive/Number Tested)					
	Negative	10 IU/mL	25 IU/mL	50 IU/mL	50,000 IU/mL
Lot #1	0/89	72/89	164/177	88/90	90/90
(%)	(0%)	(81%)	(93%)	(98%)	(100%)
Lot #2	0/90	59/90	168/180	88/89	90/90
(%)	(0%)	(66%)	(93%)	(99%)	(100%)
Lot #3	0/90	59/90	170/179	88/89	90/90
(%)	(0%)	(66%)	(95%)	(99%)	(100%)
Results By Site (Number Positive/Number Tested)					
Site #1	0/90	66/89	166/178	88/89	90/90
(%)	(0%)	(74%)	(93%)	(99%)	(100%)
Site #2	0/89	65/90	170/179	90/90	90/90
(%)	(0%)	(72%)	(95%)	(100%)	(100%)
Site #3	0/90	59/90	166/179	86/89	90/90
(%)	(0%)	(66%)	(93%)	(97%)	(100%)

Table 2
Reproducibility Results- Standard Specimen Processing Procedure

Results By Lot (Number Positive/Number Tested)					
	Negative	25 IU/mL	50 IU/mL	100 IU/mL	50,000 IU/mL
Lot #1	0/90	56/89	166/180	89/90	90/90
(%)	(0%)	(63%)	(92%)	(99%)	(100%)
Lot #2	0/90	66/89	165/179	89/90	90/90
(%)	(0%)	(74%)	(92%)	(99%)	(100%)
Lot #3	3/87	68/90	167/179	83/87	90/90
(%)	(3%)	(76%)	(93%)	(95%)	(100%)
Results By Site (Number Positive/Number Tested)					
Site #1	0/87	61/89	162/179	85/87	90/90
(%)	(0%)	(69%)	(91%)	(98%)	(100%)
Site #2	1/90	72/90	169/179	88/90	90/90
(%)	(1%)	(80%)	(94%)	(98%)	(100%)
Site #3	2/90	57/89	167/180	88/90	90/90
(%)	(2%)	(64%)	(93%)	(98%)	(100%)

Analytical Sensitivity - Dilutional Panels

The analytical sensitivity of the COBAS® AmpliScreen HCV Test, v2.0 was determined by testing 10 HCV 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, 16.7 and 5.6 HCV RNA IU/mL for the Multiprep Specimen Processing Procedure and 300, 100, 33.3 and 11.1 IU/mL for the Standard Specimen Processing Procedure. The COBAS® AmpliScreen HCV Test, v2.0 detected 16.7 HCV RNA IU/mL at a frequency greater than 90% with a lower 95% confidence limit of 86.4% using the Multiprep Specimen Processing Procedure. The assay detected 33.3 HCV RNA IU/mL at a frequency greater than 84% with a lower 95% confidence limit of 79.7% using the Standard Specimen Processing Procedure. The data are presented in Tables 3 and 4.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Multiprep Specimen Processing Procedure indicate an average 95% Limit of Detection (LOD) of 21.0 IU/mL, with lower and upper 95% confidence limits of 17.1 IU/mL and 27.8 IU/mL, respectively. The LOD of 21.0 IU/mL corresponds to approximately 57 copies/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 54.1 IU/mL, with lower and upper 95% confidence limits of 44.1 IU/mL and 71.7 IU/mL, respectively. The LOD of 54.1 IU/mL corresponds to approximately 146 copies/mL.

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

Multiprep Sample Processing Procedure				
HCV RNA Concentration (IU/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit – One-Tailed
150	219	219	100.0%	98.6%
50	220	220	100.0%	98.6%
16.7	197	218	90.3%	86.4%
5.6	30	44	68.1%	54.8%

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

Standard Sample Processing Procedure				
HCV RNA Concentration (IU/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit – One-Tailed
300	220	220	100.0%	98.6%
100	220	220	100.0%	98.6%
33.3	183	217	84.3%	79.7%
11.1	54	87	62.1%	52.7%

Analytical Sensitivity - WHO HCV International Standard

The analytical sensitivity of the COBAS® AmpliScreen HCV Test, v2.0 was also determined using the WHO HCV International Standard (96/790). The WHO HCV International Standard was serially diluted in HCV-negative plasma to final concentrations of 200, 100, 50, 25, 15, and 10 IU/mL. Each dilution was tested with two lots of the COBAS® AmpliScreen HCV Test, v2.0 using both the Multiprep and Standard Specimen Processing Procedures.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Multiprep Specimen Processing Procedure indicate an average 95% LOD of 28.8 IU/mL, with lower and upper 95% confidence limits of 20.5 IU/mL and 85.8 IU/mL, respectively.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Standard Specimen Processing Procedure indicate an average 95% LOD of 41.9 IU/mL, with lower and upper 95% confidence limits of 28.0 IU/mL and 111.8 IU/mL, respectively. Tables 5 and 6 summarize the overall results for the Multiprep and Standard Specimen Processing Procedures, respectively.

Table 5
Serial Dilution Testing Summary for Multiprep Method
Combined Input Values with Lower 95% Confidence Limit (One-Sided)

HCV RNA Concentration (IU/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit (One-sided)
200	132	132	100.00%	97.76%
100	132	132	100.00%	97.76%
50	130	132	98.48%	95.31%
25	128	132	96.97%	93.20%
15	95	132	71.97%	64.83%
10	92	132	69.70%	62.45%

Table 6
Serial Dilution Testing Summary for Standard Method
Combined Input Values with Lower 95% Confidence Limit (One-Sided)

HCV RNA Concentration (IU/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit (One-sided)
200	131	131	100.00%	97.74%
100	129	132	97.73%	94.23%
50	132	132	100.00%	97.76%
25	115	132	87.12%	81.31%
15	93	131	70.99%	63.77%
10	84	132	63.64%	56.19%

Analytical Sensitivity - CBER HCV Panel

The FDA CBER HCV Panel Members # 1-10 were processed using the Multiprep and Standard Sample Processing Procedures. Both specimen processing methods detected HCV RNA at 50 copies/mL. The Multiprep Sample Processing Procedure detected 100% of all positive members ranging from 10 - 100,000 copies/mL. The Standard Sample Processing Procedure detected 100% of all positive members ranging from 50 to 100,000 copies/mL. Both negative members of the panel were negative by both methods. The data are shown in Table 7.

Table 7
CBER HCV RNA Panel Results

CBER HCV RNA Panel (Copies/mL)	**CBER HCV Panel Member Test Results (Percent Positive)**									
	1 (1000)	**2 (Neg)**	**3 (100,000)**	**4 (10,000)**	**5 (Neg)**	**6 (500)**	**7 (200)**	**8 (50)**	**9 (10)**	**10 (5)**
Multiprep Method	100%	0%	100%	100%	0%	100%	100%	100%	100%	67%
Standard Prep Method	100%	0%	100%	100%	0%	100%	100%	100%	67%	0%

Genotype Detectability

Twenty individual plasma specimens representing Genotypes 1 and 4, sixteen plasma specimens of Genotype 2, nineteen plasma specimens of Genotype 3, four plasma specimens of Genotype 5, and eight plasma specimens of Genotype 6 have been tested. As an additional measure of the ability of the COBAS® AmpliScreen HCV Test, v2.0 to identify HCV genotypes, six Genotype 6a transcripts and one Genotype 5a HCV RNA transcript were diluted to 5 IU/PCR, and directly tested by the COBAS® AmpliScreen HCV Test, v2.0. With the exception of one sample (Genotype 2a/2c), which was below the limit of quantitation by a quantitative assay, each specimen was diluted to approximately 200 IU/mL of HCV RNA in pooled negative human plasma. Diluted samples were processed using both the Multiprep and Standard Sample Processing Procedures. The COBAS® AmpliScreen HCV Test, v2.0 detected all Genotypes at 200 IU/mL except the one sample that was not quantifiable. This sample (Genotype 2a/2c) was detected using the Multiprep Specimen Processing Procedure, but was negative when tested using the Standard Specimen Processing Procedure. This result is consistent with HCV RNA levels below the detection limit of the assay. Data are provided in Table 8.

Table 8
HCV Genotype Samples Tested

HCV Genotype/Subtype	Quantity	Reactive / Total (Multiprep)	Reactive / Total (Standard Prep)
1	8	8/8	8/8
1a	3	3/3	3/3
1b	9	9/9	9/9
2	1	1/1	1/1
2a	2	2/2	2/2
2b	10	10/10	10/10
2a/2c	3	3/3	2/3[a]
3a	12	12/12	12/12
3a	6	6/6	6/6
3e	1	1/1	1/1
4	1	1/1	1/1
4	11	11/11	11/11
4a	2	2/2	2/2
4c	3	3/3	3/3
4c/4d	2	2/2	2/2
4h	1	1/1	1/1
5a	5[b]	5/5	5/5
6a	14[b]	14/14	14/14

a One sample contained HCV RNA at a level below the Limit of Quantitation of a quantitative assay. Sample was tested undiluted.

b One Genotype 5a and six Genotypes 6a HCV RNA transcripts were included in the testing, and all yielded positive results.

Seroconversion Panels

Nine anti-HCV seroconversion panels were tested using both the Multiprep and the Standard Specimen Processing Procedures. Each specimen in each panel was tested by the Ortho HCV, version 3.0 ELISA Test system and all samples with reactive EIA results were also tested by Chiron RIBA HCV 3.0 SIA. The HCV RNA test results were then compared to the EIA test results for each specimen to determine if HCV RNA testing detected the presence of HCV infection prior to seroconversion.

The COBAS® AmpliScreen HCV Test, v2.0 detected HCV infection an average of 32 days before seroconversion for the nine seroconversion panels. The data are summarized in Table 9.

Table 9
HCV Seroconversion Study

Panel	Day Positive Ortho 3.0 EIA and Chiron RIBA 3.0	Day Positive COBAS® AmpliScreen HCV Test, v2.0	Difference COBAS® AmpliScreen vs EIA
6212	14	0	14
6224	19	0	19
6215	20	0	20
9047	28	0	28
9045	41	0	41
6225	78	39	39
6213	43	11	32
6222	40	17	23
6227	74	0*	74*
Mean Days Earlier Detection			32

** Specimen was RNA positive on Day 0, but negative on Days 22 and 24. Day 74 specimen was RNA positive again.*

Analytical Specificity - Potentially Cross Reactive and Interfering Microorganisms

The analytical specificity of the COBAS® AmpliScreen HCV Test, v2.0 was evaluated by testing a panel of microorganisms and other disease states, including 23 viral isolates, two bacterial strains and one yeast isolate. No-cross reactivity was observed with the COBAS® AmpliScreen HCV Test, v2.0. Table 10 summarizes the microorganisms studied.

Table 10
Analytical Specificity - Microorganisms Tested

Adenovirus type 2	Epstein Barr Virus	HIV-1 Subtype D
Adenovirus type 3	Hepatitis A Virus	HIV-2
Adenovirus type 7	Hepatitis B Virus (n=3)	HTLV-I
Autoimmune samples	Herpes Simplex type 1	HTLV-II
Candida albicans	Herpes Simplex type 2	Human Herpes Virus 6
Chlamydia trachomatis	HIV-1 Subtype A	Human Herpes Virus 7
Coxsackievirus B1	HIV-1 Subtype B	*Staphylococcus epidermidis*
Cytomegalovirus	HIV-1 Subtype C	Varicella-Zoster
Echovirus 1		

Up to ten individual patient plasma specimens from each of the following disease categories were spiked with low levels of HCV-positive plasma (within 2-3X the 95% LOD): HIV-1, HIV-2, autoimmune disease, EBV, CMV, and *Candida albicans*. No false negative test results were observed.

Analytical Specificity - Non-Hepatitis Samples

Twenty-five HAV- and 25 HBV-positive specimens (all HCV-negative) were tested for cross reactivity with the COBAS® AmpliScreen HCV Test, v2.0 by using both the Standard and Multiprep Sample Processing Procedures. All samples were found to be negative. No false positive test results were observed.

These samples were also spiked with low levels of HCV-positive plasma and tested using both the Standard and Multiprep Sample Processing Procedures. All samples were found to be positive. No false negative test results were observed.

Potentially Interfering Substances

Endogenous Interfering Substances

HCV-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 HCV Test, v2.0, using either the Standard or Multiprep Specimen Processing Procedure.

Exogenous Interfering Substances

HCV-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 using either the Standard or Multiprep Specimen Processing Procedure.

CLINICAL PERFORMANCE

Chronic HCV Population

Fifty-eight specimens were obtained from patients with a diagnosis of chronic HCV disease. All specimens were confirmed to be serologically positive by a licensed anti-HCV EIA followed by RIBA 3.0. The specimens were tested undiluted using the Standard Specimen Processing procedure and diluted 1:24 using the Multiprep Specimen Processing procedure. All specimens were positive in the COBAS® AmpliScreen HCV Test, v2.0 by both specimen processing procedures.

High Risk Population

Specimens were prospectively collected from a patient population being evaluated at hematology clinics for biochemical, clinical and/or histological evidence of liver disease and/or evidence of HCV infection. Specimens were tested in a blinded fashion with COBAS® AmpliScreen HCV Test, v2.0 using the Standard Specimen Processing Procedure.

Fifty-seven of 62 total specimens were positive for HCV RNA. Four specimens negative for HCV RNA were also negative for HCV antibody by both a licensed screening EIA and confirmatory assay and were excluded from the analysis. The COBAS® AmpliScreen HCV Test, v2.0 detected 57 out of 58 HCV antibody-positive specimens.

Pool Reactivity in Volunteer Blood Donors

A random selection of 8,240 pools revealed that 117 Primary Pools were reactive for an initial reactive rate of 1.42%. There were 106/117 (90.6%) positive pools that were concordant with confirmed positive serology status. None of these pools were identified as having a window period case. A total of 11 pools were found positive but were not confirmed positive by serology or by subsequent testing of individual donations by the COBAS® AmpliScreen Test, v2.0. Results are summarized in Table 11.

Table 11
Pool Reactivity in Volunteer Blood Donors

Category	Pools	Percentage
Pools Tested	8,240	100
Non-Reactive pools	8,123	98.58
Initially reactive pools	117	1.42
Initial pools with concordant serology	106	1.28
Positive pools due to window case	0	0
Initial pools with negative serology and negative individual donation AmpliScreen Testing (false positive)	11	0.13

A random selection of approximately 250,000 specimens was selected from geographically divergent sites. The results from these specimens were used to determine the specificity and sensitivity of COBAS® AmpliScreen HCV Test, v2.0. Using the antibody results, the HCV status of each specimen was determined. HCV status-negative included either: 1) anti-HCV EIA negative, regardless of other results (unless the subject was enrolled in the follow-up study and had test results that changed this assessment); or 2) anti-HCV EIA positive and RIBA negative.

HCV status-positive included either: 1) anti-HCV EIA repeat reactive and RIBA positive; or 2) anti-HCV EIA repeat reactive or HCV RNA positive upon follow-up. HCV status-unknown included anti-HCV EIA repeat reactive with RIBA indeterminate or unknown.

There were 247,998 specimens that were determined to be HCV status-negative. Of these, 247,990 were also HCV RNA-negative. The specificity of the COBAS® AmpliScreen HCV Test, v2.0 in this study was 247,990/247,998 or 99.997% with 95% confidence limits of 99.99% to 100.00%. The negative predictive value obtained by summing all the cases determined to have HCV status negative among the 248,106 COBAS® AmpliScreen HCV Test, v2.0 negative donations is estimated in this study to be 99.95% with exact 95% confidence limits (99.94%, 99.96%).

There were 243 specimens that were determined to be HCV status-positive. Of these, 203 were also HCV RNA-positive. The positive predictive value obtained by finding the percentage of specimens detected to be HCV status positive among 215 COBAS® AmpliScreen HCV Test, v2.0 positive donations is estimated to be 94.42% with exact 95% confidence limits (90.45%, 97.08%). All 243 samples in this population were included in the analysis, irrespective of HCV RNA titers. These data are consistent with previous reports that about 20% of HCV seropositive samples will have undetectable HCV RNA.

A total of 416 specimens were repeatedly reactive by EIA and of these 204 were also COBAS® AmpliScreen HCV Test, v2.0 positive. Of the 204 COBAS® AmpliScreen HCV Test, v2.0 positive specimens, none were RIBA negative.

Table 12:
COBAS® AmpliScreen HCV Test, v2.0 Results for EIA Repeatedly Reactive Specimens

EIA RR = 416							
NAT (–) = 212				NAT (+) = 204			
RIBA				RIBA			
ND	+	–	IND	ND	+	–	IND
20	43	96	53	3	200	0	1

Detection of Window Period Cases

From April 8, 1999 to December 31, 2000, approximately 7 million donations were tested. During this period there were 20 confirmed window period cases detected. A confirmed window period case is defined as an enrolled individual from whom the index donation was positive with the COBAS® AmpliScreen HCV Test, v2.0 but non-reactive by EIA for anti-HCV, and a follow-up specimen was shown to be anti-HCV EIA repeat reactive using the Abbott HCV EIA 2.0 assay and/or the Ortho HCV Version 3.0 ELISA test system and/or HCV RNA positive. The detection rate of such window period cases was 0.00029% (1 in 350,000) with a 95% confidence interval of 0.00017% to 0.00041%. In addition, four subjects with negative serology and no follow-up specimens were presumed to be window period cases, as a specimen from the plasma bag for each confirmed the index HCV RNA positive result. If these four subjects are included, the detection rate of window period cases is 0.00034% (1 in 292,000) with a 95% confidence interval of 0.00021% to 0.00049%.

Single Donation Testing Performance

A total of 2,515 blood donor specimens were tested individually in the COBAS® AmpliScreen HCV Test, v2.0 clinical trial. Of the 2,515 specimens, five were classified as HCV seropositive and were removed from the calculation of specificity. Of the 2,510 specimens tested, 2,508 were HCV RNA negative and two were HCV RNA positive.

No follow-up was conducted on these two donors and they were presumed to be false positive. The specificity of the COBAS® AmpliScreen HCV Test, v2.0 in this study was 99.92% (2,508/2,510) with a 95% confidence interval of 99.71% to 99.99%.

PERFORMANCE CHARACTERISTICS OF SOURCE PLASMA

Clinical Performance

A total of 104,448 donations from 35,905 donors were tested in the 96-member minipool format in 1,088 pools. Seven donations from 3 donors were positive for HCV RNA and negative by antibody to HCV EIA and RIBA. Two donors each donated a HCV RNA positive & anti-HCV positive sample that was tested in one 96-member minipool. The data are presented in Table 13.

Table 13
Pool Reactivity in Source Plasma Donors

Category	No. of Pools	Percentage
Pools Tested	1088	100%
Non-Reactive pools	1077	98.99%
Initially Reactive pools	11	1.01%
Initial pools containing donation with concordant serology[1]	1	0.09%
Positive pools due to window case	7	0.64%
Initially Reactive pools with negative resolution COBAS® AmpliScreen Testing (false positive)	3	0.28%

[1]Two HCV EIA positive donations in one 96-member minipool.

Of the 3 eligible donors, one donor had been previously qualified but had been absent from the collection center for more than 6 months as was reclassified to Applicant status upon return. The other 2 donors indicated their willingness to participate in the HCV follow-up study. All three donors are considered to be confirmed window cases due to subsequent donations testing positive for HCV RNA.

Additional testing on the index donation sample volume permitting was positive by both National Genetics Institute (NGI) HCV UltraQual™ reaction per primer pair and Bayer Versant® HCV Quantitation. The quantitation for one sample was 492,047 copies/mL.

Both enrolled follow-up study participants were anti-HCV positive and HCV positive by the Roche COBAS® AmpliScreen HCV Test, v2.0 upon the first study samples collected. Antibody was detected by RIBA for one of the study participants, and sample was sent out for Ortho HCV EIA 3.0 analysis, and yielded a reactive result. The specimen from the other follow-up study participant was reactive for HCV antibodies by the Abbott HCV EIA Test, v2.0.

There were 1080 pools that were used to determine the specificity of HCV RNA. Of these pools, 1077 were HCV RNA negative. The specificity of the COBAS® AmpliScreen HCV Test, v2.0 in this study was 1077/1080 or 99.7222% with 95% confidence interval of 99.19% to 99.94%.

NON-CLINICAL PERFORMANCE

Ten commercially available HCV seroconversion panels were diluted 1:96 with HCV negative human plasma and tested using the Multiprep Specimen Processing Procedure. Results were compared with test results from U.S. FDA licensed tests for anti-HCV EIA and RIBA. Five (5) of the 10 panels (50%) were never positive for EIA, two (2) of the 10 (20%) panels were never positive for ELISA and seven (7) of the 10 panels (70%) were never reactive for RIBA. The data are presented in Table 14.

Table 14
Summary of Pre-Seroconversion Detection of HCV RNA vs. HCV FDA Licensed Tests

	Days Before Abbott HCV EIA 2.0 (10 panels tested)	Days Before Ortho HCV 3.0 ELISA (10 panels tested)	Days Before Chiron RIBA 3.0 (10 panels tested)
Mean	41	29.6	35.3
Median	35	32	37
Maximum	53	41	55
Minimum	20	20	20

In 100% (10/10) of the HCV seroconversion panels tested, the COBAS® AmpliScreen HCV Test, v2.0 used with the Multiprep Processing procedure and pools of 96 specimens, identifies HCV RNA infected specimens earlier than did the U.S. FDA licensed HCV EIA, ELISA, and the RIBA assays.

NON-CLINICAL PERFORMANCE CHARACTERISTICS FOR CADAVERIC SPECIMENS

Sensitivity Study

Sixty pre-mortem EDTA plasma and fifty-eight cadaveric EDTA plasma specimens non-reactive for HCV were divided into 5 groups. Specimens within each group were spiked with HCV 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 HCV Test, version 2.0 kit lots.

The COBAS® AmpliScreen HCV Test, v2.0 using samples diluted 1:5 and the Multiprep Specimen Processing Procedure correctly detected 98.3% (59/60) pre-mortem EDTA plasma specimens and 94.8% (55/58) of cadaveric specimens spiked with HCV RNA at 3X the LOD of the COBAS® AmpliScreen HCV Test, v2.0. The results reflect the retesting of two cadaveric specimens that were inhibited on initial testing. In repeat testing, both resolved negative. The summary of the test results of this study is presented in Table 15 below.

Table 15
Summary of Sensitivity Test Results

		Pre-Mortem EDTA Plasma Specimen	Post-Mortem EDTA Plasma Specimen
Total Specimens Tested		60	58
Test Results	**+**	59	55
	–	1	3
	Inhib.	0	0
Sensitivity		98.3%	94.8%
95% Confidence Interval	**Upper**	99.9%	98.9%
	Lower	91.1%	85.6%

Specificity Study

Sixty pre-mortem and fifty-eight post-mortem specimens which were negative for HCV 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 HCV Test, v2.0.

The COBAS® AmpliScreen HCV Test, v2.0 using samples diluted 1:5 and the Multiprep Specimen Processing Procedure yielded negative results on 100% (60/60) of the pre-mortem EDTA plasma specimens, and 100% (58/58) of the post-mortem EDTA plasma specimens. The summary of results is presented in Table 16.

Table 16
Summary of Specificity Test Results

		Pre-Mortem EDTA Plasma Specimen	Post-Mortem EDTA Plasma Specimen
Total Specimens Tested		60	58
Test Results	**(+)**	0	0
	(–)	60	58
	Inhib.	0	0
Final Specificity		**100%**	**100%**
95% Confidence Interval	**Upper**	100%	100%
	Lower	94.0%	93.8%

Reproducibility Study

Twenty pre-mortem EDTA plasma and 20 individual cadaveric specimens were spiked with HCV 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 HCV Test, v2.0 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 17 below.

Table 17
Summary of Reproducibility Study Test Results - Post-Mortem versus Pre-Mortem

	Post-Mortem	Pre-Mortem
Results by Lot (# Positive / # Tested, Percent Hit Rate)		
Lot #1	120/120 100%	119/120 99.2%
Lot #2	117/120 97.5%	117/119 98.3%
Lot #3	119/120 99.2%	120/120 100%
Results by Site (# Positive / # Tested, Percent Hit Rate)		
Site #1	118/120 98.3%	120/120 100%
Site #2	118/120 98.3%	118/120 98.3%
Site #3	120/120 100%	118/119 99.2%

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