COBAS AmpliScreen™ HCV Test, version 2.0

FOR IN VITRO DIAGNOSTIC USE.

COBAS AmpliScreen HCV Test, version 2.0

<table>
<thead>
<tr>
<th>Test Kit Description</th>
<th>Tests</th>
<th>P/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>COBAS AmpliScreen Multiprep Specimen Preparation Reagents Kit</td>
<td>48 Tests</td>
<td>03139204 123</td>
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<tr>
<td>COBAS AmpliScreen Negative Human Plasma Reagent Kit</td>
<td>96 Tests</td>
<td>03184480 123</td>
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<tr>
<td>COBAS AMPLICOR™ Wash Buffer</td>
<td>500 Tests</td>
<td>20759889 123</td>
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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 from donations of whole blood and blood components for transfusion.

The test is intended for use in screening 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 HCV RNA in conjunction with licensed tests for detecting antibodies to HCV.

This assay 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 blood-borne 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 15% in Japan.2

Serological screening assays have greatly reduced, but not completely eliminated, the risk of transmitting viral infections by transfusion of blood products.3,4 Recent studies indicate that nucleic acid-based amplification tests for HCV RNA will allow detection of HCV infection earlier than the current antigen-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.5,7

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 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)5
3. PCR amplification6 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

The Multiprep Specimen Processing Procedure for preparation of mini-pool specimens consists of:

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

Reverse Transcription

The reverse transcription and amplification reactions are performed with the thermolabile recombinant enzyme Thermus thermophilus DNA Polymerase (rTth pol). In the presence of manganese (Mn²⁺) and under the appropriate buffer conditions, rTth pol produces a cDNA copy of the HCV target RNA, which is then amplified. The Multiprep Specimen Processing Procedure results in the isolation of cDNA from the target RNA in plasma.

PCR Amplification

Following reverse transcription using rTth pol, a second DNA strand is produced from the cDNA copy, resulting in the production of a double-stranded DNA molecule termed an amplicon. The COBAS AMPLICOR Analyzer automati- cally repeats this process for a designated number of cycles, each cycle effectively doubling the amount of amplicon DNA. The required number of cycles is programmed 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 deoxyuridylate, but not DNA containing deoxythymidylate. Deoxyuridylate is not present in naturally occurring DNA, but is always present in the RNA of the Multiprep Internal Control (IC). The AmpErase enzyme 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 Reagents Kit, COBAS AmpliScreen Negative Human Plasma Reagent Kit and the COBAS AMPLICOR Wash Buffer are provided as stand-alone kits to be used in conjunction with the COBAS AmpliScreen HCV Test, v.2.0, as well as with the COBAS AmpliScreen™ HIV-1 Test, v.1.5.

**COBAS AmpliScreen MultiPrep Specimen Preparation Reagents Kit**

<table>
<thead>
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<th>Kit Name</th>
<th>Tests</th>
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<tbody>
<tr>
<td>MP (–) C</td>
<td>48</td>
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<tr>
<td>MP LYS</td>
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<tr>
<td>MP DIL</td>
<td></td>
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<td>MP IC</td>
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**COBAS AmpliScreen Negative Human Plasma Reagent Kit**

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<td>NHP</td>
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**COBAS AmpliScreen HCV Test, version 2.0**

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<tbody>
<tr>
<td>HCV C, v.2.0</td>
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<td>HCV CTL</td>
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**COBAS AmpliScreen HCV Amplification Reagents, version 2.0**

<table>
<thead>
<tr>
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<td>HCV MMX, v.2.0</td>
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<td>HCV Mn²⁺, v.2.0</td>
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**COBAS AmpliScreen HCV Detection Reagents, version 2.0**

<table>
<thead>
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<th>Kit Name</th>
<th>Tests</th>
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<tbody>
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<td>CI PS1</td>
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<td>CI2</td>
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</tr>
<tr>
<td>SB1</td>
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**COBAS AMPLICOR Wash Buffer**

<table>
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<th>Tests</th>
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</thead>
<tbody>
<tr>
<td>WB</td>
<td>500</td>
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OTHER MATERIALS REQUIRED BUT SOLD SEPARATELY (MAY BE PURCHASED FROM ROCHE)

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

MATERIALS REQUIRED BUT NOT PROVIDED BY ROCHE

- Hamilton MICROLAB® AT plus 2 Pipettor or equivalent
- Refrigerated high speed centrifuge with fixed angle rotor (45 degrees, capacity for at least 24 x 1.5-mL tubes) with an RCF of 23,600 x g (Heraeus Centrifuge 17RS or Biofuge 28RS with IFA 22.1 rotor, Heraeus Biofuge Stratos with the 3331 rotor or equivalent).
- Microcentrifuge, (max. RCF 16,000 x g, min. RCF 12,500 x g) (Eppendorf® 5415C, HERMLE Z230M, or equivalent)
- Eppendorf 1.25 mL 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
- Powderfree, disposable gloves
- Isopropl alcohol, reagent grade
• Disposable, sterile, polystyrene pipettes (5 mL, 10 mL and 25 mL)
• Sterile, RNase-free, fine-tip transfer pipettes
• Pipettes (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 Reagents Kit

MP (–) C
[Multiprep (–) Control]
< 0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

MP LYS
[Multiprep Lysis Reagent]
Tris-HCl buffer
60% Guanidine thiocyanate
3% Dithiothreitol
< 1% Glycogen
Xn
< 0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

MP DIL
[Multiprep Specimen Diluent]
Tris-HCl buffer
< 0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

MP IC
[Multiprep Internal Control]
Tris-HCl buffer
< 0.01% 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 primer binding sequences and a unique probe binding region
< 0.005% Poly rA RNA (synthetic)
EDTA
< 0.1% Amaranth dye
0.05% Sodium azide

COBAS AmpliScreen Negative Human Plasma Reagent Kit

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

COBAS AmpliScreen HCV Test, version 2.0

HCV (+) C, v2.0
[HCV (+) Control, version 2.0]
< 0.001% Non-infectious in vitro transcribed RNA (microbial) containing HCV sequences
< 0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

COBAS AmpliScreen HCV Detection Reagents, version 2.0

HCV PRO v2.0
[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
CH2, v2.0
(HCV Probe Suspension 2, version 2.0)
- Sodium phosphate buffer
- 34.7% Sodium thiocyanate
- 0.2% Solubilizer

CI PS1
(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

CI2
(IC Probe Suspension 2)
- Sodium phosphate buffer
- 24.9% Sodium thiocyanate
- 0.2% Solubilizer

DN2
(Denaturation Solution)
- 1.6% Sodium hydroxide
- EDTA
- Thymol blue

CN4
(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

SB1
(Substrate A)
- Citrate solution
- 0.01% Hydrogen peroxide
- 0.1% ProClin 150

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

COBAS AMPLICOR Wash Buffer
(WB)
(10X-Wash Concentrate)
- < 2% Phosphate buffer
- < 9% Sodium chloride
- EDTA
- < 2% Detergent
- 0.5% ProClin® 300

STORAGE INSTRUCTIONS
A. Room Temperature is defined as 15 - 30°C.
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, HCV (+) C, v2.0; MP (-) C, MP DIL and NHP
   - HCV MMX, v2.0 and HCV Mn2+, v2.0
   - CH PS1, v2.0, CH2, v2.0, CI PS1 and CI2
   - CN4, SB1 and SB
D. Store DN2 at 2 - 25°C. Store WB at 2 - 30°C. DN2 and WB are stable until the expiration dates indicated.
E. Do not expose SB1, 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, HCV (+) C, v2.0, MP (-) C, MP DIL and NHP
   - HCV Mn2+, v2.0, SB, CH PS1, v2.0 and CI PS1.
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 Test System and trained in handling infectious materials should perform this procedure. Thoroughly clean and dideff 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 antibodies to HIV-1/2, HBV, HBcAg, and HCV. Testing of Negative Human Plasma by PCR methods showed no detectable HIV-1 RNA, HCV RNA or HBV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All human blood-sourced materials should be considered potentially infectious and should be handled with Universal Precautions. If spillage occurs, immediately disinfect, then wipe up with a 0.5% (final concentration) sodium hypochlorite solution (diluted bleach) or follow appropriate site procedures.

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

D. This product contains sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing a sodium azide compounds are disposed of in a plumbing system, they should be flushed and flushed with running water. These precautions are reccommended 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.

O. Material Safety Data Sheets (MSDS) are available on request. Thoroughly clean and disinfect test equipment and work area prior to use. Hypochlorite solution (diluted bleach) or follow appropriate site procedures. If spillage occurs, immediately disinfect, then wipe up with a 0.5% (final concentration) sodium hypochlorite solution (diluted bleach) or follow appropriate site procedures.

P. Do not allow MP LYS, MP MMX, v2.0, HCV Mn²⁺, v2.0, CH₂, v2.0, CI₂, DN2, CN4, SB1, SB and Working Substrate (mixed SB1 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 CH₂, v2.0 and CI₂, which contain sodium thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.

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

Q. Refer to the Pooling System Guide for use with the COBAS AmpliScreen Tests and the Operator’s Manuals for the AMPLILINK software and COBAS AMPLICOR Analyzer.

R. Follow all manufacturer’s 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.

REAGENT PREPARATION

A. MP IC, HCV (+) C, v2.0, MP (-) C, MP DIL and NHP

1. Warm MP IC, HCV (+) C, v2.0, 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.


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 disinfectanted in accordance with methods outlined in Precautions (Item A). Failure to do so may result in reagent contamination.

2. Pipette 100 µL HCV Mn²⁺, 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 Mn²⁺, v2.0 has been added to the HCV MMX, v2.0. Discard the remaining HCV Mn²⁺, 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 CH₂, 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 CI₂ 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 six instrument cycles (12 hours per cycle). Mixing occurs automatically on the COBAS AMPLICOR Analyzer.

4. Store Working Probe Suspension Detection Reagents at 2 - 8°C between instrument cycles. Remove from refrigerator 30 minutes before use on the COBAS AMPLICOR Analyzer.

E. DN2 - Denaturation Reagent and CN4 Conjugate Reagent

1. Once opened, DN2 and CN4 are stable for 30 days at 2 - 8°C, or until the expiration date, whichever comes first. Both DN2 and CN4 can be used for a maximum of six instrument cycles (12 hours per cycle).

2. Store DN2 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 SB1 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 SB1, 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.

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

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

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

E. Do not freeze whole blood.

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

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

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

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

J. Thaw frozen specimens at room temperature before using.

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

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

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

**NOTE:** The user must validate other pooling algorithms and equipment.

**PROCEDURAL NOTES**

A. **Run Size**
1. Each kit contains reagents sufficient for six 8-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 HCV Positive (+) Control must be included in each A-ring (see "Quality Control" section).

2. The Specimen Preparation and Amplification Reagents are packaged in six single-use bottles. The Multiprep Negative (–) and HCV 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 8.

B. **Equipment**
1. Prepare the COBAS AMPLICOR Analyzer and the Data Station for the AMPILINK Software for use according to instructions in the Operator’s Manual for the AMPILINK software and the Operator’s Manual for the COBAS AMPLICOR Analyzer.

2. Prepare the Hamilton MICROLAB AT plus 2 System and SUNPLUS Data Station or equivalent for use according to instructions in the Operator’s Manuals.

3. Pre-cool the high-speed centrifuge and rotor to 2 - 8°C. See operating instructions for the high speed centrifuge for details.

4. Perform manufacturer recommended maintenance and calibration on all instruments, including pipettors, to ensure proper functioning.

C. **Reagents**
1. All reagents except HCV MMX, v2.0 and HCV MMX+, 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 AMPLI LiNK 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 15º to 30ºC.

F. Pipetting

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

G. Vortexing

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

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

   NOTE: A multi-analyte (HIV-1/HCV) positive control can be made by adding 20 µL of each analyte specific (+) C to the tube labeled “MP (+) C” containing Working Lysis Reagent and NHP.

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. 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

   NOTE: A multi-analyte (HIV-1/HCV) positive control can be made by adding 20 µL of each analyte specific (+) C to the tube labeled “MP (+) C” containing Working Lysis Reagent and NHP.

   Vortex HCV (+) C, v2.0 and HIV-1 (+) C, v1.5 briefly. Tap vial to collect the solution in the base. Pipette 20 µL HCV (+) C, v2.0 and 20 µL HIV-1 (+) C, v1.5 to the tube labeled “MP (+) C” containing the Working Lysis Reagent and NHP. Cap the tube and vortex briefly.

B22. 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.

C. Reverse Transcription, Amplification and Detection

   NOTE: Amplification must begin within 45 minutes from when the first specimen or control in the A-ring is added to the Working Master Mix.
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 (CH2, v2.0), Working IC Probe Suspension Reagent (CI2), Working Substrate (SB1), Denaturation Reagent (DN2), and Conjugate (CN4).
   b. Place the CH2, v2.0 and CI2 cassettes in the test-specific reagent rack.
   c. Place DN2, CN4 and SB1 cassettes in the generic reagent rack. Record on the cassette the date when each cassette was opened.
   d. Identify the reagent racks as generic or test specific using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the Operator’s Manual for AMPLILINK software.
   e. Configure the reagent racks by entering the reagent positions and lots using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the Operator’s Manual for AMPLILINK software.
   f. Load the reagent racks onto the Analyzer using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the Operator’s Manual for AMPLILINK software. Make sure that each reagent cassette is in its assigned position and that each cassette fits tightly into its rack.
   g. Place the D-cup rack on the D-cup platform. Two D-cups are required for each A-tube and two D-cups are required for each Working Substrate cassette to allow for blanking by the COBAS AMPLICOR Analyzer, as described in the Operator’s Manual for the COBAS AMPLICOR Analyzer.
   h. Place the A-ring into the thermal cycler segment of the COBAS AMPLICOR Analyzer and close the cover on the thermal cycler segment.
   i. Load the A-ring into the COBAS AMPLICOR Analyzer using the Analyzer barcode scanner for the AMPLILINK software, as described in the Operator’s Manual for AMPLILINK software.
   j. Create an A-ring order, using the AMPLILINK software, as described in the Operator’s Manual for AMPLILINK software. As part of an overall Quality Assurance program, if desired, for each run, print the AMPLILINK A-ring Results Report and the Run Log and retain these along with 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.
   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 part of an overall Quality Assurance program, if desired, for each run, print the AMPLILINK A-ring Results Report and the Run Log and retain these along with the A-ring worklist record created for specimen processing to assist in entering the A-ring order.

QUALITY CONTROL PROCEDURES

1. At least one Multiprep (–) Control and one HCV (+) 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 at 660 nm 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 (specimen and control preparation, amplification and detection) must be repeated.
   b. Positive Control
      The absorbance for the HCV (+) C, v2.0 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 HCV (+) C, v2.0 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.

<table>
<thead>
<tr>
<th>Summary of Control Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HCV Result</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
</tr>
<tr>
<td>Positive Control</td>
</tr>
</tbody>
</table>

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

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

INTERPRETATION OF RESULTS

1. Flags and comments may be generated by the COBAS AMPLICOR Analyzer during a run. The Operator must check the runprintout(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:

<table>
<thead>
<tr>
<th>HCV Result</th>
<th>IC Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt; 0.2)</td>
<td>(&lt; 0.2)</td>
</tr>
<tr>
<td><strong>NEGATIVE</strong></td>
<td><strong>NEGATIVE</strong></td>
</tr>
<tr>
<td>(\geq 0.2)</td>
<td>(&lt; 0.2)</td>
</tr>
<tr>
<td><strong>POSITIVE</strong></td>
<td><strong>ANY</strong></td>
</tr>
</tbody>
</table>

**Interpretation**

- Specimen is negative for HCV RNA.
- Specimen is positive for HCV RNA.
- Repeat entire test procedure for invalid specimen.

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 using new, reagent lots. The entire test procedure must be performed using new, reagent lots.

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 original plasma samples.

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

Results of Pooled Donor Specimens

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 Pool - 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, for which the four associated secondary pools are negative. Positive pools resolved as negative at the secondary resolution testing level is usually a result of a contamination event to the primary pool, but theoretically may be due to a viral load below the limit of detection.


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."
- As part of an overall Quality Assurance program, you may wish to conduct additional testing to determine the cause of the positivity of the Primary and Secondary Pools, for which the associated individual samples were negative. Positive pools resolved as negative at the tertiary resolution testing level is usually a result of a contamination event to the secondary pool, but theoretically may be due to a viral load below the limit of detection.

**Results of Individual Donor Samples**

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

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

**PROCEDURAL LIMITATIONS**

1. This test has been evaluated only for use in combination with the COBAS AmpliScreen Multiprep Specimen Preparation Reagents Kit, COBAS AmpliScreen Negative Human Plasma Reagent 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. For detailed instructions on the use of the Hamilton MICROLAB AT plus 2 pipettor for the COBAS AmpliScreen Test, contact your local Roche representative to obtain a copy of the COBAS AmpliScreen Pooling System Guide.
PERFORMANCE CHARACTERISTICS

Reproducibility

The reproducibility of the Test 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, 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, 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 Tables 1 and 2 below:

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

<table>
<thead>
<tr>
<th></th>
<th>Lot #1</th>
<th>Lot #2</th>
<th>Lot #3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>95%</td>
<td>95%</td>
<td>95%</td>
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<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>95%</td>
<td>95%</td>
<td>95%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Lot #1</th>
<th>Lot #2</th>
<th>Lot #3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
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<tr>
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<tr>
<td></td>
<td>95%</td>
<td>95%</td>
<td>95%</td>
</tr>
</tbody>
</table>

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 sample 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 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% 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. This equates to an LOD of 504 IU/mL or 1368 copies/mL for an individual donor specimen tested in a pool of 24 donors.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Standard Specimen Processing Procedure indicate an average 95% LOD of 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

<table>
<thead>
<tr>
<th></th>
<th>Multiprep Sample Processing Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>90.3%</td>
</tr>
<tr>
<td></td>
<td>68.1%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>HCV RNA Concentration (IU/mL)</th>
<th>Number of Positives</th>
<th>Number of Individual Trials</th>
<th>% Positive</th>
<th>95% Lower Confidence Limit – One-Tailed</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>220</td>
<td>220</td>
<td>100.00%</td>
<td>98.6%</td>
</tr>
<tr>
<td>100</td>
<td>220</td>
<td>220</td>
<td>100.00%</td>
<td>98.6%</td>
</tr>
<tr>
<td>33.3</td>
<td>183</td>
<td>217</td>
<td>84.3%</td>
<td>79.7%</td>
</tr>
<tr>
<td>11.1</td>
<td>54</td>
<td>87</td>
<td>62.1%</td>
<td>55.7%</td>
</tr>
</tbody>
</table>

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. This equates to an LOD of 691.2 IU/mL for an individual donor specimen tested in a pool of 24 donors.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Standard Specimen Processing Procedure indicate an average 95% LOD of 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)

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

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

| HCV RNA Number of Number of % Positive 95% Lower Confidence Limit |
|-----------------------------------------------|-----------------------------|-----------------------------|-----------------------------------------------|
| Concentration (IU/mL) | Positives | Individual Trials | Positive | Limit (One-sided) |
| 300 | 220 | 220 | 100.00% | 98.6% |
| 100 | 220 | 220 | 100.00% | 98.6% |
| 11.1 | 54 | 87 | 62.1% | 55.7% |

Genotype Detectability

Twenty individual plasma specimens representing Genotypes 1 and 4, sixteen plasma specimens of Genotype 2, nineteen plasma specimens of Genotype 3, and two plasma specimens each of Genotypes 5 and 6 were tested. 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.
HCV Genotype Samples Tested

<table>
<thead>
<tr>
<th>HCV Genotype/Subtype</th>
<th>Quantity</th>
<th>Reactive / Total (Multiprep)</th>
<th>Reactive / Total (Standard Prep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>8/8</td>
<td>8/8</td>
</tr>
<tr>
<td>1a</td>
<td>3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>1b</td>
<td>9</td>
<td>9/9</td>
<td>9/9</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>2a</td>
<td>2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>2b</td>
<td>10</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>2a/2c</td>
<td>3</td>
<td>3/3</td>
<td>2/3*</td>
</tr>
<tr>
<td>3a</td>
<td>12</td>
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<td>12/12</td>
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<td>3a</td>
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<td>6/6</td>
</tr>
<tr>
<td>3e</td>
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<td>1/1</td>
<td>1/1</td>
</tr>
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<td>1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>11/11</td>
<td>11/11</td>
</tr>
<tr>
<td>4a</td>
<td>2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>4c</td>
<td>3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>4c/4d</td>
<td>2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>4h</td>
<td>1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>5a</td>
<td>2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>6a</td>
<td>2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
</tbody>
</table>

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

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 before seroconversion for nine seroconversion panels. The data are summarized in Table 9.

Table 9

<table>
<thead>
<tr>
<th>Panel</th>
<th>Day Positive Ortho 3.0 EIA and Chiron RIBA 3.0</th>
<th>Day Positive COBAS AmpliScreen v2.0</th>
<th>Difference COBAS AmpliScreen vs EIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>6212</td>
<td>14</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>6224</td>
<td>19</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>6215</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>9047</td>
<td>28</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>9045</td>
<td>41</td>
<td>0</td>
<td>41</td>
</tr>
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<td>6225</td>
<td>78</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>6213</td>
<td>43</td>
<td>11</td>
<td>32</td>
</tr>
<tr>
<td>6222</td>
<td>40</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td>6227</td>
<td>74</td>
<td>0*</td>
<td>74*</td>
</tr>
</tbody>
</table>

* 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

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

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-HCV 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/dL), triglycerides (up to 3000 mg/dL), hemoglobin (up to 1.0 g/dL), and albumin (up to 6 g/dL) were tested. These endogenous substances did not interfere with the sensitivity or specificity of the COBAS AmpliScreen 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.
REFERENCES


12. Richmond, J.Y. and McKinney, R.W. eds. 1999 Biosafety in Microbiological and Biomedical Laboratories. HHS Publication Number (CDC) 93-8395.

