COBAS AmpliScreen HBV Test

FOR *IN VITRO* DIAGNOSTIC USE.

COBAS AmpliScreen HBV Test	HBV	96 Tests	P/N: 21118323 123
COBAS AmpliScreen MultiPrep Specimen Preparation and Control Kit	MULTIPREP/CTL	96 Tests	P/N: 03272885 123
COBAS AMPLICOR Wash Buffer	WB	500 Tests	P/N: 20759899 123 ART: 07 5989 9 US: 83314

INTENDED USE

The COBAS AmpliScreen Hepatitis B Test (HBV) is a qualitative *in vitro* test for the direct detection of Hepatitis B Virus DNA in human plasma from donations of whole blood and blood components, plasma intended for transfusion or further manufacture, and organ and tissue donors.

The test is intended for use in screening individual donor samples of human plasma or pools of human plasma comprised of equal aliquots of individual donations. It is also intended for use to screen individual organ donors when plasma specimens are obtained while the donor's heart is still beating and to detect HBV DNA in blood specimens from cadaveric (non-heart beating) organ and tissue donors. This assay may be used in conjunction with serology tests for detecting HBsAg and/or antibodies to HBV.

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

SUMMARY AND EXPLANATION OF THE TEST

Hepatitis B Virus is considered to be one of the major etiologic agents that cause chronic and acute hepatitis, cirrhosis and hepatocellular carcinoma.[1-4] HBV is one of the most infectious diseases, with about 350 million chronic hepatitis B carriers worldwide.[9] The yearly new infections are about 200-300 thousand in the United States[10] and approximately 1 million in Europe.[11] The global prevalence of chronic HBV infection, as determined by immunoserology, ranges from < 2% in western countries to ≥ 8% in Asian and African countries.[12]

HBV is a partially double-stranded circular DNA virus with a genome of approximately 3,200 bases that contains four overlapping open reading frames encoding for all viral proteins.[1,5,6] As a blood-borne virus, HBV can be transmitted with a higher risk than HCV and HIV by blood and blood products.[7,8]

The presence of HBV antigens or antibodies in patients infected with HBV has led to the development of immunoserological tests that are specific for these antigens or antibodies. Implementation of these tests has reduced, but not completely eliminated, the incidence of post-transfusion hepatitis.[7,8] Currently, the most used common marker of HBV infection is the presence of HBV surface antigen (HBsAg). However, it has been reported that blood units from HBsAg-negative donors caused post-transfusion hepatitis B in the recipients[13,14] and HBV DNA was prospectively detected in the donor's blood units by PCR.[13] In theory, screening of blood donations for HBV DNA should further reduce the residual transmission risk. PCR tests should also detect viremic units donated by carriers who are in the window period, early acute infection, or late resolving infection that may not be detectable by the existing immunological assays.[13,15-17] Although current nucleic acid testing technologies do not have sufficient throughput for testing of individual units within a reasonable time, a number of proposals have been made for performing nucleic acid tests on mini-pools composed of small aliquots from many individual units.[18-20] The high sensitivity of PCR should allow potentially infectious donations contained within mini-pools to be detected.

The COBAS AmpliScreen HBV Test, an *in vitro* nucleic acid amplification test for the qualitative detection of HBV DNA in plasma, is designed to screen samples of donated human plasma by using approved pooling algorithms. The test incorporates features that meet the criteria required for screening pooled plasma: sensitivity sufficient to detect a single unit containing 1,000 copies per mL when diluted with negative units[21], at least 99% specificity to minimize the number of false positive results that need to be resolved by pool breakdown testing[21], and throughput sufficient to generate results for mini-pools representing 18,400 units of blood within 48 hours of collection.[22]

PRINCIPLES OF THE PROCEDURE

The COBAS AmpliScreen HBV Test is based on four major processes:

1. Sample Processing
2. PCR amplification of target DNA using HBV-specific complementary primers
3. Hybridization of the amplified products to oligonucleotide probes specific to the target(s)
4. Detection of the probe-bound amplified products by colorimetric determination

NOTE: The COBAS AmpliScreen HBV Test has been validated by Roche using the components and protocols listed within this package insert. The user takes responsibility to validate any changes to components other than those listed. User should refer to manufacturers' guidelines for the substituted components.

Sample Processing

Two Roche specimen processing procedures are used with the COBAS AmpliScreen HBV Test as follows:

- MultiPrep Specimen Processing Procedure is recommended for use in the testing of specimens in the mini-pool format and individual specimens from cadaveric donors.
- Standard Specimen Processing Procedure is recommended for use in the preparation of individual non-cadaveric donor samples.

NOTE: For testing of cadaveric specimens, the specimen should be first diluted 1:5 in MultiPrep Specimen Diluent (MP DIL) prior to processing using the MultiPrep Specimen Processing Procedure.

In the Standard Specimen Processing Procedure, HBV DNA is isolated directly from plasma by lysis of the virus particles with MultiPrep Lysis Reagent followed by precipitation of the DNA with alcohol. In the MultiPrep Specimen Processing Procedure, HBV 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 DNA with alcohol.

The MultiPrep Internal Control (**MP IC**), containing the HBV 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 HBV Internal Control is an DNA plasmid with primer binding regions identical to those of the HBV target sequence, a randomized internal sequence of similar length and base composition as the HBV target sequence, and a unique probe binding region that differentiates the HBV Internal Control amplicon from target amplicon. These features were selected to ensure equivalent amplification of the HBV Internal Control and the HBV target DNA.

PCR Amplification

The amplification reactions are performed with the thermostable recombinant enzyme *Thermus aquaticus* DNA Polymerase (Taq *pol*). The reaction mixture is heated to separate double-stranded DNA. As the mixture cools, primers anneal to the target DNA and in the presence of Mg^{2+} and excess deoxynucleotide triphosphates (dNTPs), the Taq *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 (uracil-N-glycosylase) enzyme is added to the COBAS AmpliScreen HBV Test. The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[23], 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 AmpErase enzyme before amplification of the target DNA. AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA rendering the DNA non-amplifiable. 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 HBV amplicon and the HBV MultiPrep 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 HBV amplicon (HBV DET) or HBV Internal Control amplicon is added to the individual D-cups. The biotin-labeled HBV and HBV 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 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 HIV-1 Test, version 1.5, the COBAS AmpliScreen HCV Test, version 2.0 and the COBAS AmpliScreen HBV Test.

NOTE: For cadaveric blood specimens, there is not sufficient MP DIL (MultiPrep Specimen Diluent) in the COBAS AmpliScreen MultiPrep Specimen Preparation and Control Kit to run 96 tests as an additional 800 µL MP DIL is used to prepare each specimen and control. Additional COBAS AmpliScreen MultiPrep Specimen Preparation and Control Kits must be ordered to run 96 tests.

COBAS AmpliScreen MultiPrep Specimen Preparation and Control Kit — MULTIPREP/CTL — **96 Tests**
(P/N: 03272885 123)

- **MP (–) C**
  [MultiPrep (–) Control]
- **MP (+) C**
  [MultiPrep (+) Control]
- **MP LYS**
  (MultiPrep Lysis Reagent)
- **MP DIL**
  (MultiPrep Specimen Diluent)
- **MP IC**
  (MultiPrep Internal Control)
- **NHP**
  [Negative Plasma (Human)]

COBAS AmpliScreen HBV Test — HBV — **96 Tests**
(P/N: 21118323 123)

COBAS AmpliScreen HBV Amplification Reagents — HBV AMP

- **HBV MMX**
  (HBV Master Mix)
- **HBV Mg^{2+}**
  (HBV Magnesium Solution)

COBAS AmpliScreen HBV Detection Reagents — HBV DK

- **BH PS1**
  (HBV Probe Suspension 1)
- **BH4**
  (HBV Probe Suspension 2)
- **BI PS1**
  (HBV IC Probe Suspension 1)
- **BI4**
  (HBV IC Probe Suspension 2)
- **DN4**
  (Denaturation Solution)
- **CN4**
  (Avidin - Horseradish Peroxidase Conjugate)
- **SB3**
  (Substrate A)
- **SB**
  (Substrate B)

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

- **WB**
  (10X-Wash Concentrate)

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

- COBAS AMPLICOR Analyzer, 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 AMPLILINK 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 HFA 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
- Powderless, disposable gloves
- Isopropyl alcohol, reagent grade
- Disposable, Sterile, Polystyrene pipettes (5 mL, 10 mL and 25 mL)
- Sterile, RNase-free, fine-tip transfer pipettes
- Pipettors (capacity 20 µL to 1000 µL, capable of providing ± 3% accuracy and precision ≤ 5%) with aerosol barrier or positive displacement RNase-free tips
- Tube racks (Sarstedt P/N 93.1428 or equivalent)
- 1.5-mL sterile, non-siliconized, conical polypropylene screw-cap tubes, (Sarstedt 72.692.105 or equivalent)
- Vortex mixer
- Hamilton Slotted Deepwell Archive Plate, 2.2 mL and Sealing Capmat
- Hamilton Slotted Intermediate Plate

REAGENTS

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

MP (–) C 8 x 0.1 mL
[MultiPrep (–) Control]

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

MP (+) C 8 x 0.1 mL
[MultiPrep (+) Control]

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

MP LYS 8 x 9.0 mL
(MultiPrep Lysis Reagent)

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

Xn 60% (w/w) Guanidine thiocyanate

Harmful

MP DIL 8 x 4.8 mL
(MultiPrep Specimen Diluent)

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

MP IC 8 x 0.1 mL
(MultiPrep Internal Control)

Tris-HCl buffer
< 0.001% Non-infectious plasmid DNA containing HBV primer binding sequences and a unique probe binding region
< 0.001% Non-infectious *in vitro* transcribed RNA (microbial) containing HCV primer binding sequences and a unique probe binding region
< 0.001% Non-infectious *in vitro* transcribed RNA (microbial) containing HIV-1 primer binding sequences and a unique probe binding region
< 0.005% Poly rA RNA (synthetic)
< 0.1% Amaranth dye
EDTA
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 HIV-1, antibody to HCV, HIV p24 antigen and HBsAg. HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods.
0.1% ProClin® 300

COBAS AmpliScreen HBV Test HBV **96 Tests**

COBAS AmpliScreen HBV Amplification Reagents HBV AMP

HBV MMX 8 x 0.7 mL
(HBV Master Mix)

Tris buffer
Glycerol
< 0.001% AmpliTaq® DNA Polymerase (Taq *pol*, microbial)
Ammonium sulfate
<0.05% dATP, dCTP, dGTP, dUTP
< 0.001% HBV-104UB, HBV-104D and HW016TBB1 primers (HBV-104UB is biotinylated)
< 0.001% AmpErase (uracil-N-glycosylase) enzyme (microbial)
< 0.5% Tween 20
0.09% Sodium azide

HBV Mg^{2+} — 8 x 0.1 mL
(HBV Magnesium Solution)

< 1% Magnesium chloride
Amaranth dye
0.05% Sodium azide

COBAS AmpliScreen HBV Detection Reagents HBV DK

BH PS1 — 1 x 100 Tests
(HBV Probe Suspension 1)

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

BH4 — 1 x 100 Tests
(HBV Probe Suspension 2)

Sodium phosphate buffer
24.9% Sodium thiocyanate
0.2% Solubilizer

BI PS1 — 1 x 100 Tests
(HBV IC Probe Suspension 1)

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

BI4 — 1 x 100 Tests
(HBV IC Probe Suspension 2)

Sodium phosphate buffer
24.9% Sodium thiocyanate
0.2% Solubilizer

DN4 — 1 x 100 Tests
(Denaturation Solution)

1.6% Sodium hydroxide
EDTA
Thymol blue

Xi — 1.6% (w/w) Sodium hydroxide
Irritant

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

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

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

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

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

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

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

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

STORAGE INSTRUCTIONS

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

B. Do not freeze reagents.

C. Store the following reagents at 2 - 8°C. Unopened, these reagents are stable until the expiration date indicated.

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

HBV MMX, HBV Mg^{2+}

BH PS1, **BH4**, **BI PS1** and **BI4**

CN4, **SB3**, **SB**

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

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

F. The following reagents are one-time use. Discard any unused portion.

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

HBV Mg^{2+}, **SB, BH PS1** and **BI PS1.**

PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE.

A. Specimens may be infectious. Use Universal Precautions when performing the assay.[30-31]. 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 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. 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 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**, **HBV MMX**, **HBV Mg^{2+}**, **BH4**, **BI4**, **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 BH4 and BI4, 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 the *Operator's Manuals* for the AMPLILINK software and COBAS AMPLICOR Analyzer for use with the COBAS AmpliScreen Tests.

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. COBAS AmpliScreen has been validated by Roche using the components and protocols listed within this package insert. The user takes responsibility to validate any changes to components other than those listed. User should refer to manufacturers' guidelines for the substituted components.

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

REAGENT PREPARATION

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

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

B. **Working Lysis Reagent**

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

C. **Working Amplification Master Mix**

1. Prepare Working Master Mix in a template-free area (e.g., in a dead air box). **Reagent preparation area must be clean and disinfected in accordance with methods outlined in *Precautions* (Item A). Failure to do so may result in reagent contamination.**
2. Pipette 100 µL **HBV Mg^{2+}** into 1 bottle **HBV MMX**. Recap **HBV MMX** bottle and mix well by inverting 10-15 times. The pink color confirms that the **HBV Mg^{2+}** has been added to the **HBV MMX**. Discard the remaining **HBV Mg^{2+}**. 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 HBV Probe Suspension: Mix **BH PS1** well by vortexing briefly to suspend the microparticles. Pipette 2.5 mL **BH PS1** into one **BH4** cassette.
2. Prepare Working IC Probe Suspension: Mix **BI PS1** well by vortexing briefly to suspend the microparticles. Pipette 2.5 mL **BI PS1** into one **BI4** cassette.
3. Both Working Probe Suspension Detection Reagents are stable for 30 days at 2 - 8°C. Working Reagents can be used for a maximum of ten instrument cycles (12 hours per cycle). Mixing occurs automatically on the COBAS AMPLICOR Analyzer.
4. Store Working Probe Suspension Detection Reagents at 2 - 8°C between instrument cycles. Remove from refrigerator 30 minutes before use on the COBAS AMPLICOR Analyzer.

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

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

F. **Working Substrate Reagent**

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

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

G. **Wash Buffer Reagent**

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

H. **70% Ethanol**

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

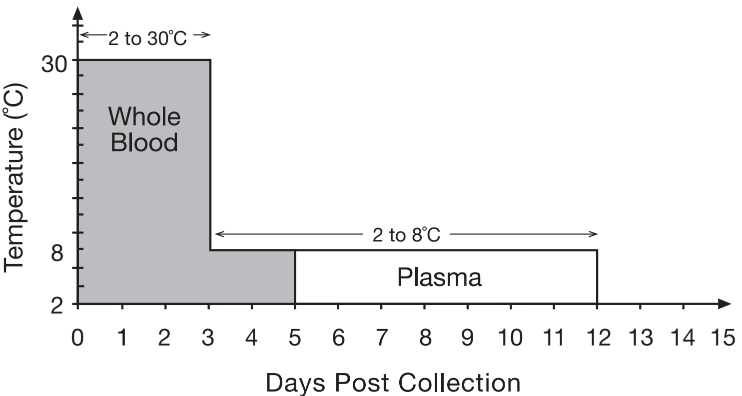
SPECIMEN COLLECTION, TRANSPORT, STORAGE AND POOLING

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

Living (Heart Beating) Blood Donor Specimens

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

B. Blood collected in EDTA may be stored for up to 72 hours from time of draw at 2 - 30°C, 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 - 24ºC. Following centrifugation of the CPD, CPDA-1, or CP2D samples at 800-1600 x g for 20 minutes, plasma may be stored at 1 - 6°C for an additional 7 days from the date the plasma was removed from the red blood cells. Plasma separated from the cells may be stored at ≤ -18ºC for up to one month.

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

E. Do not freeze whole blood.

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

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

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

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

J. Thaw frozen specimens at room temperature before using.

K. The user should validate other collection and storage conditions. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.[26]

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 HBV Test may be used in screening individual donor samples of human plasma, or pools of human plasma comprised of equal aliqouts of individual donations.
2. Pooling algorithms may require the preparation and testing of smaller Secondary Pools and/or individual specimens for follow-up testing in the event a Primary Pool tests positive.

Cadaveric Blood Specimens

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

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

P. Cadaveric blood specimens should be briefly (e.g. pulse) centrifuged prior to use to remove particulate matter.

NOTE: Cadaveric specimens should be placed at 2-8°C as soon as possible after collection. The use of excessively hemolyzed cadaveric specimens should be avoided.

PROCEDURAL NOTES

A. **Run Size**

1. Each kit contains reagents sufficient for eight 12-specimen runs, which may be performed separately or simultaneously. At least one preparation of the COBAS AmpliScreen MultiPrep Negative (–) Control and one preparation of the COBAS AmpliScreen MultiPrep Positive (+) Control must be included in each run (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 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 HBV MMX** and **HBV Mg^{2+}**, 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 15º to 30ºC.

F. **Vortexing**

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

G. **Pipetting**

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

H. **Specimen Processing**

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

I. **Decontamination**

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

INSTRUCTIONS FOR USE

The MultiPrep Specimen Processing Procedure is used for extracting nucleic acid from pooled specimens and from individual cadaveric specimens. The Standard Specimen Processing Procedure is used for extracting nucleic acid from individual non-cadaveric specimens as well as for tertiary resolution.

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:

NOTE: The performance data for the Multiprep Specimen Processing Procedure presented in the "PERFORMANCE CHARACTERISTICS" section should be taken into consideration when determining pool size. For the Roche COBAS AmpliScreen System, a pool size of 24 has been validated. The user must validate other pool sizes or configurations.

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 (HBV) and the time the Working Master Mix was prepared.

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

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

B. Specimen and Control Preparation

Performed in Pre-Amplification - Specimen and Control Preparation Area

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

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

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

Step B2.

B2. Vortex **NHP** briefly.

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

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

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

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

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

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

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

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

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

B10. Prepare Controls as follows:

a. <u>Negative Control</u>

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. <u>Positive Control</u>

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 the 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 DNA. Note that some insoluble material may remain.

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

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

Standard Specimen Processing Procedure (Individual Specimens [Non-Cadaveric])

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. <u>Negative Control</u>

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. <u>Positive Control</u>

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 the 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 DNA. Note that some insoluble material may remain.

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

Loading the A-ring

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

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

B41. Pipette 50 µL of each processed specimen and control into the appropriate A-tube containing HBV Working Master Mix. Immediately cap the A-tube and repeat this step for all 12 A-tubes to complete the A-ring loading. Use the A-ring worklist record to ensure the appropriate specimen or control is added to the correct A-tube position for each A-ring.

B42. Transfer the A-ring with sealed tubes containing the processed specimens and controls in Working Master Mix to the Amplification/Detection Area. Proceed to Part C.

NOTE: Amplification must begin within 45 minutes from when the first specimen or control in the A-ring is added to the Working Master Mix.

C. Amplification and Detection

Performed in Post-Amplification - Amplification/Detection Area

C1. Perform Daily Instrument Maintenance as outlined in the *Operator's Manual* for the COBAS AMPLICOR Analyzer including:

a. Wipe D-cup handler tip with a lint-free moist cloth and dry.

b. Wipe initialization post with a lint-free moist cloth and dry.

C2. Before each run:

a. Check waste container and empty if necessary.

b. Check Wash Buffer Reservoir and add prepared Wash Buffer if necessary.

c. Replace used D-cup racks.

d. Prime the COBAS AMPLICOR Analyzer.

C3. Instrument Loading and System Operation

a. Prepare enough of the following detection reagent cassettes to complete the workload: Working HBV Probe Suspension Reagent (**BH4**), Working IC Probe Suspension Reagent (**BI4**), Working Substrate (**SB3**), Denaturation Reagent (**DN4**), and Conjugate Reagent (**CN4**).

b. Place the **BH4** and **BI4** cassettes in the test-specific reagent rack.

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

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

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

f. Load the reagent racks onto the 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. Use the A-ring worklist record created for specimen processing to assist in entering the A-ring order.

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

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

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

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

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

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

QUALITY CONTROL PROCEDURES

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

a. Negative Control

The absorbance for the **MP (–) C** should be less than 0.2 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 0.2 and/or its associated **MP IC** is less than 0.2, the entire A-ring is invalid, and the entire test procedure for that A-ring (specimen and control preparation, amplification and detection) must be repeated.

b. Positive Control

The absorbance for the **MP (+) C** should be greater than or equal to 1.0 at 660 nm and its associated **MP IC** should be greater than or equal to 0.2 at 660 nm for the Positive Control to be valid. If the absorbance value for the **MP (+) C** is less than 1.0 and/or its associated **MP IC** is less than 0.2, the entire A-ring is invalid, and the entire test procedure for that A-ring (specimen and control preparation, amplification and detection) must be repeated.

Summary of Control Acceptance Criteria

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

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

3. External Control

If an External Control is required by the laboratory or by a regulatory agency, an External Control must be processed in the same manner as a sample. The External Control should contain a defined number of target sequence copies for HBV and the level of this control should be a multiple of the cut-off value of the test system. The HBV absorbance and the **MP IC** absorbance for the External Run Control must both be valid for the External Control to be valid. The HBV absorbance of the External Run Control should be greater than or equal to 0.2 at 660 nm and the **MP IC** absorbance for the External Run Control should be greater than or equal to 0.2 at 660 nm. If the absorbance of the 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 run printout(s) for flags and comments to verify that the run is valid. Refer to the *Operator's Manual* for the AMPLILINK software and the *Operator's Manual* for the COBAS AMPLICOR Analyzer for interpretation of flags and comments.

2. Specimen Results

   Two absorbance values are obtained for each specimen: one for the HBV 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 HBV 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:

HBV Result		IC Result		Interpretation
A_{660}	Comment	A_{660}	Comment	
< 0.2	NEGATIVE	≥ 0.2	VALID	Specimen is negative for HBV DNA.
< 0.2	NEGATIVE	< 0.2	INVALID	Invalid result. Repeat entire test procedure for invalid specimen.
≥ 0.2	POSITIVE	ANY	VALID	Specimen is positive for HBV DNA.

Invalid Test Runs

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

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

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

Invalid Specimen Results

For non-cadaveric plasma specimen(s) that are invalid, repeat entire test procedure 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 HBV DNA.

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

Results of Pooled Donor Specimens

The COBAS AmpliScreen HBV Test may be used in screening individual donor samples of human plasma, or pools of human plasma comprised of equal aliqouts of individual donations.

Testing of pooled samples for the COBAS AmpliScreen HBV Test requires a single level of testing for Primary Pools that are negative for HBV DNA. Depending on the pooling algorithm used, a positive Primary Pool may require deconstruction into smaller intermediate Secondary Pools for testing or go to immediate testing of individual donations. If using intermediate pools, a positive Secondary Pool may require further Tertiary Resolution Testing of individual donations. An example is given below.

Negative Primary Pools

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

Positive Primary Pools - Utilizing Secondary Pool Testing

When the Primary Pool is positive, prepare smaller 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 "HBV DNA Negative". For positive Secondary Pools, proceed to the section entitled ***"Positive Primary Pool, Positive Secondary Pools - Utilizing Tertiary Resolution Testing."***
- If all four Secondary Pools are negative, the individual donor specimens in that Primary Pool may be reported as "HBV DNA 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 were 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.

Positive Primary Pool, Positive Secondary Pools - Utilizing Tertiary Resolution Testing

For a positive Secondary Pool, test each of the individual donor specimens in that Secondary Pool. The individual donor specimens can 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 "HBV DNA Positive" and the remaining negative donor specimens associated with the positive Secondary Pool are reported as "HBV DNA Negative."
- If all of the individual donor specimens in that Secondary Pool test negative, the donor specimens in the Secondary Pool may be reported as "HBV DNA 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 "HBV DNA Positive."

If an individual donor specimen is negative, the negative donor specimen is reported as "HBV DNA Negative."

Results of Individual Cadaveric Specimens

If an individual cadaveric specimen is positive, the positive cadaveric specimen is reported as "HBV DNA Positive."

If an individual cadaveric specimen is negative, the cadaveric specimen is reported as "HBV DNA Negative."

For cadaveric specimens that had an initial invalid result and were repeated in duplicate, if either or both the duplicate samples are positive, the specimen is reported as "HBV DNA Positive." If both duplicate specimens are negative or if one duplicate repeat is negative and one is invalid, the specimen is reported as "HBV DNA Negative." If both replicates are invalid, it is most likely due to inhibitory substances in the specimen and results should be marked as invalid or unresolved.

PROCEDURAL LIMITATIONS

1. This test has been evaluated only for use in combination with the COBAS AmpliScreen MultiPrep Specimen Preparation and Control Kit, COBAS AMPLICOR Analyzer and the Hamilton MICROLAB AT plus 2 Pipettor for the automated preparation of plasma pools. The user takes responsibility to validate any changes to components other than those listed. User should refer to manufacturers' guidelines for the substituted components.
2. ***Heparin inhibits PCR; specimens collected using heparin as the anticoagulant should not be used with the COBAS AmpliScreen HBV Test.***
3. Reliable results are dependent on adequate specimen collection and proper transport procedures.
4. Detection of HBV DNA 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.

PERFORMANCE CHARACTERISTICS

These data apply only if the COBAS AmpliScreen HBV Test is used in combination with the COBAS AmpliScreen MultiPrep Specimen Preparation and Control Kit.

Assay Specificity

Assay specificity of the COBAS AmpliScreen HBV Test was determined by analysis of individual blood donors that were HBV seronegative by FDA licensed serological tests. Over 500 sero-negative blood donations were tested with MultiPrep Sample Processing procedure and Standard Sample Processing procedure, separately. None of the sero-negative samples gave a positive result. See Table I.

Table 1
HBV Seronegative Blood Donors

Sample Processing Procedure	N	Not detected	Positive	A_{660} Maximum	A_{660} Minimum	Mean A_{660}	Standard Deviation
MultiPrep	504	504	0	0.043	0.001	0.007	0.006
Standard	502	502	0	0.049	0.000	0.007	0.006

Analytical Specificity

The analytical specificity of the COBAS AmpliScreen HBV Test was evaluated by testing a panel consisting of the following 38 microorganisms. None of the isolates tested positive.

Adenovirus, Human Type 2	*Chlamydia trachomatis*	HCV 1a
Adenovirus, Human Type 3	*Neisseria gonorrhoeae*	HCV 1b
Adenovirus, Human Type 7	Epstein Barr Virus (Burkitt's lymphoma)	HCV 2a/2c
Cytomegalovirus (3 strains)	Epstein Barr Virus (RAJI Human Burkitt's lymphoma)	HCV 2b
Herpes Simplex type 1	Echovirus 1	HCV 3a
Herpes Simplex type 2	Echovirus 5	HIV-1 Subtype A
Hepatitis A	Coxsackievirus B1	HIV-1 Subtype B
Human Papilloma Virus, Type 6a	Varicella-Zoster	HIV-1 Subtype C
Human Papilloma Virus, Type 16	Varicella	HIV-1 Subtype D
Human Papilloma Virus, Type 18	*Propionibacterium acnes*	HIV-1 Subtype E
HTLV-I	*Staphylococcus aureus*	HIV-1 Subtype F
HTLV-II	*Staphylococcus epidermidis*	HIV-1 Subtype G

Endogenous Interference

Endogenous interference studies indicate that plasma samples derived from whole blood containing abnormally high levels 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) will not interfere with the specificity and/or sensitivity of the COBAS AmpliScreen HBV Test using either the Standard or MultiPrep Sample Processing Procedure.

Genotype Inclusivity and Sensitivity

The performance of the COBAS AmpliScreen HBV Test was evaluated by testing 14 HBV specimens representing Genotypes A through G. The HBV DNA concentration for each genotype specimen was determined by using the COBAS AMPLICOR HBV MONITOR Test. A limit of detection study was performed for each specimen with both the MultiPrep and Standard Sample Processing procedures. Twenty-two replicates were tested for each dilution level of each specimen. See Table 2 and Table 3.

Table 2
COBAS AmpliScreen HBV Test Genotype Inclusivity
MultiPrep Sample Processing Procedure

Positive Rate with MultiPrep Sample Processing Procedure				
HBV Genotype	19.6 IU/mL (100 c/mL)	9.8 IU/mL (50 c/mL)	4.9 IU/mL (25 c/mL)	2.5 IU/mL (12.5 c/mL)
A (#11090)	—	95%	100%	91%
A (#13621)	100%	100%	91%	—
B (#11158-1)	95%	95%	81%	—
B (#13373) (#6)	100%	100%	91%	—
C (#13626)	—	100%	100%	100%
C (#13629)	—	100%	100%	100%
D (#8907)	100%	95%	77%	—
D (#8909)	100%	95%	71%	—
E (#7859)	100%	91%	—	—
E (#7858)	100%	100%	100%	77%
F (#5)	100%	100%	95%	73%
F (#12468)	—	100%	95%	82%
	8.2 IU/mL (42 c/mL)	3.3 IU/mL (16.8 c/mL)	0.8 IU/mL (4 c/mL)	0.4 IU/mL (2 c/mL)
F (#12461)	100%	100%	100%	100%
	19.6 IU/mL (100 c/mL)	5.9 IU/mL (30 c/mL)	2 IU/mL (10 c/mL)	0.6 IU/mL (3 c/mL)
G (#AF160501)	100%	100%	100%	90%

Table 3
COBAS AmpliScreen HBV Test Genotype Inclusivity Standard Sample Processing Procedure

Positive Rate with Standard Sample Processing Procedure				
HBV Genotype	78.4 IU/mL (400 c/mL)	39.2 IU/mL (200 c/mL)	19.6 IU/mL (100 c/mL)	9.8 IU/mL (50 c/mL)
A (#11090)	—	100%	95%	86%
A (#13621)	—	—	95%	95%
B (#11158-1)	100%	90%	—	—
B (#13373) (#6)	100%	100%	86%	—
C (#13626)	—	100%	100%	86%
C (#13629)	—	—	100%	100%
D (#8907)	—	100%	100%	91%
D (#8909)	100%	100%	91%	—
E (#7859)	—	100%	95%	55%
E (#7858)	—	100%	100%	82%
F (#5)	—	100%	100%	95%
F (#12468)	—	100%	100%	100%
	16.5 IU/mL (84 c/mL)	8.2 IU/mL (42 c/mL)	3.3 IU/mL (16.8 c/mL)	2.9 IU/mL (15 c/mL)
F (#12461)	100%	100%	100%	100%

NOTE: Genotype G was not tested by the Standard procedure.

Analytical Sensitivity

The analytical sensitivity of the COBAS AmpliScreen HBV Test was assessed using two different strains from each of 6 HBV genotype plasmid clones (A, B, C, D, E, and F). Plasmid clones were diluted with MultiPrep Specimen Diluent to approximately 1.2, 0.78, 0.39 and 0.2 IU/PCR (6, 4, 2 and 1 copies/PCR) and tested in 22 replicates at each concentration. The COBAS AmpliScreen HBV Test can detect HBV DNA at concentrations as low as 0.39-0.78 IU/PCR (2-4 copies/PCR) with a positivity rate of greater than or equal to 95% for genotypes A through F.

Sensitivity

The sensitivity of the COBAS AmpliScreen HBV Test was determined using both the MultiPrep and the Standard Sample Processing procedures by evaluating the HBV NIBSC Working Reagents (98/780) and the HBV WHO International Standard (97/746).

For the MultiPrep Sample Processing procedure, the NIBSC Working Reagent was serially diluted to 120, 60, 30, 15, 7.5, and 4 copies/mL in HBV negative human plasma and the WHO International Standard was serially diluted to 30, 20, 10, 5, 2.5, and 1.25 IU/mL in HBV negative human plasma. Thirty-three (33) replicates at each concentration level were tested.

For the Standard Sample Processing procedure, the NIBSC Working Reagent was serially diluted to 200, 150, 100, 75, 50, and 25 copies/mL in HBV negative human plasma and the WHO International Standard was serially diluted to 40, 30, 20, 10, 5, and 2.5 IU/mL in HBV negative human plasma. Thirty-three (33) replicates at each concentration level were tested.

The COBAS AmpliScreen HBV Test demonstrates that using the MultiPrep Sample Processing procedure the limit of detection is 5 IU/mL. This equates to a detection level of 120 IU/mL for an individual donor specimen tested in a pool of 24 donors. The MultiPrep Sample Processing procedure can detect HBV DNA levels as low as 30 copies/mL on the NIBSC Working Reagent and 5 IU/mL on the WHO International Standard with a positivity rate of greater than or equal to 97%. Similarly, the limit of detection for the COBAS AmpliScreen HBV Test with the Standard Sample Processing Procedure is 20 IU/mL. The Standard Sample Processing Procedure detected 100 copies/mL on the NIBSC Working Reagent and 20 IU/mL on the WHO International Standard. See Table 4 and Table 5 below.

Table 4
COBAS AmpliScreen HBV Test Limit of Detection
(NIBSC HBV Working Reagent [98/780])

COBAS AmpliScreen HBV Test with MultiPrep Sample Processing Procedure			COBAS AmpliScreen HBV Test with Standard Sample Processing Procedure		
Copies/mL	Positive results	Positivity rate	Copies/mL	Positive results	Positivity rate
120	33/33	100%	200	31/32	97%
60	33/33	100%	150	32/33	97%
30	33/33	100%	100	32/33	97%
15	18/32	56%	75	27/33	82%
7.5	18/33	55%	50	16/32	50%
4	12/33	36%	25	13/33	39%

Table 5
COBAS AmpliScreen HBV Test Limit of Detection
(HBV WHO International Standard [97/746])

COBAS AmpliScreen HBV Test with MultiPrep Sample Processing Procedure			COBAS AmpliScreen HBV Test with Standard Sample Processing Procedure		
IU/mL	Positive results	Positivity rate	IU/mL	Positive results	Positivity rate
30	33/33	100%	40	33/33	100%
20	33/33	100%	30	33/33	100%
10	33/33	100%	20	33/33	100%
5	32/33	97%	10	27/33	82%
2.5	21/33	64%	5	22/33	67%
1.25	18/33	55%	2.5	16/33	48%

Reproducibility

The reproducibility of the COBAS AmpliScreen HBV Test was determined by testing a high titer HBV clinical specimen diluted with negative human plasma to 1961, 196.1, 19.6 and 1.96 IU/mL (10,000, 1000 and 100 copies/mL) for the MultiPrep Sample Processing procedure and to 1961, 490.2 and 78.4 IU/mL (10,000, 2,500 and 400 copies/mL) for the Standard Sample Processing procedure. An HBV negative clinical specimen and the assay's negative and positive controls were also included in the study for both processing procedures. For each processing procedure, each of two different operators tested these six samples in four replicates per day (24 tests/day) for 15 days. Thus, a total of 1,440 tests were performed with the combination of the two sample processing procedures and four different operators. All tests yielded the expected qualitative test result. No false positive or false negative results were observed.

PERFORMANCE CHARACTERISTICS FOR CADAVERIC SPECIMENS

Sensitivity Study

Sixty pre-mortem EDTA plasma and 58 cadaveric specimens non-reactive for HBV were divided into 5 groups. Specimens within each group were spiked with HBV viral target to a concentration of 3X the LOD using a different clinical viral isolate for each group. The spiked specimens were equally divided and tested between three COBAS AmpliScreen HBV Test kit lots.

The COBAS AmpliScreen HBV Test, using samples diluted 1:5 and the MultiPrep Specimen Processing procedure, correctly detected 98.3% (59/60) pre-mortem EDTA plasma specimens and 96.6% (56/58) of cadaveric specimens spiked with HBV DNA at 3X the LOD of the COBAS AmpliScreen HBV Test. The summary of the final test results of this study is presented in Table 6 below.

Table 6
Summary of Sensitivity Results

		Pre-Mortem EDTA Plasma Specimen	Post-Mortem EDTA Plasma Specimen
	Replicates	60	58
Test Results	+	59	56
	-	1	2
	Inhib.	0	0
	Sensitivity	98.3%	96.6%
95% Confidence Interval	Upper	99.9%	99.6%
	Lower	91.1%	88.1%

Specificity Study

Sixty pre-mortem and 58 post-mortem specimens that were negative for HBV DNA were divided into three groups, diluted 1:5 in MP DIL, processed using the MultiPrep Specimen Processing Procedure, and tested between 3 lots of the COBAS AmpliScreen HBV Test.

The MultiPrep Specimen Processing Procedure detected 100% (60/60) of all negative specimens in pre-mortem EDTA plasma specimens, and 100% (58/58) on post-mortem EDTA plasma specimens. The summary of results of this test is presented in Table 7 below.

Table 7
Summary of Specificity Test Results

		Pre-Mortem EDTA Plasma Specimen	Post-Mortem EDTA Plasma Specimen
	Replicates	60	58
Test Results	+	0	0
	-	60	58
	Inhib.	0	0
	Final Specificity	100%	100%
95% Confidence Interval	Upper	100%	100%
	Lower	94%	93.8%

Reproducibility Study

Twenty pre-mortem EDTA plasma and 20 individual cadaveric specimens were spiked with HBV viral target using a secondary standard to a final concentration of 3X the LOD. Each of the 20 pre- and post-mortem specimens were tested using three different COBAS AmpliScreen HBV Test lots at three different testing sites in this study. At each testing site, each specimen was tested singly in two separate runs using each of the three different kit lots (total of six valid test results for each specimen at each site). There were a total of 18 valid test results (six results per site x 3 testing sites) for each specimen.

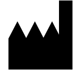
All valid reproducibility data for post-mortem and pre-mortem specimens were evaluated by calculating the percentage of correct results for each assay. The data were analyzed by lot and by testing site. The summary of results of the reproducibility study test is presented in Table 8 below.

Table 8
Summary of Reproducibility Study Test Results — Post-Mortem versus Pre-Mortem

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

REFERENCES

1. Fagan, EA and Harrison, TJ. 2000. Viral Hepatitis: A Handbook for Clinicians and Scientists, Springer-Verlag New York Inc., 89-130.
2. Beasley RP. 1982. Hepatitis B virus as etiologic agent in hepatocellular carcinoma - epidemiological considerations. Hepatology **2 (suppl)**:21S-26S.
3. Feitelson M. 1992. Hepatitis B virus infection and primary hepatocellular carcinoma. Clin Microbiol Rev **14**:257-301.
4. Mast EE and Later MJ. 1993. Epidemiology of viral hepatitis: an overview. Semin Virol **4**:273-283.
5. Robinson WS, Clayton DA, and Greenman RL. 1974. DNA of a human hepatitis B virus candidate. J Virol **14**:384-391.
6. Galibert F, Mandart E, Fitoussi F, Tiollais P, and Charney P. 1979. Nucleotide sequence of the hepatitis B virus genome (subtype ayw) cloned in E. coli. Nature **281**:646-650.
7. Kitchen A. 1998. Hepatitis B and blood safety. Vaccine **16**:s34-s37.
8. Schreiber GB, Busch MP, Kleinman SH, and Korelitz JJ. 1996. The risk of transfusion-transmitted viral infections. N Engl J Med **334**:1685-1690.
9. Zuckerman AJ. 1999. More than third of the world's population has been infected with hepatitis B virus [letter]. Br Med J **318**:1213.
10. McQuillan GM, Coleman PJ, Kruszon-Moran D, Moyer LA, Lambert SB, and Margolis HS. 1999. Prevalence of hepatitis B virus infection in the United States: The National Health and Nutrition Examination Survey, 1976 through 1994. Am J Pub Health **89**:14-18.
11. Van Damme P, Tormans G, Beutels P, and Van Doorslaer. 1995. Hepatitis B prevention in Europe: a preliminary economic evaluation. Vaccine **13(Suppl 1)**:S54-S57.
12. Maddrey WC. 2000. Hepatitis B: An important public health issue. J Med Virol **61**:362-366.
13. Larsen J, Hetland G, and Skaug K. 1990. Posttransfusion hepatitis B transmitted by blood from a hepatitis B surface antigen-negative hepatitis B virus carrier. Transfusion **30**:431-432.
14. Saraswat S, Banerjee K, Chaudhury N, Mahant T, Khandekar P, Gupta RK, and Nail S. 1996. Post-transfusion hepatitis type B following multiple transfusions of HBsAg-negative blood. J of Hepatol **25**:639-643.
15. Yotsuyanagi H, Yasuda K, Iino S, Moriya K, Shintani Y, Fujie H, Tsutsumi T, Kimura S, and Koike K. 1998. Persistent Viremia After Recovery From Self-Limited Acute Hepatitis B. Hepatology **27**:1377-1382.
16. Michalak TI, Pasquinelli C, Guilhot S, and Chisari FV. 1994. Hepatitis B virus persistence after recovery from acute viral hepatitis. J Clin Invest **93**:230-239.
17. Cabrerizo M, Bartolomé J, Caramelo C, Barril G, AND Carreño V. 2000. Molecular Analysis of Hepatitis B Virus DNA in Serum and Peripheral Blood Mononuclear Cells From Hepatitis B Surface Antigen-Negative Cases. Hepatology **32**:116-123.
18. Busch MP, Stramer SL, Kleinman SH. 1997. Evolving applications of nucleic acid amplification assays for prevention of virus transmission by blood components and derivatives. In: Garratty G, ed. Applications of molecular biology. Bethesda, American Association of Blood Banks 1997:121-73. (presented at a workshop during the 50th Annual Meeting of the AABB, October 1997, Denver, CO).
19. Mortimer J. 1997. Intersecting pools and their potential application in testing donated blood for viral genomes. Vox Sanguinis **73**:93-96.
20. Kanemitsu K, Tomono T, Murozuka T, Emura H, Yugi H, Miyamoto, and Nishioka K. 2000. National NAT screening of HBV, HCV, and HIV for blood transfusion. Vox Sanguinis **78 S1**:0081.
21. Yang Y, Xu D, Mendoza M, Lamendola M, Wu Y, Yeh S, Kung K. 2000. A prototype of Hepatitis B virus (HBV) PCR assay with a semi-automated instrument system. Transfusion 40 No.**10S**:SP207.
22. Rosenstraus M, Gutekunst K, Herman S et al. 1998. Improved COBAS AMPLICOR viral assays as a basis for mini-pool screening of viruses in blood or plasma. Infusionsther Transfusionsmed **25**:153-9.
23. Longo M.C., Berninger, M.S. and Hartley, J.L. 1990. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene **93**:125-128.
24. Richmond, J.Y. and McKinney, R.W. eds. 1999. *Biosafety in Microbiological and Biomedical Laboratories*. HHS Publication Number (CDC) 93-8395.
25. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue. Approved Guideline. CLSI Document M29-A Villanova, PA:CLSI, 1997.
26. International Air Transport Association. Dangerous Goods Regulations, 41st Edition. 2000. 704 pp.



Roche Molecular Systems, Inc., Branchburg, NJ 08876 USA
A Member of the Roche Group

Roche Diagnostics
Indianapolis, IN 46256 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800 526 1247)

Roche Diagnostics (Schweiz) AG
CH-6343 Rotkreuz

Roche Diagnostics GmbH
D-68298 Mannheim, Germany

Roche Diagnostics S.L.
E-08006 Barcelona

Roche Diagnostics
Laval, Quebec, Canada H7V 4A2
(For Technical Assistance call:
Pour toute assistance technique,
appeler le: 1-877 273 3433)

Roche Diagnostics
F-38240 Meylan

Distributore in Italia:
Roche Diagnostics SpA
Piazza Durante 11
I-20131 Milano

Distribuidor em Portugal:
Roche Farmacêutica Química, Lda
P-2700 Amadora

ROCHE, AMPERASE, AMPLILINK, COBAS, AMPLICOR, and AMPLISCREEN are trademarks of Roche.

ROCHE RESPONSE CENTER is a service mark of Roche.

Dynabeads® paramagnetic particles are licensed under patents owned by Dynal Biotech ASA, Oslo, Norway. Dynabeads® is a registered trademark of Dynal Biotech ASA, Oslo, Norway, licensed to Roche Diagnostics Corporation, Indianapolis, Indiana.
Eppendorf®, Eppendorf Multipette® and Eppendorf Combitip® are registered trademarks of Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany.
MicroAmp® is a registered trademark of Applera Corporation or its subsidiaries in the U.S. and certain other countries.
MICROLAB® is a registered trademark of the Hamilton Company.
ProClin® is a registered trademark of Rohm and Haas Company.

Copyright 2006, Roche Molecular Systems, Inc. All rights reserved.

6/2006 00058004262-04

Roche Diagnostics GmbH
D-68298 Mannheim

0088