COBAS AmpliScreen HCV Test, version 2.0

FOR *IN VITRO* DIAGNOSTIC USE.

COBAS AmpliScreen HCV Test, version 2.0	96 Tests	P/N: 3302563018
COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit	96 Tests	P/N: 3302555018
COBAS AMPLICOR™ Wash Buffer	500 Tests	P/N: 20759899123 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 RNA in plasma from individual donors of whole blood and blood components including 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. This test is not intended for use on specimens from cadaveric (non-heart beating) donors. This test is not intended for use on samples of cord blood.

Plasma from all donors may be screened as individual samples. For donations of whole blood and blood components, plasma may be tested in pools comprised of equal aliquots of not more than 24 individual donations. For donations of source plasma, plasma may be tested in pools comprised of equal aliquots of not more than 96 individual donations.

This assay 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 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® enzyme (uracil-N-glycosylase) 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
- Standard Sample Processing for preparation of individual donor samples

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, the AmpErase enzyme (uracil-N-glycosylase, UNG) 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 **96 Tests**
(P/N: 3302555018)

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 **96 Tests**
(P/N: 3302563018)

COBAS AmpliScreen HCV Amplification Reagents, version 2.0

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

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 Kit **500 Tests**
(P/N: 20759899123; 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 1.4), the COBAS AmpliScreen Pooling System Guide (Roche Pooling Methods Software, verson 1.4 and the COBAS AmpliScreen Pooling System Guide are validated to prepare pools of equal aliquots of not more than 24 individual plasma donations using Hamilton MICROLAB AT Plus Pipettor with Hamilton SUNPLUS and RUNENDE Software).

  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 — **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 Symbol*)

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

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

COBAS AmpliScreen HCV Amplification Reagents

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

Bicine buffer
16% DMSO
Glycerol
< 0.01% r*Tth* 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 (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

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 Symbol*)

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 SK53
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 Symbol*)

CN4 — 2 x 100 Tests

(Avidin-Horseradish Peroxidase Conjugate)

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

SB3 — 10 x 75 Tests

(Substrate A)

Citrate solution
0.01% Hydrogen peroxide
0.1% ProClin 150

SB — 10 x 75 Tests (10 x 5 mL)

(Substrate B)

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

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

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 — **500 Tests**

WB — 2 x 250 Tests

(10X-Wash Concentrate)

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

STORAGE INSTRUCTIONS

A. Room Temperature is defined as 15 - 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, 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** and **WB** at 2 - 25°C. **DN4** and **WB** are stable until the expiration dates indicated.

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

F. The following reagents are one time use. Discard any unused portion.
MP IC, MP (+) C, MP (–) C, MP DIL and **NHP**
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 other COBAS AmpliScreen package inserts, the COBAS 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.

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 six instrument cycles (12 hours per cycle). Mixing occurs automatically on the COBAS AMPLICOR Analyzer.
4. Store Working Probe Suspension Detection Reagents at 2 - 8°C between instrument cycles. Remove from refrigerator 30 minutes before use on the COBAS AMPLICOR Analyzer.

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

1. Once opened, **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 six 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**

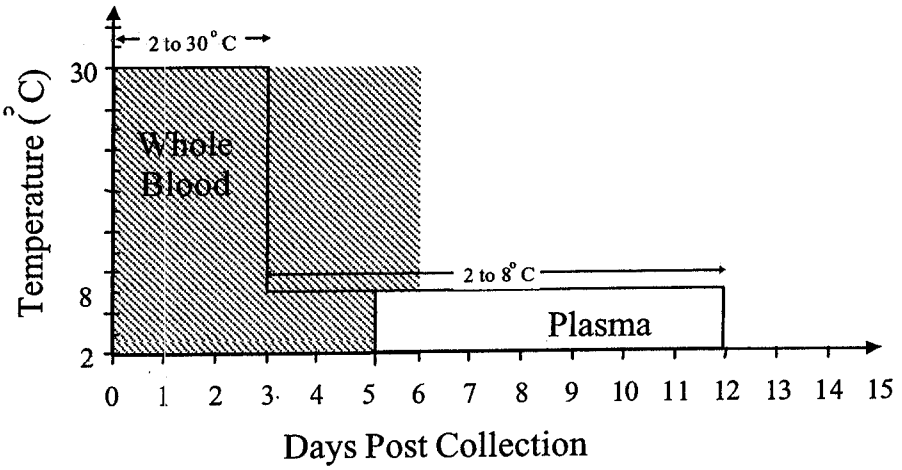
1. Prepare 70% ethanol fresh daily.
2. One mL 70% ethanol is needed for each specimen and control processed. For example, mix 11.7 mL 90% ethanol and 3.3 mL of distilled or deionized water for every 12 specimens and controls to be processed.

SPECIMEN COLLECTION, STORAGE AND POOLING

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

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

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

E. Do not freeze whole blood.

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

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

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

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

J. Thaw frozen specimens at room temperature before using.

K. The user should validate other collection and storage conditions. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.[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 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 less than 24 specimens are available, testing is performed using the individual specimens.
2. For Source Plasma, the Hamilton performs barcode scanning and pooling operations that combine aliquots from 96 individual samples into a single Primary Pool that is used for testing. Positive Primary pools are traced to the positive individual using an overlapping 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.

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 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. 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

B1. 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.

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.

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

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